Acute and Chronic Pathological Effects of Sulfur Mustard on Genito-urinary System and Male Fertility

Yunes Panahi,[1] Mostafa Ghanei,[1] Kamyar Ghabili,[2,3] Khalil Ansarin,[2] Saeid Aslanabadi,[4] Zohreh Poursaleh,[1] Samad Eslam Jamal Golzari,[5] Jalal Etemadi,[6] Majid Khalili,[7] Mohammadali Mohajel Shoja[8]

[1] Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Young Researchers Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[4] Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[5] Cardiovascular Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[6] Chronic Kidney Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[7] Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[8] Medical Philosophy and History Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Corresponding Author:

Kamyar Ghabili, MD
Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Tel: +98 914 410 6136
Fax: +98 411 337 8093
E-mail: kghabili@gmail.com

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Purpose: To review the acute and chronic pathological effects of sulfur mustard on the genitourinary system and male fertility.

Materials and Methods: We searched PubMed and Google Scholar to find studies related to the sulfur mustard-induced genitourinary effects and male infertility. Information in the abstracts of non-English related papers as well as those in the proceedings of congresses on sulfur mustard were reviewed as well.

Results: In acute phase after sulfur mustard exposure, evidences are in favor of microscopic and macroscopic renal lesions, very low androgen levels, and impaired spermatogenesis. Several years following sulfur mustard exposure, the long-term pathological effects vary from the renal function impairment to the gonadal damage, in particular, the spermatogenesis. Nevertheless, carcinogenic effect of sulfur mustard on the genitourinary system as well as the prevalence of male infertility among sulfur mustard-exposed veterans in the chronic post-exposure phase is still unclear.

Conclusion: Sulfur mustard causes both acute and chronic injuries to different parts of the genitourinary system.

Keywords: mustard gas, urogenital system, infertility

INTRODUCTION

First used by the German military at Ypres in September 1917 during the World War I, [bis (2-chloroethyl) sulfide], commonly known as sulfur mustard (SM), is an alkylating chemical agent causing many casualties among enemy forces and civilians upon exposure.[1] Later, SM was employed by the Iraqi forces against Iranian military and civilians, resulting in thousands of medical casualties in the period of 1983 to 1988.[2]

Sulfur mustard exerts direct toxic effects on the eyes, skin, and respiratory system, with subsequent systemic effects on physiological systems.[1] Apart from its acute effects, SM induces a wide range of long-term pathological effects on the skin, eyes, respiratory tract, and immune system, and in some cases on the gastrointestinal tract, cardiovascular, nervous, and genitourinary systems.[3] This review will focus on the acute and chronic pathological effects of SM on the genitourinary system as well as male fertility.

MATERIALS AND METHODS

We searched PubMed and Google Scholar from 1980 to April 2012 to find studies related to the SM-induced genitourinary complications and male infertility using the following terms: "mustard gas, sulfur mustard, vesicant gas, genitourinary, urology, urological, testicular, testes, infertility, fertility, sterility, urinary, kidney, and renal".

Information in the abstracts of non-English related papers as well as those in the proceedings of congresses on SM were reviewed as well. Checking the search results and their references, we found 39 full-text articles (31 in English, 6 in Persian, 1 in Japanese, and 1 in German), 9 abstracts, and 1 book. The articles included original animal and human studies and few case reports. Herein, all the observations and inferences we discuss apply to the SM, but not analogous agents, such as 2-chloroethyl ethyl sulfide (CEES), nitrogen mustard, etc.[4]

KIDNEY INJURY

Animal Studies

Effects of exposure to SM on the renal tissue have been investigated in a number of animal studies. Both percutaneous and inhalation exposure to SM at doses of 1 to 2 LD50 (42.3 to 84.6 mg/m^3) resulted in renal lesions characterized by congestion and hemorrhage. Histopathologically, these lesions included vascular granular degeneration with perinuclear clumping of the cytoplasm of renal parenchymal cells.[5,6] Furthermore, exposure to SM via intraperitoneal injection caused tubular necrosis and urinary epithelial cell sloughing in rats in a time- and dose-dependent manner.[7]

It is believed that oxidative stress or imbalance between the antioxidant enzymes and products of oxidative reactions plays a key role in the pathogenesis of both acute and chronic effects of SM exposure.[4,8] Few studies have investigated the oxidants/antioxidants status in the kidney tissues of the animals exposed to SM. Mouse kidneys showed changes in glutathione metabolism and oxidative stress after subcutaneous injection of butyl 2-chloroethyl sulfide (butyl mustard). Both levels of reduced and oxidized glutathione fell markedly, and after one hour, there was evidence for decreased lipid peroxidation; glutathione peroxidase and glutathione S-transferase activities increased.[9]

The imbalanced oxidants/antioxidants status in SM-exposed animals has been recently corroborated by Boskabady and colleagues.[10] Two weeks after exposure to 100 mg/m^3 inhaled SM, Guinea pigs treated with vitamin E and/or dexamethasone showed significant improvement in the pathological alterations in the kidneys.[10] In contrast, activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase in the renal tissues of the SM-exposed rats, were comparable to those of the control group twenty-four hours after dermal application of a 0.5 LD50 dose of SM in rats.[11]

Apart from the histopathological and biochemical changes in the kidneys following SM exposure through inhalation, the effect of SM toxicity might be reflected in the urinary variables as well. A time- and dose-dependent increase was noted both in blood and excretion of urinary uric acid following inhaled SM exposure in mice. However, creatine and creatinine levels increased significantly in a time-dependent manner only at higher inhaled SM doses.[12] In contrast, oral administration of SM at doses up to 0.3 mg/kg/day did not result in alterations in the levels of blood urea nitrogen and serum creatinine.[13]

Human Studies

A few studies have addressed at the renal complications of SM exposure in human victims. Most of the studies revealing the early effects of SM toxicity on the human kidneys were performed during the period that the Iraqi forces used SM against Iranians (1983 to 1988). Over the first week after SM exposure, only mild transient glycosuria, hematuria, proteinuria, and urobilinogen were detected in urinalysis. In this phase, the patients might complain of oliguria and hematuria.[14]

In contrast, a recent clinical survey on workers in Louisiana exposed to SM failed to detect any abnormalities in post-exposure urinalysis of these subjects.[15] In few cases of severe SM exposure, some degrees of renal failure determined by elevated blood urea and creatinine were notable. Nevertheless, the SM victims had no complaints except urinary incontinence (5%). They showed no significant changes in the urinalysis two months after exposure.[16]

Interestingly, renal pathology at autopsy of SM victims in the acute phase of toxicity was indicative of edema and spotty hemorrhage in the renal glomeruli, desquamation of the renal tubular epithelial cells, acute hemorrhagic nephritis, and tubules containing casts.[14,17]

Several years after SM exposure (chronic phase), no characteristic renal finding could be attributed to the SM toxicity. A self-reported history of urologic conditions and findings was taken 19 to 26 years after high-dose SM exposure. Accordingly, Soroush and coworkers reported a positive history of urinary calculi (17%), recurrent urinary tract infections (9%), benign prostatic hyperplasia (2%), and renal failure (1%) in 289 veterans. They highlighted that the frequency of nephrolithiasis and recurrent urinary tract infections in these individuals was high compared with the normal population. They failed to detect any association between recurrent urinary tract infections, urinary calculi, and other variables, such as age, time interval from exposure to the study, and type or dose of medications. Nevertheless, their study was biased in favor of several medications (eg, systemic corticosteroids), numerous hospitalizations and interventions, and the self-reported nature of the survey.[18]

On the other hand, in a case-series study, Taghaddosinejad and colleagues further delineated the renal pathology several years after SM exposure. Based on the autopsy studies, simple renal cyst and membranoproliferative glomerulonephritis (MPGN) were the most common pathological findings of the kidneys followed by acute pyelonephritis, chronic inflammatory changes in the calyces, and chronic renal failure.[19] As MPGN is an immune-mediated glomerulonephritis usually presenting in childhood or young adulthood, presentation of MPGN in SM victims older than 40 years might be attributed to this chemical warfare agent.

Interestingly, multiple intrarenal abscesses have been reported in an SM victim who received a living-unrelated renal transplant.[20] Sulfur mustard-induced immunosuppression was deemed as a factor predisposing to renal abscess formation in this patient.

CARCINOGENESIS

Literature indicates that SM may have carcinogenic effects in humans, but it is not a potent carcinogen, and perhaps its carcinogenesis depends on the duration of exposure.[1] Although increased risk of renal cell carcinoma in men occupationally exposed to SM (odds ratio, 4.6; 95% confidence interval, 1.7 to 12.5) was reported by Hu and associates,[21] no history of urogenital malignancies was stated among Iranian victims almost 20 years after high-dose SM exposure.[18] Nevertheless, a significant increase was noted in urinary bladder carcinoma several years after occupational exposure to SM.[22] Therefore, urogenital carcinogenicity of SM in humans is still ambiguous.

REPRODUCTIVE HORMONES

Animal Studies

Effects of SM exposure on the reproductive hormones in animals have been less studied. In a study by Kooshesh and coworkers on male rats, intraperitoneal injection of SM (5 and 10 mg/kg) did not lead to significant changes in the serum levels of testosterone and estradiol ten days after exposure.[23] These insignificant findings were indicative of dose-dependent decrease and increase in the serum testosterone and estradiol levels, respectively.[23] Further similar investigations with higher doses of SM exposed in different routes might result in interesting and significant findings.

Human Studies

Iranian SM victims have been studied for hormonal abnormalities since the first post-exposure week. In a study on male SM victims a week after the exposure, serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) did not change compared with the unexposed individuals. However, SM victims had significantly decreased total and free serum testosterone and dehydroepiandrosterone (DHES) levels in the first week after exposure.[24-26] A long-term study by Azizi and colleagues on young SM-exposed men showed the drop in serum levels of total and free testosterone and DHES in the first 5 weeks after exposure and the normalization of these values by the 12th week after injury.[26]

Furthermore, hormonal studies revealed small but significant increase in the serum level of LH by the 3rd week and that of FSH by the 5th week after SM exposure.[26] Further assessments in these patients delineated that administration of gonadotropin-releasing hormone (GnRH) caused no significant rise in the serum levels of FSH and LH. In addition, within three months after the SM exposure, serum levels of 17α-OH progesterone were normal in the victims.[26] Patterns of alterations in the serum levels of total and free testosterone and DHES in the SM victims between 1 to 12 weeks after exposure are illustrated in Figure 1. These studies are indicative of a transient malfunction in the Leydig cells resulting in primary testicular failure following exposure to SM.

During the period between 1 to 3 years after SM exposure, few studies aimed at assessing the reproductive hormonal status of the Iranian SM victims. In a study by Azizi and associates on 42 moderately to severely SM-exposed men aged 18 to 37 years, serum levels of testosterone, FSH, and LH were normal compared with those of the normal individuals.[26] On the contrary, Amini and Hosseinpour found markedly decreased serum levels of testosterone, but not FSH and LH alteration, in the SM victims three years after exposure.[27] The long-term effects of SM exposure on the reproductive hormones have been recently analyzed. Amirzargar and colleagues found normal serum testosterone and LH levels in 64 SM-exposed men twenty years after mild to severe injury. Nonetheless, the exposed men had higher serum levels of FSH compared with unexposed individuals in their study.[28] Although these studies lacked accurate drug history of SM veterans after exposure and resulted in controversial findings, it seems that serum levels of the reproductive hormones are within the normal range in SM-exposed men several years after the injury.

SEMEN INDICES

Animal Studies

The effects of SM exposure on the semen indices have been studied by Sasser and colleagues[29] and Kooshesh and associates.[23] Oral administration of SM at dose of 0.5 mg/kg to rats for ten days resulted in a significant two-fold increase in the total number of abnormal sperm heads and a reduction in percentage of normal sperm with unchanged percentage of sperm motility and concentration.[29] Furthermore, Kooshesh and coworkers found that intraperitoneal injection of SM at doses of 5 and 10 mg/kg into male rats significantly reduced the sperm count compared with the sham group.[23] However, this semen index was not different between the two studied SM doses.[23]

Human Studies

Data addressing the acute and subacute effects of SM exposure on semen indices and related abnormalities are lacking. One to three years following moderate to severe SM exposure, the mean total sperm count of 42 SM victims aged 18 to 37 years was 84 × 10^6/mL.[26] Azizi and colleagues also detected oligozoospermia (total sperm count <20 × 10^6/mL) in approximately one-third of the SM-exposed young men.[26] However, screening of Iranian veterans four years after

Infertility rate among sulfur mustard-exposed and unexposed veterans.

No.	Reference	Time from exposure	Infertility rate (exposed)	Infertility rate (unexposed)
1*	Amirzargar et al.[28]	4 years	22.2%	-
2*	Ketabchi[39]	8 years	23.3%	1.6%
3*	Shakeri et al.[30]	10 years	35%	-
4*	Ghanei et al.[50]	12 years	17.1%	15.1%
5	Ghanei et al.[31]	15 years	8.3%	-
6*	Amirzargar et al.[28]	20 years	22.6%	4.9%
7	Soroush et al.[51]	20 years	2.5%	-

*These studies reported the male infertility rate.

mild to severe SM exposure revealed the mean total sperm count of 172 × 10^6/mL.[28] This difference might be attributed to an enhanced spermatogenesis four years after SM exposure; however, the latter study included the mild SM-exposed victims as well, which might contribute to such discrepancy.

Long-term effects of SM exposure on the semen indices have been investigated in few studies. At least ten years after suspicious SM exposure, the results of semen analysis in 56 individuals were indicative of the sperm abnormalities in 38% of the SM victims aged <55 years.[30] Shakeri and associates reported that the most common semen abnormalities were abnormal sperm morphology (54%) and decreased sperm motility (48%).[30] Fifteen years post exposure, 10% of the SM victims had oligospermia.[31] Twenty years after mild to severe SM exposure, azoospermia and oligozoospermia were reported in nearly 30% of the exposed subjects.[28]

Furthermore, Amirzargar and coworkers noted significant decrease in all the semen indices, including ejaculate volume, sperm concentration, total sperm count, and sperm motility and morphology between four and twenty years after SM exposure. They also found that except for the sperm motility, other semen indices were significantly lower in the exposed than in unexposed casualties twenty years after exposure to SM.[28]

In a recent study on SM-injured and non-SM-injured infertile and fertile men, Safarinejad detected significant decline in semen values (sperm concentration, total sperm count, and sperm motility and morphology) of infertile SM victims twenty years after exposure.[32,33] Interestingly, fertile SM-exposed men had similar findings in their spermograms compared with their non-SM-injured fertile peers.[32] Furthermore, among SM-exposed infertile patients, an inverse correlation was found between the severity of SM exposure and sperm concentration, sperm motility, and sperm with normal morphology.[32] These findings represent progression of the gonadotoxicity of SM in the chronic phase.

It is generally agreed that the major cytotoxic effect of SM arises from DNA damage.[4] In a recent study orchestrated to investigate an association between SM exposure and sperm DNA damage two decades after injury, Safarinejad performed sperm chromatin structure assay (SCSA) on SM-injured and non-SM-injured infertile and fertile men. Accordingly, a significant increase in DNA fragmentation index was noted in SM fertile and infertile casualties in comparison with matched controls. In other words, spermatozoa from SM-injured subjects had more abnormal chromatin than their non-SM-injured counterparts.[32] This interesting finding might imply vulnerability to congenital abnormalities and genetic defects in SM-exposed veterans' offspring created by intra-cytoplasmic sperm injection technique.[32] Through intra-cytoplasmic sperm injection technique, the natural progression of sperm selection is bypassed that might result in direct access of weaker or damaged sperms to a fertile egg.[34]

SEMEN INDICES AND REPRODUCTIVE HORMONES

Azizi and colleagues found no significant difference in the

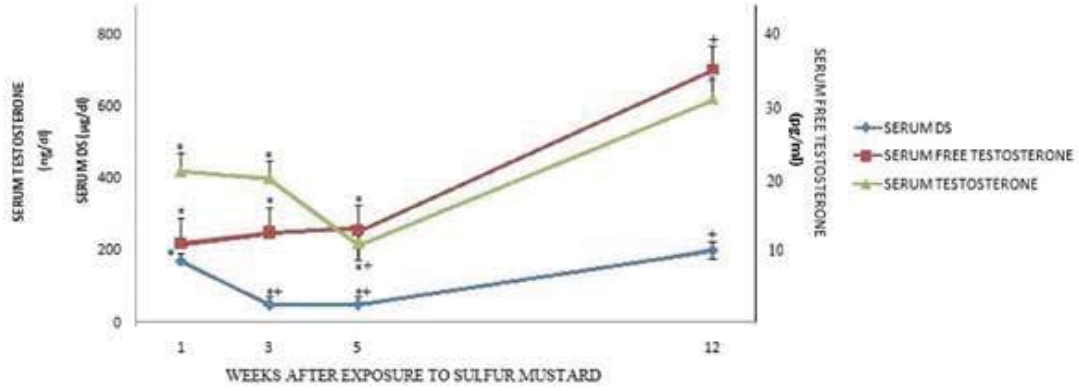

Figure 1. Serum levels of total and free testosterone and dehydroepiandrosterone sulphate (DS) in patients injured by sulfur mustard (SM) 1, 3, 5, and 12 weeks after exposure.
The vertical bars indicate ± standard error of the mean (SEM). *P < .001, as compared to normal controls. +P < .001, as compared to values in the 1st week after SM injury. Modified from Azizi and colleagues' manuscript.[26]

serum levels of testosterone, FSH, and LH between the SM-exposed subjects with (total sperm count <20×10^6/mL) or without oligozoospermia (total sperm count >20×10^6/mL).[26] In their study one to three years after SM exposure, of 29 men who had oligozoospermia, 20 had total sperm counts above 60 × 10^6/mL. Comparing this subgroup of patients with those with oligozoospermia revealed that serum FSH was significantly higher in the latter group. The serum testosterone and LH were not different between these two groups.[26] Interestingly, intensity of SM exposure and level of FSH were found as independent factors associated with the log-sperm count.[35]

Twenty years after SM exposure, low sperm concentration and abnormal sperm counts were found to be significantly associated with a high FSH level. Additionally, sperm concentration and sperm counts were positively correlated with the testosterone level in these subjects.[28] These findings indicate that a reduced sperm count is attributable to a primary testicular injury; a proof supporting the idea of SM gonadotoxicity. Furthermore, arrest of spermatogenesis in testicular biopsies of SM-exposed veterans with oligozoospermia or azoospermia (see below) rules out other pathologic causes of low semen volume, such as ejaculatory duct obstruction.

TESTICULAR HISTOLOGY

Animal Studies

Intravenous injection of SM in male mice resulted in damage to the testes with inhibition of spermatogenesis.[3] In an investigation applying intraperitoneal injection of SM in male rats, Ghahari and associates found dose-dependent alterations in the testicular tissue integrity.[36] Eight weeks after SM injection, increased distance between the seminiferous tubules, presence of necrotic forms of spermatocytes, and necrotic cells with picnotic nuclei in the lumen were detected in the SM-treated rats.[36] In another similar study, Kooshesh and coworkers reported dose-dependent decrease in the testis weight and Johnsen's score (indicative of the maturation of the seminiferous tubules) following intraperitoneal injection of SM in the male rats.[23]

Human Studies

A week after SM exposure, postmortem needle sampling of testicular tissue revealed normal histology.[16] One to three years later, Azizi and coworkers performed testicular biopsy in six young SM victims with oligospermia. The results showed testicular atrophy and complete or partial arrest of spermatogenesis.[26] At least three years after SM exposure, infertile victims showed almost total atrophy of the seminiferous epithelium with intact interstitial cells. Furthermore, the infertile azoospermic SM victims appeared to have a Sertoli cell only pattern in the testicular biopsy (Figure 2).[35] Several years after SM exposure, Amirzargar and colleagues confirmed these findings in the azoospermic exposed veterans.[28] Altogether, it seems that spermatogenesis is the main target of gonadal injury caused by SM.

SEXUAL DYSFUNCTION

Loss of libido was complained by 25% of Iranian SM victims three years after exposure.[16] However, their complaint of the loss of libido increased to 52% one year later.[37] Interestingly, an increase of libido was recorded in 9.7% of the SM victims, which had not been previously reported in the medical literature.[37] In a survey of 800 Iranian men exposed to SM, 35% and 1% of men reported decreased and increased libido, respectively.[38] Eight years after chemical warfare agent exposure mostly to SM, loss of libido was reported in one-third of the veterans; this was significantly higher than unexposed veterans.[39]

Several years following the SM exposure, erectile dysfunc-

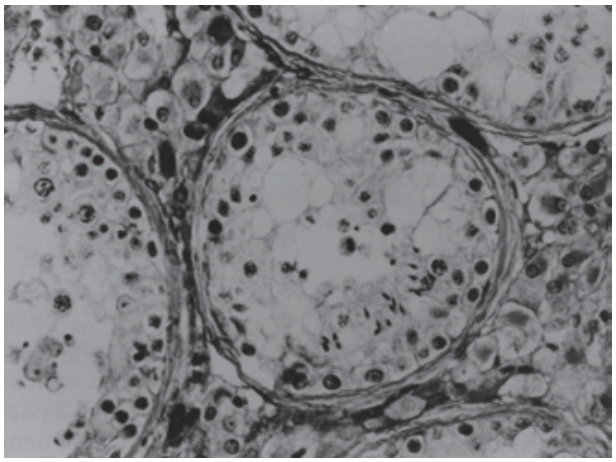

Figure 2. Testicular biopsy from an azoospermic patient exposed to sulfur mustard.
Testicular tubule is lined by Sertoli cells only. Groups of Leydig cells are present in the interstitial tissue. From Safarinejad,[35] reproduced with permission from Elsevier.

tion or impotence was detected in 9% of the victims.[37,39] Furthermore, premature ejaculation was significantly more common among the SM-exposed victims than the unexposed individuals (23.6% versus 4.3%).[39] While erectile dysfunction can be related to the decreased serum testosterone level, the premature ejaculation in SM-exposed veterans seems to be secondary to posttraumatic stress disorder. In other words, psychiatric complications of SM exposure might give rise to a wide range of sexual dysfunctions in SM victims.[40]

GENITAL LESIONS

Sulfur mustard-induced skin lesions present with erythema and subepidermal blisters within hours after exposure.[41,42] When large blisters rupture, full-thickness skin loss followed by ulceration and formation of a necrotic layer or eschar on the affected skin surface develops within a few days.[1,41] These skin lesions usually end with hyper and/or hypopigmentation during healing and this can persist indefinitely, along with scarring.[42,43]

Genital area is one of the anatomical locations associated with severe SM-induced lesions and shorter time to onset of symptoms.[1] The natural characteristic of genitalia gives rise to this susceptibility; moisture covers the thin skin with more hair follicles, leading to a facile penetration by SM.[44] Figure 3 shows the bulla formation on the genital area produced by SM within hours after exposure in 1984.

Several years after SM exposure, non-specific skin disorders, hyperpigmentation, xerosis, and scars at the sites of previous SM-induced skin injuries were the most frequent objective findings.[45-47] The SM-induced scar, incapacitating particularly in the genital area, has been reported to cause stenosis of the external urethral orifice in a number of exposed veterans (Figure 4).[48,49]

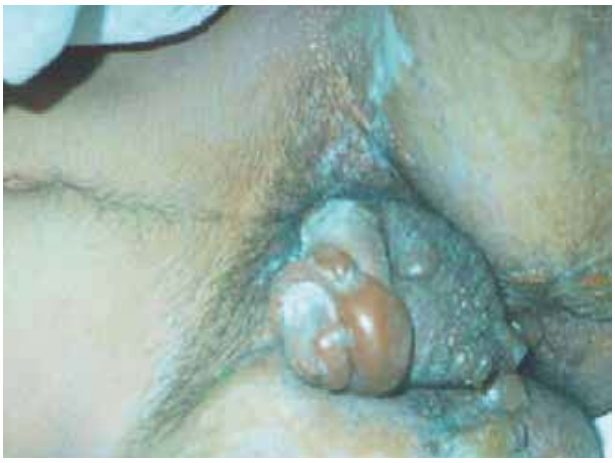

Figure 3. Bulla formation on the genitalia 4 hours after sulfur mustard exposure in the battlefield, 1984. From Emadi and associates,[49] reproduced with permission from John Wiley and Sons.

MALE INFERTILITY

Prevalence of Infertility

There is still a debate on the prevalence of infertility among SM-exposed veterans in the chronic post-exposure phase. Table summarizes the results of different studies in this regard. With definition of infertility as failure to conceive after 12 months of unprotected intercourse after marriage, the long-term prevalence of infertility among SM victims ranged from 2.5% to 35%.[28,30,31,39,50,51] The divergence of infertility rates among the SM victims might stem from numerous factors. Ketabchi studied chemical victims exposed to numerous chemical warfare agents, such as SM, hydrogen cyanide, and nerve agent, among which SM constituted the most commonly exposed agent (85%).[39]

Shakeri and colleagues found male infertility rate of 35% among the victims who had suspicious SM exposure, but not confirmed, at least ten years previously. Their study also lacked the infertility rate among unexposed individuals to be compared with that of the exposed patients.[30] Ghanei and associates concluded that their calculated infertility

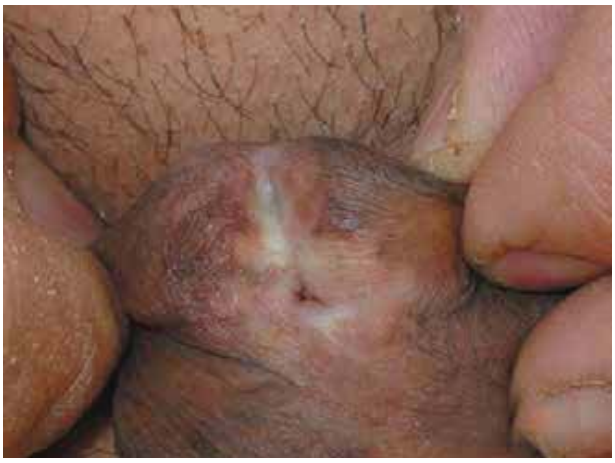

Figure 4. Hyperpigmentation, depigmentation, and meatal stenosis with ventral meatotomy on the glans of penis in a sulfur mustard victim several years after exposure. From Emadi and associates,[49] reproduced with permission from John Wiley and Sons.

rate of 8.3% among SM-exposed couples was comparable to the overall 8% prevalence of infertility among the general Iranian population.[31] Furthermore, it is believed that these controversial findings might be attributed to the variation in the extent of SM exposure of the study subjects;[27] the gonadotoxic effects of SM mostly occur in more severe SM injuries.[35] Further similar studies targeting at the infertility rate among SM victims with particular attention to the extent of SM exposure together with inclusion of the unexposed individuals seem crucial prior to reaching any reliable conclusions in this regard.

Infertility and Reproductive Hormones

The subject of reproductive hormonal status in infertile SM-exposed men has been of some researchers' interest. In the following reports, infertility has been regarded as failure to conceive after 12 months of unprotected intercourse after marriage. In a study on 81 infertile men who had been exposed to SM at least three years previously, Safarinejad found significantly higher serum levels of FSH than the upper limit of normal. However, the serum levels of testosterone and LH were within the normal range. Furthermore, the mean FSH level was significantly higher in severe oligozoospermic (sperm count $<2 \times 10^6$/mL) or azoospermic infertile subjects suffering from severe SM injuries than infertile subjects with moderate and mild injuries.[35]

Recent studies by Amirzargar and colleagues and Safarinejad on Iranian victims twenty years after SM exposure confirmed that infertile SM-exposed men had higher serum levels of FSH than fertile SM victims.[28,32] Moreover, dramatically low serum values of testosterone were not observed more frequently in infertile versus fertile SM-exposed men in the study of Amirzargar and associates.[28] These findings imply the relative resistance of the Leydig cells to SM toxicity along with the seminiferous tubule damage twenty years after SM exposure.

CONCLUSION

Sulfur mustard causes both acute and chronic injuries to different parts of the genitourinary system. In acute phase after SM exposure, evidences are in favor of microscopic and macroscopic renal lesions, very low androgen levels due to transient malfunction in the Leydig cells, and impaired spermatogenesis. Several years following SM exposure, the long-term pathological effects vary from the renal diseases to the gonadal injury, in particular the spermatogenesis. Nevertheless, carcinogenic effect of SM on the genitourinary system as well as the prevalence of male infertility among SM-exposed veterans in the chronic post-exposure phase is still unclear.

Apart from the long-term pathological effects of SM on the eyes, skin, and respiratory tract, clinicians should consider persistent consequences of SM poisoning on the genitourinary system when evaluating a patient with history of SM exposure. Nevertheless, there is a need for further detailed clinical studies focusing on the long-term effects of SM on the genitourinary system and male fertility. For instance, how SM victims have been monitored and treated over years, the effect of age after exposure, the correlation between dose and time of SM exposure with complications, and morbidity rates can constitute indispensable steps towards drawing any conclusions with regard to the chronic genitourinary complications of SM toxicity. Furthermore, information about the abortion rate, teratogenicity, and mutagenicity among infertile SM-exposed men's sibling, if SM victims achieve a pregnancy with their partners, can lead to new clinical findings in this regard.

CONFLICT OF INTEREST

None declared.

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