Microbiological Evaluation of Chronic Blepharitis Among Iranian Veterans Exposed to Mustard Gas: A Case-Controlled Study

Farid Karimian, MD, Siamak Zarei-Ghanavati, MD, Baradaran-Rafii A, MD, Khosrow Jadidi, MD, and Alireza Lotfi-Kian, PhD

Purpose: To evaluate the microbiological characteristics of eyelid margin flora in chronic blepharitis in mustard gas–exposed individuals and compare the results with those in age- and sex-matched unexposed people.

Methods: In this comparative case series, 289 patients with ocular manifestations of mustard gas exposure (case) were evaluated for signs of chronic blepharitis. Additionally, microbiological evaluation of eyelid margins was conducted in these patients and compared with results of 100 unexposed patients with chronic blepharitis (control).

Results: One-hundred fifty (52.0%) of 289 mustard gas casualties had signs of chronic blepharitis. Microbiological evaluation revealed higher isolation rates of *Staphylococcus epidermidis* (78%) and *Staphylococcus aureus* (57%) in the case in comparison to control group ($P < 0.01$). Moreover, *S. aureus* isolated from the cases exhibited greater resistance to common antibiotics compared with control group. Fungi were isolated more frequent in the case compared with controls (30% vs. 4%, $P < 0.01$), with *Cladosporium* and *Candida* species being most common in the case group.

Conclusions: Exposure to mustard gas seems to alter the microbiological flora of the eyelid margin. *Staphylococcus* spp., including antibiotic-resistant strains, and fungi were more frequently isolated in these patients. The relationship between microbial culture results and the severity of ocular surface manifestations in mustard gas–injured cases warrant further investigation.

Key Words: mustard gas, sulfur mustard, chronic blepharitis, ocular chemical injury, chemical warfare agent, microbiological study

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Mustard gas (sulfur mustard) is a potent chemical alkylating warfare agent that was initially used for military purpose in 1917 during World War I and more recently during the Iraq–Iran war. More than 100,000 Iranians have been exposed to this toxic agent, and one-third of those continue to suffer from its prolonged side effects.[1]

One of the most common complications of mustard gas exposure is ocular involvement, which can present as acute, chronic, or late onset.[2] Late-onset complications can present as persistent inflammation (chronic form) or with symptoms emerging after a latent period (delayed form).[3] Chronic blepharitis and dry eye are commonly seen in the late stage and are reported in up to 100% of mustard gas–injured patients.[4] Because of chronic nature of mustard gas keratopathy and accompanying predisposing factors including dry eye, perilimbal conjunctival ischemia, chronic tearing and photophobia, and probable compositional tear film abnormalities, chronic blepharitis can sometimes be overlooked. Etiology of chronic blepharitis is not clearly known; therefore, treatment regimens may often fail. Microbial infection may play a major role, either as a causative or adjunctive agent in chronic blepharitis; therefore, identification of microbial agents and their antibacterial sensitivities may be helpful in management of this condition.

Herein, we report the prevalence of chronic intractable blepharitis among chemical warfare victims and compare microbial profiles isolated from these patients and compare it with age-matched patients suffering from the same disease. To the best of our knowledge, this is the first report describing microbiological findings in chronic blepharitis among mustard gas–exposed patients.

PATIENTS AND METHODS

In this comparative case series, from a total of 289 male patients who had documented mustard gas exposure, those with clinical signs and symptoms of chronic blepharitis, were selected. Microbiological evaluations of the eyelid margin flora were conducted and results compared with those individuals with the same disease but no exposure to mustard gas.

Informed consent was obtained from all participants. All patients in the exposed (case) group had their exposure to mustard gas during the Iraq–Iran war between 1983 and 1988, as documented in reports of the Veterans Foundation of Iran with sufficient evidence of ocular involvement after eye examinations.

Exclusion criteria included: use of antibacterial drugs of any form, treatment of blepharitis within 3 months before sampling, any use of immunosuppressive drugs, diabetes or

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From the Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Labbafinejad Medical Center, Department of Ophthalmology, Tehran, Iran.
Reprints: Farid Karimian, Department of Ophthalmology, Labbafinejad Medical Center, Boostan 9th, Pasdaran Avenue, Tehran 166666, Iran (e-mail: karimianf@yahoo.com).

any debilitating disease, or history of contact lens wearing within 3 months before sampling. As immediate steps in the management of these patients, because of prevalent dry eye state, preservative-free tear film substitute eyedrop (Artelac; Bausch & Lomb), punctual occlusion, and finally lateral tarsorrhaphy were considered.[4] One hundred normal individuals, aged between 30–50 years, with a clinical diagnosis of chronic blepharitis, were considered as the control group.

Clinical Evaluations

Routine ophthalmic examinations were performed for all patients by an experienced ophthalmologist with specific attention paid to ocular signs of exposure to mustard gas and the presence of blepharitis. Ocular manifestations of mustard gas exposure consisted of conjunctival, limbal, and/or corneal findings. Conjunctival and limbal signs included localized vascular abnormalities (tortuosity and telangiectasis), areas of limbal ischemia, and scar. Corneal involvement presented as superficial and/or deep scarring, thinning, peripheral neovascularization, and intrastromal hyaline or lipid-like deposition.[4–6] All cases had sufficient manifestations of mustard gas ocular injuries, along with chronic blepharitis.

These clinical signs were diagnostic of chronic blepharitis: thickening of eyelids, eyelid margin vascularization and hyperkeratinization, eyelid margin notching, meibomian gland orifice inspissations and hyperemia, foamy (lipid) secretions, scaling and crusting, eyelash changes (madarosis, poliosis, trichiasis, and broken eyelash), bulbar conjunctival injection, and corneal involvement (inferior punctate erosions, pannus, and scarring) (Fig. 1). Patients in the case group had prominent signs of posterior blepharitis (chronic meibomianitis) with less striking presentation of anterior blepharitis.

Bacteriologic Evaluation

Bilateral culture sampling of the upper and lower eyelid margins was performed with sterile swabs moistened with balanced salt solution without application of topical anesthetics. The swabs were passed twice along the eyelid margin from the medial toward the lateral canthus. The swabs were immediately inoculated onto blood and chocolate agar plates for aerobic bacteria, anaerobic blood agar for anaerobic bacteria, and Sabouraud dextrose agar with chloramphenicol for fungal agents. The swab was then placed in thioglycolate broth. Corn meal agar with Tween 80 media was used for better detection of *Candida* species. Gram-stained smears were used for direct detection of bacteria. Moreover, KOH-prepared and lactophenol cotton blue–prepared direct smear microscopy were performed for fungal study.

Sabouraud dextrose agar cultures were incubated between 25 and 30°C (room temperature) for 4 weeks. Other cultures were incubated at 35°C. Anaerobic cultures were incubated in the candle jar containing 3%–10% carbon dioxide and were examined at days 2, 5, and 7.

Thioglycolate cultures were examined every day up to 7 days, and if there was any opacity in the media, it was subcultured. Bacterial colonies were identified by standard laboratory tests. Sensitivities of *Staphylococcus aureus* cultures to commonly used antibiotics were determined by Bauer–Kirby disc method in Mueller–Hinton agar.

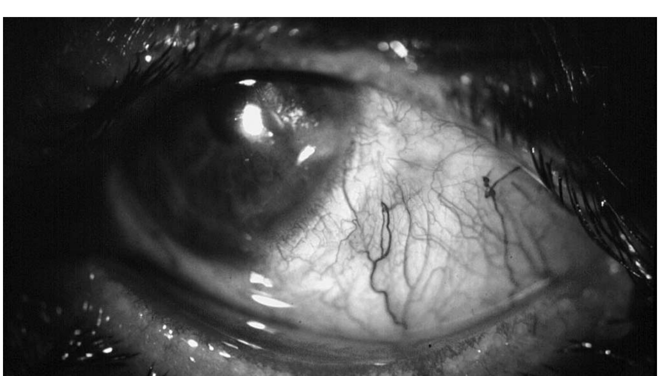

FIGURE 1. Slit lamp photograph of a mustard gas–injured patient with prominent signs of chronic blepharitis and conjunctival and limbal inflammation.

Statistical Methods

The statistical analysis was performed using SPSS 15 (IBM, Chicago, IL). The common culture-positive rates were analyzed separately in each group and compared by χ^2 test or the Fischer exact test (for an expected value smaller than 5). P values less than 0.05 were considered statistically significant.

RESULTS

In this study, 150 of 289 male mustard gas casualties (52.0%) with chronic blepharitis were enrolled (case group). The control group consisted of 100 age- and sex-matched individuals with similar eyelid disease. Mean age was 41.3 ± 7.2 (35 to 49) years in the case and 40 ± 8.6 (31 to 50) years in the control group ($P > 0.05$). All patients in both groups were of male sex.

Isolated bacteriologic culture results from the case group showed *Staphylococcus epidermidis* in 117 patients (78%), *Propionibacterium* spp. in 96 (64%), *S. aureus* in 45 (30%), *Corynebacterium* spp. in 42 (28%), *Staphylococcus saprophyticus* in 14 (9.3%), other strains of *Staphylococcus* spp. in 22 (14.6%), and *Micrococcus* spp. in 8 (5.3%) (Table 1).

The most commonly isolated bacteria from the control group in descending order of frequency were as follows: *S. epidermidis* in 57 (57%), *Propionibacterium* spp. in 51 (51%), *Corynebacterium* spp. in 32 (32%), *S. saprophyticus* in 15 (15%), *S. aureus* in 3 (3%), other strains of *Staphylococcus* spp. in 10 (10%), and *Streptococcus viridians* in 4 (4%) (Table 1). Because more than 1 organism and bacteria were isolated from each single patient, the total number of positive culture results becomes more than the patients' number, and finally the percentages exceed 100.

Negative culture results were seen in 5 (3.3%) cases and 11 (11%) in the control group. The difference in *S. aureus* and *S. epidermidis* culture-positive rates between these 2 groups was statistically significant, being higher in the exposed (case) group ($P < 0.001$) (Table 1).

The results of antibacterial susceptibility tests for *S. aureus* in the case group showed that most isolates were

TABLE 1. Frequency of Isolated Bacteria from Eyelid Margin in Case and Control Groups

Isolated Microorganism	Case Group, n (%)	Control Group, n (%)	P*
S. epidermidis	117 (78)	57 (57)	<0.01
Propionibacterium spp.	96 (64)	51 (51)	NSS
S. aureus	45 (30)	3 (3)	<0.001
Corynebacterium spp.	42 (28)	32 (32)	NSS
Staphylococcus, other species	22 (14.6)	10 (10)	NSS
S. saprophyticus	14 (9.3)	15 (15)	NSS
Micrococcus spp.	8 (5.3)	0 (0)	NSS
Fungi	45 (30)	4 (4)	<0.01

*χ^2 test.
NSS, not statistically significant.

sensitive to aminoglycosides (gentamicin and amikacin) (97%), chloramphenicol (91%), erythromycin (86%), and tetracycline (75%), whereas only 48% of *S. aureus* cases were sensitive to oxacillin, suggesting some degree of methicillin-resistance in these isolates. *Staphylococcus aureus* cultures in the control group showed sensitivity to all aforementioned antibiotics.

Among the exposed (case) group, 45 patients (30%) showed positive cultures for fungal species compared with only 4 (4%) in the control group. Direct microscopic studies were consistent with culture results in these patients. Fungal classification was limited to the genus level. *Cladosporium* spp. was the most commonly isolated fungi in both the case and control groups (8% vs. 2%, respectively) ($P < 0.05$). *Candida* spp. (including *Candida albicans*) were cultured more frequently in mustard gas–injured patients (8%) ($P < 0.05$) (Table 2). *Candida* spp. becomes as common as Cladosporium spp. if all of their species are considered together.

DISCUSSION

Sulfur mustard [bis (2-chlorethyl) sulfide] is a highly cytotoxic agent that rapidly penetrates cells and alkylates their components, especially DNA, RNA, proteins, and lipid membranes.[7] It causes further damage by forming free radicals[6,8,9] and destruction of nucleotides.[10] Moreover, mustard gas releases inflammatory mediators that may be responsible for ongoing chronic complications.[11–13] However, the exact pathogenesis of toxic effects is not clear.

TABLE 2. Fungal Species Isolated from Eyelid Margins in Case and Control Groups

Fungus Species	Exposed Case Group, n (%)	Control Group, n (%)	P*
Cladosporium spp.	12 (8)	2 (2)	<0.05
Candida spp.	12 (8)	1 (1)	<0.05
Acromonium spp.	6 (4)	0 (0)	NSS
Alternaria spp.	3 (2)	0 (0)	NSS
Stemphylium spp.	3 (2)	0 (0)	NSS
Penicillium spp.	3 (2)	0 (0)	NSS
Nigrospora spp.	3 (2)	0 (0)	NSS
Aspergillus spp.	3 (2)	1 (1)	NSS
Total	45 (30)	4 (4)	<0.01

*χ^2 test.
NSS, not statistically significant.

Although mustard gas has a relatively low acute lethal impact, it causes significant morbidity. It mainly affects the eye, skin, and respiratory system. The eye is the most sensitive organ, with ocular morbidities seen in 75%–90% of casualties.[2,14] This striking susceptibility of the eye is attributed to the affinity of the mustard gas to the lipid layer of the tear film and the rapid turnover of corneal epithelial cells.[2,6,14]

Ocular manifestations are classified into acute, chronic, and delayed onset stages.[2] Delayed onset ocular manifestations include blepharoconjunctival, limbal, and corneal involvement. Chronic blepharitis is a common complication among mustard gas–exposed patients. Javadi et al[4] reported common manifestations of the late stages of mustard gas injury that included chronic blepharitis, meibomian gland dysfunction, dry eye, limbal ischemia, stem cell deficiency, and corneal changes. They reported chronic blepharitis and dry eye in all patients in their case series. Clinical chronic blepharitis was seen in 52% of our patients. This discrepancy is probably related to patient selection in these 2 studies. Javadi et al[4] included only severe cases, describing corneal scar and opacity in 87.5% of their patients. In our study, however, we considered all range of mustard gas–injured patients including those with mild to severe toxic damage.

Although chronic blepharitis is one of the most common diseases seen by ophthalmologists, it may still remain a diagnostic and therapeutic dilemma. Based on our experience, treatment of blepharitis in mustard gas–exposed patients may be more challenging. This is related, to some extent, to a lack of data and a poor understanding of the pathogenesis of chronic blepharitis in these patients.

Thygeson[15] was the first to speculate colonization of the eyelid margins by bacteria as the pathogenesis of blepharitis and advocated antibiotics for its treatment. This study showed that results of cultures in cases of chronic blepharitis were quite different in 2 groups of mustard gas–exposed and mustard gas–nonexposed patients. *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Corynebacterium* spp. are the most common isolated bacteria from eyelid margins of patients with chronic blepharitis in this study similar to previous studies.[16,17] But here, *Staphylococcus* spp. were more common in the mustard gas–exposed group than in the control group. It is known that exotoxins from these bacteria have lipase activity,[18,19] which may contribute to some of the ocular manifestations seen in cases of mustard gas injury. However, the products of hydrolysis will again stimulate proliferation of *Staphylococcus* spp.[20]

This study showed that growth of fungi, especially *Candida* spp., was significantly more common in the eyelid margin cultures of the exposed group. Huber-Spitzy et al[21] studied patients suffering from both atopic dermatitis and chronic blepharitis. In that study, only positive cultures for *Candida* spp. were seen in patients with ulcerative blepharitis. They suggested *Candida* spp. can cause ulcerative blepharitis in the presence of severe inflammation. Chronic ocular inflammation is a constant feature in mustard gas exposure. Therefore, *Candida* spp. may potentiate both the severity and the resistance to routine treatment of chronic blepharitis seen in these patients.

Changes in the microbiological environment and the presence of unusual microbial pathogens in the eyelid margin flora of patients exposed to mustard gas may be explained by the effects of this toxin on the local immune system of the ocular surface, tear film structure, and the basal cell function in both cornea and conjunctiva (even in the meibomian glands).[11–13] This remains a speculation, until further details about this microenvironment are better clarified. Mutagenic effects of mustard gas on fungal or bacterial DNA and/or secondary activation of latent fungi or bacteria by exposure to that toxin are possible. These could result in a remarkable change in microbial flora and their sensitivity to various antibiotic agents. Effects of mustard gas can also be because of hydrolysis by-products that can alter bacterial and fungal growth and their morphologic and cultural properties. Also, increased dehydrogenase activity is accompanied by an increase in number and size of mitochondria in the fungal cells exposed to mustard gas.[22]

Chronic and recalcitrant course of blepharitis in mustard gas–exposed cases may be because of the causative microbial agents. The higher frequency of fungi isolated from these cases with chronic blepharitis may be responsible for the higher rate of treatment failure. Also, according to our results, this may be related to the relatively higher antibiotic resistance displayed by staphylococcal species in cases of mustard gas–exposed patients, where a number of isolates can also be considered methicillin-resistant *S. aureus*. Treatment in these cases must be initiated more aggressively and continued for a longer duration to achieve better results. We cannot be certain about application of antifungal agents in treatment of these patients. Finally, the relationship among the severity of blepharitis, culture results, and ocular surface presentations are issues that could be addressed in future studies.

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