



Status of serum antibodies against *Mycobacterium tuberculosis* among sulfur mustard-poisoned Iranian veterans three decades after exposure



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ABSTRACT

Background: Sulfur mustard (SM)-exposed veterans suffer from chronic respiratory complications. Many receive immunosuppressive therapy to alleviate pulmonary inflammation, which may create a favorable environment for opportunistic pathogens such as *Mycobacterium tuberculosis* (TB). Timely diagnosis and treatment of TB could improve their respiratory status. This study aimed to assess serum levels of specific anti-TB antibodies (IgM, IgG, IgA) as a screening tool to evaluate TB status in this population.

Methods: Serum anti-TB antibody levels were measured using commercial ELISA kits (Alpha Diagnostic, USA) in 35 male Iranian veterans with at least 25% disability due to SM poisoning three decades earlier.

Results: Titers of all anti-TB antibody classes (IgM, IgG, IgA) were below the cut-off value (<8 U/mL) for all veterans, indicating negative results. No borderline (8–12 U/mL) or positive (>12 U/mL) results were observed.

Conclusion: The undetectable antibody levels may reflect SM-induced dysfunction of both innate and adaptive immunity. Alternatively, the results could indicate the absence of active TB and suggest that revaccination against TB might be considered for these veterans by their physicians.

Keywords: *Mycobacterium tuberculosis*; Anti-TB antibodies; Veterans; Sulfur mustard.

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Introduction

Chemical warfare agents were first used on a large scale during World War I. Since then, they have been employed on several occasions, with the Iran–Iraq conflict representing the most devastating chemical warfare episode (1). Sulfur mustard (SM) is a vesicant chemical agent that was extensively used by Iraqi forces against Iranian military personnel and even civilians. The toxicity of SM arises from irreversible alkylation of certain proteins and nucleic acids, particularly DNA. Initially, SM forms a cyclic ethylene sulfonium ion intermediate, followed by electrophilic attack on target molecules, which can lead to inhibition of proteins, nucleic acids, and adenosine triphosphate biosynthesis (2–4).

Early and delayed complications of SM toxicity

primarily affect the eyes, skin, and respiratory tract, the three main target organs in humans (2). Both upper and lower respiratory tracts are involved in SM poisoning, potentially leading to chronic respiratory complications. In a study of 236 Iranian veterans with delayed complications of SM exposure, the most frequently affected organs were the respiratory system (78%), skin (41%), and eyes (36%). Respiratory complications are the most common cause of long-term disability among SM-exposed individuals (5). In the lungs, SM can cause bronchial mucosal damage, inflammation, fibrosis, and pneumonia (2, 5, and 6).

Chronic respiratory diseases are frequently observed in chemically injured veterans. SM-induced chronic pulmonary diseases

encompass a wide spectrum of manifestations, including chronic bronchitis, bronchiolitis, bronchiectasis, tracheal and main bronchial stenosis, airway hyperreactivity, and pulmonary fibrosis. Respiratory tract infections are among the factors that exacerbate the symptoms of chronic pulmonary diseases. The severity of these complications – presenting as chronic cough, dyspnea, and sputum production – ranges from mild to severe. A notable feature of this condition is periodic exacerbation of symptoms due to infection, increased tracheobronchial secretions, and intensified bronchospasm (7).

Chemically injured veterans often receive immunosuppressive drugs for respiratory diseases. In addition, their immune systems are already compromised by SM's immunotoxic effects. Together, these factors may create a favorable environment for opportunistic bacteria such as *Mycobacterium tuberculosis* (TB) (8–11). If active or latent TB is present in these veterans and appropriate treatment is administered, the severity of their suffering, largely related to the respiratory system, could be reduced. Because routine TB diagnosis via sputum collection can be challenging in some cases, and because addressing the health problems of chemical veterans remains a national research priority, this study aimed to measure serum anti-TB antibody levels. Positive results (in unvaccinated individuals) would prompt further diagnostic work-up for active TB and appropriate treatment, while negative results would inform decisions regarding vaccination.

Methods

Study population and participant selection

Based on medical records from the Veterans' Foundation, a list of veterans exposed to SM three decades earlier was prepared. Our primary target was to evaluate veterans with at least 40% disability due to mustard gas poisoning. However, due to logistical constraints, 35 male veterans with a minimum of 25% disability were ultimately enrolled.

Ethics approval and informed consent

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1396.58). Written informed consent was obtained from all participants.

Blood sample collection

Blood samples were collected using standard procedures. In the supine position, 10 mL of blood was drawn from the left antecubital vein. Fresh blood samples were collected in sterile tubes.

Serum separation and storage

Sera were separated by centrifugation and stored at -20°C until analysis of anti-TB antibodies using commercial ELISA kits specific for human samples (Alpha Diagnostic, USA).

ELISA procedure

According to the kit manufacturer's protocol, all ELISA reagents were brought to room temperature before analysis. The required number of strips was arranged and labeled. Then, 100 μL of control, standards, and 1:100 diluted serum samples from veterans were added to the respective wells pre-coated with TB-specific antigen and incubated for 60 minutes at room temperature. After incubation, the contents were aspirated, and the wells were washed three times. Subsequently, 100 μL of conjugated antibody reagent was added to each well and incubated for 30 minutes at room temperature. After aspiration and three washes, 100 μL of substrate-chromogen solution was added and incubated for 20 minutes at room temperature. Finally, 100 μL of stop solution was added, and the optical density of each well was read at 450 nm using a Stat Fax ELISA reader.

Results

Demographic information

Demographic and clinical information of SM-poisoned veterans is presented in Table 1.

Table 1. Demographic and clinical characteristics of SM-poisoned veterans (n=35).

Characteristic	Value
Age (years)	
Range	46 – 78
Mean $\pm$ SD	62.0 $\pm$ 8.0
Disability due to SM exposure (%)	
Range	25 – 70
Mean $\pm$ SD	49.2 $\pm$ 14.13
Year of poisoning	1983-1988
Time since poisoning (years)	
Range	28 – 33
Mean $\pm$ SD	30.0 $\pm$ 1.4
Height (cm)	
Range	157 – 182
Mean $\pm$ SD	168.6 $\pm$ 6.0
Weight (kg)	
Range	58 – 122
Mean $\pm$ SD	77.06 $\pm$ 13.31
Body mass index (BMI, kg/m^2)	
Range	21.2 – 36.8
Mean $\pm$ SD	27.0 $\pm$ 3.82

Table 2. Serum levels of anti-TB antibodies by isotype in SM-exposed veterans (n=35).

Antibody isotype	Number of veterans with titer <8 U/mL	Interpretation
Anti-TB IgM	35 out of 35 (100%)	Negative
Anti-TB IgG	35 out of 35 (100%)	Negative
Anti-TB IgA	35 out of 35 (100%)	Negative

Note:

Cut-off values according to the kit manufacturer: negative <8 U/mL, borderline 8–12 U/mL, positive >12 U/mL.

Serological findings of anti-TB antibodies

In the present study, serum levels of three isotypes of anti-TB antibodies (IgM, IgG, and IgA) were measured in 35 veterans with a history of SM exposure dating back approximately three decades. Quantitative ELISA analysis demonstrated that all participants had antibody titers below the manufacturer-defined cut-off value of <8 U/mL. According to the kit manufacturer, titers exceeding 12 U/mL are considered positive, while values between 8 and 12 U/mL are classified as borderline. None of the veterans showed titers in the borderline or positive range.

As summarized in Table 2, a separate breakdown by antibody isotype revealed that for anti-TB IgM, all 35 veterans (100%) had titers below 8 U/mL. Similarly, for anti-TB IgG, all 35 veterans (100%) had titers below 8 U/mL. Likewise, for anti-TB IgA, all 35 veterans (100%) had titers below 8 U/mL. Thus, the serological status for all three isotypes was uniformly negative across the entire study population.

In summary, all 35 chemically injured veterans exhibited anti-TB antibody titers below the diagnostic threshold for IgM, IgG, and IgA, indicating a complete absence of detectable humoral immune response against TB in this cohort.

Discussion

The present study was designed to evaluate serum levels of specific anti-TB antibodies (IgM, IgG, and IgA) in Iranian veterans who had been exposed to SM approximately three decades earlier. The rationale for this investigation stemmed from two key observations: first, SM-exposed veterans suffer from chronic and often progressive respiratory complications (2, 5, and 6); second, many of these individuals receive immunosuppressive medications to manage pulmonary inflammation, which may increase

susceptibility to opportunistic infections, including tuberculosis (8–11). The results of our study demonstrated that all 35 veterans had anti-TB antibody titers below the cut-off value (<8 U/mL), indicating a uniformly negative serological status. In the following discussion, we interpret these findings in light of the known immunotoxic effects of SM and explore their potential clinical and public health implications.

At first glance, the absence of detectable anti-TB antibodies in all participants might suggest that none of these veterans had been recently exposed to TB or developed an active or latent infection. It is also important to note that BCG vaccination status and prior TB history were not systematically recorded in our cohort. Therefore, while SM induced immune dysfunction remains a plausible explanation, we cannot exclude the possibility that these veterans were never immunologically exposed to TB antigens or that the ELISA assay lacks sufficient sensitivity in this specific population. Thus, negative serology should not be equated with the absence of latent TB infection (12).

However, this interpretation must be approached with caution for several reasons. First, the seroprevalence of anti-TB antibodies in the general Iranian population, particularly among older adults who may have been vaccinated with BCG or exposed to environmental mycobacteria, is not negligible. It seems epidemiologically implausible that a cohort of 35 middle-aged individuals with chronic respiratory disease would have had absolutely no contact with TB over three decades. Second, and more importantly, SM is a potent immunosuppressive and immunotoxic agent that has been shown to cause long-lasting damage to both innate and adaptive immune systems (8–11).

Accumulating evidence indicates that SM exposure leads to persistent abnormalities in immune cell populations and their functions. Riahi-Zanjani et al. (8) reported that SM-poisoned veterans exhibit significant alterations in innate immune parameters, including reduced phagocytic activity of neutrophils and impaired function of natural killer cells. Furthermore, the same research group demonstrated that the acquired immune system is also compromised, as evidenced by abnormal serum cytokine profiles (10) and altered lymphocyte subpopulations (11). Specifically, Balali-Mood et al. (11) found decreased levels of

CD⁴⁺ T cells and a reduced CD4/CD8 ratio in SM-exposed veterans, which can impair humoral immune responses to both recall and novel antigens.

In the context of our study, it is conceivable that the B-cell compartment, which is responsible for producing anti-TB antibodies, is functionally deficient in these veterans. Even if they had been exposed to TB (either through natural infection or BCG vaccination), their ability to mount and maintain a humoral response may be severely blunted. This could explain why the titers of all three isotypes (IgM, IgG, and IgA) fell below the detection limit. Notably, IgG is the predominant isotype generated upon secondary exposure or re-exposure to TB antigens; the absence of specific IgG is particularly striking and may reflect a failure of immunological memory, a hallmark of adaptive immunity that is often compromised after SM poisoning.

An alternative, though not mutually exclusive, interpretation is that the negative serological results genuinely reflect the absence of active or recent TB infection in this cohort. Because all participants had chronic respiratory symptoms that could be attributed solely to SM-induced lung damage (e.g., chronic bronchitis, bronchiectasis, fibrosis), none of them may have developed TB. If that is the case, then the uniformly low antibody titers, particularly the lack of anti-TB IgG, might indicate that these veterans are immunologically naive to TB and could benefit from vaccination. Although most Iranian adults receive the BCG vaccine during childhood, its protective efficacy wanes over time, and revaccination is not routinely performed. Given the negative serology, one might theoretically consider TB revaccination in these veterans. However, this remains a highly speculative suggestion. Because SM-exposed individuals may have significant, yet incompletely characterized, immune dysfunction, the use of live attenuated BCG vaccine could be potentially harmful. Therefore, any decision regarding revaccination must be individualized and made only by an experienced clinician after a thorough immunological evaluation and the exclusion of active TB. We do not recommend routine revaccination based on our findings (13). Conventional diagnostic methods for pulmonary TB include sputum smear microscopy, culture, and molecular tests such as PCR (14, 15). While

these methods are highly specific, they suffer from variable sensitivity, particularly in paucibacillary cases or when patients are unable to produce adequate sputum samples. Serological tests, including those measuring anti-TB antibodies, have been largely discouraged by the World Health Organization due to their poor performance in endemic settings (16). Nevertheless, in certain high-risk populations with known immune dysfunction, serological screening may provide complementary information. Our results suggest that in SM-exposed veterans, a negative anti-TB serology does not necessarily rule out latent TB infection because of the possibility of false negativity due to immunosuppression. Therefore, clinicians should not rely solely on serology; rather, they should consider further diagnostic work-up, including interferon-gamma release assays (IGRAs) or tuberculin skin tests (TST), along with chest imaging and, if indicated, sputum molecular testing (17).

Limitations of the study

First, the sample size was relatively small (n=35), and all participants were male, which limits the generalizability of our findings to female veterans or other SM-exposed populations with different disability grades. Second, we did not measure cellular immune responses (e.g., via IGRA or TST) that could have provided a more comprehensive assessment of TB-specific immunity. Third, the functional status of B cells and plasma cells was not directly evaluated; we only measured serum antibody levels without assessing the avidity or neutralization capacity of the antibodies. Fourth, information regarding the participants' BCG vaccination status and any history of TB treatment was not systematically collected, which could have helped distinguish between primary non-response and vaccine-induced immunity. Finally, the cross-sectional design does not allow us to determine whether the low antibody titers were a consequence of SM exposure or pre-existing immune deficits.

Recommendations for future research and clinical practice

To better understand the interplay between SM-induced immunotoxicity and the risk of TB, future prospective studies with larger cohorts are warranted. Such studies should incorporate both

humoral and cellular immune assays, including IGRAs, lymphocyte proliferation tests, and measurement of B-cell subsets (e.g., memory B cells). Long-term follow-up of veterans to monitor the incidence of active TB would also provide valuable data. From a clinical perspective, physicians caring for SM-exposed veterans with chronic respiratory symptoms should maintain a low threshold for investigating TB, using a combination of clinical, radiological, and microbiological methods rather than relying solely on serology. In selected cases where no evidence of active TB is found, and the patient's immune status is deemed adequate, revaccination against TB might be considered after careful risk-benefit analysis.

Conclusion

In summary, this study found that all evaluated SM-poisoned Iranian veterans exhibited undetectable serum levels of anti-TB antibodies (IgM, IgG, and IgA) three decades after exposure. This finding is most plausibly attributed to the long-lasting immunotoxic effects of SM on the humoral immune system. While the results may also indicate the absence of current TB infection, the possibility of false-negative serology due to immune dysfunction should not be overlooked. Therefore, additional diagnostic tests are recommended to rule out latent or active TB in these vulnerable individuals. Furthermore, the role of revaccination against TB in this population deserves further investigation. Ultimately, a multidisciplinary approach involving pulmonologists, immunologists, and infectious disease specialists is essential to optimize the respiratory health of SM-exposed veterans.

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