

Assessment of Allergic Markers and Atopic Phenotype among Sulfur Mustard Induced Small Airway Diseases with Persistent Wheezes in Iranian Veterans; Post Wartime

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 29 Mar 2014
Revised: 17 Feb 2015
Accepted: 25 Mar 2015

Keywords:
Sulfur mustard
Small airway diseases
Wheeze
Allergy
Atopic phenotype
Immunoglobulin E antibody
Iranian
Adults

ABSTRACT

Background: A wheezes is originated from small airway obstruction. Phenotypes of the wheeze have different in causal factors etiology. Persistent wheeze detects in allergic and atopic disorders. Sulfur mustard (SM) is a potential chemical warfare agent. It can develop small airway diseases (SAD) with wheezes. The aim of the study was assessed prevalence of allergic markers and atopic phenotype among sulfur mustard induced small airway diseases with the persistent wheeze in Iranian veteran's post wartime.

Methods: The study designed in two stages; diagnosis of SM induced SAD and detection of atopic subjects. Skin prick test, Total immunoglobulin E antibody's concentration in serum and peripheral blood eosinophil count applied on samples of study.

Results: Sixty-three male chemical veterans associated with SAD meet criteria of the study. Chronic obstructive pulmonary disease (COPD) and asthma diseases had prevalently the frequency in focus population. Frequency of allergic status and atopic phenotype was observed in 56% and 22% of study population, respectively. Mean age recorded 45.98 ± 9.99 SD years (ranged 35-67). The Mean \pm SD of total IgE (TIgE) concentrations and peripheral blood eosinophilia were 283.15 ± 263.86 Iu/dl (range 5-900) and 266.60 ± 240.94 . A highly relevant difference in TIgE denoted between allergic and non-allergic subgroups ($P < 0.001$). There were significant differences between allergic status with atopic phenotype ($\chi < 0.001$).

Conclusion: The study demonstrated the noticeable prevalence of allergic marker and atopic phenotype among SM induced SAD with persistent wheezes. They markedly distributed in the subgroup of bronchial asthma and COPD asthma-phenotype of target population.

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► *Implication for health policy/practice/research/medical education:* Allergic Markers and Atopic Phenotype among Sulfur Mustard Induced Small Airway Diseases

► *Please cite this paper as:* Agin Kh. Assessment of Allergic Markers and Atopic Phenotype among Sulfur Mustard Induced Small Airway Diseases with Persistent Wheezes in Iranian Veterans; Post Wartime. International Journal of Medical Toxicology and Forensic Medicine. 2015; 5(3): 117-25.

1. Introduction:

Wheeze is a continuous adventitious lung sound, and reflected as a symptom of airway's obstruct. It finds more prevalently within respiratory diseases with asthma /asthma like symptoms. Wheeze is generally presented by the patients. Incidence of wheezing illness is 43% in the middle age group (1), and the wheeze sign is 25.5% in rural population (2). Its features are detected in the clinic as transient or permanent signs.

Sulfur Mustard (SM) is a chemical warfare agent (3). It used in a large quantity in war between Iraq-Iran during 1981-1989. It caused the deaths of many military personnel as well as civilians of Iran. At least, up to 50,000 incurred chemical veterans have still been survived in Iran (4).

Respiratory system is a susceptible and the most common target organ to toxic effects of SM. Iranian's studies revealed that SM can be induced small airway diseases (SAD) (5) and asthma (6, 7). However, bronchiolitis obliterans was reported as a late pulmonary complication of SM. It can mimic clinically an asthma pattern (8).

Animal model of study showed that effect of intratracheal SM aerosol leads to asthma-like symptoms (9). Bronchial hyper-reactivity to methacholine test was detected among SM community (10). Clinical features of SAD can be manifest in asthma-like symptoms with wheezes.

The concept is that persist bronchial asthma patterns between inducing SM diseases with wheeze. Phenotypic Patterns of asthma are classified as allergic and non-atopic. A link between allergy and asthma has long been recognized (11). Atopic phenotype is an important risk factor for bronchial asthma with or without clinical symptoms (12). However, a significant fraction of atopic

subjects will be susceptible to developed asthma at some time in their live (13). Report from several studies indicated that onset of manifestation, Clinical features, reaction to stimulant and response to therapy of both phenotypes of asthma are somewhat different from each other (14-16).

The aim of the study was assessed prevalence of allergic markers and atopic phenotype among sulfur mustard induced small airway diseases with wheeze in Iranian veteran's post wartime.

2. Materials and Methods:

The study was cross sectional. It conducted with collaboration of Janbazan foundation (The Iranian Veteran's Affairs Agency) and Shahid Beheshti University of medical sciences (SBUMS), Loghman Hakim general teaching hospital, Tehran, Iran in 2004-2005.

The samples' population enrolled among SM exposed during 1983-1988. They were sequentially recruited among patients treated in the outpatient chest clinic. The study designed in two stages, I- detection of SM induced SAD with wheezes and II- diagnosis of phenotypes of atopic from the non-atopic pattern.

First step was detection of SM induced SAD diseases with wheezes (asthma/ asthma like) based on the following protocol; male, positive history of SM exposing, current wheezes symptom and wheezes over last three years.

A self-reported questionnaire administrated among targets of study. Highlight topics included demographic data, smoking habit, medications and history data of SM exposed. General information about the chemical contact included types of chemical exposed, conflict zones, clinical features of exposing during contact. However, modified asthma, asthma like symptoms and chronic bronchitis symptoms added to the questionnaire. They consisted of wheezing, chest tightness, cough, shortness attacks episode of breath during the last 12 months, using asthma medications, whether a physician even made diagnosis asthma (17) sputum, seasonal variation symptoms.

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Physician considered confirmation of SM exposure as personally, interview and recording information, review of previous medical records. The subjects of the study should have history of chemical episodes in the known areas; for more confirmation of the data. All the subjects had 100% certification of Janbazan foundation.

Pulmonary physician confirmed SAD via examination and standard chest x-ray, pulmonary function testing, and evaluation of previous medical records. American Thoracic Society (ATS) guideline (18) and global initiative for chronic obstructive lung disease (COLD) applied on the subjects (19). The exclusion criteria consist of pulmonary tuberculosis, generalized eczema and smoking habit. Medications used by the subject such as; beta-blocker therapy, using antihistaminic medications two weeks prior the study (astemizole does not use in Iran) and tricyclic anti-depressants. Steroids and beta-agonists do not interfere with a skin prick test (20).

There was a 4-6 month time lag between the first and second stages of study. All practical work in second stage was undertaken in the winter months.

Second step was diagnosis of phenotypes of atopic from the non-atopic performed through applying definition criteria of atopy. Subjects are readily to produce IgE antibodies after exposure to common environmental allergens is called as atopic (21). The second self-reported questionnaire distributed among SM induced SAD for detection of atopic disorder associated with following subjects. The term atopic allergy implies a familial tendency to be manifest such as conditions' asthma, rhinitis, urticaria, and eczema dermatitis, hay fever alone or in combination. Atopia explained by (SPT) and positive reactivity to at least one of the panels of common indigenous environmental allergens (22). In addition, it attributed with raised concentration of total Immunoglobulin E antibodies (TIgE) in the serum beyond the cut-off point value of allergy index and existent personnel and or family history of allergic disease. Non-atopic phenotype denoted assuming absence personal or family history of allergy,

negative SPT, and normal serum levels of TIgE antibodies 1.

Skin prick test (SPT) and peripheral blood eosinophil count applied on all the selected subjects with the allergic range of TIgE antibodies. They were remained from previous stage.

Vein blood samples obtained after overnight fasting. TIgE concentrations in serum determined using the Enzyme-Linked Immunosorbent (ELIZA) Assay according to the Manufacture's protocol (Padtan Elm, Iran Co Ltd). Cut-off point value was less than 182 Iu/ml in no-atopic adults. A subject was considered allergic state if the total serum IgE antibody concentration was equal or greater than 200 Iu/ml (23). Differential leukocyte count performed at the same time. SPT is a method, which measure sensitizations to common allergens throughout skin reactions. SPT includes extracted allergens applied on the left and right of the volar aspects the forearms, using a lancet (Allergopharma Joachim Ganzer KG. West Germany). Histamine dihydrochloride (1.7 mg histamine, 9 mg NaCl) and the solvent (physiological saline, 0.4% phenol) served as positive and negative controls. Skin reactivity to six standardized allergen extract's panels were done singly by same investigation and according to the instructions of European Academy of Allergy and Immunology (21). Allergen extracts include mite (*Dermatophagoides pteronyssinus*), *Alternaria Alternata*, *Cockroach Blatella Germanica*, Grasses and Tree (Blossoming). Allergen extracted solution contains an allergen-active substances. It dissolved in physiological saline containing 50% glycerol and preserved with 0.4% phenol. After testing the patient kept under medical observation for one hour. The allergen potency kept constant by recommendation of manufacture. SPT method includes several steps followed as. Skin was prepared by ethanol and waiting for 5 minutes in order to allow blood circulation of the skin to normalize. Then, one drop of each extracts dropped onto the marked skin using a pipette. Distance between two drops was 4 cm. the skin is pierced about half a

centimeter obliquely through the drops using lancet and avoid bleeding. Lancet was cleaned with a sterile swab between each test in order to avoidance allergen transfer (recommended by manufactures). Control's tests controls applied as positive and negative at the end of test series and remaining test liquid on the skin removed after 10 minutes in case of normal test reaction. Assessments of final results were read after 20 minutes.

A reaction was approving if the wheal diameter is at least 3 mm or greater than that of a negative control, accompanied by flare reaction (24). Subjects considered as an atopic if they had a positive reaction to any of the allergens tested.

All statistical calculations performed using the analyzed package program of SPSS version -18. Comparisons between means were calculated for dichotomous variables with the Chi-squared test and for continuous variables with independent -sample T test. A statistically significant result was defined as $P < 0.05$.

3. Results:

Sixty-three male subjects completed the second stage of the study out of final screening. Mean age recorded 46.96 ± 9.62 SD years. It ranged between 35-67 years; median 45 years.

Conflict sits of victim exposed included Majnon Island 42.6%, the Halabcheh 39.7% and other areas 19%.

Atopic knowledge of clinical manifestations of common atopic diseases consists of rhinitis 58.7%, conjunctivitis 31.7%,

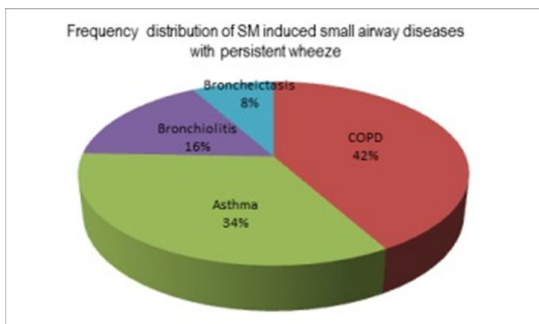


Fig. 1. It shows frequency distribution of sulfur mustard induced small airway diseases with persistent wheeze.

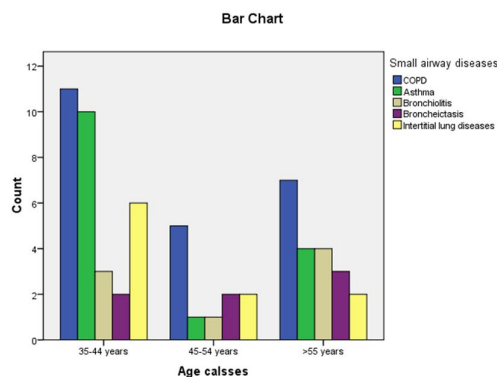


Fig. 2. It presents age distribution of sulfur mustard induced small airway diseases with persistent wheeze.

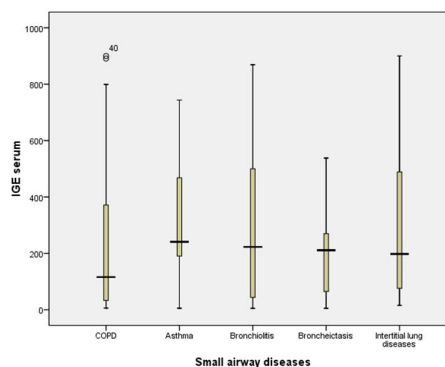


Fig. 3. It discloses distribution of means IgE levels within sulfur mustard induced small airway diseases with persistent wheeze.

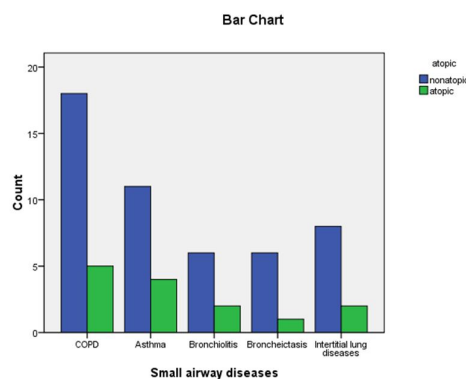


Fig. 4. It reveals frequency of atopic phenotype distribution in sulfur mustard induced small airway diseases with persistent wheeze.

urticaria 27%, eczema 19%, seasonal variation symptoms and personal 44.4% and family history 23.8%.

Fig. 1 reveals frequency of SM induced SAD with persistent wheeze. Asthma–COPD phenotype found in 36% of population

Table 1: Distribution of victims according to age group

Allergen extracts	Atopic subset in SM induced asthma
Mite Number	6.68±1.66 11
Alternaria alternate Number	6.86±1.95 7
Cockroach blatella Germania Number	5±1 3
Tree Number	6.75±0.5 4
Grasses Number	6.33±1.5 6

study. The global frequency of diseases distributed more within first class of age 51% and third class 32%. Fig. 2 shows frequency distribution of SM induced SAD with age classes.

The Mean± SD of TIgE concentrations and peripheral blood eosinophilia were 283.15±263.86 Iu/dl (range 5-900) and 266.60±240.94, respectively.

Our finding revealed significant allergic status 35 (56%) in focus sample. A highly relevant difference denoted in TIgE concentrations between allergic and non-allergic subgroups ($P<0.001$). Frequency of allergic status followed first 49% and third 34% classes of age, respectively. Number of allergic subject detected more frequency in COPD 31%, asthma 31% and interstitial lung diseases (ILD) 14%. Fig3 shows mean distribution of TIgE levels in the SM induced SAD. Concentration of TIgE in serum was higher in asthma than bronchiolitis, ILD and COPD, respectively.

Table 1 reveals characteristics of SPT resultant. The atopic phenotype observed in 14 (22%) of sample. There were significant differences between allergic status with atopic phenotype ($\chi<0.001$). Atopic phenotype detected markedly in COPD 37% asthma 22%, ILD 14% and bronchiolitis 12%. Fig. 4 presents distribution of atopic phenotype in SM induced SAD.

4. Discussion:

Wheeze is a prevalent of pulmonary symptoms. It is hallmark of small airway obstruction. Chronic wheezed disorders are seen among reversible or irreversible airway obstruction patterns (25, 26). They are found in more frequently in SAD. Wheezing has several phenotypes in link with clinical and epidemiological criteria (27). Persistent wheeze is a chronic symptom²⁵ and also observed in allergic (27), atopic (28) and chronic airway diseases (26).

Focus population revealed noticeable frequency of allergic status 56% and atopic phenotype 22% in SM induced SAD with persistent wheeze. Our finding may be an improvement with subsequent evidence.

The present study showed TIgE concentrations was meaningfully higher in allergic subjects ($P<0.001$) and atopic subsets ($P<0.001$). Following concepts may be explained effects of some causal factors on the magnitude of IgE status. They may be contributed with conditions; allergic background, atopic phenotype, exposed size of aeroallergen, gender and age.

As a rule, TIgE concentrations in atopic subjects correlate with the size of the target organ affected. Therefore, the lowest levels are observed among rhinitis, the highest in those with atopic eczema and the intermediate in asthma (29). However, effects of various exogenous environmental factors on IgE status depend to physical and chemical properties of the allergens. Current report indicated that a higher level of exposure not only causes to increase TIgE concentrations but also, to increase the risk of sensitization in susceptible people. Aside, it may be to trigger episodes of disease in sensitized subjects (30, 31). Our result indicated that marked rising of TIgE mean levels detect in the asthma, bronchiolitis and ILD diseases than COPD, separately. However, subjects with atopic phenotype were distributed markedly within COPD, asthma and ILD diseases, respectively. Current resulting has considerably overlapped with the former reports. It may be interpreted as follows.

The results of study coincided with Sapigni T, *et al*, study. They suggested that subjects

with occupational dust or gas exposure in the general population had a higher TIgE concentrations than those not exposed. It is independent of sex, age and smoking habit (32). Consequently, a portion of increasing of TIgE concentrations may be related to contacting with indoor and outdoor allergens. In addition, previous study had shown that house dust mite reactivity was more frequent in subjects seen during the winter compared to the others (33). SPT in our study was performed in winter.

Age can be the effect on the mass of IgE. Concentrations of IgE in serum were increased with rising age in both sexes (34). Male sex had high IgE concentrations than female except at birth (35). Frequency of age distribution in our study was in forth a decade. Observed results were not under influence age variation. Sample population was male and may be effective on the level of IgE concentration.

Resulting indicated meaningfully of asthma disease, raised allergic marker at allergic state and atopic phenotype in focus population. Bronchial asthma is a chronic inflammatory airway's disease, characterized with airway hyper-responsiveness. It is a heterogeneous disease and genetic (atopic) and environmental factors contribute to its initiation via inducing, enhancing, triggering, and continuance (36).

The first report of asthma was published as a forming chronic bronchitis in occupational poisoning since 1957, year in Yeprit (37). Database searching has not been shown that involving subjects to asthma among exposed SM since 2004, year. Epidemiological based medical evidence and animal study (11) have also been supported a potential role of SM effect in developing asthma. The knowledge indicated that chemical combat against Persian's soldiers was important experience of the world following World War II. Iranian scholars reported bronchial asthma between SM veterans' community apparently 16 years postwar time (13, 14, 38).

The current delaying in the report may be come from following concept. Onset of clinical features of asthma may be associated with delayed late complications of SM toxicity and occurring in a chronic state (13,

14). Pathophysiological mechanism of SM toxicity in developing bronchial asthma has not been still well understood. Inflammatory mediators, oxidative stress and abnormal protease function may be interfering in the disease's activity and progression (39). However, it may be contributed irritant function, superimposed recurrent pulmonary infections originated on SM induced diseases and immunologic stimulation effect of SM. Current concepts can be suggested as a hypothesis.

Our resulting was presented allergic asthma and atopic phenotype in SM induced SAD. The prevalence of asthma and allergic disease increased substantially all over the world during recent decades in many countries (40). Recent finding may be suggested allergic induction pathway with SM toxicity. Links reported between environmental toxic inhalation compounds with occupational asthma in current decades (41). However, database searching has not been shown a link between SM gas toxicity and aeroallergen capability.

Bronchiolitis is a risk factor in the development of asthma and allergy (42, 43). Chronic bronchiolitis detected among SM exposed veterans. Database searching did not denote a link between SM effects and allergic sensitization.

COPD reported among SM exposed veterans (44, 45). Elevated TIgE level observed within COPD population (46, 47). Asthma and COPD are different entity in diseases. However, they have to overlap in pathophysiology. It named as "overlap phenotype COPD-Asthma". Raised IgE concentration in serum of COPD patient correlated with the severity of disease (48). Aside, atopic phenotype is a risk factor in development of COPD (49). Our finding was agreement with the recent concept.

Chronic pulmonary diseases may be induced raising IgE level through a non-atopic pathway. Bacterial and fungal parasitic infections can stimulate IgE production (50). Broncheictasis may be present in the clinic with asthma features. It can be manifest with elevated IgE level and reversibility of airway obstruction (51). However, chronic pulmonary aspergillosis may be detected

among chronic respiratory diseases as; bronchiectasis (52). It can raise serum IgE level (53).

The main question is that SM can encounter as an aeroallergen. Weather SM can be provoked previously susceptible phenotype that may be presented among veterans. SM can increase the risk of asthma among atopic subjects. However, it has irritant effect or stimulated immunological pathways to an onset of asthma and exacerbation. This is a new concept, and medical evidence has not been supported the recent concept. Responses to these questions should be investigated in future studies.

5. Conclusion:

The study demonstrated the noticeable prevalence of allergic marker and atopic phenotype among SM induced SAD with persistent wheezes. They markedly distributed in the subgroup of bronchial asthma and COPD asthma- phenotype of target population.

Acknowledgments

The authors thank the participants for their help and cooperation, which made this study possible. The authors gratefully acknowledge the invaluable collaboration of Janbazan foundation, and who agreed to participate in the survey, the interviewer and nurses the staff at the pulmonary function testing laboratory of Logman Hakim hospital. The authors are grateful for support from Zand laboratory and data analysis by Ali Asghar kolahi.

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