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Association of serum immunoglobulins levels and eye injuries in sulfur mustard exposed: Sardasht-Iran Cohort Study

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ABSTRACT

In this study the associations between ocular problems and serum levels of immunoglobulins in sulfur mustard (SM) exposed population 20 years after exposure in context of Sardasht-Iran Cohort Study was explored. Serum immunoglobulins (Ig) levels including IgM, IgA, IgE, IgG, and subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) in 372 SM-exposed patients were titrated and compared with 128 unexposed controls considering their ocular problems. In exposed patients with tearing and blurring of vision, serum IgM levels were significantly lower than matched controls ($P = 0.026$ and 0.027, respectively). Serum IgM levels in exposed patients with normal ocular conditions were significantly lower ($P < 0.050$) than that of matched controls. Serum levels of IgA, IgE and IgG and IgG3 levels were not significantly different between the two groups with abnormal and normal ocular conditions. Mean serum IgG1 levels in exposed patients with normal ocular conditions were significantly higher than the matched controls ($P < 0.05$) except for tearing and photophobia. Mean serum IgG2 levels in exposed with blurring of vision and without tearing, ocular pain, photophobia, lids and bulbar conjuctival abnormalities were significantly higher than that of matched controls ($P < 0.050$). Mean serum levels of IgG4 in exposed patients with normal ocular conditions and most of the abnormal ocular conditions were significantly lower than the matched controls ($P < 0.05$). The results of the current study showed that even 20 years after SM exposure serum immunoglobulins are different from matched normal controls and the levels of IgM and IgG4 are associated with some aspects of ocular surface problems.

1. Introduction

SM with DNA alkylating activities is the most dangerous vesicant agent. Different organs such as eyes, skin, and lungs, are the main target of SM in liquid or gas forms [1]. Eyes are very sensitive to sulfur mustard (SM) gas because of the wet surfaces. In a large sample of SM casualties, in the Sardasht-Iran Cohort Study (SICS) photophobia and ocular surface discomfort (burning, itching, and redness) were the most significant symptoms and bulbar conjunctiva and limbal tissue abnormalities were the most significant signs [2,3].

The molecular mechanism(s) involved in sulfur mustard-induced ocular problems is not well known yet. Local and systemic evaluations are necessary to attain a more clear image of the mechanism(s), therefore antibody levels were checked to explore a possible relation of antibody response with ocular problems induced by sulfur mustard [4–11].

Hassan et al. in a short-time follow up on Iranian SM exposed patients showed that in short term follow up, IgM levels initially increased but

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decreased after 6 months. Serum level of IgG was initially higher than normal, but gradually returned to normal within 6 months and IgA levels were high only in less than 10% of the patients. In long term, IgM levels were elevated only in severely affected patients, IgG and IgA levels were normal but mucosal IgA was decreased significantly. IgE levels were high only in about one fifth of the patients [12]. Another study done in chronic phase of severe SM toxicity showed a significantly higher serum IgM level in study group 16 to 20 years after SM exposure [13]. These studies were not focused on the relation between immune responses and ocular complications in SM intoxicated patients.

Like SM, injuries, some ocular surface disorders such as Sjogren syndrome (SS), allergic or vernal keratoconjunctivitis and mucous membrane pemphigoid are examples of simultaneous ocular and immune system involvement. Presence of specific types of circulating immunoglobulin and ocular surface involvement are the characteristic feature of such disorders [14–18].

In previous study we have shown an association between the serum levels of inflammatory mediators and ocular injuries induced by SM [19]. The aim of the current study is to evaluate the possible association between serum immunoglobulin levels and ocular surface disorders in SM intoxicated patients.

2. Materials and methods

2.1. Study design and participants

The details of the design and methodology, including inclusion/exclusion criteria of the study have been previously described in Sardasht Iran Cohort Study (SICS, 2009). No significant difference was seen between the baseline information such as age, body mass index, marital status, and smoking habits of the two groups [2,20]. The age range of the participants was 20–60 years. A total of SM 372 individual after 20 years of exposure were compared with 128 unexposed age/gender matched controls. Special ophthalmic variables related to ocular problems including the patients' complaints were checked using slit-lamp biomicroscopy, and direct and indirect ophthalmoscopy; and then the report was completed by an ophthalmologist. The clinical evaluations were performed during 2007. Based on the previously defined classification for severity of ocular involvement [21], most of the patients were in the category of mild ocular involvement. By this definition, conjunctival vascular dilation, telangiectasia, tortuosity, segmentation, and subconjunctival hemorrhage, were considered as characteristics of the mild group.

2.2. Ethical considerations

The study was approved by the Ethical Committees of Iranian Ministry of Health and Medical Education, Ethics Boards of the Janbazan Medical and Engineering Research Center and Shahed University. The study protocol was performed according to Helsinki Declaration. Potential candidates who were willing to donate samples and sign an informed consent were recruited.

2.3. Clinical evaluation

An ocular examination chart and comprehensive questionnaire were used for every participant. An interviewer recorded the systemic and ocular history in addition to symptoms such as photophobia, ocular surface discomfort (burning, itching, and redness), foreign body sensation, tearing, pain, blurred vision, and dry eye sensation according to the volunteer' complain. A slit lamp biomicroscope (Nidek model, Gamagori, Japan) was used to evaluate the lids, tear meniscus layer, bulbar conjunctiva, limbal tissue, cornea, and anterior segment. Ocular Posterior segments were assessed using direct and indirect ophthalmoscopes (Heine K 180 Ophthalmoscope, Germany and Heine Omega 100 EN20-1 Binocular Indirect Ophthalmoscope, Germany).

2.4. Serum collection

At the time of clinical evaluations (2007) peripheral blood samples were drawn into Vacutainer tubes (BD Biosciences). Sera were separated via 20 minute centrifugation at 2000 $\times g$ (4 °C), aliquoted, and stored −80 °C and laboratory measurements were performed for 6 months.

2.5. Immunoglobulin measurement

A capture ELISA method was used to titrate immunoglobulin classes and IgG subclasses. Anti-IgG, -IgA, -IgM and -IgE (Bethyl, USA) and anti-IgG1, -IgG2, -IgG3 and -IgG4 (Sigma, St Louis, MO, USA) were coated (5 μg/mL) for 2 h at 37 °C. After washing two times using phosphate buffered saline, pH 7.2 containing 0.05% (v/v) Tween 20 (PBS-T), nonspecific sites were blocked with PBS-T containing BSA 1% (w/v) for 1 h followed by three washes with PBS-T. 100 μl of serum samples (diluted 1:10,000 for IgG and IgA, 1:6000 for IgM, 1:5 for IgE, 1:1000 for IgG1, 1:500 for IgG2 and 1:100 for IgG3 and IgG4) was added to the wells in duplicates and incubated for 1 h at RT. After washing five times with PBS-T, 100 μl of HRP-conjugated detecting antibodies specific for each immunoglobulin class (Bethyl, USA) or subclass (Sigma, St Louis, MO, USA) was added at appropriate dilutions. Plates were incubated for 1 h at RT and after washing five times, 100 μl of TMB substrate solution (Sigma, St Louis, MO, USA) was added. After 20 min incubation in the dark, the reaction was stopped using 100 μl of 5% (v/v) solution of sulfuric acid and read using an ELIZA reader (Awareness, USA) at 450 nm.

2.6. Statistical analysis

Data is presented as mean ± SD. Data analysis was performed using SPSS software, version 16 (SPSS Inc, Chicago, USA). Since the sample size was small in some subgroups, a non-parametric Mann–Whitney test was used to compare the data in the study groups. $P \leq 0.05$ was considered statistically significant. Ocular findings which were positive only in 10 cases or less, were ignored due to low statistical reliability

3. Results

Mean serum IgM level in SM exposed participants with abnormal ocular conditions of tearing and blurring of vision, was significantly lower than that of the matched controls ($P = 0.026$ and 0.027 respectively). Overall, mean serum IgM level in SM exposed participants with normal ocular conditions was significantly lower than the matched controls ($P < 0.05$) (Table 1). No statistical difference was seen in IgM level between SM-exposed participants with normal and abnormal ocular findings except in individuals with tear meniscus abnormality.

Mean serum level of IgA in SM exposed participants with ocular surface discomfort was significantly lower ($P = 0.020$) than the SM-exposed participants with no ocular surface discomfort symptom. Mean serum IgA level in controls and SM-exposed participants with photophobia was significantly higher than in participants with no photophobia in each group ($P = 0.018$ and 0.037 respectively). Overall, in normal and abnormal ocular conditions, mean serum IgA level was not significantly different between the two groups (Table 2).

Mean serum IgE level was not significantly different between the two groups with normal and abnormal ocular conditions, an exception being in SM exposed participants without tearing in which a significantly lower IgE level was seen when compared to that of the matched controls ($P = 0.026$). Mean serum IgE level in controls and those exposed with ocular surface discomfort was significantly higher than in those without this symptom ($P = 0.022$ and 0.035 respectively). Serum IgE