ORIGINAL ARTICLE

Allergen –induced IL4 levels in patients of allergic rhinitis

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ABSTRACT

**Background:** Allergic rhinitis (AR) is an important health problem in many countries. Diagnosis is critical for appropriate therapies. Diagnostic strategies that confirm the diagnosis are somewhat problematic. **Objectives:** To investigate the in vitro response to specific allergen stimulation and to compare allergen induced IL4 levels in allergic and control subjects. **Methodology:** This is a case control study involved two groups, date palm pollen allergic patient group and healthy controls. Skin prick test was performed, peripheral blood mononuclear cells were separated and cultivated in the presence and absence of date palm pollen. Levels of IL4 supernatant fluid and total serum IgE were measured by ELISA. **Results:** After specific allergen stimulation, the levels of IL4 were significantly increased in the patient group up to (67.8 pg). IL4 induced levels in patient group was markedly elevated than in control group (67.8 Vs 1.9). **Conclusion:** Allergen induced IL4 could give an extra evidence that confirm allergic rhinitis.

INTRODUCTION

Allergic diseases are highly prevalent worldwide. Allergic rhinitis (AR) and asthma are important public health problems in all countries and a burden for the medical system. Allergic rhinitis is defined by the presence of nasal congestion, anterior and posterior rhinorrhea, sneezing, and nasal itching secondary to IgE-mediated inflammation of the nasal mucosa. It is the most common form of non-infectious rhinitis, 10% and 30% of all adults and as many as 40% of children are suffering from this disease, many of them have a negative impact on their quality of life. The World Health Organization has estimated that 400 million people in the world suffer from AR, and 300 million from asthma.

Patients with AR frequently have manifestations of other allergic diseases, atopic dermatitis, conjunctivitis and asthma. More than 40% of patients with AR have asthma, and most of asthmatic patients (more than 80%) suffer concomitant rhinitis. Also, patients with rhinitis have an increased risk of developing asthma.

Once diagnosis of AR has been established and relevant trigger allergens have been identified, it is possible to recommend the appropriate therapies; allergen avoidance, allergen-specific immunotherapy and anti-IgE therapy. Diagnostic strategies that confirm the diagnosis are somewhat problematic, concise diagnosis is based on a detailed patient’s history and a diagnostic panel includes in vivo and in vitro tests.

Skin tests (intradermal tests, skin prick tests) are crucial for the identification of causative allergens and selection of line of treatment; environmental control and immunotherapy. There is no universally accepted “gold standard” test for diagnosing allergic rhinitis, although

in research studies, nasal provocation is often used as the reference standard. Skin test is an accurate diagnostic tool detection of IgE antibodies. In addition, they are convenient, simple, biologically relevant, cheap, reproducible, easy and rapid tests with high sensitivity. Performing skin tests require well trained and experienced physician, interpretation of these tests are not simple and it usually needs collaboration of medical history, laboratory work and even challenge experiments.

This study investigates the in vitro response to specific allergen stimulation and compare allergen induced IL4 levels in allergic and control subjects to explore the potential ability allergen induced IL4 to add an extra evidence of IgE mediated allergy.

METHODOLOGY

**Study design:**
This is a case control study involved two groups, a patient group and healthy controls. Patients with clinically diagnosed AR, referred to Zagazig University Allergy & Immunotherapy Unit, were selected according to these inclusion criteria: age ranging from 18-55/ mild to moderate nasal symptoms/ positive skin test to date palm pollen (Phoenix dactylifera, Pho). (The most prevalent allergen from the unit records).

**Exclusion criteria include:**
Severe nasal symptoms/ comorbid conditions such as COPD, diabetes, hypertension, cancer; autoimmunity/chemotherapy or immunosuppressive drugs/previous allergen specific immunotherapy/ pregnancy.

Detailed thorough history was taken to identify the causative exposures and the suspected allergens. History
included analysis of the predominant symptoms; duration, intensity, diurnal and seasonal variations. Environmental assessment of housing, bedding and animal contact was also involved. Written consent was taken from all study participants. Approval of the ethical committee was obtained from Faculty of Medicine, Zagazig University.

**Allergen preparation:**

Fifty grams of crude pollen were added to the Coca’s solution (500 ml) at a concentration of 1:10 in a flask with shaking in a shaker for 48 hours at room temperature. The extract was filtered through Whatman No1 filter paper, then, through a sterilized Seitez filter using membrane filter pore size 0.45 um. Finally through syringe filter pore size: 0.22 um. The sterility test of the extract was carried out by cultivation on nutrient and blood agar both aerobically and anaerobically to exclude bacterial contamination.

**Skin prick test:**

This was done by disinfection of volar aspect of the forearm skin by 70% ethyle alcohol and application of one drop of each allergen extract to the skin, at least 2 cm apart. Allergen panel included home - made extracts of : date pollen, house dust, smoke, wool, cotton, mixed fungi, and hay dust. Histamine was used as positive control and saline as negative control. In order to avoid false negative results, the patients were instructed not to receive anti-histaminics, ephedrine, epinephren, or corticostiroids for at least 4 days. The response was judged by measuring the size of the wheal after 20 min. A wheal diameter 3 mm or greater, accompanied by erythema, was defined as a positive reaction.

**Blood Sampling:**

Seven ml of peripheral blood were obtained from each study participant by venous puncture, 2 ml was dispensed into a plain tube for serum preparation and the remainder of the sample was added into preservative-free heparin containing tubes.

**Peripheral blood mono nuclear cell separation and cultivation:**

Heparinized blood was diluted 1:1 with normal saline and completely layered on an identical volume of the density gradient. The diluted blood was allowed to run down side of tubes without allowing the two solutions to mix, and then centrifuged for 30 min at 400 × g at room temperature. The PBMCs interface (Buffy coat) was carefully removed by Pasteur pipettes and was washed twice. After washing, the cell pellet was suspended in 2 ml of RPMI and the count was done using blood counting chamber. PBMCs (2x105/ml) from each participant were prepared for culture in U shaped 96-well microtiter plates. 180 ul of PBMC in complete medium added to 2 wells, the first well contained PBMCs incubated in the complete culture media alone (RPMI 1640, 10% heat-inactivated fetal bovine serum, penicillin/streptomycin 1%) , the second well contained PBMCs in the complete culture media in the presence of 20 ul of date pollen allergen (1:1000). The microtitre plate then was incubated in CO2 incubator providing 5% CO2 for 72 hours. Cell-free supernatants were harvested and centrifuged. The supernatants were stored at -20 °C. IL-4 levels were measured in the supernatants by means of specific ELISA (e Bioscience, Vienna, Austria). Total serum IgE levels were estimated (Abcam, Cambridge, UK).

**Statistical analysis:**

Statistical analysis was done using t test for two independent means to compare results of patients and controls, while t test for two dependent means was used to compare results of the each group before and after allergen stimulation. Chi square test was used to compare qualitative data.

**RESULTS**

Table (1) summarizes the characteristics of patient and control groups. The mean age was 26.9 ± 5.8 in patient group and 27.4± 5.8 in control group. There were no significant differences between the patient and control groups regarding age and sex. There was a significant difference in IgE levels in both groups, more elevated total serum IgE level was detected in patient group.

<p>| Table 1: Characteristics of the patient and control groups |
| --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Number</th>
<th>Patient</th>
<th>Control</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean± SD)</td>
<td>26.9 ± 5.8</td>
<td>27.4± 5.8</td>
<td>t- test</td>
<td>0.3</td>
</tr>
<tr>
<td>Male/Female</td>
<td>9 (41%)</td>
<td>10 (45%)</td>
<td>X²</td>
<td>1.0</td>
</tr>
<tr>
<td>Total IgE (kU/L)</td>
<td>166 ± 116</td>
<td>46 ± 26</td>
<td>t- test</td>
<td>&lt; 0.05*</td>
</tr>
</tbody>
</table>

SD, standard deviation, * significant P value
IL4 levels (Figure 1, Table 2)

**IL4 levels in unstimulated conditions:**

In the absence of antigen stimulation, cultures of allergic subjects produced low levels of IL-4 (2.8 pg/ml). Control subjects showed traces of IL4 in the unstimulated conditions, there were no significant differences between unstimulated IL4 levels between the two groups.

**Allergen induced IL4 levels:**

After specific allergen stimulation, the levels of IL4 were significantly increased in the patient group up to (67.8 pg), however, in control group, allergen induced IL4 level was showed some increase but of no statistical significance.

IL4 induced levels in patient group was markedly elevated in the patient group than in control group (67.8 Vs 1.9).

![Figure 1: Levels of IL4 (pg) in control and patient groups in the absence and presence of allergen](image_url)

**Table 2: Difference of IL4 levels in presence and absence of the allergen**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Allergic patients</th>
<th>Controls</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture medium</td>
<td>2.8</td>
<td>1.5</td>
<td>t- test</td>
<td>P&gt; 0.05</td>
</tr>
<tr>
<td>Date palm pollen</td>
<td>67.8</td>
<td>1.9</td>
<td>t- test</td>
<td>P&lt; 0.05*</td>
</tr>
<tr>
<td>Test</td>
<td>t- test</td>
<td>t- test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>P&lt; 0.05*</td>
<td>P&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant P value

**DISCUSSION**

Allergy is a very common medical problem. The major allergic diseases, allergic rhinitis, asthma, food allergies and skin allergies. Accurate diagnosis is crucial for designing the proper treatment plan. Although skin prick test is the most accurate line of diagnosis, sometimes, it cannot be done or interpreted well either due to a relative or absolute contraindication or due to lack of trained personnel. Adverse reactions and age restrictions also may be a weakness point.

IL-4 plays a critical role in allergic disease and is a key element in the initiation of atopy. By promoting differentiation to the TH2 phenotype, IL-4, together with IL-13, provides the signals required for the class switching of IgE from B cells. Also, IL-4 promotes the differentiation of monocytes into more efficient antigen-presenting cells and favors the differentiation of naïve T cells into the Th2-type. In a nasal allergen challenge study, there was an increased expression of IL4 and IL5 mRNA in tissue T lymphocytes and mast cells the T cell is the major source of these cytokines. In this work we investigated the ability of allergen induced in vitro IL4 level to be an extra evidence of IgE mediated allergic rhinitis.

There was a significant increase in the level of total IgE in patient group. This finding is similar to other reports that pointed to elevated serum level of IgE in patients with allergic diseases. Moreover, the removal of total IgE using anti-IgE therapy is very promising in allergic diseases. A significant relationship has been demonstrated in several studies between the levels of specific serum IgE and the probability of clinical rhinitis. However, any individual, atopic or not could show a demonstrable total IgE level. Parasitic infestation is a well-known cause of elevated IgE levels, which is needed to be ruled out, smoking also can enhance IgE production.

The two study groups were quite similar regarding age and sex. Both groups (patient and control) showed detectable basal levels of IL4, however, no significant difference between levels in the two groups, this finding
is similar to results of Gabrielson and colleagues. However, others had reported that all of the normal controls failed to make detectable levels of IL-4. Variations in study groups, culture conditions and ELISA kits could be the cause of such different observation.

In the present study, allergen induced IL4 levels were significantly increased in patient group compared to the unstimulated IL4 levels, and compared to the response of control group. Similar reports observed that children with atopic asthma, produced higher levels of allergen induced IL-4 than control children did either alone or in association with IL-13 production. Kasalan and colleagues, had noticed that peripheral blood mononuclear cells (PBMC) isolated from non-atopic subjects, did not produce IL-4 in response to allergen, whereas PBMC isolated from each atopic subject tested secreted measurable IL-4.

In a study conducted by Imada et al., markedly more intense IL-4, and weaker IFN-γ, production was found in the response of allergic rhinitis subjects. Cytokine protein levels measured in allergen-stimulated culture used reflect net IL-4 or IFN-γ production. Cytokine levels are influenced by consumption of cytokine and cross-regulation of cytokine gene expression among the responding cells. Another in vitro approach using dendritic cells had reported that mRNA levels of the TH2 cytokines IL-4 and IL-13 showed a sustained upregulation by pollen allergen (Bet v 1), however. DCs are generally not considered to be a source of IL-4. It had been found that the IL-4/IFN-γ ratio of allergen stimulated mononuclear cells was correlated with the clinical symptoms of seasonal allergic rhinitis and numbers of lymphocytes positive for the Th2-type cytokines IL-4 and IL-13 were significantly increased during the birch pollen season. From these findings, allergen-induced IL4 can be used to confirm diagnosis of allergy, especially when skin prick test couldn’t be applied. More investigations are needed to assess the situation in different allergens and different allergic diseases and to study the state of other cytokines (TH1 and TH2) in the in vitro allergen challenge experiment.

CONCLUSION

Allergen induced IL4 may give an extra evidence that confirm allergic rhinitis

Limitations: None

Conflict of interest:
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for its
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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