Identification Of Specific IgE In Asthmatic Patients In Iraq

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ABSTRACT
A sample of 52 patients were studied to measure IgE level by ELISA. The allergen's diagnosis is sometimes carried out by going through the medical history of people & discovering the positive results for allergen’s presence. The goal is to decrease Bronchial hyper responsiveness and initiate a program of preventive therapy with education of patients to what to avoid (i.e. according to the test performed specific IgE).

Specific IgE while carrying out blood test or skin test, and specific IgE testing is considered a confirmed test for the detection of allergy. All patients in the current sample are asthmatic attending Al-Zahraa Allergic Center in Al-Karkh Hospital. The level of IgE of 20 different allergens was measured in those patients. The result was positive for Dermatophagoides farina in 25 patients and Birch pollen was positive in 22 patients while Alder pollen was positive in 22 patients so as for Plantain pollen. This result was comparable to USA and Canada studies.

INTRODUCTION

Asthma is a longstanding common chronic inflammatory ailment of the lung’s airways; hence, categorized by inconstant & repeated symptoms, which comprise breath shortness, chest tightness, coughing and episodes of wheezing [1]. Asthma occurs by an amalgamation of environmental and genetic factors which involve exposures to allergens and air pollution. This might be categorized as non-atopic and atopic. Here atopy denotes to a susceptibility to the type I hypersensitivity reaction’s development [2].

Moreover, nor treatment is available for the symptoms of asthma, however, could be avoided by escaping triggers like irritant and allergens, or by using the inhaled long-acting beta agonists (LABA), anti-leukotriene agents and corticosteroids [3].

Asthma occurs through a mixture of intricate & partly assumed genetic & environmental communications. Such factors effect both its responsiveness & rigorousness to cure [4]. This is assumed that escalated asthma rates are because of altering epigenetics & fluctuating factors of environment [5].

Beginning afore the age of12 is possibly because of the genetic influence, whereas, onset subsequent to 12 is perhaps because of environmental influence [5]. Numerous factors of environment are connected to the exacerbation & development of asthma involving allergens & pollution of air along with allergens [6].

Further, Asthma is connected to the indoor allergens’ exposure that comprise of mold, animal dander, cockroaches and dust mites [7].

Thus, the Spirometry is thought to be superlative test which helps in Asthma management and diagnosis [7]. There are several kinds of asthma: non-allergic Asthma, Alcohol-induced Asthma, Aspirin induced, occupational and Exercise-induced Asthma [8]. Moreover, the non-allergic Asthma is termed as non-atopic or intrinsic Asthma as well; make up amongst the cases of 10%-33% indicated negative skin test against mutual allergens & normal blood IgE level. Frequently, it initiates late in life & as compared to males, females are more common [9].

IgE is essential factor in type I hypersensitivity which leads to several allergic ailments like atopic dermatitis, chronic urticarial, food allergies, allergic rhinitis, and allergic asthma. Moreover, IgE performs an essential function in reactions to allergens for instance, allergy caused by anaphylactic drug [10].

IgE-mediated allergic response are primed by IgE through the binding to receptors of Fc set up on basophils and mast cell’s surface. Likewise, Fc receptors can also be seen on the human platelets, macrophages, monocytes and eosinophils [11]. The blood of Atopic patients can contains the IgEs up to ten times than that of normal level. Precisely, the IgE can identify an allergen (for example, protein like dust mite), ragweed and grass pollen, etc consists of an exclusive long-lived contact with its high-affinity receptor FcεRI [12]. Hence, mast cells and basophils have ability to mediate the inflammatory responses, are prepared to discharge the chemicals such as some interleukins, leukotrienes and histamines [13]. Such chemicals linked to the allergic symptoms for instance, in asthma, the constriction of airways. Moreover, due to the eczema’s local inflammation, improved secretion of mucus in allergic rhinitis, as well as the escalated vascular permeability, this is supposed to permit other immune cells to attain the approach to cell tissues [14]. Hence, total IgE test calculates the complete IgE number in blood, rather than the quantity of a precise category. This could be utilized to identify body’s allergic response as compared to the precise allergy. Furthermore, this test might match the provided information by tests which discover the allergen-specific IgE [15].

Pollens are known as a fine to abrasive powdery substance which consists of pollen grains that are the seed plant’s male micro gametophytes producing sperm cells (or male gametes). As the pollen grains consists of hard coat composed of sporopollenin, it guards the gametophytes in the procedure of their movement in flowering plants from stamens to pistil, or in coniferous plant’s from male cone to female cone [16]. There are many types of pollen such as: Tree pollen like elder ash, beech, cedar, eucalyptus; Grass Pollen-Like such as Suden, salt, conary; Weed Pollen e.g. Cockle weed, goose foot, mash elder [17].

MATERIALS & METHODS
Fifty two blood sample had been collected from patients visiting Alzahraa Allergic Centers in Alkarkh Hospital and Specific IgE was measured in those samples using ELISA technique for 20 different allergens.

**Materials**
1. Start solution (buffered protein solution)
2. Anti-IgE antibody (monoclonal antibody labeled with ligand)
3. Enzyme-labelled anti ligand (ligand conjugated to alkaline phosphatase)
4. Substrate solution (5 bromo-4chloro-3indolyolphosphate and 4nitrobule tetrazolium)
5. Washing buffer (phosphate buffer, pH 7.4).

**Procedure**
1. The membrane was washed with 1ml wash buffer
2. Two hundred & fifty µl of start solution was added and mixed for 1min on 30 rpm
3. Two hundred µl of serum was added into membrane from one end and mixed for 60min on 30rpm
4. The membrane was washed with 250 µl of wash buffer and mixed for 5min on 30rpm
5. Two hundred & fifty µl of anti-IgE antibody was added and mixed for 45min on 30rpm
6. Two hundred & fifty µl of enzyme ligand-anti ligand was added and mixed for 20min on 30 rpm
7. Two hundred & fifty µl of substrate solution was added and mixed for 20 min on 30rpm and wait to dry before reading IgE level in the device.

**RESULTS & DISCUSSION**

**Distribution of Allergen among Asthmatic Patients**
In this research, we have collected blood sample of 52 asthmatic patients suffering from asthma and through the case our statistical work for the most allergen and how many patients who are allergic to this allergen and what is their rigorouses.

**Table 1: Type and percentage of Allergen among the suited patients**

<table>
<thead>
<tr>
<th>Allergens</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophagoides farina</td>
<td>25</td>
</tr>
<tr>
<td>Birch pollen</td>
<td>22</td>
</tr>
<tr>
<td>Alder pollen</td>
<td>22</td>
</tr>
<tr>
<td>Plantain pollen</td>
<td>22</td>
</tr>
<tr>
<td>Oak pollen</td>
<td>19</td>
</tr>
<tr>
<td>Mug wart pollen</td>
<td>19</td>
</tr>
<tr>
<td>Rey pollen</td>
<td>16</td>
</tr>
<tr>
<td>Dog epithelia</td>
<td>16</td>
</tr>
<tr>
<td>Dermatophagoides Pteronyssinus</td>
<td>15</td>
</tr>
<tr>
<td>Hazel pollen</td>
<td>14</td>
</tr>
<tr>
<td>Timothy Grass pollen</td>
<td>12</td>
</tr>
<tr>
<td>Cat epithelia</td>
<td>12</td>
</tr>
<tr>
<td>Horse epithelia</td>
<td>11</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>9</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>8</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>5</td>
</tr>
<tr>
<td>Penicillium notatum</td>
<td>3</td>
</tr>
<tr>
<td>Rabbit epithelia</td>
<td>1</td>
</tr>
<tr>
<td>Guinea epithelia</td>
<td>0</td>
</tr>
<tr>
<td>Hamster epithelia</td>
<td>0</td>
</tr>
</tbody>
</table>

The most allergen which noticed was Dermatophagoides farina so as birch pollen and alder pollen. These results are listed in Table 1.

The current results showed that no sensitization was noticed among the asthmatic patients; against both Guinea pig a Hamster epithelia since the Iraqi people don’t breed and care for these two animals as domestic pets. On the other hand, it was noticed that there were some types of pollen in Iraq similar to that in Canada and USA that people are allergic to like Brich, Plantain, Alder, Rey and Timothy grass pollen. However, in USA the common Ragweel is the main cause of weed allergy.
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Figure 1: A diagram shows number of asthmatic patients in Iraq for each allergen.

Classification of IgE Levels
Concentration of IgE was distributed into classes as shown in Table 2 below. In the table the classes are ranged from (0-6) [i.e. from <0.35 up to more than 100 Pg. / ml?]

Table 2: Classification of IgE concentration

<table>
<thead>
<tr>
<th>Class</th>
<th>Conc. of IgE</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.35</td>
<td>No specific antibody detection</td>
</tr>
<tr>
<td>1</td>
<td>0.35 - &lt;0.7</td>
<td>Very weak antibody concentration</td>
</tr>
<tr>
<td>2</td>
<td>0.7 - &lt;3.5</td>
<td>weak antibody concentration</td>
</tr>
<tr>
<td>3</td>
<td>3.5 - &lt;17.5</td>
<td>clear antibody concentration</td>
</tr>
<tr>
<td>4</td>
<td>17.5 - &lt;50</td>
<td>strong antibody concentration</td>
</tr>
<tr>
<td>5</td>
<td>50 - &lt;100</td>
<td>very strong antibody concentration</td>
</tr>
<tr>
<td>6</td>
<td>≥100</td>
<td>Extremely high antibody concentration</td>
</tr>
</tbody>
</table>

Distribution of Highest Allergens cases according to IgE Classes
Table 3 and figure 2 showed the frequency of IgE classes among Dermatophagoides farina allergen cases. These data illustrated that class 0 is dominant one (27 cases) while class 2 recorded in 12 cases. The highest IgE level (i.e. ≥100 Pg. / ml) was recorded in 4 cases only.

Table 3: Distribution of IgE classes within the main allergen Dermatophagoides farina

<table>
<thead>
<tr>
<th>Highest Allergen</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatophagoides farina</td>
<td>0 1 2 3 4 5 6</td>
</tr>
</tbody>
</table>

While class 2 recorded in 12 cases. The highest IgE level (i.e. ≥100 Pg. / ml) was recorded in 4 cases only.

Figure 2: Distribution of IgE class among Dermatophagoides farina
The confirmation of the measures' efficiency prevention is weak for the development of asthma. Hence, WHO (World Health Organization) suggested declining risk like perfumes, chemical irritation, air pollution, tobacco smoke & inhibits the infection of lower respiratory tract. Several other promising efforts comprise of

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restrictive smoke contact in breastfeeding, utero, & augmented exposure to huge families or daycare, however, no single one of them are extremely supported to be mentioned for such indication [18-19]. Primary pet exposure might be valuable. Therefore, the outcomes from the exposure to pets are convincing at other times & are just suggested the removal of pets from home, in case if, an individual comprise symptoms of allergies to those pets [20-22]. Dietary restrictions all through pregnancy or while breast feeding is not considered efficient, therefore, not suggested [23-24]. Thus, decreasing or eradicating the compounds might be effective as these are recognized to sensitive people from place of work. Moreover, this is not yet clear that either vaccinations of annual influenza impact the exacerbations’ risks. Though, the immunization is recommended by World Health Organization. Likewise, the ban of smoking effective to reduce the asthma exacerbations [25-26].

CONCLUSION
It is essential to perform specific IgE test on patients suffering from Asthma and Allergic rhinitis to avoid the consecutive agent when knowing this the patient can avoid the specific allergen to decrease the Bronchial irritation and mucus over secretion which causes the distressing symptoms in patient. The allergen differ in each country from the other, in western countries the main allergen can sing allergy are pet epithelia like Guinea , Hamster , Rabbit , Dog Cat , Horse While in our country it is mostly Dermatophagoides Farinac.

REFERENCES
25. Kramarz P, DeStefano F, Gargiullo PM, Davis RL, Chen RT, Mullody JP, Black SB, Shinefield HR, Bohlke