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Investigation into Causes of Allergic Diseases Using Quantitative Measurement of Allergen-Specific Ige in Serum in Al- Bayda, Libya

Abdulsalam Elfowiris[1]*, Monier Sharif [2] and Salma Bianco[3]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-Bayda- Libya
[2] Department of Pathology and Anatomy, Faculty of Veterinary, Omar Al-Mukhtar University, Al-Bayda- Libya
[3] Ministry of health, Al-Bayda- Libya

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Abstract: Allergy is a complex condition that results from different causative factors and different kinds of reactions, which caused by vibrant interactions ranged from genetic predisposition, environmental factors, food reaction, animals, insects, to a dysfunctional immune system. A public concern has grown in response to the increasing prevalence of allergy and related atopic conditions. Although the underlying mechanism of the true causes of allergy is complicated due to insufficient data and variable methodologies, Immunoglobulin E (Ig E) is commonly accepted as a specific index for allergic diseases among many indices used to test allergy. The aim of the study was to perform analysis of food allergens, inhalatory allergens, and other allergens types in patients diagnosed with an allergy by testing specific IgE to understand the risk factors, prediction, preventing, and determine the treatment. The results showed that specific IgE serum levels are significantly higher in patients sensitized to *D. pteronyssinus* and *D. farinae* allergens. However, more data and studies are needed to investigate the local allergens that cause allergy.

Keywords: Immunoglobulin E (Ig E), house dust mite (HDM), atopic dermatitis.

INTRODUCTION

Allergic diseases are a health concern for patients and practitioners that can affect the quality of life and are potentially life-threatening. The genetic, epigenetics and environmental risk factors are increased, creating more obstacles in the prevention and treatment strategies (Mastrorilli, Caffarelli, & Hoffmann-Sommergruber, 2017). At pathophysiological level allergies are a complex interaction of epithelial, mucosal, immune system, exposure and microbial in some cases.

The diagnosis of allergy mainly depends on the medical history, sensitivity test, and an oral food challenge. Recently, more specific and accurate methods are introduced for allergy diagnosis such as specific IgE, basophil activation tests, and DNA methylation signature (Bordon, 2017).

Allergic diseases include hay fever, food allergies, atopic dermatitis, allergic asthma, and anaphylaxis (Guillet, 2000). Hay fever or allergic rhinitis affects about 26% in the UK, it's a long-term case that has a considerable negative impact on quality of life and costly in health care (Porteous et al., 2013).

Food allergies are common, and their prevalence has been increased up to 10% in the last two decades (Jhamnani et al., 2018). Many foods can induce food allergy. However, certain foods are more likely to produce a more severe reaction than others; the most common accused foods include cow's milk, egg, peanut, tree nut, soy, wheat, fish, and shellfish. Milk

***Corresponding Author:** Abdulsalam Elfowiris abdulsalam.eafowiris@omu.edu.ly Department of Pharmacology and Toxicology, Omar Al-Mukhtar University

and egg allergy more common in childhood whereas peanut and tree nut allergy can occur during childhood or adulthood (Sicherer & Sampson, 2018).

There are hundreds of different allergens that can cause clinical symptoms of asthma and it is hard to identify which allergen has the most potential to cause clinical symptoms of asthma. House dust mite (HDM) is the most common type of allergen causing allergic asthma, *D. pteronyssinus, D. farina*, and *Blomia tropicalis* are the main sources of HDM allergens. Diagnosis of allergy includes skin prick testing, specific serum IgE testing, and oral food challenges (Jang et al., 2009). Many risk Factors such as family history of atopy and asthma are the main risk factors for the progress of a food allergy. Other factors including vitamin D deficiency and obesity also could be provokers for food allergy (Bordon, 2017; Boyce et al., 2011).

In general, allergies are IgE-mediated reaction that manifested symptoms ranged from pruritus to anaphylactic shock, and usually appear within minutes or delay for several hours from ingesting or contact of allergen (Fleischer et al., 2012). Symptoms are varied and affecting different systems, which include respiratory tract that leads to sneezing, congestion, rhinorrhea, wheezing, and laryngeal edema. Gastrointestinal symptoms include nausea, vomiting, abdominal pain, and diarrhea. Skin symptoms include urticaria, angioedema, flushing, or pruritus. Tachycardia and hypotension as cardiovascular symptoms (Burks et al., 2012). It is important to distinguish between allergy occurring due to food and the case of food intolerances. Food intolerances can include lactose intolerance and fructose intolerance. Histamine intolerance is nonimmunologic conditions due to foods that contain or cause a release of histamine, for instant, alcoholic beverages, ripe cheese, tomato, and smoked or processed meats (Fleischer et al., 2012; Zukiewicz-Sobczak, Wroblewska, Adamczuk, & Kopczynski, 2013). Food intolerances cannot be detected by traditional allergy testing, which includes IgE testing or skin prick testing (SPT) (Michael, 2011).

Treatment strategies are directed to strict avoidance of allergen and instant treatment of sensitivity reaction. Patients and parents of children with a food allergy should be educated to avoid allergens, read food content, and be alert for cross-contamination of food (Henson & Burks, 2012).
The aim of this study is to recognize the allergens that lead to allergic diseases using the quantitative method.

MATERIALS AND METHODS

This study was conducted at the outpatient clinic in Al-Beida, Libya. Samples were collected from 83 patients; 43 patients were females and 40 patients were males. All subjects consented to provided assent for the study, and they were suffering from different types of allergies. The samples were tested by using polycheck® allergen diagnostic test kit (Atopic 20-I). The test is based on enzymatic immunoassay for the quantitative measurement of allergen-specific IgE in serum. Each well of polycheck cassette contains allergens and standards. Allergen-specific IgE bind to the corresponding allergen after incubation of the patient's serum. Non-bound component serum was removed by washing. The anti-IgE- antibody was bound to allergen IgE complex, and the unbound was washed out. Enzyme-labeled anti-ligand binds to the immune complex. The substrate solution was added, which is specifically bound to the enzyme and convert the colourless substrate to a dark colour. The generated colour is proportional to the respective allergen-specific IgE concentration in a patient's serum. The results were interpreted by using biocheck imaging software.

Statistical differences between different groups were analyzed via single factor analysis of variance (ANOVA), followed by a non-parametric method and a calculation of median. Statistical significance was only presented when p is

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≤0.05. Statistical analyses were performed using the GraphPad Prism software.

RESULTS

According to study data, the females percentage was (51.81%), and males percentage was (48.19%). The average age of the subjects was 40.2 years old and ranged from 19 to 59 years of age (Table 1). The participated patients have one or more allergies type, such as allergic asthma, allergic rhinitis, and food allergy.Skin prick test was considered positive if the diameter of the wheal is >3 mm. The specific IgE test was considered significant if the specific IgE levels are >0.35 kU/L. The cut-off value of specific IgE testing is 0.35 - 101 kU/L. In this analytical statistic calculation, data valued <0.15 kU/L were considered as 0 kU/L, while data valued >100 kU/L were considered as 101 KU/L.

The results showed that the highest allergen sensitizations were caused by *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*, which affected about 28.92% of allergic tested patients. About 15.66% of subjects were showed sensitization to Birch-Oak mix where the lowest allergen sensiti-zation was caused by Chicken-meat (4.82%), with the exclusion of sensitization to pork, which was (0%), since pork meat is forbidden in Libya and this is indicated the high sensitivity of test (Figure 1). These percentages represented the subjects that show clear antibody concentrations (>3.5 kU/L) to extremely high antibody concentrations (>100 kU/L). It appeared that specific IgE levels varied widely among subjects. The majority of sensitized patients, for most allergens, ranged from clear to very strong category. Nevertheless, about 19 patients showed extremely high antibody concentrations to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, which were the highest levels of IgE and beyond the machine's detection range. Analysis of the difference of specific IgE serum levels was done by comparing the median value and by using a non-parametric test. The analysis showed that the house dust mite (HDM) is the most common type of allergen causing sensitization of tested patients with higher levels of specific IgE serum, which showed a statistical significance.

Table (1). Characteristic of subjects (n= 83)

Sex (male), n (%)	40 (48.19)
Sex (female), n (%)	43 (51.51)
Age (years), mean (SD)	40.2 (12.04)
Allergen prevalence, n (%)	
Chicken-meat	4 (4.82)
Flour mix	6 (7.32)
Bakers - Yeast	7 (8.43)
D.pteron+D.farinae	24 (28.92)
Birch -Oak mix	13 (15.66)
Grass-Mix	9 (10.48)
Cladosp.herb+Altern.altern.	5 (9.24)
Total-IgE	80 (96.39)

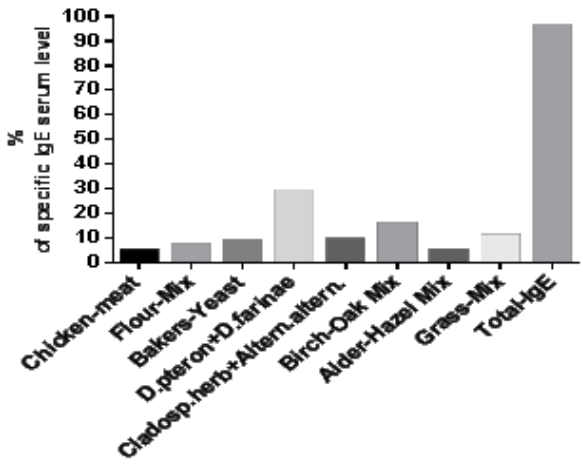

Figure (1). Distribution of specific IgE serum level.

Table(2). Quantitative specific IgE serum level: The results are represented as median, data were analysed by using Kruskal-Wallis statistic test, * P < 0.05, ****P<0.0001 and (ns) represents non-significant.

Allergen type	Median	P-value*
Chicken-meat	0.34	ns
Flour-Mix	0.28	ns
Bakers-Yeast	0.29	ns
D.pteron+D.farinae	4.90	****
Cladosp.herb+Altern.altern.	0.41	ns
Birch-Oak Mix	0.33	*
Grass-Mix	1.00	ns
Total-IgE	38.50	****

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DISCUSSION

From the result of this study, we found that the highest allergens prevalence was caused by the exposure to house dust mite (HDM) (28.92%), which is represented in the results by the *D. pteronyssinus* and *D. farinae*. Our data were consistent with previous studies that stated a large number of allergen is responsible for respiratory allergy disease. However, a huge body of literature showed that *D. pteronyssinus* and *D. farina* are the most common allergens or risk factors that lead to allergic respiratory diseases. A study in the United States using the immunoassay method found that around 38% sensitization in allergic rhinitis patients were caused by *D. pteronyssinus* and *D. farinae* (Zhao et al., 2017). Another study in Indonesia showed that *D. pteronyssinus* allergen prevalence is as high as 77.3%, followed by *D. farinae* (69.6%) (Hannaway & Roundy, 1997).

The results of this study showed that specific IgE serum levels, which is quantitively measured, is significantly higher in patients sensitized to *D. pteronyssinus* and *D. farinae* allergens, followed by Birch-Oak Mix (Table 2). Furthermore, the measurement of specific IgE levels could not be done with absolute number. Patients with IgE levels below the detection limit (<0.15 kU/L) were considered 0 kU/L, while the IgE levels above the detection limit (>100 kU/L) were calculated as 100 kU/L.

In view of the limitation of using skin prick test and the lack of accuracy of finding the allergen by using skin prick test, the quantitative measurement of specific IgE levels is very sensitive in measuring the IgE levels compared to skin prick test.

Grass pollen is one of the most important allergen sources worldwide and causes severe respiratory symptoms especially in allergic patients. Data from this study showed around (15.66%) of patients are sensitive to Birch-Oak mix, and about 10.54% have a sensitivity toward Grass-Mix. Although these percentages weren't that high, they have a huge impact on subjects health, since the released pollen from both Birch- Oak mix and Grass-Mix exacerbate allergic sensitization because they transport allergens. These allergens provoke an allergic reaction leading to inflammation. Moreover, pollen grains increase the release of bioactive lipids and enzymes that activate human neutrophils and eosinophils (Traidl-Hoffmann et al., 2003).

In general, serum total IgE is believed to reflect IgE production levels in the body. Our data showed that about (96.36%) (Table 1) of tested patients revealed an increase in total IgE ranged from a strong to extreme levels. Although the measurement of total IgE is still accepted as a tool for the assessment of allergic diseases, it is important to take in our considerations that the increase in total IgE level could not be reflected only by the hypersensitivity disorder, it may be involved in the pathogenesis of other diseases. This may explain why the data showed a very high percentage of total IgE compared to specific-IgE of allergens. Moreover, this high percentage could be due to other allergens that not detected by the polycheck cassette that was used in our study (Atopic 20-I). On the other hand, it has been well documented that the total IgE level has a role in the development of asthma (Park, Lee, & Kho, 2016).

In view of statistical analysis, the median value of specific IgE levels in subjects ranged from 0.28– 4.90 kU/L. The highest median value in this study was caused by *D. pteronyssinus* and *D. farinae* sensitization (Table 2). This number was not that high, considering negative or class 0 patients were also taken into account in the statistical analysis. On the other hand, the highest number that can be measured by the machine is limited to 100 kU/L. Thus, the median value of specific IgE levels in this study did not represent the actual IgE levels in the subject.

CONCLUSION

In summary, this study seeks to clarify the causes of allergies using quantitative serum specific IgE levels. The identification and the elimination of allergens is essential to avoid the

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triggers of allergic episodes in susceptible individuals, as well as to help in the prognosis of a proper treatment.

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ETHICS

All the data were collected after outpatient clinic permission and consent of patients.

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التحقيق في مسببات أمراض الحساسية باستخدام القياس الكمي للجلوبيولين المناعي المضاد للحساسية في مصل الدم، البيضاء، ليبيا

عبدالسلام الفويرس[1]*، منير الشريف[2] وسالمة بيانكو[3]

[1] قسم علم الأدوية والسموم، كلية الصيدلة،جامعة عمرالمختار،البيضاء، ليبيا

[2] قسم علم الأمراض والتشريح ، كلية الطب البيطرى،جامعة عمر المختار ،البيضاء، ليبيا

[3] مركز الرازي للتحاليل، البيضاء-ليبيا

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المستخلص : الحساسية هي حالة معقدة تنجم عن عوامل مسببة مختلفة وأنواع مختلفة من التفاعلات الحيوية وكذلك عامل الاستعداد الوراثي ، والعوامل البيئية ، وحساسية الغذاء ، والحيوانات ، والحشرات . ويمكن أن تنشأ الحساسية كنتيجة لخلل في الجهاز المناعي. زادت المخاوف العامة كنتيجة على زيادة انتشار الحساسية والظروف الاستشرائية المرتبطة بها. على الرغم من تعقيد وصعوبة آلية معرفة الأسباب الحقيقية للحساسية بسبب عدم كفاية البيانات. إلا أنه يتم يبقى الجلوبيولين المناعي E (Ig E) كمؤشر فعال لأمراض الحساسية بين العديد من المؤشرات التي تستخدم لاختبار الحساسية. الهدف من الدراسة هو تحليل وتحديد المواد المسببة للحساسية الغذائية والمواد المسببة لحساسية الاستنشاق وغيرها من أنواع الحساسية في المرضى الذين تم تشخيصهم بالحساسية عن طريق اختبار محددة IgE لفهم عوامل الخطر والتنبؤ والوقاية والعلاج. أظهرت نتائج هذه الدراسة أن مستويات مصل IgE الخاص بـ D. pteronyssinus و D. farinae allergen أعلى بشكل ملحوظ لدى كثير من المرضى الذين تم فحصهم، ومع ذلك مازلنا بحاجة إلى المزيد من البيانات والدراسات للتحري عن مسببات الحساسية عن طريق المهيِّجات المحلية.

الكلمات المفتاحية : الجلوبيولين المناعي (Ig E)، عثّ غبار المنزل، التهاب الجلد التأتبي.

* عبدالسلام الفويرس abdulsalam.eafowiris@omu.edu.ly قسم علم الأدوية والسموم، كلية الصيدلة، جامعة عمرالمختار، البيضاء، ليبيا