

Determination of the antigenic susceptibility of egg white proteins, ovomucoid, and ovotransferrin

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ABSTRACT

The present study aims to detect specialized E-type antibodies against ovomucoid, and ovotransferrin in the serum of individuals with egg allergy. Current study included collecting 88 samples, including 70 samples from food allergy patients and 18 control samples, during the period from September 2023 to December 2023. Age range of the samples was from 3 to 61 years. Study was conducted using ELISA technique, where the ovomucoid, and ovotransferrin proteins were prepared according to the Vidal method. Results of the current study showed that among the samples studied, 12.85% had antibodies against the egg white ovomucoid antigen, which showed a significant difference at the probability level of $P \geq 0.05$ compared to the control samples. In addition, 30% of the studied samples were allergic to ovotransferrin antigen, which also indicates a significant difference at $P < 0.05$ compared to the control samples.

1. Introduction

Allergic diseases are an abnormal response of the immune system to harmless environmental stimuli, usually proteins [1]. A food allergy is defined as an adverse effect caused by specific immune response, which frequently occurs after two hours of exposure to a specific food and can result in a sudden onset of itching, hives, or swelling of the face, tongue, or back of the throat, as well as difficulty breathing and lower blood pressure [2]. It can occur in any organ, however it most usually affects skin (urticaria) [3]. Studies indicate that food allergy have increased globally in the past decade [4]. An increase in the epidemiology of food allergy has been reported in the past few decades [5]. It appears that patients visiting hospitals due to food-related anaphylaxis have increased [6], [7].

Allergens are almost always proteins, with a protein antigen showing allergenic activity; it should stimulate IgE production, which should trigger a type I hypersensitivity response upon subsequent exposure to the same protein [8]. Although the prevalence of allergies varies from one country to another, food allergy are most often caused from milk, eggs, tree nuts, peanuts, soybeans, wheat, fish, and shellfish [9]. The majority of children with egg allergy are made aware of the allergens of egg white and not egg yolk [10]. A person's allergy can be validated by high

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levels of particular IgE or total IgE in serum, or by positive skin prick test findings [11]. The prevalence of IgE-mediated food allergies is on the rise, and it is currently estimated that they affect about 5-10% of children in the United States [12][13]. Anaphylaxis is the most serious clinical symptom of IgE-mediated food allergy. As a result, the typical symptoms of an IgE-mediated food allergy response generally appear quickly and can lead to death [14]. Food allergy may lead to a wide range of clinical symptoms, It might look as skin., gastrointestinal , respiratory , cardiac, or neurology signs and, symptoms, in isolation or in association with the same or different timing. Reactions may range from mild anaphylaxis to fatal or near fatal anaphylaxis [15]. Chicken egg allergy is one from the most common types of food allergies are becoming more common in Western countries [16]. To date, it is estimated that it affects between 1.6% and 10.1% [17], and up to 9.5% of children [13].

The burden of chicken egg allergies is heavy because can lead to allergic reactions or chronic gastroenteropathy. An elimination diet lead result in nutritional imbalance and, increase the risk of poor growth. Chicken egg allergy affects the quality of life by limiting social activities for children and parents, regardless of the intensity of symptoms [18]. Four proteins in egg whites have been identified as causing allergies: ovomucin 11%, ovalbumin 55% , ovomucoid 12%, and lysozyme 3%, named Gal d 1 to Gal d 4, respectively [19].

Allergen specific IgE test in the laboratory involves drawing blood from the patient and mixing it with the allergen. Allergy is diagnosed when specific IgE binds to an allergen and becomes insoluble. The IgE secondary antibody and allergen-specific antibody concentrations in an individual's serum are used to calculate the IgE-bound percentage of total immunoglobulin [20]. This is measure using ELISA. This laboratory approach enables high-speed, high-sensitivity experiments with a homogenous concentration of IgE in kU/I. While IgE testing may detect a wide range of allergies, it is often less sensitive than skin prick testing [21].

The work aims to detect specialized type E antibodies against the egg antigens Ovomuroid and Ovotransferrin in the serum of individuals suffering from egg allergy.

2. Methods

2.1 Sample Collection

Eighty-eight venous blood samples were collected, comprising 58 samples from females and 30 samples from males, obtained from Al-Hussein Teaching Hospital and private laboratories in Dhi Qar. After diagnosis by a specialist doctor and obtaining patient consent, venous blood was drawn using a special single-use medical syringe, with a volume of 5 ml. The blood samples were placed in test tubes without anticoagulant and centrifuged 3000 rpm for 15 minutes. to obtain serum. These samples were transferred to Eppendorf tubes and stored at -20 degrees Celsius until further use.

2.2 Detection of Food Allergies - Total IgE Test (Ovomuroid, Ovotransferrin)

The total IgE level in the sera of the subjects under study was estimated using the Sandwich Direct ELISA test, following the working method outlined in the diagnostic kit prepared by the Bioassay Technology Laboratory/China.

2.3 Detection of Allergen-Causing Antigens - IgE Test (Ovomuroid, Ovotransferrin):

The indirect ELISA test used to measure the amount of specific IgE in the sera of the subjects in the study, targeting the egg antigens Ovomuroid and Ovotransferrin. These antigens were prepared from Sigma-Aldrich, and the Specific IgE ELISA Assay was conducted according to the method described by Vidal [22].

2.4 Statistical Analysis

For the purpose of statistical analysis, the SPSS digital package was utilized. Percentages of qualitative data were calculated using the Chi-square test (X^2) at a significance level of 0.05.

3. Results

The results of this study revealed that out of a total of 70 samples, 46 showed sensitivity to IgE, constituting a rate of 66%. Conversely, the percentage of patients not sensitive to IgE was lower, at a rate of 34%.

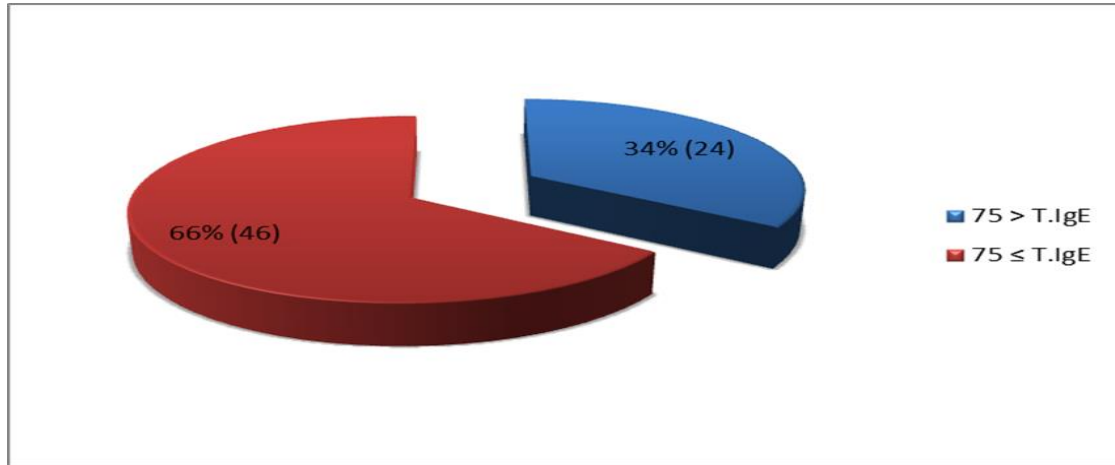


Fig.1. Results of the Total IgE ELISA Test in Patient Samples.

The findings, as depicted in Table 1, indicated that the percentage of individual's sensitive to the egg white protein ovomucoid was 12.85%. Furthermore, the percentage of females exhibiting sensitivity to the egg white ovomucoid antigen was higher compared to males, amounting to 11.42%.

Table 1. IgE ELISA Test Results for Egg White Antigen (Ovomucoid) by Gender and Age Groups.

Gender	patient samples N=70	Specific IgE	
		allergic N (%)	Non-allergic N (%)
Male	(32.85) 23	1 (1.42)	22 (31.42)
Female	(67.14) 47	8 (11.42)	39 (55.71)
Total (%)	(100) 70	9 (12.85)	61 (87.14)
Age Groups(Years)			
18 ≤	(25.71) 18	2 (2.85)	16 (22.85)
29-19	(27.14) 19	1 (1.42)	18 (25.71)
40-30	(22.85) 16	3 (4.28)	13 (18.57)
41 ≥	(24.28) 17	3 (4.28)	14 (20)
Total (%)	(100) 70	9 (12.85)	61 (87.14)

When distributing those susceptible according to age, it was noted that the third age group (40-30) had the highest sensitivity to the ovomucoid egg white antigen, with the percentage of immunoglobulin E for the ovomucoid egg white antigen being 4.28% compared to the other age groups. The number of individuals' sensitive to the egg white ovomucoid antigen was 9 of a total of 70 samples, representing a percentage of 12.85%.

Results in this study, as shown in table 2, revealed that the percentage of individual's sensitive to the egg white antigen was 30%. Additionally, the percentage of females who were sensitive to the egg white antigen ovotransferrin was higher compared to males, reaching 22.9%.

Table 2. Results of the IgE ELISA Test for the Egg White Antigen Ovotransferrin by Gender and Age Groups.

Gender N=70	patient samples	Specific IgE	
		allergic N (%)	Non-allergic N (%)
Male	(32.85) 23	5 (7.14)	18 (25.71)
Female	(67.14) 47	16 (22.85)	31 (44.28)
Total (%)	(100) 70	21 (30)	49 (70)
Age Groups(Years)			
18 ≤	(25.71) 18	7 (10)	11 (15.71)
29-19	(27.14) 19	7 (10)	12 (17.14)
40-30	(22.85) 16	3 (4.28)	13 (18.57)
41 ≥	(24.28) 17	4 (5.71)	13 (18.57)
Total (%)	(100) 70	21 (30)	49 (70)

When distributing those susceptible according to age, it was noted that the first age group (≤18) and the second age group (19-29) exhibited the highest sensitivity to the egg white antigen ovotransferrin, with the percentage of immunoglobulin E for the egg white antigen ovotransferrin being 10% for each group compared to the other age groups. The number of individual's sensitive to the egg white antigen ovotransferrin was 21 out of a total of 30 samples, representing a rate of 30%.

4. Discussion

The findings of our investigation demonstrated a rise in the concentration of total immunoglobulin E (IgE) in patients sensitive to egg white mucosal antigens (ovomucoid, ovalbumin, and ovotransferrin) at a level of ≤150 T. IgE. Their percentages were 12.9%, 12.9%, and 30%, respectively, compared to samples from non-allergic patients.

Determining the ratio of food specific IgE to quantitative IgE yields results similar to determining food specific IgE alone [23].

In their study, [24] indicated that the level of total.IgE in the blood serves as a reliable sign of allergies in youngsters. It is impacted by variables like as early weaning, early bottle feeding, passive smoking, pollen, cold, and pets, and is linked to blood eosinophilia. Total.IgE levels in the blood are also good predictors of allergies in children with asthma.

Another aspect highlighted in a study by [25]. was the association between egg allergy and total IgE antibodies in the blood. Serum total.IgE levels differed significantly between children with and without egg allergy (P ± 0.01). Their findings showed that individuals with atopic dermatitis with egg allergy had considerably higher average total blood IgE levels than patients without egg allergy (366.6 vs. 78.53 IU/ml). This implies that egg allergy may raise total blood IgE antibodies in Alzheimer's patients compared to individuals with other prevalent food allergies.

The results of a study [26] conducted on 185 samples, revealed that 78.4% had an allergy to egg whites. Based on the result of ELISA specialized IgE testing (specific IgE) for gender and age groups, a significant difference was noted between males, females, and age groups. Specifically, females in the first age group (10-20) exhibited a sensitivity to egg antigens at a rate of 100%. Furthermore, a significant difference was observed between males and females within the same age group.

A research conducted by [27] discovered strong and similar connections between total IgE (tIgE) and specific IgE (sIgE) levels in eggs, milk, peanuts, and soybeans. It emphasizes the need of considering sIgE levels in the context of tIgE when making diagnostic and therapeutic decisions in individuals with food allergies.

[28] demonstrated that among 89 patients with egg white allergy who were tested, 48 (53.9%) patients had elevated levels of total serum IgE. Additionally, the results of [29] indicated that total IgE levels for food allergy. When distributing our study samples based on age groups, the results showed that the second age group (19-29 years) had the highest incidence of food allergy among patients, at a rate of 24.3% compared to other age groups. This may be due to various risk factors, such as a healthy lifestyle, consumption of industrially processed foods, early-age inflammation, the impact of sun exposure, vitamin D deficiency, dietary fat intake, decreased consumption of antioxidants, increased use of antacids (which reduces the digestion of allergens), and obesity (resulting in a constant inflammatory state). Additionally, there may be an association with other atopic diseases [30].

The study conducted by [31] demonstrated that the highest frequency of food allergy was among allergy patients aged 13-30 years compared to those aged 31-52 years. The prevalence of food allergy was 12.35% among individuals aged 13-30 years and 10% among those aged 31-52 years. Patients were high (72.9%). [32] aimed to ascertain total and specific IgE levels in diagnosing food allergy cases in patients. Their study revealed that adults over the age of 30 years exhibited the highest percentage of food allergy (52.0%), while the lowest percentage was found among children, at 16%, with a female predominance (54.7%). Moreover, higher IgE values were found among patients (38.7%), with an average value greater than 200 IU/ml. Pediatric patients tested positive for milk, egg whites, cocoa, nuts, and wheat, and total IgE levels were elevated in these patients with various food allergens.

Another study by [33] conducted on 90 samples, observed an increase in the rate of IgE in patients with food allergies by 76.67% compared to samples from healthy individuals.

5. Conclusions

Food allergies pose a threat to an individual's health, so early and accurate diagnosis of sIgE against the egg antigens under study is crucial for patients with food allergies. The current study concludes that egg white antigens ovomucoid and ovotransferrin are highly effective antigens and have a close relationship with food allergies in the samples under study. Additionally, measuring T.iIgE is an important criterion for evaluating food allergies due to the direct relationship between the level of T.IgE and individuals sensitive to egg white antigens.

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تحديد القابلية المستضدية لبروتينات بياض البيض، المخاط البيضوي، وناقلة البيض

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معلومات البحث	المخلص
الاستلام القبول النشر	تهدف الدراسة الحالية للكشف عن الأجسام المضادة المتخصصة من النوع E ضد مستضدات البيض المخاط البيضوي، و ovotransferrin في مصل الأفراد الذين يعانون من حساسية البيض. تضمنت الدراسة الحالية جمع 88 عينة، منها 70 عينة من مرضى حساسية الطعام و18 عينة سيطرة، خلال الفترة من سبتمبر 2023 إلى ديسمبر 2023. وكانت الفئة العمرية للعينات من 3 إلى 61 سنة. أجريت الدراسة باستخدام تقنية الاليزا حيث تم تحضير البروتينات المخاط البيضوي، ovotransferrin حسب طريقة فيدال. أظهرت نتائج الدراسة الحالية من بين العينات المدروسة، كان لدى 12.85% أجسام مضادة ضد مستضد بياض البيض المخاط البيضوي، مما أظهر اختلافاً معنوياً عند مستوى احتمالية $P \geq 0.05$ مقارنة بعينات السيطرة. بالإضافة إلى ذلك، كانت 30% من العينات المدروسة تعاني من حساسية تجاه مستضد ناقله البيض، مما يدل أيضاً على وجود فرق كبير عند مستوى احتمالية $P < 0.05$ مقارنة بعينات السيطرة.
الكلمات المفتاحية	حساسية البيض، الحساسية الغذائية، الأجسام المضادة، المستضد، المخاط البيضوي، ناقله البيض.
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