



## Role of Alpha Trypticase Enzyme, Total IgE, Specific IgE & IgG4 In Patients with Food Allergy

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### ABSTRACT

**Background:** Food allergy is an abnormal adverse immune response to exposure and consumption of a variety of food containing allergens in individuals inherited atopic gene. Different types of food materials are imposed on food hypersensitivity like egg, milk, dairy products, nuts, and other foodstuffs. Different tests are used to diagnose and follow up food allergies like total IgE, Specific IgE and Skin test. **Objectives:** This study aimed to investigate the importance of IgG4 and Alpha Typticase parameters besides total and specific IgE in diagnosis food allergy cases in patients. **Patients & Methods:** The study was conducted during the period from October 2019 to January 2020 at Ramadi Teaching Hospital and Microbiology Department, College of Medicine, University of Anbar. Seventy-five (75) Patients form both sexes, adults and children were showing signs and symptoms consistent with food allergy were attending Allergy Section at Ramadi Teaching Hospital and Private Allergy and Dermatology Clinics at Ramadi city, each patient was examined and followed by a senior physician. serum from each was investigated for total IgE, Specific IgE, IgG4, and serum Trypticase contents. Fifty normal individuals resembling the patient's group were considered as the Control group and the same investigations were done for them. Data were analyzed using SPSS system. **Results:** Adult patients above 30 years old were showing the highest ratio of food allergy (52.0%) while the lowest ratio (16%) was found among children with predominance in females (54.7%) Higher values of IgE were found among patients (38.7%) with mean value more than 200IU/ml. Serum IgG4 was elevated in those with food allergy with increased total IgE in the patient group. Higher Tryptase enzyme means values (17.21 ng / ml) were found in patients sera than control individuals (9.4 ng / ml) with no effect of gender. Eight (8, 38%) of sera were showing positive specific IgE in children while eighteen (18, 71%) of adult sera were positive for specific IgE. Child patients were showing positive results for Milk, Egg white, Cacao, Nuts, and Wheat, while adults were found positive for pepper, Barely, Tomato, Citrus, and Broad beans. **Conclusion:** Food allergy represents a common health problem at any age group and mostly in adult and child females. Total serum IgE, IgG4, and Serum tryptase were elevated in these patients affected with a variety of food allergens. So They are good parameters for food allergy.

## INTRODUCTION

Food allergy is an abnormal adverse health reaction resulting from a specific immune reaction occurring after the ingestion of a particular food. Definition displays IgE-mediated food allergies, including food-induced anaphylaxis, immediate gastrointestinal hypersensitivity, acute urticaria, and oral allergy syndrome, and non-IgE-mediated food allergies, including food-induced food allergies (Joshua *et al.*, 2011 ., Burks *et al.*, 2012 ). Type-I hypersensitivity reactions are immediate allergic reactions (e.g., food and pollen allergies, asthma, anaphylaxis). Type II hypersensitivity reactions are considered cytotoxic since they contain antibodies that are unique to different tissues within the body and cause cell death in these tissues. Hypersensitivity reactions of type III are immune-complex with tissue damage caused by mediated deposition of antigen-antibody (Longo G, Berti I, Burks AW, Krauss B, Barbi E 2013). Type IV hypersensitivity reactions are delayed and cell-mediated (e.g., TB skin tests, contact dermatitis) and are the only hypersensitivity reaction involving sensitized T lymphocytes rather than antibodies (Liu *et al.* 2010., DavMartinoid *et al.*, 2014, Niederberger *et al.*, 2004). There are two well-established allergic sensitization routes. Oral allergens including peanut, egg, and milk causing G.I.T sensitization and known as class one allergens while aeroallergens including as birch pollen causing sensitization of airways and named class two allergens. The immune system responds to these allergens that cross-react to symptoms with similar food allergens as (great apple) (Campana *et al.*, 2008 ). Allergy levels vary between adults and children, children may sometimes show peanut allergies. Egg resistance in( 1- 2 %) of kids, but at age five it outperforms about( 2-3%) of kids ( Simons *et al.*, 2011 ). Food allergies sometimes begin early life while others appear later with misconceptions among a large percentage of people that they have a food allergy ( Caubet *et al.*, 2014 . , Mandal

*et al.*, 2017). Allergies develop when IgE antibody binds to food components and the problem is food protein, this binding leads to the secretion of inflammatory mediators such as histamine. A clinical medical diagnosis of food allergy achieved by taking a full medical history, skin tests, and blood investigations including food-specific IgE immunoglobulins and in some patients require oral food challenge tests ( Kronenberg, 2001 ., Ortolani & Pastorello 2006). The predisposition of peoples to food allergy was related to both genetic and environmental factors that are responsible for their susceptibility to the development of an allergic immune response to allergens( Yu *et al.*, 2011 ).The aims of this study are: To investigate the importance of IgG and Alpha Tryptase parameters beside total and specific IgE in diagnosis food allergy cases in patients with food allergy.

## MATERIALS AND METHODS

Seventy-five (75) Patients form both sexes were showing signs and symptoms consistent with food allergy were attending the Allergy Section at Ramadi General Hospital and Private allergy and Dermatology Clinics at Ramadi, West of Anbar Governorate during the period extended from October 2019 to January 2020. The study was approved by the ethical committee of the University of Anbar. Patients with food allergy and newly diagnosed cases were included in the present study and each patient was examined by a senior physician and submitted to the required clinical and laboratory investigations. Patients under the treatment of desensitization therapy were excluded. A questionnaire was done for each patient including the mentioned information and the type of food allergy was registered. Five ml. of the venous blood sample was obtained from each subject included in this study. serum samples were pooled from each blood specimen and dispensed into sterile closed capped tubes Eppendorf tubes and kept frozen at(-20c) to be used for required serological tests. Fifty (50) normal

individuals from both genders equally resembling adult test groups were included in this study as a control group. Serum specimens were taken from each one and the same investigations were done for each one. ELISA Serological tests were applied for the diagnosis of food allergy included total serum IgE and specific IgE using ELISA kit (Demeditec\Germany). Food specific IgG4, Serum tryptase enzyme, and food-specific IgE following the instructions mentioned by the manufacturing company (Shangha Yehua\China). Data were analyzed using SPSS (statistical package for the social sciences) version 22. The results for

frequencies are put in figures or tables. chi-square test was used for comparison of the variables. p value < 0.05 was considered significant (Daniel & Chad , 2013).

**RESULTS**

**Age and Gender:**

Adult patients, particularly above 30 years old were showing the highest ratio of 52.0% while the lowest ratio was 16% for children. The same observation was found among the control group (Table-1). Female patients were showing higher ratio (54.7%) than male patients (45.3%), Table 1.

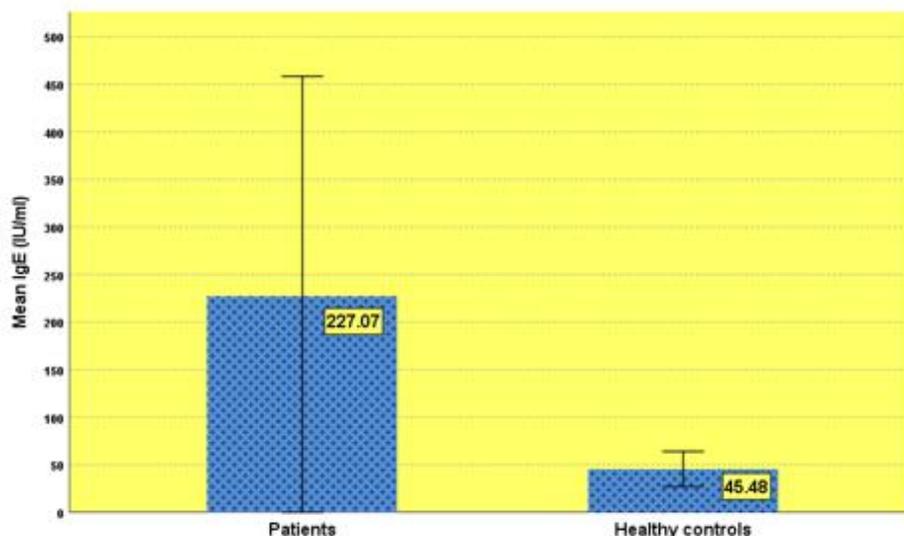
**Table 1:** Age groups and gender of patients and control groups

Age Groups		Patients		Healthy controls		P value
		No	%	No	%	
Age (years)	1---9	7	9.3	1	4.0	0.934
	10---19	11	14.7	4	16.0	
	20---29	18	24.0	8	32.0	
	30---39	14	18.7	5	20.0	
	40---49	14	18.7	5	20.0	
	50---59	6	8.0	1	4.0	
	60---69	5	6.7	1	4.0	
	Mean±SD (Range)	31.3±16.3 (1-69)		30.6±13.3 (8-62)		
Age (years)	<=17 years	12	16.0	4	16.0	0.928
	18---29	24	32.0	9	36.0	
	=>30 years	39	52.0	12	48.0	
	Mean±SD (Range)	31.3±16.3 (1-69)		30.6±13.3 (8-62)		
Gender	Male	34	45.3	12	48.0	0.817
	Female	41	54.7	13	52.0	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level						

**Total IgE:**

Patients were showing higher values of IgE (227.07±231.13) than control individuals

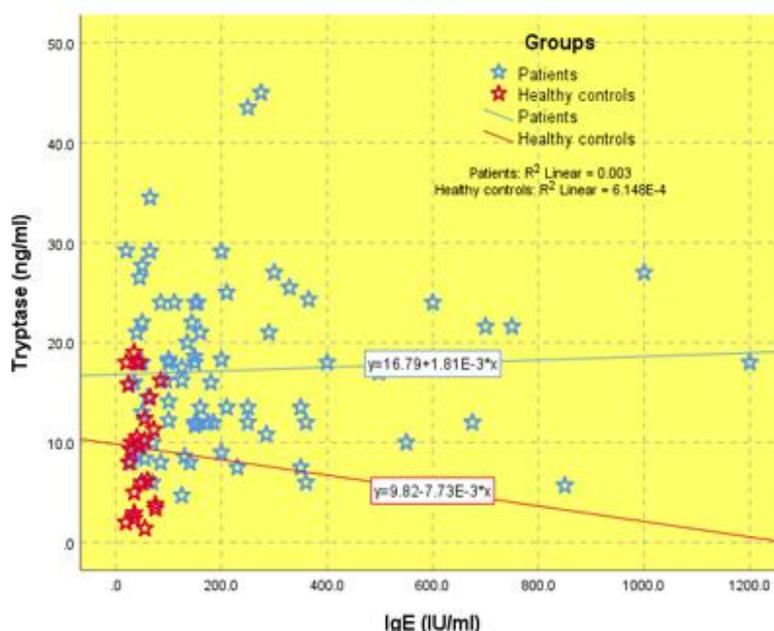
(45.48 ±18.56), twenty-nine patients (29,38.7%) were showing mean values of total IgE more than 200 IU/ ml. (Fig -1).



**Fig 1:** Total IgE mean values in patients and control groups.

The relation between Total IgE and Tryptase enzyme: Mild positive correlation ( $r = + 0.051$ ) was found between Tryptase enzyme values and Total IgE in sera of patients, the same correlation coefficient was

found between tryptase and IgG4 values in sera of patients ( $r = 0.051$ ). A strong reverse correlation ( $r = 0.09$ ) was found between Tryptase and IgE and IgG4 in sera of normal individuals (Control group) (Fig-2).



**Fig-2:** Correlation between Tryptase mean values and IgE in sera of studied individuals

### **Tryptase Enzyme:**

Patients Were showing higher mean values (17.21 ng/ml) of the Tryptase enzyme

in their sera than that of control individuals (9.4 ng / ml). Twenty-six (26, 34.7%) patients were showing mean values above 20 ng/ml of the Tryptase enzyme in their sera (Table-2).

**Table -2:** Tryptase mean values in patients and control groups.

Tryptase (ng/ml)	Patients		Healthy controls		P value
	No	%	No	%	
1.0---	-	-	3	12.0	0.0001*
2.5---	1	1.3	4	16.0	
5.0---	4	5.3	4	16.0	
7.5---	9	12.0	2	8.0	
10.0---	13	17.3	5	20.0	
12.5---	7	9.3	1	4.0	
15.0---	6	8.0	2	8.0	
17.5---	9	12.0	4	16.0	
=>20.0 ((ng/ml)	26	34.7	-	-	
Mean±SD (Range)	17.21±8.27 (4.7-45)		9.47±5.79 (1.4-19)		0.0001*

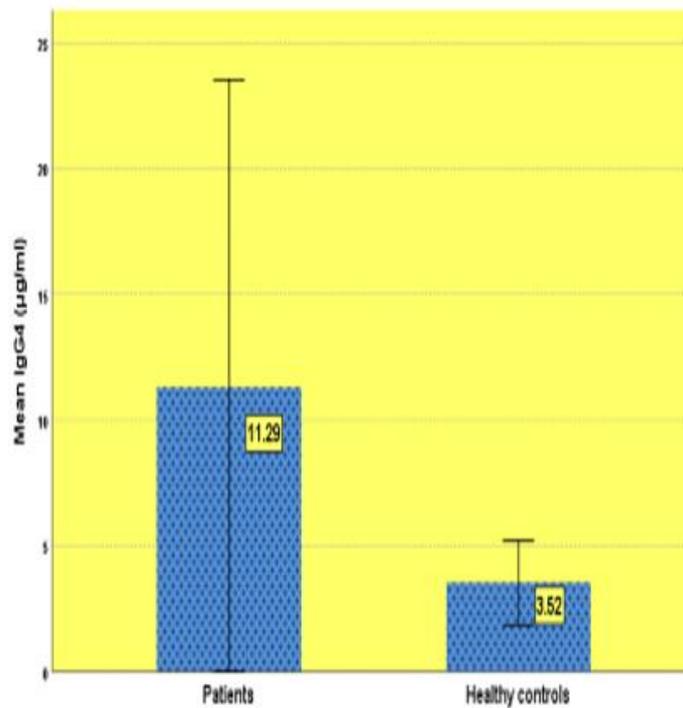
\*Significant difference between proportions using Pearson Chi-square test at 0.05 level

\*Significance of difference between two independent means using Students-t-test at 0.05 level.t

**IgG4:**

Significant difference ( p= 0.002) was found between IgG 4 mean values of patients (11.29 ±12.25 µg/ml) and that of control

individuals (3.52±1.67 µg/ml ), ( Fig -3). A non-significant difference (p= 0.534) was found between IgG4 values of adults and children in the patient group in both genders.



**Fig-3:** IgG4mean values in patients and control groups.

**Specific IgE:**

High Significant difference was found between specific IgE results of patients and control (P= 0.0001), all sera from control

individuals were showing negative results for specific IgE tests. Children were showing higher (p=0.025\*) ratio eight (8, 61.6%) of positive specific IgE for different tested food allergens IgE than adults (18, 29%), (Table 3).

**Table -3:** Distribution of specific IgE (test positivity) by Age.

Age	Positive		Negative		Total
	No	%	No	%	
<17years	8	61.6	5	38.4	13
=>17years	18	29.0	44	71.0	62
P value	0.025*				
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.					

**Type of Allergens:**

Regarding the type of tested food allergens, the majority of tested sera from child patients were showing positive results for Milk, Egg white, Cacao, Nuts, and Wheat, (7, 5, 4, 4, 3,2) for each respectively. Adult

patients were showing a high ratio of positive results of specific IgE for the following tested food allergens (pepper, Barely, Tomato, Citrus, and Broad beans) (12, 11, 10, 10, 9) for each respectively, (Table-4A & 3B).

**Table-4A:** Distribution of Specific IgE in those below 17 Years.

Type of Allergen	Percentage %
Milk	7(87.5%)
Egg White	5 (62.5%)
Sunflower Seed	1(12.5%)
Soybean	1(12.5%)
Banana	2(25%)
Tomato	2(25%)
Cacao	4(50%)
Kiwi	1(12.5%)
Broadbean	1(12.5%)
Chicken	1(12.5%)
Citrus Mix	2(25%)
Hazelnut	1(12.5%)
Peanut	4(50%)
Meat	1(%12.5)
Wheat	2(25%)
Sesame	1(12.5%)
Pepper	2(25%)
Aubergine	1(12.5%)
Walnut	3(37.5%)
Almond	1(12.5%)
Carrot	1(12.5%)
Rice	1(12.5%)
Barely	1(12.5%)

**Table-4B**-Distribution of Specific IgE in Patients older than 17 years

<b>Egg white</b>	<b>6(33.3 %)</b>
Sunflower seed	5(27.7 %)
Soyabean	4 (22.2 %)
Banana	5 (27.7 %)
Tomato	10(55.5 %)
Cacao	8 (44.4%)
Kiwi	6 (33.3 %)
Broadbean	9(50 %)
Chicken	2(11.1 %)
Citrus Mix	10(55.5 %)
Hazelnut	7 (38.8 %)
Peanut	3 (16.6%)
Meat	1(5.5 %)
Wheat	6 (33.3 %)
Sesame	1(5.5 %)
Pepper	12(66.6 %)
Aubergine	5 (27.7 %)
Walnut	4 (22.2 %)
Almond	3 (16.6%)
Barely	11(61.1 %)
Peach	6 (33.3 %)
Fig	5 (27.7 %)
Pistachionut	9(50 %)
Strawberry	1(5.5 %)
Carrot	2(11.1 %)
Apricot	7 (38.8 %)
Raspberry	4 (22.2 %)
Rice	3 (16.6%)
Potato	2(11.1 %)
Garlic/Onion	7 (38.8 %)
Olive	2(11.1 %)

### DISCUSSION

A significant proportion (52%) of patients was above 30 years old while the lowest ratio (16%) was seen in child patients. This result was incompatible with the results of (Al-jebori, 2014) who found (66.7%) cases in children and 33.3% in adults in Kerbella, Iraq. This discrepancy may be due to the difference in the sample size of studied individuals. The distribution of cases of food allergy by sex in children was higher in females while adult males showed a higher ration This is different from the results of other studies on the gender of patients with food allergy. In adult patients, the findings were higher in females than in males (65.18% and 34.82% respectively (Kelly & Gangur

2009 ). This result agreed with the findings of (Akerman ,2006) who found more food allergy in females than males in the European community. This discrepancy in the ratio of food allergy in relation to age and gender can be attributed to variability in the type of the research and its methods or variations in studied populations ( Rona *et al.*, 2007). The explanation for gender variation is might be due to sex hormones in both genders, Sex hormones may cause immune modulation in atopic patients ( Ackerman ,2006 ). Higher mean values of Tryptase of patients than healthy control people 34.7% and this agreed with the research on the role of serum tryptase in allergic reactions that indicate that patients with acute allergic reactions have elevated

serum histamine as well as less commonly seen tryptase in about 50% of patients entering Emergency Units. Some patients have elevated serum mediators like tryptase with no clinical symptoms of an extreme systemic reaction such as extreme circulatory signs of hypotension and tachycardia, and when serum tryptase levels remain normal in other patients despite elevated histamine levels indicate the fact that blood basophil is involved (Lin *et al.*, 2000).

The Serial tryptase parameter in children with food allergy reactions was more selective because the increases in tryptase rates found in anaphylaxis patients were substantially greater than in the non-anaphylaxis group (Narita *et al.*, 2006). There is no impact of gender on serum tryptase level in the group of patients and there is also an inverse association between age and tryptase level in the same group and age-advancing level in line with previous studies that find sBT levels slightly decreased with age but did not reach statistical significance (Komarow *et al.*, 2009). Total serum tryptase levels are significantly higher in younger infants compared to older infants, and in infants of the same age, serum tryptase levels that differ depending on the clinical situation, indicating the involvement of mast cells in both the physiological and allergic immune responses of young infants and, at the same time, researchers have not found any Impact of gender on sBT rates (Belhocine *et al.*, 2011). Out of the total 13 patients below 17 years old individuals included in the study, only eight (8, 61.6%) of them were positive for specific IgE, and eighteen (18, 29%) out of the total 62 adults were positive for specific IgE of different allergens checked. It reflects a major deviation from that of control people in both children and adults, and this is similar with the study showing that food allergy is more common in children than adults (Branum & Lukacs 2008). Allergy in children can be affected by many factors like type of feeding, breast feeding from non-atopic mothers decrease risk of asthma and vice versa, breast feeding from atopic mothers increase atopy in children (Friedman & Zeiger 2005 .,

Alkazemi *et al.* , 2018 ). Twenty four( 24 ) Specific IgE tests for various food allergens below 17 years of age show positive results for Milk, Egg White, Cacao, Nuts and Wheat respectively, while positive results for allergens (Pepper, Barely, Tomato, Citrus and Broad beans respectively) were observed in patients above 17 years of age This was in agreement with the study showing that children are most responsive to cow's milk, hen's egg, tree nuts, wheat, potato, tomato, and peanut respectively ( Moghtaderi *et al.*, 2012 ).

In conclusion, Food allergy represents a common health problem at any age group, and mostly in adults and child females were being more affected than males. Total serum IgE , IgG4, and Serum tryptase were elevated in patients with a food allergy, both children and adults affected wide a variety of food allergens. So they are good parameters for food allergy.

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