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Preparation of some allergens from shrimp meat and evaluated of their ability to anaphylaxis's in patients suffering from shrimp allergy in Basra province ,south of Iraq

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Abstract

one hundred and ninety sera from subjects were tested by direct and indirect ELISA test for IgE antibodies against raw and cooked shrimp antigen respectively. The result showed that the total IgE 100>IU/ml in patients had a higher rate (98.4 %) with significant difference($p<0.01$) between total IgE 100< IU/ml. distribution of shrimp antigens (raw and cooked) was determined by indirect specific IgE based ELISA, the result of this test revealed that the overall rate (57.3%, 74.2%) respectively of allergic patients were seropositive against all tested allergens. According to the sex of allergic patients, the female had a higher rate (69.2 %) of seropositivity against raw allergens was than that of males (51.2 %) with significant difference ($p<0.05$) between them. In concern to the effect of age on the seropositivity, the highest rate (60%) was observed in allergic patients (10-20)years of age, There was no significant effect ($p>0.05$) for the age of allergic patients. Also, specific IgE seropositivity against the tested cooked allergens showed a higher rate in females (84.6 %) with significant difference ($p<0.05$) compared with males. While the effect of age on the seropositivity, other age group showed higher rate (83.3 %) in the 4th age group (41-50)years another age group, there was no significant difference between other age group ($p>0.05$).

Key words : Allergens, shrimp , anaphylaxis's

1-Introduction

Food allergy is a serious public health problem and causes an abnormal reaction by the immune system in people allergic to some food allergen (Sicherer & Sampson,2006; Chafen *et al*.,2010). This response depended on the genetic predisposition of individuals and the type of food and the amount of antigen and different environmental factors(Worm *et al*.,2014). specific immunoglobulin E (IgE) antibodies play a pivotal role in the development of food allergy (Urisu *et al*., 2014) The IgE antibodies specific to allergen components or the peptide epitopes are good indicators for the identification of patients with food allergy. Seafood allergy is a common food allergy and The prevalence shellfish allergies are estimated at 0.2%,0.3%, and 0.6%, respectively Allergy to crustacean shellfish (Chafen *et al*., 2010). Adverse reactions to shellfish can be generated via immunological and non-immunological reactions, resulting from exposure to the shellfish components (Audicana &Kennedy,2008; Nieuwenhuizen *et al*., 2006). Recent advances in molecular biological techniques have enabled the efficient analysis of food allergy, Studies of shrimp allergens are the most advanced among the shellfish allergies (Leung *et al*., 2014). This study aimed to Prepare shrimp allergens extracts to be used as antigen in ELISA test which is used in the estimation of the specific IgE antibodies response against shrimp extract and determine total IgE in subjects under study and controls.

2. Materials and Methods

2.1. Patients:

A total of 190 patient's blood samples were collected during the period from July 2016 to November 2016 , **(**125 Males and 65 females) with age group from (6 - 70)years . As 190 of them were collected at random from people who live in different parts of the province of Basra , Also collected blood samples from 10 people who have no history of shrimp consumption does not suffer from allergy symptoms was considered as a control group They agreed to participate in the trial all investigated population were immunologically tested by direct and indirect ELISA.

2.2.Sampling :

From each patient 3ml of venous blood, was collected in plain tube and centrifuged for 10 minutes (1500 rpm/min) in order to botanies serum used in ELISA test .The shrimp *M. affinis* purchased from Basra local market.

2.3.Preparation of antigens

shrimp antigens were prepared according to Yadzir *et al*.(2012), The 50g from muscle shrimp meats were homogenised with 200ml PBS buffer (0.1 M , pH 7.2) , Protein extracted divided into identical weight parts to heat processing for 10min at 100 °C The other part extracted over night without cock at 4°C . The homogenate was centrifuged at 4 500 rpm for 30 min at 4°C and then at 14 000 rpm for 15 min in cooling centrifuge . The supernatant obtained was dialysis condensed against D.W for 24 h.

2.4.Determination of protein content:

protein content of each allergens extracts were determined by (Hudson and Hay , 1989) as summarized below ,Three millitter of each allergen extract were pipette in quartz cuvattes . the absorbance value was measured spectrophotometrically at 260 and 280 nm the protein concentration mg/ml=1.55 * A280-0.77* A260.The concentration of protein in the extract of raw and cooked Shrimp meat was (15mg/ml,10 mg/ml) respectively .

2.5. Total IgE estimation in serum sample .

Total IgE concentration in the sera of studied individuals was determined by a micro plate enzyme immune assay according IgE ELISA kit(Demeditec/Germany) The micro titer strips were left in the strip holder to enable the running of standards and samples . Starting with well 2 (10 µl) of standards and samples were pipette in to appropriate wells of the strips . Enzyme conjugate(200 µl) was added into each well(except well 1). The plate was covered with the enclosed foil and incubated for (30) minutes at 37°C , the incubation solution discarded and wash micro titer strips with (100 µl) diluted wash buffer . added (100 µl) of the TMB substrate solution was into micro titer strips . The plate was covered with the enclosed foil and incubated for (15) minutes 37°C in the dark . The reaction was stopped by adding (100 µl) of TMB stop solution to each well . The micro titer strips were shacked gently and read at (450) nm .

2.6. Estimation Specific IgE (manual ELISA technique):

2.6.1.Principle of ELISA procedure:

Shrimp antigens based ELISA was performed in estimation of specific IgE in the sera of studied population according to method of (Baher *et al*.,1980)

2.6.2.Chequer board titration ELISA (CB-ELISA)

To determine the optimal dilution for three reagent serum , shrimp antigen and conjugate . Chequer board was conducted as described by Baher et al. (1980).The shrimp antigen was diluted in coating buffer in a 2-fold dilution series dilutions (10 mg/ml, 5 mg/ml ,2.5 mg/ml, 1.2 5 mg/ml,0. 6 25 mg/ml,0.312 mg/ml,0.1 5 mg/ml,0.075 mg/ml,0.037 mg/ml,0.018 mg/ml).Than the first well of micro- plate was left empty for blank .Across the plate(horizontal row) , (100µl) per well of on antigen dilution was added , to the next row the second dilution wear added and soon , the serum done for the other antigen dilutions. The plate was covered with covered foil and incubated at 4°C over night .The plate was all well to reach room temperature then the cover seal removed and the plate was washed , By emptying and filling with diluted (PBS ,pH 7.2) containing (0.05 %) tween 20 immediately after filling the plate was emptied , refilled ,and allowed to sock for three minutes . this procedure was carried out two or more to give on total three washes and after the last wash the plate dried on paper towel .The pool of ten positive serum sample were diluted into the following dilution (1/2 , 1/4 ,1/8, 1/16, 1/32 , 1/64 , 1/128, 1/256, 1/512, 1/1024) ,(100µl) of each was added antigen dilution starting from second well in the first vertical row. cover seal was applied and the plate was incubated at 37°C for two hours. The plate was taken out the 37 °C incubation and washed .The conjugate anti-human IgE-HRP was added (100µl) at dilutions (1/10 ,1/100 , 1/1000 , 1/10000, 1/100000 ,1/1000000 , 1/0000000,1/00000000 , 1/000000000, 1/0000000000) to all test wells , the plate was incubated at 37 °C for one hour .The plate was taken on (37 °C)incubation and washed three times .Freshly prepared substrate solution containing tetramethylbenzidine (TMB) 50 µl was added to each well of the plate . The covered seal was appliced the plate was incubated and incubation at 37°C (X =the mean of the negative sample optical density . SD=standard deviation ofthe O.D.value any sample shows (OD)value equaled greater than cut – off value considered as positive.) for 30minutes in dark of (1MH2SO4) (50µl) was added to stop the reaction . The plate mustbe read as soon as possible by ELISA plate reader at weave length 450 nm .Depending on the results of CB ELISA , same ELISA procedure was performed on (190)serum samples. The same best selected shrimp antigens cooked and

raw 0.037, mg/ml, 0.312 mg/ml) respectively, sera (1/32 µl/ ml, 1/512) respectively, conjugate (1/000000 µl/ ml, 1/0000000000 µl/ ml) ,were used in shrimp antigens based ELISA.

2.6.3.Estimation cut-off value:

To determine the diagnostic level of the antibodies in the tested samples the cutoff value of the reaction must be determined. This can be estimated according to the method of (Llatser *et al*..,1998) . briefly ten serum samples were taken from volunteer individuals who were not exposed to shrimp antigens . these sample considered as negative control and have been tested to determine cut-off value according to the following formula:

Cut-off value=X+(3*SD).

3- Results and discussion

3-1 Estimation of total and specific IgE in allergic patients .

According to the IgE values (>100 IU/ml), the results of this study showed in table (1)decrease in the level of total IgE in allergic patients who sensitive to the shrimp antigens which prepared under study to the shrimp antigens which prepared under this study , It is found that 187(%98.4**)** where level IgE values (<100 IU/ml) but 3(%1.6**)** where level IgE values (>100 IU/ml) with highly significant difference ($p \leq 0.0001$, $X^2=166.352$). Measurement of total IgE is very important to know whether the allergic reaction is IgE mediated or non- IgE mediated before making any therapeutic and can help to early detection of allergy in infants and adults (Omenaas *etal*., 1994;Akdis *et al*.,2005), but in some study showed that measurement of total IgE was unsuitable in the diagnosis of food allergies in patients who suffering from food allergy , Mughales(2016) found %44 allergic patients had normal level IgE values, recommended several studies ,not to rely on total IgE in diagnosis of food allergies such as study Mehl et al .(2005) in German and Gharagozlou *et al.(* 2005) in Iran, also Jaber , (2011) found that (79.4%)Volunteer pigeon breeders had low level of total IgE (<25IU/ml) .

In table (2) The result showed that the overall rate of allergic patient who had positive ELISA results for raw allergens was (57.3%) . Concerning the sex of patients , in the same table the higher overall rate of sero positively (69.2**%)** was observed in females with significant difference $P \leq 0.0\ 5$ compared with males , while according to age of patients the higher overall rate (%60) of sero positivity was observed in the first age group (10-20)years compared with other age group , there was no significant difference between and between age group ($p>0.05$) . Also the results in table (3) revealed that in case of patients who were sero positive to cooked tested allergens , the higher rate of seropositivity was observed in females (% 84.6**)** $P \leq 0.0\ 5$ compared with males . Also the result of this study in same table showed that the higher overall rate **(** 83.3 % **)** of sero positivity was observed in the 4th age group (41-50)years compared with other age group , there was no significant difference between and between age group ($p>0.05$). The present study showed that the clinical allergy to shrimp allergen (raw and cooked) was detected in (%57.3 , %74.2 **)** respectively of allergic patients.Different international studies have shown that 83 % of shrimp allergic subjects had positive IgE antibodies response against some shrimp allergens in raw and cooked shrimp (Morgan *et al*.,1989). Taylor *et al*., 2000 and Jeebhay *etal*.,2001 showed that allergic symptoms results not only from ingestion of seafood, but can also be triggered by inhaling cooking vapours and handling seafood in the domestic , Gendeh *et al*.(2000) demonstrated that sensitization to shrimp allergen was %48 in residents of Kuala Lumpur City.Shellfish is one of the leading causes of food allergy in western countries suchas Europe, United States and Australia, but seems to be more prevalent in Asian countries where allergic reactions to seafood and particularly shellfish are very common among children and adults (Woods *et al*.,2001 ; Chiang *etal*.,2007). Rates of shrimp sensitivity vary among countries and communities Social conditions, customs and traditions play a major role in determining and spreading the sensitivity of shrimp among people the development of allergic reactions when swallowing food contains only shrimp, but the symptoms are also due to contact with shrimp directly or with wrappers containing shrimp derivatives in their components ,also contact with the water in which the shrimp are soaked or inhaled during cooking is a source of allergens that cause the sensitivity of shrimp to sensitive individuals, especially those involved in the processing of food and seafood (Lehrer *et al*.,1990), the study by Ayuso *et al*.,2010 found that shrimp-specific IgE levels in all subjects were relatively constant during the 24 months of the study and have higher specific IgE antibody levels, show more intense binding to shrimp peptides, and a greater epitope diversity in adults and children ,therefore suggesting that sensitization to shrimp might decrease by age.

Table (1) distribution total IgE level in allergy patient

Ex. No.	Total IgE	n.	%
190	<100	187	98.4
	>100	3	1.6
total		190	100

Table (2) distribution of raw shrimp allergen based ELISA positive results according to sex and age of allergic patients

sex	Exam No.	Raw shrimp antigen No (%)
Mal	125	64(51.2)
female	65	45(69.2)
Age group		
10-20	70	42 (60)
21-30	54	32(59.2)
31-40	30	16(53.3)
41-50	36	19(52.7)
Total	190	109(57.3)

Table (3) The distribution of cocked shrimp allergen based ELISA positive results according to sex and age of allergic patients

sex	Exam No.	cocked shrimp antigen No (%)
Mal	125	86 (68.8)
female	65	55(84.6)
Age group		
10-20	70	48 (68.5)
21-30	54	39(72.2)
31-40	30	24 (80)
41-50	36	30 (83.3)
Total	190	141 (74.2)

4-CONCLUSINS

This study has been observed that the processing shrimp by heating lead to increased in allergenicity and antigenicity of shrimp antigen , which plays an important roled in local allergic patients against shrimp.

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