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Preparation of allergens from beef and chicken meat and evaluated of their ability to anaphylaxis's in patients suffering from allergy.

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Summary

Two hundred and sixty four sera from suspected meat allergic subjects were tested by ELISA specific IgE antibodies to beef and chicken meat respectively . The distribution of beef and chicken meat (raw and cooked) allergens was determined by indirect specific IgE based ELISA. The result of this test revealed that the overall rate (84.5%) of allergic patients were seropositive against all tested allergens .Depending on the seropositiveity against allergens as one type or in association (56.4%)of allergic patients were seropositive against associated beef and chicken meat (raw and cooked),while (14.8% and 13.3%) of them were seropositve against chicken (Ch1,Ch2) and beef (Rm1,Rm2) respectively . According to sex of allergic patients ,in the female the overall rate (85.1%) of seropositiveity against all tested allergens was slightly higher than that of males (83.9%).In concern to the effect of age on the seropositiveity , the highest overall rate (90.3%) was observed in allergic patients >40 years of age , followed by the rate (85.3%) of patients in the age group (>30-40).There was no significant effect (p>0.05) for the sex or age of allergic patients , on the specific IgE seropositiveity against the tested allergens .But the seropositiveity rate was significantly variable (p>0.05) in male and females within each age group .

Key words: Allergens,beef and chicken meat, Food,type I Hypersensitivity.

1-Introduction

A food allergy is any adverse reaction to an otherwise harmless food or food component that involves the body's immune system[1]. To avoid confusion with other types of adverse reactions to foods, it is important to use the term "food allergy" or "food hypersensitivity" only when the immune system is involved in causing the reaction[2].

There are several different types of adverse reactions involving the immune system, which helps the body resist disease. In the case of food allergy, "immediate hypersensitivity" is the most clearly understood[3]. This reaction involves three primary components: food allergen, immunoglobulin E (IgE), and mast cells and basophils[4;5].Food allergen is the part of a food that stimulates the immune system of food-allergic individuals. A single food can contain multiple food allergens, the majority of which are likely to be proteins, not carbohydrates or fats[6] . People with food allergies produce increased amounts of IgE, which is an antibody in the immune system[5].

Food allergy can be further classified into immunoglobulin E (IgE)-mediated and non-IgE-mediated reactions [7;8] . Non-IgE-mediated reactions to food are caused by aberrant immune reactions to proteins in food without inducing IgE, as celiac disease induced by gluten [9;10]. IgE-mediated food allergy accounts for the majority of food allergic reactions and is characterized by the presence of antigen (Ag)-specific serum IgE antibodies [4;8].

This study aimed to Prepare beef and chicken meat allergens extracts to be used as antigen in ELISA test which is used in the estimation of the specific IgE and antigenic potency of prepared beef meat extract .

2. Materials and Methods

2.1. Patients:

A total of 264 patient's blood samples were collected during the period from March 2010 to June 2010 , (130 Males and 134 females) with age group from (6 - 70) years .The patients complaining of symptoms related to swelling or itching of the lips ,mouth and throat, nausea, vomiting , diarrhea ,eczema , redness andurticaria , attending the center of asthma and allergic disease in Basra city .They agreed to participate in the trial,all investigated population were immunologically tested by ELISA indirect .

2.2.Sampling:

From each patient 2ml 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 beefand chicken meat was purchased from Basra local market Preparation of antigen extract.

2.3.Preparation of antigens

Preparation of Beef and chicken antigens were prepared essentially as described by [11]. The150g from beef and chicken meats were freed of fat by aceton at 4 ^{0}C for 24 h. The muscle were homogenised with 0.05 M PBS buffer (0.05 M sodium phosphates + 0.15 M NaCl, pH 7.4) at a ratio of 1:1. The homogenate was extracted at 4°C for 12 -18 h and divided into identical weight parts to heat processing for 30min at 100^{o}C and the another part without cocking . Cooling was followed by centrifugation at 1000g for 20 min. The supernatant obtained was dialysis-condensed against 50% polyethylene glycol for 24 h.

2.4.Determination of protein content:

The protein content of each allergen extracts was determined by [12] 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 fresh and cooked beef meat allergy was (11.82,27.73 mg/ml) respectively and concentration of protein in the extract of raw and cooked chicken meat allergy was (12.70,10.74 mg/ml) respectively .

2.5.Specific IgE estimation (manual ELISA technique):

2.5.1.Principle of ELISA procedure:

Beefand chicken meat antigens based ELISA was performed in estimation

of specific IgE in the sera of studied population according to method of [13]

2.5.2.Chequer board titration ELISA (CB-ELISA)

To determine the optimal dilution for three reagent serum , antigen (meat beef or chicken) and conjugate . Chequer board was conducted as described by [13].The meat extract was diluted in coating buffer in serial dilutions (100 µg/ml, 50 µg/ml , 25 µg/ml , 12.5 µg/ml , 6.25 µg/ml , 3.125 µg/ml ,1.5 µg/ml ,0.7 µg/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 wae added and so on , the serum done for the other antigen dilutions. The plate was covered with covered foil and incubated at 4C^{o}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) , (100µl) of each was added antigen dilution starting from second well in the first vertical row. Than cover seal was applied and the plate was incubated at 37 oC for two hours. The plate was taken out the 37 oC incubation and washed.The conjugate anti-human IgE-HRP was added (100µl) at dilutions (1/100 , 1/200 , 1/400, 1/800 , 1/1600 , 1/3200,1/6400) , to all test wells ,the plate was incubated at(37 C^{o}) for 1 hour .The plate was taken on (37 C^{o}) 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^{o}) for 30 minutes in dark of (1M H2SO4) (50µl) was added to stop the reaction . The plate must be 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 (264)serum samples. The same best selected beef antigens cooked and raw (3.125 µg/ml, , 12.5 µg/ml) respectively, chicken antigens cooked and raw (6.25 µg/ml , 25 µg/ml) respectively , sera (1/64 µl/ ml), conjugate (1/3200 µl/ ml) ,were used in both meat beef and chicken antigens based ELISA.

2.5.3.Estimation cut-off value:

To determine the diagnostic level of the antibodies in the tested samples the cut-off value of the reaction must be determined. This can be estimated according to the method of [6] . briefly twelve serum samples were taken from volunteer individuals who were not exposed to meat 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).

X =the mean of the negative sample optical density . SD=standard deviation of the O.D.value any sample shows (OD) value equaled greater than cut – off value considered as positive.

3.Results

In table (1) ,according to type of allergen the overall rate of allergic patient who had positive ELISA results for all studied , allergens was (56.4%) of these patients were sero positive to both cooked , fresh White meat (chicken meat)and red meat(beef meat), while (13.3%) and(14.8%) of allergic patients were sero positive to cooked , fresh beef meat and chicken meat respectively .Concerning the sex of patients , in the same table the higher overall rate of sero positively (85%) was

observed in females , while according to age of patients the higher overall rate (90.3%) of sero positivity was observed in the 4th age group . Also in the results in table (1) revealed that in case of patients who were sero positive to all tested allergens , the higher rate of sero positivity was observed in females (16.9%) and in the 3th age group (69.1%), while in red meat allergic patients the higher rate of sero positivity observed in males (14.6%) and in 2nd age group (17.9%). In concern to the allergy white meat ,the higher rate of sero positivity was observed in males (18.5%) and in 4th age group (27.8%) There was no significant difference p>0.05 between (males and females) and between age group concerning the ELISA positive results.

Table (1) the distribution of beef and chicken meat based Elisa positive results in study population according to age and sex.

Variable / Sex	Exm.No.(%)	Sensitivity to allergen +Ve.No. (%)			Total +ve (%)
		[1]Rm1,[2]Rm2, [3]Ch1,[4]Ch2	Rm1,Rm2	Ch1,Ch2	
Mal	130(49.2)	66(50.8)	19(14.6)	24(18.5)	109(83.9)
Female	134(50.8)	83(61.9)	16(11.9)	15(11.2)	114(85)
Age group					
<10-20	57	31(54.4)	7(12.2)	8(14.1)	46(80.7)
>20-30	67	36(53.7)	12(17.9)	6(8.9)	54(80.5)
>30-40	68	47(69.1)	6(8.8)	5(7.4)	58(85.3)
>40	72	35(48.6)	10(13.9)	20(27.8)	65(90.3)
Total (%)	264(100)	149(56.4)	35(13.3)	39(14.8)	223(84.5)

p>0.05

[1]Rm1= Cooked beef meat [3]Ch1=Cooked chicken meat

[2]Rm2=Fresh beef meat [4]Ch3= Fresh chicken meat

According to sex of patients with in age groups (table 2) explained the distribution of the rate of sero positivity in the studded allergic patients . As was shown in this table the males showed higher rate in 2nd age group of sero positivity to red meat (23.5%) , while females of 4th age group had high rate of sero positivity to red meat (13.9%). In case of white meat the males(4th) age group and females(1st,4th) age group showed the higher rate of sero positivity against white meat (38.9%,16.7%and 16.7%)receptivity .Concerning the allergy to both allergen in the one individual , the males and females of 3th age group show higher rate of seropositivity(72.7%) and (65.7%) receptivity , this results showed significant differences according to sex with in age group(<20-30) in allergens (Rm1,Rm2) ($p < 0.05$) as compared with seropositive patients to(Rm1,Rm2Ch1,Ch2) and (Ch1,Ch2) ,while in this table showed showed significant differences according to sex with in age group(<40) in allergens showed (Ch1,Ch2) ($p < 0.05$) as compared with seropositive patients to (Rm1,Rm2, Ch1,Ch2) and (Rm1,Rm2) .Also in this table significant differences according to sex with in age group(<40) all allergens (Rm1,Rm2Ch1,Ch2) ($p < 0.05$) as compared with seropositive patients to (Ch1,Ch2) and (Rm1,Rm2) .

Table (2) The distribution of positive beef and chicken meat based ELISA according to sex with in age groups of allergic patients

Ages group	Sex	Exam. No.	[1]RM1 , [2]RM2		[3]Ch1 ,[4]Ch2		RM1 , RM2, Ch1 ,Ch2	
			positive	%	positive	%	Positive	%
<10-20	M	27	4	14.8	3	11.1	13	48.2
	F	30	3	10	5	16.7	18	60
>20-30	M	34	8	23.5	4	11.8	16	47.1
	F	33	4	12.1	2	6.1	20	60.6
>30-40	M	33	2	6.1	2	6.1	24	72.7
	F	35	4	11.4	3	9.1	23	65.7
>40	M	36	5	13.9	14	38.9	13	36.1
	F	36	5	13.9	6	16.7	22	61.1
Total	264		35	13.3	39	14.8	149	56.4

p<0.05

[1]Rm1= Cooked beef meat
[2]Rm2=raw beef meat
[3]Ch1=Cooked chicken meat
[4]Ch3= raw chicken meat

4.Discussion

Food allergy is one of the important health ,surveys show that about one-third of all adults believe they have food allergies . Yet true food allergy is estimated to affect less than two percent of the population [5] . About 4-8% percent of young children are diagnosed with food allergies, most of which are evident in the first years of life and are often outgrown [8]. Although allergic reactions can occur to virtually any food allergens , Food allergens are proteins or glycoprotein's with molecular weight ranging from 10 to 70 KDa and although any proteins is potentially allergenic , the most sever adverse reactions to food are associated with the consumption of a small number of products classified in eight main food groups responsible for about 90% of all IgE –mediated food allergies : Cow milk , eggs, fish, peanuts , soybeans, tree nuts and wheat [16] . The beef and chicken meat allergies are example of new important food allergens, published studies on beef allergy were mainly done in a population of highly atopic children suffering from atopic dermatitis and often concomitantly from milk allergy .The reported prevalence of beef allergy among children with atopic dermatitis is 1.8% to 6.5% and 13-20% in cases with concomitant milk allergy [17]..

In the present study we found the higher rate of allergic patients who had positive ELISA results for all studied allergens (Rm1, Rm2, Ch1, Ch2) was (56.4%) of these patients were seropositive to cooked and uncooked meats(beef meat and chicken meat),while(13.3%) and (14.8%) of allergic patients were seropositive to cooked and raw meats(beef meat and chicken) respectively.The high sensitization rate to beef meat and chicken meat because ,the muscles of beef meat contain bovine serum albumin 68KD Called Bos d6 (BSA) and bovine gamma globulins(BGG) are the major beef allergens ,the (BSA, BGG) are thermo labile protein and resistant digestive process ,which performs a well-known role in some cases of food hypersensitivity reactions , and although it is considered of small clinical value , the allergy to these proteins can have serious consequence[17; 18] .Other minor beef allergens are muscles proteins such as actin , myoglobin , and tropomyyosin has been shown to be heat resistant [19] .The heat treatment dose increase the allergenicity of beef in particular (BSA) is partly heat labile [20;17; 21] . Different studies have shown that the reactivity and

allergenicity of (BSA) is increase when beef protein are heat-processed.[22].reported that patients sensitized to heat –labile proteins might tolerate well cooked meat , while patients with specific IgE to heat resistant proteins tolerate neither raw nor well cooked meat [23].reported that the allergies to muscle beef proteins (actin , myoglobin , and tropomyyosin) can be reduced by various treatments (heat, homogenization and freeze-drying .Also [24] reported that meat allergy induced symptoms rang from oral allergy syndrome to server anaphylactic reaction .Many studies supported the present of clinical allergy to chicken meat .According to the reference lists of the allergens data collections (mainly based on searches Medline and food science and Technology Abstracts data cases) of the food allergic[25] found that 17 % of children with atopic dermatitis had post IgE response directed against chicken meat . [7] and [22] reported that 8 patients exhibited IgE mediated chicken meat allergy but all were asymptomatic with egg ingestion .[26] reported a case of serve chicken meat allergy , this case appeared in adulthood and without exposure to bird . [27] study was aimed to investigate the relationship between bacterial colonization and lung function tests in allergic airways disease patients .Also [28] study was to evaluate the relationship between ß-carotene as antioxidant with malondialdehyde (oxidative stress) in the serum of asthmatic patients.

In Iraq no studies have been published and focus on distribution of meat allergensassociated disease, thus we are unable to compare the present results with any previous one.

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تحضير المستضدات من لحوم الابقار والدجاج وتقييم تاثيرها في المرضى الذين يعانون من الحساسية.

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الملخص

شملت الدراسة فحص مصول (264) مريض يشتبه بإصابتهم بالحساسية الغذائية للحوم (الأبقار والدجاج) لغرض الكشف عن الأجسام المضادة من النوعالمتخصص الموجهة ضد لحم الأبقار والدجاج باستخدام اختبار ELISA. أستخدم اختبار الأليزا المعتمد على IgE المتخصص لتقدير نسبة انتشار مستضدات لحم الأبقار والدجاج (المطبوخة وغير المطبوخة). و أظهرت نتائج هذا الاختبار ان النسبة الكلية (84.5%) لمرضى الحساسية كانوا ذو ايجابيه مصليه ضد جميع المستضدات المدروسة .وبالاعتماد على الايجابية المصلية ضد جميع المستضدات قيد الدراسة سواء كان بحالة منفردة او في حالة اشتراك مع المستضد الأخر فأن (56.4%) من المرضى يملكون حساسية لجميع المستضدات المحضرة قيد الدراسة, وبنسبة (13.3%) يملكون حساسية ضد لحم الأبقار بنوعيه المطبوخ وغير المطبوخ أما نسبة الأشخاص المتحسسين إلى مستضدات الدجاج المحضرة قيد الدراسة كانت (14.8%) . و بالاعتماد على نتائج الاليزا المعتمدة على IgE المتخصصة (specific IgE) بالنسبة للجنس لوحظ ان النسبة الكلية الايجابية المصلية (85.1%) كانت في الاناث اعلى بقليل من الذكور (83.9%) .وعند اخذ تأثير العمر على الايجابية المصلية بعين الاعتبار فان اعلى نسبه كلية (90.3%) لوحظت في مرضى الحساسية في الفئة العمرية الرابعة (40>) و بنسبة (85.3%) في الفئة العمرية الثالثة (40 −30 >) ,لم يظهر تأثير إحصائي للجنس أو العمر على الايجابية المصلية ضد جميع المستضدات المفحوصة ولكن لوحظ وجود فرق معنوي (P<0.05) بين ذكور وإناث الفئة العمريه الواحدة .