SEROLOGICAL STUDY FOR SOME CHICKEN ALLERGENS IN ALLERGIC PATIENTS

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Keywords; Allergic ,Serum ,IgE

(Received 9 June 2012, Accepted 9 July 2013)

ABSTRACT

An allergic extracts from chicken feather and dropping were prepared withextraction, followed by purification using dialysis.

Total and specific IgE ELISA was performed on a total of 190 serum samples collected from allergic and healthy individuales in center of asthma and allergic diseases in Basrha city during September 2011.

ELISA test based on total IgE results revealed that the higher rate of allergic patients (47.37%) had allergy questionable while (35.26%) of them had allergy very probable and (17.37%) of them infected with allergy not probable. According to the relationship between the symptoms of allergy and total IgE based ELISA results, the higher rate of allergy very probable 95.5% was observed in symptomatic patients.

According to the results of ELISA based on specific IgE the overall rate of chicken allergens in studied patients 94.7%. Chicken dropping allergens showed the higher overall 94.7% of distribution followed by the rate of feather allergens 73.68%. Depending on the sex, age (first age group range from>15-45 and second age group range from <45-75) and type of sensitivity to all tested allergens, males and the patients of second age group showed higher rate of sensitivity (80.35% and 80% respectively), while in patients who were sensitive to single allergen (Dropping) the higher rate of sensitivity was observed in females and first age groups patients (23.88% and 15% respectively).

The estimation of IgEseropositivity in symptomatic and asymptomatic individuals resulted the symptomatic patients showed the higher rate of seropositivity (95.8%)against chicken dropping allergens, while the higher rate of seropositivity (75%) was observed against chicken feather allergens in asymptomatic patients.

According to the relationship between total and specific IgE based ELISA results, the patients with allergy very probable showed the higher rate of seropositivity for both dropping and feather allergens (98.5% and 76.1% respectively).

The positive feather and dropping based ELISA results (mean± SD) of the allergic patients sera depending of sex and age revealed different mean ± SD value of optical density in concern to sex and age of allergic patients.

INTRODUCTION

chicken (which probably originated as a jungle fowl in southwestern Asia) was one of the earliest animals to be domesticated, possibly as 4000 BC. They were popular in China and among the Greeks and Romans, and are now distributed virtually throughout the world. They form by far the most important class of poultry, raised principally for their meat and eggs. Breeders as well as workers in the chicken food processing industry are examples of groups with high risk of exposure. Other means of exposure are pillows made of chicken feathers, arts and crafts that include chicken feathers, and wing feathers used in fletching arrows. A few breeds of chicken are raised chiefly for their ornamental appearance or as pets. Direct or indirect contact with chicken allergens may cause sensitization. Allergen exposure may occur from contact with chicken feather, chicken droppings or chicken serum. Chicken droppings may contain, similarly to pigeon dropping, excreted serum protein antigens, which may have been degraded, making identification difficult. Droppings may also include bacterial endotoxin and other non-specific biological substances (1).

Allergy is one of the most wide spread diseases of the modern world . More than 25% of the population in developed countries suffer from allergies (2). A hypersensitivity reaction refers to a state of altered reactivity in which the body mounts an amplified immune response to a substance. Hypersensitivity reaction are classified into four groups (Type I, II, III, IV) each characterized by specific biological actions (3). It is since many years that immunoglobulin E was identified as a key molecule in mediating what are now considered as type 1 hypersensitivity reactions . Studies have shown a relationship between allergens and asthma and findings represent a strong association between specific immunoglobulin E antibodies or total IgE and the allergic conditions (4;5). Asthma, allergic rhinitis and allergic conjunctivitis may result following exposure to chicken feathers, epithelial cells or droppings. The allergic manifestations may present as bird fancier's asthma and as so-called bird-egg syndrome with symptoms such as rhinitis, urticaria and angioedema (6) Type1 hypersensitivity can be evaluated by several types of allergy tests as skin test which are used to test air born allergen, food ,insect stings and penicillin . Immediate-type 1 hypersensitivity also can be evaluated through serum IgE antibody testing. Although widely used in the past ,serum measurement of the total IgE level is unhelpful in the diagnosis of allergy. Of more clinical use are assay for specific IgE antibodies to suspected allergens (7). RAST was the first widely employed method of detecting IgE

antibodies in the blood that are specific for a given allergen (8). ELISA and ELISA inhibition is non radioactive method based on the same principle to detect IgE depending on the assay design of inhibition method, it is possible to measure all allergens in acrude extract or single allergens (9).

This study aimed to extract and partial purificated of chicken feather and dropping allergens **,**

detect of feather and dropping allergy in allergic patients by direct and indirect ELISA.

MATERIALS AND METHODS

Studied population

The investigated population consisted of 190 symptomatic and asymptomatic volunteer individuals including chicken breeders. The investigated population of eligible cases attending the center of asthma and allergic diseases in Basrha during September 2011. The range of volunteer ages was from 7 to 71 years, 57 of them were males and 143 were females this number choosing randomly depending on the patients of the center of asthma and allergic diseases . The symptomatic patients were complaining of symptoms related to upper and lower respiratory tract disorder or conjunctival disease or urticaria. In addition 24 blood samples were collected from control group (healthy). All investigated individual agreed to participate in the trail and tested serologically by total and specific IgE based ELISA test. Dropping and feather were collected from chicken loft and chickens wings respectively.

Preparation of the allergen extract and material sourceses

- **Blood samples:** five ml of blood was collected from each individual by vein puncture and placed in tubes without anticoagulant, allowed to clot for 15 minutes, and the serum samples were obtained by centrifugation for 15 minutes at 6000-10000 rpm stored at -20^{o}C for 3 months.

-Dropping samples: Fresh dropping (feces) were collected directly from chicken loft in clean container. According to method of(10). One per twenty 1/20 (w/vol) chicken dropping (feces) extract was prepared by dissolving 2 gm of fresh chicken feces in 40ml of 0.15 mol/L PBS pH 7.4. The mixture was left at 4^{o}C for 24 hours. The obtained extract was 3 times centrifugated at 12000 rpm for 30 minutes. The supernatant was collected and dialyzed against distilled water for 7 days. This crude chicken dropping extract was stored at -20 ^{o}C until use.

-Feather samples: Chicken wing feather were collected from different local breed of chickens. Chicken feather extract was prepared depending on the method of (11), two gm of chicken wings feather were soaked in 20 ml of 0.1 mol/L PBS pH 7.0 (1:10, w/v). Then the chicken feather extract was obtained after overnight refrigeration, Filtered through Whatman No.4 filter paper(Germany), dialyzed against 20 mmol/L ammonium bicarbonate for 72 hours and stored at

-20^{o}C until use.

Estimation of protein concentration

The protein content of each allergen extract was determined by (12) method: 3 milliliters of each allergens extract were pipette in quartz cuvatte. The absorbance value was measured spectrophotometrically at 260 and 280 nm. The protein content was calculated according to the following equation:

Protein concentration mg/ml= 1.55×A 280 -0.77 × A260. The concentration of protein in the extract of chicken dropping and feather was (1.06 and 1.28 mg/ml) respectively.

Estimation of IgE by ELISA technique

1- Total IgE estimation by direct ELISA test

T he human total IgE ELISA intended for professional use is based on direct antigen ELISA technique. Total IgE concentration in the sera of studied population was determined by the monoclonal anti –human IgE antibody which has been coated on the micro titer wells. The procedure of estimation was performed according to human ELISA kit (Germany).

2- Manual ELISA technique (specific IgE estimation)

Chicken dropping and feather antigen based ELISA was performed in estimation of specific IgE in the sera of studied population .

Chequer board titration ELISA (CB-ELISA) use to determind the optimal dilution for the three reagent serum, antigen (chicken dropping and feather extract) and conjugate, chaquer board was conducted as described by (13).Depending on the results of CB ELISA, same procedure was performed on 190 serum samples. The same best selected dilutions of dropping Ag (1/1.5625) and feather Ag (1/12.5) , sera and conjugate were used as crude in both chicken dropping and feather antigens based ELISA.

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 (14). Briefly ten serum samples were taken from volunteer individuals who were not exposed to chicken antigens. These samples 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 equal or greater than the cut off value considered as positive .

Statistical Analysis

Statistical analysis done by using SPSS software version 11, using chi sequare to Statistical significance.

RESULTS

The distribution of chicken antigens:

In table 1 the overall rate of chicken allergens in studied population was 94.7% (180/190). According to sex and age of allergic patients similar or slightly different overall rate was observed in different sex and age groups, in males, females, first age group and second age group (94.6%, 94.8%, 94.7% and 95% respectively). Depending on the type of sensitivity males and patients of second age groups showed higher rate of sensitivity to both allergens (80.35% and 80% respectively). While in patients who in were sensitive to single allergen (dropping) the higher rate of allergic sensitivity was observed in females and first age group (23.88% and 15% respectively)

Table (1): The rate of feather and dropping based ELISA seropositivity in study population according to sex and age.

Variables		Ex. No. (%)	Sensitivity to tested allergens (F + D)		Total
			Both (F+D)	Single (D)	+Ve (%)
Sex	Males	56 (29.47)	**45 (80.35)**	8 (14.28)	**53 (94.6)**
	Females	134 (70.53)	95 (70.89)	32 (23.88)	**127 (94.8)**
Total		190 (100)	140 (73.68)	40 (21.05)	**180 (94.7)**
			0.591719	2.415094	0.000211
Age group	>15-45	150 (78.95)	108 (72)	34 (22.67)	**142 (94.7)**
	<45-75	40 (21.05)	**32 (80)**	6 (15)	**38 (95)**
Total		190 (100)	140 (73.68)	40 (21.05)	**180 (94.7)**
X^2 P> 0.05			0.421053	1.561691	0.000474

*F: feather.*D: dropping.*+ve: positive.

Estimation of IgEseropositivity

specific IgE

According to table (2) the overall rates of seropositivty in symptomatic and asymptomatic individuals to both allergens was 94.7 % (180/190). The symptomatic patients showed the higher rate (95.8 % ,159/166) in chicken dropping allergens , while the higher rate of seopositivity (75% ,18/24) was observed in asymptomatic patients to chicken feather allergens.

Table (2) : The distribution of feather , dropping based ELISA results in symptomatic and asymptomatic studied population.

Symptoms	**Ex. No.**	Feather		Dropping	
		+Ve (%)	-Ve (%)	+Ve (%)	**-Ve (%)**
Symptomatic	166 (87.37)	122 (73.5)	44 (26.5)	**159 (95.8)**	7 (4.2)
Asymptomatic	24 (12.63)	**18 (75)**	6 (25)	**21 (87.5)**	3 (12.5)
Total	190 (100)	140 (73.7)	50 (26.3)	**180 (94.7)**	10 (5.3)
X^2 $P < 0.05$		0.015152	0.043689	0.375832	**4.12515**

Total IgE

According to the total IgE values, there were three types of allergy displayed in table (3) , in this table the higher rate (47.37% ,90\190) of tested patients had the type two of allergy, allergy questionable, while(35.26% ,67/190) of them had allergy very probable and (17.37% ,33/190) had allergy not probable.

Table(3) : Classification of allergy according to total IgE based ELISA results in patients.

IU/ml	Interpretation	No. (%)
>25	Allergy not probable	33 (17.37)
<25-100	Allergy questionable	**90 (47.37)**
<100	Allergy very probable	67 (35.26)
Total	……*	190 (100)
X^2 P<o.o5		**13.66704**

The relationship between total and specific IgE

In table (4) the relationship between the total IgE and specific IgE was estimated in 190 patients. The patients with allergy very probable showed higher rate of seropositivity for both allergen dropping and feather (98.5% and 76.1% respectively). In general higher rate of seropositivity to dropping allergen was observed in patients with the three types of allergy in compare to feather sensitive patients of same types of total IgE classes of allergy.

In table (5). The relationship between the total IgE and allergy symptoms was estimated in 190 patients. The higher rate of allergy very probable 95.5% (64/67) was observed in symptomatic patients. In general all types of allergy appeared in higher rates of positivity in symptomatic in compare to the rates of these types of allergy in asymptomatic patients.

Table (4) : The relationship between specific and total IgE based ELISA results.

Total IgE based ELISA Ex. No.)(	Allergy symptoms		Total No. (%)
	Symptomatic No.(%)	Asymptomatic No.(%)	
Allergy not probable	21 (63.6)	12 (36.4)	33 (100)
Allergy questionable	81 (90)	9 (10)	90 (100)
Allergy very probable	64 (95.5)	3 (4.5)	67 (100)
Total	166 (87.4)	24 (12.6)	190 (100)
X^2 P< 0.05	7.004496	**34.27937**	

Table (5) : The relationship between symptom of allergy total IgE based ELISA.

Total IgE based ELISA	Ex. No.	Specific IgE based ELISA			
		Feather No.(%)		Dropping No.(%)	
		+Ve	Ve-	+Ve	-Ve
Allergy not probable	33	25 (75.8)	8 (24.2)	30 (90.9)	3 (9.1)
Allergy questionable	90	64(71.1)	26 (28.9)	84 (93.3)	6 (6.7)
Allergy very probable	67	**51(76.1)**	16 (23.9)	**66 (98.5)**	1 (1.5)
Total	190	140(73.7)	50 (26.3)	180 (94.7)	10 (5.3)
X^2 P< 0.05	**25.96842**	0.21157	0.612727	0.32034	5.234682
Variables	**Mean ± SD of OD value**				

Table (6) : Positive feather and dropping based ELISA results (mean ±SD) of the allergic patients depending on sex and age

		No.	Feather	No.	Dropping
Sex	**Females**	95	1.573±0.214	127	1.669±0.495
	Males	45	1.441±0.715	53	1.492±0.006
Total		140	1.507±0.464	180	1.580±0.250
			6.012041		**4.690386**
Age group	**>15-45**	108	1.532±0.386	142	1.634±0.290
	<45-75	32	1.510±0.868	38	1.528±0.584
Total		140	1.521±0.627	180	1.581±0.437
P<0.05			0.74575		**3.901804**

The table-6 display the positive ELISA results as a mean ± SD of the optical density values which were recorded spectrophotometricaly by ELISA reader.

According to this table there was different mean ±SD value in concern to sex and age of tested allergic patients sera.

DISCUSSION

Detection of indoor and outdoor avian antigen and antibody might contribute to the correct diagnosis and appropriate management of bird related hyper sensitivity. Bird fancier lung (BFL) is a type of hypersensitivity pneumonitis (HP)which is induced by inhalation of avian antigens (15 ; 16)**.**

There is no standardized method to clarify the existence of avian antigen in the patients environment. Even if the patients try to avoid avian antigen, it is difficult to estimate whether avoidance is complete or incomplete as (17) reported that avian antigen persist in the house 6 months after removal of all birds. Though previous paper has reported that immunoglobulin (IgA or degraded IgA) and intestinal mucin in bird dropping and bloom are assumed to be causativeavianantigen (18). Several reports suggested that even a low exposureto wild birds (19) and unrecognized exposure to feather duvets and others (20 ; 21 ; 22 ; 23; 24) and might lead to HP.

Only one Iraqi study has been reported the distribution of pigeon antigen in pigeon fanciers and subjects with no significant contact with pigeons (25).

To conduct sensitive detection of antibody activity against avian antigens, ELISA test was used in the present study. Previous studies has reported ELISA as a method to detect avian antigens and antibodies against these antigens (26 ; 27 ; 28 ; 29 ; 30; 25).

The ELISA method which was used in the present study showed the following rates of seropositivity against avian dropping and feather allergens (94.7% and 73.7% respectively), as these seropositive patients had OD values higher than cut off value which was 0.2 in case feather allergens and 0.3 in case of dropping allergens.

The present reported rates of avian droppings and feather were in contrast with these materials rates which were reported in (25) as she found in here study on pigeon derived allergens distribution in pigeon breeder that the overall rate of seropositivity against feather (88.6%) was higher than that of droppings 64.8%. The value of seropositivity rate in avian allergic patients in relation to symptoms was estimation in the present study. A notable point of this estimation was that both symptomatic and asymptomatic allergic individuals had high value of sropositivity against droppings allergens 95.8% and 87.5% respectively. In case of the seropositivity against feather allergens the higher rate 75% was observed in asymptomatic and 73% of symptomatic were seropositivity despite of variability in

seropositivity rates in relation to symptoms which was reported in the present study the only one recently conducted Iraqi study also supported this finding as (25) confirmed the presence of variable rate of seropositive against pigeon droppings and feather in both symptomatic and asymptomatic pigeon breeder but the symptomatic showed the higher rate of seropositivity in case feather or droppings allergens.

Many studies conducted in other parts of world confirmed the seropositivity against avian allergens in both symptomatic and asymptomatic allergic individuals as the studies of (31;26) , while other studies (32 ; 33) also confirmed antibody activity against avian allergens in both symptomatic and asymptomatic but always symptomatic seropositivity rate was higher than asymptomatic.

Concerning the relationship between the type of allergens and OD values (mean ±SD), different OD values were observed in the present study in contrast with. (34) who found that higher specific IgG values against pigeon intestinal mucin in compare to pigeon serum. However, in contrary to. (34), value of specific IgG to both antigenic sources in patients with extrinsic allergic alveolitis were similar Rodrigo *et al*. (26). The explanation for these differences in the results between the present study and other could be attributed to one or more factors including the absence of standardizing measuring method for measuring specific IgE antibodies to avian allergens by ELISA testing, the variation in the type of techniques, isotype of assessing antibody and finally many researcher have found that the antibody activity may reflect sub clinical inflammation and that individuals only become symptomatic when this inflammation is advanced (31 ; 29). In concern to effect of age and sex of patients on the seropositivity rate the present study revealed that there was no effect for these variables on the seropositivity. This finding was incontrast with (25) who reported significant effect ($p > 0.05$) of these variables on the seropositivity.

According to the total IgE values there were three types of allergy. The present results revealed that 47.37% of examined patients complained questionable allergy while 35.26% of them had allergy very probable and allergy not probable appeared in 17.37% of patients. These results were in contrast with recent local study (25) who reported that higher rate 79.4% of pigeon breeders had not probable allergy and 20.6% of them had questionable allergy and no one of them had very probable allergy of the very probable allergy. Another local study (35) supported the results of present study in concern to higher rate72.9% of very probable allergy in the allergic patients.

In concern to the relationship between total and specific IgE, higher rate of seropositivity against both chicken feather and droppings were observed in patients with allergy very probable. This result was in contrast with the result of (25) who found that higher rate of seropositivity was observed in pigeon breeder who had allergy not probable. The present study also estimate the relationship between the total IgE value and allergy symptoms and revealed that higher rate of very probable allergy was observed in symptomatic patients (95.5%) and also other two types of allergy occurred in higher rates of symptomatic patients. This observation was in agreement with the results of other local study (36) which was conducted in Basrah and reported that 43% of symptomatic allergic patients had very probable allergy (>100 mg/ml). Other studies conducted in other parts of world (37 ; 38, 39 , 40) also reported that high total IgE values were observed in different rate of symptomatic allergic patients. The first study report 25% while all other studies reported that 72% of symptomatic patients had allergy very probable.

The present result is revealed that seronegative patients against both tested allergens had high total IgE value these results supported by other previous reporte of (41) who indicate that elevated total IgE can also present in parasitic and fungal infection and some non allergic disease. So that the elevated total IgE is neither sensitive nor specific. On the other hand normal level of total IgE don't preclude full allergy work to identify sensitization to allergens (41).

Farther more the present result revealed that higher rate of seropositive patients had low total IgE value (allergy not probable), this result was online with other reports of (41) who mentioned that if there is an increase in IgE antibodies against one or a few allergens this may not alter the total IgE level in the serum which will be reported in the normal range. Other studies of (41; 42) supported the present study as these studies reported that 74.02% and 96.06% of pigeon breeders who had allergy not probable showed positive results in pigeon dropping and feather based ELISA respectively.

In conclusion the seropositivity against chicken feather and dropping which was estimated by ELISA method indicate that those two material were distributed in allergic patients and play important role in the development of allergic diseases.

دراسة مصلية لبعض مستأرجات الدجاج في مرضى الحساسية

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الخلاصة

حضر المستخلص ألأرجي من ريش وبراز الدجاج بواسطة الاستخلاص الذي أعقبه التنقية بواسطة الديلزة. أجري اختبار ELISA المعتمد على الكلوبيولين المناعي نوع (E) الكلي والمتخصص على 190 عينة مصل جمعت من أشخاص أصحاء ومصابين بالحساسية في مركز الربو وأمراض الحساسية في البصرة خلال شهر أيلول سنة 2011.

بينت نتائج اختبار ELISA المعتمد على IgE الكلي أن أعلى نسبة (47,37٪) من الأشخاص كانوا مصابين بالنوع الثاني من الحساسية (Allergy questionable) بينما (35,26٪) منهم كانوا مصابين بالنوع الثالث من الحساسية (Allergy very probable) وان (17,37٪) منهم أصيب بالنوع الأول من الحساسية (Allergy not probable). واعتمادا على العلاقة بين أعراض الحساسية ونتائج اختبار ELISA المعتمد على IgE الكلي فأن أعلى نسبة (95,5٪) للحساسية من النوع الثالث لوحظت في المرضى الذين ظهرت عليهم أعراض الحساسية.

واستنادا على نتائج اختبار ELISA المعتمد على IgE المتخصص فأن النسبة الكلية لمستأرجات الدجاج في المرضى قيد الدراسة كانت (94,7٪) وان مستأرج براز الدجاج أظهر أعلى نسبة كلية (94,7٪) للانتشار وتليه نسبة انتشار مستأرجات الريش والتي هي (73,68٪). وبالاعتماد على جنس وعمر ونوع التحسس لكل المستأرجات المفحوصة فأن الذكور والمرضى من الفئة العمرية الثانية اظهروا أعلى نسبة من التحسس (80,35٪ , 80٪ على التوالي). بينما في حالة التحسس لمستأرج واحد (براز الدجاج) فأن أعلى نسبة للتحسس لوحظت في الإناث والمرضى من الفئة العمرية الأولى (23,88٪ و 15٪ على التوالي).

ونتج عن قياس الايجابية المصلية للكلوبيولين المناعي نوع E (IgE) في الأشخاص الذين ظهرت عليهم أعراض الحساسية أو لم تظهر. أن مرضى الحساسية ذوي الأعراض أظهروا أعلى نسبة من الايجابية المصلية (95.8٪) ضد مستأرج براز الدجاج بينما الأشخاص الذين لم تظهر عليهم أعراض الحساسية فأن أعلى نسبة إيجابية مصلية (75٪) لوحظت ضد مستأرجات ريش الدجاج.

واستنادا إلى العلاقة بين نتائج اختبار ELISA المعتمد على IgE الكلي والمتخصص فأن المرضى المصابين بالنوع الثالث من الحساسية (Allergy very probable) أظهروا أعلى نسبة من الايجابية المصلية ضد كلا المستأرجين البراز و الريش (98,5٪ و 76,1على التوالي).

بينت النتائج الايجابية لاختبار ELISA المعتمد على مستأرجات الريش والبراز لمصول مرضى الحساسية وبالاعتماد على الجنس والعمر قيم مختلفة للمتوسط الحسابي ± الانحراف المعياري لقيم الكثافة البصرية عند أخذ جنس وعمر المرضى بعين الاعتبار

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