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Association of HLA -DQB1*0305with Type I Hypersensitivity in beef allergic patients

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Abstract

Food allergies are important health problems.Since there are no previous studies to assess food allergies to beef in Iraq.So the aim of this study reveals food allergies by using immunological and genetic testing . Two hundred and sixty four sera from suspected meat allergic subjects were tested by in direct ELISA for specific IgE antibodies to beef.Polymerase chain reaction technique was performed to detect genetic relationship between food allergy and human leukocyte antigen classII allele(HLA -DQB1*0305).Depending on the ELISA seropositivity results,13.3% of allergic patients were seropositve against beef allergensraw and cooked andthe higher rate of seropositivity was observed in males (14.6%) and in 2nd age group patients (17.9 %) in concern to sex and age effect on the seropositivity.There werenon significant ($p>0.05$) variable values of optical density mean and standard deviation.The loss of beef allergy was not observed in all allergic patients and all of them showed the same positive raw and cooked beef specific IgE response .The results of PCR amplification showed successful binding between the specific primers of HLA- DQB1*0305 and the extracted DNA appeared as single band of expected size, 195bp .Thepositive specific IgE response to beef allergens was significantly associated with HLA DQB1*0305.

Key words :Beef meat ,HLA class II , HLA -DQB1*0305 .

1-Introduction

Food allergy is triggered by an aberrant immune response elicited by the oral administration of dietary antigens. Systemic exposure to an antigenic stimulus leads to the development of specific antibodies and of cell-mediated immunity Adverse reactions to food can either be of toxic or non-toxic reactions . Toxic reactions are due to factors inherent to a food and will thus occur in any exposed individual given an appropriate dose. Non-toxic food reactions affect only those individuals that are susceptible and can be divided in non-immune-mediated (food intolerance) or immune-mediated (food allergy) [1;2].

The Beef allergy is food allergy in both children and adults..Studies regarding this particular allergy have demonstrated a predilection toward atopic dermatitis among children[3;4].A previous report described 10 cases of food allergy caused by beef in adults, presenting various clinical manifestations, including urticaria, angioedema, anaphylaxis and gastrointestinal symptoms [5;6] . Polymorphic HLA MHC class II molecules are displayed on the surface of antigen-presenting cells (APCs)[7] .These molecules bind and present antigenic peptides to T cells bearing T-cell receptors that are then capable of recognizing the specific b ound peptide within the context of the presenting MHC class II molecule, facilitating antigen specific T -cell activation. Inherited differences inMHC class II (a-chain) and (b-chain) allelic polymorphisms place genetic constraints on the host's ability to bind and present specific antigenic peptides to T cells.Over 500 diseases are associated either with classical HLA alleles , Several studies have reported associations between HLA alleles and atopy and specific allergies[8;9]. . Worldwide reports have found that food allergy-HLA association is mainly the result of DQ molecules. Among HLA class II antigens HLADQB1 which is associated with several allergic diseases [10].

This study aimed to Prepare beef allergens extracts to be used as antigen in ELISA test which is used in the estimation of the specific IgEanstibodie response aganist beef extract . also to determine MHC class II allele(HLA -DQB1*0305) and food allergy relationships by PCR test .

2.Materials and Methods

2.1.Patients

A total of (264) patient's blood samples were collected during the period from March 2010 to Jun 2010 , (130 Males and 134 females).The range of patient's ages was from6 to 70 years,The patients complained 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 was immunologically tested by ELISA test.

2.2.Sampling

From each patient 5ml of venous blood, was collected in plain tube Two ml of collected blood was centrifuged for 10 minutes (1500 rpm/min) , to obtains serum used in ELISA test ,The remained 3ml of blood was poured in tubes containing EDTA , kept under - 18 C^{o} and later use for HLA-DQ

genotyping. The beef samples were purchased from Basra local market.

2.3.Serological study

2.3.1.Preparation of Beef antigens

Beef antigen extract was prepared essentially as described by[11].By aceton150gm ofbeef was freed of fat at 4 C^0 for 24 h.,homogenized with 0.05 M PBS (0.05 M sodium phosphates + 0.15 M NaCl, pH 7.40) at a ratio of 1:1. The homogenate was extracted at $4C^0$ for 12 - 18 h and divided into identical weight parts.One part wascooked for 30 min at 100C^ofollowed by cooling and theother part was left without cooking..Centrifugation at 1000g for 20 min was performed on both cooked and raw beef. The supernatant wasdialysed against 50% polyethylene glycol for 24 h.

2.3.2.Determination of protein content

The protein content of each allergen extracts was determined by [12] **.**Three millitters of each allergen extract were pipette in quartz cuvates .the absorbance value was measured spectrophotometrically at 260 and 280 nm.The protein content was calculated by the following equation:Protein concentration mg/ml=1.55×A280 - 0.77×A260.

2.3.4.Specific IgE estimation by ELISAtest

To determine the optimal dilution for serum , antigen (beef) and conjugate . Chequer board was conducted as described by [13] .Eight protein concentrations(µg/ml) for each tested allergens extract (100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml,6.25µg/ml,3.125µg/ml.56,1µg /ml,0.781µg/ml) were used.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 was added and so on . The plate was covered with covering foil and incubated at 4^oC overnight .Then washed by emptying and filling with PBS ,pH 7.2 containing (0.05 %) twin 20 for three times.Pool of teen serum samples 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 to each antigen dilution starting from second well in the first vertical row .Cover seal was applied and the plate was incubated at 37$C^o$for two hr, then washed as mentioned above .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 and incubated at 37 $C^o$for 1 hr.The plate was taken on (37^{o}C) incubation and washed three times as mentioned above.Freshly prepared substrate solution containing tetramethylbenzidine (TMB) 50 µl was added to each well of the plate .The covered seal was applied to the plate and incubated.at$37C^0$ for 30 minutes in the dark ,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. Thebest selected protein concentration ofbeef antigens (cooked and raw) were3.125µg/ml and 12.5µg/ml respectively. Sera (1/64µl/ml) and conjugate (1/3200µl/ml) ,were used in beef antigens based ELISA.

2.3.5.Estimation of negativity cut-off value

The cut-off value of ELISA negativity was estimated according to the method of [14] .briefly twelve serum samples were taken from nun allergic volunteer individuals according to the following formula:**Cut-off value=X+3SD**

X =the mean of the negative sample optical density.
SD=standard deviation of the negative sample optical density.

2.4.Molecular analysis:

The genomic DNA from the whole blood of 264 patients was extracted and purified according to the instructions of Wizard , Genomic DNA purification kit (protégé ,USA). For the detection of the HLA-DQB1*0305 by PCR the specific primerwere designed according to [15].As a follow forward, TGCACACCGTGTCCAACTC**,revers,** GCTACTTCACCAACGGGACC The PCR amplification mixture (25µl)includes 12.5 µl of green master mix (which contains bacterially derived Taq DNA polymerase , dNTPs , MgCl2 and reaction buffer at optimal concentration for efficient amplification of DNA templates by PCR)5 µl of template DNA ,0.5 µl of each forward and reverse primers and 6.5 µl of nuclease free water to complete the amplification mixture to 25 µl . The PCR tubes containing amplification mixture were transferred to preheated thermocyclerandstart the program as follow, 3 min at 95C^{o} for one cycle,then 40 cycle of 1minat95$C^{O,}$,1min at 58$C^{o,}$and,72C^{o} for 30 sec with one final extension of 5mpn at 72C^{o}.The results of PCR weredetected after the amplification process. 10 µl from amplification sample wasdirectly loaded in a 1.5% agarose gel containing 0.5 µl /25 ml ethidium bromide with the addition ofloading buffer and DNA size marker as standard in electrophoresis and run at 70 V , then the productswerevisualized by UV transilluminatoruntil the bromophenol blue tracking dye migrated to the end of the gel.The DNAwas observed and photographed by using gel documentation system

2.6.Statistical analysis

Statistical analysis is done by using SPSS software version 11, the chi square is used to assess. Statistical significance

3.Results and discussion

3.1.Serological study

3.2.Estimation of protein contentof allergens extracts.

The concentration of protein in the extract of raw and cooked beef was 11.82 and 27.73 mg/ml respectively

3.3.ELISA results

The seropositivity against beef was estimated by indirect ELISA test .The results of this test were displayed intable-1.In this table 13.3% of allergic patients weresignificantlydiffered ($p<0.05$) in theirseropositivity against beef (B1,B2) and the higher rate of seropositivity was observed in males (14.6%) and in 2nd age group patients (17.9 %) in concern to sex and age effect on the seropositivity.

Table (1) The distribution of beef based ELISA positive results according to age and sex ofallergic patients.

sex	.Exm No (%)	*B1,B2
Mal	130(49.2)	19(14.6)
female	134(50.8)	16(11.9)
Age group		
<10-20	57 (21.6)	7(12.2)
>20-30	67(25,3)	12(17.9)
>30-40	68(25.8)	6(8.8)
>40	72(27.3)	10(13.9)
Total (%)	264(100)	35(13.3)

p<0.05,*B1=Coked beef, *B2= Rawbeef

Food allergy remain a well-recognized problem that affects people of different age and can alter quality of life [16;17] . Among food allergies,hypersensitivity to beef ,various reports have implicated beef ingestion as cause of infantile colitis[18] , chronic diarrhea [19] , urticaria[20;21], glomerulonephritis [22],delayed anaphylaxis, angioedema or urticarial[23].In agreement with these studies clinical allergy to beef was detected in 13.3% of allergic patients in the present study . Different international studies have shown that , 83 % of beef allergic subjects had positive IgE antibodies response against bovine IgG in raw beef but this response was observed also in 24% of beef tolerant subjects [24] .[4] demonstrated that sensitization to beef varied from 7% to 26%.[25;4] mentioned that clinical reactivity to beef was lower than 12% [26;27] reported several cases of occupational contact urticaria and hand dermatitis due to beef meat.[23] mentioned that 36.4% of children were sensitive to commercial beef extract by using skin test as diagnostic test for beef allergy . While [28] studied 460 subjects and did not report any positive reaction to beef .To our knowledge evolutes studies of beef allergic patients have not been reported in Iraq .

One of the most important aims of this study is the determination of heating effect on the antigenicity , allergenicity of beef extracts and whether results from ELISA test could explain the differential reactivity of patients to raw and well cooked beef .One concern has been raised that some proteins may change their characteristics with various processing treatments [29] .In the current study the loss of beef allergy was not observed in all allergic patients and all of them showed the same positive IgEELISA results against raw and cooked beef . This finding was in agreement with previous published data . A report by [23] mentioned that the glycoprotein of food is generally implicated as the allergenic component .These glycoprotein are characteristically watersoluble ,largely heat resistant , acid stable and commonly in the molecular weight rang of 10 to 60 kd . [23] in the same study also documented the heat stability of (BSA) in PBS (pH 7.2), but this protein appeared as heat labile in the beef extract in a neutral pH.In contrast to the present results ,[30] indicated that heat may influence the antigenicity /allergenicity of beef ,he reported a patient who had anaphylactic symptoms after eating medium red beef meat but tolerated well cooked beef meat

.Thermal stability of proteins can be influenced by the pH [32] ,divalent cations,sugar content and lipid concentration of the solution ,as the lipid concentration remains high enough to destabilize the BSA protein in the beef meat extract even after the defeating procedure [21] .

Table (2) explained the significant difference($p<0.05$)in the distribution of the seropositivityrate in the studded allergic patients according to sex of patients within age groups . As was shown in this table the males in 2nd age group showed higher rate of seropositivity (23.5%) , while females of 4th age group had high rate of seropositivity (13.9%). The current study contain another important observation , that beef allergy appeared in clinically allergic patients of different age and sex in contrast to previous reports which showed that meat allergy is rarely described in children ,young and adult life [4;5;3;22] .While other studies supported the results of the present study in which clinical onset of beef allergywas appeared in mature age[21; 24].

Table(2) The distribution of positive beef based ELISA results according to sexofallergic patients within age groups

Ages group	sex	Exam. No.	B1,B2*	
			positive No.	%
<10-20	M	27	4	14.8
	F	30	3	10
>20-30	M	34	8	23.5
	F	33	4	12.1
>30-40	M	33	2	6.1
	F	35	4	11.4
>40	M	36	5	13.9
	F	36	5	13.9
Total	264		35	13.3

p<0.05,*B1=Coked beef,*B2=Rawbeef

Table (3) displayed the non significant ($p>0.05$) variable values of optical density mean and standard deviation and negativity cut off value in seropositive allergic patients of different sex and age groups according to specific IgE ELISA results .

Table (3)The optical density (Mean ± Standard deviation)and negativity cut offvalues in seropositive allergic patients tested with beef and chicken meat allergens based ELISA.

Ages group	sex	+VeNo.	OD value Mean ± SD	
			*B1	*B2
<10-20	M	4	**0.524 ±0.342262**	**0.496 0.261203**
	F	3	**0.409 ±0.020648**	**0.302 0.302692**
>20-30	M	8	**0.8881 ±0.344742**	**0.755±0.253201**
	F	4	**0.692±0.171476**	**0.384±0.316276**
>30-40	M	2	**0.698±0.024042**	**0.682±0.061518**
	F	4	**0.769 ±0.271486**	**0.725±0.121907**
>40	M	5	**0.296±0.173315**	**0.385±0.149475**
	F	5	**0.231±0.174222**	**0.201±0.107948**
Negativity cut off l	35		**0.115187**	**0.111920252**

P>0.05,*B1=Coked beef *B2=Raw beef

3.4.PCR results

The results of PCR amplification performed on the extracted DNA was confirmed by electrophoresis . The result of the successful binding between the specific primers of HLA- DQB1*0305 and the extracted DNA appeared as single band under UV illuminator ,using ethidium bromide as a specific DNA stain .Only the band with expected size, 195bpwas observed in figures (1) .

Figure (1) HLA DQB1* 0305 PCR products
Lane 1:100bp Ladder, Lane 2-8 HLA DQB1* 0305

Table(4) Theassocition between positive beef based ELISA results and HLA- DQB1*0305 according to sexofallergic patients within age groups.

ges group	sex	positive B1,B2* Exam. No.	HLA- DQB1*0305	
			positive No.	%
<10-20	M	4	4	100
	F	3	3	100
>20-30	M	8	8	100
	F	4	4	100
>30-40	M	2	2	100
	F	4	4	100
>40	M	5	5	100
	F	5	5	100
Total	35		35	100

p<0.05, *B1=Coked beef,*B2=Rawbeef

The HLADQB1*0305 was significantly (p<0.05) distributed among allergic patients who had positive IgE response directed against beef (raw or cooked).In table (4) the HLA DQB1 *0305 presented in (100%) of patients who were sensitive to beef allergens .HLA class II alleles have been implicated in susceptibility to a wide range of diseases known to have an immunological basis .Atopic allergy is one of these disease and the most probable role of these genes is the interaction between the HLA-peptide complex and T-cell receptors in setting up the allergic reaction and their pivotal role in immune response regulation combined with their extensive polymorphism [33] .In agreement with this hypothesis , the present study revealed the associationof some HLA class II alleles with beef allergic patients. The association between genes within the HLA complex and food allergy are supported by several genome -linkage studies.Cow's milk allergy was associated with HLA-DQ7(HLA-DQB1*0305) in an Italian patient sample[34] . [35] did not find any association between cow's milk allergy and HLA-A,B,BW,C or DR antigens . Studies on other food allergies have reported associations with HLA haplotypes. Peanut allergy was associated with DRB1*08, DRB1*12 and DQB1*04 in Caucasian subjects [36] . Boehncke and Coworkers also reported an association between peanut allergy and HLA-DRB1*08,[37] found asocition between carrot allergy and HAL-DRB1*12 and grass pollen allergy and HLA-DQB1*0301, whereas DRB1*01,DQA1*0101 and DQB1*0501 forming ahaplotype were decreased among birch pollen allergy associated hazel nut allergy patients .In conclusionthepositive specific IgE response to beef allergens was significantly associated with HLA DQB1*0305 andThe loss of beef allergy was not observed in all allergic patients and all of them showed the same positive raw and cooked beefspecific IgE response.

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ارتباط0305*DQB1- HLA مع النوع الاول من فرط الحساسيه في المرضى المتحسسين للحم الابقار

شيماء جبار ريسان فوزيه علي عبدالله

الملخص

تعد الحساسية الغذائية من المشاكل الصحية المهمة . و بما انه لا توجد دراسات سابقه لتقييم الحساسية الغذائية للحوم الأبقار في العراق , لذا كان الغرض من هذه الدراسة هو الكشف عن الحساسية الغذائية باستخدام الاختبارات المناعية والوراثية.شملت الدراسة فحص مصول (264) مريض يشتبه بإصابتهم بالحساسية الغذائية للحوم الأبقار لغرض الكشف عن الأجسام المضادة من نوع IgE المتخصص الموجهة ضد لحم الأبقار باستخدام اختبار ELISAغير المباشر. استخدمت تقنية تفاعل البلمرة المتعدد السلسلة للكشف عن العلاقة الوراثية بين الحساسية الغذائية و الاليل (HLA -DQB1*0305) . وبالاعتماد على نتائج الايجابية المصلية فأن 13.3% من المرضى لديهم حساسية ضد لحم الأبقار بنوعيه المطبوخ وغير المطبوخ وعند اخذ تاثير الجنس والعمرعلى النسبة الكلية للايجابية المصلية فان اعلى نسبه (14.6%) لوحظت في الذكور ومرضى الحساسية في الفئة العمرية الثانيه و بنسبة () (17.9 %)كما لوحظ أن قيم الكثافة الضوئية المقدرة بالوسط الحسابي وانحراف المعيار القياسي لا تختلف فيما بينهاإحصائيا (P>0.05) في جميع المرضى الايجابيين مصليا .لم يظهر فقدان في حساسيه لحم الابقارالمطبوح وان جميع المرضى اظهروا نتائج ايجابيه متشابهه في اختبار ELISA المعتمد على لحم الابقار المطبوخ والنئ. اظهرت نتائج تقنية تفاعل البلمرة المتعدد السلسلة وجود حزمه بحجم(195bp) ناتجه عن ارتباط مستخلص ال DNA مع بادئة الاليل 0305*DQB1- HLA و ان النتائج الموجبة لوحظت في الاليل0305*DQB1 HLAالذي ظهر بنسبة (100%)في مرضى الحساسية ذو الايجابية المصلية.ووجد ترابط محسوس احصائيا (**p<0.05**) بين الاليل0305*DQB1- HLA و استجابة IgEالموجبه ضد مستضدات لحم الابقار