

STUDY OF AMERICAN COCKROACH ALLERGY DISTRIBUTION AND ITS ASSOCIATION WITH SOME HLA- DQ AND - DR ALLELES IN ATOPIC PATIENTS IN BASRAH PROVINCE / IRAQ

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ABSTRACT

Ninety six for each healthy(n=96)and atopic,(n=96)individuals duals from the same geographical region, paired by sex and age, their sera specific IgE antibodies were estimated by enzyme linked immune sorbent assay test (ELISA) and genotyped by polymerase chain reaction based onHLA-DQB1*0602, HLA-DQB1*0604 andHLA-DRB1*12.

The specific IgE based on ELISA results revealed that Out of 96 only59 (61.5%) of atopic patients were sensitive to CR allergen. The association between sensitivity to CR allergen and age was considered to be not statistically significant ($P>0.05$).However the higher rate of CR allergens sensitivity(62.9%) was observed in first age group(<45 year) of atopic patients. In contrast the effect of sex on sensitivity to CR allergens was considered to be statistically significant ($p<0.05$) and the higher rate of sensitivity(75.6%) was observed in atopic patients males.

The overall differences in the HLA-DQB1*0602, HLA-DQB1*0604 and HLA-DRB1*12 alleles frequency between patients and controls werestatistically ($p<0.05$).According to the results of risk factors statistical analysis values(p :value = 0.0001; OR:84.3481; CI 95%: 5.065- 1404.520) HLA-DQB1*0602 allele was associated with susceptibility of patients to the CR allergy.

The overall differences in the HLA-DQB1*0602, HLA-DQB1*0604 and, HLA-DRB1*12 alleles distribution between atopic patients and controls were statistically

highly significant ($p < 0.01$) concerning the effect of the age and sex. In general the allele HLA-DRB1*12 was not observed in both atopic patients and controls in contrast HLA-DQB1*0602 was present in atopic patients only while HLA-DQB1*0604 appeared in both patients and controls with different frequencies. The older atopic patients showed higher frequency (61.8%) for the HLA-DQB1*0602 allele. In contrast higher frequency of HLA-DQB1*0604 allele occurred in younger patients (40.3%). According to sex, the higher frequency of HLA-DQB1*0602 allele was observed in males patients (31.7%) while the allele HLA-DQB1*0604 higher frequency (17.9%) was observed in the females of the control group.

The overall frequency of HLA-DQB1*0602 (60.4%) or HLA-DQB1*0604 (39.6%) as a single allele was observed in the seropositive or seronegative atopic patients. The seropositive showed higher frequency (35.6 and 15.3%) for HLA-DQB1*0602 and HLA-DQB1*0604 respectively.

INTRODUCTION

Bernton and Brown described cockroach allergy in 1967 (1) and studies carried out throughout the world have shown an association between asthma and the development of specific IgE antibodies. The role of cockroach antigens in respiratory allergies has only been studied previously (2,3). Various antigens from the two most common species, *Periplaneta americana* and *Blattella germanica*, have been identified and purified. The most important of these are the major and the secondary antigen from *Blattella germanica* (*Bla g1* and *Bla g2*) and the major antigen from *Periplaneta americana* (*Per a1*), with an extensive cross reaction between them. Prick test reactivity and blood IgE response to roach antigens are demonstrable in many asthmatics. It has also been shown that an increase in lymphocyte proliferation in response to *Bla g2* antigen is associated with high levels of *Bla g1* or *Bla g2* in homes (3).

Exposure to allergens is assumed to play an important role in asthma, as indicated by the following considerations: the association of asthma with sensitivity to common allergens in the home is strong and found in various parts of the world; an allergenic bronchial provocation induces both immediate and delayed bronchial responses in asthmatics; a decrease in the levels of exposure to allergens results in a reduction in bronchial hyperactivity (4).

Sensitivity to mites in household dust is considered to be the strongest risk factor for asthma in some studies (5), but other allergens have also been demonstrated to be significantly related to asthma, such as dog and cat epithelium, mold spores and cockroaches (CR) (6,7). The most common types of cockroaches are *Blattella germanica*, *Periplaneta americana* and *Blattella orientalis* (8). In urban areas, German cockroach is more prevalent (9). Over 20% of the world population suffers from immunoglobulin E (IgE) mediated allergic diseases such as asthma, rhino conjunctivitis, eczema and anaphylaxis (10). Airway allergy is now considered to be a disease not confined to a specific target organ but rather a disorder of the whole respiratory tract. Epidemiological evidences and clinical as well as experimental observations have suggested a link between rhinitis and asthma leading to a definition of allergic rhinobronchitis (11) or united airways diseases (UAD) [12] and the concept of 'one airway one disease'. German cockroach produce several important allergens, including Bla g 1, Bla g 2, Bla g 4, and Bla g 5, that are secreted and accumulate in the environment. Previous studies have shown that Bla g 2 is a potent allergen that elicits IgE responses in 60 to 80% of cockroach allergic patients and gives positive immediate skin tests at concentrations as low as 10–10⁻⁶ g/ ml (13,14). Many epidemiological studies have shown that 10 to 100-fold lower levels of CR allergens elicit IgE responses when compared with other common indoor allergens, such as dust mite or cat (15). Exposure to low levels of Bla g 1 and Bla g 2 has also been associated with wheezing among infants in the first 3 months of life and with increased proliferative T cell responses (16). Sensitization and exposure data suggest that Bla g 2 is an especially potent allergen. Skin tests were associated with exposure to median Bla g 2 levels of 0.32 g/ g (range 0.08–15 g/ g), whereas comparable figures for dust mite were 38 g/ g (range 2–45). This study aimed to test for specific IgE responses against the cockroaches allergens and if HLA- DRB1*12, HLA- DQB1*0602 and HLA- DQB1*0604 gene products have a general influence on the reaction to cockroaches allergens.

MATERIALS AND METHODS

Study population

The investigated population consisted of 96 apparently symptomatic and 96 healthy control volunteer individuals. The investigated population of eligible cases attending

the center for asthma and allergic diseases, AL-Mauanya and Al-Shafaa hospitals in Basra during August and September 2011. The range of symptomatic and healthy controls ages was from 16–70 and 16–69 years, respectively. Forty one of symptomatic were males and fifty five were females while forty five of healthy controls were males and fifty one were females, these numbers were chosen randomly. The symptomatic patients were complaining from symptoms related to upper and lower respiratory tract disorder or conjunctival disease or urticaria. All investigated individual agreed to participate in the trial and tested serologically by specific IgE based ELISA test and genotyped by HLA-DQ based PCR. HLA-DR genotyping.

Samples

From each studied individual 5ml of venous blood, was collected in plain tube; two ml of collected blood was centrifuged for 10 minutes (1500 rpm/ min), to obtain serum used in ELISA test, The remained 3ml of blood was poured in tubes containing EDTA, kept under - 18 °C for until use in HLA-DQB1 and HLA-DR genotyping.

Preparation Of The Allergen Extract And Material Sources

Frozen CR (*Periplaneta americana*) was previously collected from Basrah indoors. pulverized was using a mortar and pestle. Then log of CR mixture (10 g) was defatted with ethyl ether (1:5, w/ v). The allergen was extracted for 48 hours at 4°C in phosphate-buffered saline (PBS; pH 7.4) that contained 0.2% phenol. The extract was centrifuged at 13,000 ×g for 15 minutes at 4°C, and the supernatant was dialyzed extensively against distilled water. The dialyzed sample was (0.22-µm; Millipore) filtered. Thereafter, the extract was aliquot, and kept under - 18 °C until use. (17).

Estimation Of Protein Concentration

The protein content of CR allergen extract was determined by Hudson, L. and Hay, F. C. (1989) (18) method: 3 milliliters of each allergens extract were pipette in quartz cuvette. 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 = $1.55 \times A_{280} - 0.77 \times A_{260}$. The concentration of protein in the CR extract was (1.06 mg/ ml)

Detection of Specific IgE By ELISA Technique

The partially purified CR allergen extract was used as antigens in ELISA test. Chequer board titration ELISA (CB-ELISA) was used to determine the optimal

dilution for the three reagents serum, antigen and conjugate (19). Depending on the results of CBELISA, non diluted antigens, sera and conjugate were used in ELISA test to determine the diagnostic level of the IgE antibodies in the tested serum samples of 96 symptomatic and 96 healthy controls. The cut-off value was estimated according to the method of (20). Briefly ten serum samples were taken from healthy controls individuals. These samples considered as negative control and have been tested to determine cut-off value according to the following formula:

Cut-off value = $X + (3 \times SD)$ X = The mean of the negative sample optical density, SD = standard deviation of the O.D value, Any sample showed (OD) value equal or greater than the cut off value considered as positive.

PCR -based HLA-DQB1 and HLA-DRB1 Genotyping

The genomic DNA from the whole blood of 192 tested patients and healthy controls individuals was extracted and purified according to the instructions of Genomic DNA purification kit (BIONEER-USA). For the detection of the HLA-DRB1*12, HLA-DQB1*0602 and HLA-DQB1*0604 by PCR the specific primer were designed according to (21)(Table.1). The PCR amplification mixture (25 µl) 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, 1 µl of each forward and reverse primers and 5.5 µl of nuclease free water to complete the amplification mixture to 25 µl. The PCR tubes containing the amplification mixture were transferred to preheated the rmocycler (Cleavere Scientific-USA). The rmocycling parameters were as follows: denaturation at 94°C for 5min and 30 cycles of 94°C for 30s, 60°C for 45s, and 72°C for 45 s, with a final extension at 72°C for 5min. Amplified products were resolved by using 1.5% agarose gel electrophoresis containing 0.5 µl / 25 ml ethidium and DNA size marker.

Statistical analysis is done by using SPSS software version 11. To demonstrate any association between results, the exact Fisher test, T test and Pearson's chi-squared test with Yates correction were used with the limit of significance being set at 5%. The values of odds ratio (OR) and 95% confidence interval (CI) were calculated.

Table(1) HLA class II Oligonucleotide primers sequence used for PCR.

HLA-	Alleles	primer sequence (5' → 3')		SIZ bp
		Forward	Reverse	
DRB1	*12	AGTACTCTACGGGTGAGTG TT	CACTGTGAAGCTCTCCAC AG	248
DQB1	*0602	CGTGCGTCTTGTGACCAGA T	GCTGTTCCAGTACTCGGC AT	121
	*0604	CGTGTACCAGTTTAAGGGC A	GCAGGATCCCGCGGTACC	254

RESULTS

Specific IgE Based ELISA Results

The sensitivity to CR allergen was estimated by IgE based indirect ELISA test .The result of this test was displayed in Table 2 and 3.Out of 96 only59 (61.5%) of allergy symptomatic patients were sensitive to CR allergen. The association between sensitivity to CR allergen and age group was considered to be not statistically significant($p>0.05$).However the higher rate of sensitivity(62.9%)was observed in first age group(<45)allergy symptomatic patientsTable.2. This table also shows the positive ELISA results as a mean \pm SD of the optical density values which were recorded spectrophotometrically by ELISA reader. According to the relation of age and sensitivity to CR allergens ,the test of mean significance revealed that there was no significant difference at a 95% level in the mean \pm SD value of ELISA reading optic density between the two age groups of patients.

The association between sensitivity to CR allergen and sex was considered to be statistically significant ($p<0.05$) and the higher rate of sensitivity(75.6%)was observed in symptomatic allergy patients males. However there was no significant difference at a 95% level in the mean \pm SD value of ELISA reading optic density between males and females patients(Table. 3).

Table.2 The distribution of CR allergen based ELISA results according to age of allergy symptomatic patients .

Age groups (years)	Tested n.(%)	Specific IgE	
		Seropositive n.(%)	ELISA OD value: Mean \pm SD
≤ 45	62(64.6)	39(62.9)	0.3216 \pm 0.1508
>45	34(35.4)	20(58.8)	0.2861 \pm 0.0766
Total	96(100)	59(61.5)	

The difference for all patient age was not significant (χ^2 :0.030;degrees of freedom (DF):1; p-value = 0.8622) OD= Optic Density

Table 3- The distribution of CR allergen based ELISA results according to sex of allergy symptomatic patients .

Sex	Tested n.(%)	Specific IgE	
		seropositive n.%	ELISAOD value: Mean \pm SD
Males	41(42.7)	31(75.6)	0.3178 \pm 0.1617
Females	55(57.3)	28(50.9)	0.308 \pm 0.1014
Total	96(100)	59(61.5)	

patients of different sex the difference was significant (χ^2 :5.053;degrees of freedom (DF):1; p-value = 0.0246). OD= Optic Density

PCR -Based Genotyping Results

The Results Of PCR Amplification

The amplification of the extracted DNA was confirmed by electrophoresis in which the successful binding of the specific primers of HLA- DQB1*0602, HLA- DQB1*0604, and HLA- DRB1*12 with the extracted DNA appeared as single band under UV illuminator ,using ethidium bromide as a specific DNA stain .Only the band with expected size, 121 (HLA- DQB1*0602)and 254(HLA- DQB1*0604) was shown Figures (2).

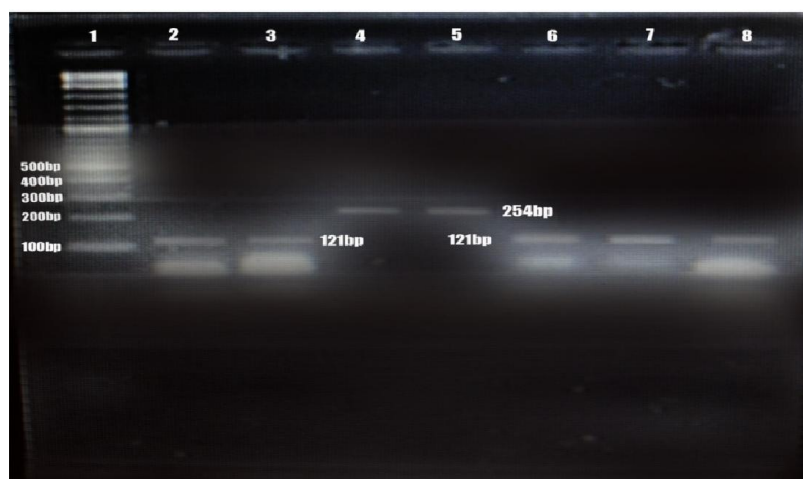


Figure 1 : PCR Products of HLA-DQB1*0602, and HLA-DQB1*0604 alleles. Lane1:ladder,Lanes:2,3,6,7,8:HLA-DQB1*0602(121bp).Lanes:4,5:HLA-DQB1 *0604 (254bp).

Associations Of HLA Alleles With Allergy

In Table-4,5 the overall differences in the HLA-DQB1*0602, HLA-DQB1*0604 and, HLA- DRB1*12 all else distribution between atopic patients and control were statistically highly significant ($p<0.01$) concerning the effect of the age and sex .In general the allele HLA-DRB1*12 was not detected in both CR sensitive patients and controls in contrast HLA-DQB1*0602 was present in allergy symptomatic patients only while HLA-DQB1*0604 appeared in both patients and controls with different frequencies .The older atopic patients showed higher frequency(61.8%)for the HLA- DQB1*0602 . In contrast higher frequency of HLA- DQB1*0604 occurred in younger patients(40.3%)(Table-4)

The distribution of HLA- DQB1*0602, HLA- DQB1*0604 and HLA-DRB1*12 genotypes according to sex of allergy symptomatic patients and controls was displayed in table-5. The overall frequency of HLA- DQB1*0602 in males and females patients was 31.7and 29.1% respectively while the HLA- DQB1*0604 higher frequency(17.9 %) was observed in the females of the control group.

In table-6the overall frequency of HLA HLA- DQB1*0602 and HLA HLA-DQB1*0604 distribution as a single allele in the seropositive and seronegative allergic patients allergic patients was 60.4and 39.6% respectively. The seropositive showed higher frequency(35.6 and 15.3%) for HLA- DQB1*0602 and HLA-

DQB1*0604 respectively compared to frequency of same alleles(HLA- DQB1*0602 and HLA- DQB1*0604)in seronegative patients (21.6 and 8.1% respectively).

Odds ratio test was performed on both allergy symptomatic patients and controls subjects. The results of this risk factors statistical analysis revealed that HLA- DQB1*0602 was associated with unacceptability to the allergy (table 7). The values of susceptibility association include p-value = 0.0001; OR:84.3481; CI 95%: 5.065- 1404.520). HLA- DQB1*0604 and HLA- DRB1*12 association was considered to be not statistically significant risk factors for protection and susceptibility to allergy.

Table-4. The distribution of HLA-DQB1 and HLA-DRB1 alleles according to atopic patients and controls age .

Age groups (years)		Tested n.(%)	HLA-DQB1		HLA-DRB1
			*0602 +ve n.(%)	*0604 +ve n.(%)	*12 +ve n.(%)
<45	P n.=96	62(64.6)	20(59.7)	7(40.3)	0
	C n.=96	72	0	7(9.7)	0
>45	P n.=96	34(35.4)	9(61.8)	5(38.2)	0
	C n.=96	24	0	3(12.5)	0

Yates' chi-square:11.712, Yates' p-value:0.0084377, df:3 P=Patients C=controls

Table-5. The distribution of HLA- DQB1 and HLA- RB1 alleles according to atopic patients and controls sex .

Sex		Tested n.(%)	HLA-DQB1		HLA-DRB1
			*0602 +ve n.(%)	*0604 +ve n.(%)	*12 +ve n.(%)
Males	P n.=96	41(64.6)	13(31.7)	5(12.2)	0
	C n.=96	57(59.4)	0	3(5.3)	0
Females	P n.=96	55(35.4)	16(29.1)	7(12.7)	0
	C n.=96	39(40.6)	0	7(17.9)	0

Yates' chi-square:11.232, P:0.01053, df:3

Table-6. The association of HLA- DQB1 and HLA- RB1 alleles with CR- based ELISA seropositivity results.

CR-based ELISA Seropositivity	Tested n.(%)	HLA-			Total
		DQB1*0602	DQB1*0604	DRB1*12	
Seropositive	59(61.5)	21(35.6)	9(15.3)	0	30(50.8)
Seronegative	37(38.5)	8(21.6)	3(8.1)	0	11(29.7)
Total	96(100)	29(60.4)	12(39.6)	0	41(42.7)

 $\chi^2:0.047, P:0.8283, df:1$
Table -7. Risk factors analysis for the association of allergy with HLA- DQB1*0602, HLA- DQB1*0604 and HLA- RB1*12 alleles.

HLA-	Patients n.=96	Controls n.=96	Odds ratio	95% confidence interval	P
DQB1*0602	29(60.4)	0	84.3481	5.065- 1404.520	0.0001
DQB1*0604	12(39.6)	10(10.4)	1.2286	0.504- 2.996	0.8213
DRB1*12	0	0	1.0000	0.019- 50.914	1.0000

DISCUSSION

Changes in life style with individuals spending more time in closed environments and a consequent increase in exposure to household allergens (22). As than other forms of allergic illnesses represent significant public health problem (23). Cockroaches have been associated with asthma in a number of regions throughout the world. Unfortunately, in contrast to the many controlled clinical studies that support the use of pharmacological treatment of asthma, there has been a relatively small amount of research devoted to the exposure to allergens and to the determination of whether removing or minimizing allergen exposure would be an important measure for controlling asthma (24). Even for dust mites, which have been the allergens most extensively studied, there is some uncertainty about whether environmental control measures would actually bring effective benefits to the control of asthma (25).

An important role is played by allergens; in some atopic respiratory disorders hence it is essential to identify them for diagnosis and proper treatment of these disorders.

In the current study the protein extract of crushed whole Cockroaches body have been used as antigen in the serological determination of hypersensitivity of studied allergic patients to these insects by performing specific IgE based indirect ELISA. This application supported by other previous studies as.,(26) who reported that protein called glutathione *S*-transferase (EC 2.5.1.18; GST). Natural *B. germanica* GST, purified from cockroach body extracts by glutathione affinity chromatography, showed excellent IgE antibody binding activity. 1.,(27) stated that the Potential cockroach allergens in household dust include whole bodies of dead insects, saliva, secretions, shed products and feces. Relevant allergens have been identified in the body and the feces of the insects .

The present study showed that there was a 61.5% higher frequency of patients with respiratory and/ or skin allergic diseases exhibit positive reactions to specific IgE for cockroaches protein extract. in agreement with data of studies which used different techniques for the assessment of exposure (26) .Beside that (28) have demonstrated that 40 to 55% of children and young adults with asthma and/ or allergic rhinitis exhibit positive reactions to the skin test and/ or specific IgE for cockroaches

The population of the present study consisted of 96 atopic patients of both sexes aged

15 to 75 years, Despite the existence of an association between allergic diseases (including asthma and rhinitis) and exposure to cockroach antigens, the present group of patients was not investigated for the presence of household cockroaches. The household species of cockroaches spend large part of the time aggregated, hidden in dark and damp places. As a result, infestation is not often apparent to the dwellers. Gelber et al. (29) demonstrated that 20% of homes studied with no visible evidence of cockroach infestation exhibited levels of *Blag2* antigens in at least one of the rooms of the house.

The purpose of the study was not to measure the levels of cockroach allergens present in the residences, but to quantitatively assess the presence of cockroaches health impact. However, we realize that it was an oversight not to have determined the number of dead cockroaches in each room of the house, a procedure that would have permitted the current data to correlate with those reported in a study by, (30) reported

that the combination of exposure and sensitivity to cockroach allergens is a risk factor for the severity of asthma in children and (31) confirmed the relationship between cockroach infestation and severity of the illness in the 31.6% (25/ 79) of the exposed asthmatic patients in the infested residences.

Although the combination of cockroach allergy and exposure to high levels of infestation can explain some aspects of these patients, a clear causal relationship between these factors has not been sufficiently demonstrated (26;30) and more information should be obtained about this relationship.

Whereas in the current study there is a lack of knowledge on the part of the population about the relationship between exposure to cockroach allergens and allergic diseases. When questioned, the large majority of persons stated that they knew nothing about this association. statistical analysis of the present data revealed that association between sensitivity to CR allergen and sex was considered to be statistically significant ($p < 0.05$) and the higher rate (75.6%) of IgE reactivity against CR allergen was observed in allergy symptomatic patients males. The high ratio of allergic males may explain this result.

In agreement,(31) who reported that asthma was diagnosed by the questionnaire in 11.8% (11/ 93) of the noncockroaches infestation residences ($P = 0.001$), no significant correlation between level of infestation and severity of the illness ($P = 0.89$) and no statistically significant effect for the age groups, the statistical analysis of the present study revealed no significant correlation between level of IgE reactivity and age ($P > 0.05$) and the higher ratio of IgE reactivity(62.9%) was observed in first age group(<45) of atopic patients.

Risk Factors Association

Allergic disorders result from an interplay between genetic, epigenetic, and environmental factors (32) . The genetic component is undoubtedly multifactorial (33). Numerous genes, some increasing susceptibility ,and others with a protective effect, together affect the development of e.g. asthma (32). One approach for studying genetic factors in allergy is to investigate candidate genes that have functions related to allergic symptoms or immunopathology (34) such as HLA class II A and B genes. HLA class II A and B genes encode for A and B chains which form a cell surface heterodimer on antigen-presenting cells. These molecules interact with antigen peptide fragments and with the T cell receptor and their essential role in the immune response together with their great polymorphism explain the association of certain

allelic variants with the susceptibility to certain immune disorders (35,36) . Independent publications have shown that certain human leukocyte antigen (HLA) alleles are strongly associated with hypersensitivity. As HLA molecules are a critical element in T-cell stimulation: It is not surprising that particular HLA alleles have a direct functional role in the pathogenesis of hypersensitivity and have reported associations between HLA alleles and atop and/ or specific allergies. Cow's milk allergy (CMA)was associated with HLA-DQ7 (HLA-DQB1*0301) in an Italian patient sample (37). However, a larger Finnish study comparing 100 CMA patients with healthy subjects did not find any association between CMA and HLA A, B, Bw, C or DR antigens(38). Studies on other food allergies have reported associations with HLA haplo types. Peanut allergy was associated with DRB1*08, DRB1*12 and DQB1*04 in Caucasian subjects (39) . Boehncke and coworkers also reported an association between peanut allergy and HLA-DRB1*08 (40). HLA class II restricted IgE production against certain part of allergens explains in part the genetic susceptibility of subjects with allergic asthma (41).

In agreement with these studies the current study confirms these finding by revealing that the overall differences in the HLA-DQB1*0602, HLA-DQB1*0604 and, HLA- DRB1*12allelesbetween patients and controls were statistically highly significant ($p<0.01$).According to the results of risk factors statistical analysis HLA-DQB1*0602allele was associated with susceptibility of patients to the allergy .

(42) disagreed with the current results as they mentioned that the HLA-DRB1*12 significantly increased in asthmatic patients(4.5% vs. 0%, P -value=0.04). HLA-DQB1*0603 and 0604 alleles were significantly higher in asthmatics than those in normal controls (10% vs. 0%, P -value= 0.0001; and 9.3% vs. 3.7%, P -value= 0.04, respectively). Conversely, HLA-DQB1*0501 and 0602 were decreased in asthmatics compared to normal controls .In conclusion Allergy to cockroaches is widely distributed in Basrah atopic patients and the HLA-DQB1*0602 allele associated with susceptibility to allergy.

دراسة انتشار ارجية الصرصر الامريكي وارتباطها مع بعض الألائل HLA-DQ و DR لمرضى الحساسية في محافظة البصرة / العراق

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الخلاصة

سنة وتسعون شخص لكل من مرضى الحساسية ومن الاصحاء من نفس المنطقة الجغرافية قسموا الى مجموعتين اعتمادا على الجنس والعمر وقدرت الاجسام المضادة IgE في مصولهم بواسطة اختبار الممتز المناعي المرتبط بالانزيم واستخدم تفاعل البلمرة المتسلسل المعتمد على الألائل HLA-DQB1*0602, HLA-DRB1*12, DQB1*0604 في تمييزهم الجيني.

كشفت نتائج اختبار الممتز المناعي المرتبط بالانزيم (ELISA) الذي يتخذ IgE الخاص اساسا لهان من مجموع 96 مصاب بالحساسية كان 59 (61.5%) منهم فقط متحسس لمستارات الصرصر. ان الربط بين التحسس لمستارات الصرصر والعمر لم يعتبر ذو معنوية احصائية ($P > 0.05$). مع ذلك لوحظت اعلى نسبة تحسس (62.9%) لمستارات الصرصر في مرضى الفئة العمرية الاولى (< 45 year). على العكس من ذلك اعتبر تأثير الجنس على التحسس لمستارات الصرصر ذو معنوية احصائية ($p < 0.05$) ولوحظت اعلى نسبة تحسس (75.6%) في الذكور من مرضى الحساسية. كان الاختلاف الشامل لتردد الألائل HLA-DQB1*0602, HLA-DRB1*12 و DQB1*0604 بين المرضى والسيطرة ذو معنوية احصائية ($p < 0.05$). اعتمادا على قيم نتائج التحليل الاحصائي لعوامل الخطورة-5.065; CI95%: 84.3481; OR: 0.0001; p -value = 1404.520) ارتبط الألائل HLA-DQB1*0602 بقابلية المرضى للإصابة بالحساسية للصرصر. كان الاختلاف الكلي لانتشار الألائل HLA-DRB1*12 and, HLA-DQB1*0604, HLA-DQB1*0602 بين مرضى الحساسية والسيطره ذو معنوية احصائية عالية ($p < 0.01$) عند اخذ تأثير العمر والجنس بعين الاعتبار. بصورة عامه لم يلاحظ الألائل HLA-DRB1*12 في كل من مرضى الحساسية والسيطره وعلى العكس من ذلك وجد الألائل HLA-DQB1*0602 في مرضى الحساسية فقط بينما ظهر- HLA-DQB1*0604 في كل من مرضى الحساسية بترددات مختلفه. اظهر المتقدمين بالسن من مرضى الحساسية اعلى تردد (61.8%) للألائل HLA-DQB1*0602 على العكس من ذلك اعلى تردد (40.3%) للألائل- HLA-DQB1*0604 ظهرت في صغار السن من المرضى. اعتمادا على الجنس لوحظ اعلى تردد للألائل- HLA-DQB1*0602 في ذكور المرضى (31.7%) بينما لوحظ اعلى تردد (17.9%) للألائل HLA-DQB1*0604 في اناث مجموعة السيطرة. لوحظ التردد الكلي لكل من HLA-DQB1*0602 (60.4%) او- HLA-DQB1*0604 (39.6%) كألائل مفرد في مرضى الحساسية ذوي المصلية الايجابية او السلبية. اظهر المرضى ذو المصلية الايجابية اعلى نسب التردد (35.6 و 15.3) الألائل HLA-DQB1*0602 و- HLA-DQB1*0604 على التوالي.

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