THE RELATIONSHIP BETWEEN HLA-DRB1*0103 AND HLA-DQA1*0201 GENOTYPES AND SPECIFIC IGE RESPONSES TO UNRELATED ALLERGENS IN ATOPIC PATIENTS

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ABSTRACT

Because of the pivotal role of the human leukocyte antigen (HLA) class II molecules in regulating the immune response and their extensive polymorphism, it is not surprising that particular HLA class II alleles have been implicated in susceptibility to allergic diseases and in restriction of the IgE responses to a variety of allergens. We investigated the relationship between HLA-DRB1*0103 and HLA- DQA1*0201 genotypes and allergies to amoxicillin(AX) and love bird (LB) and explored their restriction of IgE responses to these allergens.

Enzyme Linked Immunosorbent Assay test (ELISA) was used to examine the specific IgE antibodies in the sera of 175 subjects including 145 atopic patients and 30 healthy individuals without any allergic reaction. All of them were chosen for HLA genotyping by polymerase chain reaction . Compared with seropositive all control and seronegative individuals showed negative PCR HLA genotyping results.The higher overall rate (39.4%) of PCR positivity was observed inseropositive patients with positive PCR HLA-DQA1*0201 genotyping results .Also PCR HLA-DQA1*0201 genotyping revealed the higher rates of the PCR positivity in the males and females of the second age group in comparison with those of the first age groups but without significant difference (P > 0.05). The same result was also observed in positive PCR result for the patients with HLA-DRB1*0103 and those with both HLA -DRB1*0103 and HLA-DQA1*0201 as the non-significant higher PCR positive rates was observed in the males and females of the 2nd age groups.Also the females of the second age group showed the higher PCR positivity rates (100%,50% and30%)for HLA-DQA1*0201 and HLA-DRB1*0103 and both respectively ELISA results revealed that the overall rate of seropositivity in tested individuals to amoxicillin(AX) or love bird dropping extract (LBDE) allergens was 54.9% .The females were not significantly different (P ≥ 0.05) from males and they showed the
higher rate of seropositivity against AX (57.9%) and LBDE (58.9%) allergens. In contrast there was no significant effect \((P > 0.05)\) for the age on the seropositivity against LBDE and the patients of the 1\(^{st}\) age group showed higher rate (55.9\%) of seropositivity in comparison with rate (43.7\%) of 2\(^{nd}\) age group patients.

**INTRODUCTION**

Atopy is a syndrome characterized by exaggerated and persistent IgE responses to environmental allergens. In the general population and within families, beta-lactam (BL) antibiotics are the most frequent elicitors of drug hypersensitivity reactions (HR). Although benzylpenicillin (BP) was the first BL implicated in hypersensitivity reactions, amoxicillin (AX) now being the most frequent drug inducing reactions (1). Clavulanic acid is emerging as a drug involved in a significant number of cases (1,2). Although HRs can be produced by any of the four immunological mechanisms of Gell and Coombs, in the allergy practice we mainly deal with type I and type IV reactions. As a working classification, they can be grouped as immediate (appearing within one hour of drug intake) and non-immediate reactions (appearing more than one hour after drug intake) (3).

Early reports showed a frequency of allergic reactions to penicillins ranging from 0.7-10\%, with the frequency of anaphylaxis being 0.015-0.004\% (4). Studies carried out in large series of patients with cutaneous symptoms showed that 19\% were finally diagnosed as being allergic to BLs (5).

The specific IgE mediated type I hypersensitivity reaction initially depends on recognition of allergen derived peptides bound to antigen presenting major histocompatibility complex (MHC) class II molecules, by T helper lymphocytes (6). It became evident that many peptides bind to MHC class II molecules representing the restriction elements of T helper lymphocytes and that a high degree of variation within an antigen is tolerable to retain functional T-cell epitopes (7). Numerous studies in the general population and within families have suggested that both genetic and environmental influences are involved in the pathogenesis of atopy and asthma (8,9). A likely candidate for controlling specific IgE responses is the MHC. This region contains many genes, which might influence the pathogenesis of allergic diseases and reactions. HLA class II antigens -DR, -DP, and -DQ play the key roles in antigen presentation to CD4+ T lymphocytes via T cell receptor and influence the specificity of immune response. Indeed, different HLA products or genes may represent risk factors for, or protective factors against, the development of allergic disease (10,11,12).

Direct or indirect contact with bird allergens may cause sensitization. Bird allergens may be major components of house dust. Detection of indoor and outdoor birds antigen and...
antibody might contribute to the correct diagnosis and appropriate management of bird related hypersenstivity. Previous papers has reported that immunoglobulin (IgA or degraded IgA) and intestinal mucin in bird dropping and bloom are assumed to be causative avian antigen. Several reports suggested that even a low exposure to wild birds and unrecognized exposure to feather duvets and others (13,14, 15) might lead to hypersensitivity.

This study aimed to: test if HLA-DRB1*0103 and HLA-DQA1*0201 genes or alleles products have a general influence on the reaction to unrelated allergens, genotype for HLA-DRB1*0103 and HLA-DQA1*0201 in atopic patients and in healthy controls from Basrah populations and test for specific IgE responses against these allergens.

MATERIALS AND METHODS

Subjects
This study included 175 patients with allergies to amoxicillin (80 males and 95 females; aged ≥15 years to ≥75 years). Among them, 30 control subjects, Most subjects were eligible cases attending the center of asthma and allergic diseases in Basrah and General Altahrear Hospital during the period from March 2013 to June 2013. All patients were complaining of symptoms related to upper and lower respiratory tract disorder or conjunctival disease or urticarial and all them agreed to participate in the trail and tested serologically by specific IgE based ELISA test and PCR genotyped for HLA-DRB1*0301 and DQA1*0201.

The source material and preparation of the testing samples.

Blood: Five ml of blood were drawn from each subject for extracting DNA and separating sera after clotting for 2 hours at room temperature, the sera were separated by centrifugation for 15 minutes at 6000-10000 rpm and stored at -20°C until the in vitro tests were performed. Genomic DNA from each subject was isolated from a whole blood sample according to the manufacturer instruction(Wizard, Genomic DNA purification kit, Promega, USA)

Love bird dropping (LBDE): Fresh dropping sample was collected directly from love bird cage in clean container. According to method of(16); 1/20 (w/vol) the bird dropping extract (LBDE) was prepared by dissolving 2gm of fresh love bird feces in 40ml of 0.15 mol/L PBS pH 7.4. The mixture was left at 4°C for 24 hours. The obtained extract was centrifuged 3 times at 12000 rpm for 30 minutes. The supernatant was collected and dialyzed against distilled water for 7 days. The protein content (5.25ng/ml) was calculated by Nano drop apparatus and stored at -20°C until ELISA testing in which it was serially diluted into1/10,1/100,1/1000,1/10000 and 1/100000 and used as antigen.
Amoxicillin (AX): Amoxicillin 500 mg vials were purchased from local pharmacy and stored at 4°C until the specific IgE based ELISA test were performed. Amoxicillin test dosing made by ourselves as the therapeutic dose of amoxicillin suspension was immediately prepared by adding 2 ml of PBS aseptically to amoxicillin 500 mg vial and left for few minutes until dissolving. This suspensions was serially diluted into 1/10, 1/100, 1/1000, 1/10000 and 1/100000 and used as antigen (17).

**Specific IgE based ELISA test.**

An ELISA test for measuring the antibody response was established by chequre board (CB) titration of the antigen, sera and conjugate. The procedure for conducting the sold phase ELISA was essentially as described by (18). The best selected dilutions of Ag and sera and conjugate were used. Depending on the results of CB ELISA, ELISA test was used for evaluation of IgE antibodies in the sera from 145 atopic patients as well as 30 normal subjects. Briefly, microtiter plate (Immlon Dynatatech, Piashigen, Germany) were inoculated with 100 µl/well of a solution containing AX (2.5 mg/ml) or LBDE (5.25 ng/ml) in a carbonate bicarbonate buffer (0.2 M pH 9.6) and overnight incubated at 4°C. Plates were washed with PBS (0.145 M sodium chloride, 0.010 M phosphate buffer and 0.05% Tween 20) and incubated for 1 hour at room temperature 100 µl/well of non-diluted sera. After washing, bound antibodies were detected by adding 200 µl/well of horseradish peroxidase rabbit antihuman immunoglobulin E conjugate. After 1 hour incubation at room temperature, the reaction was developed by adding 100 µl/well of substrate solution (0.5 mg/ml O-phenylenediamine in 0.1 M citrate buffer). Optical densities were read at 490 nm in a Micro ELISA auto reader, (Human/Germany). The negativity cutoff was estimated according to the method of (19).

**PCR detection**

The genomic DNA from the whole blood of 100 patients was extracted according to Wizard, Genomic DNA purification kit (Promega, USA). For the detection of HLA-DRB1*0103 and DQA1*0201 by PCR the specific primers were designed according to (20) as a follow (HLA-DRB1*0103 forward: CCCGCTCGTTTCCAGGAT and reverse: TTGTGGCGCTTAAGTTTAAGT). While the DQA1*0201 forward: ACGGTCCCTCTGGCCAGTT and reverse CAGGATGTCAAGTTATGTTTAG. The PCR amplification mixture (25 µl) included 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), 2.5 µl of template DNA, 1.25 µl of each forward and reverse primers and 7.5 µl of nuclease free water to complete the amplification mixture to 25 µl. The PCR tubes containing amplification mixture were transferred to preheated thermocycler and start the program as follow, 3 min at 95°C for one cycle, then 40 cycle of 1 min. at 95°C, 58°C for 1 min. and, 72°C for 30 sec. with one final extension of 5 min. at 72°C.
Statistical Analysis
Statistical analysis is done by using SPSS software version 11, the chi square was used to assess Statistical significance

RESULTS

Specific IgE response estimation
According to ELISA results, table (1,2), showed that the overall rate of seropositivity in tested individuals to AX or LBDE allergens was 54.9%. The females were not significantly different (P ≥ 0.05) from males and they showed the higher rate of seropositivity against AX(57.9%) and LBDE(58.9%) allergens. In concern to the age groups, significant difference (P ≤ 0.05) between the two age groups was observed in case of seropositivity against AX and the higher rate(81.3%) of seropositivity was in the 2nd age group in comparison with the 1st age group rate (52.2%). In contrast there was no significant effect (P > 0.05) for the age on the seropositivity against LBDE and the patients of the 1st age group showed higher rate(55.9%) of seropositivity in comparison with rate (43.7%) of 2nd age group patients (Table-2).

Table (-1) The distribution of Amoxicillin allergens in study subjects according to sex and age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ex. No.</th>
<th>subjects (No) (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>seropositive (%)</td>
<td>seronegative (%)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>80</td>
<td>41 (51.2)</td>
<td>39 (48.8)</td>
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<tr>
<td>Females</td>
<td>95</td>
<td>55 (57.9)</td>
<td>40 (42.1)</td>
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<tr>
<td>Total</td>
<td>175</td>
<td>96 (54.9)</td>
<td>79 (45.1)</td>
</tr>
</tbody>
</table>

P > 0.05

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Ex. No.</th>
<th>subjects (No) (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15-45</td>
<td>159</td>
<td>83 (52.2)</td>
<td>76 (47.8)</td>
</tr>
<tr>
<td>&gt;45-75</td>
<td>16</td>
<td>13 (81.3)</td>
<td>3 (18.7)</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>96 (54.9)</td>
<td>79 (45.1)</td>
</tr>
</tbody>
</table>

P < 0.05
Table (2) The distribution of love bird dropping allergens in study subjects according to sex and age.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ex. No.</th>
<th>individuals (No)</th>
<th>Total</th>
</tr>
</thead>
</table>
|           | (%)     | seropositive     | seronegative | No. (%)
|           |         |                  |        |
| Sex       |         |                  |        |
| Males     | 80 (45.7) | 40 (50)         | 40 (50) | 80 (100) |
| Females   | 95 (54.3) | 56 (58.9)       | 39 (41.1) | 95 (100) |
| Total     | 175 (100) | 96 (54.9)       | 79 (45.1) | 175 (100) |

P > 0.05

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Ex. No.</th>
<th>individuals (No)</th>
<th>Total</th>
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<tbody>
<tr>
<td>&gt;15-45</td>
<td>159 (90.9)</td>
<td>89 (55.9)</td>
<td>70 (44.1)</td>
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<tr>
<td>&gt;45-75</td>
<td>16 (9.1)</td>
<td>7 (43.7)</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td>Total</td>
<td>175 (100)</td>
<td>96 (54.9)</td>
<td>79 (45.1)</td>
</tr>
</tbody>
</table>

P > 0.05

The result of PCR amplification

The result of PCR amplification which was performed on the extracted DNA confirmed by electrophoresis as the strands of the DNA which were resulted from successful binding between specific HLA-DRB1*0103 and HLA-DQA1*0201 primers and the extracted DNA appear as a single band under UV illuminator using ethidium bromide as a specific DNA stain. Only the bands with expected size 173bp (HLA-DRB1*0103) and 197bp (HLA-DQA1*0201) were observed in figure-1. In table-3, the higher overall rate (39.4%) of PCR positivity was observed in seropositive patients with positive PCR HLA-DQA1*0201 genotyping results. Also PCR HLA-DQA1*0201 genotyping revealed the higher rates of the PCR positivity in the males and females of the second age group in comparison with those of the first age groups but without significant difference (P > 0.05). The same result was also

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observed in positive PCR result for the patients with HLA-DRB1*0103 and those with both HLA-DRB1*0103 and HLA-DQA1*0201 as the non-significant higher PCR positive rates was observed in the males and females of the 2nd age group. Table- 3 also display the higher PCR positivity in the females of the second age group as we can see that the higher rate of PCR positivity was in HLA-DQA1*0201 (100%) and the same observation was detected in PCR results of HLA-DRB1*0103 (50%) and both alleles (30%) All control and seronegative individuals showed negative PCR HLA genotyping results.

![Image of PCR products](image)

**Figure 1**: PCR Products of HLA- DQA1*0201 and HLA- HLA-DRB1*0103
Lane: 1 ladder
Lanes: 2,3; HLA- DQA1*0201 (173bp).
Lanes: 4,5; HLA-DRB1*0103(197bp)
**DISCUSSION**

Many previous study demonstrated that the variant genes were involved in the development of allergies to penicillins through the production of specific IgE antibodies. (21,22) It is necessary to investigate the genetic background of allergies to penicillins. Being the pivotal role of the HLA class II molecules in regulating the immune response, combined with their extensive polymorphism, it is not surprising that HLA class II alleles have been implicated in susceptibility to allergic disease and reaction known to have an immunological basis. (23) Many factors appear to be very important in investigating HLA gene susceptibility to diseases the clinical phenotype must be precisely defined in relationship to a specific antigen. Indeed, reports of association with HLA antigens are restricted only to specific allergens. A study found that immune responsiveness to a highly purified *Loliumperenne* pollen component was significantly associated with DR3 and B3 HLA antigens. (24) Whereas, an association between atopy and the DR4 and DR7 HLA class II alleles suggest that these alleles are more involved in a general susceptibility to atopy rather than in a specific

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>sex</th>
<th>Ex. No.</th>
<th>DRB1*0103 V+(%)</th>
<th>DQA1*0201 V+(%)</th>
<th>Both V+(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15-45 Males</td>
<td>74</td>
<td>8 (10.8)</td>
<td>20 (27.02)</td>
<td>4 (5.4)</td>
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<tr>
<td></td>
<td>85</td>
<td>11 (12.9)</td>
<td>34 (40)</td>
<td>3 (3.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;45-75 Males</td>
<td>6</td>
<td>2 (33.3)</td>
<td>5 (83.3)</td>
<td>1 (16.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5 (50)</td>
<td>10 (100)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Total No.(%)</td>
<td>175</td>
<td>26 (14.9)</td>
<td>69 (39.4)</td>
<td>11 (6.3)</td>
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</tr>
</tbody>
</table>

P > 0.05
sensitivity to particular allergens. A previous study using the mixed lymphocyte method showed an association of HLA-A2 and HLA-DR52w antigens and delayed hypersensitivity to aminopenicillin. Few recent local studies of some HLA class II genes association with allergy and specific IgE responses conducted in Basrah province. In our study, we performed HLA-DRB1*0103 and HLA-DQA1*0201 typing by PCR, a high resolution DNA based method that allows an accurate detection of HLA class II polymorphism and, therefore, a more precise identification of a possible association with specific IgE responses. In addition, we based our results on subjects were without diseases involving HLA genes. ELISA has been described previously in the diagnosis of IgE mediated allergy. To conduct sensitive detection of antibody activity against avian antigens, ELISA test was used in the present study. Previous studies had reported ELISA as a method to detect avian antigens and antibodies against these antigens. The ELISA method which was used in the present study showed the following rates of seropositivity against love bird dropping and amoxicillin allergens (54.9% for each one), as these seropositive patients had OD values higher than cut off value which was 0.032 in case love bird dropping allergens and 0.041 in amoxicillin allergens. The presence of this seropositivity rate against avian dropping, in the present study an indicate that most likely antigenic sources might be found with those materials containing strong reacting antigens of the materials tested, only avian dropping contained such antigens reacting strongly with allergic patients sera. It would therefore appear that avian dropping is the only avian material with a full complement of major antigens concerned with avian allergic patients and should be considered as the most important source of antigenic material in this allergy. This finding was supported by other recent local studies as reported rates of seropositivity (64.8% and 94.7% respectively) against avian dropping but these rates were higher than the preset study rate and the explanation for this variability could be attributed to one or more following factors including the absence of standardizing measuring method for measuring specific IgE antibodies to avian allergens by ELISA technique, the variation in the type of techniques, isotype of assessing antibody and finally many researchers have found that the antibody activity may reflect sub clinical inflammation and that individuals only become symptomatic when this inflammation is advanced. Many studies conducted in other parts of the world confirmed the seropositivity against avian allergens in both symptomatic
and asymptomatic allergic individuals as the studies of (39,40) also confirmed antibody activity against avian allergens in both symptomatic and asymptomatic but always symptomatic seropositivity rate was higher than asymptomatic, so the first study reported antibody activity against avian allergens in 100% of symptomatic and 60% of asymptomatic and the second study reported that all symptomatic and 57% of asymptomatic were seropositive.

In conclusion, our results show the importance of HLA-DRB1*0103 and HLA-DQA1*0201 alleles in the development of allergies to studied allergens through modulating specific serum IgE response.

IgE واستجابة HLA-DRB1*0103 and HLA-DQA1*0201 الخاصة بالمستارجات عديدة الصلة في مرضى الحساسية
نتائج ظهرت نتائج ELISA بأن أعلى نسبة كلية الإيجابية المصية للأشخاص والمفحوصين بمستضدات الأموكسيسيلين وبراز طيور الحب كانت بمقادير 54.9% لم تختلف إلاناث عن الذكور إحصائيًا (P ≥ 0.05) وفي الأشخاص المفحوصين كانت نسبة الإيجابية المصية ضد الأموكسيسيلين (57.9%) مقابل نسبة إيجابية مضادات براز طيور الحب (58.9%) لا يوجد تأثير محسوس إحصائيًا (P > 0.05) للعمر على الإيجابية المصية ضد مضادات براز طيور الحيوان لمرضى الفئة العمرية الأولى ظهرت أعلى نسبة (55.9%) للإيجابية المصية بالمقارنة مع نسب مرضى الفئة العمرية الثانية (43.7%).

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