Available online at: www.basra-science-journal.org

ISSN –1817 –2695

Association of HLA DQA1 and HLA DRB1 Alleles with Asthma Patient In Basra City

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Received 3-3-2014 , Accepted 27-5-2014

Abstract

The current study were included one hundred and twenty blood sample with draw form patients with allergic asthma , they were selected randomly from the Allergy and Asthma Center in Basra cityand there were eighty sample with draw from healthy individual as control . All samples from patients and controls were tested by direct ELISA for total IgE and Polymerase chain reaction was performed to detect a genetic relationship between allergic asthma and human leukocyte antigen classII allele(HLA- DQA1 and HLA-DRB1 alleles).

Result of this study showed that the total IgE>100 IU/ml had higher rate in asthma patients at (70.8%).with significant difference($p<0.05$) between total IgE<100 IU/ml and controls. The study also included detection of some human leukocyte antigens(HLAclassII) allelesHLA DRB1*0101/2/4, HLA DQA1*0401, HLA DQA1*0101/4 by the use of PCR analysis to determine their impact on the asthma allergy. The results of this analysis revealed the HLA DQA1*0401 had higher rate at(89.2%) inasthma patients ,also this allele appeared in 100% of asthma patientswho had total IgE>100 IU/ml . All studied HLA class II alleles were significantly ($P<0.05$) distributed in the study groups .

Key words : Asthma ; HLA antigens ; IgE; genetic

1.Introduction

Asthma is a serious public health problem and chronic disease affecting on people of all ages but it most often starts during childhood and caused by combination of genetic and environmental factors.[1;2] .This disease characterized by episodes of variable airflow obstruction with clinical symptoms such as, coughing , shortness of breath and wheezing[3].

Some genetic variants may cause asthma when they are combined with specific environmental exposures.[4]. The genetic basis of allergic sensitization,including asthma, has been long recognized with the HLA being the first specific chromosomal region implicated [2]. Many chromosomal effect on serum IgE level as 5q and 11q13 [5].Furthermore, that HLA class II alleles association with IgE production in patients with allergic asthma [6] and determinants of IgE level by genetic factors are important compared with other factors such

as environmental exposures [7] . The human leukocyte antigen (HLA) complexlocated on chromosome 6p21,and may playimportantrole in the genetic basis of childhood asthma[5]**.**All genetic studies of asthma have concentrated on classical allergic asthma. Segregation analysis has indicated the presence of major genes underlying atopy and asthma, a number of chromosomal regions have been identified as containing genes which influence asthma and atopy[8].

The aim of the present study was to determine total IgE in asthma patients and controls. Also determine genetic relationship between allergic asthma and human leukocyte antigen classII allele(HLA- DQA1 and HLA- DRB1 alleles).and association between HLA-DQA1 and HLA- DRB1 alleles).and production IgE.

Material and Methods

2.1. Patients:

A total of 120 asthma patient's and 80 controls blood samples were collected from the center of asthma and allergic disease in Basra city. The patients complaining of symptoms related wheezing, chest tightness, dyspnea attending , all these patients were diagnosis by specialist physician .The patients and controls were from both sexes and their ages were from (6-45) year. They agreed to participate in the trial , all investigated population were immunologically and genetically tested by ELISA test and PCR amplification of the HLA DQA1*****0101/4, *0401, HLA and HLA DRB1*0101/2/4 alleles respectively .

2.2.Sampling

Blood samples were collected from all asthma patients and controls in plain tube. 3 ml of collected blood were centrifuged for 10 minutes (1500 rpm/min) , in order to obtain serum used in ELISA test ,The remained 2 ml of blood were poured in tubes containing EDTA and kept in - 18 ^{o}C and used later for HLA-DQA1 and HLA-DRB1 genotyping .

2.3.Serological study

2.3.1.Total IgE estimation in serum sample .

The total IgE concentration in the sera of studied individuals was determined by a micro plate enzyme immune assay according IgE ELISA kit(monobind.inc /USA).Sufficient microtiter strips were left in the strip holder to enable the running of standards and samples . Starting with well 2, (10 µl) of standards and samples were pipetted in to appropriate wells of the strips . Enzyme conjugate(200µl) was added into each well(except well 1),and mixed thoroughly for (15) seconds. The plate was covered with the enclosed foil and incubated for (30) minutes at room temperature . Washing : The incubation solution was discarded , the well was rinsed 3 time with (300 µl) diluted wash buffer was removed.Promptly (100µl) of the TMB substrate solution was pipette into the rinsed wells (including well 1). The plate was covered with the enclosed foil and incubated for (15) minutes at room temperature in the dark . The reaction was stopped by adding (100µl) of TMB stop solution to each well (including well 1). The micro titer strips were shacked gently and read at (450) nm (against the substrate blank) within (60) minutes from the stopping

2.4.Molecular analysis:

The genomic DNA was extracted from the whole blood of 120 patients and 80 control and purified according to the instructions of Wizard , Genomic DNA purification kit (protege ,USA). Genotyping of the DQA1 and DRB1 alleles was carried out by PCR reaction in (Thermocycler , Thermo USA). Genotypes were amplified by PCR the specific primer were designed according to[9]AsafollowHLA-QA1***0101/4**as fallow forward,**CATGAATTTGATGGAGATG AG ,** revers, **ATGATGTTCAAGTTGTGTTTTGC**(14 9pb) ,***0401** As a follow forward,**ACCCATGAATTTGATGGGC,** revers**,ACATACCATTGGTAGCAGCA**(194pb) andHLADRB1***0101/2/4,** As a follow forward, **CCGCCTCTGCTCCAGGAG,revers, TTGTGGCGCTTAAGTTTGAAT**(194p b) . the 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 thermocycler andstart the program HLA-DQA1 as follow, 5 min at 96C^{o} for one cycle,then 40 cycle of 1min at 96C^{O,}, annealing tempreture 1min at (53C^{o}) for HLA-DQA1* **0101/4** and (60 C^{o}) for HLA-DQA1***0401** ,72C^{o} for 1 sec with one final extension of 10 mpn at 72C^{o}. but the program of DRB10101/2/4 as follow 5 min at 95C^{o} for one cycle,then 35 cycle of 20 sec at 95C^{O,}, annealing tempreture 20 sec at (58C^{o}),72C^{o} for 20 sec with one final extension of 10 min at 72C^{o} .The results of PCR weredetected after the amplification process. 10 µl from amplification sample was directly loaded in a 1.5% agarose gel containing 0.5 µl /25 ml ethidium bromide with the addition of loading buffer and DNA size marker as standard in electrophoresis and run at 70 V , then the products were visualized by UV transilluminatoruntil the bromophenol blue tracking dye migrated to the end of the gel .The DNA was observed and photographed by using gel documentation system

2.5. 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

The result showed that the total IgE level >100 had higher rat (70.8%) with significant difference between total IgE level >100 and control,table (1).

The pathogenic mechanisms involved in occupational asthma remain to be determined by Immediate type I immunoglobulin E (IgE)-mediated hypersensitivity was involved in asthma caused by environment factors induced asthma allergy [10].The study by [7] showed that increasing total serum IgE level was associated with respiratory and asthma disease .Also another study showed that the development of allergic diseases and inflammation is sensitization to allergen and production of IgE showed the important role of IgE in asthma[11] .

Table (1) distribution total IgE level in asthma patients and control.

Stat	Ex. No.	IgE<100IU/ml	IgE>100 IU/ml
Asthma patients	120	35(29%)	85(70.8%)
Control	80	65(81.3%)	5(18.8%)
Total	200		

P<0.05

Also the present study showed in table(2) that HLADQA1*0104 had higher rat at (89.2%) with significant different in other alleles under study ,also in the same table showed that the allelic frequency of HLA DQA1 and HLA DRB1 alleles had higher rat in asthma patients compared to normal controls with significant difference between them ($p<0.05$).

Allergic asthma Stronger associations with human leukocyte antigen (HLA) class II genes. It seems that HLA-DR typing will ever be of prognostic relevance for have been seen with asthma [12;13] Genome-wide screening studies have identified multiple chromosomal containing susceptibility genes for asthma such as 2q, 3p, 5q, 6p21 and 12q23[14;15] . The study by [16] showed that some susceptibility allele were associated with asthma . Also [8] showed that susceptibility factor are association in asthmatic . another study by [17] showed that Asthma is the result of a complex interaction between environmental factors and genetic variants that confer susceptibility. Nevertheless, this finding has been consistently replicated in independent populations of European ancestry and also in other ethnic groups. Thus, chromosome 17q21 seems to be a true asthma susceptibility locus. Other genes that were identified in more than one GWAS are IL33, RAD50, IL1RL1 and HLA-DQB1.[18]found a decreased frequency of HLA-DQB1*03 allele in childrenwith asthma compared with control, and suggesting that HLA-DQB1*03 may be protective allele against the development asthma. Also [19] studied the distribution of both HLA-DR and HLA-DQ in asthma patients and found that the risk of epidemic asthma associated with the DRB1*13 gene.

Table (2) . HLA class II allele frequencies in patients with Asthma and control.

HLA class II alleles	Allergic Asthma N=120 (%)	Control N=80 (%)
HLADQA1*0101/4	88 (73.3%)	6(7.5%)
HLA DQA1*0401	107(89.2%)	1(1.25%)
HLADRB1*0101/2/4	100(83.3 %)	8(10%)

P<0.05

In the second part of this study ,we analyzed the association of HLA class II alleles with the low or high level of total IgE production in asthmatic . The result study in table (3) found HLADRB1*0301/4 most frequencies in asthma patients with low level IgE<100 at rat (100%). while the result study in the same table showed that HLADQA1*0101/4, HLA DQA1*0401 and HLADRB1*0101/2/4 most frequencies in asthma patients with high level IgE>100 at rat(100 %,91.8% and 94.1%) respectively with significant different compared with asthma patients with low level IgE<100 .

The role of genetic factors in asthma and atopy is unquestionable. It was initially postulated from the observation of familial clustering, and twin studies have subsequently shown that there is a genetic element to asthma susceptibility, with heritability of the condition estimated at between 0.36 and 0.77 [20;21] . Also[22] showed that HLA class II genes are controller on the IgE immune response to asthma .Studied by[23;24] suggest that chromosome Ilql3 is region contains the important candidate gene FceRI-fJ (the P chain of the high affinity receptor to IgE). Associated with this region was originally seen in families with severe atopy, and polymorphism within the gene has been associated with asthma .The study by Movahedi, *et al*. (2008) found that HLA-DRB1*0301 and DRB1*0701 alleles might be associated with low level of IgE production while DRB1*0101 and 1401, HLA-DQB1*0301 and DQA1*0505 are associated with high total serum IgE level.

Table (3). HLA class II alleles frequencies in asthmatic patients and total serum IgE levels

HLA class II alleles	Allergic Asthma IgE> 100IU N=35	Allergic Asthma IgE< 100IU N=85
HLADQA1*0101/4	10 (28.6 %)	78 (91.7 %)
HLA DQA1*0401	22(62.9 %)	85 (100 %)
HLADRB1*0101/2/4	20(57.1 %)	80 (94.1 %)

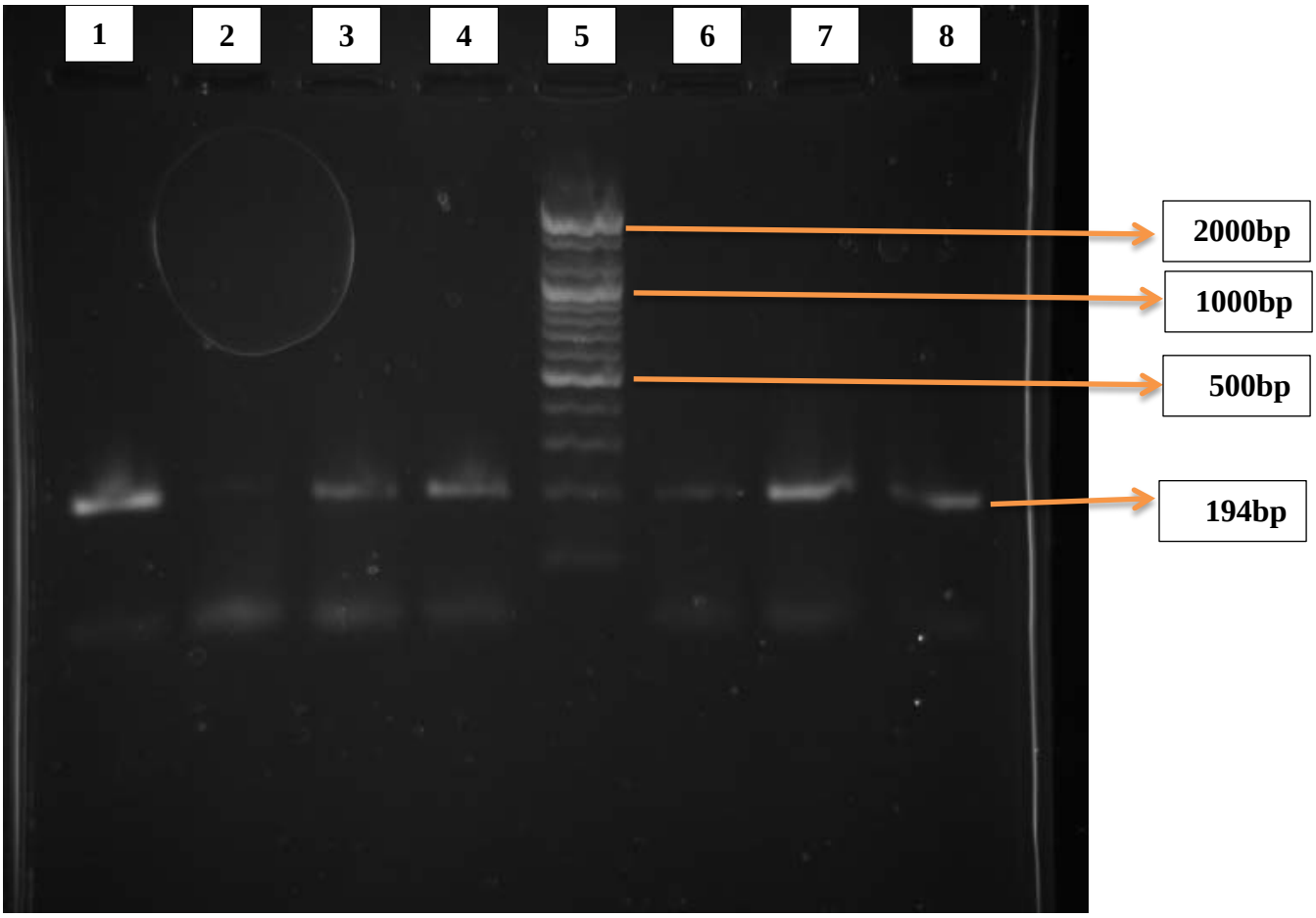

Figure (1) HLA DQA1* 0401 and HLA DRB1* 0101/2/4 PCR products
Lane 5:100bp Ladder, Lane 1-4 HLA DQA1* 0401
Lane 6-8 HLA DRB1* 0101/2/4

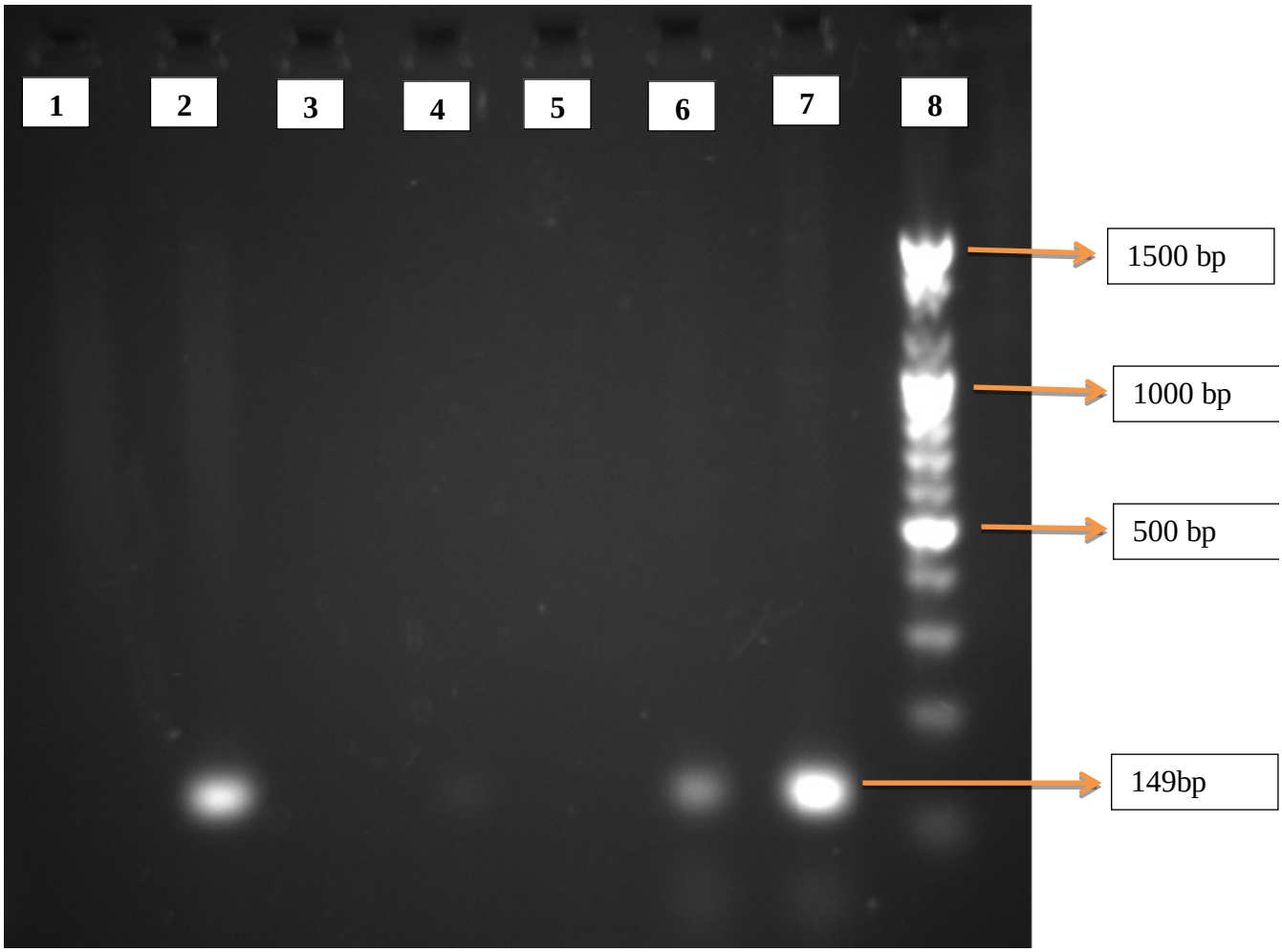

Figure (2) HLA DQA1* 0101/4PCR products
Lane 8:100bp Ladder, Lane(2,6,7)HLA DQA1* 0101/4

Conclusion:

In view of the present results show that some of the HLA-DRB1and HLA DQA1 alleles might be implicated in susceptibility to allergy and asthma and serum IgE production.

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علاقة اليلات معقد التوافق النسيجي HLA DQA1 and HLA DRB1 مع مرضى حساسية الربو في محافظة البصرة

شيماء جبار ريسان

الخلاصة

شملت الدراسة الحالية (120) عينةدم للمرضى الذين يعانون من الربو التحسسي جمعت عشوائيا من مركز الحساسية و الربو في مدينة البصرة و (80) عينة من الاشخاص الاصحاء (عينات سيطرة) . تم اختبار جميع العينات من المرضى و عينات السيطرة بأستخدام اختبار ELISA المباشر لقياس مستوى الاميونواكلوبيولين نوع)IgEواختبار PCR للكشف عن العلاقة الوراثية بين الحساسية والربو وبعض اليلات مستضد الكريات البيض البشرية (HLA– DQA1 و HLA– DRB1)

أظهرت نتائج هذه الدراسة أن معدل IgE> 100 وحدة دولية / مل في مرضى الربو هو (70.8 ٪) . مع مع وجود فرق معنوي (ع < 0.05) بين IgE< 100 وحدة دولية / مل وعينات السيطره. شملت الدراسة الحالية أيضا الكشف عن مستضد الكريات البيض البشرية (HLAclassII) DRB1 * 0101/2/4، HLA DQA1 * 0401، HLA DQA1 * 0101 / 4 باستخدام ال PCRلتحديد تأثيرها على حساسية الربو. وكشفت نتائج هذا التحليل أن HLA DQA1 * 0401 كان أعلى معدل في (89.2 ٪) في المرضى الذين يعانون من الربو ، كما ظهر هذا الأليل نسبة 100 ٪ في مرضى الربو الذين كان معدلIgE> 100 وحدة دولية / مل. وان جميع الأليلاتHLA IIاظهرت فرق معنوي(P< 0.05) توزع بين مجموعات الدراسة.