The Association between Human Leukocyte Antigen-DRB1 and Vitiligo

Galawish A. Abdullah
Mohammed Y. Abbas
Hyam Raouf
Batool M. Mahdi
Haider H. Zalzala
Laheeb A. Abid
Zena Nehad

1* Al-Kindy College of Medicine, Iraq
(MBChB, FICMS) Dermatology
Department of Medicine
E-mail: galawish_ahmed@yahoo.com
2* Al-Kindy College of Medicine, Iraq
(MBChB, FICMS) Dermatology
Department of Medicine
E-mail: mohammedyassin1975@gmail.com
3* Al-Kindy College of Medicine, Iraq
(MBChB, MSc) HLA typing research Unit
4* Al-Kindy College of Medicine, Iraq
(MBChB., M.Sc.FICMS).Clinical Immunology
Director of HLA research unit
E-mail: abas_susan@yahoo.com
5* Al-Kindy College of Medicine, Iraq
(MBChB, FICMS)HLA typing research Unit
6* Al-Kindy College of Medicine, Iraq
Pharmacist
(BS, Diploma) HLA typing research Unit
7* Al-Kindy College of Medicine, Iraq
Chemist
(BS) HLA typing research Unit

Abstract
Human leukocyte antigen (HLA) as part of the immune system has a role in the disease process. Genetic factors play an important role in susceptibility to vitiligo. Our aim in this study is to assess the relationship between HLA-DRB1 alleles frequency in Iraqi patients with vitiligo compared with a healthy control group using the PCR-SSOP method. The patient group consisted of forty Iraqi Arab Muslims patients with vitiligo that consulted the dermatological department in Al-Kindy teaching hospital from September 2013 to June 2015 were assessed for HLA genotyping for HLA-DRB1. A control group consisted of thirty healthy volunteers among the staff of AL-Kindy.
European Journal of Medicine, 2015, Vol.(10), Is. 4

College of medicine that did not have vitiligo or family history of vitiligo. HLA genotyping for HLA-DRB1 was performed for each patient and for the control persons using PCR with sequence-specific-oligonucleotide primers. Results showed an increase in the frequency of HLA genotype DRB1* 07:0101 (P value= 0.026) and DRB1* 11:0101 (p-value = 0.016) in patients with vitiligo compared with healthy controls. In conclusions, our results suggest an association between HLA-DRB1* 07:0101 and DRB1* 11:0101 and susceptibility to vitiligo.

Keywords: vitiligo, genetic, PCR.

Introduction
Vitiligo is a common, multifactorial depigmenting disorder of the epidermis and hair follicles, manifesting clinically as white depigmented patches with natural margin or hyperpigmentation. It is the most common pigmentation disorder affecting 0.5–1 % of the world population. [1]

The disease pathogenesis is unknown. But it has been made clear that genetic and immunological factors play a significant role in its developing [2, 3].

Of these, autoimmune hypothesis remains most widely accepted because of frequent occurrence of other autoimmune disease in vitiligo cases, the presence of auto reactive T cells in the vitiliginous lesions and peripheral circulation, and the presence of circulating autoantibodies in the sera of patients. [3, 4, 5]

Clinically characterized by milky-white macules with fairly homogenous depigmentation and a well-defined border. Vitiligo is classified into generalized (vulgaris, acrofacial, and mixed), and localized (focal, segmental, and mucosal) types. [6]

Major histocompatibility complex (MHC) represents a gene region that the histocompatibility molecules responsible for antigen presentation to the immune system. In humans, MHC is located in the short arm of chromosome 6 and it is called HLA (human leukocyte antigens) system. The genes in the HLA system have been classified into three regions: Class I, II and III.

Class I comprises HLA – A, B and C loci which modify the classical histocompatibility molecules expressed on the surface of all nucleated cells.

Class II region is composed by HLA-DR, DQ and DP loci, and class III that includes other molecules such as tumor necrosis factor, proteins, C4,C2 and factor B of the complement system, heat shock protein and 21-hydroxylase enzymes. [7, 8]

Given the polymorphism of the HLA system, its association is highly variable, as a result depending on the genetic load, an individual may present a higher or lower risk of developing a certain disease. So the aim of this study is to investigate whether there is an association between HLA class II DRB1 and Vitiligo.

Patients and methods
The study consisted of forty Iraqi Arab Muslims patients who had vitiligo that consulted the dermatological department in Al-Kindy teaching hospital from September 2013 to September 2015 were assessed for HLA typing class II DRB1.

The study took place at the HLA typing research unit, AL-Kindy college of Medicine, Baghdad University. The second control group consisted of thirty healthy volunteers’ age and sex matched among the staff of Al-Kindy College of medicine that did not have vitiligo or other autoimmune diseases and had negative family history for vitiligo.

The age of the patients ranged from 6-45 years with a mean age of 35.

The ethical committee of Al-Kindy College of medicine, Baghdad University approved the study; all samples were obtained with informed consent in accordance with the Al-Kindy Teaching hospital declaration.

HLA genotyping
Peripheral venous blood samples from patients and control groups were collected in ethylene diaminetetraacetic acid containing tubes and then stored at -20°C until testing for class II HLA-DRB1 using the polymerase chain reaction (PCR). Sequence specific primer (SSOP) method. Genomic DNA was extracted using promega DNA extraction Kit (promega corporation, fitchbury, Wisconsin USA). All DNA was stored at-20°C until testing. Locus and allele-specific amplification
of genomic DNA was performed for DRB1. Amplification and Hybridization was performed using a panel of sequence-specific.

Oligonucleotide probes (SSOP) using HLA-DRB1 amplification and hybridization kits (SSO HLA type DRB1 plus and mastermix for HLA type DRB1 Amp plus kits-Innogenetics-Belgium) using automated method by Autolipa-48 Innogenetics-Belgium. The results were interpreted using LiRas version-5.0 software-Innogenetics-Belgium.

**Statistical analysis:**
The distribution of HLA alleles in the patient and control group was compared using chi-square for continuous variables using minitable version 15 software in each comparison, the odds ratio (OR) along with the 95% confidence interval (95% CI) was used. A P-value less than 0.05 were considered statistically significant.

**Results**
Patient group with vitiligo and control group were typed for identification of the HLA-DRB1 alleles using DNA-based methodology (PCR-SSOP). Allele’s frequencies of HLA-DRB1 for vitiligo patients and control group are shown in Table 1.

There was an increased frequencies of HLA-DRB1* 07:0101 in patients with vitiligo than control group (P-value = 0.026, odd ratio = 3.285, CI= 1.151 – 9.378)

Also there was an increased frequencies of HLA-DRB1* 11:0101 in patients with vitiligo than control group (P-value = 0.016, odd ratio = 3.631, CI= 1.271 -1 0.370).

**Discussion**
Association of MHC alleles with a disease gains importance because of the antigen-presenting function of the MHC. The peptides presented by the MHC molecules have allele-specific motifs. The affinity of the peptide to be particular MHC molecule is determined by the amino-acid residues present in peptide-binding groove. Shared amino acids in the peptid-binding pockets have been demonstrated in autoimmune diseases [8].

In this study, a significant increase was found in frequency of HLA-DRB1* :07 and HLA-DRB1* 11 in patients with vitiligo than control group, that is in agreement with singh et al [9], who reported an increase in HLA-DRB1*07:01, HLA-A*33:01 & HLA-B*44:03 in patients with vitiligo than the controls in North India and in Gujarat. Also, our results agreed with that reported increased frequency of DRB1*07:01 in vitiligo patients from Slovakia (Buc etal, 1996) [10] and in china (Ren etal, 2009) [11]. Also a study on Turkish Vitiligo showed increase frequency of DRB1*03, DRB1*04 & DRB1*07 (Tastan et al. [12].

Genetic models of vitiligo also show a positive association among DQB1*0303, DQ1*0503 & DRB1*0901 alleles with vitiligo susceptibility. [13]

A study found increased frequency of HLA-B7, B15, BW6, CW6, CW6, DRB4*0101 and a decrease frequency of HLA-A9, B5, DQ1, DRB3*010101 in Saudi patients with vitiligo. [14]

Other study in Omani patients with vitiligo found that the frequency of HLA-DR7 was significantly increased. [15]

The inheritance pattern of vitiligo does not follow the simple mendelion pattern and its mode of heredity suggests that it is a polygenic disease. Vitiligo seems to be a complex hereditary disease governed by aset of recessive alleles situated at several unlinked autosomal loci which may be involved in the generation of oxidative stress, melanin synthesis, autoimmunity etc that could collectively confer the vitiligo phenotype. [16] The presence of circulating antimelanocyte and antikeratinocyte antibodies in the sera of Vitiligo patients, the selective destruction of melanocytes might be due to antibody reactivity directed to the antigens preferentially expressed on pigment cells or from an antibody response against antigens expressed on a variety of cell types that might selectively destroy melanocytes because they are intrinsically more sensitive to immune mediated injury than other cells [17] many studies have indicated a role for both cellular [18], And humoral immunity in the pathogenesis of vitiligo [19, 20].

Histological evidence further supports an autoimmune etiology. Vitiligo lesions have an infiltrate of inflammatory cells, particularly cytotoxic and helper T cells and macrophages, this infiltrate is most prominent in the perilesional skin just prior to the clinical appearance of vitiligo. More over vitiligo is epidemiologically associated with increased risk of several other autoimmune
diseases, both in patients & their relatives. Suggesting that these autoimmune diseases involve shared susceptibility genes. [21]

The major contribution of the study of HLA and vitiligo has been done to show the genetic aspects of the disease, further more this study has contributed a great deal to a better in sight in the pathogenesis of the disease.

The differences in the association of HLA antigens in Vitiligo in our study with other studies can be attributed to racial group variations, religion, sample size and methods used in the study. Discrepancy of our results as compared to other studies may be due to a racial factor and normal distribution of HLA antigens in different populations.

**Conclusions**

Vitiligo is associated with HLA-class II DRB1* 07:0101 and DRB1* 11:0101 which has a role in the etiopathogenesis of the disease.

**Acknowledgments**

We would like to thank Iraqi Research and Development Department-Ministry of Higher Education and Scientific Research for their support. Our thanks extended to the Baghdad University and Al-Kindy College of Medicine who facilitated this study.

**Table 1: Human leukocyte antigens (HLA-DRB1) alleles frequencies in patients with vitiligo and healthy control groups**

<table>
<thead>
<tr>
<th>HLA-DRB1* alleles</th>
<th>Patient No=40</th>
<th>Control No=30</th>
<th>Odd ratio</th>
<th>95% CI</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>02:0301</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>03:0101</td>
<td>3</td>
<td>7.5</td>
<td>4</td>
<td>13.33</td>
<td>0.527</td>
</tr>
<tr>
<td>03:0102</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>03:1701</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>13.33</td>
<td>Na</td>
</tr>
<tr>
<td>03:1101</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3.33</td>
<td>Na</td>
</tr>
<tr>
<td>04:0201</td>
<td>3</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>04:2201</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>07:0101</td>
<td>20</td>
<td>50</td>
<td>7</td>
<td>23.33</td>
<td>3.285</td>
</tr>
<tr>
<td>08:0101</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>08:0201</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>08:0701</td>
<td>5</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>11:0101</td>
<td>21</td>
<td>52.5</td>
<td>7</td>
<td>23.33</td>
<td>3.631</td>
</tr>
<tr>
<td>11:0701</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>11:2201</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>12:0901</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>13:0501</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>Na</td>
</tr>
<tr>
<td>13:1801</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>23.33</td>
<td>Na</td>
</tr>
<tr>
<td>13:2201</td>
<td>7</td>
<td>17.5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>14:0101</td>
<td>3</td>
<td>7.5</td>
<td>2</td>
<td>6.66</td>
<td>1.135</td>
</tr>
<tr>
<td>14:0201</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>26.66</td>
<td>Na</td>
</tr>
<tr>
<td>15:0101</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>16:0101</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>Na</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>Na</td>
</tr>
</tbody>
</table>

Na = not applicable
References:
12. Tastan HB, Akar A, Orkunoglu FE, Arca E and Inal A. Association of HLA class I antigens and HLA Class II alleles with Vitiligo in a Turkish population; pigment cell Res. 2004; 17:181-4.