EFFECT OF HLADRB1 ON DEVELOPMENT OF ALOPECIA AREATA

Galawish A. Abdullah¹, Mohammed Y. Abbas², Batool M. Mahdi³, Hyam Raouf⁴, Haider H. Zalzala⁵, Laheeb A. Abid⁶, Zena Nehad⁷

1. Al-Kindy College of Medicine, Baghdad-Iraq
2. Department of Medicine, Al-Kindy College of Medicine, Baghdad-Iraq
3. Director of HLA research unit, Al-Kindy College of Medicine, Baghdad-Iraq
4. HLA typing research Unit, Al-Kindy College of Medicine, Baghdad-Iraq
5. HLA typing research Unit, Al-Kindy College of Medicine, Baghdad-Iraq
6. Pharmacist, HLA typing research Unit, Al-Kindy College of Medicine, Baghdad-Iraq
7. Chemist, HLA typing research Unit, Al-Kindy College of Medicine, Baghdad-Iraq

*Corresponding Authors Email:
1. galawish_ahmed@yahoo.com, 2. mohammedyassin1975@gmail.com, 3. abas_susan@yahoo.com

(Received: October 05, 2015; Accepted: November 08, 2015)

ABSTRACT

Background: Alopecia areata (AA) is among the most highly prevalent human organ specific autoimmune diseases, also known spot baldness. The genetic basis of AA is largely unknown and the role of any potential environmental contributors is also unclear. Evidence supporting a genetic basis for AA depends on the heritability in first-degree relatives.

Aim of the study: confirm the genetic burden of HLADRB1 in the development of AA formation in Iraqi Arab Muslims patients

Patients and methods: A case control comparative study included forty unrelated Iraqi Arab Muslims patients with AA (30 women and 10 men) aged 6-45 years (mean age 35) were included in this study. The control group was comprised of 30 healthy unrelated sex and age matched volunteers. Genomic DNA was extracted. 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.

Results: There was an increased frequency of HLADRB1*11:01:01 in patients with AA compared with healthy controls (p P< 0.026, odd ratio=3.285, 95% CI: 1.151 – 9.378). The other allele HLADRB1* 16:01:01 was also increased in AA patients and not detected in control group. The other DRB1 allele groups (*03,*014) failed to achieve statistical significance.

Conclusions: There is a significant association between AA and HLADRB1*11:01 in Iraqi Arab Muslims patients.

Keywords: Alopecia areata, HLA, genetic.

INTRODUCTION

Alopecia areata (AA) is among the most highly prevalent human organ specific autoimmune diseases, also known spot baldness leading to disfiguring hair loss from some or all areas of the body due to the body's failure to recognize its own self antigens (hair follicle )and destroy its own tissues and subsequent autoimmune attack [1,2]. The genetic basis of AA is largely unknown and the role of any potential...
environmental contributors is also unclear\(^3\). Evidence supporting a genetic basis for AA depends on multiple researches, including the heritability in first-degree relatives\(^4\), twin studies\(^5\) and, from family-based linkage studies\(^6,7\). There are several genetic loci responsible for AA. These include loci on chromosome 2q33.2 containing CTLA4, chromosome 4q27 containing IL-2/IL-21, chromosome 6p21.32 containing the HLA, chromosome 6q25.1 containing the ULBP genes, chromosome 10p15.1 containing IL-2RA (CD25), and chromosome 12q13 containing Eos (IKZF4) and ERBB3\(^8\). There is also an association with genes controlling the activation and proliferation of regulatory T cells (Treg cells), cytotoxic T lymphocyte-associated antigen 4 (CTLA4), interleukin (IL)-2/IL-21, IL-2 receptor A (IL-2RA; CD25), Eos (Ikaros family zinc finger 4; IKZF4), genes expressed in the hair follicle itself (PRDX5 and STX17), region within the ULBP (cytomegalovirus UL16-binding protein) on chromosome 6q25.1, encoding activating ligands of the natural killer cell receptor NKG2D\(^9\). Genetic studies of AA have focused on HLA antigens due to immunological aspects of the disease\(^10\). Several studies have focused on association between AA and HLA typing that deals with chromosome 6p21.32, some of them deals with prognosis, recurrence and extent of the disease. One study demonstrated that the frequency of HLA-DRB1*1104 was significantly increased in all sorts of AA\(^11\). Another study reported that HLA-DQ*0602 and HLA-DQ*0603 were associated with AA in 80% of patients with AA, independently of the phenotype, and in 92% of individuals with total or universal AA\(^12\). Xiao et al\(^13\) (2006) evaluated Chinese individuals, 192 with AA and 252 controls and found higher frequency of HLA-A*02 and A*03 in patients than in controls. The importance of HLA-DRB1 that belongs to the HLA class II beta chain. The class II molecule is a heterodimer consisting of two chains an alpha (DRA) and a beta chain (DRB), both anchored in the membrane of the cell wall. HLA DRB1 plays a central role in the immune system by presenting peptides derived from extracellular proteins and the Class II molecules are expressed in cell wall of antigen presenting cells B lymphocytes, dendritic cell and macrophages. The beta chain is approximately 26-28 kDa and is encoded by 6 exons. Exon one encodes the leader peptide; exons 2 and 3 encode the two extracellular domains; exon 4 encodes the transmembrane domain; and exon 5 encodes the cytoplasmic tail. Within the DR molecule the beta chain contains all the polymorphisms of HLA that specifying the peptide binding specificities. Allelic variants of DRB1 are linked with many diseases\(^14\).

In our study we try to confirm the genetic burden of HLA*DRB1 in the development of AA formation in Iraqi Arab Muslims patients which manifests itself in greater significance of hereditary burden and greater power of association with genetic markers of the disease.

**Patients and methods:**

A case control comparative study included forty unrelated Iraqi Arab Muslims patients with AA (30 women and 10 men) aged 6-45 years (mean age 35) were included in this study. The patients were recruited from the outpatient of Department of Dermatology, Al-Kindy Teaching Hospital at Baghdad -Iraq between September -2013 to June -2015. Patients represent a group of newly diagnosed patients, while the other samples were collected retrospectively. Clinical data of all patients were obtained, including age at onset; severity of alopecia was assessed according to the AA investigational assessment guidelines\(^15\). 34 patients were categorized as having patchy AA S1 (less than 25% hair loss) to S4 (75-99% hair loss), 3 had alopecia totalis (AT) 100% scalp hair loss without loss of body hair, 2 had alopecia totalis/universalis (AT/AU) 100% scalp hair loss with variable loss of body hair and 1 had alopecia universalis (AU) 100% loss of both scalp and body hair. There was a family history of AA in 18/40 patients (45%), defined as having at least one first or second degree relative with AA.

The control group was comprised of 30 healthy unrelated sex and age matched volunteers among the staff of Al-Kindy College of Medicine that did not have a history of AA or other autoimmune diseases.

The Scientific and Ethical Committee of Al-Kindy Medical College and Al-Kindy Teaching Hospital had approved the study. Informed consent was obtained from all patients and control group.

**HLA genotyping:** Peripheral venous blood samples from patients and control groups were collected in ethylenediaminetetraacetic acid-containing tubes and then stored at -20°C until testing for class II- HLA-DRB1. Genomic DNA was extracted using Promega DNA extraction Kit -USA.
DNA product was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light. 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**

Statistical analysis was done using Minitab version. 3.0 software. The distribution of HLA alleles in patients and control groups were compared using chi-square for continuous variable. In each comparison, the odds ratio (OR) along with the 95% confidence interval (95% CI) was used. Gene. P-value less than 0.05 was considered statistically significant.

**RESULTS:**

In our study, the DRB1 locus was examined in a sample of 40 AA patients as compared to a sex- and age matched control group.

<table>
<thead>
<tr>
<th>HLA-DRB1* alleles</th>
<th>patients with AA No.=40</th>
<th>Healthy control group No.=30</th>
<th>Odd ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>02:0301</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>03:0101</td>
<td>4</td>
<td>4</td>
<td>13.33</td>
<td>0.722</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.165 – 3.156</td>
<td>0.665</td>
</tr>
<tr>
<td>03:0102</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>03:1101</td>
<td>0</td>
<td>1</td>
<td>3.33</td>
<td>na</td>
</tr>
<tr>
<td>03:1701</td>
<td>0</td>
<td>4</td>
<td>13.33</td>
<td>na</td>
</tr>
<tr>
<td>04:0201</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>04:03:01</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>07:0101</td>
<td>0</td>
<td>7</td>
<td>23.33</td>
<td>na</td>
</tr>
<tr>
<td>08:0101</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>08:0201</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>11:0101</td>
<td>20</td>
<td>7</td>
<td>23.33</td>
<td>3.285</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.151 – 9.378</td>
<td>0.026</td>
</tr>
<tr>
<td>11:0301</td>
<td>0</td>
<td>4</td>
<td>13.33</td>
<td>na</td>
</tr>
<tr>
<td>11:22:01</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>11:6701</td>
<td>0</td>
<td>4</td>
<td>13.33</td>
<td>na</td>
</tr>
<tr>
<td>12:0901</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>13:01:01</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>13:0501</td>
<td>0</td>
<td>2</td>
<td>6.66</td>
<td>na</td>
</tr>
<tr>
<td>13:1801</td>
<td>0</td>
<td>7</td>
<td>23.33</td>
<td>na</td>
</tr>
<tr>
<td>14:0101</td>
<td>3</td>
<td>2</td>
<td>6.66</td>
<td>1.135</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.177 – 7.258</td>
<td>0.893</td>
</tr>
<tr>
<td>14:0201</td>
<td>0</td>
<td>8</td>
<td>26.66</td>
<td>na</td>
</tr>
<tr>
<td>15:01:01</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>16:01:01</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>na</td>
</tr>
</tbody>
</table>

na = not applicable

Table 1: Human leukocytes antigens (HLA-DRB1) alleles frequencies in patients with AA and healthy control groups.
sample using a DNA-based methodology PCR-SSOP method. Table 1 summarizes the results of the HLA-DRB1 allele frequencies obtained in AA patients and controls. There was an increased frequency of HLA-DRB1*11:01:01 in patients with AA compared with healthy controls (p < 0.026, odd ratio=3.285, 95% CI: 1.151 – 9.378). The other allele HLADR81*16:01:01 was also increased in AA patients and not detected in control group. The other DRB1 allele groups that have been tested (*03,*014) failed to achieve statistical significance.

AA is a T cell mediated autoimmune response leads to abnormal keratinization and hair breakage within the follicles [16]. The precise etiology of AA is unknown but its association with HLA especially HLA-DRB1 has been described in many studies [17]. Our study showed that AA had a significant association with HLA*11:01:01 in Iraqi Arab Muslims patients. Our study was in agreement with Colombe et al. [18] who reported that HLADR81*11:04 is more frequent with early onset and sever patchy AA. Entez et al [19] reported that HLA-DRB1*03 was confer a protective effect and the DRB1*04 allele group was confirmed as a risk factor for the development of AA with the allele DRB1*0401 accounting for the greatest proportion of the effect in a Belgian-German population. This indicates that genetic effects of the HLA system are strongest in familial cases of AA. Other studies demonstrated the relation with HLA-A1, HLA-B62, HLA-DQ1, and HLA-DQ3 [20]. Studies were done in Polish and Turkish AA patients were also showed the protective effect of DRB1*03 [21,22]. Other studies were done in UK, North American White and Danish reported the risk conferring role of DRB1*04 in AA [23,24]. There are several studies have focused on the association of HLA and AA; some of them correlating prognosis, protective effect, predisposing effect, extent, chance of recurrence and family history with HLA typing [25]. These studies report that HLADQB1*03 allele was presented in 80% of all patients with AA, independently of the phenotype, and in 92% of individuals with total or universal AA [26]. As mentioned above, there are several studies conducted in patients with AA, which showed a predisposition to develop AA in cases with HLADRB1*03, HLA-DRB1*04, HLA-DQB1*06, HLADR81*13, HLADRw52a, DQ7, HLADQB1*03, HLA-DRB1*11 [27].

The differences in the results of many studies done among populations racially and ethnically distinct. The Brazilian population is genetically very different from Caucasians, Africans and Native Americans [28]. This fact may explain the differences found in the studies. A comparison of several studies with our study using samples of diverse ethnic backgrounds reveals differences in the important allele as well as varying allele frequencies between populations. In addition to that, this study was conducted with a small sample. Moreover, it was difficult to collect some relatives of the patients, especially the second degree, which refused to participate in the study. These two factors were found limitations in this study.

Since no specific gene variant has yet been identified as a cause of AA despite positive associations in each study, there is a need to identify associated alleles in each particular population.

CONCLUSIONS:
There is a significant association between AA and HLADR81*11:01 in Iraqi Arab Muslims patients. This study was conducted with a small sample. We suggest further studies with larger sample.

Conflict of interest: There is no conflict of interest

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.

Competing Interests: The authors have declared that no competing interest exists.

REFERENCES


