

Studying Profile Of HLA-DQB1 In Patients With Alopecia Areata In Basrah Province

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الخلاصة

تضمنت الدراسة 100 فرد ، 50 شخص مصاب بداء الثعلبة و 50 من الأفراد الأصحاء. لم يكن هناك فرق معنوي بين المرضى المصابين بداء الثعلبة والأصحاء من ناحية الجنس و الفئات العمرية. درست أليلات HLA-DQB1 في هؤلاء المرضى والأصحاء، كان هناك فرق معنوي سلبي في الأليل HLA-DQB1*020101 و الأليل DQB1*030201 في المرضى المصابين بداء الثعلبة مقارنة بالأصحاء (P < 0.05 ، OR = 0.76 ، 95% CI = 0.65 - 0.89) و (P < 0.05 ، OR = 0.72 ، 95% CI = 0.61 - 0.86) على التوالي. الأليلات HLA-DQB1*030101 ، HLA-DQB1*060101 و HLA-DQB1*060201 اظهروا نسبة تكرار عالية وذات مغزى ايجابي في المرضى المصابين بداء الثعلبة مقارنة بالأصحاء (P < 0.05 ، OR = 1.23 ، 95% CI = 0.97 - 1.56) و (P < 0.05 ، OR = 1.46 ، 95% CI = 1.05 - 2.02) على التوالي. لم يكن هناك فرق معنوي في أليلات HLA-DQB1 في المرضى الذين لديهم تاريخ عائلي لمرض داء الثعلبة والذين ليس لديهم تاريخ عائلي لهذا المرض. أظهرت النتائج وجود مغزى ايجابي بالنسبة لتناظر أليلات HLA-DQB1 للمصابين بداء الثعلبة مقارنة بالأصحاء. (P < 0.005 ، OR = 0.05 ، 95% CI = 0.01 - 0.23) . كانت نسبة تكرار HLA-DQA1*0103 – HLA-DQB1*0301 عالية مقارنة بارتباط HLA-DQB1 – HLA-DQA1 في الأليلات الأخرى.

Abstract

A total of 100 individuals were included in this study, 50 patients with alopecia areata and 50 healthy controls. There was no significant difference between alopecia areata patients and controls according to gender and age groups. No significant difference between alopecia areata patients and controls according to age groups. HLA-DQB1 alleles were studied in those patients and compared with HLA-DQB1 in healthy controls. A significant decreased frequency of HLA-DQB1*020101 and HLA-DQB1*030201 alleles in patients with alopecia areata comparing with healthy controls (P < 0.05, OR= 0.76, 95% CI= 0.65-0.89) and (P < 0.05, OR= 0.72, 95% CI= 0.61-0.86) respectively. HLA-DQB1*030101, HLA-DQB1*060101 and HLA-DQB1*060201 showed significant increased frequencies in alopecia areata patients comparing with healthy controls, (P < 0.05, OR= 0.24, 95% CI= 0.06-1.10), (P < 0.05, OR= 1.23, 95% CI= 0.97-1.56) and (P < 0.05, OR= 1.46, 95% CI= 1.05-2.02) respectively. There was no significant difference in HLA-DQB1 alleles in patients with family history of alopecia areata and patients without family history of alopecia areata. Patients with alopecia areata showed increased homozygosity in HLA-DQB1 when compared with controls (P < 0.005, OR= 0.05, 95% CI= 0.01-0.23). Combination of HLA-DQA1*0103 – HLA-DQB1*0301 showed higher frequency in comparison with other HLA-DQA1-HLA-DQB1 alleles combinations.

Aim of the present study:

To know if there is association between HLA-DQB1 alleles and alopecia areata, and which allele play a protective role and which allele might be a risk factor to the disease. Also to study the significant combination between HLA-DQA1 and HLA-DQB1 alleles.

Introduction

Alopecia areata (AA) is one of the oldest clinically documented diseases. It is characterized by the rapid onset of hair loss in a sharply defined area. Any hair-bearing surface can be affected, but the most noticeable surface is the scalp (1)(2). Papadopoulos *et al.* classified alopecia areata according to the extent of hair loss into: localized or patchy AA (LAA), alopecia totalis (AT), with complete loss scalp hair, and alopecia universalis (AU) with total body hair loss (3). Many Studies confirmed that the disease is autoimmune (4). Although increased levels of T helper1 (Th1) cytokines in lesional AA skin have been reported, Th2 immune response is also incriminated in the pathogenesis of AA (5). Alopecia areata can be used to dissect the contributing roles of immune privilege, immunogenetics, and neuroendocrine factors in the initiation and propagation of autoimmune disease. More systematic and widespread exploitation of this autoimmunity model in the immunology community, therefore, will not only further promote understanding of AA pathogenesis and the development of better AA management strategies but will also be widely relevant to the general study of autoimmunity (6). The human leukocyte antigen (HLA) super-locus is a genomic region that encodes the six classical transplantation HLA genes and at least 132 protein-coding genes that have important roles in the regulation of the immune system as well as some other fundamental molecular and cellular processes (7). They encode peptides involved in

host immune response and are associated with a variety of infectious, autoimmune, and inflammatory diseases (8) and (9). The MHC genomic sequence template has been used extensively to investigate single nucleotide polymorphism (SNP) and gene expression (10) and (11). The expression of particular HLA alleles may be associated with the susceptibility or resistance to some diseases (12). Many studies have shown that certain HLA loci are associated with alopecia areata but with ethnic distribution (13);(14) and (15). No report has ever been published about association of HLA alleles with alopecia areata in Iraq, so results of the present study compared with studies done in other countries.

Materials and Methods:

This study included a total of 50 patients with age groups from (15- 68) years, with alopecia areata attending Basrah General Hospital and private clinic and a total of 50 healthy controls with age groups from (15-68) years during the period between 2012 –2013 who have been studied in our previous study (16). Diagnosis was confirmed by the clinical examiner prior to collecting blood samples and written informed consent was obtained from all participants. DNA which extracted from whole blood samples of patients and normal control was achieved with Wizard ® Genomic DNA purification kit (Promega Company/USA). These DNA samples subjected to HLA-DQB1 genotyping (Table.1).

Table 1. Frequencies of DNA samples extracted and typed for HLA-DQB1 from blood samples of patients and controls:

Typed for HLA-DQ	DNA samples extracted from alopecia araeta patients	DNA samples extracted from controls	Total 100
	N (%) 50	N (%) 50	
DQB1	17 (34%)	50 (100%)	67

PCR Amplification and Sequencing of HLA-DOB1 Gene:

The PCR Amplification of HLA-DQB1 gene was done in the Cell Research Unit, college of Science, University of Basrah according to the protocol of a previous study (17). The primer sequences used were as follows: (1) exon 2 of DQB1 gene (BioNeer Corporation, USA) forward: 5-TCCCCGCAGGATTTCGTG-3; and reverse: 5-GGCGACGACGCTCACCTC-3. The GoTaq Green Master Mix 2 X (Promega Corporation, USA) preparation was according to the following equation: 2X Mix 92%, 4% of each upstream and downstream primer. For each tube; 25 μ l

Master Mix was added, 5 μ l DNA (45 ng/ μ l), and 20 μ l autoclaved D.W. Each PCR reaction was performed in a final volume 50 μ l. PCR was carried out under the following conditions: an initial denaturation step (96 $^{\circ}$ C for 5 minutes) was followed by 44 cycles consisting of (denaturation at 96 $^{\circ}$ C for 1 minute, primer annealing at 57 $^{\circ}$ C for 1 minute and extension at 72 $^{\circ}$ C for 1 minute. The final extension was continued for 10 minutes at 72 $^{\circ}$ C. The purification and sequencing – PCR of the amplified HLA-DQB1 gene was done in {Korea, Bioneer sequencing laboratories}{49-3, Munpyeong-Dong, Daedeok-Gu, Daejeon 306-220, Korea} (Figure. 1-3

Figure. 1: Sequences of DQA1 homozygous gene.

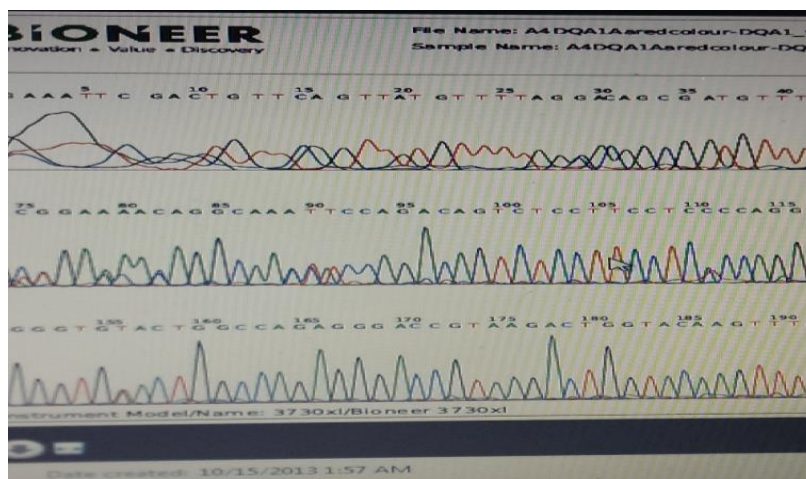


Figure. 2: Sequences of DQB1 homozygous gene.

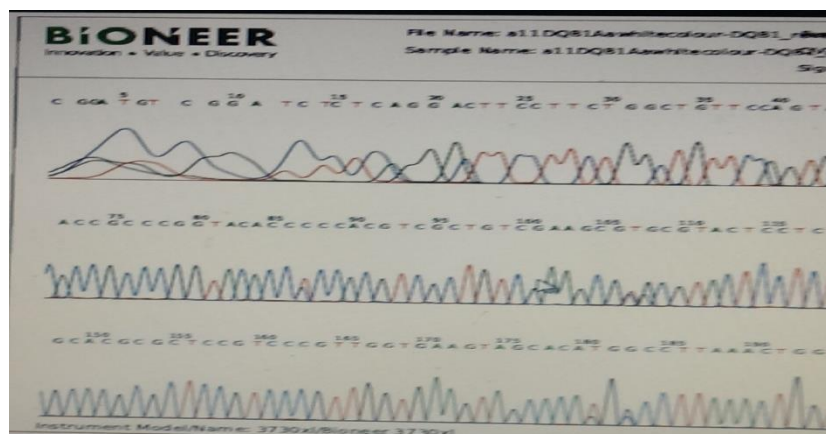
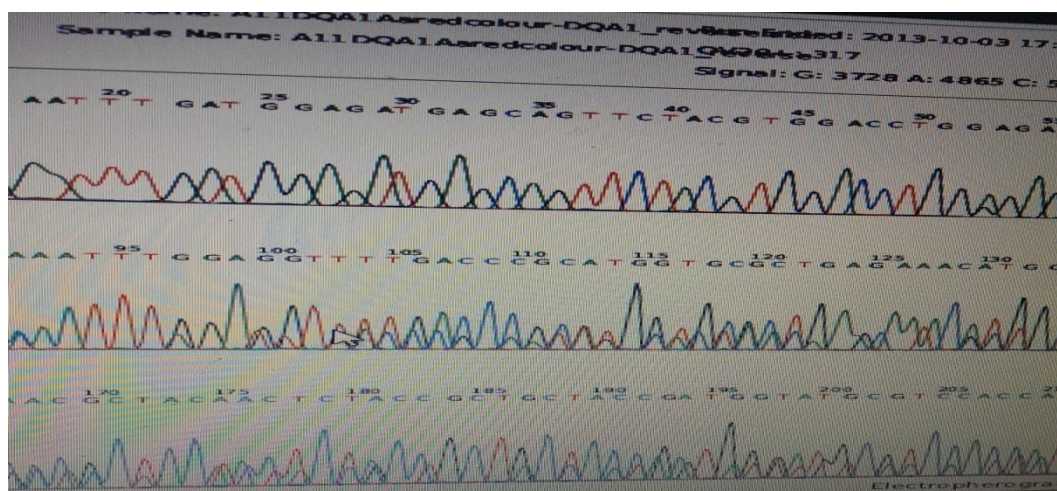


Figure. 3: Sequences of DQA1 heterozygous gene



Genotyping study of HLA-DQA1 Gene:

Analysis of HLA-DQA1 Genotyping was done for all included patients and corresponding controls as reported in our previous study (16).

Statistical Analysis:

For qualitative variables, frequency data were summarized as percentage. Statistical significant of differences between two groups was tested by Pearson Chi-square (χ^2) with Yates' continuity correction. Risk was estimated using Odds ratio (OR) and 95% confidence interval (95% CI). P-value was determined by Fisher's exact test, P-value of (< 0.05) was considered statistically significant. Data were analyzed using SPSS program for window (Version 15).

Results:

Distribution of alopecia areata patients & controls according to Gender and age groups:

Table .2 showed that out of 50 patients with alopecia areata, 27(54%) were males and 23(46%) were females. For control group, out of 50 healthy controls, 23(46%) were males and 27(54%) were females. The results showed no significant differences between males and females when compared with control groups $\chi^2=0.04$; $P=NS$; $OR= 0.73$; $95\% CI= (0.33-1.59)$. Results in table.2 showed that out of 50 patients with alopecia areata, 39(78%) were from age group $15 > 45$) and 11(22%) were from age group > 45). For controls group, out of 50, 34(68%) were from age group $(15 > 45)$ and 16(32%) were from age group (> 45) . These results showed no significant association between age groups when compared with controls $\chi^2= 1.27$; $P=NS$; $OR= 0.60$; $95\% CI= (0.25-1.47)$.

Table .2 Distribution of patients with Alopecia areata and controls according to gender and age groups.

Variables		Alopecia areata patients N=50	Controls N=50	χ^2	P	OR	95% CI
Gender	Male	27(54%)	23(46%)	0.64	NS	0.73	0.33-1.59
	Female	23(46%)	27(54%)				
Age group	(15 > 45)	39(78%)	34(68%)	1.27	NS	0.60	0.25-1.47
	(> 45)	11(22%)	16(32%)				

Genotype frequency of HLA-DQB1 in alopecia areata patients and controls:

Genotype frequencies of HLA-DQB1 alleles were studied in 50 patients with alopecia areata and compared with 50 controls. out of 50 DNA samples subjected to HLA-DQB1 genotyping, only 17 samples showed results and for controls group, 50 DNA samples showed results. Table 3, indicated that HLA-DQB1*020101 allele was absent in alopecia areata patients and present in 12 out of 50 controls, the frequencies of the allele were 0% and 24%, respectively. The decreased allele frequency in alopecia areata was statistically significant ($\chi^2 = 4.69$, $P < 0.05$, OR= 0.76, 95% CI= 0.65-0.89) as compared with controls. HLA-DQB1*030201 allele was absent in alopecia areata patients and present in 14 out of 50 controls, the frequencies of the allele were 0% and 28% respectively. The decreased allele frequency in alopecia areata patients was statistically significant ($\chi^2 = 5.69$, $P < 0.05$, OR= 0.72, 95% CI= 0.61-

0.86). Also results indicated that HLA-DQB1*030101 allele was present in 5 out of 17 alopecia areata patients and in 5 out of 50 controls, the frequencies of the allele were 29.41% and 10%, respectively. The increased allele frequency in alopecia areata was statistically significant ($\chi^2 = 4.26$, $P < 0.05$, OR= 0.24, 95% CI= 0.06-1.10) as compared with controls. Results in table 3. Indicated that HLA-DQB1*060101 allele was present in 3 out of 17 alopecia areata patients and absent in controls, the frequencies of the allele were 17.65% and 0%, respectively. The increased allele frequency in alopecia areata was statistically significant ($\chi^2 = 9.82$, $P < 0.05$, OR= 1.23, 95% CI= 0.97-1.56) as compared with controls. Also HLA-DQB1*060201 allele was present in 5 out of 17 alopecia areata patients and absent in controls. the frequencies of the allele were 29.41% and 0%, respectively. The increased allele frequency in alopecia areata was statistically significant ($\chi^2 = 16.91$, $P < 0.05$, OR= 1.46, 95% CI= 1.05-2.02) as compared with controls.

Table . 3 HLA-DQB1 genotype frequency of Aa patients and controls

HLA-DQB1	Aa Patients (n=17)		Controls (n=50)		χ^2	P	OR	95% CI
	No	%	No	%				
020101/0202/0204	0	0 %	12	24%	4.69	< 0.05	0.76	0.65-0.89

030101/030104/0309/0321/0322/0324/030302	5	29.41%	5	10%	4.26	< 0.05	0.24	0.06-1.10
030201	0	0 %	14	28%	5.69	< 0.05	0.72	0.61-0.86
030302	0	0 %	6	12%	2.11	NS	0.89	0.79-0.98
0402	0	0 %	1	2%	0.33	NS	0.98	0.94-1.02
050101	1	5.88%	3	6%	0.001	NS	0.96	0.09-9.91
050201	1	5.88%	1	2%	0.75	NS	0.31	0.02-5.20
050301	1	5.88%	0	0 %	3.17	NS	1.07	0.94-1.21
060101/060103	3	17.65%	0	0 %	9.82	< 0.05	1.23	0.97-1.56
060201	5	29.41%	0	0 %	16.91	< 0.05	1.46	1.05-2.02
060301/061401	0	0 %	0	0 %	N/A	N/A	N/A	N/A
060401/0634	0	0 %	0	0 %	N/A	N/A	N/A	N/A
060801	0	0 %	0	0 %	N/A	N/A	N/A	N/A
0609	0	0 %	0	0 %	N/A	N/A	N/A	N/A

enotype frequencies of HLA-DQ of patients with and without family history of alopecia areata:

Genotyping of HLA-DQB1 was studied in patients with family history of alopecia areata and compared with patients without family history of alopecia areata. Results shown in Table.4, indicated that HLA-DQB1 alleles showed no significant difference between patients with family history of alopecia areata and patients without family history of alopecia areata.

Table. 4 HLA-DQB1 genotype frequency of individuals with and without family history of Aa

HLA-DQB1 allele	Individuals with Family History of Aa		Individuals without Family History of Aa		χ^2	P	OR	95% CI
	No=6	%	No=11	%				
020101/0202/0204	0	0%	0	0%	N/A	N/A	N/A	N/A

030101/030104/0309/03 21/0322/0324/030302	2	33.33%	3	27.27%	0.07	NS	1.33	0.16-11.50
030201	0	0 %	0	0 %	N/A	N/A	N/A	N/A
030302	0	0 %	0	0 %	N/A	N/A	N/A	N/A
0402	0	0 %	0	0 %	N/A	N/A	N/A	N/A
050101	0	0 %	1	9.09%%	0.58	NS	1.10	0.91-1.33
050201	1	0 %	0	0 %	1.95	NS	0.83	0.58-1.19
050301	0	0 %	1	9.09%%	0.58	NS	1.10	0.91-1.33
060101/060103	1	16.67%	2	18.18%	0.006	NS	0.90	0.06-12.58
060201	2	33.33%	5	45.45%	0.24	NS	0.60	0.08-4.76
060301/060401	0	0%	0	0%	N/A	N/A	N/A	N/A
060401/0634	0	0%	0	0%	N/A	N/A	N/A	N/A
060801	0	0%	0	0%	N/A	N/A	N/A	N/A
0609	0	0%	0	0%	N/A	N/A	N/A	N/A

Homozygosity of HLA-DQB1 in patients with alopecia areata and controls:

HLA-DQB1 homozygosity was studied in patients and controls. Results shown in Table.5, indicated that out of 17 patients, 15 were homozygous and 13 out of 50 controls, were homozygous, with frequencies of 88.24% and 26% respectively. Patients with alopecia areata showed increased homozygosity in HLA-DQB1 when compared with controls.

Table . 5 HLA-DQB1 genotypes homozygosity in Alopecia areata patients & controls

HLA-DQ Homozygosity	Cases			
	Patients with alopecia areata		Controls	
	No	%	No	%

DQB1	Homozygous	15	88.24%	13	26%
	heterozygous	2	11.76%	37	74%
	Total	17	100%	50	100%

$$\chi^2 = 20.20, P < 0.005, OR = 0.05, 95\% CI = 0.01-0.23$$

Frequency of HLA-DQA1 / HLA-DQB1 alleles in alopecia areata patients:

Table. 6 indicated the frequency of HLA-DQA1 / HLA-DQB1 alleles in alopecia areata patients. HLA-DQA1 genotyping results in our previous study (16), used for combination with present results of HLA-DQB1 genotyping. The combination results revealed that (HLA-DQA1*0103 – HLA-DQB1*0301) was present in 6 out of 17 patients (35.29%). (HLA-DQA1 *0103 – HLA-DQB1 *060101) and (HLA-DQA1*0104 – HLA-DQB1*030101) were present in 3 (17.65%). (HLA-DQA1*0104 – HLA-DQB1*050101) and (HLA-DQA1*0104-HLA-DQB1*060101) were present in 2 (11.76%). Also HLA-DQA1*0103 – HLA-DQB1*060201 was present in 1 (5.88%) patient. HLA-DQA1*0103 – HLA-DQB1*0301 showed higher frequency in comparison with other alleles combination.

Table. (6): Frequency of HLA-DQA1 / HLA-DQB1 alleles in Alopecia areata patients

HLA-DQA1-DQB1	Alopecia areata pateints	
	N=17	(%)
HLA-DQA1 *0103 – HLA-DQB1 *060101	3	17.65%
HLA-DQA1*0103 – HLA-DQB1*0301	6	35.29%
HLA-DQA1*0103 – HLA-DQB1*060201	1	5.88%
HLA-DQA1*0104 – HLA-DQB1*030101	3	17.65 %
HLA-DQA1*0104 – HLA-DQB1*050101	2	11.76%
HLA-DQA1*0104-HLA-DQB1*060101	2	11.76%

Discussion:

Currently available evidence suggests that alopecia areata can be considered a T-cell-mediated autoimmune disease in which the gradual loss of protection provided by immune privilege of the normal hair follicle plays an important role (18) and (19). The results of the present study showed that the frequency of males infected with alopecia areata was 54% while females frequency was 46%. These

results indicated that there was no significant difference between males and females when compared with control group ($\chi^2 = 0.04$; $P = NS$; $OR = 0.73$; $95\% CI = (0.33-1.59)$). A study done on a sample of Tunisian patients (20) indicated that the frequencies of males and females was (48% and 52%) respectively. While male to female ratio was 1.6:1 in a study done in Turkey (21). Our results indicated that there was no significant difference in

frequencies of age groups when compared with controls ($\chi^2 = 1.27$; $P = \text{NS}$; $\text{OR} = 0.60$; $95\% \text{ CI} = (0.25-1.47)$). In the present study, 100 DNA samples were extracted from blood samples obtained from patients and controls and then subjected to PCR for amplification of HLA-DQB1 gene. The Master Mix 2 X preparation was according to the following equation which was developed in Medical Microbiology laboratories (NML) in Winnipeg, Canada: 2X Mix 92%, 4% of each upstream and downstream primer. This method showed very good results after PCR and the bands on agarose gel were very clear, (DQB1 $\approx$ 300 base pairs). The minimum concentration of the amplified DNA samples was 45 ng/ul and the maximum concentration of the amplified DNA samples was 50 ng/ul. Out of 50 DNA samples subjected to HLA-DQB1 genotyping, only 17 samples showed results. Transportation of the PCR product from Iraq to Korea in July without using liquid nitrogen, might be the reason for only 17 samples gave results after sequencing. In a previous study (17) transportation of the samples to Canada was in January, only some samples did not show results. The results of the present study showed that the frequency of males infected with alopecia areata was 54% while females frequency was 46%. These results indicated that there was no significant difference between males and females when compared with control group ($\chi^2 = 0.04$; $P = \text{NS}$; $\text{OR} = 0.73$; $95\% \text{ CI} = (0.33-1.59)$). Studying genotype frequency of HLA-DQB1 in alopecia areata patients and controls showed a significant decreased frequency of HLA-DQB1*020101 allele in alopecia areata patients ($\chi^2 = 4.69$, $P < 0.05$, $\text{OR} = 0.76$, $95\% \text{ CI} = 0.65-0.89$) as compared with controls. These results do not agree with results of a study done by Chowdhury (22). Also HLA-DQB1*030201

allele showed a significant decreased frequency in alopecia areata patients ($\chi^2 = 5.69$, $P < 0.05$, $\text{OR} = 0.72$, $95\% \text{ CI} = 0.61-0.86$) as compared with controls, which indicates that these two alleles might have a protective role in alopecia areata. Also results indicated statistically significant increased frequency of HLA-DQB1*030101 allele in alopecia areata patients ($\chi^2 = 4.26$, $P < 0.05$, $\text{OR} = 0.24$, $95\% \text{ CI} = 0.06-1.10$) as compared with controls. These results agree with a study done by Nazila who reported positive association of HLA-DQB1*0301 with AT/AU (23). Also results of the present study showed a significant increased frequency of HLA-DQB1*060101 and HLA-DQB1*060201 alleles in alopecia areata patients ($\chi^2 = 9.82$, $P < 0.05$, $\text{OR} = 1.23$, $95\% \text{ CI} = 0.97-1.56$) and ($\chi^2 = 16.91$, $P < 0.05$, $\text{OR} = 1.46$, $95\% \text{ CI} = 1.05-2.02$) respectively as compared with controls. The significant increased allele frequencies of HLA-DQB1*030101, HLA-DQB1*060101 and HLA-DQB1*060201 alleles indicates that these alleles might be a risk factor in alopecia areata patients. These results agree with results of two studies done on Chinese Hans (24) and (25) which showed association between this allele and alopecia areata in Chinese Hans. Genotyping of HLA-DQB1 was studied in patients with family history of alopecia areata and compared with patients without family history of alopecia areata. Results of the present study indicated that there was no significant difference between patients with family history of alopecia areata and patients without family history of alopecia areata. HLA-DQB1 homozygosity was studied in patients and controls. Results indicated that patients with alopecia areata were homozygous in HLA-DQB1 when compared with controls. The combination results found that HLA-DQA1*0103 – HLA-DQB1*0301 was present in 6 out of 17 patients (35.29%). (HLA-DQA1*0103 – HLA-DQB1*060101)

and (HLA-DQA1*0104 – HLA-DQB1*030101) were present in 3 (17.65%). (HLA-DQA1*0104 – HLA-DQB1*050101) and (HLA-DQA1*0104-HLA-DQB1*060101) were present in 2 (11.76%). Also HLA-DQA1*0103 – HLA-DQB1*060201 was present in 1 (5.88%) patient. HLA-DQA1*0103 – HLA-DQB1*0301 showed higher frequency in comparison with other alleles combination. The overall findings in this study support hypothesis that the HLAs class II are major susceptibility determinants for AA and may be useful in distinguishing among AA severity phenotypes.

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