Possible association of HLA class-I Molecules with autoimmune Hepatitis in Iraqi patients

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Summary:

Background: genetic factors were considered to play a possible role the development of autoimmune hepatitis.

Patients and methods: polymerase chain reaction-sequence specific primers (PCRSSP) was the method used to asses HLA-typing of 100 blood samples of 60 AIH patients and 40 healthy normal controls.

Results: comparison between AIH patients and healthy controls showed several antigens deviations in their frequencies. HLA-A*113 (A1/-/Null) observed to play a possible risk factor in this disease while significant loss of HLA-A*2 allele were clearly observed which prompt us to believe that it could act as a protective factor, on the other hand, increased frequency of HLA-B*8 & B*14 were statistically significant in AIH which is most likely to be considered as a rather risk factor, while most HLA-B*16 were lost which led us to think of being acting as a rather protective factor.

Conclusions: this finding reflects a preliminary picture that HLA antigens might play a role in AIH susceptibility and further studies are worth being carried out.

Key words: AIH, HLA antigen, PCR-SSP.

Introduction:

Autoimmune hepatitis (AIH) is the third most common inflammatory chronic liver disease in man preceded by HBV infection and HCV infection (1, 2). According to several observations, attention has been focused on the possibility that genetic background could play a crucial role in the susceptibility to AIH.The importance of participation genetic factors in the disease is currently based on the increased risk of autoimmune disease in first- degree relatives of patients with AIH however few reports denoted that 15% of siblings and 10% of offspring (3, 4) were observed, while increased frequency of specific genetic markers as certain human leukocyte antigen (HLA) has been reported in patients group other than in general population (5). Therefore; several studies in different areas were undertaken to test the possibility of association between this disease and one or several of the HLA- Ags, as suggested by Czaja and associates (5, 6). Positive association with the Ag (A1) was reported in Caucasian patients (7), while another study reported other Ags (1.6.7).

Patients and Methods:

The present study included 60 Arab, Iraqi AIH patients (42 females and 18 males), attending The Gasteroentestinal and Hepatology Teaching Hospital,. Baghdad Teaching Hospital and Al-Yarmook Teaching Hospital in a period between November 2006 and July 2007.Their age raged between 4-62 years, compared with 40 healthy individuals (age and sex matched). Both groups were typed for HLA-class II (DRB & DQ) antigens.

Laboratory investigation:- The basic material for typing with histo type/DNA-SSP kit is purified DNA. The test procedure was done by using the Sequence Specific Primers (SSP). This method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3´-end of both primers.

Results:

The frequency of distribution of various class I HLA-Ags for two studied groups were presented in tables (1and 2). At HLA-A locus, comparison between AIH patients and healthy control showed several antigens deviation in their frequencies. It was found that the presence of HLA- A*113 (A1/-/Null) formed an etiological, risk factor for the disease, with (P value<0.001). While HLA-A*02010101-22, 24-33, 36-45, 47, 49-54, 57-61, 63, 64, 66-69, 71-77 , 79-86 (A2 /Low A2 / A203 / - / A210 / Null) (8.3% Vs 87.5 %) represented a protective factor with (P value<0.01). Within HLA-B locus , B*140201,0202,03,04,0601,0602 (B14) was found with increased frequencies in patients (15.0% Vs 5.0%) than healthy group with P value 0.009, besides B *0806 (B8) which more significant than (B14) (P value <0.007). In contrast B *3804 (B16) occurs in decreased frequencies in patients

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compared to healthy control groups with non significant differences (P value <0.028).

Table-1: Observed numbers and percentage frequencies of HLA-A alleles (serotypes and genotypes) in AIH patients and controls.

HLA-A Alleles		Patients (No. = 60)		Controls (No. = 40)	
Serotype	Genotype	No.	%	No.	%
*A1/ - / Null	A*113	16	26.6	2	5.0
**A2 /Low A2 / A203 / - / A210 / Null	A*02010101-22, 24-33, 36-45, 47, 49-54, 57-61, 63, 64, 66-69, 71-77 , 79-86	5	8.3	35	87.5
A2 / -	A*234, 3501, 3502, 56, 62	1	1.6	4	10.0
A2 / -	A*0255	2	3.3	4	10.0
A2 / -	A*278	N.D.	N.D.	4	10.0
A3/ Null / -	A*03010101-07, 09-11N, 13-16	3	5.0	1	2.5
A11 / - / Null	A*110101 - A*1116, 20, 21N-23	5	8.3	2	5.0
A11 / - / Null	A*2303 / A*2424	4	6.6	1	2.5
A23(9) / - / Null	A*2301,02,04-07N, 08N,10,11N,12	1	1.6	2	5.0
A24(9) / Low A24/ A2403 / A9 / -	A*24020101-07 , 09N, 10, 11N, 13-15, 17-20, 22, 23, 25-28, 30-35, 36N, 37-40N, 41, 43-45N, 46-48N, 49-53	2	3.3	3	7.5
A24(9) / -	A*2408, 21, 29, 42	N.D.	N.D.	1	2.5
A25(10) / -	A*250101, 03, 04	4	6.6	1	2.5
A26(10), A10, Null	A*260101-0104, 03, 05, 0701-08, 10-12, 14-18, 21-25N, 26	3	5.0	3	7.5
A26(10)	A*2602 ,04, 06	1	1.6	N.D.	N.D.
A26(10)	A*2609	N.D.	N.D.	1	2.5
A26(10)	A*2613, 19	1	1.6	1	5.0
A29(19)	A*29010101-06 ,08N-13	N.D.	N.D.	2	5.0
A29(19) / Null / -	A*2907	N.D.	N.D.	1	2.5
A30(19) / -	A*300101-A*3004, 06-14L, 15	4	6.6	2	5.0
A31(19) / -	A*310102, 02, 05, 06, 08 09, 11, 12	N.D.	N.D.	1	2.5
- / A31(19)	A*3103, 04	N.D.	N.D.	1	2.5
- / A31(19)	A*3107, 10	N.D.	N.D.	2	5.0
A34(10) / -	A*3401-A*3406	N.D.	N.D.	1	2.5
A66(10) / -	A*6601 - A*6604	4	6.6	N.D.	N.D.
A66(10)	A*6602	1	1.6	1	2.5
A10	A*6603	1	1.6	N.D.	N.D.

N.D.: Not detected

P value=0.01 ** P value=0.001*

Table-2: Observed numbers and percentage frequencies of HLA-B alleles (serotypes and genotypes) in AIH patients and controls.

HLA-B Alleles		Patients (No.= 60)		Controls (No.= 40)	
Serotype	Genotype	No.	%	No.	%
B7, B703, -	B*070201 - 04, 08, 10, 15, 16,21-23, 25, 26, 28-30, 33, 35, 37, 39, 41, 42	1	1.6	N.D.	N.D.
B7 / -	B*0707	2	3.3	1	2.5
B7	B*0720	1	1.6	N.D.	N.D.
B8, - Null	B*080101, 0102, 04,05, 08N, 10, 11 , 13, 15, 18, 19N, 20, 22-24	6	.6.6	1	2.5
*B8	B*0806	18	30.0	2	5.0
B13, -	B*1301 ,06, 11 , 12, 13	1	1.6	N.D.	N.D.
**B14, -	B*140201,0202,03,04,0601,0602	9	15.0	2	5.0
B62(15)	B*150102	N.D.	N.D.	1	2.5
B62(15)	B*1507	1	1.6	N.D.	N.D.
B18	B*1809	1	1.6	2	5.0
B27, -	B*2701, 02 0401, 0402, 0502-0509, 10, 13-17, 19, 23, 25, 28-30	1	1.6	3	7.5
B35, -	B*3519, 47	1	1.6	N.D.	N.D.
B35, -	B*3534	3	5.0	1	2.5
B37, Null	B*3701 , 03N, 04	N.D.	N.D.	2	5.0
B38(16), -, B16	B*3801 , 0201 , 0202, 04, 06-09, 11	1	1.6	1	2.5
B39(16), -	B*3903, 14	N.D.	N.D.	1	2.5
B39(16)	B*3904, 12	1	1.6	N.D.	N.D.
B60(40), Null, -	B*400101 - 0104, 1401 -1403 ,22N , 25 , 30, 31 , 33, 34, 42, 43, 45, 49, 54, 55	N.D.	N.D.	1	2.5

B61(40), -	B*400201 - 0203, 04, 060101 , 060102, 0602, 09, 11 , 15, 16, 18, 24, 27, 29, 32, 37, 40, 44, 50, 53, 56-58	1	1.6	2	5.0
B60(40)	B*4013	1	1.6	N.D.	N.D.
B60(40)	B*4021	1	1.6	N.D.	N.D.
B41, -	B*4101-07	2	3.3	1	2.5
B44(12), -	B*440301,0302,04,07,13,26,28,30,32,35,36,38,39	3	5.0	2	5.0
B44(12)	B*4410	N.D.	N.D.	1	2.5
B44(12)	B*4437	2	3.3	1	2.5
B45(12), -	B*4504	1	1.6	1	2.5
B47 , -	B*47010101, 010102, 04, 05	1	1.6	2	5.0
† B16	B*3803	2	3.3	20	50.0
B5, B51(5),	B*5116, 21, 31, 34, 36	N.D.	N.D.	1	2.5
B53, -	B*530101-0103, 02, 04, 10	1	1.6	2	5.0
B54(22) / -	B*5403	1	1.6	N.D.	N.D.
B55(22) / -	B*5504, 17	1	1.6	N.D.	N.D.
B56(22)	B*5508	1	1.6	N.D.	N.D.
B55(22). B56(22)	B*5614	3	5.0	2	2.5

N.D.: Not detected * P=0.007 **P=0.009 † P =0.028 (Not significant)

Association of HLA-Ags among different types of AIH: The frequencies of each allele for Class-I loci appeared in the table 3 and 4 below:

Table-3: Observed numbers and percentage frequencies of HLA-A alleles (serotypes and genotypes) in different AIH types.

HLA-A Alleles		AIH-1 (No. = 35)		AIH-2 (No.=15)		AIH-3 (No.=10)	
Serotype	Genotype	No.	%	No.	%	No.	%
*A1/ - / Null	A*113	14	40.0	1	6.6	1	10.0
**A2 /Low A2 / A203 / - / A210 / Null	A*02010101-22, 24-33, 36-45, 47, 49-54, 57-61, 63, 64, 66-69, 71-77 , 79-86	2	5.7	2	13.3	1	10.0
A2 / -	A*234, 3501, 3502, 56, 62	1	2.8	N.D	N.D	N.D	N.D
A2 / -	A*0255	1	2.8	N.D.	N.D.	1	10.0
A2 / -	A*278	N.D.	N.D	N.D	N.D	N.D	N.D
A3/ Null / -	A*03010101-07, 09-11N, 13-16	3	8.5	N.D	N.D	N.D	N.D
A11 / - / Null	A*110101 - A*1116, 20, 21N-23	3	8.5	1	6.6	1	10.0
A11 / - / Null	A*2303/ A*2424	2	5.7	2	13.3	N.D	N.D
A23(9) / - / Null	A*2301,02,04-07N, 08N,10,11N,12	1	2.8	N.D	N.D	N.D	N.D
A24(9) / Low A24/ A2403 / A9 / -	A*24020101-07 , 09N, 10, 11N, 13-15, 17- 20, 22, 23, 25-28, 30-35, 36N, 37-40N, 41, 43-45N, 46-48N, 49-53	1	2.8	1	6.6	N.D	N.D
A24(9) / -	A*2408, 21, 29, 42	N.D.	N.D.	N.D	N.D	N.D	N.D
A25(10) / -	A*250101, 03, 04	2	5.7	1	6.6	1	10.0
A26(10), A10, Null	A*260101-0104, 03, 05, 0701-08, 10-12, 14-18, 21-25N, 26	1	2.8	1	6.6	1	10.0
A26(10)	A*2602 ,04, 06	1	2.8	N.D.	N.D.	N.D.	N.D.
A26(10)	A*2609	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A26(10)	A*2613, 19	1	2.8	N.D.	N.D.	N.D.	N.D.
A29(19)	A*29010101-06 ,08N-13	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A29(19) / Null / -	A*2907	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A30(19) / -	A*300101-A*3004, 06-14L, 15	3	8.5	N.D.	N.D.	1	10.0
A31(19) / -	A*310102, 02, 05, 06, 08 09, 11, 12	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
- / A31(19)	A*3103, 04	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
- / A31(19)	A*3107, 10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A34(10) / -	A*3401-A*3406	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
A66(10) / -	A*6601 - A*6604	1	2.8	2	13.3	1	10.0
A66(10)	A*6602	1	2.8	N.D.	N.D.	N.D.	N.D.
A10	A*6603	N.D.	N.D.	N.D.	N.D.	1	10.0

N.D.: Not detected * P=0.001 **P=0.05

Among A-locus allelic Ag; A*113 (A1/ - / Null) was observed in high frequency in type 1-AIH in comparison with type 2 and 3 0f the disease (40% Vs. 6.6% and 10.0%) respectively. However, other genotypes showed remarkable similarities of allelic frequencies between different types of AIH patients.

Table-4: Observed numbers and percentage frequencies of HLA-B alleles (serotypes and genotypes) in different AIH types.

HLA-B Alleles		AIH-2 (No.=15)			AIH-3 (No.=10)	
Serotype	Genotype	No.	No.	%	No.	%
B7, B703, -	B*070201 - 04, 08, 10, 15, 16,21-23, 25, 26, 28-30, 33, 35, 37, 39, 41, 42	1(2.8%)	N.D.	N.D.	N.D.	N.D.
B7 / -	B*0707	1(2.8%)	N.D.	N.D.	1	10
B7	B*0720	1(2.8%)	N.D.	N.D.	N.D.	N.D.
B8, - Null	B*080101, 0102, 04,05, 08N, 10, 11 , 13, 15, 18, 19N, 20, 22-24	3(8.4%)	N.D.	N.D.	N.D.	N.D.
B8*	B*0806	13(3.5%)	N.D.	N.D.	5.0	50
B13, -	B*1301 ,06, 11 , 12, 13	1	N.D.	N.D.	N.D.	N.D.
B14, -**	B*140201,0202,03,04,0601,0602	1	7	46.6	1	10
B62(15)	B*150102	N.D.	N.D.	N.D.	N.D.	N.D.
B62(15)	B*1507	N.D.	N.D.	N.D.	1	10
B18	B*1809	N.D.	1	6.6	N.D.	N.D.
B27, -	B*2701, 02 0401, 0402, 0502-0509, 10, 13-17, 19, 23, 25, 28-30	1	N.D.	N.D.	N.D.	N.D.
B35, -	B*3519, 47	1	N.D.	N.D.	N.D.	N.D.
B35, -	B*3534	1	1	6.6	1	10
B37, Null	B*3701 , 03N, 04	N.D.	N.D.	N.D.	N.D.	N.D.
B38(16), -, B16	B*3801 , 0201 , 0202, 04, 06-09, 11	1	N.D.	N.D.	N.D.	N.D.
B39(16), -	B*3903, 14	N.D.	N.D.	N.D.	N.D.	N.D.
B39(16)	B*3904, 12	N.D.	N.D.	N.D.	1	10
B60(40), Null, -	B*400101 - 0104, 1401 -1403 ,22N , 25 , 30, 31 , 33, 34, 42, 43, 45, 49, 54, 55	N.D.	N.D.	N.D.	N.D.	N.D.
B61(40), -	B*400201 - 0203, 04, 060101 , 060102, 0602, 09, 11 , 15, 16, 18, 24, 27, 29, 32, 37, 40, 44, 50, 53, 56-58	N.D	1	6.6	N.D	N.D
B60(40)	B*4013	1	N.D.	N.D.	N.D.	N.D.
B60(40)	B*4021	N.D.	1	6.6	N.D.	N.D.
B41, -	B*4101-07	1	N.D.	N.D.	1	10
B44(12), -	B*440301,0302,04,07,13,26,28,30,32,35,36,38,39	3	N.D.	N.D.	N.D.	N.D.
B44(12)	B*4410	N.D.	N.D.	N.D.	N.D.	N.D.
B44(12)	B*4437	1	1	6.6	N.D.	N.D.
B45(12), -	B*4504	N.D.	N.D.	N.D.	1	10
B47 , -	B*47010101, 010102, 04, 05	1	N.D.	N.D.	N.D.	N.D.
B16	B*3803	1	N.D.	N.D.	1	10
B5, B51(5),	B*5116, 21, 31, 34, 36	N.D.	N.D.	N.D.	N.D.	N.D.
B53, -	B*530101-0103, 02, 04, 10	N.D.	1	6.6	N.D.	N.D.
B54(22) / -	B*5403	1	N.D.	N.D.	N.D.	N.D.
B55(22) / -	B*5504, 17	N.D.	N.D.	N.D.	1	10
B56(22)	B*5508	N.D.	N.D.	N.D.	1	10
B55(22) / B56(22)	B*5614	1	1	6.6	1	10

N.D.: Not detected * P=0.003 **P=0.012

Many of B-locus Ags were considered to be an etiological factors, yet B*0806 (B8) showed high frequency among type-1 and 3-AIH patients with significant differences (P value < 0.003).In addition, B*140201,0202,03,04,0601,0602 (B14) which revealed less significant difference in it's frequency among different types of AIH patients with (P value < 0.012), table -4.

Discussion:

During the past 30 years remarkable advances have occurred in the understanding of the epidemiology, natural history and pathogenesis of chronic liver disease, including AIH.

The role of genetic factors in the etiology of AIH was documented many decades ago. As a result, the investigative efforts were focused on the genetic markers of susceptibility to this disease. Moreover, the high familial incidence of AIH suggests the possibility of a linkage or an association of disease with MHC. Whittingham and colleagues have reported a family study compatible with the linkage of AIH with MHC (8), however, this study is the first report on the association of HLA class I , II and III alleles with AIH in Iraqi patients.

In the present work, there was a significant association of HLA-A*1 with AIH patients (p= 0.001). This result is in agreement with what reported by some researchers regarding significant statistical association of AIH with A*1in comparison with healthy control group (5).

Different results regarding this association was reported, results differ in different population. For instance Strettell and co-workers (5) reported the association of class I HLA-Ag with AIH and observed an increase frequency of HLA-A1 in Caucasians patients (7), other study reported by Menna and collegues revealed an increased frequency of HLA-A2 and HLA-A3 and decreased HLA-A1 in Argentina patients (9).

On the other hand, our results has shown that there was a significant loss in the HLA-A*2 alleles when compared with the healthy control group. These alleles could be considered as protective markers.Regarding the presence of HLA-B Ag and it's proposed association with AIH, extensive studies were conducted on this locus, to give contradicting results. In this study the increasing in frequency of HLA-B8 and B14 were statistically significant in AIH patients (p=0.007, 0.009) respectively compared to healthy control. This result is at variance with other study (10) which lack such association between B locus and this autoimmune liver disease, while other scientist reporting positive association with antigens of HLA-B. For instance, Amarapurkar and associates noticed that B27 was increased significantly in the AIH cases (11), other investigator reported the positive association with B3 (12). Furthermore, these results showed that most HLA-B*16 alleles in the AIH patients seem to be lost; again this alleles may act as a rather protective factors in AIH.

Moreover, this study showed that the risk group of class I, HLA-A locus fall into specificity A1 allele which fall in high percentage in type 1-AIH compared to type 2 and 3 of the disease (40.0% Vs. 6.6 % and 10.0%) respectively. While at B-locus, B8 exclusively present in type 1 and 3-AIH but not in type 2 of the disease. B*14, on the other hand, are the characteristic genetic marker of type-2 of the disease. The result obtained from the present study come in agreement with a study reported by Boberg (13), but in disagreement with other study which reported that Class I alleles have no effect in determining the susceptibility to AIH (14).

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