Possible Association of HLA-DR and DQ Molecules with Autoimmune Hepatitis in Iraqi Patients

Eman Sh. AL-Obeidy* **BSc, PhD**
Khalida M. Mousawy ** **MBChB, PhD**
Raghad J H AL-Akayshi *** **CABM, FICMS (GE & H)**
Laith Abdalelah ****** **MBChB, FIBMS (Immun.)**

Summary:

Background: Human leukocyte antigen (HLA) is the most polymorphic genetic system in man. The genes of this region seem to influence susceptibility to certain diseases.

Patients and methods: Polymerase chain reaction-Sequence Specific Primers PCR-SSP is the method used to asses HLA-typing of 100 blood samples of 60 AIH patients and 40 healthy normal controls.

Results: An increased frequency of HLA-DR3, DR4 and DR7 was observed for patients group versus control group with P-value (0.0001, 0.05, and 0.001) respectively, while DR*0211 (DR2) may be formed the basis for protection against the disease. HLA-DQ on the other hand, yielded on association in Iraqi patients with AIH.

Conclusions: This finding demonstrated that HLA-DR3, DR4 and DR7 might play a role in AIH susceptibility.

Fac Med Baghdad 2010 Vol. 52, No. 1
Received April 2008
Accepted Aug. 2008

Key words: autoimmune hepatitis, Human leukocyte antigen (HLA), PCR –SSP assay.

Introduction:

The specific causes of autoimmune hepatitis (AIH) are still mysterious, nevertheless; environmental, genetic, familial factors found to play a crucial role in the development of this autoimmune liver disease (1). AIH occurs three to four times more commonly in relatives of patients who have this disease than in the general population and this excess persist even if the clearly hereditary forms are excluded (2,3).
Attempts for identifying the genes involved in predisposition to this disease have a step forward when tissue typing for HLA class II Ag of Caucasian patients showed that 60-70% of patients with AIH were HLA-DR4 positive by cellular or serological techniques compared with 20-25% of control population (4). While, other groups observed that the presence of these alleles is associated with higher frequency of remission during corticosteroid treatment (5).

Patients and Methods

Patients: 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

Table-1 below revealed the importance of DR-alleles through their frequencies in AIH patients in comparison with healthy controls. As shown, DR*0306 (DR3), DR*0401 (DR4) and DR*0704 (DR7) found in high frequencies in patients compared to healthy control groups with (P value <0.0001, 0.05, and 0.001) respectively. While DR*0211 (DR2) may be formed the basis for protection against the disease; It's presence formed significant difference between patients and healthy individuals (P value < 0.008). In spite of presence of DR*0401 (DR4) in high frequency (20.0% Vs.5.0%); its importance as a risk factor was less than DR*0306 (DR3), and DR*0704 (DR7).

*Dept. of Virology, Medical Laboratory, Medical City
** College of Medicine / University of Baghdad.
*** Gasteroentestinal and Hepatology Teaching Hospital.
**** Al-Karama Teaching Hospital.

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

HLA-DR Alleles		AIH patients (No. = 60)		Healthy control (No. = 40)	
Serotype	Genotype	No.	%	No.	%
DR2*	DR*0211	3	5.0	32	80.0
DR 1, -	DR*010101,0102,0201-0204,04-13	3	5.0	N.D.	N.D.
DR 1	DR*010103	1	1.6	N.D.	N.D.
DR4***	DR*0401	12	20.0	2	5.0
DR 7****	DR*0704	15	25.0	3	7.5

N.D.: Not detected
* P=0.008
** P=0.0001
*** p=0.05
****p=0.001

A survey of the distribution of HLA-DQ frequency yielded no evident association between DQ Ag and AIH patients, except **for DQB1*0101,02** (DQB1) which considered as a protective factor since it present in highly significance among healthy control group (P value < 0.005), (table-2).

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

HLA-DQB Alleles		AIH Patients (No. = 60)		Healthy Controls (No. = 40)	
Serotype	Genotype	No.	%	No.	%
DQB 7(3) /	DQB1*0304,14	1	1.6	4	10.0
*DQB1	DQB1*0101,02	2	3.3	28	70.0
DQB5(1)	DQB1*050101-050302,0504	4	6.6	2	5.0
DQB 6(1)	DQB1*060101-0103	1	1.6	1	2.5
DQB 6(1) / 1 /	DQB1*0602-18,20-22,24-26N	4	6.6	2	5.0
DQB 2	DQB1*020101-0102,0202-04	4	6.6	2	5.0

N.D.: Not detected
* P=0.005

Association of HLA-Class II Ags among different types of AIH:Frequencies of Class-II HLA were shown in table-3 which included the percentages of these molecules among the three types of AIH patients. HLA-DR considered as an essential factor in AIH mechanism; thus DR*0306(**DR3)** showed highly significant difference (P value < 0.0001) between the type 1, 2 and 3 of the disease, in addition to DR*0401 **(DR4)** and **DR*0704 (DR7)** with (P value 0.001 and 0.05) respectively.

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

.

HLA-DR Alleles		AIH-1 (No. = 35)	AIH-2 (No. = 15)	AIH-3 (No.=10)
Serotype	Genotype	No. (%)	No. (%)	No. (%)
*DR3	0306	25(71.4)	4(26.6)	9(90.0)
DR4**	DR*0401	11(31.3)	N.D (N.D.)	1(10.0)
***DR 7,-	DR*0704	1(2.8)	12(80.0)	2(20.0)

N.D.: Not detected
* P=0.0001
** P=0.001
***P=0.05

So from all what had been mentioned previously it appeared that DR3 formed a big significant difference between patients and the normal persons since it's P value was <0.0001.

Discussion:

HLA genes have been known for a long time and the whole of the HLA complex has been sequenced, so why we still doing HLA association studies? One of the many reasons is to identify disease- specific susceptibility (risk) and protective markers (alleles that were lost in patients) that can be used in immunogenetic profiling, and risk assessments although their demonstration have no role in the diagnosis or treatment of the disease**.**The association of HLA-DR3 or other HLA-DRB1 alleles with AIH had now been studied in nearly every population. Similarly, the fact that the presence of HLA-DRB alleles affects the course and outcome of AIH has likewise been seen in all studies (1,6).Like other studies, our results showed that there is a strong association between certain HLA-DRB1 alleles and AIH. This evidence brought in hands when we estimated the frequency distribution of HLA-DR*3, DR*4 and DR*7 alleles groups that formed an etiologic risk factor with (P value 0.0001, 0.05 and 0.001) respectively. Strong association with the absence of HLA-DR*2 was observed that might be considered as protective markers. The same allele (DR*2) was seen to be associated with AIH in patients in other nearby and far country, as for example which reported on Turkish and on Japanese patients (7, 8).Regarding to the fact says that DRB1 locus is the principle susceptibility region of MHC in patients with AIH, several different alleles of DR*3 and DR*4 has been identified in patients with this disease from different populations. For instance Donaldson and co-workers reported the association of DRB1*0301 and DRB1*0401 in Northern European while DRB1*0404 allele was reported in Mexican and DRB1*0405 in Japanese patients with AIH (8, 9, 10). Interestingly, our study showed that DRB1*0306 (DR3) and DRB1*0401 (DR4) was the

risk alleles associated with AIH in Iraqi patients. The reason for such allelic variation is still mysterious; it may be due to gene drift, when some genes get associated together by chance or by gene flow which is the result of admixture between different populations (1).Some researchers noted an increased frequency of HLA-DR*7 in AIH, and they suggested a possible causal role of this antigen in this disease (1,12), while other study denoted that there was no differences for DR*7 between healthy control and AIH patients, who were Australian born (12). Interesting finding of this study was the higher expression of DR*0704 (DR7) in AIH patients which account for (25.0% Vs 7.5%) in healthy controls. HLA-DQ antigen, were encoded by genes within the HLA-class II region, have not been studied extensively in AIH. A recent study revealed for the first time that the HLA-DQ locus may hold a real promise to define class II susceptibility to AIH and demonstrated a significant increased frequency of HLA-DQB*2 and DQB*6 in patients from Canda (13). But other scientists were unable to confirm the last observation (14, 15). However, their importance remains lesser than HLA-DR that is demonstrated in different ethnic groups. The present investigation failed to demonstrate such association. Indeed, the rarity of HLA-DQ in the study population suggested that it might be protective against the disease in Iraqi patients. The apparent paradox that a genetic factor increasing susceptibility in one population while acting as protective elements in another is still mysterious. Susceptibility in Iraqi patients may be mediated by a protective function encoded by HLA-DQB against a region-specific etiologic trigger (eg, a virus or environmental toxin). Over the last decades a large body of evidence has accumulated linking HLA-DR, particularly DR*3 to the predisposition to AIH. In the present study, HLA-DR*3 alleles were detected in all types of AIH, thus suggesting that this genetic molecule may be a generic marker of the disease. They exhibit 71.4%, 26.6%, 90.0% of type 1, 2 and 3 respectively. This result was lower than that for abroad studies (85%, 45.6% and 94.0%) (16). the explanation for this result is related to the large size sample of the last study. Regarding DR*4 frequency, it was stated that these Abs were observed in about 43% of patients with AIH (17). Our results has shown that there was a significant loss in HLA-DR*4 alleles in type 2 and 3-AIH. By contrast they occur in high frequency in patients with type-1 of the disease. However, this allele showed to have smaller frequency as well as it had smallest P value (0.05) than DR*3, hence this Ag is considered as the one with strongest association with risk of having AIH. This suggests that this allele may have a secondary effect in predisposing to develop type 1-AIH but obscured by the increased frequency of HLA-DR*3 in this disease.Obviously, the results in this work (table-3) were in agreement with the recent study (9) in which HLA-DR*7 exhibit high percentage in type 2-AIH but loss or greatly decreased in type 1 and 3 of the disease. However, the present investigation dose not agrees with that study from United Kingdom or USA (5, 18).So, generally the development of AIH would depend upon the expression of the susceptible HLA alleles and absence of the protective alleles, along with the environmental factors that trigger the autoimmune process. This fact was confirmed recently by Czaja (1).

References:

1-Czaja AJ. Current concepts in autoimmune hepatitis. Annals of Hepatology 2005;4 (1): 6-24.

2- Kalliopi Zachou, Eirini Rigopoulou. Autoantibodies and autoantigens in autoimmune hepatitis: important tools in clinical practice and to study pathogenesis of the disease. J. of Autoimmune Disease 2004;1: 420-442.

3- Waldenstrom J. Leber, Blutproteine und Nahrugseiweisee. Dtsch Gesellsch Verdstroffw 1950; 15: 113-119.

4- Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson

PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis:

human leukocyte antigens DR4 and A1-B8-DR3 are independent

risk factors. Hepatology 2002; 13: 701-706.

5- Czaja AJ, Donaldson PT. Genetic susceptibilities for immune expression and liver cell injury in autoimmune hepatitis. Immunol Rev

2000; 174: 250-259.

6- McFarlane IG. Definition and classification of autoimmune hepatitis. Semin Liver Dis 2008; 22: 317-324.

7- Vazquez MN, Alaez C, Olivo A et al.,. MHC class II sequence of susceptibility and protection in Mexican with autoimmune hepatitis . J Hepatol 2005; 28: 985-990.

8- Al-Khalidi J, Al-Muzairai I. Autoimmune hepatitis among the Arab population of Kuwait. Kuwait Med J 2000;30: 229-232.

9-Seki T, Kiyosawa K, Inoko H, Ota M. Association of autoimmune hepatitis with HLA-Bw54 and DR4 in Japanese patients. Hepatology 1990; 12: 1300-1304.

10- Qiu D-K, Ma X. Relationship between human leukocyte antigen-

DRB1 and autoimmune hepatitis type I in Chinese patients. J. Gastroenterol Hepatol 2003; 18: 63-67.

11- Ad'hiah, A. H., Mitchell, J. Papiha. All types of complement components C4, C3, C2 and BF in the population of Tasmania and northeast England. Gene. Geogr 1996; 10: pp93-103.

12- Czaja AJ, carpenter HA, Moore SB. Clinical and HLA phenotypes of type 1-autoimmune hepatitis in North American patients. Liver Int. 2006; 50: 233.

13- Seki T, Kiyosawa K, Inoko H, Ota M. Association of autoimmune hepatitis with HLA-Bw54 and DR4 in Japanese patients. Hepatology 1990; 12: 1300-1304.

14- Djilali SI, Renous R, Caillat ZS, Debray D, Alvarez F. Linkage disequilibrium between HLA

class II region and autoimmune hepatitis in pediatric patients. J Hepatol 2004; 48: 320.

15- Obermayer-straub P, Strassburg CP. Manns MP. Autoimmune hepatitis. J. Hepatology 2007; 42(1 suppl): 181-197.

16- McFarlane IG. Definition and classification of autoimmune hepatitis. Semin Liver Dis 2008; 22: 317-324.

17- Czaja AJ. Autoimmune liver disease. Audio-Digest Gastroenterology 2002; 16: 8-15.

18- Schifferli JA, Steiger G. Difference in the biological properties of the two forms of the fourth component of human complement (C4). Clin Exp Immunol 2003; 63: 473-7.