Association of HLA-DR3, DR4, and B27 gene in autoimmune hepatitis patients

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الخلاصة

التهاب الكبد المناعي الذاتي هو مزمن، مناعي او اضطراب الكبد الالتهابي, المسببات غير معروفه. ويعتقد حتى الآن أن يكون من امرض المناعة الذاتية. ويعتقد أن واحدة من العوامل الوراثية هي جينات هلا DR3-وDR4 . هدفت الدراسة الحالية إلى التحقيق في ترددات جينات هلا DR3-و DR4 و هلا B27-بين تفاعلات تفاعل البوليميراز المتسلسل لجينات هلا. تراوحت أعمار المرضى من 7-70 سنة في مجموعة المرضى، ومن 8-67 سنة في مجموعة الضوابط الصحية. في سياق التنميط الجيني للجينات هلا، وجد أن DR3 و DR4 و B27 اختلفوا في تردداتهم بشكل ملحوظ بين مرضى التهاب الكبد المناعي الذاتي التي تخلق نسبة عالية من المسببات 0.504 و 0.583 و 0.129 على التوالي مقارنة مع الضوابط الصحية، والOR ل 7.35DR3 ، و 8.0 ل DR4 و 2.23 ل B2 وتفاوتت تواتر هذه الجينات في المرضى المصابين بمرض التهاب الكبد المناعي اختلفا معنويا بين مجموعة المرضى ل DR3 مقارنة بالمجموعة السليمة التي كانت 58.3٪ و 16٪ على التوالي، وكانت عالية جدا اختلفت معنويا بين مجموعة المرضى ل DR4 مقارنة مع المجموعة الصحية التي 66.7٪ و 20٪ على التوالي، في حين لم تختلف معنوية بين مجموعة المرضى مقارنة مع مجموعة صحية ل B27 والتي 23.3٪ و 12٪ على التوالي.

Abstract

Autoimmune hepatitis (AIH) is a chronic, immunologically mediated inflammatory liver disorder of unknown etiology. AIH is so far thought to be an auto-immune disease. One of the genetic predisposing factors is

thought to be HLA-DR3 and DR4 genes. The present study aimed at investigation the frequencies of HLA-DR3, DR4 and HLA-B27 genes among the Real-time PCR were used for the HLA-genes. The age of the patients were ranged from 7- 69 years in AIH group and from 8-67 years in healthy controls group. On the context of genotyping of HLA- genes, DR3, DR4, and B27 were found to be differed in their frequencies significantly among AIH patients that creating high etiological fraction of 0.504, 0.583, and 0.129 respectively compared to healthy controls, with odd ratio (OR) 7.35 for DR3, high OR 8.0 for DR4 and 2.23 for B27. The frequencies of these genes in AIH patients, highly significant differed between patient group, for DR3 compared to healthy group which 58.3% and 16% respectively, and very high significantly differed between patient group for DR4 compared to healthy group which 66.7% and 20% respectively, while no significant differed between patient group compared to healthy group for B27 which 23.3% and 12% respectively.

Introduction

The first description of a chronic form of hepatitis in young women was by Jan Waldenstrom in 1950 (1). Later, the disease was associated with other autoimmune diseases and was termed “lupoid hepatitis” because of the presence of antinuclear antibodies and lupus erythematosus cells (2). These observations led to the idea that the foundation of this disease was a loss of immunological tolerance. The term Auto Immune Hepatitis (AIH) in its current meaning was introduced by Mackay and colleagues in 1965 when the concept of autoimmunity was acknowledged at an international meeting (3).

Women are affected more frequently than men with a sex ratio of around 4:1(4). In women a bimodal age pattern is usually seen, one in the late teens and one around the menopause but it should be stressed that disease can develop in all age groups and both genders (4).

Autoimmune hepatitis (AIH) is a serious autoimmune liver disease that is characterized by a progressive destruction of the liver parenchyma and the development of chronic fibrosis (5). An estimated 100,000 to 200,000 persons are currently affected by AIH in the USA (6) and, according to the World Health Organisation, AIH has an annual incidence of approximately 2 in 100,000 individuals and a prevalence 15 cases per 100,000 persons worldwide (7).

Autoimmune hepatitis (AIH) is an immune mediated, chronic inflammatory disease of unknown aetiology, mainly affecting the hepatocytes. It was first defined in 1950 by Waldenstrom when he described a chronic hepatitis in young woman which eventually lead to liver cirrhosis (8). There are particularly strong associations within the HLA-DR locus (9), with the HLA-DR3 and -DR4 molecules conferring susceptibility to AIH-1 in Europe and North America. The associations with HLA -DR3 and -DR4 are considered strong enough to contribute to the diagnosis of AIH according to the revised diagnostic scoring system designed by the International Autoimmune Hepatitis Group (IAIHG) (10). Anomalous presentation of human leukocyte antigen (HLA) class II on the surface of hepatocytes, possibly due to genetic predisposition or acute liver infection, causes a cell-mediated immune response against the body's own liver, resulting in autoimmune hepatitis (11).

Materials and Methods

Two study groups have been enrolled in this study, the first was composed of Sixty Iraqi autoimmune hepatitis patients, include (19) males and (41) females, had been clinically diagnosed as autoimmune hepatitis by physicians, biochemical tests, and immune assay tests, with an age range of (7-69) years with an a mean age (40.6) year old. The second group was consisted of 50 healthy control individuals (15 males and 35 females) for age (10-67) years old, sex and ethnic back ground (Iraqi Arabs) were selected who had no history or clinical evidence of hepatitis or any chronic disease and obvious abnormalities according to laboratory findings of biochemical tests and immune assay tests who were considered as control. Individuals of both groups were subjected for the occurrence of HLA-DR3, HLA-DR4, and HLA-B27 as well as described by (12). A real time PCR was used to amplify the gene using the primer mentioned in table (1), which designed using HLA-alleles specific sequence from NCBI-GenBank 111database (HLA-DR3: Genbank code: NT-167244.2), HLA-DR4: Genbank code: AH002824.2 and HLA-B27 Genbank code : M12967.1) and primer 3 plus design online, they were provided by Bioneer Company, Korea as shown in the following table (1).

Table (1): primers sequence with orientation and the PCR product size

HLA allele specific primer	Sequence		PCR Size
HLA-DR3	F	TTGTTGGGGTTCACAAGTGG	80bp
	R	AAGCCACAAGCCTGTTTTCC	
HLA-DR4	F	ATCCAGGCAGCATTGAAGTC	124bp
	R	ACTGTTTCCAGCATCACCAG	
HLA-B27	F	AATCTGCATGTTCGCTGTGC	97bp
	R	TCAACACCAAATGGGCACAG	

Genbank code: HLA-DR3: NT-167244.2, HLA-DR4: AH002824.2, and HLA-B27: M12967.1

Data were translated into a computerized database structure. Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences) in association with Microsoft excel 2010. To measure the strength of association between 2 categorical variables, such as the presence of certain HLA genotype and disease status the odds ratio (OR) was used.

Result and discussion

Autoimmune hepatitis (AIH) is an inflammatory condition of the liver that can affect patients of all ages, sexes, and races (13), as shown in table (2) the current study revealed an age range of 7-69 years in autoimmune hepatitis patients with mean age (mean= 40.6 ± SD 14.9), whereas the age range of healthy group were (10-67) years with mean age (36.7 ± SD 15.0), which selected matched and this result are comparable to Mauss (14). Moreover, it was observed in this study, that the mean age of the disease was (40.6) years which, is to some extent comparable to (39.2±11.2) years reported by Mauss (15).

Table (2): Distribution of studied groups by age and gender

Parameters	Healthy controls	Autoimmune hepatitis	P (t-test)
Age (years)			0.17 [NS]
Range	(10 to 67)	(7 to 69)	
Mean	36.7	40.6	
SD	15.0	14.9	
SE	2.1	1.9	
N°	50	60	

Gender	N	%	N	%	P(Chisquare) = 1 [NS]
Female	34	68	41	68.3	
Male	16	32	19	31.7	
Total	50	100	60	100	

SD: Standard deviation, N: number and NS: no significant.

Thus in Iraq the incidence of AIH is in younger age patients which might be attributed to environmental factors, malnutrition and stress or due to the fact that the life span of Iraqi are lower than that for European (16). Also table (2) showed there was no obvious statistical significant difference in mean age between cases and controls.

Data of the current study showed a female predominance, where the female/male showed 41(68.3%)/19(31.7%) for the case group while the mal showed 16(32%) and 19(31.7%) for the control and case, this comparable with Baranov and Sabri (17, 18). variations in age may be related to the differences in race and genetic factor in addition to environmental differences. The explanation for predominance of AIH among female more than females may be due to the effect of hormonal differences which activate Th2 and subsequently enhance autoantibodies production (19).

There are specific alleles of HLA-class II that associated with susceptible for development of the AIH disease. The frequency distribution of class II HLA-DR3, DR4 & B27 alleles for patients as compared with healthy control group in (%, OR, P, EF) are shown in (table 3).

Table (3): The risk of having AIH disease compared to controls in the presence of selected positive HLA-DR3, DR4 & B27.

	Healthy controls (N= 50)	AIH patients (N= 60)				

HLA gene	N	%	N	%	OR	95% CI OR	P	EF
HLA-DR3								
Negative	**42**	**84.0**	**25**	**41.7**				
Positive	**8**	**16.0**	**35**	**58.3**	**7.35**	**(2.95 -18.3)**	**<0.001**	**0.504**
HLA-DR4								
Negative	**40**	**80.0**	**20**	**33.3**				
Positive	**10**	**20.0**	**40**	**66.7**	**8.00**	**(3.33 – 19.2)**	**<0.001**	**0.583**
HLA-B27								
Negative	**44**	**88.0**	**46**	**76.7**				
Positive	**6**	**12.0**	**14**	**23.3**	**2.23**	**(0.79 - 6.33)**	**0.131[NS]**	**0.129**

A survey of the distribution of HLA-DR3, HLA-DR4 and HLA-B27 genes frequency yielded evidence of positive association between class II alleles and AIH disease. For DR3, there was a high significant difference in the frequency of this gene, that is, 58.3% vs. 116.0%, with OR: 7.35, and EF: 0.504 in comparison with healthy control, there was statically difference (P <0.001). The second gene, DR4 investigated in this study has also showed a very high significant difference as it is expressed in high frequency in AIH disease patients compared with control group; 66.7 vs. 20.0% with OR: 8.0, and EF: 0.707, the (P <0.001).

Moreover, B27, are found in low frequencies in patients of AIH disease compared to healthy control groups. The percentages of these genes among AIH disease patients were 23.3% vs. 12.0% with OR 2.23, and etiological fraction (EF 0.4), there is no significant difference (p 0.131). This result was incompatible with Andreas (20), who detected HLA-DR3 and HLA-DR4 in (38%) of German AIH patients and (30%) for –DR3 and (23%) for –DR4 in Italian patients.

This study is comparable to Ma and Qiu (21) in case of HLA-DR4, Furumoto (22), were the frequency of the HLA-DR4 was significantly higher in AIH than in control individuals (59.7 % vs. 41.8 %, P < 0.001),

the Odds ratio (95 % Cl) was 2.14 (1.51–3.04), and also nearly comparable with Hassan (2013) in the HLA-B27 Odds Ratio, (Confidence Interval) which 0.39(0.02-0.75), and p value(0.504).

As shown in table (4), after adjusting for the other two tested HLA genes, the presence of HLA-DR3 significantly increased the risk of having the disease by 14.7 times. A positive HLA-DR4 significantly increased the risk of having autoimmune hepatitis by 16.8 times after controlling for the remaining two HLA genes included in the model. A positive HLA-B27 marginally increased the risk of having autoimmune hepatitis by 2.2 times after controlling for the remaining two HLA genes included in the model.

The model was statistically significant and accurately predicted the group membership of subjects (controls Vs cases) with 79.1% accuracy.

HLA-DR3 associated with younger age at presentation, diminished response to therapy and more frequent liver failure requiring liver transplantation as compared to HLA-DR4 (23).

Table (4): Multiple logistic regressions with the risk of being a case (autoimmune hepatitis) as the dependent (outcome) variable and selected HLA phenotype as the explanatory variables.

HLA- gene	Partial OR	95% confidence interval	P
Positive DR3	14.7	(4.4 to 48.8)	<0.001
Positive DR4	16.8	(5.3 to 53.7)	<0.001
Positive B27	2.2	(0.56 to 8.8)	0.25[NS]

Overall predictive accuracy = 79.1%
P (Model) < 0.001

These results are in compatible with Amarapurkar (24) who studied HLA Genotyping autoimmune hepatitis in western India and attended

strongly significant association of autoimmune hepatitis was found amongst HLA-B27.

As in other autoimmune diseases, there are primary associations with the HLA class I B8 and class II DR3 and DR52a loci. There is also a secondary association with HLA-DR4 in white patients and a primary association with HLA-DR4 in Asians. With the use of more sophisticated molecular techniques, genotyping has confirmed the disease association with specific loci in the HLA-DR region and identified specific amino acid sequences in the light chains of the HLA-DR beta molecules as more specific markers (25).

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