Association of HLA-DR3, and some autoantibodies in autoimmune hepatitis patients

Abdulrazzaq Abaulla Taher *
Hammadi A. Alhilali **
Osama T. Al-Obeidy ***

*** MSc. Ministry of health / Al-Diwanyia Teaching Hospital**
**** Professor. Collage of medicine/ University of Al-Qadisiya**
*****Assi. Prof. Collage of medicine/ University Al-Qadisiya**

الخلاصة

التهاب الكبد المناعي الذاتي هو مزمن، مناعي او اضطراب الكبد الالتهابي. المسببات غير معروفة. ويعتقد أن أيه حتى الآن أن يكون مرض المناعة الذاتية. ويعتقد أن واحدة من العوامل الوراثية جينات هلا .DR3-وقد هدفت الدراسة الحالية إلى التعرف على ترددات جينات هلا-DR3وبعض الأجسام المضادة لدى مرضى التهاب الكبد المناعي الذاتي باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي في الجينات هلا وتقنية إلاليزا للأجسام المضادة الذاتية. تراوحت أعمار المرضى من 7-70 سنة في مجموعة المرصى، ومن 8-67 سنة في مجموعة الضوابط الصحية. في سياق التنميط الجيني لل هلا DR3-وجد أنها تختلف في تردداتها بشكل ملحوظ بين المرضى (ET) عالية 0.504، مقارنة مع الضوابط الصحية، مع نسبة غريبة (OR) 7.35. وتفاوتت تواتر هذه الجينات في المرضى التي اختلفت معنويا بين مجموعات المرضى مقارنة بالمجموعة الصحية (58.3٪) و (16٪) على التوالي.

وتختلف التركيزات المتوسطة للأجسام المضادة ANA, LKM اختلافا كبيرا (P <0.001) بين مريض التهاب الكبد المناعي الذاتي (20.9 U /مل)، و (7.5 U /مل) على التوالي.

Key ward

Autoimmune hepatitis**;** HLA-DR3; ANA**;** LKM

Abstract

One of the genetic predisposing factors is thought to be HLA-DR3 genes. The present study aimed at investigation the frequencies of HLA-DR3 genes and some autoantibodies among the autoimmune hepatitis patients by using Real-time PCR were used for the HLA-genes and

ELISA technique for autoantibodies. 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- DR3genes were found to be differed in their frequencies significantly among AIH patients that creating high etiological fraction of 0.504, compared to healthy controls, with odd ratio (OR) 7.35. The frequencies of these genes in AIH patients, highly significant differed between patient groups compared to healthy group which (58.3%) and (16%) respectively.

The median concentrations of ANA and LKM autoantibodies has significantly different ($P < 0.001$) between autoimmune hepatitis patient (20.9 U/ml), and (7.5 U/ml) for ANA and LKM respectively.

Introduction

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 (1). It is characterized by the morphological changes of interface hepatitis on liver biopsy, hypergammaglobulinaemia and the presence of high circulating ANA in the serum. It is more common in women (around 75%) and can affect at any age from young to elderly (2). Blood tests can show signs of hepatitis with raised alanine transaminase (ALT, U/L) (usually less than 500 U/L), aspartate transaminase (AST, U/L) and occasionally bilirubin (mmol/L). Typical immunology profile in AIH patients are raised Ig G with positive antinuclear antibody (ANA), anti-smooth muscle antibody ASMA (in type 1 AIH) and anti- Liver-kidney-microsomal LKM (in type 2 AIH). Viral hepatitis, toxins, drugs should be excluded in patient presenting with acute or chronic form of

hepatitis. Men with AIH appear to have a higher relapse rate and younger age of disease onset (3). Cirrhosis at presentation (4) and presence of SLA antibodies are poor predictive outcomes in type 1 AIH patients (5).

There are particularly strong associations within the HLA-DR locus, 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) (6).

Aim of study

In Iraq, the autoimmune hepatitis is highly increasing compared with the last two decades. Shedding light on the prevalence of certain HLAs and the validation some auto-antibodies among Iraqi patients with autoimmune hepatitis was the aim of this study.

Patients and Methods

Two study groups have been enrolled in this study, the first was composed of Sixty autoimmune hepatitis patients from Al-Diwanyia province include (19) males and (41) females, had been clinically diagnosed as autoimmune hepatitis by clinical signs , 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 apparently healthy. Individuals of both groups were subjected for ELISA to evaluate the

ANA and LKM ready to use kits from (AESKULISA / Germany) were used for this purpose as instructed by the manufacture.

A portion of subject's blood was investigated for the occurrence of HLA-DR3, as described by Gersuk (7). 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,) and design online, they were supplied by Bioneer Company, Korea.

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

HLA allele specific primer	Sequence		PCR Size
HLA-DR3	F	TTGTTGGGGTTCACAAGTGG	80bp
	R	AAGCCACAAGCCTGTTTTCC	

Genbank code: HLA-DR3: NT-167244.2

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

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 (8).

Moreover, the mean age at disease onset was (40.6) years which, is to some extent comparable to (39.2±11.2) years reported by Mauss (9). 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 (10). Also table (2) showed there was no obvious statistical significant difference in mean age between patients and controls because the small sample size.

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.

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 (11, 12). 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 (13).

The different parameters of anti- ANA antibody over the two studied groups are presented in (table 3). It shows statistically significant differences in the median level of this auto-antibody (P 0.001) among the two studied groups. It raised in AIH patients (20.9 U/ml) compared to healthy control group (4.8 U/ml). Moreover, the level range of Anti-ANA was (0.2 to 164.2 U/ml) in AIH group and (0.02 to 20.4 U/ml) among healthy control individuals.

Table (3): Different (anti-ANA) level parameters among study groups.

Anti- Nuclear antibody (ANA U/ml)	Study group		P value
	Healthy controls	Autoimmune hepatitis	< 0.001
Range	(0.02 to 20.4)	(0.2 to 164.2)	
Median	4.8	20.9	
Inter-quartile range	(2.6 to7.5)	(8.35 to59.8)	
N。	50	60	

Using ROC curves to evaluate the diagnostic value of this auto-antibody revealed a considerable ROC value (0.655) for this class of auto-antibody when used as test to predict AIH differentiating them from healthy control (table 5).

The median titers of LKM for the two study groups evaluated in this work are shown in table (4) and figure (1). There were significant

differences in the median, range, and inter-quartile range of this autoantibody between the two groups studied. The median range in health group was (3.6U/ml) ranged in (0.2-16.8U/ml) while the median of case group was (7.5 U/ml) ranged in (0.3-104.3 U/ml). The results of the present study revealed highly significant differences (p 0.01) comparing the two groups. The level (positives) of LKM autoantibody among the AIH patients is 38(63%).

AL-Obeidy *et al*., (2009), the LKM was (16.44%), Mauss *et al*., (2013) (14, 15).

Table (4): Different (anti-LKM) level parameters among study groups.

Anti-Liver kidney antibody (LKM U/ml)	**Study group**		**P value**
	Healthy controls	**Autoimmune hepatitis**	**0.01**
Range	**(0.2 to 16.8)**	**(0.3 to 104.3)**	
Median	**3.6**	**7.5**	
Interquartile range	**(1.7 to 8.3)**	**(1.7 to 18.99)**	
N	**50**	**60**	
Mean rank	**46.9**	**62.7**	

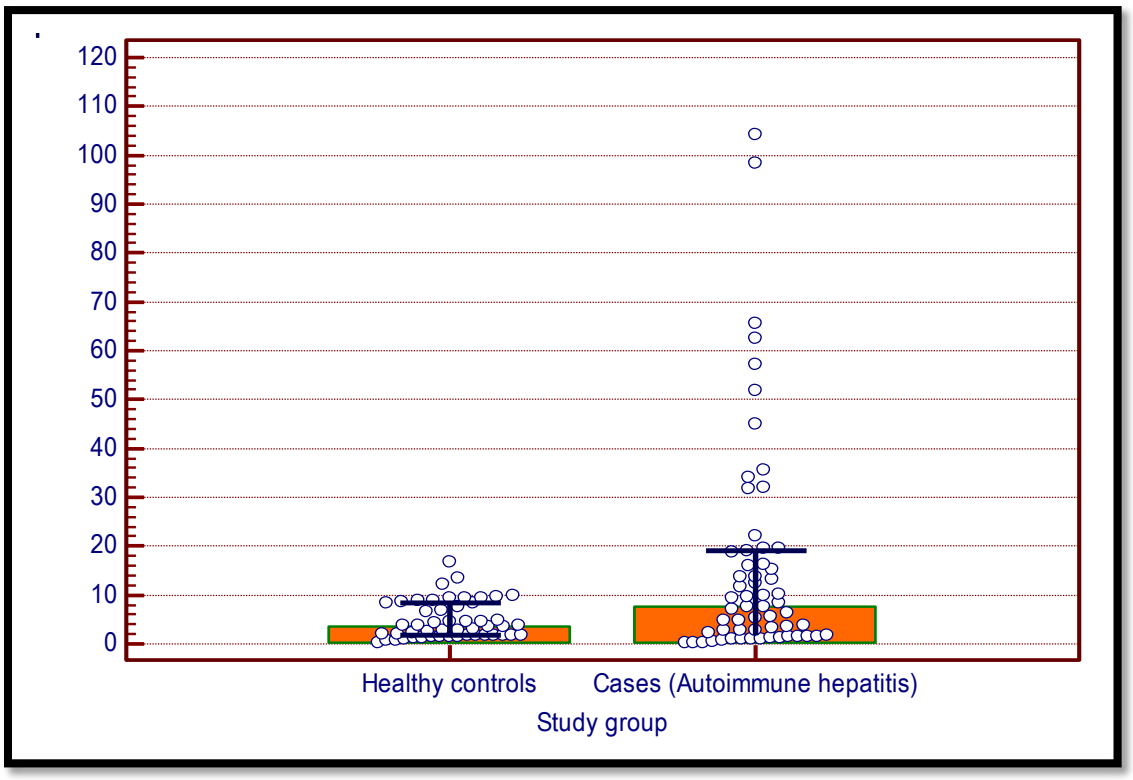

Figure (1): Dot diagram with error bars showing the case-control difference in median (with its inter-quartile range) of LKM.

The diagnostic and differentiating value of these two auto-antibodies has been also calculated using ROC curves. In the differentiation of autoimmune hepatitis patients from healthy control, this test has revealed on ROC area value of (0.644, p 0.01, table 5).

Table (5): ROC area when used as test to predict a diagnosis autoimmune hepatitis differentiating them from healthy controls.

Auto-antibody (U/ml)	**ROC area**	**P**
ANA	**0.804**	**<0.001**
LKM	**0.644**	**0.01**

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 alleles for patients as compared with healthy control group in (%, OR, P, EF) are shown in (table 6).

Table (6): 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-DR3	N	%	N	%	OR	95% CI OR	P	EF
Positive	8	16	35	58.3	7.35	(2.95 -18.3)	<0.001	0.504
Negative	42	84.0	25	41.7				

A survey of the distribution of HLA-DR3 genes frequency yielded evidence of positive association between class II alleles and AIH disease. There was a high significant difference in the frequency of this gene, which is, 58.3% vs. 16.0%, with OR: 7.35, and EF: 0.504 in comparison with healthy control, which statically significant difference (P <0.001), and this disagreeing with Ngu (16)

As shown in table (7), after adjusting for the tested HLA genes, the presence of HLA-DR3 significantly increasing the risk of having the disease by 14.7 times. 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 (14).

Table (7): Multiple logistic regressions with the risk of being a case (autoimmune hepatitis) as the dependent (outcome) variable is HLA-DR3

HLA- gene	Partial OR	95% confidence interval	P
Positive DR3	14.7	(4.4 to 48.8)	<0.001

Overall predictive accuracy = 79.1%

P (Model) < 0.001

Conclusion

1- 1- The HLA-DR3 gene is common predisposing factor for AIH patients

2- The ANA, and LKM, autoantibodies are significantly increased, which were observed in AIH patients than healthy controls.

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