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

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Abstract

AIH 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

In current study the age range of 7-69 years in autoimmune hepatitis patients with mean age (40.6), this result was just similar to that of Mauss *et al*., (2013). Moreover, it was, for some extent comparable to (39.2±11.2) years reported by (1).

Thus in Iraq the incidence of AIH is in younger age patients which might be attributed to environmental factors, malnutrition and stress or even due to the fact that the life span of Iraqi are lower than that for European (2).

The female was predominant in this study (68.3%) and this comparable with other studies where women represent (73.4%) in (3),

Also the ratio of male to female which was (1:2.8) in this work which is nearly comparable to that of (1:2.7) reported by (4). However, the ratio 1:2.4 and 1:3 had been reported by abroad studies (4).

These variations may be related to the differences in race and genetic factor in addition to environmental differences. The explanation for predominance of AIH among females more than males may be due to the effect of hormonal differences which activate Th2 and subsequently enhance autoantibodies production (5).

In general, regarding the laboratory diagnosis of autoimmune diseases, the detection of autoantibodies alone doesn't mean the presence of neither the disease, nor it is absence exclude it so far. However, these tests, i.e., autoantibodies detection, may accomplish the clinical feature of the case, as do the biochemical tests. The routinely used tests during the course of autoimmune hepatitis were investigated in this study (6).

To shed light the supportive value of these autoantibodies and on possible association with more candidate genetic factors, as well median concentration of this autoantibody ($p < 0.001$) among autoimmune hepatitis patient and the level of ANA antibodies among AIH patients in this study was (75%) have referred to the positive level of ANA antibodies in 76% of patients with AIH group (7).

Al have reported similar results, where the ANA was 80% positive and (2009). However, lower frequencies have been reported by (2013), where it occurred in only (17.6%) and where it occurred in only (13.33%) in AIH patients (8).

There were significant differences in the median concentration of this autoantibody ($p < 0.005$) between autoimmune hepatitis patient and healthy control (9).

As with the occurrences of other autoantibodies during the course of diverse autoimmune diseases, such materials may be/may be not developed, and if developed, there is a certain extent at which they produced. In other words, some controversial between different studies regarding the presence of anti-SLA, some are in concordance with ours like this differences may be due to small number of samples (10).

The significant differences of LKM among AIH patients ($p= 0.01$) when compared with health control group in this study is disagree with this difference because the small sample size and variation of prevalence of auto-immune diseases among AIH patients in this study (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,

HLA allele	Sequence		PCR
HLA-DR3		TTGTTGGGGTTCACAAGTGG	80bp
		AAGCCACAAGCCTGTTTTCC	
HLA-DR4		ATCCAGGCAGCATTGAAGTC	124bp
		ACTGTTTCCAGCATCACCAG	
HLA-B27		AATCTGCATGTTCGCTGTGC	97bp
		TCAACACCAAATGGGCACAG	

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

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 (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

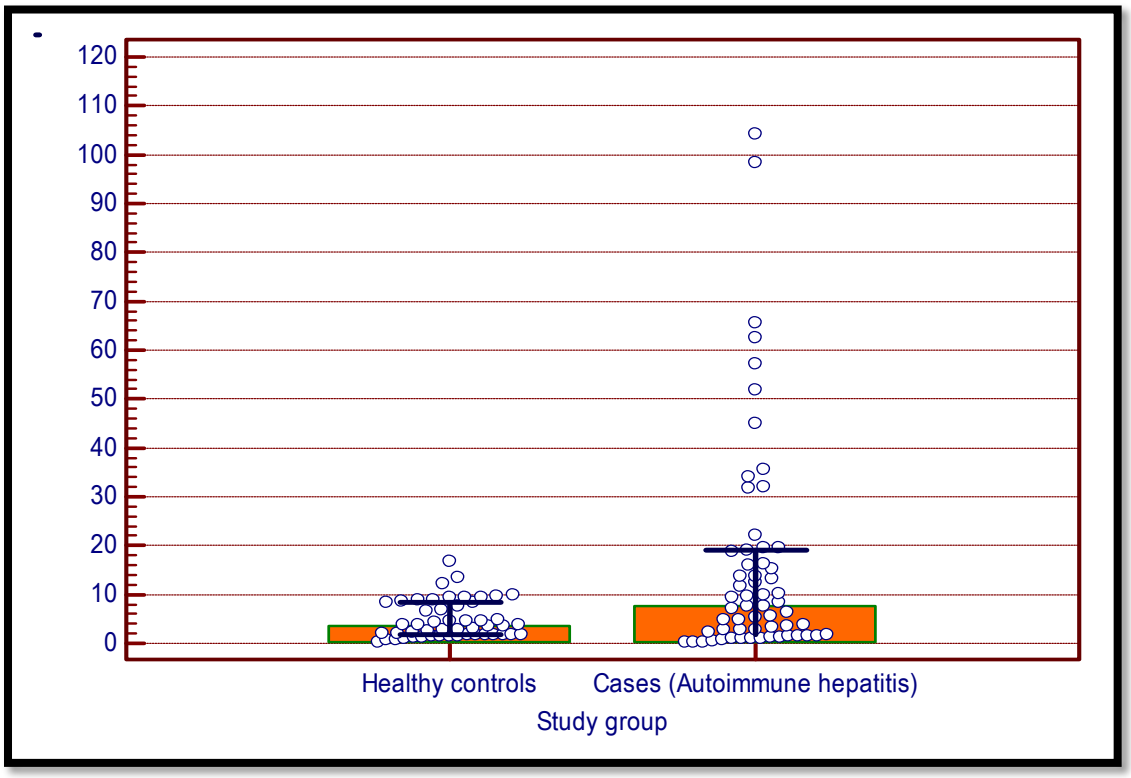
Parameters	Healthy controls		Autoimmune hepatitis		P (t-test)
Age (years)					0.17 [NS]
Range	(10 to 67)		(7 to 69)		
Mean	36.7		40.6		
SD	15.0		14.9		
SE	2.1		1.9		
N。	50		60		
Gender	N	%	N	%	P(Chisquare) = 1 [NS]
Female	34	68	41	68.3	
Male	16	32	19	31.7	
Total	50	100	60	100	

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 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 (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 (25).

References

1- Waldenstrom J. (1952). Liver, blood proteins and food proteins. Dtsch Z Verdau Stoffwechselkr; 12:113–121.

2- Cowling DC, Mackay IR, Taft LI. (1956). Lupoid hepatitis. Lancet; 271: 1323-1326 [PMID: 13386250].

3- Mackay IR, Weiden S, Hasker J. (1965). Autoimmune hepatitis. Ann N Y Acad Sci; 124: 767-780 [PMID: 5214838 DOI: 10.1111/ j.1749-6632.1965.tb19000.x].

4- Gronbaek L, Vilstrup H, Jepsen P. (2014). Autoimmune hepatitis in Denmark: incidence, prevalence, prognosis, and causes of death. A nationwide registry-based cohort study. J Hepatol; 60: 612-617 [PMID: 24326217 DOI: 10.1016/j.jhep.2013.10.020].

5- Czaja, A.J. (2015). Diagnosis and management of autoimmune hepatitis. Clin. Liver Dis., 19, 57–79. [CrossRef] [PubMed].

6- Czaja, A.J. (2006). Autoimmune hepatitis—Approach to diagnosis. MedGenMed 8, 55. [PubMed].

7- World Health Organization. (2016). Dept. of Protection of the Human Environment; Inter-Organization Programme for the Sound Management of Chemicals. Principles and Methods for Assessing Autoimmunity Associated with Exposure to Chemicals. World HealthOrganization:Geneva,Switzerland,2006.Availableonline:http:/ /www.who.int/iris/handle/10665/43603 (accessed on 23 November 2016).

8- Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al. (2010). Diagnosis and management of autoimmune hepatitis. American Association for the Study of Liver Diseases. Hepatology; 51:2193–2213.

9- Donaldson PT. (2004). Genetics of liver disease: immunogenetics and disease pathogenesis. Gut; 53:599–608.

10- Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. (1999). International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol; 31:929–938.

11- Czaja AJ, Carpenter HA, Moore SB. (2008). HLA DRB1as a risk factor for type 1autoimmune hepatitis in North American patients. Digestive disases and sciences; 53 (2):522-8.

12- Gersuk, V. H. and Nepom, G. T. (2007). A real-time PCR approach for rapid high resolution subtyping of HLA-DRB1*04. J Immunol Methods. 317 (1-2): 64-70.

13- Baranov AA, MD, Kaganov BS, MD, Gundobina OS, MD, Zainudinov ZM, MD. (2003). "AIH in children." Internat.l Pediat18: 1803-1813.

14- Mauss S., F. Berger, A. Schober, G. Moog, R. Heyne, C. John, S. Pape, D. Hueppe, H. Pfeiffer-Vornkahl and U. Alshuth. (2013). Screening for autoantibodies in chronic hepatitis C patients has no effect on treatment initiation or outcome: Journal of Viral Hepatitis, 20: e72–e77.

15- Manns MP. & Petra Obermyer-Straub. (2000) "Basic mechanism in autoimmune hepatitis." Am. J. Gasteroentrol.; 90: 1206- 1211.

16- Al-Obaidi E-Sh. (2008). "Molecular Typing by Polymerase Chain Reaction Sequence Specific Primer of Human Leukocyte Classes in Iraqi Autoimmune Hepatitis" A thesis submitted to the College of Medicine / University of Baghdad, in partial fulfillment of the requirements for the degree of PhD/ Microbiology.

17- Baranov AA, MD, Kaganov BS, MD, Gundobina OS, MD, Zainudinov ZM, MD. (2003). "AIH in children." Internat.l Pediat18: 1803-1813.

18- Sabri JH. (2003). "The diagnostic role of liver biopsy in grading staging and etiology of chronic hepatitis." A thesis submitted to the scientific council of the pathology in partial fulfillment of the requirements for the degree of fellowship of the Iraqi. Commission for Medical Specialization in Pathology.

19- Goldsby RA, Kindt TJ & Oaborne BA. (2000)" Autoimmunity " In: Kuby Immunology." 4th Ed. Freeman W.H. & Company, NY PP: 497-516.

20- Andreas Teufel, Markus Worns, Arndt Weinmann, Catherine Centner, Anja Piendl, Ansgar W Lohse, Peter R Galle, Stephan Kanzler.(2006). Genetic association of autoimmune hepatitis and human leucocyte antigen in German patients: World Journal of Gastroenterology ISSN 1007-9327: World J Gastroenterol. September 14; 12(34): 5513-5516.

21- Ma Xiong and De-Kai Qiu.(2001). Relationship between autoimmune hepatitis and HLA-DR4 and DRβ allelic sequences in the third hypervariable region in Chinese. World J Gastroenterol; 7 (5):718-721.

22- Furumoto Yohei, Toru Asano, Tomonori Sugita, Hiroshi Abe, Yoshimichi Chuganji, Kazuhiko Fujiki, Akihiko Sakata and Yoshio Aizawa.(2015). Evaluation of the role of HLA-DR antigens in Japanese type 1 autoimmune hepatitis BMC Gastroenterology. 15:144.

23- Ashima Makol, Kymberly D.Watt, and Vaidehi R. Chowdhary.(2011). Autoimmune Hepatitis: A Review of Current Diagnosis and Treatment: Hindawi Publishing Corporation Hepatitis Research and Treatment Volume, Article ID 390916, 11 pages.

24- Amarapurkar DN, Patel ND, Anjali D Amarapurkar, SR Kankonkar. (2003). HLA Genotyping in Type-1 Autoimmune Hepatitis in Western India: JAPI • Vol. 51 • October.

25- Krawitt, M.D. Edward. (2010). Autoimmune hepatitis medical progress review article. Downloaded from www.nejm.org at libraries of the univ of colorado on may 4: Vol. 334 No. 14.