A comparison of the prevalence of autoantibodies in individuals with chronic hepatitis C and those with autoimmune hepatitis

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Introduction:

Hepatitis C virus (HCV)has been implicated in the development of a variety of autoimmune phenomena, some of which are well documented and include a panel of auto-antibodies shared with autoimmune hepatitis (AIH).(1)

The autoantibodies that characterize autoimmune hepatitis may occur in conjunction with antibodies to hepatitis A, B, and C viruses (2,3). The concurrence of these markers not only confounds the diagnosis but has rekindled speculation that autoimmune hepatitis is caused by a virus (4,5,6,7).Additionally, anecdotal reports have indicated that administration of interferon to patients with autoimmune hepatitis may exacerbate their disease (8,9), whereas administration of corticosteroids to patients with true viral infection is ineffective if not deleterious(10). Although deterioration of patients with autoimmune markers during interferon therapy is undoubtedly rare (11), its occurrence in some patients has justified wariness for the drug. These concerns have underscored the importance of distinguishing autoimmune hepatitis with viral markers from chronic viral hepatitis with viral marker from chronic viral hepatitis with autoimmune features.

Aim of the study:

To define the pattern and prevalence of autoantibodies in Iraqi patients with chronic hepatitis C in comparison to those with autoimmune hepatitis (AIH).

Patients and Methods: -

Fifty patients (37 male, 13 female), their age ranged between 19-69 years with chronic hepatitis C (HCV), and fifty patients with AIH attending The Teaching Hospital for Gastroenterology and liver disease in a period between November 2003 and July 2004 were enrolled in this study, compared with 30 healthy individuals (age and sex matched).

Laboratory investigation:-

SMA, AMA, Abs were detected on serial dilutions of sera by indirect immunoflurescent technique (IIF) on rat liver-kidney- stomach (triple substrate) cryostat section; for ANA, slides of mouse liver were used. Significant titer for ANA, SMA, and AMA Abs were ≥ 1:20, ≥1:40, and ≥1: 40, respectively. Positive results were recognized by presence of specific pattern of flurescence. Anti-ds DNA and organ specific autoantibodies (anti-parietal cell, anti-thyroglobulin, and anti-thyroid microsomal Abs) were detected using Enzyme-Linked Immunosorbent Assay (ELISA) technique, concentration (≥12.5 U/ml) were considered positive. Euro line methods for presence of SLA/LP autoantibodies were done and positive results were recognized by presence of visible line. Positive and negative controls were included at all stages according to the manufacturers' instructions and to confirm the validity of the test.

Euro immune has supplied the above kits company, Germany.

Serum antibodies to HCV were measured by third generation ELISA-based screening test that uses antigen coated beads with an antibody coupled with an enzyme to produce florescent end product that is proportional to the amount of bound antibody. Patients with positive results were retested using a more specific test, a RIBA-based test that allows for the detection of antibodies against specific HCV antigens. Furtherly tested for the presence of HCV RNA by PCR-based test.

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Quantitation of serum Igs and C3, C4 of the study groups were carried by single radial immundiffusion (SRID) test and the concentration were expressed as mg/dl. Biochemical test (GOT, GPT, TSB and Alkaline Phosphatase) were performed using commercially available kits (Randox-UK).Forty HCV and thirty eight AIH patients underwent liver biopsy.

Results:

All patients sera (n=50) tested positive for antibodies to hepatitis C virus (HCV) by ELISA, RIBA, and PCR.Autoimmune hepatitis patients (n=50) and healthy control group were negative for HCV antibodies by ELISA.

There was significant difference in the mean age of patients with AIH, since 76% were young(8-39)years, while 19.2% with intermediate age and the rest 4.1% fall in old age compared to those with HCV infection, as 17.9% were young (19-40) years while 82.1% fall in old age(50-69)years

There were 45 females and 5 males with AIH compared to 37 male and 13 female with HCV infection. Table-1

Autoantibodies (ANA, SMA, AMA, anti-ds DNA and SLA/LP):-

These entire Abs were not present in patients with HCV infection but just in few patients in comparison to AIH patients. Compared with AIH, HCV-associated ANA and SMA exhibited ANA-homogenous pattern and SMA-anti actin antibodies at a lower prevalence (24% with titer <1:80 Vs.80% with titer 1:80-640. $P<0.01$) and (6% with titer >1:80 Vs. 84%, with titer 1:320-640. $P<0.01$) respectively, while AMA was observed in 8(16%) of AIH patients compared to 1 (2%), in the sera of HCV patients ($P<0.0001$), while, the concomitant positivity of ds-DNA and SLA/LP Abs were observed only in AIH patients sera and never in the sera of HCV patients. All healthy control group are negative as shown in table-2. There was also a significant difference between the prevalence of organ specific autoantibodies(anti-thyroglobulin, anti-thyrid microsomal and anti-parietal cell Abs) among HCV patients (10.2%) compared to the AIH patients
(31.25%) ($P<0.05$).

The Igs concentration were measured in mg/100ml, our results pointed out to a significant changes in the level of IgG between AIH and HCV infection patients and in comparison with healthy subjects . Since a higher value of mean serum IgG level (2447.36±248.95) with significant differences in AIH patients ($P<0.001$) compared to (1535±636) and (1169.56 ± 248.32) for HCV infection and healthy subjects respectively. Furthermore, IgM and IgA level were higher than those in healthy control though statistically not significant, while there was no difference in their level between AIH and HCV infection patients.

On the other hand, higher serum gamma globulin was observed among patients with AIH patients in comparison to those with HCV infection.

In the present study, several important biochemical parameters that are of value in diagnosis of liver diseases were evaluated in comparison to healthy control.

Table-3 revealed a significant elevation of serum AST, ALT and TSB in comparison to healthy control ($P<0.001$), alkaline phosphates were usually 1 to 2 folds elevation, though was not statistically significant.

An interesting, significant increase of mean serum AST, ALT, and TSB level in AIH patients as compared to HCV infection.

Table- 1 Age and gender distribution of the studied groups

Groups		Healthy controls (N=30)	AIH patients (N=50)	HCVpatients(N=50)
Parameters		No (%)	NO (%)	NO (%)
Age (years)				
X±SD		31±17.7	27.2 ± 9.44	34.82 ± 10.7
Range		9-67	8-62	19-68
Gender	Male	12	5	37
	Female	18	45	13
	Total	30	50	50

Table 2: The results of different test in AIH compared to other groups.

Test	AIH (n=50)		HCV patients (n=50)		Healthy control group (n=30)	
	No	%	No	%	No	%
SLA/LP	4	8	-	-	-	-
SMA	42	84	3	6	-	-
AMA	8	16	1	2	-	-
Ds-DNA	5	10	-	-	-	-
ANA	40	80	12	24	-	-

Table 3: The biochemical parameters findings between AIH compared to HCV and healthy control group.

Biochemical parameters	AIH (n=50)	HCV (n=50)	Healthy control (n=30)	P value
SGOT	79.63±32.9 45-142	47.35±21.8 30-80	14.0±2.5 12-20	<0.001
SGPT	79.7±31.62 24-160	39.1±14.6 12-55	13.5±3.5 10-19	<0.001
Alkaline phosphates	137±25.1 80-195	142±29.3 75-210	71.1±5.8 62-82	>0.05
TSB	9.5±4.6 0.7-18.0	3.96±1.67 1.5-7.7	0.4±0.3 0.1-0.9	<0.001

Discussion:

The observation in this study that the mean age was lower among patients with AIH since it was shown to be (27.2 ± 9.44), while it was (34.82 ± 10.7) among patients with HCV infection. These finding was almost comparable to other abroad studies (12, 13).

Regarding the sex differences, unlike AIH, there were no significant differences in the prevalence of positive autoimmune markers among men and women with chronic HCV. In keeping with the experience of most centers, a high percentage of our patients with chronic HCV were men.

The detection of serum autoantibodies is presumptive evidence for AIH and other autoimmune disorders. Therefore, when compared with autoantibody profile of HCV patients, AIH – associated ANA, SMA, and AMA exhibited a higher prevalence (81% vs. 24%, 85% vs. 6%, and 16% vs. 2%) respectively while, the concomitant positivity of ANA-H and SMA-AA showed in AIH patients and never in the sera of HCV infection patients. In chronic hepatitis C, serum autoantibodies are common, but their subspecificities are distinct from those occurring in AIH. Whereas the absence of ANA-H and/or SMA-AA dose not exclude AIH, the characterization of ANA and SMA may help to discriminate between the two conditions.

The prevalence showed in the present study for autoantibodies are substantially in line with previous reports (14, 15), which used the same IFA screening dilution employed here. However, the current study indicated the presence of negative association of healthy control with the above autoantibodies.

The exact significances of the appearance of auto antibodies such as ANA,,AMA,SMA during the course of viral hepatitis is not known at present, however, most reports agree that autoantibody positivity dose not influence either clinical or biochemical profile of chronic hepatitis C (12).

Immunoglobulin G was significantly higher in AIH patients, possible explanation of the above data was since AIH, characterized by the presence of many kinds

of autoantibodies majority of them are of class IgG which result in elevation of IgG concentration in the patients sera while, HCV infection characterized by presence of one or 2 types of autoantibodies so the elevation in the total concentration though it is present but not as high as in AIH which was proved by the electrophoresis picture .

Hypergammaglobulinemia is well accepted to be distinct feature of AIH, thus our result is in agreement with many investigators who collectively cited that serum level of γ -globulin rises in patients with AIH in comparison to HCV infection and healthy controls (13, 16). The explanation for this finding that the increased synthesis of Abs due to increased number of plasma cell in marrow, and even in liver itself.

With the availability of highly sensitive assay techniques, some chemical tests become standard laboratory procedures in clinical practice for diagnostic and prognostic purposes. Therefore, in the present study biochemical parameters including serum AST, ALT, alkaline phosphates, and total serum bilirubin were selected, there was significant association of aminotransferase with AIH , since this study showed that the highest concentration of aminotransferase (AST and ALT) as well as total serum bilirubin were observed among patients with AIH in comparison to those with HCV infection, and this is probably due to the fact that AIH are aggressive form of the disease since the level of serum aminotransferase reflect severity of disease.

Although AIH and chronic HCV have no pathognomic histopathological features to differentiate, but we revealed that moderate to severe plasma cell infiltration of the portal tracts is more common in patients with AIH while, portal lymphoid aggregates, steatosis, and bile duct damage are more common in patients with chronic HCV infection. Thus our result is quite similar to other abroad studies(17,18).

Despite the prevalence of autoimmune markers in our patients with chronic HCV, treatment with IFN did not worsen hepatitis, and response to therapy was the same in those without.

The fore mentioned findings reinforce the belief that in most instance , AIH is easily distinguished from chronic viral hepatitis since patients with AIH are more commonly women than those with chronic viral hepatitis, and they have higher serum levels of aspartate aminotransferase, bilirubin, gamma-globulin, IgG, alkaline phosphates, and higher frequency of multilobular necrosis on histologic examination than with chronic viral hepatitis.

References:

1-Zauli D,Cassani F,Bianchi FB.Auto-antibodies in hepatitis C.Biomed pharmacother.1999 jun;53(5-6):234-41

2-Czaja AJ.Autoimmune chronic hepatitis- a specific entity? The negative argument.J Gasteroenterol Hepatol .1990 ; 5:343.

3-Czaja AJ,Carpenter HA, Santrach PJ, etal> Evidence against hepatitis viruses as important causes of sever AIH in united states. J Hepatol. 1993;18: 342.

4-Magrin S, Craxi A, Fiorentino G, etal. Is autoimmune chronic active hepatitis a HCV-related disease ? J Hepatol . 1991 ;13 :56.

5-Cassani F, Muratori L, Manotti P, etal. Serum autoantibodies and the diagnosis of type-1 AIH in Italy : a reappraisal at the light of hepatitis C virus infection . Gut. 1992;33 :1260

6- McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vargani D, Williams R. Hepatitis C Virus antibodies in chronic active hepatitis: Pathogenetic Factor or False positive result. Lancet 1990; 335: 754-760.

7-Bayraktar Y, Bayraktar M, Guraker A, Hassanein TI, Van Thiel DH. A comparison of the prevalence of autoantibodies in individuals with chronic hepatitis C and those with autoimmune hepatitis: The role interferon in the development of autoimmune disease. Hepatogastroenterology 2003; 44: 417-425.

8- Cianciara J, Laskus T. Development of transient AIH during interferon treatment of chronic hepatitis B. Dig Dis Sci 1995; 40: 1842-1844.

9-Vento S, Cainelli F: Is there a role for viruses in triggering AIH? Autoimmune Rev 2004; 3(1) 61-70.
10- Sezaki H, Arase Y, Tsubota A, Suzuki Y, Kobayashi M, Saitoh S. Type C-chronic hepatitis patients who had autoimmune phenomenon and developed jaundice during interferon therapy. J Gastroenterol 2003; 38(5): 493-500.
11- Czaja AJ. Autoimmune hepatitis and viral infection. Gasteroentrol Clin North Am 1994; 23: 547-561.
12-Cassani F, Cataleta M, Valentini P, Muratori P, Giostra F, Francesconi R, Muratori L, Lenzi M, Bianchi G, Zauli D, Bianchi FB. Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. Hepatology. 1997 sep; 26 (3):561-6.
13- Manns MP, MD, Petra Obermyer-Straub, PhD. Basic mechanism in autoimmune hepatitis. Am J Gasteroentrol 2000; 90: 1206-1211.
14-Pawlotsky JM, Ben YM, Andre C, Voisin MC, Intrator L, Roudot TF,Deforges L. Immunological disorders in C virus chronic active hepatitis : a prospective case- control study. Hepatology. 1994;19:841-848.
15-Al-Hamadani AH. Viral hepatitis type D and it relation to some markers and typing Ph.D. thesis submitted to the college of medicine, Al-Mustansiriya University. 2001.
16- Meyer Zum Buschenfelde KH, Lohse AW, Gerken G, Treichel U, Lo HF, Mohr H, Grosse A, Dienes HP. The role of autoimmunity in hepatitis C infection.1995; 22(1 Suppl):93-6.
17-Unnithan VR, David C W. Autoimmune hepatitis.E-medicin.2004.
18-Czaja AJ. Autoimmune liver disease.1996;2:1259-1292.