

A comparison of the prevalence of anti-Liver/Kidney Microsome antibody type-1(LKM-1) in individuals with chronic hepatitis C and those with autoimmune hepatitis

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Summary:

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Background: Autoantibodies are frequently found in patients with chronic hepatitis C, which suggests that HCV elicit an immune response in the host. The relationship of type-2 AIH to chronic hepatitis C (HCV) is an interesting and as yet unresolved problem. Importantly, antibodies to liver/ kidney microsome type-1 (LKM1), the serologic marker of type 2-AIH, have been recognized in serum of some patients with chronic hepatitis C.

Methods: Anti-liver kidney microsome type 1 (LKM-1) autoantibodies were studied by indirect immuno florescence assay (IIF) and confirmed by immunoblot in the serum of 73 Iraqi patients with autoimmune hepatitis (AIH) in comparison with 50 patients control (HCV infection) and 50 healthy individuals.

Results: anti-LKM 1 with high titers ($\geq 1/160$) present in serum of all patients with AIH-2, whereas they present in titer ($\leq 1/80$) in serum of 19 patients with chronic HCV, while all healthy control group are negative.

Conclusion: 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 counters with chronic viral hepatitis.

Introduction:-

Autoimmune hepatitis (AIH) is a self-perpetuating hepatocellular inflammation of mysterious aetiology, characterized by the presence of interface hepatitis on histologic examination, hypergammaglobulinaemia, and circulating autoantibodies, which in most cases, respond to immunosuppressive treatment (1,2).

Three types of AIH have been proposed based on distinctive immunoserologic markers (2).

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 AIH is caused by a virus (4,5,6,7). The relationship of type-2 AIH to chronic hepatitis C (HCV) is an interesting and as yet unresolved problem. Importantly, antibodies to liver/kidney microsome type-1 (LKM1), the serologic marker of type 2-AIH, have been recognized in serum of some patients with chronic hepatitis C (8).

This has important therapeutic implications because inappropriate treatment of autoimmune chronic hepatitis with interferon- α may exacerbate liver disease (9). Conversely, corticosteroid therapy for chronic hepatitis C may enhance HCV replication (10,11), which could worsen underlying liver disease.

Aim of the study:

To define the prevalence and clinical significance of anti-LKM 1 Abs in Iraqi patients with chronic hepatitis C in comparison to those with autoimmune hepatitis type-2 (AIH-2).

Subject and Methods: -

Patients: fifty patients (37 male, 13 female), their age ranged between 19-75 years with chronic hepatitis C (HCV), and seventy-three patients, their age ranged between 8-62 years 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 50 healthy individuals (age and sex matched).

Laboratory investigation: -

Anti-smooth muscle antibody (SMA), anti-mitochondrial antibody (AMA), and anti-liver/kidney microsome antibody type -1 (anti-LKM 1) Abs were detected on serial dilutions of sera by indirect immunofluorescent technique (IIF) on rat liver-kidney- stomach (triple substrate) cryostat section; for anti-nuclear antibody (ANA), slides of mouse liver were used. Significant titer for ANA, SMA, AMA and anti-LKM 1 Abs were $\geq 1:20$, $\geq 1:40$, $\geq 1:40$ and $\geq 1:80$ respectively. Positive results were recognized by presence of specific pattern of fluorescence. Euro line methods for presence of soluble liver antigen/liver pancreas antibody (SLA/LP) and anti-LKM 1 Abs (as confirmatory test) were done and positive results were recognized

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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 fluorescent 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. Further tested for the presence of HCV RNA by PCR-based test. Quantitation of serum Igs of the study groups were carried by single radial immunodiffusion (SRID) test and the concentration were expressed as mg/dl. Biochemical test (AST, ALT, TSB and Alk. Phosphates) were performed using commercially available kits (Randox-UK).

Fourteen HCV and thirteen 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=73) and healthy control group (n=50) were negative for HCV antibodies by ELISA.

It was shown that, 49(67%) of patients had type 1(ANA and/or SMA were positive) whereas, 16 (22%) had type 2 (anti-LKM 1 Abs were Positive), and 8 (11%) had type 3-AIH Anti-SLA/LP Abs were positive), figure-1.

In this work, there was significant difference in the mean age of patients with AIH compared to those with chronic HCV infection as well as healthy control group, 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, since, 17.9% were young 19-39 years while 82.1% fall in old age 50-75 years. There were 8 males and 11 females with HCV infection were develop anti-LKM 1 in comparison to 12 females and 4 males with AIH (table-1).

Interestingly, this study showed that anti-LKM 1 with high titers ($\geq 1/160$) present in serum of all patients with AIH-2, whereas they present in titer ($\leq 1/80$) in serum of 19 patients with chronic HCV, while all healthy control group are negative.

The Igs concentrations were measured in mg/dl. our result pointed out to a significant changes in the level of IgG between AIH and HCV patients and in comparison with healthy subjects . Since a higher value of mean serum IgG level (2447.36 \pm 248.95) with significant differences found in AIH-2 patients (P<0.001) compared to (1535 \pm 636) and (1169.56 \pm 248.32) for HCV

patients and healthy subjects respectively. On the other hand, the serum IgA level in type 2-AIH patients (62.14 \pm 22.18) was significantly lower than those in chronic HCV patients and in healthy control (283.8 \pm 38.3) and (191.94 \pm 68.83) respectively (P<0.001)(table-2).

Furthermore, IgM level in studied groups were higher than those in healthy control though statistically not significant, while there was no difference in their level between AIH and HCV patients (P>0.05).

On the other hand, higher serum gamma globulin was observed among patients with AIH-2 patients in comparison to those with HCV infection, figure-2.

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

In addition our study shows a significant increase of mean serum AST, ALT, and TSB level in AIH-2 patients as compared to HCV patients.

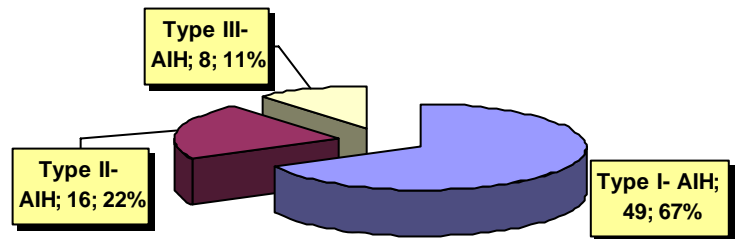


Figure 1: The frequency distribution of AIH types.

Table- 1 Age and gender distribution of the studied groups

Groups	Healthy controls (N=50)	AIH-2 patients (N=16)	HCV patients (N=18)
Parameters	No (%)	NO (%)	NO (%)
Age (years)	31\pm17.7	27.2 \pm 9.44	43.3 \pm 16.1
X\pmSD Range	9-67	8-62	19-75
Gender	Male	4 (25)	11 (57.8)
	Female	15 (30)	8 (42.2)
	Total	50	16

Figure-2: The level of γ -globulin (g/dl) of the studied groups.

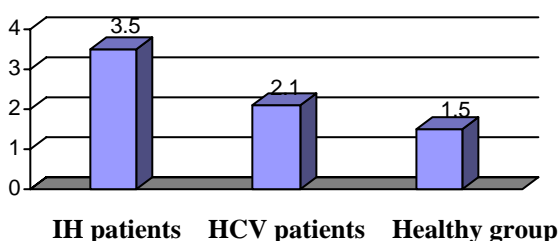


Table 2: The biochemical parameters findings between AIH compared to HCV and healthy control group.

Biochemical parameters	AIH-2 (n=16)	HCV (n=19)	Healthy control (n=50)	P value
AST	79.63±32.9 45-142	47.35±21.8 30-80	14.0±2.5 12-20	<0.00 1
ALT	79.7±31.62 24-160	39.1±14.6 12-55	13.5±3.5 10-19	<0.00 1
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	<0.00 1

As it is shown in table-3, additional autoimmune disorders often occur in patients with AIH-2 compared to those present in patients with chronic HCV infection. IDDM, autoimmune thyroiditis, vitiligo, alopecia, and nail dystrophy were the most common associated disease that detected in patients with type 2-AIH, whereas other diseases were less frequent.

Some patients with AIH-2 had more than one extrahepatic disorder.

Table-3: The distribution of concurrent immune diseases in the studied groups.

Immune diseases	Chronic HCV (n=18)		AIH Type II (n=16)		Healthy Group (n=50)	
	No	%	No	%	No	%
Glomerulonephritis	1	5.5	-	-	-	-
Gravis	1	5.5	-	-	-	-
AI thyroiditis	-	-	4	25.0	-	-
Vitiligo	-	-	3	18.75	-	-
Psoriasis	-	-	1	6.25	-	-
IDDM*	1	5.5	4	25.0	-	-
Alopecia	-	-	2	12.5	-	-
Nail dystrophy	-	-	2	12.5	-	-

*P<0.01 (Significant).

Regarding the histological characteristics in patients with chronic HCV compared to AIH-2 patients the present study revealed that, (18%) with portal lymphoid aggregate (44%) with bile duct damage and (38%) with steatosis, in contrast, no sever chronic hepatitis was recorded for any HCV

patients, whereas sever lobular inflammation, moderate to sever piecemeal necrosis, plasma cell infiltration of the portal tract and broad areas of parenchymal collapse are typical of AIH.

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 (43.3± 16.1) 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 LKM-1 Abs 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 anti-LKM 1 Abs, exhibited a higher prevalence (100% vs. 36%) . Its well known that, autoantibodies are common in chronic hepatitis C, but their subspecificities are distinct from those occurring in AIH.

Although only a few patients with HCV develop AIH, these patients appear to have a genetic predisposition that makes them more likely to develop AIH, compared to HCV-infected individuals without that predisposition. In such cases, the liver cells are damaged not only by the virus but also by the body's own immune system (14).

However; LKM-1 antibodies in viral infection recognize more conformational and diverse epitope than those in genuine AIH (15). This distinction is relevant because it form the basis for mutually exclusive therapeutic strategies of immunosuppression in AIH, and interferon in chronic virus hepatitis (16,17).

The prevalence showed in the present study for autoantibodies are substantially in line with previous reports (16, 17), 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 anti-LKM 1 Abs 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 was proved by the electrophoresis

picture. On the other hand, IgA concentration is proportionally associated with its consumption due to alternative pathway complement activation.

Hypergammaglobulinemia is well accepted to be a 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 is that the increased synthesis of Abs in AIH due to increased number of plasma cells in marrow, and even in liver itself.

With the availability of highly sensitive assay techniques, some biochemical 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 phosphatase, and total serum bilirubin were selected, there was significant association of aminotransferases 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 an aggressive form of the disease since the level of serum aminotransferase reflects severity of disease. One clue to distinguish AIH from chronic viral hepatitis may be the coexistence of AIH with other diseases of immune or autoimmune features. This fact was very clear in this study since, 11 patients with type 2-AIH had concurrent immunologic disease, including IDDM 25.0%, autoimmune thyroiditis 25.0%, vitiligo 18.75%, and psoriasis 6.25% while alopecia and nail dystrophy, 12.5% for each, are another extrahepatic manifestation associated with this type. Thus our result is quite similar to other abroad studies (17, 18).

Although AIH and chronic HCV have similar histologic features, our study 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 (18, 19). 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. Finally, our findings reinforce the belief that in most instances, 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 phosphatase, and higher frequency of multilobular necrosis on histologic examination than counters with chronic viral hepatitis.

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