Original Article

Frequency and Significance of Antibodies to Asialoglycoprotein Receptor in Patients with Autoimmune Hepatitis

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Summary:

Background:
The asialoglycoprotein receptor (ASGP-R) is a liver-specific glycoprotein of the cell membrane. They expressed on the surface of the liver cells Its main function is the internalization ofasialoglycoproteins by binding a terminal galactose residue to coated pits.

The aim of the study is to evaluate the prevalence and clinical relevance of ASGPR in AIH.

Methods:
The study was performed on 73 Iraqi patients with chronic active hepatitis (CAH) of unknown cause, attending the teaching hospital for gastroenterology and liver disease in a period between November 2003 and July 2004. Anti-liver membrane antibodies were studied by ELISA technique.

Results:
ASGPR Abs were detected in the sera of patients with type 1, 2 and 3 -AIH, but never in the sera of healthy group.

Conclusion:
It was concluded that the levels of anti-ASGP-R autoantibodies vary according to the inflammatory activity of the disease. In addition, liter of this Abs decreased significantly in response to immunosuppression, while they reappear when the disease has relapsed. Also, these autoantibodies may be diagnostically helpful when other autoantibodies are not detected and AIH is suspected.

Introduction:

Autoimmune hepatitis (AIH) is an unresolving, hepatocellular inflammation of unknown cause that is characterized by the presence of periportal hepatitis on histologic examination, hypergammaglobulinemia , and various autoantibodies to cellular structure (1,2). It is well known that, ANA, anti-smooth muscle Abs (SMA) and anti-LKM1 Abs constitute the standard repertoire of autoantibodies that are assessed in AIH (3,4).

New autoantibodies has been described in the hope that they will reveal critical pathogenic mechanisms or have prognostic value. Among this Abs is anti-asialoglycoprotein receptor (ASGPR) (5). It has been reported that, this receptor is preferentially expressed on the surface of periportal liver cells where piecemeal necrosis is found as a marker of severe inflammatory activity in patients with AIH (6). This finding may suggest a possible immunopathogenetic involvement of anti-ASGP-R autoantibodies in AIH (7). The general presumption is that target of potentially tissue-damaging autoreactions in AIH must be liver-specific and available to the immune system in vivo (e.g. expression on the surface of hepatocytes). So far, ASGP-R is the only target-autoantigen that has been positively identified and fulfils these criteria (6,7). Additional support to this emerged from the determinations of anti-ASGPR autoantibodies in consecutive AIH patients (7).

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Patients and Methods: -

Patient : a seventy three patients (20 male, 53 female) with chronic active hepatitis (CAH.) of unknown cause, attending The Teaching Hospital for Gastroenterology and liver disease in a period between November 2003 and July 2004.Their age ranged between 10-57 years, compared with 50 healthy individuals (age and sex matched).

Both groups were subjected to serological detection of ANA, SMA anti-liver/kidney microsome type l(anti-LKM 1), anti-liver cytosle type (anti LC1) and soluble liver antigen/liver pancreas (SLA/LP) Abs by IIF, ELISA and Euro line method.

Laboratory investigation: -

1 ANA, SMA and anti-ASGPR were detected using Enzyme-Linked Immunosorbent Assay (ELISA) technique used human IgG Fc as the antigen coated the microwells plate and isotype-specific horse antibodies coupled to radish peroxidase; result v/ere expressed as the optical density. Indirect immunofluorescent technique (IIP) technique on HPE-2 cell line as a confirmatory test for ANA and SMA were done: sera were screened at a dilution of 1:20 and 1:40 for ANA and SMA respectively in phosphate buffer saline (PBS) and positive results were recognized by presence of a specific pattern.

2- Anti-LKM-1 and LC-1 Abs on the other hand, were detected using the mosaic basic profile-2: rat liver, kidney, stomach substrate and positive result was associated with positive immunofluorescence staining of the third portion of proximal renal tubules and negative staining of the distal tubules, the hepatocytes are homogeneously stained for anti-LKM 1 whereas, anti-LC 1 characterized by homogenous staining of the cytoplasm and the IF staining spared the juxtavenous hepatocyte . Euroline method (as a confirmatory test for presence Anti-LKM I and LC-I Abs) and for detection anti-SLA/LP Abs were used: The test kit contains test strips coated with parallel lines of antigens, which have been purified by affinity chromatography. Euro immune has supplied the above kits company, Germany. Disease activity was monitored every 14 days by clinical examination, liver function tests, and by measurement of serum immunoglobulins and autoantibodies.

Results:-

As it is shown in figure 1, 49(67%) of patients had type 1 whereas, 16 (22%) had type 2, and 8 (11%) had type 3 AIH.

The autoantibodies (ANA, SMA, LKM-I, LC-I, SLA/LP, and anti-ASGPR) were not present in healthy control just in few patients in comparison to AIH patients (table-1).

Regarding the frequency distribution of autoantibodies in different types of AIH, there was a significant increase in the frequency of SMA was showed in 28 (57.14%) patients of type-1, in comparison to those with type 3 of the disease 3(37.5%) , P<0.001 .

On the other hand, the frequency distribution of anti-ASGPR Abs were 77.5% , 56.2% , and 62.5% in type 1,2 , and 3-AIH respectively (P<0.001).

It has been noticed that, SLA/LP Abs exhibit 2.04% , and 100% of patients with type I ,and 3 respectively (P<0.05).

Finally but not least important, LKM-I, and LC-I Abs (markers of type 2- AIH) represent 75% and 62.5% respectively (table-1).

Table 1: Correlation between different autoantibodies in studied groups

Test	AIH Type I (n=49)		AIH Type II (n=16)		AIH Type III (n=8)		Healthy Control Group	
	No.	%	No.	%	No.	%	No.	%
ANA	22	44.9	-	-	-	-	1	2.0
SMA*	28	57.14	-	-	3	37.5	3	6.0
Anti-ASGPR*	38	77.5	9	56.2	5	62.5	-	-
SLA/LP**	1	2.04	-	-	8	100	-	-
Anti-LC1	-	-	10	62.5	-	-	-	-
Anti-LKM1	-	-	12	75.0	-	-	-	-

* P<0.001 (highly significant)

** P<0.05 (significant)

Concerning the correlation between anti-ASGPR Abs and disease activity, figure-2 revealed that titer of these antibodies highly affected by treatment (response after immunosuppression and AST normalization). However, reactivation of disease, with increase serum AST level, was confirmed histologically, occurred in 45 of the 52 patients after treatment withdrawal. On the other hands, on the basis of the anti ASGPR Findings and the outcome of treatment withdrawal, the 52 patients could be divided into three groups. Group (A) composed of 30 patients were anti-ASGPR positive before the start of treatment withdrawal and all relapsed within 9 to 18 weeks. Group (B) composed of (12) patients in which reactivation of disease occurred within 10-18 weeks in all these patients who were anti-ASGPR negative before treatment withdrawal but they developed antibodies during reduction of the corticosteroid, whereas group (C) comprising (10) patients who were anti-ASGPR negative before treatment withdrawal, corticosteroid were completely withdrawn without evidence of relapse. Anti-ASGPR did not develop in any of these patients and they remained in remission without treatment during the period of observation. Analysis of age, sex, clinical features, serum immunoglobulin and antibody status and initial response to treatment did not identify any feature that distinguished patients in group C from those in group A and B.

Discussion:

It is generally accepted that type I is the most common form of AIH, however; in the present study type I -AIH showed to represent 67% of the cases while type 2 and 3 represent 22% and 11% respectively. These findings are in contrast with abroad studies who revealed that type-1 represent 80%. While type 2 and 3-AIH represent 4% and 3% respectively (7, 8), however we couldn't compare this result with other studies carried in Iraq, simply because it is the first time that this disease have been studied in details.

It has been reported that, the detection of serum autoantibodies is presumptive evidence for AIH and other autoimmune disorders. Therefore, when compared with autoantibody profile of healthy control, AIH - associated ANA, and SMA. exhibited a higher prevalence (44.9% vs. 2.0% and 42.4% vs. 6.0% respectively while, the concomitant positivity of LKM-I, LC-I, and SLA/LP were observed only in AIH patient's sera, and never in the sera of healthy control group. The prevalence showed in the present study for autoantibodies are substantially in line with previous reports (9), which used the same technique employed here.

Over the last decade a large body of evidence has accumulated linking anti-asialoglycoptotem receptor (ASGP-R) to the pathogenesis of AIH, since these antigens are both liver specific and expressed on the surface of the hepatocyte (10). However, several abroad studies declared that anti-ASGPR Abs may be useful to predict relapse of AIH after withdrawal of treatment as well as to monitor the activity of disease (11, 12).

In the present study anti-ASGPR Abs were detected in all types of AIH, thus suggesting that the Abs may be a generic marker of the disease. They exhibit 77.5%, 56.2%, and 62.5% of type 1,2 and 3-AIH respectively with titer $\geq$ 1/400 (Sig. titer = 1/100).

The result of the present study clearly demonstrates the value of anti- ASGPR Abs in predicting the outcome of treatment withdrawal, since reactivation of disease, confirmed histologically by the presence of piecemeal necrosis, occurred in every patients of group (A&B) who was anti-ASGPR positive, irrespective of whether these antibodies had been present before treatment or had developed during reduction or after cessation of treatment. In contrast, remission was sustained in all patients who remained anti-ASGPR negative. These finding was almost comparable to other abroad studies (13, 14).

The appearance of anti-ASGPR may reflect early parenchymal liver cell damage or, alternatively, an initial manifestation of reactivation of an autoimmune process.

This study revealed that anti-ASGPR concentration correlate strongly with AST levels before and during immunosupressive treatment, the close correlation between anti-

ASGPR concentration and AST level during the different phases of the liver disease points to a direct involvement of anti-ASGPR autoreactivity in the process of liver targeted autoimmune attack.

The fore mentioned findings indicate that anti-ASGPR may be the generic marker for the disease and the monitoring of this Abs is of practical value in the treatment of AIH. In addition, patients who are anti- ASGPR positive, despite apparently complete remission of disease, should remain on low-dose corticosteroid until these Abs disappear from the serum. It is also indicated that, serum anti-ASGPR should be measured at regular intervals and treatment should be re-introduced if these antibodies reappear.

References:

1. *McFarlane IG: Autoimmune hepatitis: diagnostic criteria, subclassifications, and clinical features. Clin Liver Dis 2002, 6:605-621.*
2. *McFarlane IG: Definition and classification of autoimmune hepatitis. Semin Liver Dis 2002, 22:317-324.*
3. *Czaja AJ, Freese DK: Diagnosis and treatment of autoimmune hepatitis. Hepatology 2002. 38:479-49.*
4. *Kim WR. Brown Jr . RS Terrault NA, et al. Burden of liver disease in the United States: summary of a workshop. Hepatology 2002, 38 :227-242.*
5. *Hokari A, Zeniya M. Autoantibodies in autoimmune hepatitis. Rinsho Byori 2003; 124: 70-75.*
6. *Ferrari R, Pappas G, Agostinelli D, Muratori P, Muratori L, Lenzi M, Verucchi G, Cassani F, ChoodoF, Bianchi B. Type I autoimmune hepatitis: pattern of clinical presentation and differential diagnosis of the "acute" type. Q J Mod 2004; 97:407-412.*
7. *Garcia Leiva J, Rios Vaca A, Torre Delgadillo A. Antibodies and physiopathogeny ofAIH. Rev Invest Clin 2003; 55(5): 577-582.*
8. *Manns M. Autoimmune liver diseases. Clinics in Lab Med 1998; 8:281-301.*
9. *Strassburg CP, Manns MP: Autoantibodies and autoantigens in autoimmune hepatitis. Semin Liver Dis 2002, 22:339-351.*
10. *Treichel U, McFarlane BM, Seki T, Krawitt EL, Alessi N, Stickel F, McFarlane IG, Kiyosawa K, Furuta S, Freni MA: Demographics of anti-asialoglycoprotein receptor autoantibodies in autoimmune hepatitis. Gastroenterology 1994,107:799-804*
11. *Czaja AJ, Pfeifer KD, Decker RH, Vallari AS: Frequency and significance of antibodies to asialoglycoprotein receptor in type I autoimmune hepatitis. Dig Dis Sci 1996, 41:1733-1740.*
12. *Czaja AJ. Diagnosis, Prognosis, and treatment of classical autoimmune chronic active hepatitis. Gastroenterology 2002; 8: 143-149.*
13. *Harry R. Hill, MD. AIH: Markers to aid in the diagnosis of autoimmune liver disease. Immunology 2002; 7: 115-122.*
14. *McFarlane IG, Hegarty JE, McSorley CG et al., Antibodies to liver specific protein predict outcome of treatment withdrawal in AIH. Lanet 2002; 2: 954-960.*