

Anti-Liver membrane Antibody predict outcome of Autoimmune Hepatitis

Eman Sh. AL- Obeidy* Ph.D

Batool A. Al-Haidary **Ph.D

Akram A. Najeeb*** F.I.C.M.S, D.M.

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Summary

Background: Autoantibodies against liver membrane are the specific autoantibodies found in AIH. Cytochrome P450 1A2 has been identified as the target autoantigen for these autoantibodies

The aim of the study : is to evaluate the prevalence and clinical relevance of LMA 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 IFA technique.

Results: LMA was detected in the sera of 28(57.14)and 3(37.5%) patients with type 1 and type 3 AIH respectively, but never in the sera of healthy group .

Conclusion: It was concluded that LMA is present in type-1 and type-3 AIH but not in sera of patients with type-2 or in healthy control group.

Introduction :

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

Three types of AIH have been proposed based on immuno-serologic markers [3]. Though, (IAHG) has not endorsed these types as distinct clinical or etiologic entities [4,5]. Nevertheless, type 1 and type 2 have different clinical features, whereas type 3-AIH is similar to type 1 except for its autoantibodies profile.

Intriguing clues into the pathogenesis of AIH come from the observation that circulating autoantibodies are prevalent in patients with this disorder. Among the autoantibodies described in these patients are antibodies to nuclei (ANA), SMA, anti-LKM, SLA/LP antibodies, as well as antibodies to the liver specific asialoglyco-protein receptors (or hepatic lectin) and other hepatocyte membrane protein. Although some of these provide helpful diagnostic markers, yet their involvement in the pathogenesis of AIH has not been established, except for anti-ASGP-R and anti-LKM 1, since many of these autoantibodies is frequently found in many form of chronic liver disease [6,7].

One of the most important Abs that detected in patients with AIH is liver membrane antibody (LMA). Cytochrome P450 1A2 (CYP1A2), was identified as a target antigen for these antibodies [8, 9], which is a 35KD protein expressed on the hepatocyte membrane. The major clinical value of anti-LM antibodies in AIH may be to reclassify patients with cryptogenic hepatitis as having AIH [10].

This study is a trial to determine the diagnostic role of LMA in evaluating patients with AIH.

Material and methods:-

Seventy three sera samples have been collected from AIH Iraqi patients' during the period between November 2003 and July 2004 when they sequentially admitted to the Teaching Hospital of Gastroenterology and Liver Disease. They include (20 males and 53 females) with age range between (10-57) years and the results of investigations were compared with those for 50 apparently healthy individuals as a control group who matched them with age and sex . Both groups are undergone the following tests to detect autoantibodies. These tests include ANA, SMA, LKM-1 beside LMA using Immunofluorescent Antibody Assay (IFA) and also Euro-line technique (Euroimmune, Germany) has been applied Anti-SLA/LP detection. In addition, liver-function test SGOT, SGPT, Alkaline Phosphatase and TSB applied for all the sera samples. Serum gamma globulin was estimated by protein electrophoresis.

Sequential serum sample from all patients were studied from onset throughout follow up to quantitatively evaluate LMA concentration during drug induced remission of the liver disease.

* *Ph.D. immunology, Medical City.*

** *Ph.D., Immunology / Assist Prof./ College of Medical & Health Technology/ University of Baghdad.*

*** *Gastroenterology and Liver Disease / Teaching Hospital*

Results:-**I- Clinical and Demographical AIH Picture of Patients:**

The demographical picture of AIH patients has been listed in Table 1.

Table 1: Clinical and Demographical picture of CAH patients

Clinical & demographical Picture	CAH Patients	Control Group
Age range (years)	10-60	10-57
Males: Females	20 53	20: 30
Type I AIH	49	-
Type II AIH	16	-
Type III AIH	8	-
Total	73	50

This table reveals that the incidence is prominent feature of females than males and majority of them are suffering of type I -AIH. This fact is clear in figure 1, since the majority of patients (67%) complain of type 1 whereas, (22%) had type II, and only (11%) had type III AIH.

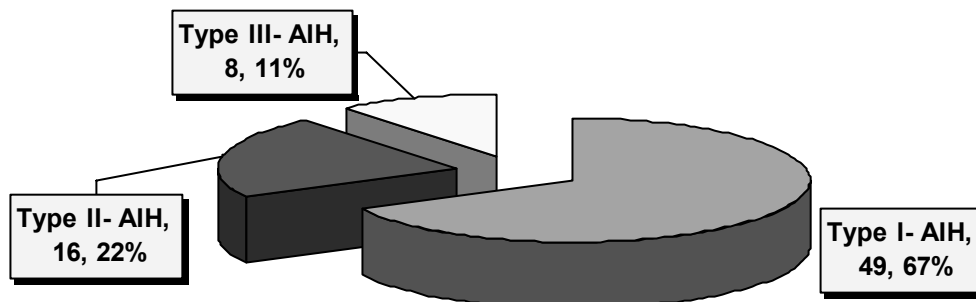


Figure -1: The frequency distribution of AIH types.

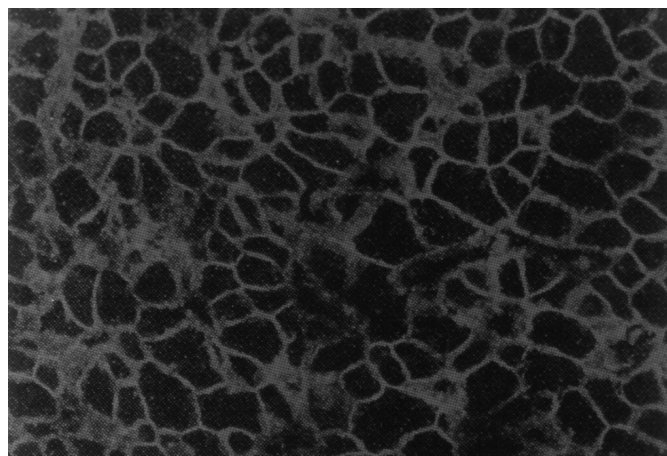


Figure-2: Indirect immunofluorescence showing LM Abs on primate liver cryostat section.

II- Effect of Age on the incidence of AIH:

The distribution of AIH patients according to their age groups are shown in Table 2. This table reveals that the majority of patients (54.8%) are at age range between 20-39 years most of them are females (72.6 %).

Table 2: Distribution of the studied groups according to age group

<i>Age Groups (years)</i>	<i>AIH Patients*</i>	<i>Control Group</i>
< 20	16 (21.9)	6 (12)
20-29	20 (27.4)	15 (30.0)
3-39	20 (27.4)	15 (30.0)
40-49	7 (9.6)	12 (24.0)
50-59	7 (9.6)	12 (24.0)
≥ 60	3 (4.1)	-
Sex: Females	53 (72.6)	30 (60.0)
Males	20 (27.4)	20 (40)
Total	73 (100.0)	50 (100.0)

* = No. between brackets represents the percentage

III- Effect of Age on the Incidence of AIH subtypes:

The effect of age on the incidence of AIH disease is shown in Table 3. In this table it seems to be that the most susceptible ages are (34.82±10.7), (27.2±9.44) and (31.25±6.52) years for type I, type II and type III respectively. The majority of type-I AIH (30.6%) are between the age (30-39) years while (34.75%) of type-II patients who are (20-29) years old. In general this is the same age group most of the type-III AIH patients (62.5%) belong to.

Table 3: The age distribution of AIH patients by subtypes.

Age (years)	AIH-I (n=49)		AIH- II (n=16)		AIH- III (n=8)	
	No	%	No	%	No	%
<20	12	24.49	4	25.0	-	-
20-29	8	16.33	7	34.75	5	62.5
30-39	15	30.6	2	12.5	3	37.5
40-49	6	12.24	1	6.25	-	-
50-59	5	10.20	2	12.5	-	-
≥60	3	6.12	-	-	-	-
Total	49		16		8	100
Mean ±SD	34.82±10.7		27.2±9.44		31.25±6.52	
Range	8-62		10-57		20-50	
P value using ANOVA test, P<0.03 (Significant)						

In this work, 56 AIH patients (76.7%) were young 8-39 years, while 14 (19.2%) with intermediate age and the rest 3 (4.1%) fall in old age, on the other hand there was significant difference in the mean age of patients with different types of AIH as presented in table 3, since patients with type 2-AIH are more younger than those with type 1 and 3.

There were 53 females and 20 males with female to male ratio 2.6:1.

IV- Correlation between LMA and Disease Activity:

Severity of AIH has been studied through out the liver function test. Table 4 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 fold elevation though was not statistically significant.

Table 4: The biochemical parameters findings between 3 types of AIH compared to healthy control group.

Biochemical parameters	AIH patients (n=73)	Healthy control (n=50)	P value
SGOT	79.63±32.9	14.0±2.5	<0.001
	45-142	12-20	
SGPT	79.7±31.62	13.5±3.5	<0.001
	24-160	10-19	
Alkaline phosphates	137±25.1	71.1±5.8	>0.05
	80-195	62-82	
TSB	9.5±4.6	0.4±0.3	<0.001
	0.7-18.0	0.1-0.9	

Regarding serum protein electrophoresis, higher serum gamma globulin was observed among patients with AIH (3.5 ± 1.9) in comparison to (1.2 ± 0.3) in healthy control group ($P<0.001$).

Interestingly, this study showed that LMA were present in sera of patients with type 1 (28 patients 57.14%) and 3-AIH (3 patients 37.5%) but not in sera of those with type 2 ($P<0.001$).

All healthy control group as well as patients control were negative.

In regarding to correlation between LM Abs and disease activity, figure-3 revealed that titer of these antibodies highly affected by treatment (response after immunosuppression and ALT normalization).

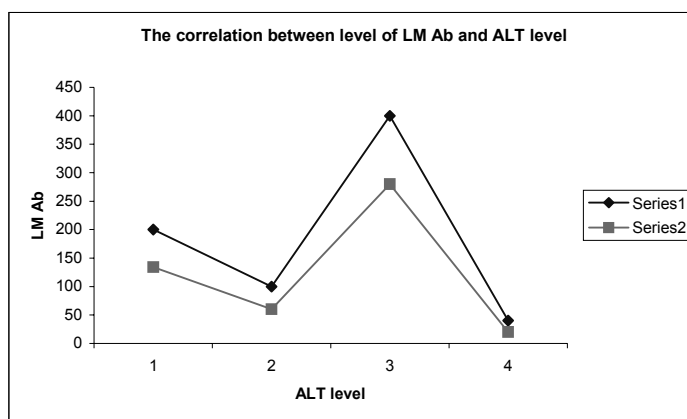


Figure 3: The correlation between level of LM Ab. and disease activity.

Interestingly, our data revealed that, LMA concentration seemed to faculae with ALT level, at onset, during disease remission, during reactivation of liver necroinflammation, after immuno-suppression tapering or withdrawal

Discussion:-

This study describes the behavior of humoral autoimmune reactions, namely LMA in the clinical setting of AIH, before and during immunosuppressive treatment. In our patients the pharmacological treatment, which is mandatory to contain a rapidly progressive liver disease, not only effectively controlled the hepatic damage through generalized immunosuppression which led to ALT reduction and normalization, but also abated the circulating level of LMA resulting in its disappearance.

Regarding LM Abs frequency, it was stated that these Abs were observed in about 83% of patients with AIH (183). Hence; serum level of these Ab could be used as a marker for both prognostic and post treatment monitoring (182).

In this study, 28 (57.14%) patients of type 1, and 3 (37.5%) of type 3 had LMA positive, on the other hand these result showed that there was a correlation between the level of these Abs and disease activity.

The observation that humoral LMA auto reactivity correlates strictly with hepatocyte injury suggests that LM antigen may be an important liver specific target of the autoimmune attack.

Our data showed that LMA concentration correlates strongly with ALT levels before and during immunosuppressive treatment in patients with AIH. In addition, ALT flares following immunosuppression tapering or withdrawal were characterized by an increase in LMA concentration.

The close correlation between LMA concentration and ALT level during the different phases of the liver disease points to direct involvement of LMA auto reactivity in the process of liver targeted autoimmune attack.

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