
Non-organ Specific Autoantibodies (ANA & SMA) in Type 1-Autoimmune Hepatitis

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Abstract:

Background: Autoantibodies against nuclear antigen and smooth muscle characterize type 1-autoimmune hepatitis. Antinuclear antibodies directed against functional and structural component of the cell while smooth muscle antibodies exhibit reactivities against actin and Non-actin components.

Objective: Aim of this study is to evaluate the prevalence and clinical relevance of antinuclear antibodies and smooth muscle antibodies in autoimmune hepatitis -1.

Methods: The study was performed on 73 Iraqi patients with chronic active hepatitis of unknown cause, attending the teaching hospital for gastroenterology and liver disease in a period from November 2006 through July 2007. : Anti-nuclear antibodies and anti-smooth muscle antibodies were measured by Enzyme Linked Immunosorbant Assay and Indirect Immunofluorescence technique.

Results: Antinuclear and Smooth muscle antibodies were detected in the sera of 49 patients (67.0%) with autoimmune hepatitis, but never in the sera of healthy group just in one patients (0.5%) in comparison to Auto immune hepatitis patients with the frequency (30.6%) was highest among the age group of 30-39 years.

Conclusion: Antinuclear Antibodies and Smooth muscle antibodies are present in identified patients with autoimmune hepatitis-1, smooth muscle antibodies are more prevalent than antinuclear antibodies in this type and patients with this type have a bimodal age distribution.

Key words: Antinuclear antibodies, anti-smooth muscle antibodies, autoimmune hepatitis type-1, Enzyme Linked Immunosorbant Assay, Indirect Immunofluorescence Technique

Introduction:

Autoimmune hepatitis (AIH) is an idiopathic hepatitis characterized by histologic evidence of chronic liver inflammation, auto-antibodies, and increased serum gammaglobulins. However, the clinical manifestations of the disease have been well characterized since its initial description by Waldenstrom in 1950 ^[1].

Three types of AIH have been proposed based on immuno serological markers. Type 1-AIH is characterized by the presence of anti-nuclear antibodies (ANA) and /or anti-smooth muscle antibodies (SMA) ^[2]. It is the most common form of the disease worldwide; furthermore, 70% of patients with this type are women younger than 40 years and more than 30%

have concurrent immune diseases ^[2]. Recent study showed that, asialoglyco-protein receptor is a candidate autoantigen of this type because it resides on the hepatocyte surface ^[3, 4]. SMA is traditionally found in AIH and is directed against cytoskeletal proteins, including actin, troponin, tubulin and vimentin.

These antibodies are present in 87% of patients with the disease and are accompanied by ANA in 54% of patients ^[2]. High-titer SMA occurs in classic autoimmune-type lipoid hepatitis. They are not specific for AIH; rather, they occur in other liver diseases as

well as various infection and rheumatologic disorders ^[4].

This study was planned to investigate the prevalence and their clinical relevance of antinuclear antibodies and smooth muscle antibodies in autoimmune hepatitis-1.

Patients & Methods:

Patient a: seventy three patients (20 male, 53 female) with chronic active hepatitis (CAH) of unknown cause, were selected from those attending The Teaching Hospital for Gastroenterology and liver disease in a period between November 2006 through July 2007. The results of investigations were compared with that of 50 healthy individuals' volunteers. Both groups were subjected to serological detection of ANA and SMA by Indirect Immunofluorescent (IIF) and Euro line method.

Laboratory investigation:

ANA and SMA were detected using Enzyme-Linked Immunosorbant Assay (ELISA) technique using human IgG Fc as the antigen coated the micro-wells plate and isotype-specific horse antibodies coupled to radish peroxidase; result were expressed as the optical density. Indirect Immunofluorescent technique (IIF) technique on HPE-2 cell line and rat stomach substrate respectively as a confirmatory test were done; sera were screened at a dilution of 1:20 and 1:40

respectively in PBS with fluorescent polyvalent antiglobulin and positive results were recognized by presence of a specific pattern. Euro immune has supplied the above kits company, Germany.

All the results statistically analyzed by application of ANOVA test ^[5].

Results

The results showed that the mean age of patients was (34.82 ± 10.7) years with a male to female ratio was 1:2.7 as shown in **table 1**. This study revealed that highest frequency of AIH patients (30.60%) was among the age

group 30-39 years with significant difference in comparison with other age groups.

Antinuclear antibodies (ANA) and smooth muscle antibodies (SMA) were detected in 49 sera samples of patients (67.0%) with CAH of unknown cause, while they were not detected in healthy controls group, just in one with non-significant titer; in comparison to AIH patients. The frequencies of the distribution of ANA and SMA Abs are shown in **figure-1** in which the isolative presence of ANA or SMA represent 22.45% and 55.1% respectively, while the coexistence of both Abs represent 22.45%.

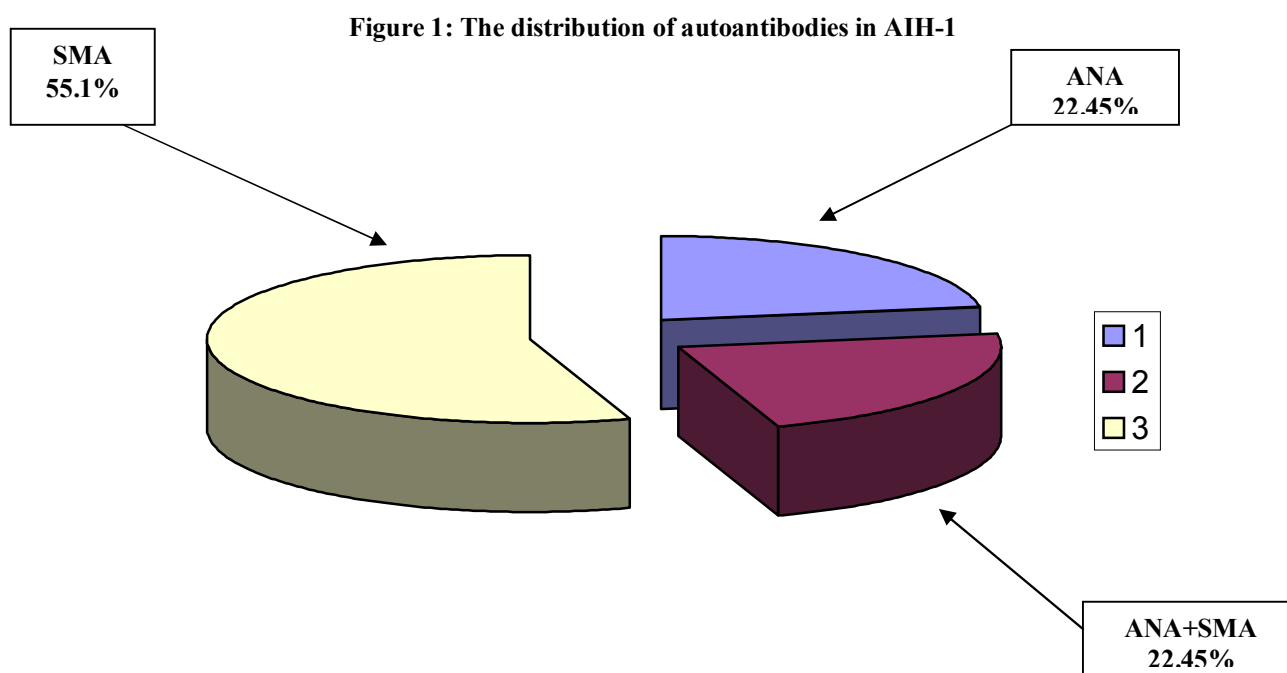


Table 1: Distribution of the studied groups according to age and gender

Age (years)	AIH- I (n=49)		Healthy control (n=50)	
	No	%	No	%
<20	12	24.49	4	8.0
20—29	8	16.33	18	36.0
30—39	15	30.6	17	34.0
40—49	6	12.24	10	20.0
50—59	5	10.20	1	2.0
≥60	3	6.12	-	
Mean ± SD	34.82 ± 10.7		30.6 ± 10.1	
Range	8-62		10-59	
Gender	AIH- I (n=49)		Healthy control (n=50)	
	No	%	No	%
Female	36	73.4	28	56.0
Male	13	26.6	22	44.0
Total	49	100%	50	100%

* P (ANOVA) <0.001

Moreover, coexistence of another autoimmune disease (AID) has been observed in 15 out of the 49 patients as shown in **table 2**. This table revealed that, rheumatoid arthritis

(RA), ulcerative colitis, autoimmune thyroiditis, and synovitis are the most common associated diseases.

Table 2: The distribution of concurrent immune diseases in AIH-1.

Immune diseases	AIH Type I (n=49) No.	%
SLE♣	1	2.04
RA♦	3	6.12
Glomerulonephritis	1	2.04
Celiac disease	1	2.04
Gravis	1	2.04
AI thyroiditis●	2	4.08
Ulcerative colitis	3	6.12
Synovitis	2	4.08
IDDM*	1	2.04

♣ = Systemic Lupus Erythematosus, Autoimmune Thyroiditis,

♦ = Rheumatoid Arthritis, * = Insulin-Dependent Diabetes Mellitus

● =

Discussion

Data of the current study showed that the majority of Type I AIH patients were females (73.4%), which is comparable with other studies where women represent (78%) [6,7]. The male to female ratio in this study was 1: 2.7, which is nearly comparable to that of local study [8]. However, the ratio 1:2.4 and 1:3 respectively had been reported by abroad studies [7,8]. 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 female more than males may be due to the effect of hormonal differences which activate TH₂ and subsequently enhance autoantibodies production [9].

Moreover, it was observed in this study, that the mean age of the disease was (34.82±10.7) years which, is to some extent comparable to (39.2±11.2) years reported by Manns MP. & Petra Obermyer-Straub (2000) [7]. On the other hand, data showed that patients with type 1 has two-peak incidence of age (8-20) and (30-39) years. This result disagrees with other studies, in which the maximum incidence of type 1-AIH observed among age (10-20) years and (45-70) years [10]. 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 [11].

The detection of serum autoantibodies is presumptive evidence for AIH and other autoimmune disorders. Therefore, when compared with autoantibody profile of healthy control, ANA and SMA are exclusively present in serum of patients with type-1 AIH. However, the prevalence showed in the present study for autoantibodies are substantially in line with previous reports [12], which used the same IIF and ELISA screening dilution employed here. Although, it is quite accepted that ANA is the traditional basis for the diagnosis of type 1-AIH, together with SMA, this study is not exclusive, yet ANA was detected in only (22.4%) while SMA were detected in (55.1%) and they are associated with ANA in (22.45%). These results are compatible with other abroad and local studies [6, 13], who revealed that type 1-AIH are associated with ANA (20.4%, 35.6%) and SMA (79.6%, 64.4%) respectively.

One clue to diagnose overlapping of autoimmune disease (AIH) may be the coexistence of other disease with immune or autoimmune features. This fact was very clear in this study since, 15 patients with type 1-AIH had main concurrent immunologic disease,

including autoimmune thyroiditis, RA, ulcerative colitis and synovitis. Thus our result is quite similar to other abroad studies [7,14].

It is clearly that these autoantibodies are of an importance in diagnosis so as in discriminating between the AIH subtypes.

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