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The Diagnostic Value of ANA and Anti-SMA in Suspected Patients with Autoimmune Hepatitis

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Abstract

BACKGROUND: Autoimmune hepatitis (AIH) is an unknown chronic disease characterized by hepatocellular inflammation with a tendency to progress to cirrhosis. AIH can present with symptoms of acute hepatitis with symptoms of chronic liver disease. AIH occurs globally; it is more commonly found in females. Autoantibodies such as antinuclear, smooth muscle, liver kidney microsome, and soluble liver antigen are used to aid in the diagnosis of AIH, which presents with a variety of symptoms that also contribute to the classification of AIH.

AIM: Evaluation of anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) positivity in hepatic diseases, gastrointestinal diseases, viral hepatitis, and extra-hepatic diseases, providing better markers for the early diagnosis of AIH-type 1.

MATERIALS AND METHODS: The study included 207 individuals. 62.4% of them were female. Regarding the diagnoses, we grouped them into 4 groups: hepatic diseases (n = 73), viral hepatitis B and C (n = 54), gastrointestinal diseases (n = 34), and extra-hepatic diseases (n = 46). Serum levels of ANA and anti-SMA were measured using an indirect immunofluorescence method following the manufacturer's instructions (Aesku Diagnostics, Germany). Fluorescence intensity was interpreted semi-quantitatively based on negative control (0) and positive control (+4).

RESULTS: The positivity of ANA and anti-SMA resulted as follows: In hepatic diseases 34.2% and 48%, in viral hepatitis B and C, ANA positivity was 14.8% and SMA positivity was 22.2%; in gastrointestinal diseases, ANA and SMA positivity were, respectively, 11.8% and 20.6%; and in extrahepatic diseases, positivity of ANA resulted in 32.6% and SMA in 26%. When compared to the viral hepatitis patient group, the ANA specificity for hemagglutination inhibition (HAI) was 85.2% and that of anti-SMA was 77.8%. The analysis of 46 extrahepatic patient groups provided an ANA specificity of 67.4% and an anti-SMA specificity of 74% for HAI. The comparison to gastrointestinal disease showed that ANA specificity for HAI was 88.2% and anti-SMA specificity was 79.4%.

CONCLUSION: Diagnosing AIH is difficult because the clinical spectrum ranges from an asymptomatic presentation to an acute and severe disease. So in all cases, AIH must be suspected. Both males and females can develop AIH, but the disease is more common in females. Based on our diagnostic parameters, we can say that ANA and anti-SMA provide moderate sensitivity for AIH, but they are more specific to AIH type 1.

Introduction

Autoimmune hepatitis (AIH), a progressive inflammatory hepatopathy and an important cause of end-stage liver disease [1]. The exact cause of AIH is unknown [2]. It is believed to be due to a combination of environmental, genetic, and immunologic factors. A few environmental triggers, such as medications and infections, have been associated with the development of AIH [3]. This causes the cells in the body that usually attack foreign invaders to start attacking the liver. This leads to inflammation and liver damage. AIH occurs globally, affecting children and adults of all ages and both sexes, although it is more commonly found in females [4]. The mode of presentation of AIH is diverse and highly variable [5]. Although the majority of adult patients manifest progressive fatigue, nausea, vomiting, abdominal pain, jaundice, amenorrhea, weight loss, and occasionally arthralgia [5]. Complications of portal hypertension, such as gastrointestinal bleeding or hypersplenism, can sometimes be apparent. Approximately 25% of patients are completely asymptomatic and are diagnosed after the discovery of abnormal liver function tests. Finally, 30–40% of patients, particularly children and young adults, present with symptoms and signs mimicking those of acute hepatitis due to other causes [6]. Irrespective of the mode of presentation, histological evidence of cirrhosis is found in at least 30% of patients, indicating that subclinical disease has been present for some time [7]. AIH can occasionally develop during pregnancy, as with other autoimmune diseases. Autoantibody detection should proceed as soon as a diagnosis of AIH is suspected [8], [9]. Not only does positivity weigh towards AIH diagnosis, but autoantibody profiles enable the distinction between AIH type 1 and AIH type 2. Type 1 is more common and is usually diagnosed in adults [10], [11]. Type 2 is more common in children and often involves a more severe disease process. Early detection of AIH is key, as early and effective treatment is associated with better patient outcomes [12].

Aim of the study

Evaluation of anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (anti-SMA) positivity in hepatic diseases, gastrointestinal diseases, viral hepatitis, and extra-hepatic diseases provides better markers for the early diagnosis of AIH-type 1.

Table 1: All diagnoses for serological testing are divided into four groups

Diagnoses	n (%)
Hepatic diseases	73 (35.3)
Acute hepatitis	34 (16.5)
Chronic hepatitis	17 (8.2)
Cirrhosis	22 (10.6)
Viral hepatitis	54 (26.1)
Hepatitis B viral	13 (6.3)
Hepatitis C viral	41 (19.8)
Gastrointestinal diseases	34 (16.4)
Ascites	7 (3.4)
Gastrointestinal bleeding	7 (3.4)
Mechanical jaundice	4 (1.9)
Abdominal colic	4 (1.9)
Hepatomegaly/splenomegaly	3 (1.4)
Ulcerative colitis	2 (1)
Pankreatitis	3 (1.4)
Chronic diarrhea	3 (1.4)
Acute gastritis	1 (0.5)
Extra hepatic diseases	46 (22.2)
Systemic autoimmune diseases	14 (6.8)
Organ-specific autoimmune diseases	12 (5.8)
Viral infection	16 (7.7)
Hematological diseases	4 (1.9)
Total	207 (100)

Materials and Methods

In this study, we included 207 individuals who were sent to our laboratory of immunology, Hospital Center “Mother Teresa” for serologic testing during the time period from 2018 to 2020. All diagnoses were divided into four main groups. These included acute and chronic hepatitis (n = 73) and gastrointestinal diseases (n = 34). The control groups were viral hepatitis B and C (n = 54) and extra-hepatic diseases (n = 46) (Table 1). Serum levels of ANA were measured using an immunofluorescence (IIF) method following the manufacturer’s instructions (Aesku Diagnostics, Germany). This kit uses Hep-2 cells as substrate antigens. SMA auto-antibodies and immunoglobulin G were also measured using an IIF method (Aesku Diagnostics, Germany). This kit uses liver, kidney, and stomach rat tissue as a substrate antigen.

Statistics

For statistical analysis, the results of ANA and SMA antibodies were recorded as continuous variables and categorical data. The diagnostic parameters such as sensitivity, specificity, and positive and negative predictive values (positive predictive values and negative predictive values) for each assay were determined with respect to both viral hepatitis B and C and the extra-hepatic diseases. Statistical test analysis was carried out using SPSS software version 18.

Results

The mean age of all patients was 43.3 ± 17.0, and 136 (62.4%) of them were females. The serum testing of 73 patients with hepatic diseases showed 25 positive ANA and 35 positive SMA results, reflecting a diagnostic sensitivity of 34.2% for ANA and 48% for anti-SMA. The testing of 54 viral hepatitis patients led to 8 (14.8%) positive ANA results and 12 (22.2%) positive SMA results. In extra-hepatic diseases, ANA and anti-SMA resulted in 15 (32.6%) and 12 (26.1%), respectively. In gastrointestinal diseases, positive ANA resulted in 11.8% and anti-SMA resulted in 20.6% (Table 2). When compared to the viral hepatitis patient group, the ANA specificity for AIH was 85.2% and that of anti-SMA was 77.8%. The analysis of 46 extra-hepatic patient groups provided an ANA specificity of 67.4% and an anti-SMA specificity of 74% for AIH. The comparison to gastrointestinal disease showed that ANA specificity

Table 2: Age, gender data, ANA and anti-SMA seropositivity in the hepatic and gastrointestinal patients and in two control group

Patients’ characteristics	Hepatic diseases	Gastrointestinal diseases	Viral hepatitis diseases	Extra hepatic diseases
Age mean (SD*)	49.6 (10.6)	48.3 (12.1)	43.1 (11.4)	46.2 (13.4)
Female/male (n)	57/16	27/7	26/28	35/11
ANA positive (n) (%)	25 (34.2%)	4 (11.8%)	8 (14.8%)	15 (32.6%)
Anti-SMA (n) (%)	35 (48%)	7 (20.6%)	12 (22.2%)	12 (26%)
Total	73	34	54	46

*SD: Standard deviation, ANA: Anti-nuclear antibodies, SMA: Smooth muscle antibodies

Table 3: Diagnostics parameters of ANA and anti-SMA in patients with hepatic diseases, in reference to viral hepatitis patient group

Antibodies	Hepatic diseases (n = 73)	Viral hepatitis (n = 54)	Sensitivity	Specificity	Positive predictive values	Negative predictive values
ANA	25	8	34.3%	88.2%	75.8	48.9
Anti-SMA	35	12	48%	78%	74.5	52.5

ANA: Anti-nuclear antibodies, SMA: Smooth muscle antibodies

Table 4: Diagnostics parameters of ANA and anti-SMA in patients with hepatic diseases, in reference to extrahepatic patient group

Antibodies	Hepatic diseases (n = 73)	Extrahepatic diseases (n = 46)	Sensitivity	Specificity	Positive predictive values	Negative predictive values
ANA	25	15	34.3%	67.4%	62.5	39.2
Anti-SMA	35	12	81.4%	74%	74.5	80.9

ANA: Anti-nuclear antibodies, SMA: Smooth muscle antibodies

Table 5: Diagnostics parameters of ANA and anti-SMA in patients with hepatic diseases, in reference to gastrointestinal patient group

Antibodies	Hepatic diseases (n = 73)	Gastrointestinal diseases (n = 34)	Sensitivity	Specificity	positive predictive values	negative predictive values
ANA	25	4	34.3%	88.2%	86.2	38.5
Anti-SMA	35	7	48%	79.4%	83.3	41.5

ANA: Anti-nuclear antibodies, SMA: Smooth muscle antibodies

for AIH was 88.2% and anti-SMA specificity was 79.4% (Tables 3-5).

Discussion and Conclusion

Diagnosing AIH is difficult, as it is frequently accompanied by non-specific symptoms like fatigue, nausea, jaundice, abdominal pain, and arthralgia. The clinical spectrum ranged from an asymptomatic presentation to acute and severe disease [5]. So in all cases, AIH must be suspected. Although both males and females can develop AIH, the disease is more common in females, with a gender ratio of 3.6–1 (Table 2). The anti-SMA positivity resulted in 22.2% viral hepatitis. Anti-SMA antibodies that are detected in hepatitis B virus and hepatitis C virus hepatitis usually do not react with F-actin [13]. So AIH type 1 may be developed after the infection with hepatitis B or C [14]. All viral hepatitis needs to be considered when making a hemagglutination inhibition diagnosis. Individuals with AIH type 1 may have other autoimmune disorders such as rheumatoid arthritis, ulcerative colitis, or other autoimmune diseases. This was demonstrated when we compared the hepatic disease patient group with the extra-hepatic patient group, and ANA specificity resulted in 67.4%, but SMA specificity (74%) was no different. The ANA distribution pattern and antigens detected in AIH type 1 are different from those seen in primary biliary cirrhosis but can overlap viral hepatitis. Our study showed that ANA specificity resulted in 88.2% when we compared hepatic diseases with viral hepatitis and gastrointestinal diseases, so we can say that ANA represents an important diagnostic criterion of AIH. Based on anti-SMA positivity (26%) in the extra-hepatic patient group, we can say that AIH patients may have a history of autoimmune disease, may have concomitant autoimmune diseases, or will develop them during follow-up [15]. In our study, anti-SMA antibodies react with F-actin, which is thought to be the most specific for AIH [16]. In conclusion, ANA and anti-SMA provide moderate sensitivity for AIH, but they are more specific to AIH type 1 [17].

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