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Investigation of patients with and without anti-filamentous-actin staining patterns in terms of clinical and laboratory data in the ASMA positive population

ASMA pozitif popülasyonda anti-filamentöz-aktin boyanma paternleri olan ve olmayan hastaların klinik ve laboratuvar verileri açısından incelenmesi

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ABSTRACT

Objective: Autoimmune hepatitis (AIH) is a chronic liver disease characterized by increased inflammation formation in hepatocytes, which can progress to liver failure if not treated. The aim of this study was to compare patients with and without anti-F-actin staining pattern in the anti-smooth muscle antibodies (ASMA) positive population in terms of the presence of type 1 AIH using clinical and laboratory data.

Methods: The study included 240 ASMA positive patients, 120 of whom had anti-F-actin staining and 120 without. After treating HEp-20-10 cells with samples from the patients, tests were done to check for anti-filamentous-actin (F-actin) staining and ASMA positivity using indirect immunofluorescence.

Results: Antinuclear antibody (ANA) positivity was 45.8% in patients with an anti-F-actin staining pattern and 29.2% in those without (p=0.008). ASMA titers were higher in the anti-F-actin staining group than in the group without anti-F-actin staining (p<0.001). Laboratory findings showed higher AST, ALT, and

ÖZET

Amaç: Otoimmün hepatit (OİH), hepatositlerde artmış inflamasyon oluşumu ile karakterize, tedavi edilmediği takdirde karaciğer yetmezliğine kadar ilerleyebilen kronik bir karaciğer hastalığıdır. Bu çalışmanın amacı anti-düz kas antikorları (ASMA) pozitif popülasyonda anti-F-aktin boyanma paterni olan ve olmayan hastaları klinik ve laboratuvar verilerini kullanarak tip 1 OİH varlığı açısından karşılaştırmaktır.

Yöntem: Çalışmaya 120'sinde anti-F-aktin boyanması olan ve 120'sinde olmayan 240 ASMA pozitif hasta dahil edilmiştir. HEp-20-10 hücreleri hastalardan alınan örneklerle muamele edildikten sonra, indirekt immünofloresan kullanılarak anti-filamentöz-aktin (F-aktin) boyanması ve ASMA pozitifliğini kontrol etmek için testler yapılmıştır.

Bulgular: Antinükleer antikor (ANA) pozitifliği anti-F-aktin boyanma paterni olan hastalarda %45,8 iken olmayanlarda %29,2 idi (p=0,008). ASMA titreleri anti-F-aktin boyanma paterni olan grupta anti-F-aktin boyanma paterni olmayan gruba kıyasla daha yüksek bulunmuştur

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immunoglobulin G values in the anti-F-actin group (*p* values <0.001, 0.002, and 0.006, respectively). Among type 1 50 AIH patients, 60% of those with an anti-F-actin staining pattern tested positive for ANA, while only 40% of those without this pattern did (*p*=0.123). In type 1 AIH patients, the anti-F-actin staining pattern associated with greater AST, ALT, GGT, total bilirubin, and CRP (*p* values <0.001, <0.001, <0.001, 0.015, 0.015, respectively), but albumin was lower (*p*=0.009). The research focused on young females aged 15 to 25 who had high levels of anti-F-actin antibodies, making up 48.7% of type 1 AIH cases. 25% of anti-F-actin-like staining patients were 15-25-year-old women. 66.6% of 15-25-year-old women had type 1 AIH. The group with anti-F-actin staining showed no link between actin titer and ANA positivity (*p* = 0.210), whereas type 1 AIH was considerably higher with a higher titer (*p*<0.001). As the ASMA titer grew, the number of patients with type 1 AIH dramatically increased (*p*<0.001). As actin titer increased, ASMA titer also considerably increased (*p*<0.001). A diagnosis of type 1 AIH was found in 20.2% of patients with anti-F-actin titers of 1/160, while 56.5% of patients with titers of 1/320 and higher had a diagnosis (*p*<0.001).

Conclusion: Anti-F-actin antibodies can be found in various diseases, including both liver and non-liver diseases, usually at low levels, but they are of great importance for the diagnosis of type 1 AIH, especially in young women.

Key Words: Autoimmune hepatitis, anti-smooth muscle antibody, actin

(*p*<0.001). Laboratuvar bulguları anti-F-aktin grubunda daha yüksek AST, ALT ve immünoglobulin G değerleri göstermiştir(p değerleri <0.001, 0.002 ve 0.006, sırasıyla). 50 Tip 1 OİH hastaları arasında, anti-F-aktin boyanma paterni olan hastaların %60'ı ANA pozitifliği gösterirken, olmayanların oranı %40 bulunmuştur (*p*=0,123). Tip 1 OİH hastaları arasında, anti-F-aktin boyanma paterni daha yüksek AST, ALT, GGT, total bilirubin ve CRP ile ilişkili bulunmuştur (p değerleri sırasıyla <0,001, <0,001, <0,001, 0,015, 0,015), ancak albümin daha düşük bulunmuştur (*p*=0,009). Araştırma, yüksek düzeyde anti-F-aktin antikorlarına sahip olan ve tip 1 OİH vakalarının %48,7'sini oluşturan 15-25 yaş arası genç kadınlara odaklanmıştır. Anti-F-aktin benzeri boyanan hastaların %25'i 15-25 yaş arası kadınlardan oluşmuştur. 15-25 yaş arası kadınların %66,6'sında tip 1 OİH saptanmıştır. Anti-F-aktin boyaması olan grupta aktin titresi ile ANA pozitifliği arasında bir bağlantı görülmezken (*p*=0.210), tip 1 OİH'te aktin titresi daha yüksek bulunmuştur. (*p*<0.001). ASMA titresi arttıkça, tip 1 OİH'li hasta sayısı dramatik bir şekilde artmıştır (*p*<0,001). Aktin titresi arttıkça ASMA titresi de önemli ölçüde artmıştır (*p*<0.001). Anti-F-aktin titresi 1/160 olan hastaların %20,2'sine tip 1 OİH tanısı konulurken, 1/320 ve üzeri titreye sahip hastaların %56,5'ine tanı konulmuştur (*p*<0,001).

Sonuç: Anti-F-aktin antikorları, hem karaciğer hem de karaciğer dışı hastalıklar dahil olmak üzere çeşitli hastalıklarda genellikle düşük seviyelerde bulunabilir, ancak özellikle genç kadınlarda tip 1 OİH tanısında büyük önem taşımaktadır.

Anahtar Kelimeler: Otoimmün hepatit, anti düz kas antikoru, aktin

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic liver disease characterized by increased inflammation in hepatocytes, which can progress to liver failure if not treated. The incidence is 1-2 per 100,000, and the prevalence is 11-17 per 100,000. 60% to 80% of cases are female (1). Common laboratory findings include elevated blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, as well as

hypergammaglobulinemia due to immunoglobulin G (IgG) type antibodies (2). AIHs can be split into two kinds based on the autoantibodies identified in serum. Type 1 AIH is identified by the presence of antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA). It accounts for 80% of AIHs. ANA and/or ASMA positivity occurs in 70-80% of cases. Anti-liver antigen/liver-pancreas antigen antibodies (anti-SLA/LP) can be found in some AIH patients who do not have ANA or ASMA antibodies. This disease, termed as type 3 AIH in older literature, shares clinical characteristics with type 1 AIH and is treated accordingly. Type 2 AIH is identified by the presence of anti-liver kidney microsomal antibodies-1 (anti-LKM-1) and anti-liver cytosolic antigen-1 (anti-LC-1) antibodies. The majority of individuals with type 2 AIH are children or young adults. IgA deficiency is widespread among these patients (3). Patients with increased liver enzymes and negative viral serology should be investigated for autoimmune liver disorders using ANA, anti mitochondrial antibody (AMA), ASMA, and LKM testing. These tests should be performed using the IIF method, with kidney, stomach, and liver tissues utilized to assess AMA, ASMA, and LKM. IgG conjugate should be utilized in IIF tests, and the company's initial dilution recommendations should be considered (4).

The presence and distribution of ANA are evaluated in HEp-2 cells. However, ANA-positive samples may stain the nuclei of stomach, kidney, and liver sections (5). Although the most prevalent ANA pattern in patients with type 1 AIH is homogenous, other patterns can be found, including fine and coarse speckled. The presence of ASMA was determined in rat or mouse stomach sections. Smooth muscle cells in the tunica muscularis and lamina muscularis mucosa stain in ASMA+ stomach sections. The staining occurs in the interior section of the muscle cells and gives the appearance of "dried soil or bark". This image is quite peculiar to ASMA. It is typically accompanied by staining of contractile interglandular fibrils in the tunica mucosa. ASMA's most common target antigen is filamentous actin (F-actin). The ASMA pattern generated by F-actin antibodies is known as "ASMA, type actin". F-actin is a microfilament in the cytoskeleton that causes linear fibrillar staining in the cytoplasm of HEp-2 cells in the presence of anti-actin antibodies. However, this image is insufficient to establish the presence of F-actin antibodies. If monkey liver is used, bile canaliculi in the liver may stain in the presence of anti-f-actin antibodies and be referred to as "bird footprints" (6).

Anti-LKM antibodies are examined in kidney sections. It is worth noting that in the presence of anti-LKM antibodies, which indicate type 2 AIHs, the rat kidney's proximal tubules are stained, but the distal tubules are not. AMA against multiple mitochondrial antigens is used to diagnose primary biliary cholangitis (6). Moreover, although antisoluble liver antigen, a nonconventional, specific, type 1AIH marker (7), has been included in the revised scoring system as an additional parameter, the subspecificity of ASMA (e.g., antiactin ASMA) is still not included (8).

The aim of this study was to examine patients with and without anti-F-actin staining patterns in ASMA positive populations for the presence of type 1 AIH using clinical and laboratory data.

MATERIAL and METHOD

This study retrospectively reviewed the data of patients who tested positive for the ASMA pattern and were referred to the Medical Microbiology Laboratory of Ankara Bilkent City Hospital from February 2019 to July 2023. The study included 240 patients, comprising 120 with an anti-F-actin staining pattern and 120 without. Anti-F-actin staining and ASMA positivity were assessed by indirect immunofluorescence assays on HEp-20-10 cells after treatment with patient sera throughout testing. In cases of duplicate samples from the same patient, only the initial serum sample was incorporated into the study, while the subsequent samples were excluded. Individuals below the age of 18 were omitted. Clinical data encompassing patient

age, gender, and diagnosis were gathered. The ANA IIF test was conducted on the identical slide utilizing a kit (Euroimmun AG, Lubeck, Germany) that included Hep-20-10/liver/kidney/stomach tissues. This kit also facilitated the investigation of the existence of AMA, LKM, and ASMA. Scanning was conducted using an initial dilution of 1:100 as advised by the manufacturer. The fluorescence intensity at the screening dilution was qualitatively assessed, ranging from 1 positive (1/100) to 4 positive (1/3200), utilizing a EUROSTAR III fluorescent microscope at 40x magnification. The localization of ASMA with F-actin specificity was demonstrated through its distinctive fluorescence pattern: in the stomach, the muscularis mucosa and the vascular axis of the lamina propria of the gastric mucosa were stained; in the liver, the submembrane actin of hepatocytes exhibited the classic "honeycomb" pattern; in the kidney, the walls of blood vessels, as well as intracellular fibrils of renal tubules and mesangial cells of the glomerulus, were stained.

The Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (IBM SPSS Inc., Chicago, IL) was employed for statistical analysis. The Kolmogorov-Smirnov test was employed to determine the normal distribution of the data. Non-normally distributed numerical variables were presented as median, while normally distributed numerical variables were given as mean and standard deviation. The t-test for independent samples was employed to assess numerical variables exhibiting normal distribution, whereas the Mann-Whitney U test was utilized for numerical variables lacking normal distribution in two-group comparisons. The Chi-square test or Fisher's exact test was employed for the comparison of categorical data, as appropriate. In statistical analyses, a *p*-value of 0.05 considered significant.

The study was approved by the Ankara Bilkent City Hospital Non-Interventional Clinical Research Ethics Committee (Date: 12.07.2023 and Number: E2-23-4480). The research was performed in compliance with the Declaration of Helsinki and publishing ethics.

RESULTS

In our study population, 57.9% of ASMA-positive patients with and without anti-F-actin staining pattern were female. The mean age of males and females was 50±19.1 and 52.1±18.6 years, respectively. 134 (55.8%) patients were from gastroenterology, 77 (32%) from internal medicine, 13 (5.4%), 10 (4.1%) from intensive care, and 6 (2.5%) from neurology. While 64.2% of patients with an anti-F-actin staining pattern were female, 51.7% of patients without an anti-F-actin staining pattern were female (p=0.050). In addition, the mean age of the first group was 49 ± 20.3 years, while the mean age of the second group was 53.5 ± 17 years (p = 0.065). ANA positivity was present in 45.8% of patients with an anti-F-actin staining pattern, while ANA positivity was present in 29.2% of patients without an anti-F-actin staining pattern (p=0.008). ASMA titers were higher in the group with the anti-F-actin staining pattern compared to the group without the anti-F-actin staining pattern (p<0.001). When comparing laboratory data between the two groups, AST, ALT, and immunoglobulin G levels were higher in the anti-F-actin staining pattern group (p-values <0.001, 0.002, and 0.006, respectively) (Table 1).

We compared patients diagnosed with autoimmune hepatitis from the groups with and without anti-F-actin staining patterns. 68.3% of the group with the anti-F-actin staining pattern were female, while 65% of the other group were female (p=0.797). The mean age of the patients with the anti-F-actin staining pattern was 47±22.2 years, while the mean age of the other group was 54±15.9 years (p=0.209).

Of the patients diagnosed with type 1 AIH, 61% of patients with an anti-F-actin staining pattern were ANA-positive, while 40% of patients without an anti-F-actin staining pattern were ANA-positive (p=0.123). In the patients diagnosed with type 1 AIH, AST, ALT, GGT, total bilirubin, and CRP were higher in the group with the anti-F-actin staining pattern than in the other group (p-values <0.001, <0.001, <0.001, <0.001, 0.015, 0.015, respectively),

while albumin was lower (p=0.009). (Table 2).

The study focused on young females aged 15 to 25 years who had high levels of anti-F-actin antibodies, making up 48.7% of type 1 AIH cases. Female patients aged 15-25 years also comprised 25% of the total number of patients with anti-F-actin-like staining. 66.6% of females aged 15-25 years were diagnosed with type 1 AIH.

Table 1. Baseline clinical and laboratory data of ASMA positive patients with and without anti-f-actin-like staining

	Patients with anti-F-actin-like staining	Patients without anti-F-actin-like staining	p value
Demographic			
Female, n (%)	64.2%	51.7%	0.050
Age, years	49±20.3	53.5±17	0.065
ANA positivity (%)			
ANA (+)	45.8%	29.2%	0.008
ANA (-)	54.2%	70.8%	
ASMA titers (n,%)			
1 positive	45 (37.7%)	104 (86.7%)	<0.001
2 positive	44 (36.7%)	14 (11.7%)	
3 positive	25 (20.8%)	0	
4 positive	6 (5%)	2 (1.7%)	
AIH			
Presence of AIH	41 (34.2%)	20 (16.7%)	0.002
Absence of AIH	79 (65.8%)	100 (83.3%)	
Laboratory data			
AST (U/L)	34 (5-1720)	26 (3-979)	<0.001
ALT (U/L)	45 (6-1357)	30 (5-507)	0.002
ALP (U/L)	97 (37-986)	92 (37-694)	0.298
GGT (U/L)	56.5 (6-1081)	34 (6-623)	0.052
Neutrophils ($\times10^9$/L)	7.25 (0.460-14.6)	6.98 (2.04-22.1)	0.997
Albumin (g/L)	42.5 (20.3-55.5)	44 (16.6-51.9)	0.117
Total bilirubin (mg/dL)	0.640 (0.120-17.8)	0.600 (0.230-19.3)	0.246
CRP (mg/L)	10.3 (3-85)	15.5 (2-140)	0.062
ESR (mm/h)	20 (3-85)	15.5 (2-140)	0.101
Ig M (g/L)	1.14 (0.203-6.75)	1.04 (0.181-2.93)	0.396
Ig G (g/L)	14.1 (6.18-79.2)	12.6 (2.19-43.5)	0.006
Ig A (g/L)	2.41 (0.06-9.8)	2.29 (0.50-14)	0.337

Table 2. Baseline clinical and laboratory data of autoimmune hepatitis patients with anti-F-actin-like staining and without anti-f-actin-like staining

	Autoimmune hepatitis patients with anti-F-actin-like staining	Autoimmune hepatitis patients without anti-F-actin-like staining	*p* value
Demographic			
Female, n (%)	68.3%	65%	0.797
Age, years	47±22.2	54±15.9	0.209
ANA positivity (%)			
ANA (+)	39%	61%	0.123
ANA (-)	60%	40%	
Laboratory data			
AST (U/L)	148 (12-1720)	25.5 (10-65)	<0.001
ALT (U/L)	189 (11-1357)	25.5 (8-65)	<0.001
ALP (U/L)	140 (37-584)	89.5 (57-171)	0.054
GGT (U/L)	107 (9-1081)	22 (11-157)	<0.001
Neutrophils (×10^9/L)	7.15 (0.460-14.6)	6.52 (2.22-15.2)	0.585
Albumin (g/L)	40.3 (20.3-50.5)	44 (20.1-49.4)	0.009
Total bilirubin (mg/dL)	1.34 (0.255-17.8)	0.740 (0.420-2.30)	0.015
CRP (mg/L)	12.2 (0.500-153)	1.19 (0.500-50.2)	0.015
ESR (mm/h)	26.5 (4-85)	18 (3-73)	0.313
Ig M (g/L)	1.10 (0.213-6.75)	0.991 (0.418-2.93)	0.644
Ig G (g/L)	18.6 (7.38-40.5)	13.3 (4.53-43.5)	0.052
Ig A (g/L)	2.59 (1.03-9.8)	1.99 (1.10-8.16)	0.265

In the group with the anti-F-actin staining pattern, there was no significant correlation with ANA positivity as the actin titer increased (p=0.210), while the presence of type 1 AIH was significantly higher with increasing titer (p<0.001). Also, in the overall group of patients, the number of people with type 1 AIH was significantly higher as the ASMA titer increased (p<0.001). In addition, ASMA titer increased significantly as actin titer increased (p<0.001). While 20.2% of patients with anti-F-actin titers of 1/160 had a diagnosis of type 1 AIH, 56.5% of patients with titers of 1/320 and higher had a diagnosis of type 1 AIH, and high anti-F-actin titers were associated with type 1 AIH (p<0.001).

The ANA patterns in the group with anti-F-actin staining patterns were mixed pattern 10%, speckled pattern (AC 4/5) 22.5%, homogeneous pattern (AC-1) 6.6%, nucleolar pattern (AC 8/9/10), 4.1%, DFS pattern (AC-2) 0.9%, and envelope pattern(AC-11/12) 2.5%, and ANA positivity was detected in 46.6% of patients. In the group without anti-F-actin staining pattern, ANA patterns were mixed pattern 5.8%, speckled pattern (AC-4/5)12.5%, homogeneous pattern (AC-1) 1.6%, nucleolar pattern (AC-8/9/10) 7.5%, nuclear dotted pattern (AC-6/7) 0.9%, and envelope pattern (AC 11/12) 0.8%, and ANA positivity was detected in 29.1% of patients. LKM and AMA positivity were not found in our patients.

The distribution of diseases in the group with and without F-actin staining pattern are given in Table 3 and Table 4, respectively.

Table 3. Distribution of diseases in the group with the anti-F actin like staining pattern

Disease	Number
Type 1 AIH	41
Other liver problems (such as liver cirrhosis, HBV hepatitis, liver abscess, hepatosteatosis, or high liver function tests)	22
Chronic renal failure	9
Malignity	7
Diabetes	6
Infections (such as bronchitis, acute gastroenteritis, or pneumonia)	5
Interstitial lung disease	3
Stroke	3
Ulcerative colitis	3
Chronic gastritis	3
Other diagnoses (such as Amyloidosis, eosinophilia, hypothyroidism, vertigo, urticaria, rheumatoid arthritis, or chronic obstructive pulmonary disease)	7
Non-specific symptoms such as headache, abdominal pain, and joint pain.	11

Table 4. Distribution of diseases in the group without anti-f actin-like staining staining pattern

Disease	Number
Other liver diseases (such as liver cirrhosis, HBV hepatitis, hepatosteatosis, increased liver function tests)	29
Type 1 AIH	20
Malignity	7
Chronic renal failure	4
Chronic obstructive pulmonary disease	4
Diabetes	3
Infection (such as acute gastroenteritis, HIV, osteomyelitis)	3
Hypothyroidism	3
Celiac disease	2
Other diagnoses (such as acute pancreatitis, atherosclerotic heart disease, atrophic gastritis, anemia, dermatitis, deep vein thrombosis, Guillain-Barre syndrome, pituitary adenoma, idiopathic thrombocytopenic purpura, proteinuria, pulmonary hypertension, cystic fibrosis,urticaria, ulcerative colitis, gallstones, and heart failure.)	16
Non-specific symptoms such as headache, abdominal pain, arthralgia, and dyspepsia	29

DISCUSSION

ASMA, which is directed against the actin protein, is a diagnostic marker for AIH type 1 (9). AIH type 1 is a rare disease. To address this issue, we conducted a single-center study to assess the diagnostic usefulness of anti-F-actin antibodies. The biological starting point was to determine the existence of ASMA, which reacts specifically against actin. The measurement of anti-F-actin is critical for further characterizing ASMA. However, no "gold standard" approach is currently available. Presumably this is one of the reasons why most centers continue to employ ASMA reactivity in rodent stomachs (10).

AIH was enhanced when only young females were considered alone: 66.6% of the 15- to 25-year-old women studied (with anti-F-actin antibodies) suffered from type 1 AIH. When looking at the levels of anti-F-actin antibodies, high levels (≥1/640) were more likely to be linked to type 1 AIH, while low levels (≤1/160) were more often found in conditions that are not AIH. These data were similar to a multicenter study conducted in France (11).

IIF is a fundamental component of the immunoserology of AIH and is the standard technique for detecting non-organspecific autoantibodies, such as ASMA, as recently delineated in a consensus statement (12). Nevertheless, the interpretation of the immunofluorescence patterns is contingent upon the observer's experience, particularly in the assessment of ASMA reactivity. A significant number of laboratories are not accustomed to defining ASMA patterns, as our laboratory is (13,14). The ASMA-tubuler/glomerular pattern is highly predictive of type 1 AIH, with a sensitivity of approximately 80% and a specificity exceeding 90%. Conversely, the ASMA-vascular pattern is regarded as less specific (15). The immunomorphological expression of an autoreaction targeting F-actin is the ASMA-tubular/glomerular pattern, as previously demonstrated (13). Consequently, a diagnostic instrument that is both dependable and user-friendly for the detection of anti-F-actin reactivity would be highly beneficial in the context of the diagnosis of type 1 AIH (16).

However, the type 1 AIH specific target of ASMA that is responsible for the VGT pattern remains elusive (17). Nevertheless, numerous studies indicate that ASMA may be targeting actin in its polymerized filamentous form, rather than its monomeric G actin form, through the VGT pattern. Nevertheless, purified F-actin does not react with approximately 20% of AIH-specific ASMA-positive cases, and anti-F-actin positivity is observed in diseases that are distinct from type 1 AIH (12,18, 19). ASMA are not specific to AIH, as they have been reported in advanced liver disease of other etiologies, infectious diseases, and rheumatic disorders, similar to ANA (20). However, in our laboratory, ASMA is exclusively reported as either actin or non-actin. Regrettably, we are unable to provide a report of ASMA based on vascular, glomerular, or tubular staining.

In accordance with a previous retrospective study, approximately 20% of type 1 AIH patients who were ASMA positive lacked anti-F-actin reactivity (21). Nevertheless, a study found a highly significant correlation between anti-F-actin antibody and ASMA-T/G, which further confirms the ASMA-T/G pattern's actin specificity, its strong association with type 1 AIH, and its significant diagnostic value (22). AIH was present in 34.2% of the patient group with anti-F-actin-like staining in our study, while it was present in 16.7% of the patient group without anti-F-actin staining (p=0.002).

The presence of anti-F-actin antibodies in a study of type 1 autoimmune hepatitis patients was linked to elevated IgG levels, yet it did not correlate with any other clinical, laboratory, histological, or immunogenetic parameters, indicating it does not delineate a specific subset of the disease among type 1 autoimmune hepatitis patients (22). This observation differs from that of Czaja et al., who identified a substantial association between anti-

actin reactivity and the HLA-B8 and DR3 alleles, correlating with a poorer prognosis (18). The elevated prevalence of HLA DR3 in North American patients, coupled with the diverse methodologies employed to identify anti-actin reactivities, may elucidate this inconsistency (23). The kinetics of anti-F-actin antibody emergence and decline during therapy and follow-up align with a recent research (24). In our investigation, laboratory data comparisons between the two groups revealed elevated AST, ALT, and immunoglobulin G levels in the group exhibiting the anti-F-actin staining pattern. Patient follow-up was not conducted in our study.

In a study, with respect to anti-F-actin antibody titers, 58% of patients with levels of 1/640 or higher had type 1 AIH, whereas 22% did not (p<0.0001) (17). In our study, while 20.2% of patients with anti-F-actin titers of 1/160 had a diagnosis of type 1 AIH, 56.5% of patients with titers of 1/320 and higher had a diagnosis of type 1 AIH, and high anti-F-actin titers were associated with AIH (p<0.001), and these data are consistent with our study.

ANA, the first autoantibody associated with AIH (25), is easily identifiable and exhibits a nuclear staining in the kidney, stomach, and liver sections. The pattern is typically homogeneous, particularly in the liver, with a coarsely or finely speckled pattern occurring less frequently (26). HEp2 cells, which are distinguished by their prominence of nuclei, should be employed to obtain a more precise specification of the nuclear pattern. Nevertheless, they should not be employed for screening purposes due to their high positivity rate in healthy adult and pediatric populations (27,28). In AIH, the target antigens of ANA are not entirely defined and are heterogeneous.

The presence of novel methodologies employing recombinant nuclear antigens and immunoassays is anticipated to enhance the characterization of ANA target antigens, while simultaneously evaluating their diagnostic specificity and potential involvement in the pathophysiology of type 1 autoimmune hepatitis (29). The mechanism behind ANA generation in AIH remains unclear, however it has been associated with the release of nuclear components due to hepatocyte injury and/or a breakdown of B cell tolerance to certain nuclear components (30). Antinuclear antibodies (ANA) are found not only in various autoimmune disorders, including systemic lupus erythematosus (SLE), Sjögren syndrome, and systemic sclerosis, but also, at low titers, in patients with viral hepatitis, drug-induced hepatitis, and both alcoholic and non-alcoholic fatty liver disease; therefore, while regarded as diagnostic markers, ANA lack specificity for autoimmune hepatitis (AIH).

In a previous study including anti-F-actin antibodies, twenty-eight patients exhibited liver illness, including viral hepatitis C. Other disorders were also linked to these antibodies. Connective tissue diseases (e.g., systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis) accounted for 20 cases (12%), along with autoimmune endocrine disorders (e.g., thyroiditis, pernicious anemia) and inflammatory bowel diseases (e.g., Crohn's disease, celiac disease) (11). In our study, 34.1% of patients were diagnosed with type 1 autoimmune hepatitis (AIH), 18.3% with various hepatic disorders, and 7.5% with autoimmune conditions including type 1 diabetes, celiac disease, pernicious anemia, rheumatoid arthritis, idiopathic thrombocytopenic purpura, and ulcerative colitis.

We also found anti-actin antibodies in patients with ulcerative colitis, as well as celiac disease. Our results agree with those published in a previous study (31). Using both ELISA and IIF on fibroblast cells, 46% of patients with celiac disease presented antimicrofilament antibodies (IgA). Moreover, in another study, they described two cases of adults with celiac disease with an atypical antibody pattern: no antiendomysium or antigliadin could be found in their sera, but the authors detected antithyroid antibodies and anti-gastric parietal cells as well as antiactin antibodies (32). It is well known that hepatitis may be associated with celiac disease,

autoimmune thyroiditis, or pernicious anemia. The results of our study are in line with these findings.

Our study's limitations include the fact that it was a single-center study, vessel, tubule, and glomerulus evaluation was not possible during ASMA evaluation, there were no anti-LKM positive individuals, and we only had patients with type 1 AIH.

In conclusion, anti-F-actin antibodies can be found in numerous disease settings, including both liver and nonliver diseases, although mainly at low levels, but they are of significant interest to the diagnosis of type 1 AIH, particularly in young women.

ETHICS COMMITTEE APPROVAL

* The study was approved by the Ankara Bilkent City Hospital Non-Interventional Clinical Research Ethics Committee (Date: 12.07.2023 and Number: E2-23-4480).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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