Evaluation of patients with positive anti-mitochondiral antibody and normal alkaline phosphatase levels for primary biliary cholangitis

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Abstract

Primary Biliary Cholangitis (PBC) is a chronic cholestatic liver disease typically diagnosed by elevated cholestatic liver enzymes and a positive anti-mitochondrial antibody (AMA) test. The clinical importance of AMA positivity in patients with normal cholestatic liver enzymes is unclear. The aim of this study was to determine the relationship between PBC and AMA positivity detected in individuals with normal cholestatic enzyme levels. The files of patients with AMA and/or AMA-M2 positivity between 2009 and 2018 and whose alkaline phosphatase (ALP) levels were below upper limit of normal (ULN) at initial admission were retrospectively analyzed. The ALP levels were normal in all patients. All patients had AMA positivity demonstrated by indirect immunofluorescence (IIF) or AMA-M2 positivity demonstrated by ELISA. A total of 16 patients underwent liver biopsy and seven (43.75%) showed changes consistent with those with PBC. A total of 12 patients were diagnosed with PBC and were treated and followed up with this diagnosis.

People with AMA positivity and normal cholestasis enzyme levels are closely associated with PBC. Some of these patients were diagnosed with PBC as a result of biopsy and some were diagnosed by clinical and laboratory findings during follow-up.. The patients with an AMA titration of 1/20 were not associated with PBC. In our study, results similar to the studies confirmed by biopsies were obtained. In this regard, there is a need for prospective and retrospective studies with longer follow-up periods. (Acta gastroenterol. belg., **2024**, 87, **282-286).**

Keywords: primary biliary cholangitis, alkaline phosphatase.

Introduction

Primary biliary cholangitis (PBC) is a chronic, nonsuppurative, and cholestatic disease of the liver with granulomatous inflammation, mostly observed in women. The diagnosis of PBC is based on established criteria that include cholestatic liver tests, a positive antimitochondrial antibody (AMA) test, and diagnostic or compatible liver biopsy findings (1). The disease has a varying clinical course. If untreated, PBC can progress to advanced fibrosis, cirrhosis, and might require a liver transplantation. With the use of ursodeoxycholic acid (UDCA) in the treatment, a considerable improvement in disease survival has been achieved (2). It usually presents with symptoms such as pruritus and malaise, but most patients are diagnosed in the asymptomatic period. Elevated cholestasis enzymes – alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) – together with AMA positivity are diagnostic for PBC. Liver biopsy is not essential for the diagnosis but has an important role in determining the prognosis of the disease. (3). Liver biopsy should be performed when the diagnosis of PBC is uncertain or where another superimposed diagnosis (AIH or non-alcoholic steatohepatitis) is suspected. if a liver biopsy is performed, histologic evidence of PBC, such as non-suppurative cholangitis and destruction of small or medium-sized bile ducts, is highly suggestive of the diagnosis of PBC (4)

Anti-mitochondrial antibody (AMA) is observed in 90%-95% of the patients (5) AMA has a high specificity for PBC in people with elevated cholestatic enzymes (6). The incidence in the general population is approximately 1%. However, the information about the relationship between AMA positivity and PBC in individuals with normal cholestasis enzyme values is unclear (7). There are very few studies on this subject. Although some studies reported that AMA positivity is predictive of PBC, others reported differently.

We think it is more likely that AMA positivity at high titrations in patients with these characteristics reflects the early stages of PBC. Improvements in the course of the disease will be evident with the detection of the disease at earlier stages and with early treatment. In this study, our aim was to determine the relationship between PBC and AMA positivity in patients with normal cholestatic enzyme levels, to evaluate the clinical and laboratory findings of these patients during follow-up, and to determine the treatments received, if any, and their efficacy.

Materials and methods

Patient group

The files of the patients with positive AMA and/or positive AMA-M2 detected for various reasons; admitted to Ege University, Faculty of Medicine, Gastroenterology Clinic between 2009 and 2018; and whose ALP levels were below the ULN at the time of initial admission

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Submission date: 05/06/2023
Acceptance date: 02/05/2024

were retrospectively analyzed. A total of 39 patients were analyzed. Thirteen patients who did not meet the study criteria were excluded.

Inclusion criteria

1. Age >18 years.
2. Detection of normal ALP levels at the time of initial admission.
3. Positive AMA (by IIF) and/or positive AMA-M2 (by ELISA).
4. Identifying sufficient data.

Exclusion criteria

1. Detection of high levels of ALP levels than previous examination.
2. Previous use of UDCA.
3. Negative AMA and AMA-M2.
4. Lack of sufficient data

Method

The sex of the patients, reasons for AMA examination, AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT, serum total protein, serum albumin levels, platelet counts, AMA (by IIF method), AMA-M2 (by ELISA), anti-nuclear antibody (ANA) (by IIF), liver biopsy and pathology results, if any, the most recent diagnoses, and treatments administered were recorded. The treatments received during the follow-up of the patients were divided as UDCA, corticosteroid, corticosteroid + azathioprine, and azathioprine groups and recorded. Criteria for the diagnosis of PBC: 1) ALP at least ≥1.5 times above the ULN and/or GGT at least ≥5 times above the ULN 2) AMA titer of ≥1/40 (by IIF) and/or positive AMA-M2 (by ELISA) 3) Histologic evidence of PBC (nonsuppurative destructive cholangitis and intralobular bile damage). The follow-up periods of the patients until the date of analysis were recorded. The Death Notification System was used for deceased patients. The follow-up periods of these patients were calculated with the date found in this system. The cause of death of the deceased patients were recorded.

Immunological methods

Anti-human immunoglobulin fluoresced by IIF was used for AMA. AMA-M2 was analyzed by ELISA by using E2 component of pyruvate dehydrogenase complex and 2-oxogluterate dehydrogenase complex. AMA was analyzed in all patients except for three. In these three patients, only AMA-M2 antibody was analyzed.

Histological analysis

Liver biopsy was performed percutaneously under ultrasound guidance by experienced gastroenterologists. All samples were placed in paraffin blocks. They were stained with H&E (hematoxylin and eosin) and Masson's trichrome. The samples were examined by experienced hepatopathologists who did not know the patients' history.

Table 1. — **Reasons for requesting AMA examination and number of patients**

Reasons for requesting AMA examination	Number of patients (%)
Transaminase elevation	8 (%30.76)
Pruritus	5 (%19.23)
Sicca syndrome	2 (%7.69)
Cirrhosis	3 (%11.53)
Allergy	2 (%7.69)
Arthralgia	1 (%3.84)
Fatty liver and elevated transaminases	2 (%7.69)
Interstitial pulmonary fibrosis	1 (%3.84)
Suspicion of a mass in the liver	1 (%3.84)
Rheumatoid Arthritis	1 (%3.84)
Total	26 (%100)

Result

Analysis of demographic and laboratory results

The mean age of the patients included in our study was 56.8 ± 14.9 years, and 23 patients were women (88.46%) and three were men (11.54%).The most common reason for requesting AMA examination was transaminase elevation. In ten of 26 patients (38.45%), transaminases were elevated. In five of the patients with elevated transaminases, transaminases were found to be two times higher than ULN. Of these patients, four were diagnosed as AIH and one as nonalcoholic steatohepatitis (NASH). The reasons for an AMA examination in patients are summarized in Table 1. ALP levels were found to be normal in all patients. AST and ALT levels were normal in 17 patients (65%) and two times below ULN in 21 patients (80.76%), GGT was normal in 13 patients (50%) and two times below ULN in 24 patients (92%), and thrombocytopenia (<150 × 10^9/ml) was seen in four patients (15.38%). Analysis of AST, ALT, serum total protein, serum albumin levels, ALP, GGT, and platelet count values of the patients at the time of initial presentation is shown in Table 2.

Analysis of AMA levels

Of the 26 patients included in the study, three were AMA negative and AMA-M2 +2 or +3 positive. Twenty-three patients were AMA positive by IIF.There were five patients (19.23%) with AMA titration levels of <1/40.

Table 2. — **Laboratory parameters and analyses in patients**

Parameters (unit)	Mean	SD	Median	Distribution range (min-max)
AST(U/L)	63	98	23	(5-480)
ALT (U/L)	76	118	23.5	(8-500)
ALP (U/ L)	75	21	77	(17-110)
GGT (U/L)	35	27	24	(10-99)
T. protein (g/dL)	7.5	0.8	7.5	(6.3-9.2)
Albumin (g/dL)	4.4	0.5	4.5	(3.4-5.1)
PLT×10^9 / mL	241	96	237.5	(71-451)

Table 3 . — **Liver biopsy pathology results and distribution of numbers and percentages of patients**

Liver biopsy pathology results	Number of patients (n) (%)
PBC	4[1] (%25)
PBC + AIH overlap	2 (%12.5)
PBC + NASH	1 (%6.25)
Autoimmune hepatitis	1 (%6.25)
Chronic hepatitis + cirrhosis	1 (%6.25)
Acute hepatitis	1 (%6.25)
Minimal hepatitis	1 (%6.25)
Steatohepatitis	1 (%6.25)
Steatosis	1 (%6.25)
Non-specific	1 (%6.25)
Normal	2 (%12.5)
[1]All patients exhibit Stage 1 PBC.	

The remaining patients had titers of ≥1/40 (80.77%).

The most common AMA titration level found in these patients was 1/160. A total of four patients had <1/160 titration value positivity.

Analyses related to Biopsy, Diagnosis, Treatment, and Follow-up

Four of the patients who underwent biopsy were found to have PBC and two were found to have PBC + AIH overlap syndrome. Two patients were completely normal and one patient was found to have cirrhosis. According to the pathology results of the biopsies performed in patients with AMA positivity, a total of seven patients (four with PBC, two with PBC + AIH, and one with PBC + NASH) were found to have changes consistent with those with PBC.

Two patients who were diagnosed with overlap syndrome as a result of biopsy were accepted as PBC because they did not fulfill the diagnostic criteria. The patient diagnosed as PBC + NASH, presented with pruritus complaints during follow-up and they were followed up for PBC.

The treatments received by the patients during follow-up were divided into UDCA, corticosteroid, corticosteroid + azathioprine and azathioprine groups and recorded. A total of 13 patients were followed up with UDCA, three with corticosteroid + azathioprine, one with corticosteroid, and one with azathioprine. None of the patients were found to have used second-line treatments. In the UDCA group, no progression in disease course was observed in any patient during follow-up.

Only three of the patients died. One patient had signs of decompensated liver cirrhosis at the beginning of follow-up and died because of complications of decompensated liver cirrhosis, one patient died because of complications during dilatation of achalasia, and one died because of existing coronary artery disease and cardiovascular problems.

Five patients with AMA positivity (1/20) and three with AMA negativity and AMA-M2 positivity could not be diagnosed with PBC. Of the remaining 18 patients with AMA positivity at a titer of ≥1/40, 12 (66.66%) were diagnosed with PBC and followed up. A total of 7 of 12 patients were diagnosed with PBC by liver biopsy. In the remaining 5 patients, cholestatic enzymes increased during follow-up and were accepted as PBC. Of the six patients in whom PBC was not diagnosed, DILI was considered as a preliminary diagnosis in two patients, and non-specific and acute changes were observed in the pathology results of their biopsies. Of the remaining four patients, two patients had strong AMA and AMA-M2 positivity and were considered to have PBC. However, no data could be obtained because the patients refused biopsy.

Discussion

In this study, we presented new evidence showing that patients' having an AMA titration ≥1/40 and normal ALP values reflect early stages of PBC. The literature reveals only few studies on patients with these characteristics. In Turkey, this study was conducted for the first time.

In our study, 26 patients with an AMA titration ≥1/20 or isolated AMA-M2 positivity were evaluated. All patients had normal ALP enzyme levels. Among these patients,

PBC could not be diagnosed in five patients with an AMA titration of 1/20 and in three patients with isolated AMA-M2 positivity. Although AMA and AMA-M2 positivity together have high sensitivity and specificity for diagnosis, none of the patients with isolated AMA-M2 positivity were diagnosed with PBC in our study. Isolated AMA-M2 positivity should be considered with suspicion in terms of PBC. The remaining 18 patients had AMA positivity at a titer of ≥1/40. Twelve of these patients (67%) were diagnosed with PBC. In seven of the patients diagnosed with PBC, histologic changes related to PBC were detected in liver biopsy. The remaining five patients were diagnosed with PBC during follow-up. Early stage histologic changes were found in all patients diagnosed with PBC by liver biopsies. On the contrary, in patients with an AMA titration <1/40, PBC diagnosis should not be considered.

There are few studies that examined the association of people with normal cholestatic enzyme levels and AMA positivity with PBC. The earliest study on this subject was conducted in the UK in 1986 by Mithincson et al.In this study which included 29 patients with positive AMA 1/40 or more and normal cholestatic enzymes, all 12 patients who underwent liver biopsy were diagnosed with PBC. Sixteen of the remaining 17 patients were followed up for a median of 4 years and 11 of these patients were diagnosed with PBC during follow-up (8). In 1996, the 10-year follow-up results of this study were announced. In addition to the 22 patients in the previous study, 7 more patients who met the study criteria were included in the study. 28 of 29 patients underwent liver biopsy and 24 patients were diagnosed with PBC (9). The study showed that an AMA titration of ≥1/40 is considerable in terms of PBC despite the absence of clinical and laboratory findings for the disease. The fact that the follow-up period was 17.8 years and most of the patients had symptoms of PBC during the follow-up period suggests that strong AMA positivity is a predictor of PBC development. When compared with our study, the number of patients included is similar; both were conducted retrospectively in a single center; and revealed a strong relationship between PBC and AMA positivity in patients with normal cholestasis enzymes. The fact that the follow-up period was 17.8 years and most of the patients had symptoms of PBC during the follow-up period suggests that strong AMA positivity is a predictor of PBC development.

In a case series study published by Berdichevski et al., 4 of the liver biopsies performed in 6 patients with AMA positive and normal cholestatic enzymes were associated with PBC. There was also an association between high serum IgM levels and PBC. Since serum IgM levels were not measured in our study, we cannot comment on this issue (10). Another national, multicenter, and prospective study was published in France in 2017. The study used data from a total of 63 laboratories in France. 720 patients with AMA ≥1/40 positivity were divided into three groups: Patients previously diagnosed with PBC, patients with newly diagnosed PBC, and patients without any diagnosis. A group of 229 patients without any diagnosis was analyzed, and 92 patients were followed up for a mean 4.0 ± 1.8 years. PBC developed in nine of these patients (10%). None of the diagnosed patients underwent liver biopsy. These patients were diagnosed with PBC with the development of symptoms consistent with those with PBC or an increase in cholestasis enzymes during follow-up. (11). The lack of biopsy confirmation of the diagnoses in the study is an important deficiency. In addition, in the UK study, it was observed that the majority of these patients developed symptoms of PBC in long-term follow-up.In our study, liver biopsy was performed in 16 patients (61%). Histologic changes consistent with PBC were found in seven patients who underwent biopsy. All patients were AMA ≥1/40 positive. Therefore, we suggest that in patients with high AMA titrations and normal cholestatic enzyme levels, early histologic changes consistent with PBC may be detected by liver biopsy.

In two patients with DILI, the AMA titration level was found to be high. The biopsies performed in these patients showed non-specific and acute changes. It is known that AMA positivity is observed in 30% of drug-related hepatitis (12). There are several hypotheses about the cause of AMA positivity in these patients. The most likely reason seems to be that the immune system creates antibodies in these patients because the drug that causes hepatitis has similar epitopes with PBC (13). It should be kept in mind that AMA positivity at high titrations may be seen in these patients. In patients with high transaminases, normal ALP, and high AMA positivity, drug history should be questioned.

It is an intriguing question whether diagnosing PBC at an earlier stage and starting UDCA treatment at an earlier stage can improve the prognosis of PBC. Previous studies have shown that UDCA achieved better responses in patients with early stage PBC. In 2013, 67 patients with PBC in China were administered UDCA and followed up for two years. Symptom, laboratory, and histologic responses were observed to be better in early stage patients. In patients with advanced PBC, histologic response with UDCA was found to be weaker (14). It is also known that UDCA delays histologic progression and prolongs survival period. (15). However, UDCA has not been reported to be beneficial in correcting advanced stage fibrosis. Therefore, early diagnosis and treatment is crucial in PBC.

In the 2017 EASL guidelines, biopsy is not recommended for diagnosis in patients with only AMA positivity, and only follow-up of cholestasis enzymes is recommended (3). However, our study showed that when liver biopsy was performed in these patients, histologic changes consistent with PBC were observed in a considerable proportion. Therefore, UDCA treatment was initiated and followed up in all of these patients diagnosed with PBC. No clinical or laboratory abnormalities were detected in these patients during

the follow-up period. However, we cannot comment on whether this is due to the natural course of the disease or the result of UDCA treatment. Thus, long term follow-up studies are required.

Since PBC-specific antibodies (anti-gp120, sp100, and p62) were not studied in our hospital laboratory, no data could be obtained for these antibodies. Therefore, the presence of antibodies and their association with PBC could not be evaluated.

We can use the analogy of an "iceberg" to describe the PBC. The symptomatic group is the tip of an "iceberg." On the immersed side, there is the "silent group" with normal cholestatic enzyme levels and PBC histology and the "asymptomatic" group with elevated cholestatic enzymes. The immersed part may transition toward the visible side of the iceberg in the future. Therefore, we think that initiation of UDCA treatment in the "silent group" will prevent the progression of the disease.

Conclusion

We found that people with AMA positivity and normal cholestasis enzyme levels were closely associated with PBC. At least two-thirds of patients with an AMA titration ≥1/40 were diagnosed with PBC. Some of these patients were diagnosed with PBC as a result of liver biopsy and some were diagnosed by clinical and laboratory findings during follow-up. In the light of this information, we think that these patients are in the early stage of the disease and we suggest that histologic changes can be detected in more patients with PBC by performing liver biopsy. In patients with an AMA titration ≤1/40, PBC diagnosis should not be considered. Women contributed close to 90% to the patient population in our study and this finding is similar to the proportions seen in patients with PBC in literature. Although the rate of AMA positivity in general population screenings is around 1% and 10% among these patients are predicted to develop PBC, there are few biopsy-confirmed, prospective, or retrospective studies with long follow-up periods. We believe that further studies will confirm our findings.

Although AMA-M2 is a more specific test for PBC, PBC could not be diagnosed in patients with isolated AMA-M2 positivity in our study. Therefore, isolated AMA-M2 positivity should be regarded with suspicion for PBC. It should be kept in mind that AMA titration may be high in drug-related hepatitis.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding Statement

None.

Conflict of interest

The authors declare no potential conflicts of interest.

Ethics approval statement

This study was approved by the Ege University Ethics Committee with decision dated 16.08.2018 and numbered 2018 / 08 -15.

Author contributions

HIE, USA, ND: Methodology, Writing-Original draft preparation, Writing-Reviewing and Editing, Critical Review, HİE, USA: Conceptualization, Methodology, Writing-Original draft preparation, Writing-Reviewing and Editing, Critical Review. ND: Statistical analysis, Software, Visualization, Investigation, Validation, Writing-Original draft preparation, Critical Review. The final manuscript was reviewed and approved by all authors.

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