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• *BRIEF REPORTS* •

High concentration of antimitochondrial antibodies predicts progressive primary biliary cirrhosis

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Abstract

AIM: To evaluate the serum concentration of antimitochondrial antibodies (AMAs) as a prognostic indicator of progressive primary biliary cirrhosis (pPBC).

METHODS: Serum concentrations of AMA subtypes (anti-M2, anti-M4, and anti-M9), biochemical indices of liver function and Mayo risk factor (MRF) were determined in 30 women with diagnosed primary biliary cirrhosis (PBC) selected among 348 females with elevated alkaline phosphatase but without signs of hepatic decompensation. They were followed up for 5 years for possible development of hepatic decompensation.

RESULTS: Anti-M2 concentration was significantly correlated with bilirubin and albumin levels as well as MRF, whereas anti-M4 was significantly correlated with albumin level, prothrombin time and MRF. During the 5-year follow-up, progressive PBC (pPBC) was diagnosed in 3 among 23 patients available for evaluation. These 3 patients were positive for both anti-M2 and anti-M4. Anti-M2 serum concentration exceeded 1 300 RU/mL in patients with pPBC and only in 1 among 20 non-progressive PBC persons (5%). Anti-M4 serum concentration exceeded 400 RU/mL in 2 of the progressive patients and none in the non-progressive group. In contrast, anti-M9 serum concentration was below 100 RU/mL in all patients with pPBC, and higher than 100 RU/mL in 11 women (55%) among the non-progressive group.

CONCLUSION: Females with elevated alkaline phosphatase and high anti-M2 and anti-M4 concentrations are at a high risk for developing pPBC. Quantitative AMA detection should be considered as a method for early diagnosis of pPBC.

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INTRODUCTION

Primary biliary cirrhosis (PBC) is an autoimmune disease of unknown etiology leading to progressive destruction of small intrahepatic bile ducts and liver cirrhosis. It is characterized by female predominance (90%) with most cases observed between the ages of 40 and 60[1,2]. PBC incidence in different parts of the world is estimated to be 4-58 cases/million per year that can be affected by diagnostic efficacy[3-7]. Antimitochondrial antibodies (AMAs) are highly specific hallmarks for PBC diagnosis, but their prevalence in the general population and its relationship to the development of PBC are not well known[8]. Nine different types of AMAs have been described in non-hepatic and hepatic disorders. Among them only anti-M2, anti-M4, anti-M8, and anti-M9 are considered specific for PBC[9]. Retrospective studies have reported that subtypes of AMAs can discriminate between a benign and a progressive course of the disease[9,10]. However, it was recently reported that profiles can predict PBC prognosis[11]. All these studies focused on qualitative evaluation of AMA subtypes whereas quantitative measurement was not investigated for PBC management.

This study was to evaluate the serum concentration of different types of AMAs as a prognostic indicator of progressive PBC (pPBC).

MATERIALS AND METHODS

Patients

Thirty women aged between 30 and 65 years with diagnosed PBC were selected among 348 females with elevated alkaline phosphatase (ALP) activities at admission to the Liver Unit of the Department of Infectious Diseases, Medical University of Bialystok in 1999. PBC diagnosis was established according to American Association for the Study of Liver Disease (AASLD) Practice Guidelines and the presence of AMAs, elevated ALP and normal bile ducts on ultrasound[12]. All 30 patients demonstrated normal activities of serum alanine transaminase and no clinical signs of liver disease decompensation (jaundice, pruritus, ascites, encephalopathy, and shank edema). Further evaluations performed in PBC patients included quantitative measurement of serum concentration of AMA subtypes (anti-M2, anti-M4,

and anti-M9). Mayo risk factor (MRF) usually applied for estimation of PBC patient survival probability was also determined. Among these 30 patients, only 23 became available for evaluation during the 5-year follow-up. Patients who demonstrated clinical signs of hepatic decompensation were identified as having pPBC. In contrast, patients without any signs of decompensation during the follow-up were diagnosed as non-progressive PBC (nPBC). Baseline results were analyzed for the diagnosis of pPBC or nPBC within the follow-up period. Ethical approval for the study was obtained from the Bioethical Committee of the Medical University of Bialystok.

AMA measurement

Serum concentration of AMA subtypes was determined by ELISA (Euroimmun, Lubeck, Germany) for human autoantibodies of the IgG class against the mitochondrial antigens M2 (pyruvate dehydrogenase complex), M4 (sulfite oxidase) and M9 (glycogen phosphorylase). For qualitative evaluation, serum samples with extinction values exceeding the recommended cut-off level (20 RU/mL) for at least one subtype of AMAs were considered positive. Quantitative determination of AMA subtype serum concentration was performed using calibration sera provided by the manufacturer.

Liver function tests

Bilirubin and albumin concentrations were determined using a Cobas Mira instrument (Roche) and the prothrombin time (PT) was detected using Kselmed K-3002 (Poland). MRF was calculated according to the formula of Dickson *et al*.[13], based on the following variables: age, bilirubin and albumin concentration, PT, presence of peripheral edema and diuretic treatment.

Statistical analysis

Values were expressed as mean±SE. The significance of difference was calculated by two-tailed Student's *t*-test. For correlation analysis, the Pearson product moment correlation was performed. $P<0.05$ was considered to be statistically significant.

RESULTS

Among the screened 348 women, quantitative evaluation of AMA demonstrated positive results in 30 (8.6%). Anti-M2 was demonstrated in 13 (3.7%), including 5 (1.4%) with accompanying anti-M4. The remaining 8 women (2.3%) were positive only for anti-M2. In the other 5 (1.4%) women, the only anti-M9 was demonstrated. Anti-M4 without any other AMA was present in sera of 12 women (3.4%, Table 1). Quantitative evaluation demonstrated significantly higher concentrations of all three types of AMAs in persons with at least one type of AMAs present in qualitative evaluation (Table 2). As demonstrated in Table 3, anti-M2 concentration was significantly correlated with bilirubin and albumin levels as well as MRF, whereas anti-M4 was significantly correlated with albumin level, PT, and MRF. Anti-M9 was not correlated to any of these parameters.

During the 5-year follow-up, clinical signs of hepatic decompensation were demonstrated in 3 among 23 patients available for evaluation, and they were recognized as having pPBC. The remaining 20 patients were diagnosed as nPBC. All three women were positive for both anti-M2 and anti-M4. Anti-M2 serum concentration exceeded 1 300 RU/mL in all pPBC women and only in 1 among 20 nPBC patients (5%). Anti-M4 serum concentration exceeded 400 RU/mL in 2 pPBC patients and in none of the nPBC patients. Anti-M9 serum concentration was below 100 RU/mL in all pPBC patients, and was higher than 100 RU/ml in 11 nPBC patients (55%). As demonstrated in Table 4, the mean concentrations of anti-M2 and anti-M4 were about five times higher in pPBC patients than in nPBC patients, however statistical significance was shown only with respect to anti-M2. The mean concentration of anti-M9 was two-fold higher in nPBC patients (Table 4). As demonstrated in Table 5, the difference found in both groups in relation to biochemical indices of liver injury and MRF was not statistically significant.

Table 1 Profiles of AMAs in 348 screened women

	n	%
Anti-M2	8	2.3
Anti-M4	12	3.4
Anti-M9	5	1.4
Anti-M2+Anti-M4	5	1.4
Any AMA	30	8.6

Table 2 Serum concentrations of different types of AMAs in women with positive (at least one type of AMAs present) or negative AMAs (mean±SE)

	Qualitative AMAs		*P*
	Positive	Negative	
Anti-M2 (RU/mL)	378.1±96.8	6.1±1.3	3×10^{-15}
Anti-M4 (RU/mL)	140.5±27.7	18.1±1.9	2×10^{-17}
Anti-M9 (RU/mL)	112.5±25.9	35.8±3.8	2×10^{-7}

Table 3 Correlation between AMA concentration and biochemical indices of liver injury and Mayo risk score in asymptomatic AMA positive women

	Anti-M2	Anti-M4	Anti-M9
Bilirubin	0.410[a]	0.339	0.011
Albumin	–0.500[a]	–0.406[a]	0.119
PT	0.321	0.514[a]	0.002
Mayo risk score	0.415[a]	0.572[a]	–0.088

[a]$P<0.05$.

Table 4 Serum concentrations of different types of AMAs in patients with pPBC or nPBC during 5-year follow-up (mean±SE)

	pPBC	nPBC	*P*
Anti-M2 (RU/mL)	1 399±48	265±81	6×10^{-12}
Anti-M4 (RU/mL)	474±149	103±17	0.09
Anti-M9 (RU/mL)	49±15	120±28	0.03

Table 5 Serum concentrations of bilirubin and albumin, PT, and MRF value in patients with pPBC or nPBC during 5-year follow-up (mean±SE)

	pPBC	nPBC	*P*
Bilirubin (mg%)	2.2±1.1	0.76±0.1	0.241
Albumin (g%)	2.7±0.2	3.5±0.1	0.027
PT (s)	17.0±0.8	14.7±0.2	0.056
Mayo risk score	7.2±0.8	4.8±0.2	0.058

DISCUSSION

According to AASLD Practice Guidelines, the diagnosis of PBC can be made in AMA positive patients with biochemical evidence of cholestasis, and normal biliary system in ultrasound examination. In these patients, a liver biopsy is not essential to make the PBC diagnosis[12].

The prevalence of AMAs in the general population is usually estimated below 1%. According to recent studies AMAs are detected in 0.64% Japanese and 0.16% Chinese workers who have an annual health check[8]. Sakugawa *et al*.[14], demonstrated that 6 AMA among 122 women with elevated γ-glutamyl transpeptidase levels (4.9%) are positive for AMAs. The prevalence of AMAs in our study, that varied from 3.7% (anti-M2 positive) to 8.6% (any positive AMA subtype), was due to inclusion of Caucasian women aged 30-65 years with elevated ALP[3-6]. However, the purpose of this study was not to demonstrate AMA prevalence, but to capture as many asymptomatic AMA positive women as possible.

Progressive PBC can be predicted even at its early stages when antibodies against M2 and M4 are present in patient sera[9]. In our study the three patients with PBC progression during follow-up, were positive for both anti-M2 and anti-M4, suggesting that the presence of anti-M2 plus anti-M4 profile in asymptomatic persons can predict pPBC. Concentrations of these antibodies were significantly higher in pPBC than in nPBC. However, this profile demonstrated that in PBC patients, it is not possible to predict fatal prognosis, and the proportion of patients, who died of liver disease or transplantation does not differ among the AMA profiles[11].

Anti-M2 serum levels in PBC patients are closely associated with the degree of liver insufficiency[15]. There is a significant correlation between Child-Pugh and Mayo scores and both are useful for the estimation of survival probabilities[16]. Our study also confirmed this observation with respect to asymptomatic AMA positive women, because there was a significant correlation between serum concentrations of anti-M2 or anti-M4 and bilirubin, albumin or PT values. Moreover, in this association we also demonstrated regarding the MRF. Prince *et al*.[17], have shown that patient age, alkaline phosphatase, albumin, and bilirubin as well as Mayo prognostic score at diagnosis can independently predict survival. However, according to Krzeski *et al*.[18], the Mayo model overestimates death risk in PBC patients, and serum bilirubin concentration appears to be the only variable of prognostic importance. Corpechot *et al*.[19], demonstrated that elevated bilirubin and decreased albumin are predictive factors for cirrhosis development under ursodeoxycholic acid treatment. We demonstrated a significant difference only regarding albumin among possible biochemical and clinical predictive factors. However, the most important factor for pPBC development is the high level of anti-M2 and anti-M4 antibodies in sera of asymptomatic persons. Similar results but without quantitative AMA evaluation are demonstrated by Kisand *et al*.[20]. According to our results, serum concentration of anti-M2 exceeding 1 300 RU/mL and anti-M4 exceeding 400 RU/mL are critical for detection of pPBC. However, border-line levels need to be validated in a larger number of patients.

In conclusion, persons with elevated ALP and high concentrations of anti-M2 and anti-M4 are at a high risk of developing pPBC. Quantitative detection of these antibodies should be considered as a method for early diagnosis of pPBC.

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