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Screening of anti-mitochondrial antibody subtype M2 in residents at least 18 years of age in an urban district of Shanghai, China

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Abstract. – OBJECTIVE: To analyze the prevalence of anti-mitochondrial antibody subtype M2 (AMA-M2) in a healthy population, and characterize the clinical features and risk factors of primary biliary cirrhosis (PBC).

PATIENTS AND METHODS: AMA-M2 was screened by enzyme-linked immunosorbent assay (ELISA) in 19012 residents who received health checkup in Xuhui District of Shanghai. Other relevant liver biochemical markers and responses to questionnaires were reviewed and analyzed.

RESULTS: Total 133 residents (about 0.73% of the 19012 residents) were detected AMA-M2, including 33 males (0.40%) and 100 females (0.94%). PBC was confirmed in 25 residents. Pollution and household smoking were found to be related to PBC. In addition, the prevalence of M2 antibody was found significantly higher in the residents living near viaducts, especially the intersection region of multiple viaducts, than those who lives in other regions.

CONCLUSIONS: Although the incidence of PBC is low, it is not a rare disease, and PBC may be associated with the living environment and genetic factors.

Key Words:
Primary biliary cirrhosis, Anti-mitochondrial antibody subtype M2, Screening.

Introduction

Primary biliary cirrhosis (PBC) is an immune-mediated, progressive, and non-suppurative inflammatory disease of bile duct with uncertain etiology. PBC is usually complicated with intrahepatic cholestasis and the damage of intrahepatic bile ductules, eventually leads to liver fibrosis and cirrhosis[1]. The main clinical manifestations of PBC are pruritis, chronic obstructive jaundice and hepatosplenomegaly, and liver failure and signs of portal hypertension are main features at advanced stage[2]. Among the serum markers of PBC, anti-mitochondrial antibody (AMA) plays an important role, of which anti-mitochondrial antibody subtype M2 (AMA-M2) is specific for PBC. The specificity is up to 95%[3,4]. The AMA-M2 antibody may present in the patients with potential PBC several years or even more than 10 years before the development of abnormal biochemical, histological changes and clinical symptoms[5]. Therefore, mass screening for the AMA-M2 antibody is important in improving positive rate, facilitating early diagnosis, promoting early treatments of PBC, and reducing the occurrence of malignant complications.

This study aims to analyze the prevalence of AMA-M2 in a healthy population, the clinical characteristics, and risk factors of PBC, to summarize the screening results of AMA-M2 and other relevant liver biochemical markers, and to response to questionnaires in 19012 subjects who received health examination in Xuhui District of Shanghai.

Patients and Methods

Patients

From January 2012 to June 2014, 19012 residents who lived in Longhua, Kangjian and Lingyun communities in Xuhui District of Shanghai were randomly selected, including 8310 (43.7%) males and 10702 (56.29%) females. The age distribution of the patients is shown in Table I.

Blood Sample Collection

The blood samples of all the patients were drawn in the morning. About 5 ml blood from each patient was put into a centrifuge tube, fol-

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Table I. Number of AMA-M2-positive subjects by gender assayed by enzyme-linked immunosorbent assay.

Age group (years)	Male			Female			Overall		
	Number of samples	AMA-M2 positive, n	Positive rate, %	Number of samples	AMA-M2 positive, n	Positive rate, %	Number of samples	AMA-M2 positive, n	Positive rate, %
20-29	2873	0	0.00	1946	0	0.00	4819	0	0.00
30-39	462	0	0.00	311	1	0.32	773	1	0.13
40-49	188	0	0.00	153	0	0.00	341	0	0.00
50-59	420	3	0.71	844	19	2.25	864	22	1.74
60-69	1861	3	0.16	2803	24	0.86	5424	27	0.58
70-79	1485	14	0.94	2624	32	1.22	4009	46	1.12
80-89	928	10	1.08	1850	23	1.24	2528	33	1.19
≥ 90	93	3	3.23	161	1	0.62	254	4	1.57
Total	8310	33	0.40	10702	100	0.94	19012	133	0.70

AMA-M2, anti-mitochondrial antibody subtype M2.

lowed by centrifugation at 3000 rpm for 10 minutes. Then the supernatant was collected and stored at -80°C in the refrigerator.

Reagents and Methods

The serum sample was diluted to 1:200 for the test by enzyme-linked immunosorbent assay (ELISA) Kit (Shanghai Kexin Biotech Co., Ltd, Shanghai, China). Biochemical markers were determined using AU400 automatic biochemical analyzer (Olympus Optical Co., Ltd, Shizuoka, Japan). Four-parameter logistic regression was used to calculate the concentrations of AMA-M2 in the test samples. Concentrations equal to or greater than 25 RU/ml were regarded as positive. The questionnaire was designed by clinical experts on liver disease, which has shown good reliability and validity.

Design and Scientific Testing of the Questionnaire

Design of the questionnaire: China National Knowledge Infrastructure (CNKI) Chinese periodical full-text databases and PubMed were searched for full-text of relevant journal publications. "Primary biliary cirrhosis" was used as the search field and "influence factor", "morbidity", etc. were used as key words. "Exact" was selected as match mode to retrieve the articles related to the clinical diagnosis and epidemiological studies of primary biliary cirrhosis (PBC). A total of 215 articles were retrieved. The full-text of all the articles were downloaded and reviewed manually, of which 42 articles were included after review. Morbidity and related risk factors that mentioned in the 42 articles were extracted. A total of 37 items were proposed to form a draft of the questionnaire.

Expert Confirmation and Modification of the Questionnaire

A meeting was held for experts to argue and confirm the content of the questionnaire. Three clinical experts in the field of liver diseases from Shanghai Longhua Hospital and Public Health Clinical Center were invited to the meeting. After discussion and consultation with the experts, 5 obscure items were deleted and 2 items were combined. "Clinical Epidemiological Survey of Primary Biliary Cirrhosis" questionnaire was finally developed as a formal edition that contains 31 items.

Scientific Evaluation of the Questionnaire

A small sample pilot clinical survey was conducted in Longhua Hospital and Public Health Clinical Center. The questionnaires were administered to 104 PBC patients, and 101 patients responded to the questions. The average time to complete the questionnaire was 8 minutes. The responses to the questions were processed and analyzed using IBM SPSS Statistics Version 19.0 (IBM Corp., Armonk, NY, USA). Cronbach α coefficient of the questionnaire was 0.78 (greater than 0.7), which indicates the content measured by each item is homogeneous. Therefore, the questionnaire has shown adequate internal consistency and reliability. Principal component analysis was used to extract the number of factors from the correlation coefficient matrix of items. According to Kaiser rule of thumb, 4 factors were extracted initially after varimax rotation, and their cumulative variance contribution rate was 56%. Generally, if the rating scale can explain more than 50% of the variation and each item has enough strength of load (0.4) in the cor-

responding factor, the rating scale is regarded as having favorable structural validity. From the study results, this scale can be considered to have favorable structural validity.

According to the adequate reliability and validity results of the questionnaire, it can be concluded that the development of the survey questionnaire is scientific, rational and feasible. It can be used as a questionnaire for an epidemiological survey of primary biliary cirrhosis (PBC).

Diagnostic Criteria

PBC was diagnosed according to the American Association for the Study of Liver Diseases (AASLD) guidelines (2009) on PBC diagnosis[5].

Statistical Analysis

Statistical analysis was conducted using SPSS Version 19.0 software (IBM Corp., Armonk, NY, USA). A p-value of < 0.05 was considered as statistically significant. Measurement data were expressed as mean ± standard deviation (mean ± SD). One-way ANOVA was used for comparison of means between two independent samples. Least significant difference was used for comparison between groups. Univariate analysis was used to screen risk factors for suspected PBC.

Results

Health Screening Analysis

Distribution of AMA-M2 Antibody Prevalence in the Study Population

ELISA was used to screen AMA-M2 in the 19012 residents. AMA-M2 was positive in 133 patients (0.73%), including 33 males (0.40%) and 100 females (0.94%). The male/female ratio was about 1:3. Quantitative distribution of positive result for men and female in each age group is shown in Table I. The distribution of AMA-M2 concentration and corresponding age groups are shown in Figure 1.

Serological and Immunological Markers in AMA-M2-Positive Patients

All AMA-M2-positive patients were referred to Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine for diagnosis and treatment. According to the diagnostic criteria of AASLD, a total of 25 were confirmed, including 2 men and 23 women (1:11.5). The results of laboratory biochemical tests for 133 AMA-M2-positive patients are shown in Table II. Age distributions, liver functions and M2 antibody concentrations for the 25 confirmed cases are shown in Table III. Correlations between markers for all positive subjects are shown in Table IV.

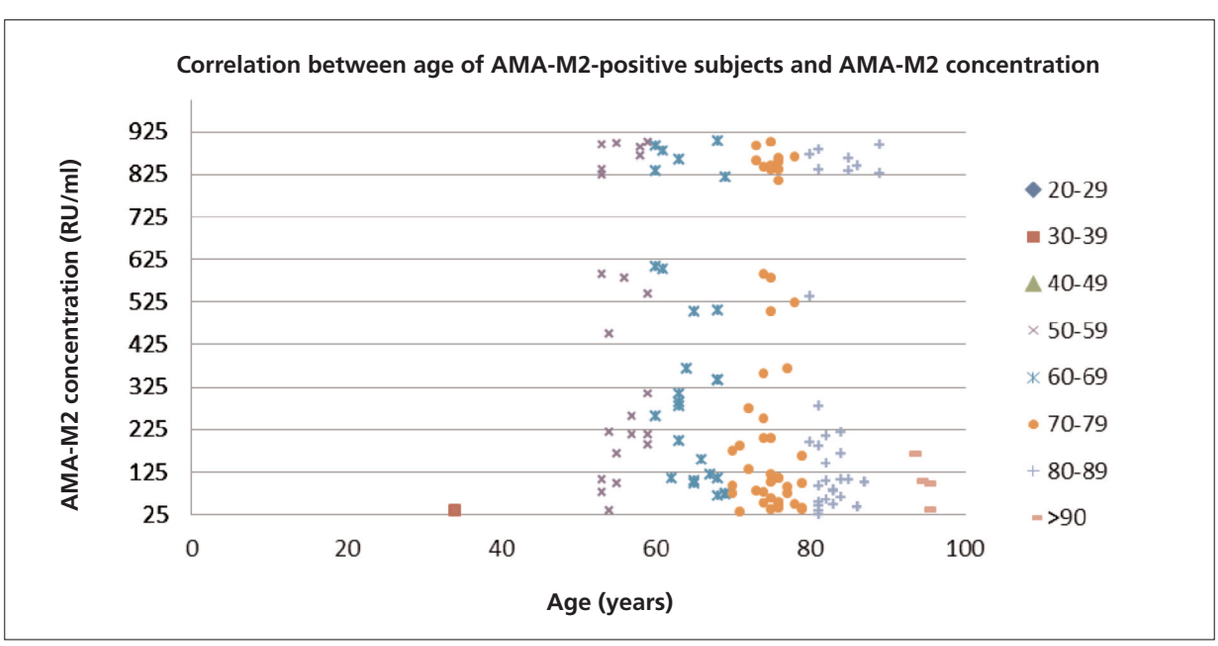

Figure 1. Distribution of AMA-M2 concentration and age of 133 AMA-M2-positive subjects. AMA-M2, anti-mitochondrial antibody subtype M2

Table II. Results of biochemical tests for 133 AMA-M2-positive subjects.

	Observed value	Reference range	Mean ± SD
HDL-C, mmol/l	0.64-2.65	0.91-1.60	1.36 ± 0.35
Triglyceride, mmol/l	0.61-5.59	0.51-1.7	1.69 ± 0.91
LDL-C, mmol/l	1.45-4.97	0-4.14	3.12 ± 0.82
Conjugated bilirubin, μmol/l	1.00-17.00	1.7-6.8	4.17 ± 2.42
Total cholesterol, mmol/l	2.64-7.88	2.1-5.7	5.26 ± 0.91
Glucose, mmol/l	4.3-15.24	3.9-6.1	5.84 ± 1.41
Total bilirubin, μmol/l	4.5-38	3.4-17.1	13.63 ± 5.47
Aspartate aminotransferase, U/L	6.3-98	0-40	30.03 ± 15.34
Alanine aminotransferase, U/L	5-117	0-40	39.78 ± 32.61
Alkaline phosphatase, U/L	24-506	40-150	112.82 ± 94.78
Gamma glutamyltranspeptidase, U/L	9-603	5-55	52.71 ± 70.86
AMA-M2, RU/ml	25.48-902	< 25	350.63 ± 321.55

AMA-M2, anti-mitochondrial antibody subtype M2; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Clinical Symptoms and Imaging Examination

Clinical pictures of PBC were mainly characterized by fatigue, pruritus, arthritis, and jaundice. Twenty-five patients with confirmed PBC were further followed up. Their clinical symptoms varied greatly. They also received an imaging examination. Their clinical symptoms, comorbidities and imaging examination results are shown in Table V and Table VI.

Questionnaire Survey on PBC Prevalence

Related Risk Factors of PBC

In the general population, the incidence of PBC is low. Twenty-five cases of PBC were confirmed in this screening survey, which was slightly higher than those reported in the literature. On this basis, a matched case-control study was designed to screen PBC-related risk factors. Control subjects were selected according to the ratio of 1:4 and were matched to cases in terms of ethnicity, gender and age. It can be seen that two factors such as pollution and household smoking, were significantly associated with PBC (Table VII). Univariate case-control study of risk factors for primary biliary cirrhosis in female population showed no correlation with PBC (Table VIII).

Geographical Distribution of PBC

The residences of the 133 AMA-M2-positive patients was reviewed and marked on the map (Figure 2). Red dots represent the dwelling of each patient. It is clear that the density near viaducts, especially intersection region of multiple viaducts, is significantly higher than that in other regions. This directly reflects that the prevalence of PBC is closely related to the living environment. However, no statistical analysis was done.

Table III. Liver function test results of 25 patients with confirmed primary biliary cirrhosis.

Age group (years)	Number of confirmed PBC		Liver function test (U/L)					AMA-M2 concentration (RU/ml)
	Male	Female	Total	ALT	AST	ALP	GGT	
50-59	0	4	4	78.20 ± 9.24	42.93 ± 6.84	308.75 ± 67.46	124.00 ± 80.28	> 800
60-69	0	4	4	98.75 ± 11.09	45.25 ± 19.97	296.25 ± 101.51	90.00 ± 53.71	> 800
70-79	1	6	7	89.43 ± 24.76	28.43 ± 13.62	276.71 ± 130.89	147.14 ± 101.72	> 800
80-89	1	9	10	90.20 ± 40.55	39.33 ± 20.71	269.70 ± 105.09	136.80 ± 174.41	> 800

PBC, primary biliary cirrhosis; AMA-M2, anti-mitochondrial antibody subtype M2; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltranspeptidase.

Table IV. Correlation between biochemical markers in 133 AMA-M2-positive subjects.

		TBIL	CBIL	ALT	AST	ALP	GGT	GLU	TC	TG	HDL-C	LDL-C	AMA-M2
TBIL	Pearson correlation	1	0.798**	0.147	0.347**	0.139	0.391**	0.051	0.119	0.032	0.113	0.080	0.089
	Sig. (2-tailed)		0.000	0.090	0.000	0.112	0.000	0.558	0.171	0.713	0.196	0.363	0.308
DBIL	Pearson correlation	0.798**	1	0.067	0.338**	0.087	0.419**	0.068	0.004	-0.113	0.066	-0.013	0.018
	Sig. (2-tailed)	0.000		0.440	0.000	0.321	0.000	0.434	0.965	0.195	0.452	0.878	0.836
ALT	Pearson correlation	0.147	0.067	1	0.464**	0.701**	0.383**	-0.051	0.063	0.127	0.102	-0.020	0.509**
	Sig. (2-tailed)	0.090	0.440		0.000	0.000	0.000	0.557	0.473	0.145	0.241	0.819	0.000
AST	Pearson correlation	0.347**	0.338**	0.464**	1	0.282**	0.514**	-0.023	0.079	-0.132	0.256**	0.002	0.174*
	Sig. (2-tailed)	0.000	0.000	0.000		0.001	0.000	0.790	0.364	0.131	0.003	0.981	0.045
ALP	Pearson correlation	0.139	0.087	0.701**	0.282**	1	0.582**	-0.072	0.052	0.117	0.116	-0.082	0.532**
	Sig. (2-tailed)	0.112	0.321	0.000	0.001		0.000	0.411	0.554	0.181	0.185	0.350	0.000
GGT	Pearson correlation	0.391**	0.419**	0.383**	0.514**	0.582**	1	-0.062	0.091	0.057	0.145	-0.020	0.234**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000		0.480	0.296	0.512	0.097	0.817	0.007
GLU	Pearson correlation	0.051	0.068	-0.051	-0.023	-0.072	-0.062	1	-0.063	-0.152	0.078	0.001	0.042
	Sig. (2-tailed)	0.558	0.434	0.557	0.790	0.411	0.480		0.473	0.081	0.374	0.992	0.628
TC	Pearson correlation	0.119	0.004	0.063	0.079	0.052	0.091	-0.063	1	0.152	0.410**	0.831**	-0.102
	Sig. (2-tailed)	0.171	0.965	0.473	0.364	0.554	0.296	0.473		0.081	0.000	0.000	0.244
TG	Pearson correlation	0.032	-0.113	0.127	-0.132	0.117	0.057	-0.152	0.152	1	-0.426**	-0.048	0.086
	Sig. (2-tailed)	0.713	0.195	0.145	0.131	0.181	0.512	0.081	0.081		0.000	0.581	0.322
HDL-C	Pearson correlation	0.113	0.066	0.102	0.256**	0.116	0.145	0.078	0.410**	-0.426**	1	0.215*	0.026
	Sig. (2-tailed)	0.196	0.452	0.241	0.003	0.185	0.097	0.374	0.000	0.000		0.013	0.770
LDL-C	Pearson correlation	0.080	-0.013	-0.020	0.002	-0.082	-0.020	0.001	0.831**	-0.048	0.215*	1	-0.181*
	Sig. (2-tailed)	0.363	0.878	0.819	0.981	0.350	0.817	0.992	0.000	0.581	0.013		0.037
AMA-M2	Pearson Correlation	0.089	0.018	0.509**	0.174*	0.532**	0.234**	0.042	-0.102	0.086	0.026	-0.181*	1
	Sig. (2-tailed)	0.308	0.836	0.000	0.045	0.000	0.007	0.628	0.244	0.322	0.770	0.037	

*Correlation is significant at 0.05 level (2-tailed); **Correlation is significant at 0.01 level (2-tailed); TBIL, total bilirubin; CBIL, conjugated bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltranspeptidase; GLU, glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AMA-M2, anti-mitochondrial antibody subtype M2.

Table V. Clinical symptoms of 25 patients with confirmed primary biliary cirrhosis.

Symptoms and imaging finding	Subjects, n	Patients, n	Percentage, %
Pruritus	25	10	40.0%
Dry mouth and eyes	25	10	40.0%
Urinary tract infection	25	3	12.0%
Jaundice	25	1	4.0%
Rough gallbladder wall	25	2	8.0%

Table VI. Comorbidity of 25 patients with confirmed primary biliary cirrhosis.

Comorbidity	Subjects, n	Patients, n	Percentage, %
Arthritis	25	6	24.0
Hyperlipidemia	25	3	12.0
Coronary artery disease	25	3	12.0
Hypertension	25	5	20.0
Fatty liver	25	2	8.0
Cataract	25	1	4.0

Table VII. Univariate case-control analysis of risk factors for primary biliary cirrhosis.

Factor	Case group (n = 25)		Control group (n = 100)		χ^2 or corrected χ 2	*p*	OR (95% CI)
	Yes	No	Yes	No			
Quality of life (X1)	24	1	95	5	0.000	1.000	1.263 (0.141-11.323)
Bad mood (X2)	4	21	14	86	0.000	1.000	1.170 (0.349-3.921)
Quiet (X3)	20	5	82	18	0.000	1.000	0.878 (0.291-2.651)
Pollution (X4)	**8**	**17**	**6**	**94**	**5.537**	**0.019***	**4.947 (1.440-16.999)**
Toxin (X5)	5	20	7	93	2.541	0.111	3.321 (0.956-11.537)
Urinary tract infection (X6)	5	20	18	82	0.000	1.000	1.139 (0.377-3.438)
Smoking (X7)	1	24	6	94	0.536	0.464	0.653 (0.075-5.683)
Household smoking (X8)	**6**	**19**	**19**	**95**	**6.785**	**0.009***	**6.000 (1.660-21.687)**
Higher education (X9)	4	21	21	79	0.313	0.576	0.717 (0.222-2.315)
Viral hepatitis (X10)	3	22	2	98	2.930	0.087	6.682 (1.053-42.409)
Rheumatic diseases (X11)	2	23	9	91	0.000	1.000	0.879 (0.178-4.350)
Autoimmune diseases (X12)	3	22	5	95	0.676	0.411	2.591 (0.575-11.665)
Metabolic disease (X13)	13	12	46	54	0.289	0.591	1.272 (0.529-3.059)
Lifestyle (X14)	22	3	89	11	0.000	1.000	0.906 (0.233-3.529)
Exercise (X15)	8	17	39	61	0.418	0.518	0.736 (0.290-1.868)
Osteoporosis (X16)	14	11	49	51	0.392	0.531	1.325 (0.549-3.199)
History of drug use (X17)	7	18	33	67	0.230	0.632	0.790 (0.300-2.077)
Alcohol drinking (X18)	1	24	4	96	0.000	1.000	1.000 (0.107-9.360)

*$p < 0.05$.

Table VIII. Univariate case-control study of risk factors for primary biliary cirrhosis (risk factors in female population).

Factor	Case group (n = 23)		Control group (n =92)		Corrected χ^2	*p*	OR (95% CI)
	Yes	No	Yes	No			
Abortion history (X1)	8	15	14	78	3.376	0.066	2.971 (1.061-8.319)
Pruritus in pregnancy (X2)	3	20	8	84	0.057	0.812	1.575 (0.383-6.475)
Menopause (X3)	20	3	80	12	0.000	1.000	1.000 (0.258-3.883)
Birth control pills (X4)	2	21	3	89	0.327	0.568	2.825 (0.444-17.992)

Result: The four factors above are not statistically significant.

Figure 2. Geographical distribution *vs*. incidence of primary biliary cirrhosis.

Discussion

In recent years, the incidence of PBC has been reportedly increasing year by year worldwide1. A study in the United States showed that during 1975-1995, the overall incidence of PBC was 27/1000000 per year, and the incidence in female and male populations were 45/1000000 and 7/1000000 per year. In 1995, the corresponding incidence was 402/1000000, 654/1000000 and 121/1000000, respectively. A study in Canada showed that during 1996-2002, the overall age/sex-adjusted incidence of PBC was 30.3/1000000 per year, and the incidence in female and male populations were 48.4/1000000 and 10.4/1000000 per year and prevalence were 100/1000000 in 1996 and 227/1000000 in 20022[6]. PBC was more prevalent in some areas of Northern Europe and North America; however, it was rarely seen in East Asia, Africa and Australia[7,8].

The present study showed that the prevalence of AMA-M2 antibody in the 19012 patients who received health examinations in Shanghai Xuhui District was 73/10000 (ELISA method), specifically 101/10000 women. The overall prevalence was slightly higher than the prevalence of 0.5% in 1530 subjects who received health examination in North Italy, and higher than the number of 0.16% (Immunospot assay) in 5011 subjects who received health examination in Shanghai. The prevalence in general adults who received health examination in south China was 492/1000000, of which the prevalence in women over 40 years old was up to 1558/1000000 (ELISA method)[9]. The different prevalence of AMA-M2 reported in the literature was mainly due to the differences of assay methods, gender and age distributions of population groups, and geographic regions. PBC was prevalent in people over 45 years old. Twenty-five confirmed PBC patients in this study were above 50 years of age, which were consistent with those reported cases in the literature.

So far, the pathogenesis of PBC is still unclear, and may be the interaction between genetic and environmental factors[10,11]. Familial clustering provided good evidence for its genetic susceptibility and high degree of consistency, and was seen in the identical twins[12-15]. In the first-degree relatives of PBC patients, the risk of developing PBC was 18.1 times higher than that in general population[16]. Environmental factors such as chemical factors and bacterial infections play a key role in breaking the immune tolerance of the individuals with genetic susceptibility[17,18]. Female hormones may be associated with the pathogenesis of PBC[9]. Alvaro et al[19] used the his-

tochemical method to study the liver biopsy specimens in the different histologic stages of PBC patients. It was found that estrogen receptor alpha (ER-α) was expressed at low level in PBC patients and disappeared in patients at the higher histological stage. These findings indicate that estrogen deficiency can contribute to the progress toward decrease of bile ducts in such diseases. Relevant studies found that estrogen can promote the responses of type 1 T helper (Th1) cells and increase the susceptibility to developing cell-mediated autoimmune diseases[20]. Two studies from Invernizzi et al[21,22] indicate that inadequate X-gene haploid might be the key factor for the predominance of autoimmune diseases in the female population. X -type monomer was more common in peripheral T and B lymphocytes than in other blood cells, and did not present in male fetuses. According to combined facts that PBC mainly affects postmenopausal women and the susceptibility of PBC might be associated with X-linked genes.

Early pathological changes in PBC can cause obstruction of small bile ducts, ductules and cholestasis, which result in the increase of relevant enzymes alkaline phosphatase (AKP) and gamma glutamyltranspeptidase (GGT) at early stage[23]. About 80% of PBC patients developed complications of other autoimmune diseases, including CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome, scleroderma, rheumatoid arthritis, dermatomyositis, mixed connective tissue diseases, autoimmune thyroid diseases, in particular, Sjogren's syndrome, accounting for about 75% of the patients. Among the 25 confirmed PBC patients in this study, 3 had other autoimmune diseases (2 with Sjogren's syndrome, 1 with rheumatoid arthritis). All patients had abnormally high levels of alanine transaminase (ALT) and AKP, 10 patients (40%) had elevated aspartate aminotransferase (AST) levels and 19 patients (76%) had elevated GGT levels. Correlation analysis showed that AMA-M2 concentration in AMA-M2-positive patients was significantly associated with ALT, alkaline phosphatase (ALP) and GGT ($p < 0.01$), as well as AST and low-density lipoprotein cholesterol (LDL-C) ($p < 0.05$). Meanwhile, for the AMA-M2 positive patients without abnormal liver function, the possibility of PBC cannot be completely ruled out if a liver biopsy is not available. They should be actively followed up to monitor the auto-antibodies and changes of liver function.

Research suggests that genetic factors may increase the susceptibility to PBC, but genetic factors alone are not enough to cause the disease. Studies have shown that smoking, toxic waste pollution, urinary tract infection due to E. coli and other environmental factors may be associated with the incidence of PBC, but the application of hair dyes, cosmetics such as nail polish and infection of retrovirus and Chlamydia pneumoniae were less associated with PBC[24]. Epidemiological studies have shown that recurrent urinary tract infections may be a factor contributing to PBC in female population[25]. Three PBC patients in this study had a urinary tract infection. Studies have shown that urinary tract infections[26,27] and smoking[28] were the most important risk factors for PBC. Furthermore, it has been reported that reproductive history[28,29], topical use of steroids[26-28] and the frequent use of cosmetics[25,28-30] were significantly associated with the incidence of PBC. Survey on the working environment of the 25 PBC patients showed that 5 patients had ever worked in coating, painting, chemical reagents and other chemical-related industries, which suggested that long-term exposure to toxic and harmful substances may also be a possible cause of PBC. Questionnaire survey found that environmental pollution and household smoking were causative factors of PBC. One hundred and thirty-three AMA-M2-positive patients lived near viaducts (especially intersection region of multiple viaducts) had a significantly high prevalence of M2 antibody, which was consistent with the relevant literature. This reflected the inductive effect of environment on PBC.

Conclusions

PBC is not a rare disease. Large sample and multicenter screening can better understand the incidence of the diseases and improve the prognosis of PBC patients. Comprehensive health questionnaire is helpful for better understanding the risk factors of PBC in our country and is of great significance for disease prevention.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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