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RESEARCH ARTICLE

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Epidemiological survey of antinuclear antibodies in healthy population and analysis of clinical characteristics of positive population

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Abstract

Background: In China, the incidence of autoimmune diseases is gradually increasing. To decrease the misdiagnosis rate of autoimmune diseases, we conducted an epidemiological investigation about the presence of antinuclear antibody (ANA) in healthy populations and analyzed the clinical characteristics of healthy population with both high titer of ANA and positive anti-SSA and AMA-M2.

Methods: Serum ANA titers were detected by indirect immunofluorescence (IIF), and other 15 types of ANA-specific antibodies were detected by line immunoassays.

Results: In 25 110 individuals for routine examination, the positive rate of ANA titer >1:100 was 14.01%, of which the positive rate of female (19.05%) was higher than that of male (9.04%; $P < 0.01$). The positive rate of ANA titer >1:320 was 5.93%, of which the positive rate of female (8.68%) was higher than that of male (3.21%; $P < 0.01$). The specific antibodies were detected in 1489 of ANA-positive people with titer >1:320, and the top three detected antibodies were anti-Ro-52 (212), AMA-M2 (189), and anti-SSA (144). The abnormal rate of blood routine test, liver function test, and other clinical indicators in AMA-M2–positive population was significantly different from those in the control group. The abnormal rate of blood routine test, liver function test, and immune index in anti-SSA–positive population was higher than those in control group.

Conclusion: There was a high prevalence of ANA positive in healthy population. To avoid misdiagnosis, those who had symptoms of abdominal discomfort, pruritus, or fatigue with abnormal results of blood routine and liver function test should be examined for ANA, AMA-M2, anti-SSA as early as possible.

KEYWORDS

AMA-M2, antinuclear antibody, anti-SSA antibody, clinical characteristics, misdiagnosis

1 | INTRODUCTION

Antinuclear antibodies (ANA) are a spectrum of immunoglobulins that react with various nuclear and cytoplasmic components of karyocytes and are majorly produced by plasma cells. ANA family includes more than 15 types of specific antibodies, such as anti-SSA and AMA-M2 antibodies, which have important values for clinical diagnosis. Previous studies have shown that AMA-M2 and anti-SSA antibodies could be detected

in the patients with primary biliary cholangitis (PBC) and systemic lupus erythematosus (SLE) several years before the disease onset.[1-3] ANA can directly act on the mitosis of karyocyte and stagnate the mitotic cycle at different stages, leading to the disorder of DNA synthesis and protein production, and which may further block the metabolism of various tissues, and even cause morphological and structural changes and the loss of function. Therefore, the vital tissues and vigorous organs for body metabolism have become the targets of ANA attack, such as liver, hematological system, epithelial tissue, and so on, which are also the initial sites of clinical symptoms in ANA-positive patients. Unfortunately, these symptoms are rarely connected to abnormal ANA by doctors in basic hospitals, which usually lead to a high rate of misdiagnosis in population. Therefore, we conducted an epidemiological investigation of ANA in healthy population and further explored the clinical characteristics in individuals with serum-positive AMA-M2 and anti-SSA antibodies, so that we could provide necessary data for clinical practice.

2 | MATERIALS AND METHODS

2.1 | Subjects

We collected samples from 25 110 residents who received health checkup in Baoding NO.1 central hospital, Baoding, Hebei, China, from January 2015 to June 2018. These participants included workers, peasants, cadres, students, kindergartens, and so on. The age range of the subjects was from 4 months to 93 years old.

The subjects were healthy individuals and patients diagnosed by AID, and those who may cause ANA positive in chronic active hepatitis, tuberculosis, and chronic infection have been excluded. In order to ensure the specificity of the results and exclude the effects of some interference factors, such as the elderly, some drugs, malignant diseases, and so on, we only chose the individuals with ANA titer >1:320. On the premise of the patient's voluntary, 15 specific antibody were also analyzed, including anti-SSA and anti-M2. The control group was negative for antibody detection, and the sex ratio was basically the same to that of the positive population. The study was approved by the Ethics Committee of Baoding NO.1 central hospitals, and informed consent was acquired from each individual.

2.2 | Detection of antibody

Blood samples (3 mL) were collected from 25 110 residents who received health checkup. Serum was separated by centrifugation at 900 *g* for 5 minutes. ANA were tested by indirect immunofluorescence on HEp-2 cells according to the manufacturer's instructions (Euroimmun AG). As a result, 1489 positive samples were further used by line immunoassay (LIA; Euroimmun AG) for 15 specific autoantibodies (anti-Ro52, anti-nRNP, anti-Sm, anti-SSA, anti-SSB, anti-Scl-70, anti-Jo-1, anti-CNEPB, anti-dsDNA, anti-PCNA, anti-His, anti-Nuc, anti-RIB, anti-M2, and anti-PMScl-70). The serum for LIA test was diluted 1:100. EUROBlotMaster (Euroimmun AG) and EUROLineScan (Euroimmun AG) were used to complete the operation and for test result interpretation, respectively.

2.3 | Statistical analysis

Statistical analysis was performed using SPSS Version 19.0 software (IBM Corp.). $P < 0.05$ was considered as statistically significant. Comparison of counting data between groups was performed using chi-square test.

3 | RESULT

3.1 | Positive rate of ANA in healthy population

A total of 25 110 residents were detected for ANA, including 12 640 males and 12 470 females. The positive rate of ANA titer >1:100 was 14.01% (3519/25 110), of which 9.04% of males (1143/12 640) and 19.05% of females (2376/12 470) was positive. The positive rate of ANA titer >1:320 was 5.93% (1489/25 110), of which 3.21% of males (406/12 640) and 8.68% of female (1083/12 470) was positive (Table 1). The difference in positive rates between genders was statistically significant.

3.2 | Distribution of ANA-specific antibodies in 1489 patients with ANA titer >1:320

A total of 1489 positive samples with ANA >1:320 population were further tested by line immunoassay (LIA) for 15 specific autoantibodies. The positive rate of 15 specific antibodies was 44.29% (659/1489). The top three antibodies were anti-Ro-52 (212), AMA-M2 (189), and anti-SSA (144) (Figure 1).

3.3 | Statistical analysis of PBC-related laboratory indicators in AMA-M2–positive patients

By detecting 15 specific antibodies, we found the high positive rate of AMA-M2 with a total of 189 of AMA-M2–positive cases, including 39 males and 150 females. Samples from 40 males and 160 females with negative ANA were randomly selected as controls. Our data demonstrated that the abnormal rate of blood routine, liver function, and other clinical indicators in 189 of AMA-M2–positive cases was significantly different from that in the control group (Table 2). We detected the biochemical indexes of liver function and found that alkaline phosphatase (ALP) was elevated in 70 patients, and the diagnostic rate of PBC was 37.4% (Table 3).

3.4 | Analysis of laboratory test indicators for anti-SSA antibody-positive population

The positive rate of anti-SSA antibody was high in the detection of 15 specific antibodies. A total of 144 of cases were positive in

TABLE 1 Distribution of antinuclear antibody-positive population by sex

ANA titer	Male, n (%)	Female, n (%)	χ^2	*P* value
ANA >1:100	1143 (9.04)	2376 (19.05)	522.06	<0.01
ANA >1:320	406 (3.21)	1083 (8.68)	337.05	<0.01

Abbreviation: ANA, antinuclear antibody.

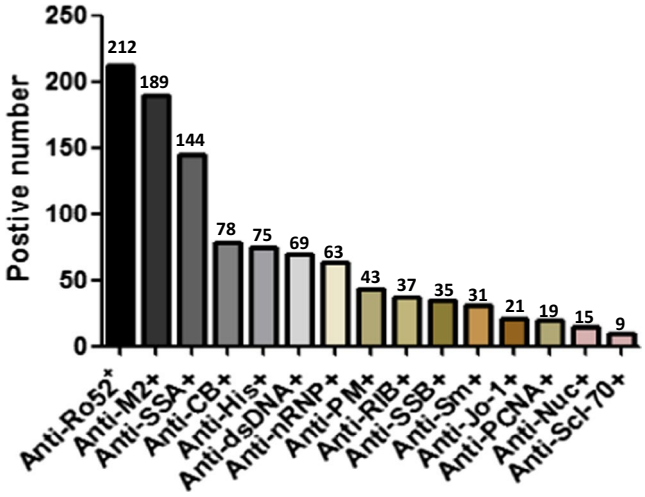

FIGURE 1 Distribution of antinuclear antibody (ANA)-specific Antibody in positive population. 15 types of ANA-specific antibodies were detected by line immunoassays

anti-SSA antibody, including 36 males and 144 females. 30 males and 120 females with negative ANA were randomly selected as control group. Compared with the control group, there were significant decreased blood formed element and increased erythrocyte sedimentation rate (ESR) in anti-SSA antibody-positive group. The abnormal rates of liver function, IgG, C3, C4, and rheumatoid factor (RF) in anti-SSA antibody-positive group were significantly different from those in the control group (Table 4).

4 | DISCUSSION

In this study, we retrospectively analyzed the positive rate of ANA in healthy population aged 4 months to 93 years old. The results showed that the positive rate of ANA titer >1:100 was 14.01%, which was basically consistent with Satoh's ANA test in healthy people over 12 years old in the United States.[4] The positive rate of females with ANA titer >1:100 was 2.08 times that of males, and positive rate of females with ANA titer >1:320 was 2.67 times that of males. It is suggested that female patients were at high risk of autoimmune diseases.

We detected specific antibodies in population with high titer of ANA positive and found that the top three positive antibodies were anti-Ro-52, AMA-M2, and anti-SSA antibodies, respectively. In this study, we focused on AMA-M2 and anti-SSA antibodies which had high specificity in autoimmune disease (AID).

Anti-mitochondrial antibody (AMA) is an autoantibody against mitochondrial inner membrane lipoprotein in cytoplasm. It is divided into nine subtypes, in which M2 subtype is a serological marker of PBC and plays an important role in the diagnosis of PBC. The specificity of AMA-M2 to PBC is up to 95%.[5,6] 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.[7,8] In this study, we analyzed the clinical symptoms associated with PBC in AMA-M2–positive population. The PBC is usually divided into four stages: the first stage is preclinical, generally only AMA positive in serum or bile duct epithelium. Only a small part of the AMA-M2–positive population did not find abnormal indicators and did not feel uncomfortable, which may be in the preclinical period, but it also should be paid great attention. It has been reported that 29 of AMA-positive population have no clinical symptoms. However, after 18 years of follow-up, the results show that 83% of them have abnormal liver function, 76% of them have fatigue and pruritus.[3] The second stage is liver dysfunction, which is mainly characterized by the increase in ALP, gamma-glutamyltranspeptidase (GGT), and other enzymes. In this investigation, we found that ALP, GGT, aspartate aminotransferase (AST), alanine aminotransferase (ALT), TB, and DB were increased in 80 cases of AMA-M2–positive group, which was significantly higher than that of

TABLE 2 Distribution of laboratory test results of primary biliary cholangitis-related indicators in AMA-M2–positive population and control group

Detection index	189 AMA-M2 positive, n (%)	200 AMA-M2 negative, n (%)	χ^2	*P* value
Abnormal blood routine[a]	87 (46.03)	16 (8.0)	72.2	<0.01
Abnormal liver function[b]	80 (42.32)	22 (11.0)	49.3	<0.01
Abdominal discomfort[c]	91 (48.15)	7 (3.5)	102.78	<0.01
Gallbladder lesion[d]	57 (30.16)	19 (9.5)	26.38	<0.01
Allergy	27 (14.29)	13 (6.5)	6.38	<0.05
Fatigue	46 (24.34)	5 (2.5)	40.68	<0.01
Pruritus	13 (6.88)	3 (1.5)	7.13	<0.01
Jaundice	4 (2.12)	0		

[a]Abnormal blood routine: Single decrease and mixed decrease in erythrocyte, leukocyte, and platelet.
[b]Abnormal liver function: Single increase and mixed increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), conjugated bilirubin (CBIL).
[c]Abdominal discomfort: including ventosity, celiakia, ructus, and intermittent sicchasia.
[d]Gallbladder lesion: including gallstones, post-cholecystectomy, and thickened gallbladder wall.

TABLE 3 Distribution of liver function biochemical indicators in AMA-M2–positive population

Detection index	ALT	AST	GGT	ALP	CBIL
ALT	56	52	57	61	5
AST		62	33	32	1
GGT			71	35	0
ALP				70	4
CBIL					6

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBIL, conjugated bilirubin; GGT, gamma-glutamyltranspeptidase.

negative group. 70 cases of AMA-M2–positive group with elevated ALP could be diagnosed as PBC, which met the diagnostic criteria of PBC in Europe and America in 2009.[9,10] The diagnostic rate of PBC in AMA-M2–positive group was 37.4%. It is of great significance for patients to be diagnosed in this period to control the disease progression and prevent misdiagnosis. The third stage is the clinical symptom stage, in which the patients have typical symptoms such as fatigue and pruritus. In AMA-M2–positive group, 46 cases showed fatigue symptoms, but 30 of them had blood formed element decrease such as RBC, WBC, and PLT. There was no definite conclusion as to whether fatigue was caused solely by anemia or whether both of them were inherent symptoms of PBC. Pruritus occurred in 13 positive individuals, but all of them were indirect or seasonal. The fourth stage is decompensated stage, which is characterized by jaundice, ascites, and encephalopathy. Once the patients go through this phase and develop jaundice, the disease rapidly deteriorates. The average time from this period to death or liver transplantation is 4 years. We only found jaundice in 4 patients in this survey. It is related to the fact that the subjects of our study were healthy population rather than patients. Therefore, mass screening for the AMA-M2 antibody in those who had abnormal symptoms of blood routine and liver function and abdominal discomfort, allergic, and fatigue is important for improving diagnosis rate, facilitating early diagnosis, promoting early treatments of PBC, and reducing the occurrence of malignant complications.

Anti-SSA antibody is an immunoglobulin produced by the immune system in response to a group of small ribonucleic acid proteins in autologous cells. Many studies have confirmed that this antibody is an invasive autoantibody.[11,12] A study of patients with SLE found that anti-SSA antibodies can adhere to the surface of granulocytes in patients with SLE and activate the complement system to cause granulocyte destruction.[13] Other studies have shown that when the separated lymphocytes from patients with neurological lupus were cultured with serum containing autologous anti-SSA antibody, the apoptotic rate increased significantly. As we found in this study, the blood components of anti-SSA antibody-positive population decreased significantly compared with the control group, which is likely to be caused by the antibody itself. Previous studies have shown that the prominent manifestation of humoral immune abnormalities in patients with pSS is hyperglobulinemia, especially the increase in IgG. Hyperglobulinemia promotes the increase in erythrocyte sedimentation rate (ESR).[14] In this study, the immunological indexes such as IgG, C3, C4, and RF in anti-SSA–positive population were also shown to be obviously abnormal. In addition, we found that the rate of abnormal liver function in anti-SSA antibody-positive group was significantly higher than that in control group. The target antigen of anti-SSA antibody is a small ribonucleic acid protein, which is closely related to cell mitosis and protein synthesis. Studies have shown that the antibody may be involved in the transcriptional regulation process. When the anti-SSA antibody binds to the antigen site, the function of the antigen would be blocked, and the downstream protein synthesis is also interrupted. Liver, as a vigorous organs of the body, has become the main target of anti-SSA antibody. It is suggested that the population with abnormal blood routine, liver function index, and immune index in clinical laboratory should be detected for anti-SSA as soon as possible in order to avoid misdiagnosis of the disease.

The limitation of this study is that there is no in-depth study of anti-Ro-52 antibodies. Anti-Ro-52 antibody can exist in a variety of

TABLE 4 Distribution of laboratory test indicators in anti-SSA antibody-positive population and control group

	144 Anti-SSA positive, n (%)	150 Anti-SSA negative, n (%)	χ^2	*P* value
Abnormal blood routine[a]	72 (50.0)	18 (12.0)	49.95	<0.01
Increased ESR	84 (58.33)	10 (6.67)	90.17	<0.01
Abnormal liver function[b]	59 (40.97)	15 (10)	37.42	<0.01
Increased IgG	19 (13.19)	3 (2.0)	13.30	<0.01
Decreased C_3 and C_4	24 (16.67)	4 (2.67)	16.71	<0.01
Increased RF	29 (20.14)	6 (4.0)	18.25	<0.01

Abbreviations: ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.
[a]Abnormal blood routine: single decrease and mixed decrease in erythrocyte, leukocyte, and platelet.
[b]Abnormal liver function: single increase and mixed increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), conjugated bilirubin (CBIL).

diseases, although it does not have high specificity, but as an antinuclear antibody with high positive rate, it is usually combined with anti-SSA antibody, which is of auxiliary diagnosis and differential diagnosis value.

5 | CONCLUSION

Based on the epidemiological statistical analysis of healthy population, this study revealed the distribution of antinuclear antibody in male and female in healthy population and the clinical characteristics of AMA-M2 antibody and anti-SSA antibody. It improves the clinicians' understanding of antinuclear antibody detection, is conducive to promoting the popularization of antinuclear antibody detection, and is convenient for early diagnosis and control of the disease.

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