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ORIGINAL ARTICLE

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Primary biliary cirrhosis in female subjects with sicca-associated antibodies

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Abstract The aim of this study is to clarify the time course of primary biliary cirrhosis (PBC) in subjects possessing anticentromere antibodies (ACA), anti-Ro, and/or anti-La antibodies, and who used alkaline phosphatase (ALP) as a serological marker for PBC. Female subjects ($n = 165$), who had at least one of ACA, anti-Ro, and/or anti-La, were enrolled in this study. Groups A (ACA alone, $n = 44$), B (anti-Ro alone, $n = 54$), E (anti-Ro and anti-La, $n = 52$), and DFG (ACA with anti-Ro and/or anti-La, $n = 14$) were analyzed. Healthy females ($n = 65$) were used as a control. The frequencies of the PBC in groups A (13.6%) and DFG (14.3%) were higher than those in groups B (1.9%) and E (0.0%). The ALP levels increased with age in groups A and DFG and slightly increased with age in groups B and C, and the control group. After correcting for age by analysis of covariance, a comparison of ALP levels among the groups not having anti-M_2 was as follows: group A ≒ group DFG > group B ≒ group E ≒ the control group. The subjects with ACA might thus have PBC more frequently than either those with anti-Ro and/or anti-La, or the control subjects.

Key words Alkaline phosphatase · Anticentromere antibodies · Anti-La antibodies · Anti-Ro antibodies · Primary biliary cirrhosis

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease, most often diagnosed in middle-aged women, and it is associated with systemic sclerosis (SSc) and/or Sjögren's syndrome (SS). Serologically, PBC is characterized by the presence of anti-mitochondrial antibodies (AMA). Alkaline phosphatase (ALP) was most commonly used for the diagnosis of PBC.[1] Anti-Ro (SSA) and anti-La (SSB) antibodies are closely associated with SS.[2] These autoantibodies are occasionally detected in patients with PBC.[3,4] Anticentromere antibodies (ACA) were first described in association with limited cutaneous systemic sclerosis (lSSc).[5] Subsequently, ACA was also found to be associated with sicca symptoms[6–8] and PBC.[7,9] Powell et al.[10] proposed to designate this association as "PACK syndrome" (PBC, ACA, CREST syndrome, and keratoconjunctivitis sicca).[10] We described earlier the time course of the salivary production rate, as measured by the Saxon test, in subjects with ACA, anti-Ro, and/or anti-La (sicca-associated antibodies).[8]

The predictive role of ACA in PBC patients was described by Nakamura et al.[9] ACA was most significantly associated with hypertension-type progression. However, they did not mention the case of a patient with ACA not having PBC. The time course of PBC in a subject having any of sicca-associated antibodies has not been clarified. If a subject who reveals no apparent symptoms has ACA, then PBC might become overt after several years. In addition, the fact as to whether PBC progresses with age in individuals having ACA has not yet been evaluated. The objective of this study is to clarify the time course of PBC in subjects having ACA, anti-Ro, and/or anti-La in a manner similar to that used in our earlier study.[8] For this purpose, we mainly used ALP as a serological marker for PBC.

Subjects and methods

The subjects with sicca-associated antibodies and control subjects

A total of 165 female subjects, who had at least one of the three sicca-associated antibodies (ACA, anti-Ro, and/or anti-La), visited our rheumatology practice three times or more, and had not been undergoing medical treatment when they visited our rheumatology practice for the first time, were enrolled in this study. Precisely, when the subjects suffering from dry mouth, dry eyes, and/or lSSc visited our practice, the sicca-associated antibodies and serum ALP

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Table 1. Exclusion criteria

Conditions affecting ALP level	Drug affecting ALP level
Drug-induced hepatitis	Fibrates
Heart failure	Ursodeoxycholic acid (UDCA)
Neoplasms	
Pregnancy	Other autoantibodies
Rheumatoid arthritis	Anti-Jo-1
Sarcoidosis	Anti-RNP
Bone fractures	Anti-Scl-70
Osteomyelitis	Anti-Sm
Parathyroid disease	
Thyroid disease	Sex
Inflammatory bowel diseases	Male
Obstructive bowel disease	
Acquired immunodeficiency disease (AIDS)	
Chronic infections	
Fever (>37.3°C)	

ALP, serum alkaline phosphatase

Table 2. Definition of each group according to the seropositivities for sicca-associated antibodies

Disease group	ACA	Anti-Ro	Anti-La	*n*
Group A	(+)	(–)	(–)	44
Group B	(–)	(+)	(–)	54
Group C	(–)	(–)	(+)	1
Group D	(+)	(+)	(–)	7
Group E	(–)	(+)	(+)	52
Group F	(+)	(–)	(+)	1
Group G	(+)	(+)	(+)	6

Subjects are all females
ACA, anticentromere antibodies; (+), positive; (–), negative

level were examined. In the subjects that visited our practice for other reasons, the seropositivities for the sicca-associated antibodies and ALP level were also examined. If a subject showed positivity for at least one of the sicca-associated antibodies, then seropositivity for the other autoantibodies including anti-RNP, anti-Scl-70, anti-Jo-1, anti-Sm, anti-mitochondria M_2 (anti-M_2), and anti-double-stranded DNA IgG (dsDNA) was also examined. To eliminate the effects of other autoantibodies (anti-RNP, anti-Scl-70, anti-Jo-1, or anti-Sm), the subjects with these autoantibodies were thus excluded in the present study. The exclusion criteria are shown in Table 1, thus affecting incorrectness of the ALP level. The subjects were classified into seven groups (groups A to G) according to their seropositivities for ACA, anti-Ro, and/or anti-La (Table 2). Groups A, B, and E contained a sufficient number of the subjects (n = 44, 54, and 52, respectively), to render these groups suitable for statistical analyses. Groups D, F, and G (those subjects having ACA with anti-Ro and/or anti-La; n = 7, 1, and 6, respectively) were analyzed as a single group (group DFG, n = 14) because each group contained seven or fewer subjects. Sixty-five healthy females were used as the control group.

Laboratory methods

The seropositivity for antinuclear antibodies (ANA) was examined by an indirect immunofluorescence kit using HEp-2 cells (Fluoro-HEPANA test, MBL, Nagoya, Japan). Anticentromere protein B (CENP-B), anti-La, anti-RNP, anti-Scl-70, anti-Sm, anti-M_2, anti-Ro, and anti-dsDNA antibodies were titrated by enzyme-linked immunosorbent assay (ELISA) kits using recombinant protein (MESACUP-2 test, MBL), affinity-purified native antigen (anti-Ro, MESACUP-2 test, MBL), or DNA antigens (anti-dsDNA, Recombigen ELISA anti-dsDNA kit, Nippon DPC, Chiba, Japan). Anti-Jo-1 was titrated by a double-immunodiffusion kit (ENA-4 test, MBL). The readings ≥16.0 index for anti-CENP-B, ≥25.0 index for anti-La, ≥22.0 index for anti-RNP, ≥24.0 index for anti-Scl-70, ≥30.0 index for anti-Sm, ≥7 index for anti-M_2, ≥20.0 index for anti-Ro, ≥10.0 IU/ml anti-dsDNA, or ≥18.0 index for anti-Jo-1 were considered to be positive. The ALP levels were examined at least three times within 3 months. An elevation of all the measurements of the ALP levels >340 IU/l was defined as a "continuous elevation of ALP level." The "ALP level" in the following analysis was defined as the mean of the three measurements of the ALP levels.

Diagnostic criteria

A diagnosis of PBC was made according to the diagnostic criteria of PBC established in 1992 by the Intractable Liver Disease Research Project Team in Ministry of Health and Welfare (presently, Ministry of Health, Labor, and Welfare) of Japan.[11] The criteria were as follows: (1) patients with histopathological features of chronic nonsuppurative destructive cholangitis (CNSDC) and laboratory test results compatible with PBC; (2) patients positive for AMA or anti-pyruvate dehydrogenase complex (PDC) with histopathological features compatible with PBC but not showing CNSDC; or (3) patients positive for AMA or anti-PDC whose clinical symptoms and clinical course are highly characteristic despite the absence of a histopathological examination. The diagnosis of autoimmune hepatitis (AIH) was made according to the 1999 revised scoring system for the diagnosis of AIH as proposed by the International Autoimmune Hepatitis Group;[12] SS, the classification criteria proposed by Vitali et al.;[13] lSSc, the classification proposed

by LeRoy et al.;[14] systemic lupus erythematosus (SLE), the American College of Rheumatology (ACR) 1982 classification criteria (modified in 1997);[15] rheumatoid arthritis (RA), ACR 1987 classification criteria.[16] The diagnosis of primary sclerosing cholangitis (PSC) is generally made by a histological examination, cholangiography, and/or magnetic resonance imaging cholangiography.

Statistical analysis

Comparisons were made between the groups of variables using the Mann–Whitney *U* test, an analysis of covariance (ANCOVA), and the Tukey–Kramer post hoc test. Correlations were analyzed using Pearson's correlation coefficient. A multiple regression analysis was used to examine the association between the autoantibodies and the specific parameter. *P* values of less than 0.05 were considered to be significant.

Results

The demographic features and the numbers in each group

The demographic features and the numbers of subjects among the four groups and the control group are shown in Table 3. The frequencies of a continuous elevation of the ALP level in the ACA-positive groups were high: group A 25.0% and group DFG 35.7%, whereas in ACA-negative groups they were low: group B 5.6% and group E 3.8%. In a similar manner, the frequencies of PBC in the ACA-positive groups were high: group A 13.6% and group DFG 14.3%, whereas in the ACA-negative groups they were low: group B 1.9%, and group E 0.0%. The frequencies of AIH among the four groups were comparable (1.9%–14.3%). There was no subject with PSC in any group. The frequency of SS was 100% in group DFG, whereas the frequencies in groups A, B, and E were comparable (57.4%–80.8%). Nevertheless, the frequency of primary SS varied remarkably among the latter three groups. The frequencies of lSSc were high in the ACA-positive groups: group A 63.6% and group DFG 50.0%, whereas those in the ACA-negative groups were low: group B 3.7% and group E 0.0%.

Analysis of the ALP levels

The 10 subjects with anti-M_2 (8 corresponds to group A; 1, group B; 1, group DFG) were excluded from groups A, B, and DFG. The ALP levels slightly but significantly increased with age in the control group. Those increased with age in groups A and DFG. Those slightly increased with age in groups B and C (Fig. 1). The ALP levels among the groups A, B, E, DFG, and the control group were compared following correction for age by ANCOVA. The ALP levels in group A were higher than those in group B and the control group, with statistical significance ($P < 0.05$ and $P < 0.01$, respectively), or group E without statistical significance ($P = 0.050$). Those in group DFG were higher than those in group B, group E, and the control group ($P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively).

Demographic features in subjects with a continuous elevation of the ALP level

There were 21 subjects demonstrating a continuous elevation of ALP level in this study (Table 4); 9 subjects were diagnosed to have PBC (subject nos. 1–9), in which 4 subjects were diagnosed on the basis of pathological examination. Meanwhile, 12 subjects were diagnosed to not have PBC (subject nos. 10–21). There was no significant difference between the ALP level in the subjects diagnosed with PBC (438 ± 130 IU/l) and that in subjects not diagnosed with PBC (430 ± 98 IU/l). Subject no. 8 was diagnosed to have PBC on the basis of pathological examinations. However, she did not have anti-M_2.

Table 3. Demographic features of the subjects, and distribution among the four groups and the female control

	Group A (ACA alone) (*n* = 44)	Group B (Ro alone) (*n* = 54)	Group E (Ro + La) (*n* = 52)	Group DFG (ACA + Ro and/or La) (*n* = 14)	*P**	Female control (*n* = 65)
Age (years, mean ± SD)	59.3 ± 10.2	50.6 ± 13.9	52.2 ± 13.4	56.2 ± 15.1	<0.01	50.3 ± 12.0
ALP levels (IU/l, mean ± SD)	287 ± 94	222 ± 103	223 ± 73	311 ± 96	<0.001	212 ± 57
Continuous elevation of ALP level, no. (%)[a]	11 (25.0)	3 (5.6)	2 (3.8)	5 (35.7)	<0.001	0 (0.0)
Primary biliary cirrhosis, no. (%)	6 (13.6)	1 (1.9)	0 (0.0)	2 (14.3)	<0.01	0 (0.0)
Autoimmune hepatitis, no. (%)	1 (2.3)	1 (1.9)	5 (9.6)	2 (14.3)	NS	0 (0)
Primary sclerosing cholangitis, no. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS	0 (0)
Sjögren's syndrome, no. (%)	31 (70.5)	31 (57.4)	42 (80.8)	14 (100.0)	<0.01	0 (0.0)
Primary, no.	2	21	29	3	<0.001	0
Secondary, no.	29	10	13	11	<0.001	0
Limited cutaneous systemic sclerosis, no. (%)	28 (63.6)	2 (3.7)	0 (0.0)	7 (50.0)	<0.001	0 (0.0)
Systemic lupus erythematosus, no. (%)	3 (6.8)	14 (25.9)	7 (13.5)	1 (7.1)	<0.05	0 (0.0)

Subjects in this table are all females
ALP, serum alkaline phosphatase level
* *P* value was calculated among groups A, B, E, and DFG
[a] All the three measurements of the ALP level were more than 340 IU/l

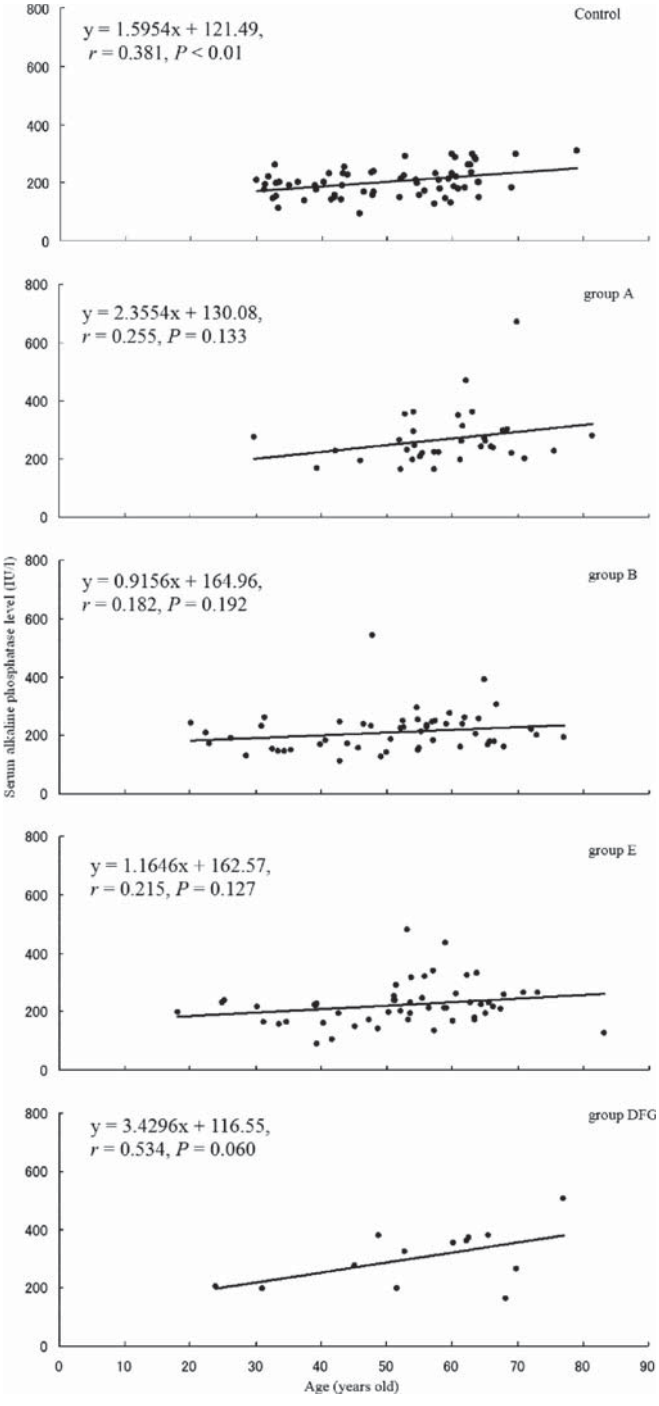

Fig. 1. Relationships between the serum alkaline phosphatase levels and age in the 65 female controls and groups A, B, E, and DFG. The subjects with anti-M_2 (8 corresponds to group A; 1, group B; 1, group DFG) were excluded from groups A, B, and DFG. A reference range of alkaline phosphatase was 100–340 IU/l

Discussion

Patients diagnosed as having PBC often reveal no apparent symptoms. Nevertheless, even though they are asymptomatic, most of the patients with PBC show cholestatic pictures in the laboratory findings (e.g., the elevation of ALP levels and γ-glutamyl transpeptidase, GGT, levels).[17] Among the parameters, Kaplan et al.[1] and Poupon et al.[18] described that they used the ALP level, and not the GGT level, for the diagnostic criteria of PBC. Other researchers have also used the ALP level in the studies of PBC.[19] Therefore, in the present study, we also used the ALP level as a parameter for chronic cholestatic diseases. PSC is a rare disease, which is usually observed in young men,[18] and in this study, no subject with PSC was observed (Table 3).

The mean ALP levels thus appeared to be different between male subjects and female subjects.[20] As a result, the male subjects were excluded in this study (Table 1). ALP is primarily derived from three sources: liver, bone, and in some instances, intestinal tract. The ALP levels in healthy men and women increase with age by bone ALP,[20–23] but not by liver ALP or intestinal ALP.[21] This might be the reason why the ALP levels slightly but significantly increased with age in the control group (Fig. 1).

Enzyme-linked immunosorbent assay is highly sensitive for the detection of anti-M_2. However, anti-M_2 measured by ELISA is not detected in all the PBC patients.[24] Muratori et al.[3] reported that of the 19 AMA-negative patients out of the 97 patients with PBC that were diagnosed on the basis of a liver biopsy, 15 were ANA-positive, 3 were ACA-positive, and 1 was anti-RNP-positive. According to their data, if a female patient shows an asymptomatic ALP elevation along with a positive finding for ANA or some autoantibodies such as ACA, even though she does not demonstrate AMA, she might have asymptomatic PBC. In the present study, after correcting for age by ANCOVA, a comparison of ALP levels among the groups (not having anti-M_2) was as follows: ACA alone (group A) ≒ ACA with anti-Ro and/or anti-La (group DFG) > anti-Ro alone (group B) ≒ anti-Ro and anti-La (group E) ≒ the control group. These results indicate that the subjects with ACA might thus have PBC more frequently than those with anti-Ro and/or anti-La or the control subjects, regardless as to whether anti-M_2 is present. Moreover, the ALP levels increased with age in groups A and DFG and slightly increased with age in groups B and C, and the control group (Fig. 1).

According to the diagnostic criteria of PBC, the seropositivity for AMA or PDC, or histopathological examination is imperative.[11] In the present study, there was no significant difference between the ALP level in subjects diagnosed with PBC (438 ± 130 IU/l) and that in subjects not diagnosed with PBC (430 ± 98 IU/l). Subject no. 8 was diagnosed with PBC on the basis of a pathological examination. She did not have anti-M_2 (Table 4). Nakanuma et al.[25] described AMA-negative patients with PBC to be asymptomatic; nevertheless, they have a liver histology of PBC at stage I with relatively mild lymphocytic piecemeal necrosis. These results indicated that (1) a mild grade of PBC might not be necessarily associated with anti-M_2, or that (2) anti-M_2 might thus become positive earlier than the clinical symptoms of PBC becoming overt.

The frequencies of SS, lSSc, and PBC in ACA-positive subjects (groups A and DFG) were 77.6%, 60.3%, and 13.8%, respectively, whereas those of SS, lSSc, and PBC in anti-Ro- and/or anti-La-positive subjects (groups B and E)

Table 4. Demographic features in the subjects with a continuous elevation of the ALP level

Subject	Age (years)	Group	ACA	M2	Pathology of PBC	ALP (IU/l)[a]	PBC	AIH	PSC	SS	lSSc	SLE
1	75	A	+	+		392	+			+	+	
2	58	A	+	+	+	405	+			+	+	
3	67	A	+	+		356	+			+	+	
4	63	A	+	+		404	+			+	+	
5	61	A	+	+	+	373	+			+		
6	55	A	+	+		373	+				+	
7	45	B		+	+	764	+			+	+	
8	77	D	+		+	506	+			+	+	
9	67	F	+	+		371	+			+	+	
10	54	A	+			360				+	+	
11	52	A	+			354				+	+	
12	69	A	+			671				+	+	+
13	62	A	+			470				+	+	
14	63	A	+			362				+		
15	64	B				392				+		
16	47	B				543						+
17	53	E				480				+		
18	59	E				436				+		
19	48	G	+			378				+	+	
20	62	G	+			362				+		
21	60	G	+			353				+	+	

ACA, anticentromere antibody; M2, antimitochondria M2 antibody; ALP, serum alkaline phosphatase level; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; SS, Sjögren's syndrome; lSSc, limited cutaneous systemic sclerosis; SLE, systemic lupus erythematosus

[a] Mean of three times measuring

were 68.9%, 1.9%, and 0.9%, respectively (Table 3), thus indicating that the difference in the frequencies with respect to lSSc and PBC was remarkable between these two groups. Miyawaki et al.[7] analyzed 120 patients with ACA, and thus showed the frequencies of SS, SSc, and PBC to be 33.3%, 70.0%, and 7.5%, respectively; they also noted the comparable frequencies of SSc and PBC in ACA-positive subjects in comparison with those in the present study. Most of the subjects with PBC and ACA were complicated with SS (87.5%, seven of eight, Table 4), thus indicating that a common immunological etiology might exist (play a role) between these two conditions. Histologically, the characteristic lesions of PBC demonstrate an asymmetric destruction of the bile ducts within the portal triads.[1] In contrast, the lesions of SS consist of a focal, periductal mononuclear cell infiltrate, a loss of acinar cells, and the relative preservation of ductal cells.[26] Nakamura et al.[9] described that positive ACA was associated with more severe ductular reaction in PBC patients. Avouac et al.[6] showed that ACA was associated with SS on the basis of a histological examination. There is also a possibility that there is a shared antigen between the excretory ducts of the liver and salivary gland in subjects with ACA.

Acknowledgment We declare that there is no conflict of interest in this article.

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