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Liver damage in primary biliary cirrhosis and accompanied by primary Sjögren's syndrome: a retrospective pilot study

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Abstract

Introduction*: Primary biliary cirrhosis (PBC) and primary Sjögren's syndrome (pSS) have been referred to as "generalized autoimmune epithelitis". Indeed, the pathogenic mechanisms, clinical features, and optimal therapeutic approaches for them are not yet fully defined.*

Material and methods: *A retrospective analysis was carried out on clinical data obtained from 302 inpatients newly diagnosed with PBC, pSS, or the coexistence of PBC and SS between May 2011 and December 2014. Forty-two patients with abnormal hepatic function were divided into the PBC group (n = 17), the coexistent group (PBC accompanied by SS, n = 13), and the pSS group (n = 12). Their clinical symptoms, laboratory data, and pathological features were collected and analyzed when they were first diagnosed. The clinical and laboratory data were collected at 0, 1, and 3 months after treatment.*

Results: *Of the 42 patients with abnormal liver function, 4 were male and 38 were female patients. Compared with the patients in the PBC group, the patients in the other 2 groups were more likely to have an elevated erythrocyte sedimentation rate (ESR) and serum immunoglobulin G (IgG) levels. Abnormal serum immunoglobulin M levels (IgM) were more frequent in the PBC group. Corticosteroids were effective in normalizing elevated liver enzyme levels in patients with SS and in those with coexistent conditions.*

Conclusions: *This pilot study suggests that patients with PBC, pSS, and PBC/SS coexistence and having liver function abnormality share similar symptoms, but have different pathogenesis and prognosis.*

Key words: *autoimmune liver disease, primary biliary cirrhosis, Sjögren's syndrome.*

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Introduction

Primary biliary cirrhosis (PBC) is a chronic form of cholestatic liver disease with a probable autoimmune etiology characterized by destruction of the biliary epithelial cells lining the intrahepatic bile ducts. Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease that primarily affects the exocrine glands epithelia with subsequent sicca syndrome. Correspondingly, a certain degree of coexistence between pSS and PBC has already been reported [1-3]. Characterized by the immune-mediated tissue injury, particularly in biliary and exocrine gland epithelia, the term *autoimmune epithelitis* has been proposed as an alternative name for PBC and Sjögren's syndrome (SS).

Material and methods

A total of 302 inpatients newly diagnosed as PBC, PBC + SS, and pSS were included into the study at the Drum Tower Hospital between May 2011 and December 2014. The inclusion criteria were as follows: patients with liver function abnormalities (elevated serum alkaline phosphatase [ALP], γ-glutamyl transpeptidase [γ-GT], alanine aminotransferase [ALT], or aspartate aminotransferase [AST], or conjugated bilirubin level). Drug-induced alteration, alcoholic liver disease, or viral infection was excluded. Diagnosis of pSS was made according to the 2002 American-European consensus criteria [4]. A PBC diagnosis was established if the patients met at least 2 of the following criteria: positivity for antimitochondrial antibody (AMA), elevated cholestatic enzyme levels, and presence of histological lesions of PBC [5, 6]. A diagnosis of PBC with SS (PBC + SS) was established if the patient fulfilled the two above-mentioned sets of criteria simultaneously. No other comorbid autoimmune disease was observed. Finally, 42 patients (4 men and 38 women; 17 patients with PBC, 12 patients with pSS, and 13 patients with PBC +

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SS) were included. To exclude autoimmune hepatitis, test results (indirect immunofluorescence [IIF], ALD kit, Euroimmun) should be negative for smooth muscle antibody and antibodies against soluble liver antigen (anti-SLA).

All the patients were assessed for clinical symptoms, including fever, Raynaud's phenomenon, feebleness, anorexia, sicca, abdominal distension, jaundice, pruritus, and insomnia. The serum liver function profiles, serum immunoglobulin level, antinuclear antibody (ANA) level (considered positive if the titer was 1/100 or higher; Euroimmun, IIF), serum concentrations of 25-OH vitamin D (commercial kit, 25-OH vitamin D assay, electrochemiluminescence immunoassay, Roche Cobas e411), while erythrocyte sedimentation rate (ESR) and rheumatoid factors (RF) were detected. Titers of AMA and its anti-M2 fraction were measured (a titer of 1/80 or higher was considered positive, Euroimmun, IIF).

Follow-up assessments were conducted for initial treatment. At 1 and 3 months after treatment, none of the patients was lost to follow-up.

Statistical analysis

Results were presented as mean ± SD. According to the type and distribution of the data, statistical significance was estimated by using the Student's *t* test, One-way ANOVA, Wilcoxon test, or 2×2 χ^2, when 2 cells (50%) had an expected count of < 5, the Fisher's exact test was used. In the above-mentioned analysis, $p < 0.05$ was considered as statistically significant. All analyses were performed by using the SPSS Statistics 11.5 software (SPSS Inc.).

Results

Population

Of the 42 patients (38 women and 4 men; mean age: 50 ±9 years; range: 29-71 years) were enrolled in the study, the mean time from onset of symptoms/abnormal liver function test to diagnosis was 13 ±10 months (range: 2-60 months). Most (52.4%) of the patients were asymptomatic. Fatigue, loss of appetite, or abdominal distension was most often observed in the patients (47.5%, 40.3%, and 36.6%, respectively; data on clinical symptoms not available). The demographic and autoantibody profiles were similar between the 3 groups (Table 1). Needle liver biopsy was conducted in two patients, of whom one had AMA-negative PBC and the other had pSS with an elevated ALP level. None of the patients died or developed hepatic failure during the 3-month follow-up period.

Laboratory features

An elevated serum ALP level was observed in almost all patients with PBC, while no significant differences in biochemical indexes (serum levels of ALT, AST, ALP, γ-GT, lactate dehydrogenase, total bilirubin, conjugated bilirubin, and albumin) and immunoglobulin levels were observed between the 3 groups (Table 2). The IgM levels of the patients with PBC were higher than those of the patients with pSS (3.34 ±2.41 g/l vs. 1.19 ±0.91 g/l, $p < 0.05$). The ESR levels of the patients with PBC were lower than those of the PBC + SS group (14 ±13.49 mm/1 h vs. 41.23 ±22.51 mm/1 h, $p < 0.05$) and pSS group (14 ±13.49 mm/1 h vs. 52.08 ±24.48 mm/1 h, $p < 0.05$; Table 2).

Among the 24 ANA-positive sera tested, 14 (58.33%) were reactive against only one of the antigen profiles and 10 (41.67%) were reactive against multiple profiles. The ANA profiles identified most frequently were the cytoplasmic (50%) and speckled patterns (37.50%). The cytoplasmic pattern was more frequently observed in the PBC group; and speckled pattern, in the pSS group (Table 3).

The frequencies of abnormal values of immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), RF, and 25-OH vitamin D were analyzed. Elevated IgM was less common in the pSS group than in the other 2 groups ($p < 0.05$), and elevated IgG levels were rare in the PBC group, unlike in the other groups ($p < 0.05$). No significant differences in the occurrence of abnormal levels of IgA, RF, and 25-OH vitamin D (referring to the

Table 1. Baseline variables of patients in the three study groups

Variables	PBC group (*n* = 17)	PBC + SS group (*n* = 13)	pSS group (*n* = 12)	*P*
Sex (male/female)	2/15	1/12	1/11	0.924
Age (years)	47.18 ±8.74	51.15 ±10.02	53.25 ±10.63	0.225
Duration of disease (mo)	16.76 ±14.22	10.85 ±5.53	10.17 ±5.75	0.062
Liver biopsy	1/17	0	1/12	NA
AMA(+)	16/17	13/13	0/12	NA
AMA-M2	12/17	10/13	0/12	NA
ANA(+)	7/17	7/13	10/12	0.075
Liver related death	0	0	0	NA

NA – not applicable

Table 2. Comparison of laboratory values between the three study groups

Variables	PBC group	PBC + SS group	pSS group	*P1*	*P2*	*P3*	*P*
Alanine aminotransferase (ALT) (U/l)	97.51 ±78.92	158.05 ±111.64	131.76 ±95.05	0.090	0.491	0.343	0.227
Aspartate aminotransferase (AST) (U/l)	72.43 ±56.59	74.73 ±71.43	98.1 ±69.10	0.924	0.375	0.302	0.543
Alkaline phosphatase (ALP) (U/l)	275.38 ±275.62	281.75 ±183.09	131.45 ±61.72	0.934	0.076	0.072	0.128
γ-glutamyl transpeptidase (γ-GT) (U/l)	252.46 ±202.72	304.99 ±186.31	275.42 ±145.55	0.441	0.689	0.741	0.740
Lactate dehydrogenase (LDH) (U/l)	208.29 ±91.89	164.00 ±80.81	213.18 ±122.35	0.229	0.220	0.896	0.377
Total bilirubin (μmol/l)	22.66 ±23.58	39.22 ±42.30	27.86 ±17.15	0.134	0.339	0.641	0.314
Conjugated bilirubin (μmol/l)	11.99 ±16.55	12.26 ±7.39	12.39 ±11.85	0.952	0.857	0.804	0.968
Serum albumin (g/l)	36.37 ±5.52	36.27 ±4.94	35.48 ±7.49	0.964	0.742	0.695	0.916
Serum globulin (g/l)	31.01 ±9.26	31.97 ±6.79	27.58 ±5.24	0.731	0.155	0.237	0.322
Immunoglobulin M (IgM) (g/l)	3.34 ±2.41	2.57 ±2.76	1.19 ±0.91	0.352	0.111	0.012	0.041
Immunoglobulin A (IgA) (g/l)	2.77 ±1.86	2.95 ±1.23	2.60 ±1.52	0.754	0.891	0.778	0.949
Immunoglobulin G (IgG) (g/l)	15.68 ±7.06	16.28 ±5.52	14.44 ±5.32	0.090	0.406	0.425	0.233
Rheumatoid factor* (RF) (IU/ml)	19.43 ±17.60	39.52 ±41.28	48.21 ±67.18	0.232	0.643	0.125	0.258
Serum 25OHD (ng/ml)	16.81 ±10.23	12.43 ±7.30	14.30 ±6.42	0.166	0.582	0.435	0.371
Erythrocyte sedimentation rate (ESR) (mm/1 h)	14 ±13.49	41.23 ±22.51	52.08 ±24.48	0.001	0.183	0.000	0.000

P1: PBC group vs. PBC + SS group; P2: PBC + SS group vs. SS group; P3: PBC group vs. SS group
** RF were detected in 36 patients*

Table 3. Prevalence of serum antinuclear antibodies profile in three groups

ANA profile (*n*)	Cytoplasmic	Speckled	Centromeric	Homogeneous	Nucleolar	Nuclear membranous	Nuclear dot
PBC group [*n* (%)]	6 (85.71)	2 (28.57)	2 (28.57)	1 (14.29)	0	1 (14.29)	0
PBC + SS group [*n* (%)]	4 (57.14)	2 (28.57)	1 (14.29)	1 (14.29)	0	0	0
pSS group [*n* (%)]	2 (20)	5 (50)	2 (20)	2 (20)	1 (10)	1 (10)	1 (10)
Total [*n* (%)]	12 (50)	9 (37.5)	5/24 (20.83)	4/24 (16.67)	1/24 (4.17)	2/24 (8.33)	1/24 (4.17)

Table 4. Comparison of laboratory abnormalities between three study groups

Variables (normal range)	Frequency of abnormality value (above normal range)			*P1*	*P2*	*P3*	*P*	$P_{1\text{ vs. }2+3}$	$P_{1+2\text{ vs. }3}$
	PBC group [*n* (%)]	PBC + SS group [*n* (%)]	pSS group [*n* (%)]						
IgA (0.7-3.3 g/l)	3 (17.65)	6 (46.15)	5 (41.67)	0.666	0.688	0.218	0.357	0.096	0.235
IgM (0.5-2.2 g/l)	9 (52.94)	5 (38.46)	1 (8.33)	0.713	0.028	0.036	0.024	0.102	0.031
IgG (8-16 g/l)	2 (11.76)	6 (46.15)	4 (33.33)	0.049	0.531	0.198	0.094	0.047	0.663
RF (< 20)	2/14 (14.29)	6/13 (46.15)	4/9 (44.44)	NA	NA	NA	NA	0.076	0.443
25OHD normal (> 31 ng/ml)	5 (29.41)	2 (15.38)	2 (16.67)	NA	NA	NA	NA	NA	0.402
deficiency (< 20 ng/ml)	7 (41.18)	4 (30.77)	7 (58.33)	0.713	0.821	0.462	0.230	0.346	0.734
insufficiency (20-30 ng/ml)	5 (29.41)	7 (53.85)	3 (25)	NA	NA	NA	0.844	0.859	1.000

$P_{1\text{ vs.}2+3}$: PBC group vs. combine of PBC + SS group and pSS group
$P_{1+2\text{ vs.}3}$: combine of PBC group and PBC + SS group vs. pSS group
NA – not applicable (> 55% of the cells had expected counts less than 5, so the assumptions are not met and the results may not be appropriate)

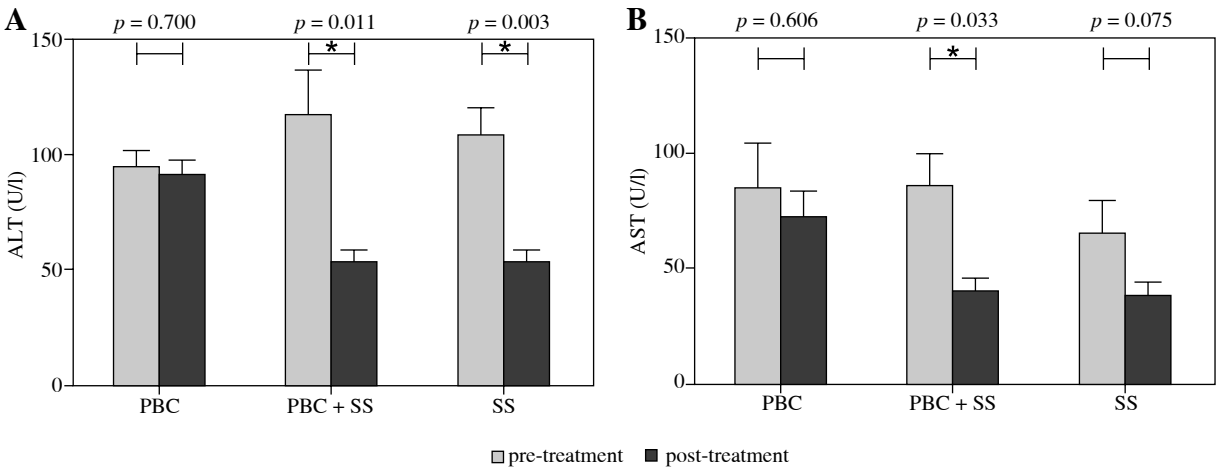

Fig. 1. The serum ALT, AST levels of three groups pre- and post-corticosteroids

three statuses: deficiency, insufficiency and normal) were observed between the 3 groups (Table 4).

According to 25-OH vitamin D levels, the indications of PBC, PBC + SS, and pSS, respectively, included the following: deficiency in 7 (58.3%), 7 (63.6%), and 8 patients (72.7%); insufficiency in 5 (41.7%), 4 (36.4%), and 3 patients (27.3%); and normal in 5 (41.7%), 2 (18.2%), and 1 patient (9.1%), respectively.

Treatment comparison

Diammonium glycyrrhizinate (150 mg/day for 1 week by intravenous drip and then 150-mg capsule orally thrice a day for 3 weeks) and ursodeoxycholic acid (UDCA; 13-15 mg/kg/day) were administered initially to the patients. Liver function tests and immunological assays were performed again after a month. Most of the laboratory indexes (mainly ALT and AST levels) were decreased, except in 3 patients with PBC, 4 patients with PBC + SS, and in 6 patients with pSS. Corticosteroids were additionally administered at 0.5 mg/kg/day to these patients, after which the amount was tapered slowly to a maintenance dose (methylprednisolone 4-8 mg/day). As a result, the elevated serum ALT and AST levels of the PBC + SS group or pSS group were decreased in response to the corticosteroid administration (Fig. 1).

Discussion

Liver involvement is considered as the most common non-exocrine complication in pSS [7, 8]. In particular, PBC was diagnosed in 7-9% of patients with pSS [3, 9]. The most common autoimmune disorder associated with PBC is SS, whereas 26-73% of patients with PBC present with sicca symptoms such as dry mouth and eyes [1, 10]. Meanwhile, SS is commonly (69-81%) found in patients with PBC [11-13]. In a study by Matsumoto *et al.* [14], liver histological examination revealed that the incidence of lymphoid non-suppurative cholangitis in the PBC with pSS group was greater than that in the PBC group. Accordingly, only few studies have examined the difference in clinical symptoms between patients with PBC, PBC accompanied by SS, and pSS status. In our study, rigorous inclusion and exclusion criteria were applied; therefore, AMA-negative cases associated with PBC in SS might have been excluded. By contrast, Hatzis *et al.* [3] reported that 7% of patients with pSS had definite (AMA-negative) PBC.

With the present criteria, early biochemical markers in the patients with PBC included an elevated ALP level. However, no significant differences in liver enzyme levels, including ALP levels, were observed between the 3 groups in this study. Although a higher level of ALP was also observed in the PBC or the PBC + SS group, this was not statistically significant. This suggests that the mean abnormal ALP level in each group could not reflect the abnormal ALP level in these cases. Meanwhile, in some of the patients with pSS, the ALP levels might be high. This means that in these patients, bile duct damage could be serious. Compared with the other 2 groups, the PBC group less likely had faster ESR. Unlike PBC, an organ-specific disease, pSS is one of the most prevalent systemic autoimmune diseases that affect the parenchymal organs (the kidney, lung, and liver) or hematological and peripheral nervous systems, indicating that it could be associated with a greater inflammatory reaction.

Immunoglobulin-mediated mechanisms are involved in the pathogenesis of PBC. IgM is the first immunoglobulin formed after primary immunization and appears first in the bloodstream during infection. It inhibits the process of dendritic maturation and increases the phagocytosis of cells undergoing apoptosis, a key phenomenon in the pathogenesis of PBC [15, 16]. Moreover, the activation of toll-like receptor 9 in B cells stimulated with bacterial

DNA results in increased IgM production [17]. Thus, environmental, rather than genetic, factors play a critical role in the elevation of serum IgM levels in PBC. In the study by Taylor *et al.* [18], IgM levels were lower in patients with AMA-negative PBC than in those with AMA-positive PBC. Although the increase in aminotransferase activity was reportedly associated with elevated IgG levels and reflects the degree of periportal and lobular necrosis and inflammation, an elevated IgG level was demonstrated to be less common in the patients with PBC in our study. Most of the cases in this study were newly diagnosed. Yet in the early stage of disease, inflammatory infiltrates might not be present, which may be the reason that the elevated IgG level was not common in PBC. Secretory IgA plays a major role in preventing microorganisms and foreign proteins from penetrating the mucosal surface [19]; thus, the absence of IgA on mucosal surfaces could facilitate the absorption of many environmental antigens. In our study, neither deficient nor elevated serum IgA levels were detected in the three groups, suggesting that a plasma IgA level may have been less significantly associated with the pathogenesis of liver diseases in the three groups.

Previous studies reported that the involvement of vitamin D metabolism in the pathogenesis of PBC was controversial [20-22]. We found that 25-OH vitamin D status was similar in the 3 groups. Nevertheless, a previous study suggested that most pSS patients with leukocytopenia could benefit from vitamin D supplementation [23].

In the present study, we found that the most frequent ANA profile identified was the cytoplasmic pattern. In fact, undefined anti-cytoplasmic staining was associated with a high frequency and wide range of specific autoantibodies, including AMA, anti-SLA, and liver pancreas [24, 25]. These findings suggest that the anti-cytoplasmic pattern may correlate with autoimmune liver disease. Furthermore, it remains uncertain whether some of the patients in our study would have autoimmune hepatitis as a complication in the future.

Glucocorticoids act as broad immunosuppressors by inhibiting NF-κB and prostaglandin productions by means of negative glucocorticoid responsive elements; therefore, they are indicated for the treatment of many autoimmune diseases [26]. Steroid therapy usually leads to a rapid improvement in clinical symptoms, and laboratory and morphological findings in patients with SS. However, as a treatment of choice for UDCA non-responders, glucocorticoids, except budesonide, were shown to have little benefit to improve the liver biochemistry profiles of patients with PBC.

Better understanding of the underlying immunological mechanisms in these patients would provide additional information of the three statuses described herein.

The authors declare no conflict of interest.

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