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CLINICOLABORATORY CHARACTERISTICS OF JAPANESE PATIENTS WITH PRIMARY BILIARY CIRRHOSIS-AUTOIMMUNE HEPATITIS OVERLAP

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Abstract: To clarify the clinicolaboratory characteristics of patients with primary biliary cirrhosis (PBC)-autoimmune hepatitis (AIH) overlap, we analyzed their clinicolaboratory findings and compared them with those of patients with AIH or PBC retrospectively. We analyzed the laboratory findings of 177 patients that diagnosed 103 PBC and 74 AIH patients at our department during the period from January 1990 to April 2005. Of 103 PBC patients with a diagnosis of PBC, we identified 10 cases (9.7%) of PBC-AIH overlap (2 male, 8 female; mean age 56.5 years). PBC preceded AIH in 2 patients, and both diseases occurred simultaneously in the other 8 patients. There is no patients AIH preceded PBC. Positive frequency of anti-smooth muscle antibody (ASMA), IgG and IgM levels were significantly higher in patients with overlap than in those with AIH or PBC. Ursodeoxychoric acid (UDCA) was administered to all 10 patients initially, and later an immunosuppressant, prednisolone or azathioprine, was added in 6 patients. Two of the 10 patients died of liver failure 5 and 12 years after diagnosis, respectively. Both patients had been treated by either prednisolone or UDCA alone. We conclude that in patients with PBC-AIH overlap, the clinical characteristics of both PBC and AIH exist in an enhanced manner.

Key words: primary biliary cirrhosis, autoimmune hepatitis, overlap

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INTRODUCTION

There are many variants of autoimmune liver disease, such as primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), and primary sclerosing cholangitis (PSC)[1-3]. PBC-AIH overlap, one of the variants, is characterized by the overlapping clinical, biochemical, immunological, and histological features of PBC and AIH, and its pathogenesis and treatment are not well understood. The frequency of this overlap varies in different reports probably because of differences in the subjects and diagnostic criteria used[4-6]. In this study, we analyzed the clinicolaboratory findings of PBC-AIH overlap and compared them with those of Japanese patients with PBC or AIH retrospectively.

PATIENTS AND METHODS

Study population

A total of 177 patients that diagnosed with PBC ($n=103$) and AIH ($n=74$) at our department during the period from January 1990 to April 2005 were included in the study. PBC was diagnosed by either histological examination of liver biopsy specimens or clinical findings of anti-mitochondrial antibodies (AMA) and cholestatic dysfunction of the liver followed by jaundice or pruritus, based on the 'Criteria for diagnosis of PBC in Japan' by the Study Group for Autoimmune Hepatitis, a subdivision of the Research Group for Intractable Hepatitis sponsored by the Ministry of Health and Welfare of Japan[7]. Patients with PBC-AIH overlap were identified according to the criteria reported by Chazouillères *et al.*[4]. Briefly, for the diagnosis of each disease, presence of at least 2 of the 3 accepted criteria was required. PBC criteria were the following: (1) alkaline phosphatase (ALP) levels at least twice the upper limit of normal values (ULN) or γ glutamiltranspeptidase (GGT) levels at least five times the ULN, (2) a positive test for AMA, and (3) a liver biopsy specimen showing florid bile duct lesions. AIH criteria were the following: (1) alanine aminotransferase (ALT) levels at least twice the ULN, (2) serum immunoglobulin G (IgG) levels at least twice the ULN or a positive test for smooth muscle antibodies (ASMA), and (3) a liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis. In addition, we analyzed the laboratory findings of 74 patients with AIH diagnosed by the international criteria[8] and compared them with those of patients with PBC-AIH overlap.

Serological tests and HLA typing

Autoantibodies to antinuclear (ANA), AMA and ASMA were detected by indirect immunofluorescence using HEp-2 cells or frozen sections of rat kidney. AMA-M2 was detected using a commercially available ELISA kit (MBL, Nagoya, Japan). HLA typing of peripheral leukocytes from patients with PBC-AIH overlap

was performed by microlymphocytotoxicity assay.

Histopathological assessment

We used the METAVIR score[9], which is based on lobular and interface inflammation, to evaluate inflammatory activity on a scale ranging from 0 to 3 : A0, no histological activity ; A1, mild activity ; A2, moderate activity ; and A3, severe activity. Fibrosis was also graded by using the METAVIR score on a five-point scale : F0, no fibrosis ; F1, portal fibrosis without septa ; F2, few septa ; F3, numerous septa without cirrhosis ; and F4, cirrhosis.

Statistical analysis

Results were expressed as mean±SD. Statistical analysis of the data was performed using the Student's t-test, Mann-Whitney analysis of variance (ANOVA) and χ^2-test for independence. Differences with a P value <0.05 were considered significant.

RESULTS

According to the criteria, 10 patients (9.7 %) of the 103 study participants were diagnosed with PBC-AIH overlap. PBC preceded AIH in 2 patients, and both diseases occurred simultaneously in the other 8. Data from laboratory or clinical findings were obtained at the time of diagnosis of PBC-AIH overlap. Median follow-up was 7 years (4-14). Biochemical response of the AIH component was defined by ALT levels lower than twice the ULN.

Clinicolaboratory data of the 10 patients with PBC-AIH overlap are given in Table 1. All patients had elevated ALT and ALP or γ-GTP levels and were positive for ANA and AMA or AMA-M2. Both levels of IgG and IgM were also elevated. Three patients were negative for ASMA. HLA-DR4 was positive in 3 of the 4 patients. Histopathological assessment of liver biopsy specimens showed

Table 1. Clinicolaboratory data of 10 patients with PBC-AIH overlap

Number	Age	Gender	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	T-Bil (mg/dl)	IgG (mg/dl)	IgM (mg/dl)	ANA (x)	AMA (x)	AMA-M2	ASMA (x)	HLA-DR
1	43	F	1,209	405	309	8.1	2,581	1,117	1,280	20	52.4	160	4, 8
2	68	F	211	412	465	0.7	3,985	1,152	80	80	21	<40	nt
3	55	F	251	1,490	272	0.6	3,044	511	80	80	17	40	1, w6
4	55	F	85	596	209	0.7	3,830	1,840	640	320	nt	<40	nt
5	52	M	111	1,337	970	2.2	4,069	722	80	320	42.8	160	nt
6	61	F	180	377	37	3.1	4,371	1,449	1,280	80	nt	160	nt
7	54	F	1,378	781	1,092	2.2	2,630	526	80	<20	37	80	4
8	49	M	713	528	159	8.6	2,360	198	640	<20	124	40	4, w6
9	71	F	203	632	209	2.1	3,510	320	10,240	<20	26	<40	nt
10	55	F	831	583	889	1.3	2,660	324	10,240	<20	28	40	nt

nt : not tested

Table 2. Clinicolaboratory data of patients with PBC-AIH overlap, PBC alone and AIH alone

	RBPBC-AIH	PBC	AIH
No. of patients	10	93	74
ASMA frequency (%)	70.0[a,b]	15.2	47.0
ALT (IU/L)	571±481[a]	55±41	410±374
ALP (IU/L)	714±389[a,b]	557±340	390±225
IgG (mg/dl)	3,304±735[a,b]	2,033±650	2,907±964
IgM (mg/dl)	816±549[a,b]	559±298	331±125

[a] Significantly different from that of PBC alone.
[b] Significantly different from that of AIH alone.

Table 3. Clinicopathological findings and outcome in 10 patients with PBC-AIH overlap

No.	Histology of the liver			Initial treatment		Onset	Prognosis
	cholangitis	activity	fibrosis	PSL (mg/day)	UDCA (mg/day)		
1	+	A2	F3	4	300	PBC → AIH	alive
2	+	A2	F2	0	600	simultaneous	alive
3	+	A3	F2	10	600	simultaneous	alive
4	+	A3	F3	20	600	simultaneous	alive
5	+	A2	F2	30	0	simultaneous	deceased (liver failure)
6	+	A2	F2	0	300	simultaneous	deceased (liver failure)
7	+	A3	F3	20	600	PBC → AIH	alive
8	+	A1	F1	30	600	simultaneous	alive
9	+	A3	F4	20	600	simultaneous	alive
10	+	A3	F2	0	600	simultaneous	alive

moderate or severe interface hepatitis in all patients.

Table 2 gives the laboratory parameters of the 3 patient groups: overlap, PBC and AIH. From the table 2, it can be seen that patients with overlap showed the characteristics of both patients with AIH, who have markedly elevated ALT and IgG, and patients with PBC, who have elevated cholestatic enzymes and IgM levels. In addition, patients with overlap had the high frequency of positive ASMA, IgG and IgM levels significantly higher than patients with AIH or PBC.

As shown in Table 3, PBC preceded AIH in 2 patients, and both diseases occurred simultaneously in the other 8. There is no patients AIH preceded PBC. UDCA was administered to 9 of 10 patients initially, and later an immunosuppressant, prednisolone or azathioprine, was added in 6 patients. In these 6 patients, the ALT levels fell below twice the normal. Two (Cases 5, 6) of the 10 patients with overlap died of liver failure 5 and 12 years after diagnosis, respectively. Both patients had been treated by either prednisolone or UDCA alone. Six patients treated with UDCA and immunosuppressive therapy showed a good response with normalization or near normalization of the ALT and ALP levels. In 2 patients

(Cases 2, 10) treated with UDCA alone, the ALT level rose transiently, but was normal most of the time.

DISCUSSION

This study showed that in patients with PBC-AIH overlap, who represented 9.7% of patients with PBC, the clinical features of both PBC and AIH exist in an enhanced manner. In the past, the frequency PBC-AIH overlap has been reported to be between 5% and 19%, and also it varied according to the diagnostic method and criteria used[4-6]. Chazouillères *et al.*[4] recognized patients with PBC-AIH overlap in 9.2% of patients with PBC on the basis of the criteria they had established. We used their criteria in this study, and the obtained frequency was almost equal to theirs.

The positive frequency of ASMA is reported to be about 60% in patients with AIH, and 20% in those with PBC; in our patients with PBC-AIH overlap, however, it was 70% (7/10), a value significantly higher than that in patients with AIH (47%) or PBC (15.2%). In AIH, the specific autoantigen of ASMA is F-actin, and its immunoglobulin class of autoantibody is present in high titer mainly in class IgG, although a high component of IgM is detected in patients with PBC-AIH overlap[10]. There is a possibility that a high component of IgM was detected in our patients with PBC-AIH overlap because their IgM levels were elevated. Furthermore, the serum levels of IgG and IgM were significantly higher in patients with PBC-AIH overlap than in those with PBC alone or AIH alone in this study. The only data we found in the literature for comparison are those of Lohse *et al.*[11], who report the mean levels of IgM and IgG (540 and 2,520 mg/dl) similar to the level of patients with PBC alone or AIH alone in this study. Our values (968 and 3,506 mg/dl), which are higher than theirs, may be due to racial differences. We suspect that the characteristics of both PBC and AIH are enhanced in Japanese patients with PBC-AIH overlap.

Histological progress is so rapid in patients with PBC-AIH overlap that in many patients, liver cirrhosis develops in a short time. Proper treatment of PBC-AIH overlap is still unknown, and whether it requires immunosuppressive therapy in addition to UDCA remains controversial. Joshi *et al.*[12] reported that response to UDCA therapy in patients with strictly defined PBC-AIH overlap was similar to that in patients with PBC. Many investigators report that immunosuppressive therapy is useful when UDCA therapy is ineffective[4,11]. In fact, liver failure developed in our two patients who received prednisolone or UDCA alone. Steroid therapy for PBC has been assumed to be ineffective; however, because there are such patients with PBC-AIH overlap, combined use of UDCA and an immunosuppressive agent is necessary, although some patients show good response to monotherapy. In patients who show histological progress, AIH is often aggravated transiently in the course of PBC. In treating them, it is necessary to select therapy after ascertaining which of

PBC and AIH is predominant in the patient.

In the patients in this study, PBC preceded AIH, or both diseases occurred simultaneously, and in no patient AIH preceded PBC[13]. Thus, PBC-AIH overlap may not be an independent disease entity. It should be regarded as a subgroup of PBC. Lohse *et al.*[11] suggested that the features of AIH are more marked than those of PBC in patients with PBC-AIH because of their genetic susceptibility, evidenced by the AIH-characteristic HLA type B8, DR3, and DR4. In our data, HLA-DR4, which Japanese patients with AIH, was positive in 3 of the 5 patients. To clarify the pathogenesis and long-term outcome of this disease, further accumulation of cases is necessary.

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