Hepatology Research

Hepatology Research 2013; **43**: 502–507 doi: 10.1111/j.1872-034X.2012.01108.x

Original Article

Disease recurrence plays a minor role as a cause for retransplantation after living-donor liver transplantation for primary biliary cirrhosis: A multicenter study in Japan

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Aim: To clarify the role of disease recurrence as a cause of graft loss after living-donor liver transplantation (LDLT) for primary biliary cirrhosis (PBC), we investigated explant grafts, as well as the native liver and liver biopsy specimens, of patients who underwent retransplantation.

Methods: Of 516 patients who underwent LDLT for PBC and were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation.

Results: Seven patients undergoing retransplantation later than 6 months after primary liver transplantation (LT) were enrolled. All seven patients were female, with ages ranging from 34–57 years, and Model for End-Stage Liver Disease scores ranging 10–28. The right lobe was used as graft in one and the left lobe in six. The initial immunosuppression regimen was tacrolimus in six and cyclosporin in one. The period between the primary LT and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation. The primary causes of primary graft loss revealed by histological examination of the explant livers were chronic rejection in three, portal thrombus and/or steatohepatitis in three and outflow block in one. PBC recurrence was observed in 3 and the stage was mild in all.

Conclusion: PBC recurrence has a small impact as a cause of graft loss after LDLT.

Key words: histology, living-donor liver transplantation, primary biliary cirrhosis, recurrence, retransplantation

INTRODUCTION

PRIMARY BILIARY CIRRHOSIS (PBC) is a major indication for liver transplantation (LT). Because autoimmune mechanisms possibly contribute to the etiology of PBC, the possibility of recurrence after transplantation and the impact on the clinical course have been reason for considerable concern. Rates of recurrence have been reported to range 9–35% in deceased-donor LT in Western countries.[1] In living-donor liver transplantation (LDLT) in Japan, the rates have been reported to range 1–40% on the basis of histological evidence.[2–6] However, this range is not reliable because routine liver biopsy is not standard. Furthermore, the impact of recurrence on the clinical course is unclear. The proportion of grafts lost due to disease recurrence was reported to be 2% 10 years after transplantation by Rowe *et al.*[7] On the other hand, Charatcharoenwitthaya *et al.* reported that recurrent PBC was not associated

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Conflict of interest: none.
Received 6 August 2012; revision 9 September 2012; accepted 12 September 2012.

with death or retransplantation.[8] There have been no reports of graft failure secondary to recurrent PBC in Japan, either.[2–6]

The difficulty of performing histological diagnosis of recurrent PBC using needle biopsy specimens is a barrier for studying the impact of recurrent PBC, although histological examination is the gold standard.[6,9,10] Heterogeneity of histological changes is a major hurdle for diagnosis on the basis of needle biopsy specimens. To overcome this problem, we conducted a multicenter study using whole hepatic grafts explanted during retransplantation for PBC.

METHODS

OF 516 PATIENTS who underwent LDLT for PBC and who were registered in the Japanese Liver Transplant Registry, nine patients (1.7%) underwent retransplantation. The demographic data of the recipients and primary donors and information on the clinical courses were obtained.

A current author (Y. N.) performed histological investigation of the native liver, the liver biopsy specimens if present, and the explant grafts. The diagnosis of acute cellular rejection (ACR) and chronic rejection was made according to the Banff criteria.[11,12] Staging of PBC was based on the Nakanuma staging system.[13]

This study was approved by the Ethical Committee of Tokyo Women's Medical University as the central office of the multicenter study, or at each institution if necessary, and it conforms to the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008).

RESULTS

OF THE NINE patients who underwent retransplantation, two died within 6 months after retransplantation. One died due to graft failure secondary to severe acute rejection and another due to small-for-size syndrome. In both cases, we examined the clinical courses and explanted livers, and confirmed the diagnoses. We enrolled the remaining seven patients in this study.

The demographic and operative data of the recipients and primary donors and the clinical courses are shown in Table 1. All patients were female and had histories of pregnancies. Human leukocyte antigen DR8 was detected in all recipients except no. 5 and in the donors of recipients no. 3, 6 and 7. The donor was the patient's husband in two cases, son in three, sister in one and mother in one.

Primary immunosuppression was performed with a triple regimen consisting of calcineurin inhibitor, steroids and antimetabolites (azathioprine, mizoribine) in three patients, and calcineurin inhibitor and steroids in four patients. The calcineurin inhibitor was tacrolimus in all patients except no. 6 in which cyclosporin was converted to tacrolimus 1 year after transplantation.

All patients were treated with ursodeoxycholic acid (UDCA) and no. 1 and 7 with bezafibrate prior to primary transplantation. All patients were given UDCA after transplantation and only no. 3 was given bezafibrate transiently.

Patients 1, 4, 6 and 7 continued to complain of fatigue even after transplantation. Postoperative complications are shown in Table 1. The period between the primary transplantation and retransplantation ranged 11–120 months, with a median of 36 months. Three patients survived and four patients died due to poor graft functions or complications after retransplantation.

Histological findings of the native liver, the liver biopsy specimens and the explant grafts are summarized in Table 2. The stage of PBC of the native liver was 4 in all patients except no. 7. The primary causes of primary graft loss were chronic rejection in three (no. 2, 3 and 6), portal thrombus in one (no. 7), nonalcoholic steatohepatitis (NASH) in one (no. 4), portal thrombus and NASH in one (no. 5), and outflow block in one (no. 1). Briefly, submassive necrosis from ischemic etiology and liver cirrhosis of chronic congestive etiology were observed in no. 1. Foamy cell arteriopathy, duct loss with degenerative epithelial damage with severe cholestasis, and centrilobular and C-C and P-C bridging fibrosis were observed in no. 2. In both patients 4 and 5 with NASH, the stage had progressed from stage 2 in the biopsy specimens to stage 3 in the explanted livers.[14] Portal vein thromboembolism and altered intrahepatic circulation was also observed in no. 5. Marked centrilobular necrosis and hemorrhage with mild inflammation and fibrosis and portal venopathy with repeated thromboemboli were observed in no. 7.

Recurrence of PBC was observed in no. 2, 6 and 7 in the specimens of on-demand needle or wedge biopsies and confirmed in the explanted livers (Figs 1–3). Histological progression of PBC was very mild or mild and the recurrence was not the main cause of graft failure. We evaluated: (i) mononuclear inflammatory infiltrates; (ii) formation of lymphoid aggregates; (iii)

Table 1 Demographic data, operative data and clinical courses

Patient no.	1	2	3	4	5	6	7
Age (years)	52	40	34	37	47	47	57
Time from diagnosis to LT (months)	22	3	60	55	65	132	99
AMA	>320	80	40	80	NA	Negative	160
Anti-M2 (mg/dL)	1859	1550	NA	NA	NA	NA	152
IgM (mg/dL)	1037.8	172.8	426	115	340	NA	524
IgG (mg/dl)	1945.7	884.2	1774	1373	2921	NA	180
ANA	640	±	Negative	±	Negative	320	NA
Child–Pugh score	7	8	11	12	12	14	10
MELD score	10	11	17	24	22	28	11
Primary donor							
Relation	Husband	Mother	Husband	Sister	Son	Son	Son
Age (years)	50	60	34	47	19	20	23
Sex	Male	Female	Male	Female	Male	Male	Male
Operative variables							
Blood type combination	Compatible	Identical	Identical	Compatible	Compatible	Compatible	Identical
GRWR	1.00	0.95	0.88	0.77	1.07	0.58	0.90
Graft type	Left	Right	Left	Left	Left	Left	Left
Operation time (min)	751	550	665	615	730	680	870
Cold ischemic time (min)	82	38	56	53	111	95	131
Warm ischemic time (min)	53	44	33	40	38	45	41
Blood loss (g)	2400	2470	850	10 320	6190	8005	4500
Postoperative complications	Hemoperitoneum, biliary stenosis, ACR, hepatic vein stenosis	Biliary stenosis, ACR, EBV infection	Chronic rejection	ACR	ACR Artery-portal shunt	Biliary leakage and stenosis	Portal vein thrombosis
Time of retransplantation (months)	39	24	36	88	120	20	11
Outcome of retransplantation	Dead (49 days)	Alive	Dead (59 days)	Alive	Alive	Dead (15 days)	Dead (284 days)
Causes of death	Lung bleeding		Graft failure			Graft failure	Graft failure

ACR, acute cellular rejection; AMA, antimitochondrial antibody; ANA, antinuclear antibody; EBV, Epstein–Barr virus; GRWR, graft recipient weight ratio; Ig, immunoglobulin; LT, liver transplantation; MELD, Model of End-stage Liver Disease; NA, not applicable.

Table 2 Histological findings of the native liver, biopsy specimens and explanted liver

Patient no.	1	2	3	4	5	6	7
PBC staging of native livers							
Stage	4	4	4	4	4	4	2
Bile duct loss	3	3	3	3	3	2	1
Fibrosis	3	2	3	3	3	3	1
Orcein deposition	3	2	3	3	3	2	1
Hepatitis activities	1	1	1	0	0	1	1
Cholangitis activities	0	0	0	0	0	0	0
Needle biopsies	Congestion at 6 months	Suspected rPBC (duct loss and hepatitis) at 20 months	No biopsy	rPBC (cholangitis) and NASH at 71 months	rPBC (cholangitis and granuloma) and NASH at 90 months	No biopsy	ACR at 9 months
Main diagnosis	Outflow block	Chronic rejection	Chronic rejection	NASH	PVT and NASH	Chronic rejection	PVT
PBC recurrence	No	Mild (mild chronic cholangitis)	No	Mild (focal duct damage and portal fibrosis)	Mild (focal duct loss and portal inflammation)	No	No

ACR, acute cellular rejection; NASH, non-alcoholic steatohepatitis; PVT, portal vein thrombosis; rPBC, recurrence of PBC.

epithelioid granuloma; and (iv) bile duct damage according to Neuberger's criteria for the diagnosis of recurrent PBC based on liver histology.[15] In patient no. 2, biopsy showed (i) and (iv) (probable recurrence) and the explanted liver showed (i), (ii) and (iv) (definite recurrence); in no. 6, biopsy showed (i), (ii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence); and in no. 7, biopsy showed (i), (iii) and (iv) (definite recurrence), and the explanted liver showed (i), (ii) and (iv) (definite recurrence).

Case report of three patients with histological diagnoses of recurrent PBC

Patient no. 2 had refractory ACR requiring steroid pulse therapy on postoperative day (POD) 12, 36, 43, 97, 103, 420 and OKT3 monoclonal antibody on POD 434. Liver dysfunction associated with biliary dilatation developed 20 months after LDLT and we performed hepaticojejunostomy and wedge liver biopsy, which revealed suspected recurrence of PBC. Immunosuppression consisted of tacrolimus (3.0 mg/day), steroid (5 mg) and mizoribine (50 mg). Immunoglobulin M was 136, antimitochondrial antibody (AMA) 80 and anti-M2 152 mg/dL. Aggressive liver failure developed despite increased immunosuppression thereafter. She underwent retransplantation 24 months after LDLT.

In patient no. 4, alkaline phosphatase (ALP) began to increase 65 months after LDLT and liver dysfunction developed thereafter. Liver biopsy was performed 71 months after LDLT. Immunosuppression consisted of tacrolimus (2.0 mg/day) and steroid (5 mg). Aspartate aminotransferase (AST) was 44, ALP 432, γ-glutamyltransferase (γ-GT) 17, total bilirubin 1.7 mg/dL, AMA 80 and AMA-M2 155 mg/dL. Tacrolimus was changed to Neoral (Cyclosporine; Novartis, Basel, Switzerland), and mycophenolate mofetil (MMF) (2000 mg/day) was added. Ascites developed 1 year after and liver failure developed. She underwent retransplantation 88 months after LDLT.

In patient no. 5, liver dysfunction developed (AST, 82 IU/L; ALP, 685 IU/L) 50 months after LDLT and was successfully treated with steroid pulse therapy. Liver dysfunction developed and liver biopsy was performed 90 months after LDLT. Total bilirubin was 1.2 mg/dL, AST 57 IU/L, ALP 585 IU/L and γ-GT 48 IU/L. AMA and M2 were not measured. Immunosuppression consisted of tacrolimus only (4.0 mg/day), and MMF (2000 mg) was added thereafter. Portal hypertension started to develop. Radiological examinations yielded a diagnosis of artery-portal shunt of segment 3 of the graft. Shunt

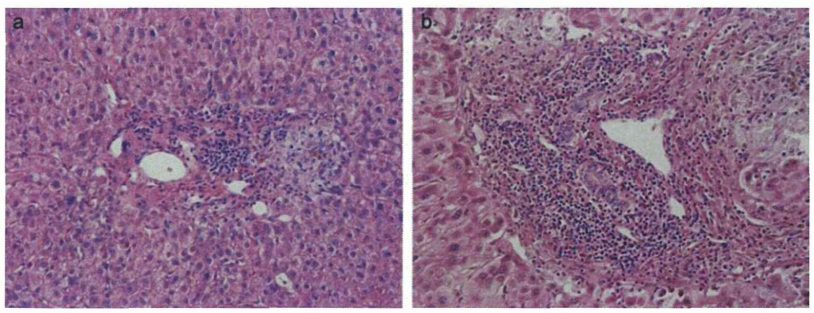

Figure 1 Histological findings of patient no. 2. (a) Wedge liver biopsy at postoperative month 20. Suspected recurrence of primary biliary cirrhosis (PBC) with bile duct loss and mild lobular and portal hepatitis. (b) Second explant liver (allograft). Suspected recurrence of PBC with moderate portal hepatitis and minimal bile duct damage (hematoxylin–eosin, original magnification ×200).

occlusion using metallic coils failed and led to liver failure. She underwent retransplantation 120 months after LDLT.

DISCUSSION

HISTOLOGICAL EXAMINATION IS the gold standard for recurrent PBC. Hubscher *et al.* reported the histological features to be mononuclear portal inflammation, portal lymphoid aggregate, portal granulomas and bile duct damage.[9] These findings are observed also in complications other than recurrent PBC. Lymphoid aggregate can be observed in chronic hepatitis, and bile duct damage and/or vanishing bile duct can be observed in chronic rejection or in the end stage of chronic cholangitis. Foamy cell arteriopathy, which is another specific feature of chronic rejection, is seldom observed on needle biopsy. Duct loss without portal granuloma suggests chronic rejection. The current study focusing on explanted allografts was conducted to avoid these uncertain factors.

Recently, late cellular rejection, chronic hepatitis, and de novo autoimmune hepatitis were discussed as causes of late liver allograft dysfunction.[16] Haga *et al.* reported perivenular lymphoplasmacytic infiltration in a case of their series, which simulated autoimmune hepatitis rather than typical PBC. In our series, ANA was strongly positive prior to primary transplantation in two patients but there were no such findings.

The incidence of recurrent PBC increased along with long-term follow up. Montano-Loza *et al.* studied the cumulative probability of PBC recurrence after LT.[17] Their histological study was not based on protocol biopsy. The overall 5- and 10-year probability of recurrence was 13% and 29%, respectively, in their series. They analyzed risk factors for recurrence and the clinical impacts. Although PBC transplant recipients receiving cyclosporin have a lower risk of disease recurrence, the development of recurrent PBC had no impact on long-term patient survival during 10 years of follow up. The incidence in LDLT based on protocol biopsy was 40% during 10 years of follow up.[3] Besides the increasing incidence, progression of recurrent PBC is still a concern, although progression of recurrent PBC was slow within 10 years of follow up in our series. In Japanese registries of LT, some cases of mortality after 10 years have been reported but information about their causes is not available.[18] A precise study of these cases is required to reveal the risks including recurrence in long-term follow-up.

Protocol biopsies for early diagnosis of recurrent PBC may not be essential to improve clinical courses of

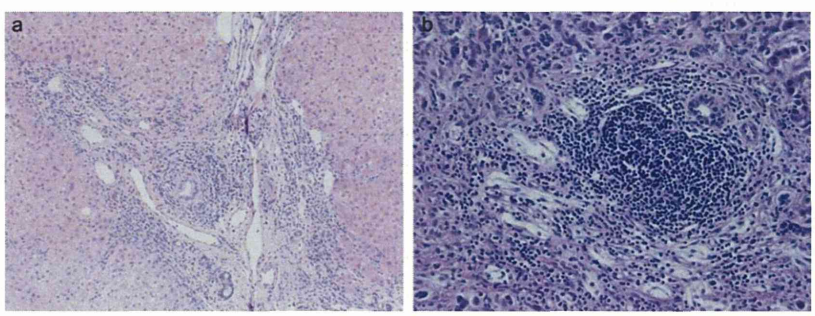

Figure 2 Histological findings of patient no. 4. (a) Needle liver biopsy at postoperative month 71. Recurrence of primary biliary cirrhosis (PBC) with non-suppurative cholangitis and moderate portal hepatitis and fibrosis. (b) Second explant liver (allograft). Suspected recurrence of PBC with focal duct damage and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×150; [b] ×200).

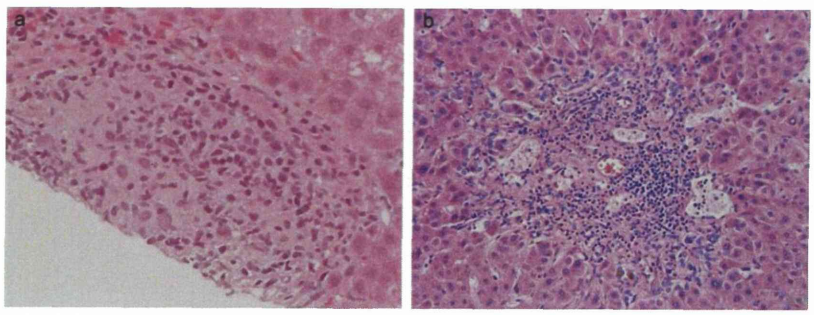

Figure 3 Histological findings of patient no. 5. (a) Needle liver biopsy at postoperative month 90. Recurrence of primary biliary cirrhosis (PBC) with focal cholangitis and epithelioid granuloma. (b) Second explant liver (allograft). Suspected recurrence of PBC with bile duct loss and portal inflammation (hematoxylin–eosin, original magnifications: [a] ×250; [b] ×200).

patients after LT for PBC. Timely biopsies and suitable radiological examinations, when hepatic chemistries deteriorate, are important to improve the clinical course within 10 years after transplantation.

ACKNOWLEDGMENTS

THIS STUDY WAS supported by a Health Labor Sciences Research Grant awarded to The Intractable Hepato-Biliary Disease Study Group in Japan.

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Transition of Serum Alkaline Phosphatase Isoenzymes during Liver Regeneration in Humans

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Original Papers, Liver

Key words: Alkaline phosphatase isoenzymes; Liver regeneration; Living donor liver transplantation; Normal liver function

Abbreviations

ALP alkaline phosphatase

POD postoperative days

LRR liver regeneration rate

CT computed tomography

HG high regeneration group

LG low regeneration group

ABSTRACT

Background/ Aims: Serum alkaline phosphatase (ALP) levels tend to increase after hepatectomy, however, no previous examinations have yet focused on the relationship between liver regeneration and the individual ALP isoenzymes levels.

Methodology: Forty living liver transplantation donors who underwent hemi-hepatectomy were herein investigated. We evaluated the serum ALP levels and ALP isoenzyme levels preoperatively and postoperatively. The liver regeneration rate (LRR) was calculated using volumetry. According to the LRR, we divided the donors into two groups, consisting of a high regeneration group (HG) and a low regeneration group (LG).

Results: The total serum ALP levels increased gradually after hepatectomy and peaked on postoperative days (POD) 14. ALP-1 was not detected in any donor preoperatively; however it was detected after hepatectomy, peaking on POD 7. The serum ALP-2 level increased after hepatectomy, reaching a peak level on POD 14. The ALP-2 levels gradually increased after hepatectomy and reached peak levels on POD 14 in both groups. However, the ALP-2 level on POD 14 was significantly higher in HG than LG.

Conclusions: The serum ALP- 2 levels after POD 14 might therefore be a useful indicator of favorable liver regeneration following hepatectomy, especially in patients who have a normal liver function.

INTRODUCTION

The liver is one of the few organs that has the ability to regenerate in the human body. Several indices are used as markers of liver regeneration, including alpha-fetoprotein and interleukin-6 (1,2). Alkaline phosphatase (ALP), which is a nonspecific phosphomonoesterase usually linked to the external cell surface via glycosyl phosphatidylinositol, may also be applicable as a marker of liver regeneration. Partial hepatectomy is known to cause an increase in the activity of ALP (3). Several reports have discussed the relationship between the total serum ALP levels and liver regeneration (4). In 1950, Burke et al. (3) reported that ALP is present in the cellular membrane and is associated with liver regeneration. Osada et al. (5) described the monitoring of the postoperative levels of ALP, and suggested that ALP may be a convenient indicator to predict liver failure after hepatectomy. However, the total serum levels of ALP during liver regeneration are not specific, and using the total ALP level as a marker of liver regeneration is controversial. We therefore focused on the levels of individual ALP isoenzymes in this study.

ALP determinations were first applied to the investigation of bone disease on a theoretical basis by Robinson (6). ALP consists of several isoenzymes in humans.

ALP-1 originates in the liver (biliary system and hepatocyte), ALP-2 the liver (intrahepatic bile duct), ALP-3 in the bone, ALP-4 in the placenta, ALP-5 in the small intestine and ALP-6 in the IgG-bound ALP (7). Therefore, measuring the fraction of each isoenzyme in patients with elevated ALP is considered to be a useful diagnostic tool (8). For example, an elevation of the ALP-1 or 2 levels can indicate the existence of an obstruction or malignant tumor in the biliary system (9). The serum ALP-3 level also increases under certain normal conditions, especially during the growth period (10).

To our knowledge, there have not been any studies which examined the relationship between liver regeneration and serum ALP isoenzymes. To clarify the transition of serum ALP isoenzymes during liver regeneration, we analyzed the ALP isoenzymes expression levels of donors who underwent a hemi-hepatectomy for living donor liver transplantation. We herein report the results of our examination of living liver transplantation donors who had normal liver function; a normal range for both the serum transaminase levels and total bilirubin levels, a normal coagulation profile and a negative hepatitis B and C status. We believe that our results may reflect the physiological phenomenon of liver regeneration in humans.

PATIENTS AND METHODS

Forty donors who underwent hemi-hepatectomy for living liver transplantation, including 17 right lobe grafts and 23 left lobe grafts, at our institution between April 2004 and December 2009 were included in this study. Right or left lateral section graft donors were excluded. To clearly determine the relationship between liver regeneration and isoenzyme serum levels of ALP, we evaluated the serum ALP levels and ALP isoenzyme (ALP-1, ALP-2, ALP-3, ALP-4, ALP-5) levels preoperatively, and again on postoperative days (POD) 3, 7, 14, and 28. The liver regeneration rate (LRR) (LRR = regenerated liver volume of POD 28/remnant liver volume of POD 0) was calculated using volumetry based on computed tomography (CT). We divided the donors into two groups according to their LRR, a high regeneration group (HG; LRR$\geqq$ 1.5) and a low regeneration group (LG; LRR$<$1.5). We analyzed each isoenzyme level of ALP at each time point, and also compared the changes of each isoenzyme between the HG and LG groups postoperatively.

STATISTICAL ANALYSIS

The Mann-Whitney *u*-test was used for statistical analysis. P values < 0.05 were regarded as statistically significant.

RESULTS

Twenty-five donors were male and 15 were female, with a median age of 32 years-old (range: 20-63y). Seventeen donated right lobe grafts and 23 donated left lobe grafts. The postoperative total serum ALP levels of all patients are shown in **Figure 1**. The total serum ALP levels increased gradually after the hepatectomy, and reached peak levels on POD 14. Concerning the individual isoenzymes of ALP **(Figure 2)**, ALP-1 was not detected in any donor preoperatively; however it was present after the hepatectomy, peaking on POD 7 (10.5% of total ALP) and gradually decreasing thereafter. ALP-2 levels also elevated after the hepatectomy and reached peak levels on POD 14 (68.7% of total ALP).

We also compared the serum ALP levels and ALP isoenzyme levels between the high regeneration group (HG) and the low regeneration group (LG). Sixteen

patients were included in the HG and twenty-four patients were included in the LG. There were no significant differences in the donors' ages between the two groups. In the HG, the resected liver volume was significantly higher than in the LG (62.0% *vs* 35.1%). The percentage of resected patients in the HG group was significantly higher than that in the LG group **(Table 1)**. The total serum ALP levels in the HG tended to be higher than the LG **(Figure 3)**. The ALP-2 levels gradually increased after hepatectomies and reached peak levels on POD 14 in both groups. However, the level of ALP-2 on POD 14 was significantly higher in the HG than the LG **(Figure 4)**. The other ALP isoenzyme levels were not significantly different between the patients in the HG and the LG.

DISCUSSION

The regenerative capacity of the liver has been known for hundreds of years, but the mechanisms underlying liver regeneration are still unclear. We have shown that the levels of each ALP isoenzyme change during liver regeneration. One of the interesting points presented in this study is that we examined donors involved in living liver transplantation. This enabled us to elucidate the mechanisms of liver regeneration,

because it presents an ideal method for monitoring liver regeneration *in vivo*. Previous reports about liver regeneration in humans have focused on dysfunctional livers, including cases of hepatocellular carcinoma (11).

Mori et al. (12) described that ALP activity increased in rat hepatocyte culture after the cell density reaches confluency. They also showed that the induced ALP activity is mainly localized at the apical surface membrane of the cells. These results suggest that during the regeneration period, the hepatocyte produces ALP on its membrane. In our study, we showed that serum ALP-2 levels increased after hemi-hepatectomy in living liver transplant donors. Moreover, the levels of ALP-2 in the HG were higher than those in the LG. We hypothesize that ALP-2, which is synthesized in the liver, increases during the regeneration period in tandem with the increase in liver growth.

One the other hand, Kaplan et al. (4) hypothesized that serum ALP levels increase after hepatectomy because the increased portal or hepatic venous pressure might cause swelling around the intrahepatic vascular, which in turn compresses the intraparenchymal billiary tract mimicking an obstructive process. However, we also observed that even if serum ALP-1 was not detected preoperatively, it could be detected after the hepatectomy. There is a possibility that pseudo-billiary tract obstruction after

the hepatectomy causes the increase in ALP-1. More investigations are needed to clarify the mechanisms underlying the increases in both of the isoenzymes and their role in level regeneration.

Concerning the LRR, the serum ALP-2 levels on POD 14 were significantly higher in the HG than in the LG, although there was a peak at POD 14 in both groups, and a gradual decrease thereafter in both groups. The serum ALP-2 levels may therefore reflect the speed of liver regeneration or the amount of regenerated liver.

The isoenzyme levels of ALP, with the exception of ALP-1 and ALP-2, were not affected by liver resection, possibly because these isoenzymes do not originate from the hepato-biliary tract. This phenomenon also supports our hypothesis that serum levels of ALP-1 or ALP-2 may be a marker for liver regeneration.

In conclusion, the ratio of each fraction of ALP isoenzymes changes in a time-dependent manner, after the hemi-hepatectomy in living liver transplant donors, who have normal remnant livers. It might therefore be possible to use the ratio of ALP-2 levels preoperatively and at various times postoperatively as an indicator of favorable liver regeneration following hepatectomy, especially in patients who have a normal liver function.

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