Table 3 Acute cellular rejection, immunosuppressive therapy and outcome of 16 patients

Case	No. of ACR episodes	Immunosuppressive therapy for ACR			Maintenance immunosuppressant			Latest laboratory date			Outcome/follow-up period (years)
		Steroid recycle	OKT3	MMF	CNI (trough)	CS (dose)	MMF	AST/ALT	ANA	IgG (mg/dL)‡	
1	1 (POD 8)	1	(−)	(−)							Died/1.0
2	0	0	(−)	(−)	FK 6.7	Prednisolone 10 mg†	(−)	25/21	(−)	1021	Alive/9.6
3	1 (POD 24)	1	(−)	(+)	CyA 128	2 mg	(−)	33/35	(−)	1468	Alive/9.2
4	3 (POD 15)	3	(+)	(+)	FK 5.3	2 mg	(−)	16/13	(−)	860	Alive/9.1
5	1 (POD 12)	1	(−)	(+)	FK 3.4	2 mg	(−)	20/19	(−)	1377	Alive/8.5
6	1 (POD 14)	1	(−)	(−)	FK 6.6	4 mg	(−)	21/39	(−)	759	Alive/8.4
7	0	0	(−)	(−)	FK 5.2	4 mg	(−)	24/20	×40	905	Alive/7.6
8	0	0	(−)	(−)	FK 5.5	2 mg	(−)	21/15	×40	1047	Alive/7.4
9	0	0	(−)	(−)	FK 3.9	4 mg	(−)	16/16	(−)	765	Alive/6.9
10	3 (POD 17)	3	(−)	(+)							Died/0.1
11	0	0	(−)	(−)	FK 5	2 mg	(−)	23/16	×40	1087	Alive/5.2
12	2 (POD 10)	2	(−)	(+)	CyA 112	2 mg	(−)	18/11	×40	1472	Alive/4.6
13	0	0	(−)	(+)	FK 5.3	2 mg	500 mg	11/9	×320	981	Alive/4.6
14	1 (POD 26)	1	(−)	(−)	FK 6.9	2 mg	(−)	17/12	×40	1108	Alive/4.5
15	1 (POD 10)	1	(−)	(+)	FK 7.4	6 mg	1000 mg	13/8	(−)	692	Alive/1.6
16	2 (POD 42)	2	(−)	(+)							Died/0.2

†Case 2 required prednisolone 10 mg/day for arthritis.
‡Normal range: 870–1700 mg/dL.
ACR, acute cellular rejection; ALT, alanine transaminase; ANA, antinuclear antibody; AST, aspartate transaminase; CNI, calcineurin inhibitor CS, corticosteroid; CyA, cyclosporin A; FK, tacrolimus; IgG, immunoglobulin; MMF, mycophenolate mofetil; OKT3, muromonab-CD3; POD, postoperative day.

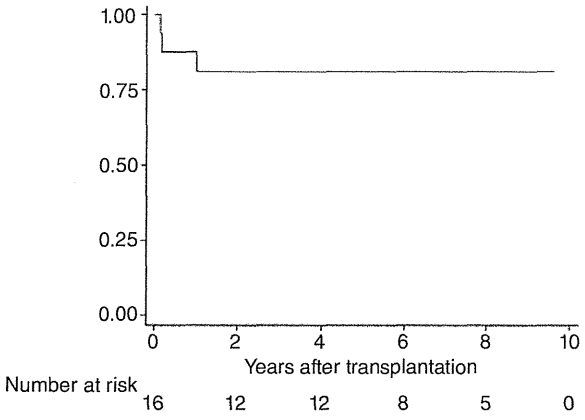

Figure 1 Cumulative survival after living-donor liver transplantation in patients with autoimmune hepatitis. The 1- and 5-year overall survival rates were 87.5% and 81.2%, respectively.

findings, none of 13 patients experienced recurrence of AIH during the follow-up period of 6.0 years (range, 0.1–9.6). At the end of the follow up, the serum IgG levels were within the normal range in all cases (mean ± standard deviation, 1041 ± 261 mg/dL), and antinuclear antibody was positive in six cases (Table 3).

DISCUSSION

AMONG ADULT LDLT recipients in our program, 16 patients (4.3%) underwent LDLT for end-stage liver disease secondary to AIH. Most of the patients were referred at the end stage after several years of treatment, and lacked the initial biopsy specimens for us to review. However, explant liver showed interface hepatitis and other changes compatible with AIH, and we were able to confirm the diagnosis.

The post-transplantation course was excellent, and survival was equivalent to that of other indications. None of the 13 recipients who survived showed liver dysfunction at the end of the follow up. Although serum antinuclear antibodies remained positive in six of 13 cases (46%) post-transplantation, the transaminase and IgG levels remained within the normal range under a maintenance dose of immunosuppressive therapy after a median follow-up period of 6.0 years (range, 0.1–9.6). Clinical recurrence was not seen among our studied population.

There is a possibility of histological recurrence, however, as we lacked protocol biopsies. Duclos-Vallee *et al.* reported that seven of 17 patients (41%) with AIH showed histological recurrence between 1 and 5 years after liver transplantation.[15] Four of these patients were diagnosed with recurrent AIH by protocol biopsy with normal liver function test results, and severe clinical recurrence occurred more than 10 years after liver transplantation.

Nonetheless, the frequency of recurrent AIH after liver transplantation is 22%, ranging 0–36% in different programs.[6,8,11,14,15,26–29] Risk factors for recurrent diseases have been inconsistently reported, including pre- and post-transplantation factors. After liver transplantation, suboptimal immunosuppression is generally thought to be a risk for recurrent AIH.[30]

Whether withdrawal of corticosteroid therapy causes recurrent AIH, however, remains controversial.[29,31] Recurrent AIH among liver transplant recipients should be treated with resumption of corticosteroids or immunosuppressants.[32,33] Narumi *et al.* reported that recurrence was frequently precipitated by reducing steroids.[14] Devlin *et al.* reported a case of recurrent AIH that occurred 10 years after liver transplantation and 8 years after corticosteroid withdrawal.[33] Prados *et al.* reported that patients with recurrence had received less immunosuppressive treatment.[26] Cattan *et al.* used cyclosporin A-based triple therapy on all 16 recipients with AIH and none developed recurrent AIH.[27] These results suggest that careful follow up is required while reducing the immunosuppressive regimen, or after reducing it, because recurrence can occur long term after transplantation. Our series may be valuable regarding the maintenance of corticosteroid therapy, because all patients in our study were maintained on corticosteroids and calcineurin inhibitors over the long term; it could be a reason why our recipients avoided clinical recurrence.

The incidence of ACR is higher among liver transplantation recipients with AIH than among those with non-AIH diseases.[11,14] In the current study, we found that more than 60% of recipients experienced pathologically-confirmed ACR, especially those who were recipients of livers from non-blood-related donors, and those with AIH alone. However, significant factor associated with occurrence of ACR was not found in this study. The relationship between ACR and survival or recurrence has not been reported, and requires further investigation.

Genetic factors are reportedly involved in the pathogenesis of autoimmune liver disease. HLA-DR3 and -DR4 are frequently seen in Caucasian patients with AIH. Wright *et al.* reported that recurrent AIH was more frequent in HLA-DR3 positive recipients of HLA-DR3 negative grafts.[8] Others also reported that HLA-DR3 was a risk factor for recurrent AIH.[9,15,16] The present study

included only Japanese recipients, however, and none of them had HLA-DR3. Six recipients had HLA-DR4, which is thought to be a common allotype among Japanese AIH patients.[34,35] There are almost no HLA-DR3 positive patients in the Japanese population and HLA-DR4 is more commonly observed.[34–36] Genetic variation might be a reason for the discrepancy with previous reports. A significant difference in clinical manifestations, such as age at the diagnosis, treatment response and associated autoimmune disease is reported. It may be speculated that the reported recurrence rate of AIH after liver transplantation is that in patients with susceptible genetic background. The recurrence rate of AIH among the Asian population may differ from that in Europe or the USA.

In conclusion, we report the long-term results of LDLT in 16 patients with AIH and AIH–PBC overlap syndrome. Clinical recurrence of AIH was not detected, even after 6 years follow up among our recipients. Although we lack protocol pathological evaluation, this stable post-transplantation course may be partially due to the continuation of corticosteroid maintenance therapy. Further studies are warranted to clarify genetic variations of the post-transplantation course in patients with autoimmune liver disease.

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Liver Regeneration and Venous Collateral Formation in the Right Lobe Living-Donor Remnant: Segmental Volumetric Analysis and Three-Dimensional Visualization

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Background. In left lobe (LL) living-donor liver transplantation (LDLT), hepatic venous congestion (HVC) caused by ligation of the middle hepatic vein tributaries is unavoidable in the right lobe (RL) donor remnant.
Methods. To clarify the impact of HVC on liver regeneration and venous collateral formation (VCF), we used three-dimensional computed tomography to examine the volumes of total/segmental liver and HVC and the degree of VCF; preoperative data were compared with data obtained on postoperative day (POD) 35 in 13 LL LDLT donors.
Results. On POD 35, the congestion rate decreased from 32.5% to 1.6% and the total liver regeneration rate was 81.7%. Preoperatively, the anterior sector-to-RL volume ratio was significantly lower, and the posterior sector-to-RL volume ratio was significantly higher than postoperatively (56.7% vs. 52.9%, $P<0.01$, and 36.9% vs. 41.5%, $P<0.01$, respectively). There was no correlation between degree of HVC and liver regeneration. Obvious VCF was found in five (38.5%) cases. The RL and posterior sector volume per square meter of body surface area in the VCF group were significantly lower than that in the non-VCF group (412 cm^3/m^2 vs. 492 cm^3/m^2, $P<0.01$, and 140 cm^3/m^2 vs. 190 cm^3/m^2, $P<0.01$, respectively). The preoperative congestion rate and liver regeneration rate were not significantly different between the groups.
Conclusions. Reconstruction of the middle hepatic vein tributaries in the RL donor remnant might not be necessary in LL LDLT, because the HVC improved dramatically by POD 35 regardless of the development of VCF.

Keywords: Congestion, Hepatic vein, Left lobe graft, Living-donor liver transplantation, Reconstruction.

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Since the first study in 1989, living-donor liver transplantation (LDLT) has been widely accepted worldwide as the treatment of choice for end-stage liver failure (*1*). Although the use of the right lobe (RL) as a graft has been increasingly successful, the problem of donor safety exists. In LDLT, it was reported that the incidence of donor complications based on 1841 donors in Japan was significantly higher in donors of the RL than in donors of the left lobe (LL) and the left lateral segment (*2*). In addition, operative mortality for RL donors was estimated to be as high as 0.5%–1.0% (*3*). We have previously reported that LL LDLT was a feasible treatment modality for ensuring minimal mortality and morbidity in donors (*4*) and that the number of biliary complications was significantly lower in LL LDLT than in RL LDLT (*5*). Donor safety is the highest priority in LDLT. Therefore, to minimize the risk to donors, LL LDLT may be an ideal option in LDLT. However, because the grafts usually include the middle hepatic vein (MHV) to improve the venous drainage in LL LDLT, hepatic venous congestion (HVC) in the right anterior sector caused by deprivation of drainage from the MHV tributaries is unavoidable in the RL donor remnant; this can lead to territories with outflow obstruction bearing the risk of insufficient liver regeneration (*6, 7*).

In the preoperative evaluation of donor livers, HVC estimates are based on three-dimensional computed tomography (3D-CT). In RL LDLT, the operative decision for the reconstruction of the MHV tributaries on the recipient side depends on the degree of HVC. However, there is no consensus with regard to the optimal reconstruction strategy on the donor side in LL LDLT. Although it has been reported that drainage of the anterior sector might be

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dependent on intrahepatic venous collaterals between the MHV tributaries and the right hepatic vein (RHV) in the later postoperative phase (*8*), it is unclear how much anastomosis would develop postoperatively. Furthermore, it is still not clear as to how the HVC would influence liver regeneration and venous collateral formation (VCF) in the later postoperative phase.

The purpose of the present study was to better understand liver regeneration and VCF in the RL donor remnant in LL LDLT. We assessed total and segmental donor liver regeneration by comparing 3D-CT data obtained preoperatively with that obtained on postoperative day (POD) 35. We also determined the degree of VCF on POD 35 and examined how the HVC had influenced liver regeneration and VCF.

RESULTS

Preoperative and Postoperative Right Lobe Volume, Hepatic Venous Congestion Volume, Congestion Rate, and Liver Regeneration Rate

The mean (SD) preoperative 3D-CT estimated volumes of the whole liver, the RL, and the HVC were 1207 (40) cm^3 (range, 1029–1491), 801 (126) cm^3 (range, 593–1070), and 260 (81) cm^3 (range, 84–414), respectively. The mean (SD) postoperative volumes of the RL remnant and the actual congestion on POD 35 were 986 (135) cm^3 (range, 765–1232) and 15 (12) cm^3 (range, 0–34), respectively. The mean (SD) congestion rate decreased from 32.5% (10.7%) (range, 14.2%–59.4%) to 1.6% (1.3%) (range, 0.0%–3.4%) on POD 35. The mean (SD) liver regeneration rate on POD 35 was 81.7% (5.8%) (range, 70.1%–92.8%) (Table 1). There was no correlation between the preoperative congestion rate and the liver regeneration rate.

Comparison Between the Moderate and Severe Hepatic Venous Congestion Groups

Among the 13 LL LDLT donors, there were five (38.5%) cases in the moderate HVC group and eight (61.5%) cases in the severe HVC group. There was no significant difference in the rate of complications greater than Clavien grade 1 between these two groups (20.0% vs. 25.0%, *P* value is not significant [NS]); in addition, the liver regeneration rate on POD 35 did not differ significantly between the groups (83.8% vs. 80.4%, *P* value is NS). Postoperative liver function tests such as serum aspartate aminotransferase, alanine aminotransferase, total bilirubin, and prothrombin time were not significantly different between the two groups (Fig. 1A–D).

Preoperative and Postoperative Right Lobe Donor Volume: Segmental Volumetric Analysis

The mean (SD) preoperative estimated volumes of the anterior sector and the posterior sectors of the RL were 450 (71) cm^3 (range, 362–569) and 297 (81) cm^3 (range, 168–429), respectively. The mean (SD) volume ratio of the anterior sector to the RL was 56.7% (8.2%) (range, 44.0%–72.1%), and the mean (SD) volume ratio of the posterior sector to the RL was 36.9% (7.4%) (range, 23.1%–50.6%). The mean (SD) preoperative estimated volumes and mean (SD) segment-to-RL volume ratios were as follows: 163 (66) cm^3 (range, 108–357) and 20.5% (6.9%) (range, 11.9%–37.9%) in segment V, 286 (58) cm^3 (range, 212–400) and 36.2% (7.3%) (range, 22.5%–48.5%) in segment VIII, 129 (60) cm^3 (range, 46–229) and 16.3% (7.1%) (range, 5.9%–28.4%) in segment VI, and 168 (66) cm^3 (range, 92–277) and 20.6% (6.2%) (range, 11.5%–32.7%) in segment VII, respectively. The mean (SD) estimated volumes of the

TABLE 1. Summary of each liver parameter before and after surgery

	Preoperative	Postoperative (POD 35)
Whole liver volume, mean (SD), cm^3	1207 (40)	—
RL volume, mean (SD), cm^3	801 (126)	986 (135)
HVC volume, mean (SD), cm^3	260 (81)	15 (12)
Congestion rate,[a] mean (SD), %	32.5 (10.7)	1.6 (1.3)
Regeneration rate,[b] mean (SD), %	—	81.7 (5.8)
Segmental liver volume, mean (SD), cm^3		
Anterior sector	450 (71)	517 (73)
Segment V	163 (66)	172 (76)
Segment VIII	286 (58)	346 (60)
Posterior sector	297 (81)	413 (102)
Segment VI	129 (60)	175 (72)
Segment VII	168 (66)	238 (103)
Sector-to-RL volume ratio, mean (SD), %		
Anterior sector	56.7 (8.2)	52.9 (7.3)
Segment V	20.5 (6.9)	17.3 (6.5)
Segment VIII	36.2 (7.3)	35.6 (7.2)
Posterior sector	36.9 (7.4)	41.5 (6.9)
Segment VI	16.3 (7.1)	17.8 (7.2)
Segment VII	20.6 (6.2)	23.7 (8.2)

HVC, hepatic venous congestion; POD, postoperative day; RL, right lobe.
[a] Congestion rate (%) was calculated as HVC volume divided by RL volume.
[b] Regeneration rate (%) was calculated as postoperative RL volume on POD 35 divided by preoperative whole liver volume.

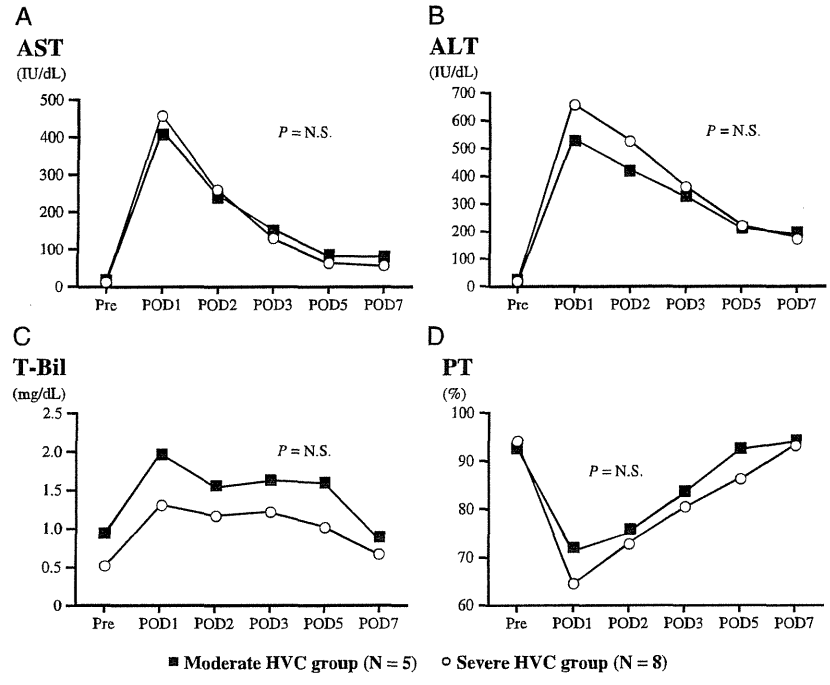

FIGURE 1. Postoperative serial change in liver function tests in the moderate and severe HVC groups. Postoperative liver function tests such as serum AST, ALT, T-Bil, and PT were not significantly different between the two groups. A, AST. B, ALT. C, T-Bil. D, PT. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HVC, hepatic venous congestion; NS, not significant; POD, postoperative day; PT, prothrombin time; T-Bil, total bilirubin.

anterior sector and the posterior sector on POD 35 were 517 (73) cm^3 (range, 396–650) and 413 (102) cm^3 (range, 238–573), respectively. On POD 35, the mean (SD) volume ratio for the anterior sector to the RL remnant and the posterior sector to the RL remnant was 52.9% (7.3%) (range, 38.6%–62.0%) and 41.5% (6.9%) (range, 31.1%–55.9%), respectively. The mean (SD) estimated volumes on POD 35 and mean (SD) segment-to-RL volume ratios were as follows: 172 (76) cm^3 (range, 112–390) and 17.3% (6.5%) (range, 10.9%–34.4%) in segment V, 346 (60) cm^3 (range, 260–445) and 35.6% (7.2%) (range, 22.9%–47.6%) in segment VIII, 175 (72) cm^3 (range, 54–300) and 17.8% (7.2%) (range, 4.8%–29.4%) in segment VI, and 238 (103) cm^3 (range, 124–407) and 23.7% (8.2%) (range, 14.0%–39.7%) in segment VII, respectively (Table 1).

On POD 35, the anterior sector did not atrophy but became enlarged, regardless of the degree of HVC, and of course, the posterior sector became enlarged. However, the ratio of the anterior sector volume to the RL volume on POD 35 was significantly lower, and the ratio of the posterior sector volume to the RL volume on POD 35 was significantly higher than preoperatively (56.7% vs. 52.9%, $P<0.01$, and 36.9% vs. 41.5%, $P<0.01$, respectively) (Fig. 2A, B). According to detailed segmental volumetric analysis, on POD 35, the ratio of segment V volume to the RL volume was significantly lower and the ratio of segment VII volume to the RL volume was significantly higher than preoperatively (20.5% vs. 17.3%, $P<0.05$, and 20.6% vs. 23.7%, $P<0.01$, respectively); however, there were no significant differences in this volume ratio for segments VIII and VI (36.2% vs. 35.6%, P value is NS, and 16.3% vs. 17.8%, P value is NS, respectively) (Fig. 2C–F).

Comparison Between the Venous Collateral Formation Group and the Non-Venous Collateral Formation Group

Among all 13 cases, obvious VCF between the MHV tributaries and the RHV was found in 5 (38.5%) cases (Fig. 3A–E), in which 1 (7.7%) case simultaneously developed intrahepatic venous anastomoses between the MHV tributaries and the inferior right hepatic vein (IRHV) (Fig. 3E). The comparison between the VCF group and the non-VCF group is summarized in Table 2. There was no significant difference in the rate of complications greater than Clavien grade 1 between the two groups (20.0% vs. 25.0%, P value is NS). Postoperative liver function tests were not significantly different between the two groups. Additionally, there was no significant difference in the preoperative congestion rate and the liver regeneration rate on POD 35 between the VCF and the non-VCF groups (35.9% vs. 30.4%, P value is NS, and 80.1% vs. 82.6%, P value is NS, respectively). The preoperative RL volume per square meter of body surface area (BSA) in the VCF group was significantly lower than that in the non-VCF group (412 cm^3/m^2 vs. 492 cm^3/m^2, $P<0.01$). Although the volume per square meter of BSA of the anterior sector was not significantly different between the VCF and non-VCF groups (250 cm^3/m^2 vs. 266 cm^3/m^2, P value is NS), the volume per square meter of BSA of the posterior sector was significantly lower

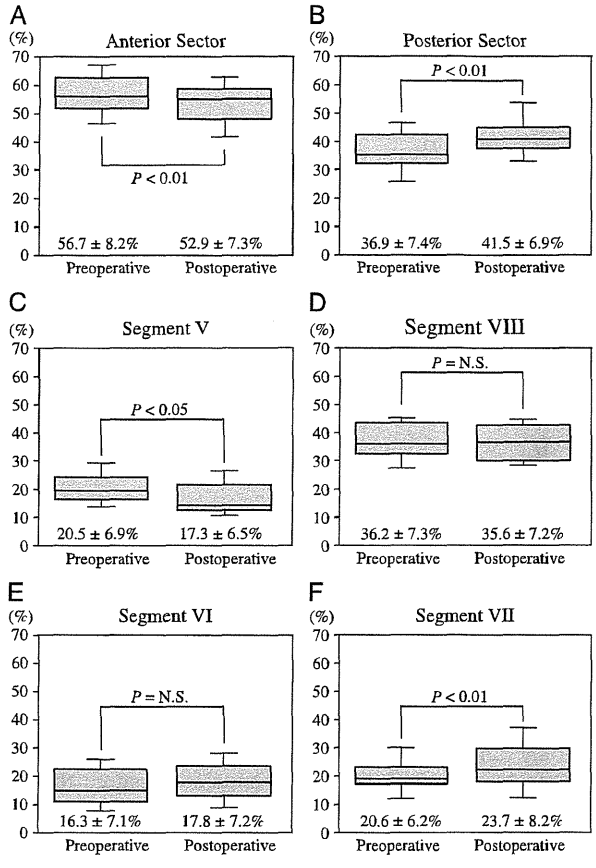

FIGURE 2. Comparison of preoperative and postoperative segmental liver-to-RL volume ratios. A, Anterior sector. B, Posterior sector. C, Segment V. D, Segment VIII. E, Segment VI. F, Segment VII. The postoperative anterior sector-to-RL volume ratio was significantly lower than preoperatively, and the postoperative posterior sector-to-RL volume ratio was significantly higher than preoperatively ($P<0.01$ and $P<0.01$, respectively). Postoperatively, the segment V-to-RL ratio was significantly lower, and the segment VII-to-RL ratio was significantly higher than preoperatively ($P<0.05$ and $P<0.01$, respectively). There were no significant differences in this ratio for segments VIII and VI, preoperatively and postoperatively. The liver segment-to-RL volume ratio is represented by box-and-whisker plots. The data (%) are shown as the mean value±standard deviation. The line in the box represents the median; the upper and lower lines of the box represent the 75th and 25th quartiles. The upper and lower lines outside of the box represent the 90th and 10th quartiles. NS, not significant; RL, right lobe.

in the VCF group than in the non-VCF group (140 cm^3/m^2 vs. 190 cm^3/m^2, $P<0.01$).

DISCUSSION

LDLT is an established procedure for the treatment of patients with end-stage liver disease, especially in Japan and other Asian countries, where deceased donors are not often available. In Western countries, LL LDLT has not generally been recognized as a feasible procedure because of the problem of graft size. The initial experience related to LL grafts demonstrated a higher incidence of small-for-size syndrome graft failure and recipient complications. Consequently, RL grafts have been used routinely at many centers (*9–11*). However, although the use of the RL as a graft has been increasingly successful, the problem of donor safety still exists. We have previously reported that the outcomes of LL LDLT were comparable with those of RL LDLT, although small-for-size syndrome occurred more often in LL LDLT. In addition, the overall donor morbidity rates were comparable between LL and RL, whereas postoperative liver function tests and hospital stay were significantly improved in LL donors (*12*). Donor safety should be the highest priority. Therefore, LL LDLT is considered the first choice in our institution.

In LL LDLT, the incidence of HVC in the right anterior sector caused by deprivation of drainage from the MHV tributaries is unavoidable. Left hepatectomy of the liver is a standard procedure in oncological liver surgery. Consequently, not much attention has been paid so far to the HVC of the remnant and reconstruction of the MHV tributaries is not usually performed. Indeed, even if transient liver dysfunction has occurred, HVC has been known to improve, with the liver returning to an almost normal level of function at POD 30 (*7*). In cases of HVC in the early postoperative phase, Doppler ultrasonography can show an absence of venous blood flow and reversed flow, indicating that the portal vein (PV) may be acting as a drainage vein owing to the presence of an acute hepatic outflow obstruction. However, by POD 7, Doppler ultrasonography can show a normal hepatopetal flow in the anterior PV (*13*). Therefore, in the later postoperative phase, drainage of the right anterior sector is believed to be dependent on the intrahepatic venous anastomoses between the MHV tributaries and the RHV (*8, 14*). Indeed, several reports have demonstrated that the collaterals can develop within several days after LDLT (*15, 16*). However, it is unclear as to how much anastomosis can develop postoperatively. Furthermore, it is still not clear as to how the HVC would influence liver regeneration and VCF in the later postoperative phase. Donor safety should be the highest priority as emphasized before. Death of donors can have a negative impact in various areas. After the death of a donor in New York in 2002, the frequency of LDLT was reduced by 51% in that city and by 21% in the United States as a whole (*17, 18*). Therefore, we find it difficult to understand such a phenomenon with regard to the RL donor remnant.

In liver transplant recipients receiving an RL graft, the reconstruction of the MHV tributaries has been performed using interposition grafts to prevent HVC. Cheng et al. (*19*) reported that there was no clinically significant difference in recipient outcome between the recipients who showed occlusion of the interposed graft and those recipients whose interposition grafts remained patent; however, graft regeneration was lower in the occluded group than that in the patent group. Whether the interposition grafts have remained patent is not considered to be clinically significant in the later postoperative phase, because the intrahepatic venous network between the MHV tributaries and the RHV is generally present (*8, 14*). However, this venous network has not been established yet in the early postoperative phase.

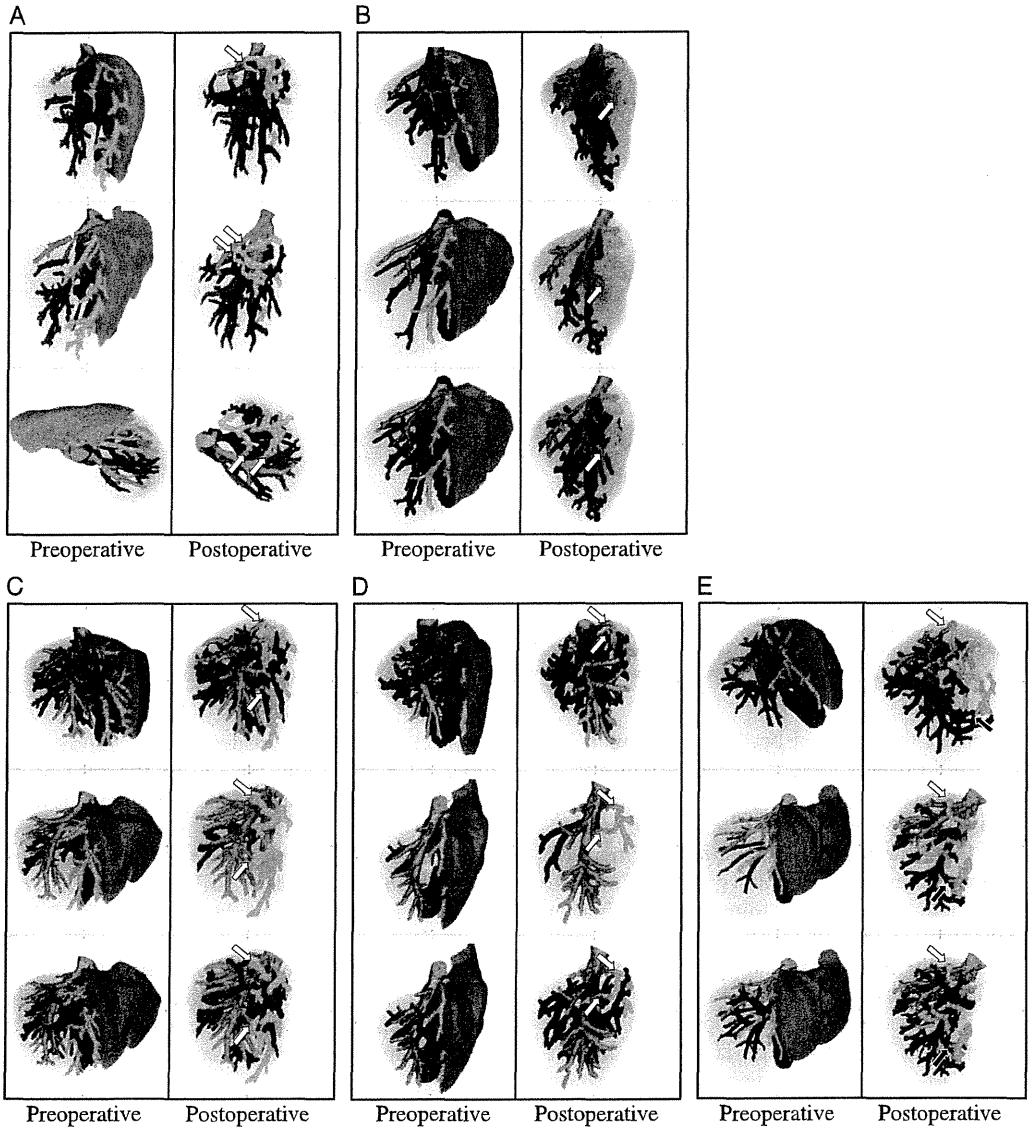

FIGURE 3. 3D-CT images of VCF visualization. Among all 13 cases, VCF between the MHV tributaries and the RHV (*white arrows*) was found in 5 cases (A–E). Of these cases, one simultaneously developed intrahepatic venous anastomoses between the MHV tributaries and the IRHV (*black arrows*) (E). The left and right sides of the figure represent preoperative and postoperative 3D-CT images, respectively. The RHV and IVC are colored aqua blue. The MHV tributaries, IRHV, and PV are colored yellow, red, and dark blue, respectively. 3D-CT, three-dimensional computed tomography; IRHV, inferior right hepatic vein; IVC, inferior vena cava; MHV, middle hepatic vein; PV, portal vein; RHV, right hepatic vein; VCF, venous collateral formation.

Therefore, to prevent liver dysfunction during this early period and eventual graft failure, the concept of the reconstruction of the MHV tributaries is an accepted modality (*8, 20*). We have previously reported that the MHV tributaries should be reconstructed in transplant recipients if the calculated HVC is more than 20% (*20*). However, there are no criteria for the reconstruction of the MHV tributaries in the RL remnant of donors in LL LDLT, and the reconstruction of the MHV tributaries has not usually been performed. The reasons for this are as follows: (1) the reconstruction procedure is difficult as it should be performed in situ and not on a back table; (2) it is necessary to create an additional wound to obtain the interposition graft; (3) because the imbalance between inflow and outflow can be mild in donors as compared with recipients (*21*), the impact of the congestion on the liver is believed to be mild in comparison to the impact on the recipients; (4) liver function will return to almost normal levels at POD 30 regardless of the degree of HVC (*7*); and (5) the collaterals between the ligated MHV tributaries and the RHV can develop within several days after LDLT (*15, 16*). However, it is still unclear as to the amount of intrahepatic venous

TABLE 2. Comparison between VCF group and non-VCF group

	VCF group (n=5)	Non-VCF group (n=8)	*P*
Preoperative factor			
Congestion rate,[a] mean (SD), %	35.9 (7.5)	30.4 (1.8)	NS
Serum AST level, mean (SD), IU/dL	21 (4)	19 (13)	NS
Serum ALT level, mean (SD), IU/dL	25 (6)	19 (20)	NS
Serum T-Bil level, mean (SD), mg/dL	0.8 (0.4)	0.8 (0.3)	NS
Serum PT level, mean (SD), %	94 (6)	93 (9)	NS
RL volume/BSA, mean (SD), cm^3/m^2	412 (28)	492 (22)	<0.01
Anterior sector volume/BSA, mean (SD), cm^3/m^2	250 (25)	266 (45)	NS
Posterior sector volume/BSA, mean (SD), cm^3/m^2	140 (31)	190 (37)	<0.01
Postoperative factor			
Regeneration rate,[b] mean (SD), %	80.1 (3.3)	82.6 (6.4)	NS
Complications greater than Clavien grade 1, n (%)	1 (20.0)	2 (25.0)	NS
Peak serum AST level, mean (SD), IU/dL	480 (156)	400 (110)	NS
Peak serum ALT level, mean (SD), IU/dL	680 (348)	523 (196)	NS
Peak serum T-Bil level, mean (SD), mg/dL	1.6 (0.4)	2.0 (1.0)	NS
Bottom serum PT level, mean (SD), %	67 (2)	70 (10)	NS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; HVC, hepatic venous congestion; NS, not significant; POD, postoperative day; PT, prothrombin time; RL, right lobe; T-Bil, total bilirubin; VCF, venous collateral formation.

[a] Congestion rate (%) was calculated as HVC volume divided by RL volume.

[b] Regeneration rate (%)was calculated as postoperative RL volume on POD 35 divided by preoperative whole liver volume.

collateral development there would be, and how much influence the HVC would have on liver regeneration and VCF in the later postoperative phase.

Scatton et al. (*6*) reported that in the LL donor remnant without an MHV, the regeneration rate of segment VI was lower and the regeneration rate of segments II and III were higher in the global congestion group at 1 month after hepatectomy. Similarly, in this series, the ratio of the anterior sector volume to the RL volume calculated on POD 35 was significantly lower than that calculated preoperatively, whereas this ratio for the posterior sector on POD 35 was significantly higher than preoperatively. However, the anterior sector did not atrophy and became enlarged regardless of the degree of HVC. In the present study, among the 13 cases, obvious VCF between the MHV tributaries, the RHV, and the IRHV was found in 5 (38.5%) cases on POD 35. In contrast to what we had expected, the preoperative congestion rate was not significantly different between the VCF group and the non-VCF group. The fact that the congestion rate decreased from 32.5% to 1.6% on POD 35, and that there was no correlation between the preoperative congestion rate and the liver regeneration rate, might suggest that tiny intrahepatic anastomoses could develop in all cases, even though they could not be visualized using 3D-CT. Preoperative RL volume per square meter of BSA in the VCF group was significantly lower than that in the non-VCF group. Furthermore, the volume per square meter of BSA of the anterior sector was not significantly different between the groups, and that of the posterior sector was significantly lower in the VCF group. From these facts, it is reasonable to assume the following: (1) the smaller the RL donor remnant is, the more overloaded it will become owing to PV inflow; (2) the posterior sector will be more affected by PV inflow, because the anterior branch may be acting as a drainage vein owing to an acute hepatic outflow obstruction; (3) the greater the PV inflow overload is, the more VCF there will be; (4) in the case of obvious VCF, overload may be caused not only by outflow block but also by extra inflow.

In conclusion, in LL LDLT, although the HVC caused by ligation of the MHV tributaries is unavoidable in the RL donor remnant, the HVC had improved dramatically by POD 35 regardless of the development of obvious VCF. There was no correlation between the preoperative congestion rate and the liver regeneration rate. Therefore, the reconstruction of the MHV tributaries in the RL donor remnant may not be necessary in LL LDLT.

MATERIALS AND METHODS

Patients

From May to November 2009 at Kyushu University Hospital, 13 patients underwent LL LDLT. A total of 13 donors were thus the subject of this study. The donors included 11 men and two women. Their median age was 34 years (range, 21–53) and their median body mass index was 22.3 kg/cm^2 (range, 17.8–25.9). Median values estimated using preoperative 3D-CT for total liver volume, extended left and caudate lobe volume, and RL volume were 1189 cm^3 (range, 1029–1491), 409 cm^3 (range, 322–492), and 792 cm^3 (range, 593–1070), respectively. For all donors, 3D-CT was performed preoperatively and on POD 35.

Three-Dimensional Reconstruction and Volumetry

The procedures used have been described elsewhere (*7, 22, 23*). Briefly, multidetector helical CT (MDCT) images were obtained using 2-mm-thick slices represented on CT machines. Enhancement was achieved using an intravenous bolus injection of nonionic contrast medium (Iopamion, Schering, Erlangen, Germany) at a speed of 5 mL/sec. Two types of 3D-CT software were used to achieve 3D reconstruction of the liver, HVC area, and portal and hepatic venous branches from the MDCT data. One type of 3D-CT software was ZIO M900 (Zio Software Inc, Tokyo, Japan), with which it was possible to freely fix the cutoff line. The other was liver segmentation software (Hitachi Medico, Tokyo, Japan), which was used to calculate

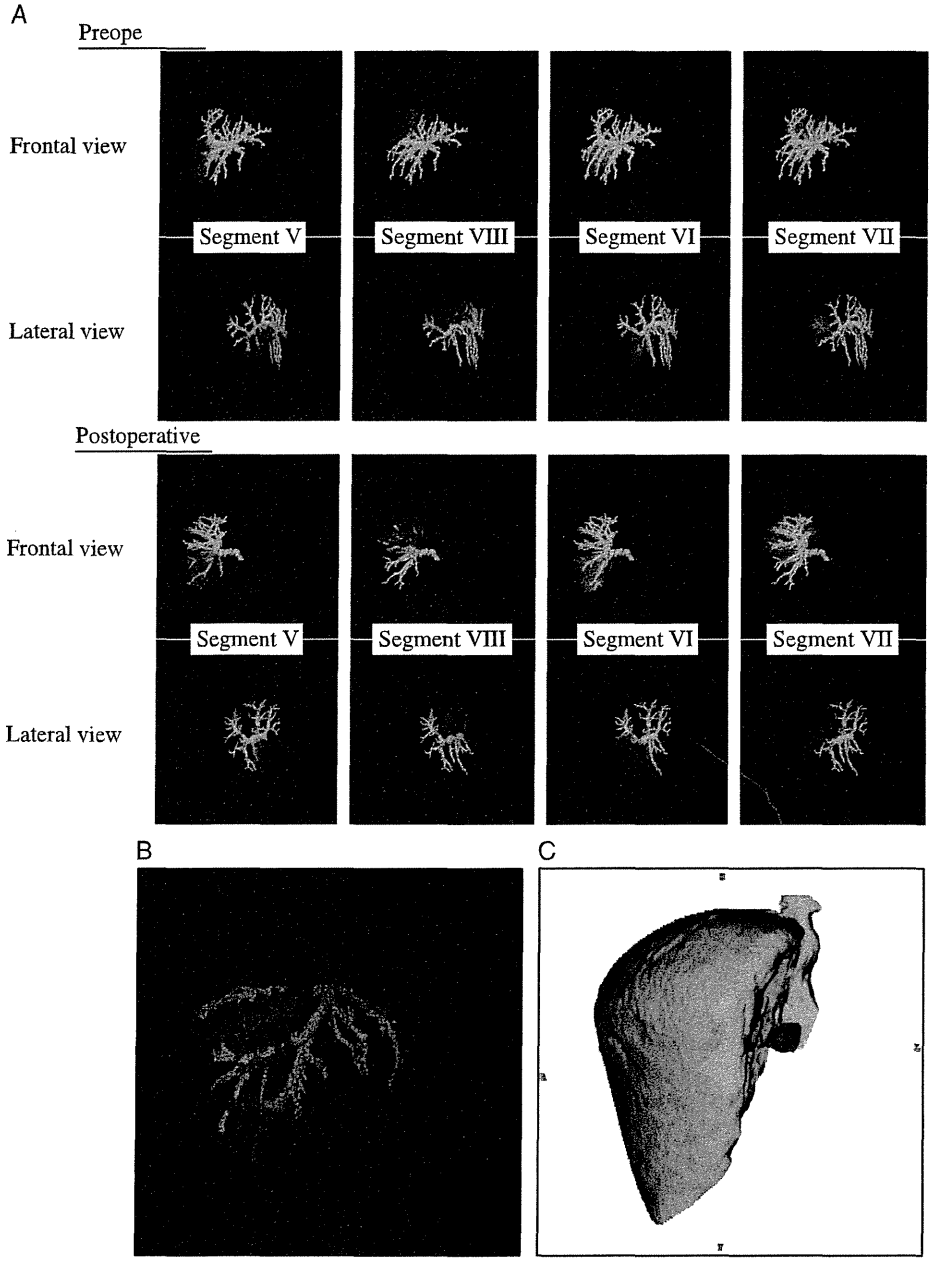

FIGURE 4. 3D-CT images of preoperative and postoperative segmental liver volumes and HVC. A, Preoperative and postoperative segmental liver volumes were calculated using liver segmentation software. Each segmental liver volume was calculated automatically from each PV branch territory and is described in frontal and lateral views. PV and each segmental PV branch are colored green and pink, respectively. The segmental liver volumes are colored light orange. B, Preoperative HVC volume of the MHV tributaries was automatically calculated from each hepatic venous branch using liver segmentation software. HV and the MHV tributaries are colored aqua blue and pink, respectively. Preoperative HVC volume is colored light orange. C, Postoperative HVC volume of the actual congestion area on POD 35 was rendered by two-phase CT using ZIO M900. It was calculated using the difference in attenuation between the congestion area and the noncongestion area. IVC and PV are colored aqua blue and dark blue, respectively. Postoperative HVC volume is colored purple. 3D-CT, three-dimensional computed tomography; HVC, hepatic venous congestion; IVC, inferior vena cava; MHV, middle hepatic vein; POD, postoperative day; PV, portal vein.

the liver volume and the volume of each vessel's (both portal and hepatic venous branches) territories from their diameter and length.

Total and Segmental Liver Volumes, the Ratio to the Right Lobe, and the Liver Regeneration Rate

Total and segmental liver volumes were calculated using liver segmentation software. The volume of the RL was calculated from the right PV territory, and the segmental liver volume of each PV branch was calculated automatically (Fig. 4A). Each volume ratio was calculated as follows: volume of a given segment divided by RL volume (%). The liver regeneration rate was calculated as follows: postoperative RL volume on POD 35 divided by preoperative whole liver volume (%).

Hepatic Venous Congestion Volume and the Congestion Rate

The preoperative HVC volume of the MHV tributaries was automatically calculated from each hepatic venous branch using the liver segmentation software (Fig. 4B). The 3D image reconstructed using this software could reflect the actual congestion volume. The postoperative HVC volume of the actual congestion area on POD 35 was rendered by two-phase CT using ZIO M900 software (Fig. 4C). The CT findings showed that the congestion area had become hyperattenuated because of poor drainage of the contrast medium (*24*). The postoperative HVC volume on POD 35 was calculated using the difference in attenuation between the congestion area and the noncongestion area. The detailed procedures have been described elsewhere (*7*). The congestion rate was calculated as follows: HVC volume divided by RL volume (%). The 13 LL LDLT donors were divided into two groups depending on the degree of congestion rate as previously described (*7*); the congestion rate of the moderate HVC group ranged from 10% to 30%, and that of the severe HVC group was greater than 30%.

Venous Collateral Formation Visualization

Postoperative VCF visualization on POD 35 was obtained from the MDCT data using ZIO M900 software. Detection of the connection between the MHV tributaries, the RHV, and the IRHV using the 3D-CT software was defined as "obvious VCF." Therefore, cases in which the MHV tributaries were patent, and in which the collateral connection could not be found, were not recognized as VCF. The 13 LL LDLT donors were divided into two groups: the VCF group and the non-VCF group.

Evaluation of Postoperative Clinical Parameters

Postoperative liver function tests such as serum aspartate aminotransferase, alanine aminotransferase, total bilirubin, and prothrombin time were measured on PODs 1, 2, 3, 5, and 7. Complications were classified according to Clavien's classification (*25*).

Graft Selection

The criteria for graft selection have been described elsewhere (*7, 8*). Briefly, an LL graft was initially considered as a graft with respect to donor safety. An RL graft was selected when an LL graft was insufficient for the recipient and the remnant liver volume of the donor was greater than 35%.

Surgical Procedure

The surgical procedures for donors have been described elsewhere (*4, 5, 8*). Briefly, donor hepatectomy was performed with intermittent inflow occlusion under the hanging maneuver. In LL grafts, the MHV was procured with the liver graft. Therefore, the MHV tributaries were ligated under hepatectomy. None of the MHV tributaries were reconstructed on the donor side.

Statistical Analysis

Statistical analysis was performed using Student *t* test and chi-square test. The data were considered significant when the *P* value was less than 0.05. All analyses were performed with the use of StatView software (Version 5.0, Abacus Concepts, Berkeley, CA).

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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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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

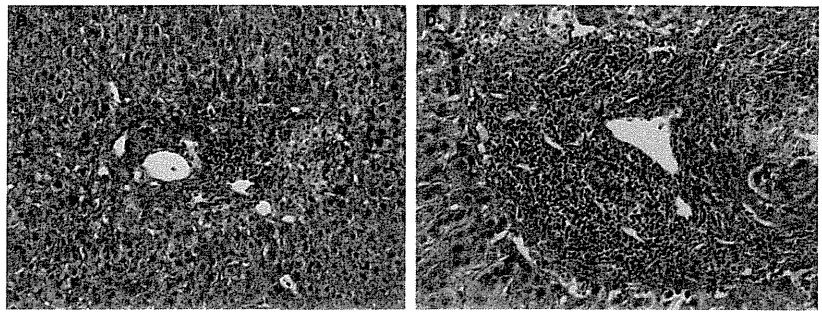

Figure 1 Histological findings of patient no. 2. (a) Wedge liver biopsy at postoperative month 20. Suspected recurrence of primary biliary cirrhosis (PBC) with bile duct loss and mild lobular and portal hepatitis. (b) Second explant liver (allograft). Suspected recurrence of PBC with moderate portal 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

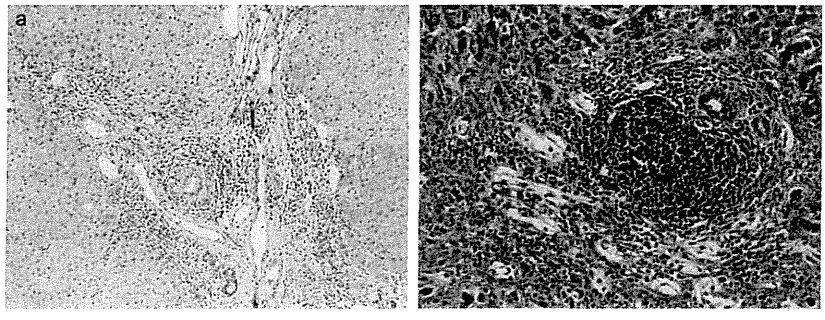

Figure 2 Histological findings of patient no. 4. (a) Needle liver biopsy at postoperative month 71. Recurrence of primary biliary cirrhosis (PBC) with non-suppurative cholangitis and moderate portal hepatitis and fibrosis. (b) Second explant liver (allograft). Suspected recurrence of PBC with focal duct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

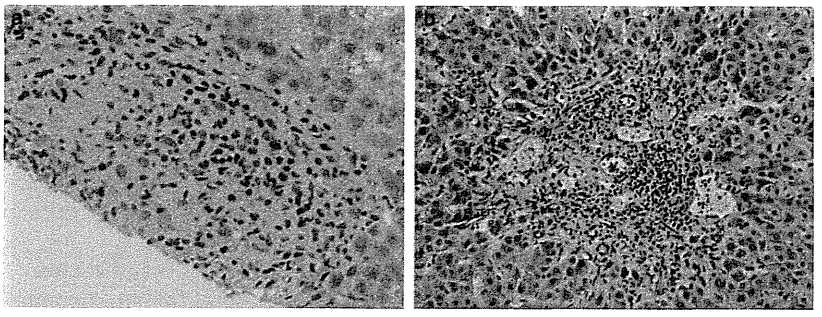

Figure 3 Histological findings of patient no. 5. (a) Needle liver biopsy at postoperative month 90. Recurrence of primary biliary cirrhosis (PBC) with focal cholangitis and epithelioid granuloma. (b) Second explant liver (allograft). Suspected recurrence of PBC with bile duct loss and portal inflammation (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.

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Invited Review

The Current Endeavors to Understand the Pathogenesis of Intractable Liver Diseases

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The death due to liver diseases accounts for more the 35,000 cases every year in Japan for decades. Among these liver diseases, the Ministry of Health, Labor, and Welfare of Japan has named both fulminant hepatitis and primary biliary cirrhosis (PBC) as intractable liver diseases, since the precise mechanism of these diseases are unclear. Accordingly, there are no effective medical treatments other than liver transplantation toward these diseases. However, still the number of the liver transplantation performed in Japan is small. Thus, we have focused on the pathogenesis of these two intractable conditions. The fulminant hepatitis is a distinct form of acute hepatitis, and hepatitis B virus infection accounts for 20~30% of this lethal condition. Only tiny proportions of patients with acute HBV infection develop fulminant hepatitis (less than 10%). It has been widely believed both viral and host factors contribute for fulminant hepatitis, although still unknown factors are expected to be involved. On the other hand, PBC is a chronic progressive cholestatic liver disease. Clinical features of PBC include female predominance (80 to 90%), the presence of antimitochondrial antibody (up to 95%), and elevated serum levels of immunoglobulin M. Eventually, patients with PBC will develop liver failure due to biliary cirrhosis in spite of medical interventions. Immune-mediated processes are believed to be responsible for the pathogenesis, although the precise mechanism is yet to be determined. In this review article, our endeavors to understand the mechanism of these intractable liver diseases are discussed.

Key words: fulminant hepatitis; hepatitis B virus; intractable liver diseases; liver failure; primary biliary cirrhosis
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For past 30 years, the death due to liver diseases accounts for more than 30,000 Japanese every year. Among these deaths, the hepatocellular carcinoma (HCC) has been major cause of liver related death in Japan (Ueno et al. 2009) In the early time of this period, the Hepatitis C virus (HCV) was not discovered, and thus so-called non-A non-B hepatitis was the most possible cause of HCC in Japan. Soon after the discovery of HCV in the end of 1980s, the majorities of non-A non-B hepatitis were proven to be actually HCV-related liver diseases. Moreover, the introduction of preventive measures to avoid iatrogenic HCV transmission, namely transfusion related infections, dramatically decreased newly infection in early 1990s (Amarapurkar et al. 2009). Thus, the most important clinical issues were focused on how to manage and treat the people with established HCV infections. Fortunately, the recent progress of treatment of HCV made possible for eradication of virus from human body in nearly half of the genotype 1b type (difficult to treat genotype) and more than 80% of other genotypes (Kogure et al. 2008; Kumada et al. 2010a). Also, chronic hepatitis B virus (HBV) infection is responsible for approximately 10% of HCC in Japan (Mikami et al. 2007). Before the year of 2000, there are number of the cases with liver failure due to chronic HBV infection. However, after the introduction of nuleot(s)ide analogues, the amount of HBV levels were controllable in the majority of the cases, which has been demonstrated as the decreased number of liver failure derived from chronic HBV infection (Kumada et al. 2010b; Inoue et al. 2011a, 2011c) (Fig. 1). Given these observations, the challenge for researchers studying liver diseases has been shifted to intractable liver diseases, rather than controllable common diseases. Currently, the Japanese Ministry of Health, Labor, and Welfare has named both fulminant hepatitis and primary biliary cirrhosis (PBC) as intractable liver diseases, and give financial supports as the grant in aid for intractable liver diseases. In this review article, the current trends and efforts to understand the pathogenesis of these two diseases are provided.

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Dr. Yoshiyuki Ueno is a recipient of the 2010 Gold Prize, Tohoku University School of Medicine.

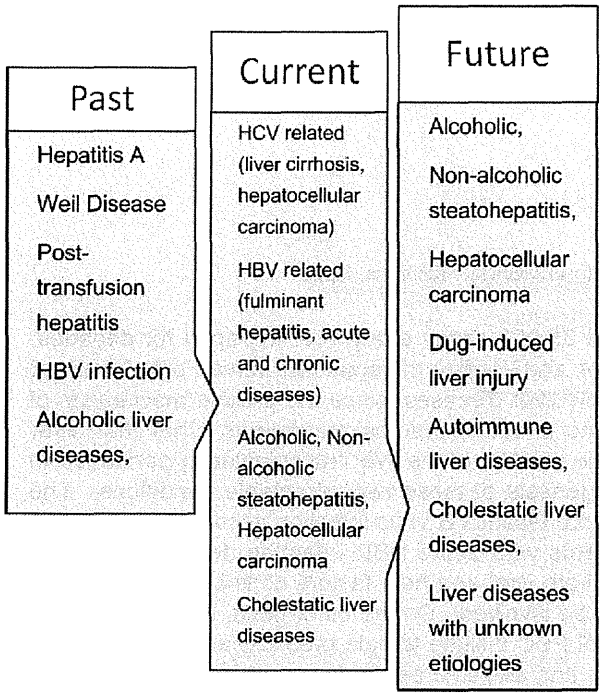

Fig. 1. Trends in the morbidity of liver diseases in Japan.
HAV: hepatitis A virus, HBV: hepatitis B virus

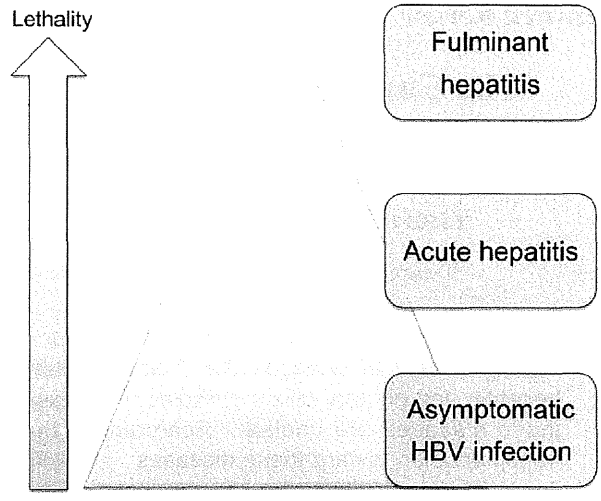

Fig. 2. Scheme of acute HBV infection.
Only tiny portions of patients with acute HBV infection develop fulminant hepatitis.

Fulminant Hepatitis

Fulminant hepatitis (FH) is a strange form of acute hepatitis. In Japan, the term 'fulminant hepatitis' is used for fatal form of acute viral hepatitis. This is very characteristic, since most of Western country uses the term 'fulminant hepatic failure', including drug-induced acute liver toxicities such as acetaminophen overdoses. Although the term has not been uniformed globally, this acute condition often demonstrates serious clinical courses, leading to more than 30-40% of mortality in acute form. In Japan, thus acute viral infection, especially acute hepatitis B virus (HBV) infection is a major cause of this fatal disease. HBV infection is one of the most common viral diseases affecting humans. HBV causes a diverse group of liver diseases such as acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Acute infection of HBV sometimes leads to fulminant hepatitis B (FHB), which is lethal during a short period in many cases. Indeed, we have experienced this emergent condition several times, which has been successfully treated with liver transplantation (Inoue et al. 2005). In fact, only a tiny proportion of patients with acute hepatitis B will develop FHB, in which the precise mechanism of pathogenesis has not been clarified (Fig. 2). Both viral factors and human (host) factors are believed to be important for the pathogenesis of FHB. Several mutations in the Core promoter and Precore region of HBV, especially a mutation at 1896 in Core region, were reported to be associated with the development of FHB. However, some conflicting results have been described about specific viral factors for the pathogenesis of FHB. Accordingly, there are still unknown mechanisms in regard to the pathogenesis of FHB.

In the year of 2000, seven patients with FHB were referred to Tohoku University Hospital, Sendai. More importantly, these cases were supposed to be derived from a single person who had been persistently infected with a specific HBV strain. Thus, in these specific cases of FHB, viral factors rather than host factors seemed to be more important for developing FH, since six of seven proven infected person with this HBV strain developed fulminant hepatitis. Unfortunately, five of six patients with this FHB deceased with acute liver failure. Thus, we have tried to clarify the viral factors to cause FHB from this series of FHB (Nagasaki et al. 2008).

First, we have performed the full-genome sequencing with this HBV strain to find out specific mutations. We have found that this viral strain belongs to HBV genotype Ba, which is reported to be typical in Asian region, and different from genotype Bj, which is dominant genotype of acute HBV infection in Japan. Next, we have done in vitro experiments to further investigate the mechanism of pathogenesis of FHB induced by this specific HBV strain (Inoue et al. 2009). To do this, we constructed vectors consisting of x1.3 length full-genome cDNA insert of HBV strain, as well as mutated inserts at various positions. Using this in vitro experimental system, we have found that i) the mutation found in this HBV strain caused dramatic increase of viral replication, ii) replicated virion accumulated in cytoplasm of hepatocytes, implicating potential cytotoxicity due to these accumulations, and iii) the mutation in pre-Core region and Core region of HBV caused enhanced transcriptional activities (Inoue et al. 2011b). Taken together, we have shown that specific HBV stain that caused series of FHB in the year of 2000 in Sendai area showed 1) potent