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Original Article

Chronologic changes of explanted liver volume and the use of ursodeoxycholic acid in patients with end-stage primary biliary cirrhosis

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Aim: The clinical presentation of Primary biliary cirrhosis (PBC) at the time of liver transplantation (LT) may have changed, due to the long-term use of ursodeoxycholic acid (UDCA). The aim of this retrospective study was to investigate whether the clinical characteristics of LT recipients with PBC have changed over the years.

Methods: Of all 421 adults undergoing LT from 1997 to 2012 at our center, we included 85 recipients with PBC into the present study. The 85 recipients were divided into three groups according to the year LT was performed: group 1 (1997–2001, $n = 29$), group 2 (2002–2005, $n = 29$) and group 3 (2006–2012, $n = 27$).

Results: There were no significant differences in sex, recipient age, Model for End-Stage Liver Disease score, updated Mayo risk score for PBC, or liver-related complications except for esophageal varices among the three groups. Patients in group 1 were complicated with esophageal varices less frequently than those in the other two groups. In older cases, the ratio of explanted liver volume to standard liver volume (ELV/SLV) was significantly higher, and the duration of pre-LT UDCA treatment was significantly shorter. The duration of UDCA treatment was significantly correlated with ELV/SLV.

Conclusion: Recent LT patients were characterized by more frequent portal hypertension and more severe liver atrophy, with longer UDCA therapy prior to LT, which might have prevented the somewhat rapid progression of liver failure characterized by hepatomegaly with insignificant fibrosis or portal hypertension.

Key words: explanted liver, hepatomegaly, liver transplantation, living donor liver transplantation, primary biliary cirrhosis

INTRODUCTION

PRIMARY BILIARY CIRRHOSIS (PBC) is a chronic and cholestatic liver disease characterized by inflammatory destruction of the intrahepatic bile ducts that is thought to be autoimmune mediated.[1,2] The mechanism underlying the development of PBC remains controversial.[3] Cholestatic liver cirrhosis leading to hepatic failure is the most severe clinical manifestation of PBC, resulting in death or requiring liver transplantation (LT).[2,4] Potential subtypes of PBC disease progression, which are hepatic failure type and portal hypertension type, were recently proposed, and are represented by the presence or absence of autoantibodies, such as anti-gp210 and anticentromere.[5–7]

First introduced into clinical practice in the 1990s, the efficacy of ursodeoxycholic acid (UDCA) for suppressing the disease progression of PBC is well established. Although the mechanisms of action of UDCA are not clarified, the prognosis of PBC has improved since the introduction of UDCA, which decreases the need for LT.[8,9] Some patients with progressive PBC, however, still require LT despite adequate treatment with UDCA.[10] We hypothesized that there would be some different or

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stepwise trends in the pretransplant characteristics of recipients with PBC according to when LT was performed, presumably because UDCA might have been administrated to PBC patients at an earlier stage in more recent LT recipients.

The main scope of this retrospective, single-center study was to examine the above hypothesis by comparing the detailed characteristics of explanted livers at LT as well as the clinical characteristics among patients who underwent LT for PBC at our center.

METHODS

Subject

USING A PROSPECTIVELY collected database in our institution, we retrospectively reviewed all consecutive patients who underwent LT between January 1996 and March 2013. Among them, we included patients who received LT for end-stage PBC, and they were divided into three groups according to when the LT was performed: group 1 (1997–2001), group 2 (2002–2005) and group 3 (2006–2012). Patients with overlapping non-immune-related liver disease such as viral or alcoholic hepatitis were excluded.

Retrospective review of the records of all LT recipients at the University of Tokyo was approved by the University of Tokyo Institutional Review Board. Often, patients were referred from other hospitals or clinics. The treatment strategy for PBC prior to LT, such as the use of UDCA or bezafibrate, was continued after referral to our center unless unnecessary. These treatment strategies were recorded as pre-LT therapy.

All candidates for LT in our center received upper gastrointestinal endoscopy to evaluate the presence and/or significance of esophagogastric varices immediately prior to living donor LT (LDLT), or before or after being listed as a deceased donor LT (DDLT) candidate. The presence of ascites and hepatocellular carcinoma (HCC) was evaluated by contrast enhanced computed tomography or magnetic resonance imaging immediately (within 1 month) pre-LT. The Model for End-Stage Liver Disease (MELD) score,[11] updated Mayo risk score for PBC[12] and M2 antimitochondrial antibody titer were also recorded from the laboratory data pretransplantation.

Histopathological evaluation of explanted liver

The size of the explanted liver was evaluated by calculating the ratio of the explanted liver volume (ELV) to standard liver volume (SLV) (ELV/SLV). The calculation formula for SLV (mL) was 706.2 × body surface area (m^2) + 2.4.[13] Explanted liver specimens were reviewed by one of the experienced pathologists at our center and scored according to Scheuer's classification (stage 1–4),[14] and explants in stage 1–3 were labeled as "non-cirrhotic". Diagnostic criteria of PBC–autoimmune hepatitis (AIH) overlap syndrome was based on the clinical guideline published by the European Association for the Study of the Liver,[15] namely, at least two of the three following criteria for PBC and AIH, respectively, should be present: PBC ([i] alkaline phosphatase, >2 upper limit of normal [ULN] or γ-glutamyltransferase, >5 ULN; [ii] antimitochondrial antibody, >1:40; and [iii] liver biopsy specimen showing florid bile duct lesions) and AIH ([i] alanine aminotransferase, >5 ULN; [ii] immunoglobulin G, >2 ULN or a positive test for anti-smooth muscle antibodies; and [iii] liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis). Other findings, such as the presence of HCC, were also noted.

Statistical analysis

We used SPSS version 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data. Differences between groups were analyzed by the Mann–Whitney *U*-test or Kruskal–Wallis test for continuous variables and the χ^2-test for categorical variables. $P < 0.05$ was considered significant.

RESULTS

Patient characteristics

OF ALL THE 421 adult LT recipients from January 1996 to December 2012 at our center, we included 86 recipients with PBC in the present study. One patient who had overlapping HCV infection was excluded. The remaining 85 patients included in the present study were divided into three groups according to when the LT was performed, as noted above: group 1 (1997–2001, $n = 29$), group 2 (2002–2005, $n = 29$) and group 3 (2006–2012, $n = 27$). Three patients in group 3 underwent DDLT, whereas the remaining 82 patients underwent LDLT. Four patients had overlapping AIH (one in group 1, one in group 2, two in group 3, $P = 0.20$).

The details of the characteristics of all three groups are shown in Table 1; older cases were complicated with esophageal varices less frequently (70% in group 1 vs greater than 90% in the other two groups, $P = 0.019$),

Table 1 Patient characteristics at LT ($n = 85$)

	Group 1 ($n = 29$)	Group 2 ($n = 29$)	Group 3 ($n = 27$)	*P*-value
Age (years)	53 (35–64)	50 (37–66)	56 (32–64)	0.19
Female sex	23 (79)	26 (90)	26 (96)	0.14
MELD score	20 (9–32)	19 (11–39)	17 (9–36)	0.69
Updated Mayo risk score	9.3 (6.1–11.8)	9.3 (5.3–12.9)	9.5 (7.2–13.1)	0.21
Total bilirubin (mg/dL)	14 (2–30)	10 (2–39)	8 (2–36)	0.55
INR	1.3 (1.1–2.4)	1.5 (1.1–3.2)	1.2 (1.2–3.0)	0.27
Ascites	17 (59)	16 (55)	20 (74)	0.15
HE	5 (17)	4 (14)	5 (19)	0.84
HCC	2 (7)	0 (0)	1 (4)	0.34
Esophageal varices	20 (70)	26 (90)	26 (96)	0.019

Unless otherwise indicated, data are given as the number (%) or the median (range).
HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; INR, International Normalized Ratio; LT, liver transplantation; MELD, Model for End-Stage Liver Disease.

although no significant difference in other factors, including MELD score or updated Mayo risk score, which represent the severity of illness, was detected.

Treatment for PBC pre-LT

The duration of UDCA treatment until LT was significantly shorter in older cases (Fig. 1a). The dose of UDCA per bodyweight (BW) was not significantly different among the three groups (Fig. 1b), whereas the cumulative dose of UDCA per BW until LT was significantly higher in recent cases (Fig. 1c). The use of bezafibrate tended to be more frequent in recent cases, but did not reach statistical significance (19% in group 1, 26% in group 2 and 33% in group 3, $P = 0.6$).

Association among liver volume, histopathological stage and UDCA treatment by years

Based on calculations of ELV/SLV, there was a significant difference in the size of the explants between groups; significantly larger explants were extirpated in older cases (Fig. 2). The rate of non-cirrhotic explants was also significantly greater in older cases (35% in group 1, 7% in group 2 and 12% in group 3, $P = 0.03$).

Considering the association between volume and histopathological stage of the explanted liver, the volume of cirrhotic explants (median ELV/SLV, 1.04 [range, 0.45–2.43]) was significantly smaller than those of non-cirrhotic explants (median ELV/SLV, 1.50 [range, 0.70–2.03]) ($P = 0.01$).

The duration of UDCA treatment prior to LT correlated significantly with ELV/SLV ($r^2 = 0.151$, $P = 0.001$, Fig 3a), whereas the cumulative UDCA dose per BW until LT was less closely associated with ELV/SLV ($r^2 = 0.09$, $P = 0.008$, Fig. 3b).

The explants of the patients with overlapping AIH ($n = 4$) were all cirrhotic, whereas 18% of the patients without overlapping AIH ($n = 81$) had non-cirrhotic explants. There was no statistical difference in ELV/SLV, however, between patients with or without AIH (median, 1.09 [range, 0.66–1.30]; and median, 1.08 [range, 0.45–2.43], respectively, $P = 0.56$).

DISCUSSION

IN THIS RETROSPECTIVE study, we present our experience that the clinical manifestations of PBC at the time of transplantation have drastically changed over the years. UDCA was initiated at an earlier stage in patients who underwent LT in more recent years. In parallel with this transition, explanted livers have become smaller, more severely shrunken, with more fibrosis and accompanied more frequently by esophageal varices. Based on these findings, we propose the following hypothesis: (i) early introduction of UDCA therapy could prevent rapid development of cholestatic liver failure with hepatomegaly, which was the dominant clinical manifestation of LT recipients with PBC in the 1990s or earlier 2000s, that is, prior to the introduction of UDCA; and (ii) even with adequate treatment with UDCA with or without bezafibrate, however, some patients develop liver cirrhosis, which eventually leads to life-threatening portal hypertension and liver atrophy (cirrhosis) and the requirement for LT in later 2000s.

An observation that may support our notion is that, in the published work before the era in which UDCA treat-

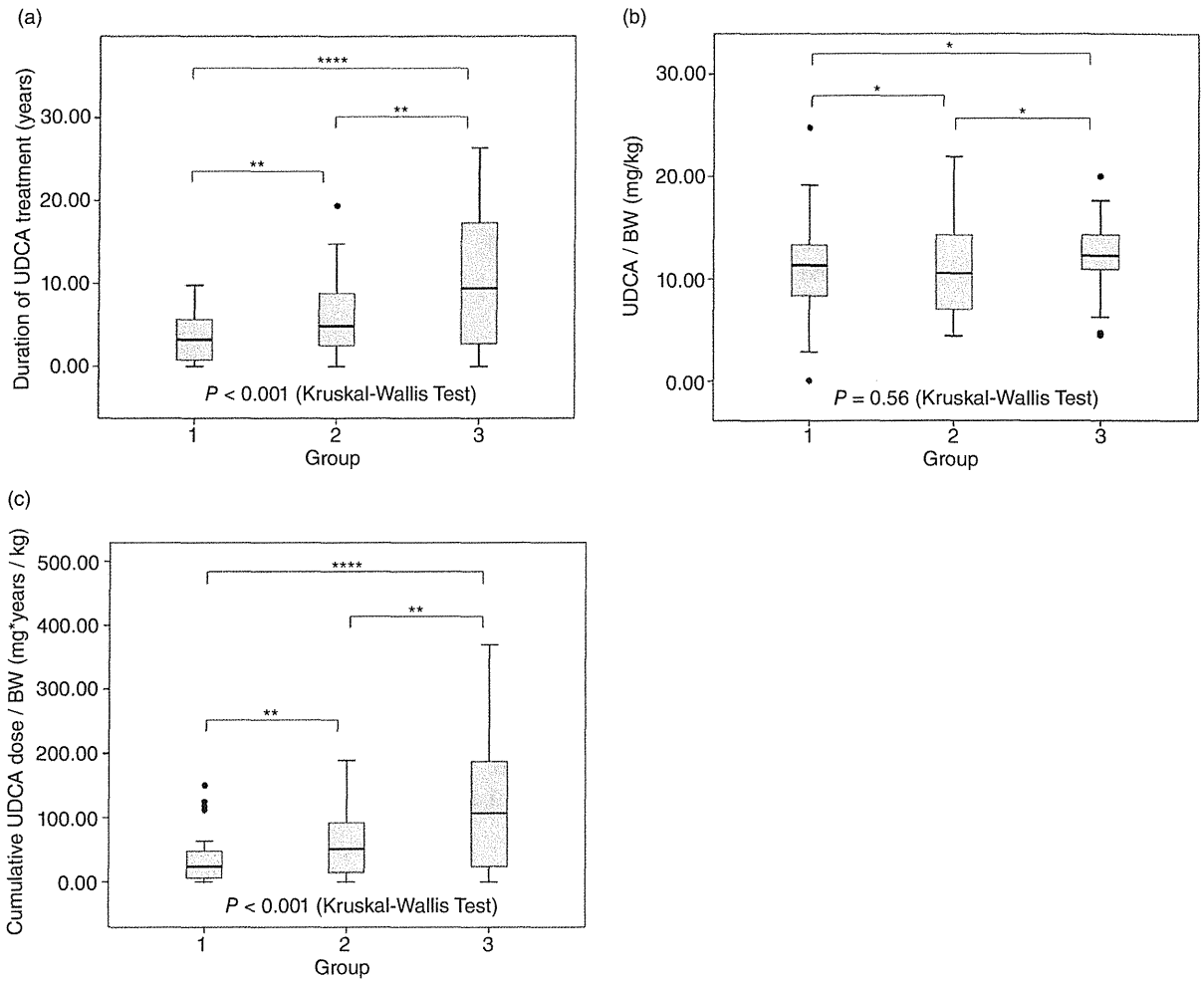

Figure 1 Box plots of (a) duration of ursodeoxycholic acid (UDCA) treatment (years), (b) UDCA dose per bodyweight (mg/kg) and (c) cumulative UDCA dose/bodyweight (mg × years/kg), for each group. The top and bottom of the boxes represent the first and third quartiles, respectively. The boxes enclose the interquartile range, with the median value denoted by the horizontal line (*P = n.s., **$P < 0.05$, ***$P < 0.01$, ****$P < 0.001$).

ment became a common practice, hepatomegaly (as well as portal fibrosis and cirrhosis) in PBC patients was accepted as a factor predicting a worse prognosis.[16,17] Hepatomegaly, however, is seldom mentioned as a prognostic factor of PBC in more recent published work.[18,19] Our current study revealed a stronger correlation between ELV/SLV with the duration of UDCA treatment than with the cumulative UDCA treatment per BW. This may indicate that the introduction of UDCA at an earlier stage is indeed beneficial and an important factor for preventing rapid progression of the disease.

Our findings are also consistent with several recent studies indicating that the prognosis of PBC improves with the introduction of UDCA, especially in those with an early treatment response.[18,20–23] Further, interestingly, presumably due to the general acceptance of UDCA for the management of PBC after this evidence was reported, the need for LT for PBC has dramatically decreased,[8,9] although authors understand that the benefit of UDCA seems controversial as there are publications doubting the influence of UDCA on patients' survival or time to LT.[24–26] In addition, LT still remains a last resort for those developing end-stage liver disease due to PBC despite adequate treatment with UDCA,[27] and there are additional areas for further dedicated studies to identify such patients.[28]

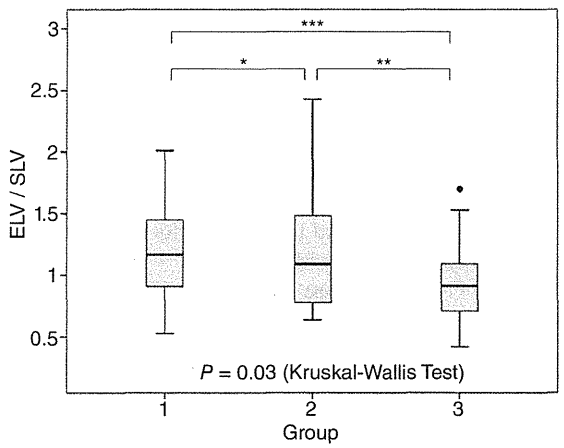

Figure 2 Box plots of the ratio of explanted liver volume to standard liver volume (ELV/SLV) for each group. The top and bottom of the boxes represent the first and third quartiles, respectively. The boxes enclose the interquartile range, with the median value denoted by the horizontal line (*P = n.s., **$P < 0.05$, ***$P < 0.01$).

Based on the histological findings of PBC, two major mechanisms are involved in the progression of PBC.[29] The first is bile duct destruction, which leads to chronic cholestasis and the development of cirrhosis with a biliary pattern. The second is interface hepatitis, which also leads to cirrhosis, the pattern of which resembles cirrhosis following patterns of chronic viral hepatitis. More recently, Nakamura *et al.* reported that the pattern of disease progression in PBC patients may be strongly associated with specific autoantibodies, such as anti-gp210 or anticentromere.[5,6] They proposed two different types of clinical manifestations of disease progression in PBC patients; one is a "hepatic failure type" progression, which is represented by positive anti-gp210 antibodies, and the other is a "portal hypertension type" progression, which is represented by positive anticentromere antibodies. As the group proposed that the "hepatic failure type" progression is characterized by rapid development of cholestatic liver failure,[30] this type could reach significant bilirubinemia and high International Normalized Ratio (i.e. high MELD score) enough to consider LT ahead of the establishment of cirrhosis, which may be correspondent with the characteristics of our older cases. In addition, several groups have proposed that some single nucleotide polymorphisms are related to the development of PBC.[28,31] Analyzing such genetic backgrounds in relation with the potential benefit of UDCA, as indicated in our study, may be beneficial.

In conclusion, recent LT patients were characterized by frequent portal hypertension, significant liver atrophy and fibrosis, and longer UDCA therapy prior to LT. According to these findings, we hypothesized that the early introduction of UDCA prevented comparatively rapid development of liver failure characterized by non-cirrhotic hepatomegaly and insignificant portal hypertension; however, it remains a challenge to identify and treat patients who will slowly develop liver

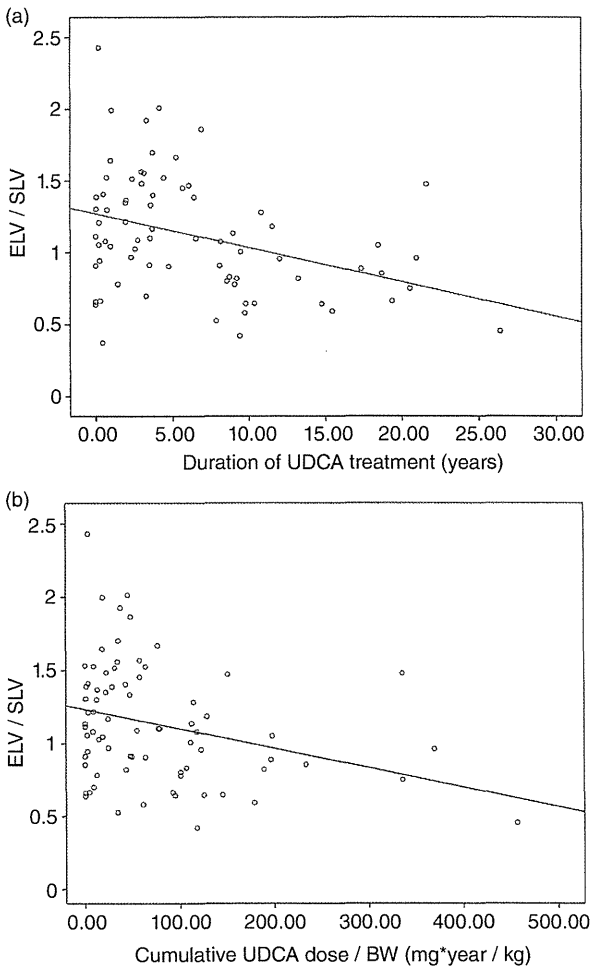

Figure 3 Relationship between the ratio of explanted liver volume to standard liver volume (ELV/SLV) and (a) duration of ursodeoxycholic acid (UDCA) treatment (years) and (b) cumulative UDCA dose/bodyweight (mg × years/kg). The duration of UDCA treatment correlated significantly with ELV/SLV ($r^2 = 0.151$, $P = 0.001$), whereas the cumulative UDCA dose until LT was less strongly correlated with ELV/SLV ($r^2 = 0.09$, $P = 0.008$).

failure requiring LT. Further studies are strongly warranted to examine our theory in a larger and prospective patient cohort, as well as the immunological or genetic corroborations.

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Original Article

Living donor liver transplantation for non-alcoholic steatohepatitis: A single center experience

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Aim: The number of patients referred for liver transplantation (LT) with non-alcoholic steatohepatitis (NASH) continues to increase, but information about living donor liver transplantation (LDLT) for NASH is scarce. We conducted this study to document the details of LDLT for NASH in a Japanese LT center.

Methods: Among all LDLT recipients in our institution from March 1996 to March 2013 ($n = 425$), we identified seven patients that underwent LDLT for NASH.

Results: Of all the seven recipients, most of the patients (86%) were obese. The median follow-up period post-LDLT was 5.3 years. All were alive at the last follow-up. Recurrent NASH was detected in one patient (14%), and no recurrent hepatic steatosis was detected among the remaining six recipients on prospectively performed ultrasonography. No significant comorbidities were observed following donor surgery among the respective living donors during the follow-up period. We also retrospectively reviewed 22 patients with NASH-related end-stage liver disease (ESLD) who were evaluated but rejected for LDLT during the same period. The reasons for rejection for LDLT were presumably associated with the nature of NAFLD/NASH in either potential recipients or donors.

Conclusion: The post-transplant outcome of LDLT for NASH-related ESLD in our institution was feasible, although the sample size was small. Further studies in a larger patient cohort are warranted to investigate the long-term outcome of LDLT for NASH, both for recipients and living donors.

Key words: living donor liver transplantation, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease

INTRODUCTION

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) has recently become one of the most common etiologies of liver disease worldwide, although it depends on the geographic area: Asian countries (16.9%) are estimated to have a lower prevalence than the Middle East (34.7%) or Europe/North America (23.2%).[1] The recent report by Kojima *et al.* supports the estimation that the proportion of NAFLD in the Japanese population is also increasing.[2]

Non-alcoholic steatohepatitis (NASH), a progressive form of NAFLD,[3] is a risk factor for the development of liver cirrhosis and/or hepatocellular carcinoma (HCC) that may require liver transplantation (LT).[4–7] The proportion of patients undergoing LT due to NASH-related liver disease has recently drastically increased in the United States, and the outcome of deceased donor liver transplantation (DDLT) for NASH is estimated to be comparable to that of other etiologies.[8,9]

Information regarding living donor liver transplantation (LDLT), however, remains limited. Several recent studies reported that genetic polymorphisms could be related to the establishment of NASH;[10–15] therefore, NASH is potentially heritable. Thus, the safety of liver

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resection in relative living donors of recipients with NASH and the long-term outcome of recipients receiving such grafts, in particular, require further clarification.

The present study was performed to describe the details of patients with NASH-related end stage liver disease (ESLD) who were evaluated for LDLT in our institution: we documented the characteristics and outcomes of patients transplanted for NASH-related ESLD, including both recipients and living donors, as well as the patients rejected for LDLT.

METHODS

Patients

USING A PROSPECTIVELY collected database in our institution, we retrospectively reviewed all consecutive patients who underwent LDLT from April 1996 to March 2013. The retrospective review of records of all liver transplant recipients at the University of Tokyo was approved by the University of Tokyo Institutional Review Board.

Pre-transplant diagnosis of NASH was based on the histopathologic findings pre-LT. In addition, extensive serologic testing and exclusion of significant alcohol consumption (>10 g/day) and hepatotoxic medications were performed to exclude non-NASH liver disease. Moreover, we reviewed all recipients that were diagnosed with cryptogenic cirrhosis (CC), among whom patients meeting the following definition were re-classified as having NASH-related cirrhosis and included in the present study: patients with explant histopathology compatible with NASH based on a retrospective and blind review by an experienced pathologist, in addition to obesity (defined as a body mass index [BMI] greater than 25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity[16]), diabetes mellitus (DM, defined as HbA1c $\geq$ 6.5 and/or medication for DM), hyperlipidemia (defined as serum triglycerides greater than 150 mg/dL, low-density lipoprotein cholesterol greater than 140 mg/dL, high-density lipoprotein cholesterol less than 40 mg/dL and/or medication for hyperlipidemia), and/or hypertension (defined as blood pressure greater than 130/85 mmHg and/or medication for hypertension). The histopathologic features of NASH include steatosis, and hepatocyte injury, such as ballooning, lobular inflammation, and/or pericellular/perisinusoidal fibrosis.[3] All patients were followed until April 2013. We also retrospectively reviewed all patients referred for ESLD due to NASH who were evaluated but rejected for LDLT in our pre-LT assessment clinic during the same period.

We instructed obese recipients to control their diet and increase physical activity pre- and post-transplant. Our selection criteria for living donors and surgical techniques for LDLT are described elsewhere.[17,18]

Laboratory tests

Serum alanine aminotransferase (ALT; in IU/L), alkaline phosphatase (ALP; IU/L), and creatinine (mg/dL) at the latest follow-up were evaluated. Estimated glomerular filtration rate (eGFR; mL/min per 1.73 m^2) was calculated using the Japanese 2009 version of the equation developed by the Modification of Diet in Renal Disease (MDRD) study group.[19]

Immunosuppression

As previously reported, the immunosuppression regimen post-LDLT comprised steroid induction with tacrolimus or cyclosporin A.[20] The doses of each drug were gradually tapered for 6 months after LDLT. Methylprednisolone was tapered from 3 mg/kg on the first postoperative day to 0.05 mg/kg at the sixth postoperative month, and a maintenance dose of 2 to 4 mg of methylprednisolone was continued in all patients, both NASH and non-NASH recipients.

Evaluation of graft injury

Protocol biopsy is not performed at our center. Liver biopsy is indicated for patients with elevated liver function test results, after excluding biliary tract complications and infection. Histopathologic assessment of recurrent NASH was documented based on the NASH Clinical Research Network (NASH CRN) scoring system.[21] Abdominal imaging such as by computed tomography, magnetic resonance imaging, or ultrasonography was performed at least annually post-LDLT. After inclusion of the current study population, all LDLT recipients for NASH prospectively underwent abdominal ultrasonography focusing mainly on hepatic steatosis, as well as transient elastography (TE) by Fibroscan (Echosens, Paris, France) at the last follow-up. The liver stiffness measurement (LSM) was considered valid only when at least eight acquisitions were successful with a success rate of at least 60% and the ratio of the interquartile range to the median value was larger than 30%. LSM operators were blinded to the clinical data.

Statistical analysis

We used the SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data.

Table 1 Characteristics and surgical factors of the seven recipients

Case	Sex	Age	Pre-transplant variables							
			Initial diagnosis	BMI	MELD	C-P Score	HCC	DM	HL	HTN	ICU stay
1	M	67	CC	33	14	8	Yes	No	No	No	No
2	M	56	CC	28	13	9	Yes	No	Yes	No	No
3	F	40	CC	23	27	10	No	Yes	No	No	Yes
4	F	55	CC	26	21	13	No	Yes	No	No	Yes
5	F	61	NASH	30	24	13	No	No	Yes	No	Yes
6	F	61	CC	27	19	12	No	No	No	No	No
7	F	62	CC	26	17	12	Yes	Yes	No	No	No

Case	Pre-transplant variables					
	Ascites	Splenomegaly	HE	EGV	Creatinine (mg/dL)	eGFR (mL/min per 1.73 m^2)
1	No	Yes	No	Yes	0.9	61
2	Yes	Post splenectomy	No	Yes	0.73	64
3	Yes	Yes	Grade 2	Yes	0.70	68
4	Yes	Yes	Grade 2	Yes	0.61	74.4
5	Yes	Yes	Grade 1	Yes	0.92	48.6
6	No	Yes	Grade 1	Yes	0.91	41.5
7	Yes	Yes	Grade 1	Yes	0.90	49

CC, cryptogenic cirrhosis; C-P, Child-Pugh; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; EGV, esophagogastric varices; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; HL, hyperlipidemia; HTN, hypertension; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis.

Differences between groups were analyzed by the Mann–Whitney *U*-test for continuous variables and the χ^2 test for categorical variables. *P*-values <0.05 were considered significant.

RESULTS

Characteristics and surgical factors of the recipients

OF ALL 425 recipients transplanted from April 1996 to March 2013, we identified seven patients (1.6%) that underwent LDLT for NASH: one was diagnosed with NASH pre-transplant based on liver biopsy, which was performed 18 months prior to LDLT, and 6 of 18 who were considered to have CC at LT met the diagnostic criteria for NASH-related cirrhosis, as described in the Methods section (none of these six recipients had not received liver biopsy pre-LT). The histopathologic findings of all seven explants were compatible with NASH-related cirrhosis.

Of these seven patients, six were obese (BMI >25). Three patients required intensive care pre-transplant. HCC was detected pre-transplant (and confirmed in explants as well) in three patients; two within the Milan criteria and one within the Tokyo Criteria,[22] but beyond the Milan criteria. Other details of the seven patients are included in Table 1.

Recipients with NASH experienced significantly more total blood loss than non-NASH recipients: median 6040 (range, 3960–53 135) mL vs 4950 (range, 630–81 450) mL ($P = 0.02$). Nevertheless, recipients with NASH experienced the similar warm ischemic time (median 60 [range, 32–85] min), cold ischemic time (median 107 [range, 76–203] min), surgical duration (median 808 [range, 698–1245] min), or total hospital stay post-LT (median 45 [range, 23–98] days), compared to non-NASH recipients (*P*-values are 0.47, 0.36, 0.78 and 0.90, respectively).

Post-transplant outcomes

The median follow-up period for the seven patients was 5.3 (1.3–11.0) years. Acute cellular rejection was observed only in case #1 at 47 days post-transplant, and was treated successfully with increased immunosuppression and steroid recycling. None of the HCCs detected pre-transplant recurred.

The detailed characteristics of the seven patients at the end of the follow-up are summarized in Table 2; all

Table 2 Last follow-up post-living donor liver transplantation (LDLT) of seven patients

Case	BMI	DM	HL	HTN	Creatinine (mg/dL)	eGFR (mL/min/1.73 m^2)	ALT (IU/L)	ALP (IU/L)
1	28	No	No	Yes	0.90	62.3	13	144
2	23	Yes	No	Yes	1.42	40.0	23	69
3	17	No	No	No	1.02	46.4	8	18
4	23	Yes	Yes	Yes	0.73	62.5	65	226
5	24	No	Yes	Yes	1.24	34.2	25	512
6	28	No	No	Yes	0.99	44.1	13	190
7	24	Yes	No	Yes	0.77	58.1	26	291

IS	LKC in ultrasound	Fibroscan (kPa)	Recurrent NASH by liver biopsy	Outcome/follow-up period (years)
FK + MMF	no	6.9	NA	Alive/11.0
CyA + CS	no	2.6	NA	Alive/9.8
CyA + CS	no	6.1	NA	Alive/6.5
FK + CS	yes	5.2	NA	Alive/5.3
FK + MMF + CS	no	4.4	Yes	Alive/4.3
CyA + MMF + CS	no	3.1	NA	Alive/2.5
FK + MMF	no	8.8	NA	Alive/1.3

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HL, hyperlipidemia; HTN, hypertension; IS, immunosuppression; LKC, liver-kidney contrast; NA, not available; NASH, non-alcoholic steatohepatitis.

seven were alive. Median BMI values of the seven recipients at the last follow-up were 23.7 (range, 17.3–28.5) which showed median increase rate of 12.5 (range, 3.8–28.1)% compared to their minimum BMI values since LDLT (median 22.2 [range, 15.6–25.2]). Of the seven patients, three (43%) had DM, two (29%) had hyperlipidemia, and six (86%) had hypertension. Corticosteroids were gradually tapered and maintained in all cases; in most cases, 2 mg or 4 mg of methylprednisolone was administrated orally at the last visit. Median serum creatinine and eGFR levels were 0.99 (range, 0.73–1.42) mg/dL and 46.4 (34.2–62.5) mL/min per 1.73 m^2, respectively, except in case #1 who underwent living donor kidney transplantation for calcineurin inhibitor toxicity 6.7 years post-LDLT. The renal function in case #1 at the last follow-up was preserved (creatinine: 0.9 mg/dL, eGFR62.3 mL/min per 1.73 m^2).

Recurrent NASH post-LDLT

Except for one patient (case #4), none showed liver-kidney (LK) contrast in the prospectively performed ultrasonography; median LSM of those six patients by TE was 4.4 (range, 2.6–8.8) kPa. Ultrasonography performed in case #4 5.3 years after LDLT showed an LK contrast with an LSM of 5.2 kPa by TE, and this patient has had a consistently abnormal ALT (above 36 IU/L in our institution) for approximately 3 years post-LDLT except during the peri-operative period. This recipient subsequently underwent a liver biopsy, which revealed recurrent NASH with macrovesicular hepatic steatosis (40%), Mallory's hyaline, ballooning degeneration, predominantly neutrophilic inflammation, and perisinusoidal fibrosis. Based on the NASH CRN scoring system, the NAFLD activity score was 5 (steatosis = 2, lobular inflammation = 1 and hepatocellular ballooning = 2) and fibrosis stage was 1B. This patient developed DM and hypertension soon after LDLT was performed and hyperlipidemia 38 months post-LDLT, although she was never obese (BMI >25) since immediately after the LDLT. The clinical course of case #4 is shown in Figure 1.

Donor characteristics

The details of the seven respective donors are shown in Table 3. None of them were obese at the time of live donation. Three patients underwent liver biopsy before the donation according to our criteria of liver biopsy for potential living donors,[23] and none of them showed significant steatosis or any other liver injury/fibrosis. All seven donors tolerated and recovered from the liver resection without significant comorbidities. After a median follow-up period of 1.8 (range, 0.3–9.9) years,

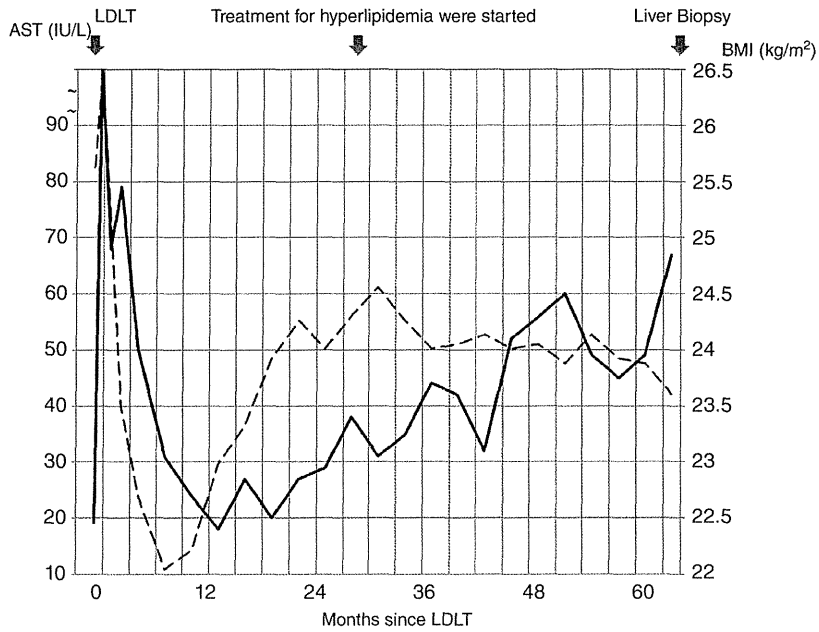

Figure 1 Clinical course of a recipient who experienced recurrent nonalcoholic steatohepatitis (NASH) (case #4). This patient never had a body mass index (BMI) >25 since immediately after the living donor liver transplantation (LDLT), although she developed diabetes mellitus and hypertension soon post-LDLT and hyperlipidemia 38 months after LDLT. Serum aspartate aminotransferase (AST) was consistently above the normal range (36 IU/L in our institution) for approximately 3 years post-LDLT except during the perioperative period. A liver biopsy was performed 5.4 years post-LDLT, which revealed recurrent NASH (NAS = 5, fibrosis stage = 1b). —, AST (IU/L); – –, BMI (kg/m^2).

two donors (cases #2 and 3) developed hyperlipidemia that has been successfully managed by diet control and increased physical activity, but none of the others suffered significant health problems.

Patients evaluated but rejected for LDLT

In total, 22 patients with ESLD due to NASH were evaluated but rejected for LDLT from April 1996 to March 2013. The characteristics of those 22 patients are summarized in Table 4. Mean BMI was 30 (range, 24–44). Of these 22, 11 (48%) had DM, three (13%) had hyperlipidemia, and five (22%) had hypertension. The reasons for rejection included donor issues (n = 12: four were obese with significant LK contrast in the ultrasonography, one was under treatment for DM, two were beyond the volumetric criteria of our institution, five withdrew their willingness to donate, recipient issues (n = 7: two died during evaluation; two were older than 65 years, which is beyond the criteria of our institution; one had an extrahepatic malignancy; one had HCC beyond the Tokyo criteria; one had too early stage of liver disease to transplant); and others (n = 3, due to cancellation of the evaluation clinic and lost to follow-up). Of the 22 patients rejected for LDLT, four patients were listed for DDLT, but died while awaiting transplantation.

Table 3 Characteristics of each of the seven donors

Case	Age	Sex	BMI	Donor relation	Number of HLA mismatch†	Preoperative liver biopsy
Case 1	34	Male	24	Son	3	NA
Case 2	54	Female	22	Spouse	4	NA
Case 3	36	Male	24	Spouse	4	Steatosis: 2–3%
Case 4	58	Male	21	Spouse	3	NA
Case 5	36	Female	18	Daughter	3	NA
Case 6	28	Male	22	Son	2	Steatosis: <1%
Case 7	64	Male	23	Spouse	2	Steatosis: <1%

†HLA-A, -B and -DR loci were used to calculate total mismatch score of 0–6.
BMI, body mass index; HLA, human leukocyte antigen; LDL, living-donor liver transplantation; NA, not available.

Table 4 Characteristics of the patients with NASH referred but rejected for liver transplantation (LT) evaluation ($n = 22$)

		n (%)
Age (median)		57 (32–70)
Male Sex		13 (56)
BMI (median)		30 (24–44)
MELD (median) score		17 (9–23)
Child–Pugh score		11 (8–13)
HCC		2 (9)
DM		11 (48)
HL		3 (13)
HTN		5 (22)
Reason for rejection	Donor issue	12 (55)
	Recipient issue	7 (32)
	Other reasons	3 (14)

BMI, body mass index; DM, diabetes mellitus; HCC, hepatocellular carcinoma; HL, hyperlipidemia; HTN, hypertension; MELD, Model for End-Stage Liver Disease.

DISCUSSION

SEVEN PATIENTS UNDERWENT LDLT for ESLD related to NASH in our institution from April 1996 to March 2013. The detailed characteristics of these seven recipients reveal an excellent survival rate; 100% during the median follow-up period of 5.3 years. None of the seven recipients showed liver dysfunction at the end of the follow-up, although one patient developed recurrent NASH with mild fibrosis.

Non-alcoholic steatohepatitis as an indication for LT is dramatically increasing in parallel with the increasing proportion of obesity and metabolic syndrome worldwide,[8,24] consistent with the theory that central obesity and insulin resistance are the most important risk factors for NASH or NAFLD.[25] The outcome post-LT has been regarded to be poorer than other indications due to technical difficulties of the transplant surgery itself or the higher rate of postoperative complications.[26] Recent large retrospective studies, however, indicated that post-transplant survival of recipients with NASH is comparable to that of patients with all other liver diseases.[8,9]

The number of patients with NAFLD/NASH in Japan has increased over the last 20 to 30 years.[2,27] A similar trend was observed in our patient cohort; no LT was performed on patients with NASH among 27 LDLT recipients before 2000. Patients with NASH accounted for two (0.7%) of 285 LDLT recipients between 2000 and 2006, and five (4.5%) among 111 LDLT recipients from 2007 to April 2013.

The reported rate of recurrent NASH following LT (mostly DDLT) ranges from 8% to 33%.[9,28–32] Of these studies, Malik *et al.* reported that 13 patients received LDLT from 1997 to 2008 in their institution, although they did not document the specific outcome focusing on LDLT.[31] Here we showed that recurrent NASH following LDLT was observed in one recipient (14%) proven by liver biopsy. As none of the other recipients showed any signs of recurrent NASH/NAFLD, such as abnormal liver function tests or LK contrast in the ultrasonography, we have not performed further investigations, especially liver biopsy, which might be harmful considering the risk-benefit ratio.[33] The diagnostic criteria for recurrent NASH are uncertain, and vary among previously published reports. Indeed, Agopian *et al.*[34] defined recurrent NASH based on histopathologic as well as radiologic findings.[9] TE was recently introduced to the LT setting, predominantly to assess recurrent hepatitis C post-transplant. Rigamonti *et al.*[35] showed that TE adequately identified the presence or absence of liver injury by nonviral graft diseases, including steatohepatitis. Using receiver operating characteristic curve analysis, they identified two cutoffs of LSM for the diagnosis of graft damage: 5.3 kPa with 100% sensitivity and 7.4 kPa with 100% specificity. In the present study, of all seven recipients, six patients, including case #4 with biopsy-proven recurrent NASH, had LSM < 7.4. The LSM in four of those six was <5.3. On the other hand, TE in case #7 showed a somewhat higher LSM (8.8 kPa), but we found no other signs of graft injury, such as abnormal serum ALT/ALP or LK contrast on ultrasonography, which supports our current view not to consider liver biopsy in this patient. We do, however, realize the potential importance of paying continuous attention to the clinical course of such a patient/graft, as well as the transition of LSM, which might reflect the progression of graft injury.

Bhagat *et al.*[30] reported that recipients receiving LT for NASH did not develop more de-novo DM but did develop more de-novo hypertension than recipients undergoing LT for alcoholic liver disease (16% vs. 29% [$P = 0.22$], 35% vs. 61% [$P = 0.04$], respectively). Likewise, in our present study, de-novo DM post-transplant was detected only in one patient and was not insulin-dependent, under a maintenance dose of 2 to 4 mg of methylprednisolone used in all recipients. On the other hand, de-novo hypertension occurred in six of seven recipients in our patient cohort. One patient (case #1) underwent living donor kidney transplantation after LDLT, and five of the remaining six recipients showed impaired renal function with an eGFR <60 mL/min per 1.73 m^2 following LDLT, indicating that 86% (6/7) recipients developed chronic kidney disease with

stage ≥ 3,[19] although three patients had eGFR < 60 mL/min per 1.73 m^2 at LDLT. Renal dysfunction in these cases might be associated with NASH, but it is always a challenge to determine the exact cause of renal dysfunction post-transplant, due to several factors such as the general use of calcineurin inhibitors. It is also important to keep an eye on obesity post-LDLT as all the seven recipients in our patient cohort have gained weight since LDLT, although five (71%) of the seven recipients were not obese (BMI > 25) at the last follow-up.

Several recent reports suggest that genetic polymorphisms are related to the development of NASH.[10–14] Kawaguchi *et al.* reported in 2012 that the progression of NASH in the Japanese population was strongly associated with the genetic polymorphisms of the human PNPLA3 gene.[15] According to those fascinating findings, it could be hypothesized that living related donors would develop NASH in the future or that recurrent NASH post-LDLT from related donors would be higher than DDLT or LDLT from unrelated donors such as spouses. In the present study, however, the patient who developed recurrent NASH received the graft from her husband, although the number of the patients included is too small to lead to robust conclusions. This patient had a consistently abnormal ALT level for almost 3 years post-LDLT, but the elevation was somewhat marginal (below 60) and fluctuated such that we did not perform liver biopsy until it became higher (>60). Thus, the actual time point when the recurrent NASH occurred is unknown. We also did not strongly suspect this patient had recurrent NASH as her BMI was below 25. This fact suggests that recurrent NASH should be suspected when the ALT is consistently elevated, despite the absence of obesity, and liver biopsy should be considered.

In our institution, none of the seven donors included had obesity or marked hepatic steatosis at the donor surgeries, and the operations were performed safely without significant surgical or postoperative complications, although two donors developed hyperlipidemia during the follow-up period. In addition to a larger study to answer this important question, a prospective study to investigate the long-term outcome of living donors related to recipients undergoing LDLT for NASH is strongly required.

In our patient cohort, more than 70% of the potential recipients with NASH failed to undergo LDLT due to donor issues; of these, 40% due to obesity with significant LK contrast in the ultrasonography and 40% due to a graft size mismatch. It might have been difficult to find a suitable donor from the graft size perspective as most patients with NASH are significantly obese. Thirty-two percent (7/22) of the patients were rejected for LDLT due to recipient issues, especially too late or too sick to transplant, which might reflect the difficulty in diagnosing and screening for NASH at its earlier stages, partly because NASH was not, until recently, well recognized among physicians.

The present study is a summary of our experience of LDLT for NASH-related ESLD. In conclusion, the outcome of LDLT for NASH was excellent without patient or graft loss, although the sample size was small. Further studies are warranted to investigate the outcome of LDLT for NASH in a larger patient cohort. It is also important to follow up living donors who provide grafts to recipients with NASH to observe the occurrence of obesity, metabolic syndrome, and/or NAFLD prospectively.

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De Novo Malignancies After Adult-to-Adult Living-Donor Liver Transplantation With a Malignancy Surveillance Program: Comparison With a Japanese Population-Based Study

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Background. Organ transplant recipients have an increased incidence of malignancy. Race differences in a variety of malignancies are observed among the general population, but de novo malignancies after adult-to-adult living-donor liver transplantation (LDLT) have not been compared with those from a Japanese population-based study.
Methods. The subjects were 360 adult LDLT recipients who survived more than 1 year after transplantation. An annual medical checkup and screening examinations were performed as follows: abdominal computed tomography or magnetic resonance imaging, upper gastrointestinal endoscopy, and total colonoscopy and immunochemical fecal occult blood test every 1 to 2 years. Complete blood count, liver function tests, and several tumor markers were checked every 1 to 3 months after LDLT.
Results. Mean follow-up period was 7.5±3.4 years. During the follow-up period, 27 de novo malignancies were diagnosed in 26 recipients. Colorectal cancer was the most commonly detected malignancy. The overall mortality of the recipients with de novo malignancies was similar to the findings of the Japanese general population-based study (standardized mortality ratio=0.9). Overall, the incidence of cancer was significantly higher in transplant recipients than in the Japanese general population (standardized incidence ratio=1.8). The 5-year estimated survival rate of recipients with de novo malignancies was 81% and those of recipients without malignancies was 93% ($P<0.0001$).
Conclusions. Colorectal malignancies predominated in Japanese liver transplant recipients. Although de novo malignancies correlated with a poor prognosis, the standardized mortality ratio was 0.9 compared with that of subjects of a Japanese population-based study.

Keywords: Living donor, Liver transplantation, De novo malignancy, Japanese.

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Since the Shinshu Group reported the first successful adult-to-adult living-donor liver transplantation (LDLT) in 1993 (*1*), the number of LDLT procedures for adult patients has increased in eastern Asia. Adult-to-adult LDLT is now an established treatment option for end-stage liver disease and the number of long-term survivors after LDLT continues to increase. Transplant recipients, however, exhibit a high incidence of malignancy (*2, 3*). The incidence of de novo malignancies in liver transplant recipients is approximately 10% at 10 years (*4*), and the risk of de novo malignancies is threefold to sevenfold higher than that in the normal population (*5*). Race differences in a variety of malignancies are observed among the general population (*6*), but de novo malignancies after adult-to-adult LDLT compared with a Japanese population-based study (*7*) have not been investigated. Additionally, malignancy surveillance programs after liver transplantation and the prognosis after a de novo malignancy diagnosis have not been fully examined.

In the present study, we aimed to describe de novo malignancies after adult-to-adult LDLT and compare their mortality and incidence with the findings of a Japanese population-based malignancy study.

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RESULTS

Mean follow-up was 7.5±3.4 years. During the follow-up period, 27 de novo malignancies were diagnosed in 26 liver transplant recipients (Table 1). Colorectal cancer was the most commonly detected malignancy (n=8) followed by gastric cancer and carcinoid (n=3 and 1, respectively), posttransplantation lymphoproliferative disorder (PTLD; n=3), leukemia (Langerhans cell sarcoma was included; n=3), skin cancer (Bowen's disease was included; n=2), oral and esophageal cancer (n=2), prostate cancer (n=2), renal cell cancer (n=2), and breast cancer (n=1). Among these, 7 of 27 (26%) recipients died from the de novo malignancy (Table 1). All but one gastrointestinal tract malignancy was diagnosed by screening endoscopy: esophageal cancer (1 of 1 [100%]), gastric cancer (one carcinoid; 4 of 4 [100%]), and colorectal cancer (7 of 8 [88%]). Seven of 13 (54%) were diagnosed with stage I (according to the tumor-node-metastasis classification) stomach or colorectal cancer. Among these, 5 of 7 (71%) were treated with endoscopic submucosal dissection. In total, 18 of 27 (59%) of de novo cancers were diagnosed as limited local, and surgical procedure including endoscopic submucosal dissection was applied to treat these cancers (Table 1).

Mean age of recipients diagnosed with de novo malignancy was 56 years. When expressed in terms of incidence per 100 person-years by age groups at the time of adult-to-adult LDLT, the rates were 0.6, 1.1, 0.2, 1.0, and 4.2 in the age groups of 18 to 29, 30 to 39, 40 to 49, 50 to 59, and 60 to 67 years, respectively (Fig. 1).

The subject of the study had similar sex and age distribution ratio with those of the Japanese population-based study. In our study, 59% (16 male, 11 female) was male and 89% (n=3 for the 20–39 years old and n=24 for the 40–74 years old) of de novo malignancy recipients were 40 and more than 40 years old at end of this study. Among the malignancy patients in a Japanese population-based study,

TABLE 1. Clinical characteristics of the 27 patients with de novo malignancies

	Age, sex	Primary disease	Diagnosis	Duration to diagnosis (yr)	Age at de novo malignancy	Treatment	Prognosis (death=1)
1	51/M	PBC	Oral	4.5	56	Radio	1
2	63/M	FHF	Esophageal	2.4	65	Chemo	1
3	64/M	PBC	Gastric	11.7	76	Resection[a]	0
4	51/M	PBC	Gastric	3.9	55	Resection[a]	0
5	52/M	LC (HBV), HCC	Gastric	1.8	54	ESD[a]	0
6	63/F	FHF	Gastric (carcinoid)	7.7	71	ESD[a]	0
7	64/F	FHF	Colorectal (cecum)	8.9	73	ESD[a]	0
8	62/F	PBC	Colorectal (ascending colon)	8.3	70	Resection[a]	0
9	54/F	PBC	Colorectal (ascending colon)	7.2	61	Resection[a]	0
10	60/M	LC (HBV)	Colorectal (ascending colon)	2.0	62	Chemo	1
11	57/F	AIH	Colorectal (sigmoid)	10.5	67	Resection[a]	0
12	55/F	LC (HBV), HCC	Colorectal (sigmoid)	4.5	60	Resection[a]	0
13	61/F	LC (HCV), HCC	Colorectal (rectal)	7.4	68	ESD[a]	0
14	56/M	LC (HCV), HCC	Colorectal (rectal)	5.6	62	ESD[a]	0
15	37/F	PBC, HCV	Breast	3.4	40	Resection[a]	0
16	63/M	LC (HCV), HCC	Prostate	5.4	68	Resection[a]	0
17	57/M	LC (HCV), HCC	Prostate	3.9	61	Resection[a]	0
18	39/F	PBC	RCC	4.1	43	Resection[a]	0
19	23/F	BA	RCC	1.6	24	Resection[a]	1
20	53/F	PBC	Skin (SCC)	14.7	68	Resection[a]	0
21	53/M	LC (HCV), HCC	Skin (Bowen's disease)	7.4	60	Resection[a]	0
22	25/M	PSC	PTLD	8.1	33	Resection+ chemo	0
23	62/M	LC (HCV), HCC	PTLD	6.0	68	Chemo	0
24	56/M	LC (HCV), HCC	PTLD	3.3	59	Chemo	1
25	49/F	PBC, HCC	Leukemia (Langerhans cell sarcoma)	4.4	53	Chemo	1
26	30/M	LC (HCV+HIV)	Leukemia	3.2	33	Chemo	0
27	60/M	FHF (drug)	Leukemia (acute myelogenous)	1.4	61	Chemo	1

[a] De novo malignancy was diagnosed as limited local.

AIH, autoimmune hepatitis; BA, biliary atresia; Chemo, chemotherapy; ESD, endoscopic submucosal dissection; FHF, fulminant hepatic failure; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LC, liver cirrhosis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PTLD, posttransplantation lymphoproliferative disorder; RCC, renal cell carcinoma; Radio, radiotherapy; SCC, small cell carcinoma.

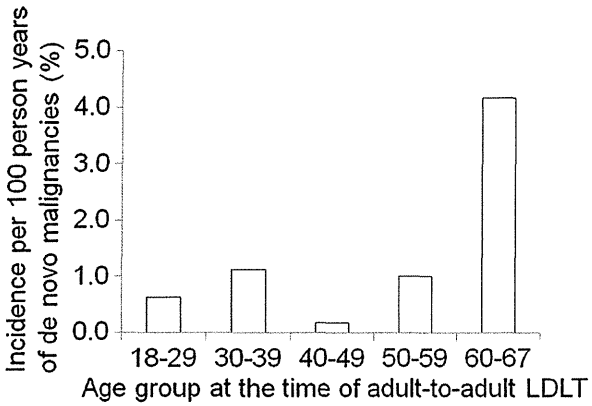

FIGURE 1. Incidence per 100 person-years by age groups at the time of adult-to-adult LDLT. Malignancies occurred most frequently in those 60 to 67 years old at liver transplantation (4.2/100 person-years) followed by those 30 to 39 years old (1.1) and then those 50 to 59 years old (1.0). LDLT, living-donor liver transplantation.

58% (253,210 male, 183,587 female) was male and 95% (n=22,312 for the 20–39 years old and n=414,485 for the 40–74 years old) of malignancy patients was 40 and more than 40 years old.

Overall mortality of transplant recipients with de novo malignancies was similar to findings of the Japanese general population-based study (standardized mortality ratio [SMR] = 0.9; 95% confidence incidence [CI], 0.4–2.0). Overall, the incidence of malignancy was significantly higher in transplant recipients than in the Japanese general population (SIR=1.8; 95% CI, 1.3–2.7). The risk of malignancy was slightly higher in female transplant recipients (SIR=1.9; 95% CI, 1.0–3.4) than in male recipients (SIR=1.8; 95% CI, 1.1–2.9; Table 2). The risk of malignancy was significantly higher in younger recipients than in the Japanese general population: 20 to 29 years old (SIR=48.0; 95% CI, 6.9–335.1), 30 to 39 years old (SIR=8.6; 95% CI, 2.2–34.1), and 40 to 49 years old (SIR=2.5; 95% CI, 0.6–9.9). The risk of malignancy was similar in older recipients: 50 to 59 years old (SIR=1.1; 95% CI, 0.4–3.0), 60 to 69 years old (SIR=1.1; 95% CI, 0.6–1.9), and 70 to 74 years (SIR=1.0; 95% CI, 0.4–2.5; Table 3). Malignancy sites or types with a significantly elevated SIR were as follows: head and neck (SIR=3.7; 95% CI, 0.5–26.6), esophagus (SIR=16.9; 95% CI, 2.4–17.9), stomach (SIR=1.6; 95% CI, 0.6–4.3), colorectal (SIR=3.5; 95% CI, 1.8–7.0) (*8*), prostate (SIR=2.2; 95% CI, 0.6–8.9), kidney (SIR=6.4; 95% CI, 1.6–25.4), malignant lymphoma (SIR=7.6; 95% CI, 2.5–23.6) (*9*), and leukemia (SIR=15.1; 95% CI, 4.9–46.9) but not breast (SIR=0.9; 95% CI, 0.1–6.4; Table 4).

TABLE 2. Total mortality rates and SMRs with 95% CI and total, male, and female IRs (×100,000) and SIRs with 95% CI

	n	IR (×100,000)	SIR	95% CI
Total mortality	7	259	0.9	0.4–2.0
Total incidences	27	963	1.8	1.3–2.7
Male	16	1085	1.8	1.1–2.9
Female	11	850	1.9	1.0–3.4

CI, confidence interval; IR, incidence rate; SIR, standardized incidence ratio; SMR, standardized mortality ratio.

TABLE 3. IRs (×100,000) and SIRs with 95% CI according to age

Age, yr	n	IR (×100,000)	SIR	95% CI
20–29	1	169	48.0	6.9–335.1
30–39	2	78	8.6	2.2–34.1
40–49	2	60	2.5	0.6–9.9
50–59	4	45	1.1	0.4–3.0
60–69	14	115	1.1	0.6–1.9
70–74	4	172	1.0	0.4–2.5

CI, confidence interval; IR, incidence rate; SIR, standardized incidence ratio.

The 3-, 5-, and 10-year estimated survival rates of recipients with de novo malignancies were 93%, 81%, and 57%, respectively, and those in recipients without de novo malignancies were 95%, 93%, and 92%, respectively (P=0.0001). The cumulative incidence of de novo malignancies at 3, 5, and 10 years after transplantation was 2%, 5%, and 10%, respectively. After de novo malignancies were diagnosed, the 1-, 3-, and 5-year estimated survival rates were 81%, 69%, and 61%, respectively.

DISCUSSION

Continuous improvements in surgical techniques and immunosuppression regimens have greatly improved the long-term results of LDLT. We reported the cause of death in 176 adult-to-adult LDLT recipients in 2005 with a median follow-up period of 2.8 years and concluded that recurrent primary disease, infection, and surgical complications in bile duct anastomosis impact the long-term outcome (*10*). Similar to the long-term findings of deceased-donor liver transplantation recipients, however, de novo malignancies were the main cause of death. According to previous reports (*11–15*), the overall risk of malignancy is two to four times higher in transplant recipients than in an age- and sex-matched population. In our

TABLE 4. IRs (×100,000) and SIRs with 95% CI according to site or type of malignancy

Malignancy	n	IR (×100,000)	SIR	95% CI
Head and neck	1	4	3.7	0.5–26.6
Esophagus	1	4	16.9	2.4–17.9
Stomach	4	15	1.6	0.6–4.3
Colorectal	8	30	3.5	1.8–7.0
Breast	1	8	0.9	0.1–6.4
Prostate	2	14	2.2	0.6–8.9
Kidney	2	7	6.4	1.6–25.4
Skin	2	7	6.4	1.6–25.4
Malignant lymphoma	3	11	7.6	2.5–23.6
Leukemia	3	11	15.1	4.9–46.9

CI, confidence interval; IR, incidence rate; SIR, standardized incidence ratio.

cohort, the risk of malignancy was similar to that in previous reports. Compared with the results of the general Japanese population-based study, however, the standardized mortality ratio (SMR) was 0.9.

Regarding the evaluation of the extent of the malignancy at diagnosis, in our cohort, 18 of 27 (67%) of de novo malignancies were diagnosed as limited local. According to the general Japanese population-based study, although there were no detailed stage data by the tumor-node-metastasis classification, 35% of malignancy was limited local, 21% was invaded to adjacent organ or lymph node metastasis, and 14% had distant metastasis at diagnosis (the remaining 30% was unknown) (*7*). One of the reasons for early malignancy diagnosis in our cohort might be malignancy screening rate. The rate of malignancy screening of Japanese general population was 21% to 26%, which was disclosed in public by Ministry of Health, Labour and Welfare of Japan (*16*). The present study was biased by the small number of patients; however, a malignancy surveillance protocol might reduce mortality in this cohort.

Younger recipients had high risk for de novo cancer in our study. There was a difference of type of malignancy between the Japanese population-based study and our cohort. In the Japanese population-based study, in the younger population (20–39 years old), the most frequent malignancy was uterus, and the second to fifth most frequent were breast, stomach, colorectal, and thyroid cancer, respectively, which accounts for 70% of the younger malignancy population (*7*). In our study, three younger (20–39 years old) recipients developed malignancy. The type of malignancy of these recipients consisted of breast cancer, PTLD, and renal cell cancer. It is well known that younger recipients have risk (*17*) of PTLD in solid organ transplant recipients. However, further study is needed because of the small number of younger recipients in our study.

Malignancy types differ between races. In a western study, Buell and colleagues reported that nonmelanocytic skin cancers are the most commonly reported de novo malignancy in solid organ transplant recipients, with the incidence varying in proportion to the degree of sun exposure (*18–20*). In Asian countries, including our study, skin cancer is less frequent. There are only a few reports from Asian countries. In a Korean liver transplant recipient study, stomach cancer was most frequent with a relative risk more than 10-fold higher than that in the general Korean population (*21*). In a Japanese population of renal transplant recipients, the most frequent malignancy was stomach and colorectal cancer when native renal cell cancer was excluded (*22*). In our cohort, the most frequent malignancy was colorectal cancer. The next most common malignancies were stomach cancer and malignant lymphoma. Two of the recipients in our cohort were diagnosed with skin cancer. Colorectal and stomach cancers might be the main malignancies in Asia. On the contrary, Penn reported that the average time to first malignancy was 5.0 years (*23*). In recent reports, Harwood reported skin cancer in organ transplant recipients with a 22-year prospective study. In their report, the median time to first skin cancer was 7.6 years (≥60 years old) to 24.1 years (30–39 years old) (*24*). In our study, mean follow-up time was 7.5 years. Our study may still underrepresent skin cancer risk.

Despite the high risk of de novo malignancy for recipients during the follow-up period, there is no consensus regarding the appropriate malignancy screening program after liver transplantation. Herrero and colleagues suggested that deceased-donor liver transplantation recipients should be screened periodically for malignancies common to the general population, which may result in timely detection of de novo malignancies (*25*). Our screening methods that focus on gastrointestinal and colorectal cancers might be suitable because prognosis after diagnosis with malignancy was relatively favorable (61% at 5 years). Our findings regarding the prognosis seem to be higher than that in previous reports. Herrero (*5*) reported that the 5-year prognosis after diagnosis of de novo malignancy in 51 liver transplant recipients diagnosed with a noncutaneous malignancy was approximately 40%. Age at diagnosis of malignancy is inversely related to the ratio of PTLD as a de novo malignancy (*26*). In our cohort, none of the younger recipients (<55 years old) who were diagnosed with malignancy had colorectal or stomach cancer. The three youngest recipients were diagnosed with renal cell cancer (24 years old), PTLD (33 years old), and Burkitt's leukemia (33 years old). Our screening methods might thus not be suitable for younger recipients.

The incidence of colon cancer in liver transplant recipients was initially thought to be similar to that in the general population (*27, 28*). A meta-analysis study, however, reported a relative risk of 2.6 for colorectal cancer in post–deceased-donor liver transplantation patients compared with an age-matched general population (*29*). Primary sclerosing cholangitis is an important high-risk factor for colorectal cancer. For example, Vera and colleagues (*30*) found a 5% incidence of colorectal cancers in recipients with primary sclerosing cholangitis versus 0.6% for recipients without nonprimary sclerosing cholangitis. Nicolaas and colleagues reported that overall transplant recipients (nonprimary sclerosing cholangitis) have an increased risk for colorectal cancer compared with the general population (relative risk: 1.8) (*29*). Thereby, they concluded that nonprimary sclerosing cholangitis transplant recipients do not need an intensified screening strategy for colorectal cancer. Based on our findings of a relatively high rate of colorectal cancer and of malignancy in recipients more than 60 years of age, we think that an active malignancy surveillance program for colorectal cancer might be needed for liver transplant recipients, especially those more than 60 years old and Asian. In the present study, 88% of colorectal cancers were diagnosed by screening colonoscopy. This study is a single-institution experience and a relatively small cohort. Further studies with a larger cohort of Japanese and/or Asian recipients are needed.

Colorectal malignancies predominated in Japanese liver transplant recipients. Although de novo malignancies correlated with a poor prognosis, the SMR was 0.9 compared with the Japanese population-based study.

MATERIALS AND METHODS

Between January 1996 and July 2012, 412 adult-to-adult LDLTs were performed at the University of Tokyo Hospital. The subjects of the present study were 360 adult LDLT recipients who survived more than 1 year after transplantation and had no previous diagnosis of malignancy, excluding hepatocellular carcinoma, at the time of transplantation. The indications

for transplantation-included hepatitis B or C–related cirrhosis (n=161), cholestatic liver disease (n=98), fulminant hepatic failure (n=38), biliary atresia (n=19), alcoholic liver cirrhosis (n=11), metabolic diseases (n=10), and others (n=23). Mean model for end-stage liver disease score (*31*) was 14.8±7.6. Mean recipient age was 49 years when transplantation was performed. The number of male and female recipients was 192 and 168, respectively. The mean age was 56 years when this study was performed. The age distribution was as follows: 20 to 29 years (3%; n=10), 30 to 39 years (8%; n=30), 40 to 49 years (13%; n=47), 50 to 59 years (28%; n=99), 60 to 69 years (41%; n=149), and 70 to 79 years (7%; n=25).

Screening examinations were performed as a first step in evaluating recipient candidates to exclude malignancy. Abdominal computed tomography or magnetic resonance imaging, upper gastrointestinal endoscopy, total colonoscopy, and several tumor markers (e.g., carcinoembryonic antigen, carbohydrate antigen 19-9, and prostate specific antigen tests) were examined. When malignancy other than hepatocellular carcinoma was found, preparation for transplantation was discontinued and treatment was started.

The transplantation procedure and donor selection criteria are described elsewhere (*32, 33*). All survivors were followed in our outpatient clinic through the end of July 2012. Mean follow-up period was 7.5±3.4 years.

The study protocol was approved by the University of Tokyo Ethics Committee (No. 2317).

Immunosuppression

Basic immunosuppressive agents, tacrolimus and methylprednisolone, were used. The target trough serum level of tacrolimus was 15 to 20 ng/mL in week 1 after transplantation. Simultaneously, methylprednisolone (20 mg/kg) was used before the anhepatic phase of surgery. Six months after surgery, the target tacrolimus trough level was gradually decreased from 8 to 5 ng/mL. At the same time, the dose of methylprednisolone was subsequently reduced to the maintenance level (0.05 mg/kg) (*34*). In patients who developed reversible posterior leukoencephalopathy syndrome as a side effect (*35*), tacrolimus was replaced with cyclosporine therapy. Acute and chronic rejection was diagnosed using the Banff schema classification (*36, 37*). When acute rejection was diagnosed, patients were treated with a bolus of intravenous methylprednisolone.

Malignancy Surveillance Program After Liver Transplantation

For patient management in the outpatient clinic, we recommend that patients undergo an annual medical checkup provided by their company or municipal government in accordance with the ordinance of the Ministry of Health, Labor, and Welfare of Japan. These medical checkups include a chest X-ray, gastrointestinal X-ray examination, stool occult blood for patients more than 40 years old, and/or breast physical examination (palpitation and mammography), and uterine cervical smears in women more than 40 years old. Additionally, we performed screening examinations as follows: abdominal computed tomography or magnetic resonance imaging, upper gastrointestinal endoscopy, and total colonoscopy and immunochemical fecal occult blood test every 1 to 2 years. Complete blood count and liver function tests with tumor markers (carcinoembryonic antigen, carbohydrate antigen 19-9, and prostate specific antigen tests) were performed every 1 to 3 months after LDLT. The diagnosis of de novo malignancy was based on histologic examination of obtained biopsies or surgical specimens of the tumors. The date of malignancy diagnosis was defined as the date of initial pathologic confirmation.

Statistical Analysis

As for incidence per 100 person-years of de novo malignancies by age group at the time of adult-to-adult LDLT, person-year was calculated at the end of July 2012 (total of 2705 person-years). When de novo malignancy was developed, patient was classified based on the age at the time of liver transplantation.

The estimated malignancy incidence and incidence rate in the Japanese general population was adopted from published data (*7*). The ratio of observed to expected number of malignancies, the SMR, and the SIR were calculated by dividing the observed number of LDLT recipients with malignancies by the expected number of malignancy patients (actual number of recipients multiplied by mean follow-up period divided by malignancy mortality (or incidence) rate of the 2006 Japanese population-based study (*7*). The 95% CI of SMR and SIR were determined using the Poisson distribution with Excel 2010 software (Microsoft Japan, Tokyo, Japan).

For comparison with a Japanese population-based malignancy study, we obtained published data available on a Web site (http://ganjoho.jp/professional/statistics/index.html) (*7*). Kaplan–Meier life table analysis with a log-rank test was used to assess whether de novo malignancies significantly affected posttransplantation patient survival using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Data are expressed as mean±standard deviation. $P<0.05$ was considered to be statistically significant.

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