cautery and an ultrasonic surgical aspirator.[7] The Pringle maneuver was performed in group A, with intermittent inflow occlusion, repeated 15-min occlusion and 5 min of reperfusion. Intraoperative blood loss, serum lactate level just after hepatectomy and postoperative liver functions in the donors, and recipient outcomes were reviewed.

Immunohistochemistry of HO-1 and TLR4

Liver tissues were obtained from segment 4 according to Couinaud's nomenclature using a 16-G needle just before removal of the liver during donor surgery. Formalin-fixed paraffin-embedded (4-μm) sections were used for hematoxylin–eosin staining, and immunohistochemistry staining with HO-1 and Toll-like receptor 4 (TLR4) was performed using a high-temperature antigen-unmasking technique. The sections were boiled in pH 6.0 ethylenediaminetetraacetic acid buffer solution at 95 C for 40 min. After 10 min of blocking with 0.1% H_2O_2/methanol, the sections were incubated for 30 min at room temperature in a humidified chamber with primary antibodies. As the primary antibodies, anti-HO-1 rabbit antihuman polyclonal antibody (dilution 1:100; Lifespan Biosciences, Seattle, WA, USA) and anti-TLR4 rabbit antihuman polyclonal antibody (dilution 1:70; Bioworld Technology, Newmarket Suffolk, UK) were used. After a 30-min reaction with the primary antibodies, the slides were reacted with Histofine Simple Stain RAT MAX PO (MULTI) (Nichirei Bioscience, Tokyo, Japan) at room temperature for 30 min. The slides were visualized with H_2O_2 and 3,3′-diaminobenzidine-tetrachloride solution at room temperature for 5 min and stained in hematoxylin for 1.5 min. The coverslips were mounted with 90% glycerol containing 1 mg/mL *P*-phenylenediamine.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*-test. We considered $P < 0.05$ to be statistically significant.

RESULTS

Characteristics of the patients

TWENTY CONSECUTIVE LIVING donors who underwent left hepatectomy including the middle hepatic vein (MHV) without the caudate lobe from September 2009 to January 2011 were prospectively divided into two groups, those who underwent (group A, $n = 10$) and those who did not undergo (group B, $n = 10$) the Pringle maneuver during hepatectomy. These donors were divided into these two groups alternatively, so that this study is not randomized, but a controlled clinical trial. The characteristics of both donors and recipients are listed in Table 1. Only the sex of the recipient was significantly different between the groups, possibly due to the small sample size.

Outcomes of the donor surgery

As shown in Table 2, the median blood loss was significantly less in group A than in group B, both in total (303 g [range, 170–480] in group A vs 720 g [range, 360–2200] in group B, $P < 0.05$) and during hepatectomy (85 g [range, 10–132] in group A vs 420 g [range, 170–1200] in group B, $P < 0.01$). Intraoperative serum lactate level after hepatectomy was not significantly different between the groups (2.2 mmol/L [range, 1.0–4.2] in group A vs 2.2 mmol/L [range, 1.0–3.4] in group B). The median alanine aminotransferase (ALT) level was significantly higher on postoperative day 1 in group A than in group B (689 IU/L [range, 329–1279] in group A vs 321 IU/L [range, 281–644] in group B, $P < 0.05$), but the difference was not significant at 7 days after surgery and was normalized in both groups A and B at the last follow up (Fig. 1). The median levels of total bilirubin and prothrombin time international normalized ratio (PT-INR) were not significantly different throughout the postoperative course between the groups. With regard to the postoperative complications, one minor bile leakage which was defined as grade I in Clavien's classifications was seen in one patient from group A. All donors are currently doing well with normal liver functions at a median follow-up period of 26 months (range, 17–32) in group A and 23 months (range, 16–31) in group B, respectively (Table 2).

Outcomes of the recipients

The graft functions in the recipients are shown in Figure 2. The median ALT level was significantly higher on postoperative day 1 in group A than in group B (408 IU/L [range, 396–419] in group A vs 219 IU/L [range, 159–917] in group B, $P < 0.05$), but this difference had disappeared at 7 and 28 days after transplantation. Although there was no significant difference

Table 1 Characteristics of donors and recipients

	Group A Pringle(+)	Group B Pringle(−)	
n	10	10	
Donors			
Age	31 (20–56)	36 (31–55)	NS
Sex, M/F	8/2	7/3	NS
Recipients			
Age	58 (27–70)	61 (46–69)	NS
Sex, M/F	8/2	2/8	$P < 0.05$
Original diagnoses			
C-LC	7	5	
PBC	0	2	
B-LC	1	0	
Alcoholic-LC	1	1	
FHF	1	0	
Caroli disease	0	1	
MELD	15 (7–27)	14 (7–31)	NS
Blood type combination			
Identical/compatible	8	10	NS
Incompatible	2	0	
Graft weight (g)	373 (306–459)	387 (310–467)	NS
Estimated residual liver volume in the donor (%)	32 (25–38)	31 (27–34)	NS
GV/RSLV (%)	40.4 (31.1–53)	41.8 (32–54.4)	NS

Data are presented as median (range). B-LC, hepatitis B-related liver cirrhosis; C-LC, hepatitis C-related liver cirrhosis; FHF, fulminant hepatic failure; GV, graft volume; MELD, Model for End-Stage Liver Disease; NS, no significant change; PBC, primary biliary cirrhosis; RSLV, recipient standard liver volume.

in median total bilirubin at 1 day and 28 days after transplantation, this parameter was significantly lower on postoperative day 7 in group A than in group B (3.2 mg/dL [range, 1.6–17.0] in group A vs 9.1 mg/dL [range, 4.6–18.4] in group B, $P < 0.05$). The median levels of PT-INR were not significantly different throughout the postoperative course between the groups. As for the outcomes for the recipients associated

Table 2 Outcome of the donors

	Group A Pringle(+)	Group B Pringle(−)	
Duration of surgery (min)	407 (325–454)	388 (343–515)	NS
Duration of liver resection (min)	72 (51–83)	75 (64–102)	NS
Blood loss (g)	303 (170–480)	720 (360–2200)	$P < 0.01$
Blood loss during liver resection (g)	85 (10–132)	420 (170–1200)	$P < 0.01$
Serum lactate after hepatectomy (mmol/L)	2.2 (1.0–4.2)	2.2 (1.0–3.4)	NS
Complications			
Clavien classification			
I	1	0	
II	0	0	
III	0	0	NS
IV	0	0	
V	0	0	

NS, no significant difference.

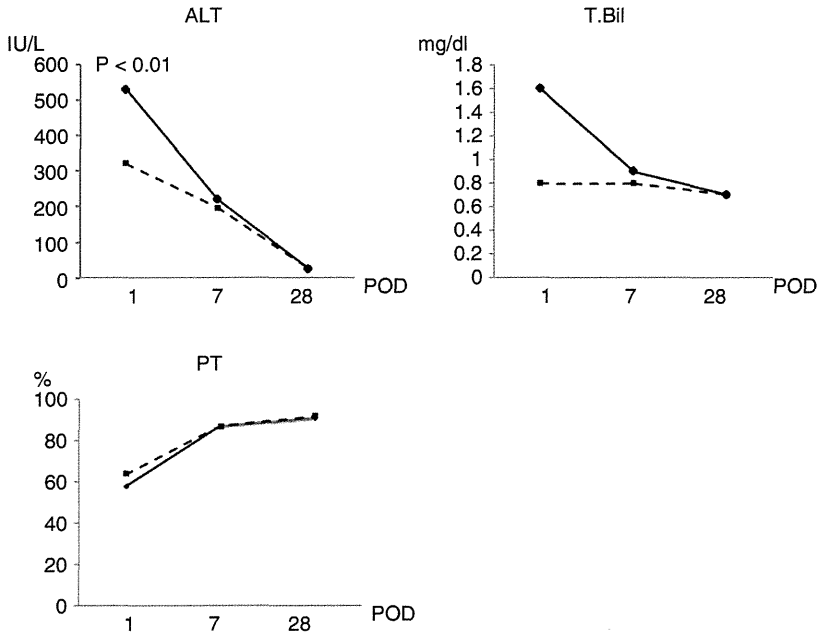

Figure 1 Serial changes of liver function tests after surgery in the donors, with (solid line) or without (dotted line) Pringle maneuver during donor surgery. ALT, alanine aminotransferase; POD, postoperative day; PT, prothrombin time; T.Bil, total bilirubin.

with these donors, the recipients for eight of 10 of the donors in each group survived with good graft function with a median follow-up period of 20 months (range, 11–26) in group A and 19 months (range, 10–25) in group B.

Immunohistochemistry of HO-1 and TLR4 in the graft

In the immunohistochemistry of the graft just after hepatectomy, the expressions of both HO-1 and TLR4 in

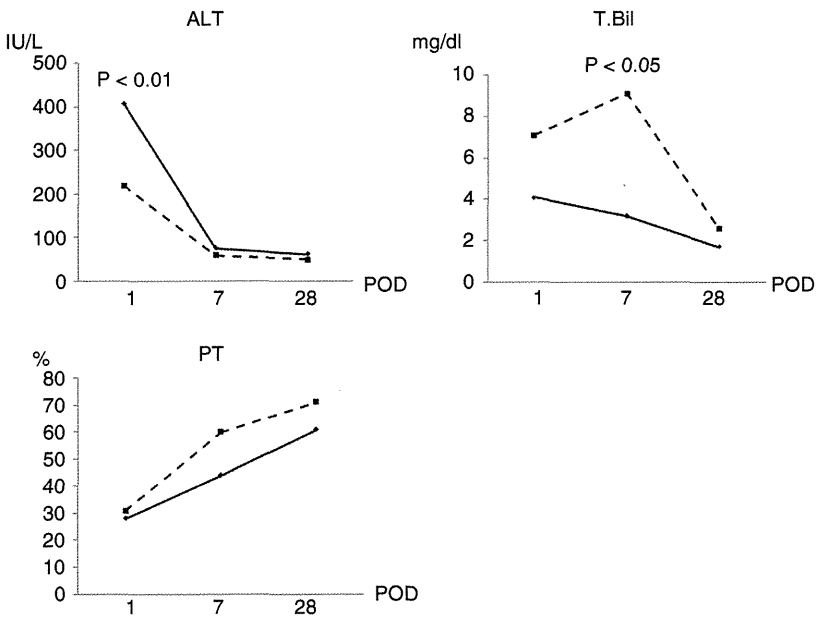

Figure 2 Serial changes of liver function tests after transplantation in the recipients, who received the graft from the donors with (solid line) or without (dotted line) Pringle maneuver during donor surgery. ALT, alanine aminotransferase; POD, postoperative day; PT, prothrombin time; T.Bil, total bilirubin.

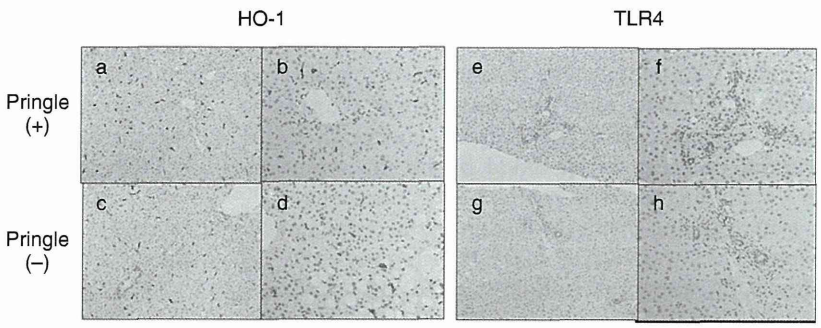

Figure 3 Immunohistochemistry of (a–d) heme oxygenase-1 (HO-1) and (e–h) Toll-like receptor 4 in the graft just before the removal of the graft in donor surgery. The expressions of HO-1 and TLR4 were not different between the Pringle group and the non-Pringle group. HO-1 was stained in the sinusoid, while TLR4 was stained in the portal triad in both groups (original magnification: [a,c,e,g] ×50; [b,d,f,h] ×100).

group A were similar to those in group B. HO-1 was stained in sinusoids, while TLR4 was stained in the portal triad in both groups (Fig. 3).

DISCUSSION

THIS STUDY SHOWED that the Pringle maneuver was safely applied in living donor hepatectomy, without causing severe injury in either the graft or the remnant liver in the donors. Although there has been no official tracking of whether the Pringle maneuver is generally adopted in living donor hepatectomy, most transplant centers do not utilize this technique, probably due to the concern that ischemic injury of the graft may occur, despite the fact that the safety of the Pringle maneuver has been proven in hepatectomy, even for cirrhotic livers.[1–3] Several single-arm retrospective studies showed that the Pringle maneuver could be safely applied in living donor hepatectomy,[10,11] and Imamura *et al.* mentioned that the graft function may be even better with ischemic preconditioning.[6] Recently, Park and colleagues also reported in their prospective randomized study that the Pringle maneuver was safely applied in living donor transplantation using a right lobe graft, with a graft-to-recipient bodyweight ratio of more than 0.9% and less than 30% steatosis.[12] They evaluated several cytokines that should be related to ischemic liver injury or liver regeneration, and none of them were significantly different between the groups with or without the Pringle maneuver. In our center, left lobe graft with MHV is the first line in adult-to-adult LDLT, so we evaluated cases of left lobe graft only. According to our study, even though these left lobe grafts were generally smaller in volume (median, 40–41% of recipient standard liver volume) than right lobe grafts, the graft function seemed to be as good as that of right lobe grafts.

However, although the final outcomes of the liver function in both the donors and recipients were not significantly different between the groups with or without the Pringle maneuver, hepatocyte injury during donor surgery seemed more severe in the Pringle group than in the group that did not undergo the Pringle maneuver because the ALT level was significantly higher in the Pringle group immediately after surgery in the donors. The ALT level result in our study was generally higher than in the previous reports.[6,10–12] This difference may be related to the fact that our procedure in liver resection was a two-surgeon technique using saline-linked electric cautery. We previously showed that our technique can be safely applied in living donor hepatectomy and with less blood loss and bile leakage than in the cases using ultrasonic surgical aspirator alone.[7] We believe that the high ALT level in the current study is related to the hepatocyte damage due to transient heat injury, which might have been enhanced by ischemic injury. In regard to the serum lactate level just after hepatectomy, there was no significant difference between the groups. Pietsch *et al.* showed in their study that serum lactate level during liver resection was significantly higher in the cases with Pringle maneuver than that in those without Pringle maneuver.[13] The result is not similar to that in our study, possibly because of absolutely normal liver of living donor in this study. Also, the mean level of lactate was 2.6 mmol/L in the Pringle group in their study, not so different from that in our study (median level of 2.2 mmol/L). However, we propose that as long as the possibility of more severe hepatocyte damage exists, the decision to use the Pringle maneuver should be made with caution in living donor hepatectomy, because donor safety is the top priority in LDLT. The indication of the Pringle maneuver should be based on the balance between the benefit (reduction of blood loss) and the

risk (possible severe hepatocyte damage) in the donor, because there seemed to be no obvious benefit for the recipient. Accordingly, our current policy is that we adopt the Pringle maneuver only in cases involving slight anemia before the surgery, emergency LDLT without time for saving blood before the surgery or uncomfortable bleeding during liver transection.

With regard to the liver function in the recipient after surgery, ALT was higher in the Pringle group the day after LDLT, while total bilirubin was higher in the non-Pringle group at day 7 after LDLT. In principle, this result may be related to the small sample size, and liver function after LDLT is easily affected by many factors, including blood flow, rejection, biliary stricture, infection, and so on. The only significant difference in patient characteristics was sex in the recipient. The number of female recipients was higher in the group with Pringle maneuver, possibly because of small sample size. Some studies have showed that the sex mismatch (female to male) is one of the significant factors related to poor outcome in LDLT.[14,15] In our study, the majority of donors were male in both groups, but sex mismatch may be related to the higher bilirubin level at day 7 after LDLT. In any case, we found that the Pringle maneuver seemed to have no beneficial impact on recipient liver function.

Heme oxygenase is the rate-limiting enzyme involved in heme metabolism. The inducible form, HO-1, is expressed in response to various stimuli, including peroxide, heat, hyperoxide, endotoxin and inflammatory cytokines. HO-1 is well recognized as one of the factors protecting hepatocyte against ischemic–reperfusion injury with its antioxidant, maintaining microcirculation and modulating the cell cycle and anti-inflammatory functions.[4,5] Several animal studies showed that HO-1 was expressed significantly more in the liver with ischemic preconditioning, but in our study, HO-1 was expressed in the livers that did not undergo the Pringle maneuver to the same degree as in those that underwent the Pringle maneuver. Because HO-1 is expressed by various stimuli as mentioned above, surgical intervention itself may induce HO-1 in a clinical situation, including heat injury with saline-linked cautery.

Toll-like receptor 4 belongs to the interleukin-1 receptor family, and triggers host inflammatory responses that are mediated by macrophages, neutrophils and complement.[16,17] Shen *et al.* reported that TLR4 may function as a putative HO-1 repressor in hepatic ischemic–reperfusion injury in their study using TLR4 knockout mice with 90 min of warm ischemia and 6 h of reperfusion.[18] In the current study, the expression of TLR4 in the Pringle group was found to be similar to that in the non-Pringle group in liver tissue on the basis of immunohistochemistry. Like HO-1, TLR4 is expressed by various stimuli, so that at least in the clinical setting in the current study, there was no significant difference in the expression of both HO-1 and TLR4 with or without the Pringle maneuver. Finally, we could not show any beneficial impact of the Pringle maneuver on the graft function in relation to ischemic preconditioning.

There are several limitations in this study, with the main one being that the number of cases was too small. A randomized control trial with sufficient number of cases should be performed to show the actual efficacy of the Pringle maneuver in LDLT, both in the donor and the recipient.

In conclusion, the Pringle maneuver was safely applied in LDLT, but the only obvious benefit was the reduction of blood loss in the donor surgery. Because the hepatocyte injury may be severe after the Pringle maneuver, this technique should be adopted in selected cases in which it is a priority to reduce the blood loss during donor surgery.

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E-Cadherin Expression in Hepatocellular Carcinoma Treated With Previous Local Treatment in Patients Undergoing Living Donor Liver Transplantation

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ABSTRACT

Background. The aim of this study was to evaluate the influence of previous local treatment on the E-cadherin (E-cad) expression in cases of hepatocellular carcinoma (HCC) after living donor liver transplantation (LDLT) within the Milan criteria.

Methods. Seventy-four of 204 patients with HCC underwent LDLT between 1997 and 2014. Previous local treatment for HCC was performed for 121 lesions in 47 patients (47/74, 63.5%). Histological and immunohistochemical E-cad expression analyses were conducted on the basis of the whole-liver histological examination technique.

Results. The interval to LDLT after the initial and last treatments was 24 months (2–206) and 10.5 months (1–58), respectively. Preoperative imaging showed necrosis in 92 (92/121, 76.0%) lesions caused by the effects of local treatment, whereas the histological examinations revealed viable HCC cells in 22 (22/92, 23.9%) lesions, demonstrating well or moderate differentiation without vascular invasion. Immunohistochemically, the expression of E-cad was maintained in 17 viable (17/22, 77.3%) lesions. There were no signs of malignant transformation or sarcomatous changes in the HCCs treated with previous therapy. The recipients who maintained an E-cad expression in the lesion with local treatment showed no recurrence or distant metastasis after LDLT.

Conclusions. HCC cells remained in approximately 20% of the evaluated lesions, even those exhibiting necrosis on imaging of the explanted cirrhotic liver. However, the expression of E-cad was maintained in most of these lesions. Furthermore, there were no significant differences in the rate of recurrence after LDLT between the patients who did and those did not receive previous local treatment for HCC.

LIVING DONOR LIVER TRANSPLANTATION (LDLT) for hepatocellular carcinoma (HCC) within the Milan criteria (M-C) is a widely accepted optimal therapy in Japan. The administration of local treatment before LDLT is often used as bridging therapy and for downstaging. On the other hand, local treatment for HCC occasionally induces sarcomatous changes and/or malignant transformation [1]. E-cadherin (E-cad) mediates cell-cell adhesion by associating with catenin. A previous study showed that a reduced function or the downregulation of E-cad is closely related to the progression and recurrence of HCC [2]. The aim of this study was to evaluate the effects of local treatment on the outcomes of patients with HCC undergoing LDLT within the M-C and to assess the expression of E-cad in the previously treated lesions.

METHODS

Seventy-four of 204 patients with HCC underwent LDLT between 1997 and 2014. Previous local treatment for HCC was performed for 121 lesions in 47 patients (47/74, 63.5%). Histological and immunohistochemical E-cad expression analyses were performed

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Table 1. Tumor Factors in the Patients Treated With and Those Without Previous Local Therapy for HCC

	Previous Local Treatment (+) (n = 47)	Previous Local Treatment (–) (n = 27)	*P* Value
AFP (ng/mL) (median, range)	16.6 (0.9–1569)	17.7 (0.8–993.7)	.6533
PIVKA-II (mAU/mL)	46 (5–535)	61 (8–4953)	.1946
Preoperative imaging			
Tumor number	1 (0–3)	1 (1–3)	.6127
Tumor size (cm)	2.0 (1.0–5.0)	2.3 (0.7–4.8)	.4636
Histological examination			
Tumor number	2 (0–36)	2 (1–8)	.6604
Tumor size (cm)	2.0 (0.4–4.5)	2.3 (0.7–5.0)	.4005
Differentiation	Well or moderate	Well or moderate	
Vascular invasion (vp, vv)	5/47 (10.6%)	3/27 (11.1%)	.8339

Abbreviations: AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K antagonist II; vp, portal vein invasion; vv, hepatic vein invasion.

according to the whole-liver histological examination (WLHE) technique [3].

RESULTS

Forty-seven patients were enrolled in this study. Seventy percent of the patients were male, and the median age was 58 years (33–72). Fourteen patients (30%) were seropositive for hepatitis B antigens (HBs-Ag), 1 (2%) was seropositive for HBs-Ag and hepatitis C antibodies (HCV-Ab), 31 (66%) were seropositive for HCV, and 1 (2%) was seronegative for both HBs-Ag and HCV-Ab. The median Child-Pugh score was 9 (5–13), and the median model of end-stage liver disease (MELD) score was 13 (4–40). The median levels of serum alpha-fetoprotein and serum protein induced by vitamin K absence II were 16.6 (0.9–1569) and 46.0 (6.0–535), respectively. The local treatments administered for HCC before LDLT included transarterial chemoembolization/percutaneous ethanol injection treatment/radiofrequency ablation/microwave coagulo-necrotic therapy/hepatectomy in 33/17/24/1/11 patients, with overlapping therapies in each case. The interval to LDLT after the initial and last treatments was 24 months (2–206) and 10.5 months (1–58), respectively. Previous local treatment for HCC was performed for 121 lesions in 47 patients (47/74, 63.5%). There were no significant differences in preoperative tumor markers, tumor number, and tumor size between the patients treated with and those without previous local therapy under univariate analysis (Table 1). Only 1 recipient in each group had recurrence after LDLT; multivariate analysis between 2 groups was statistically unreliable. The preoperative images showed necrosis in 92 lesions (92/121, 76.0%) caused by the effects of previous local treatment.

The histological examinations showed 22 viable lesions (22/92, 23.9%) remaining in or around the necrotic areas, diagnosed as regions of complete necrosis on imaging. The

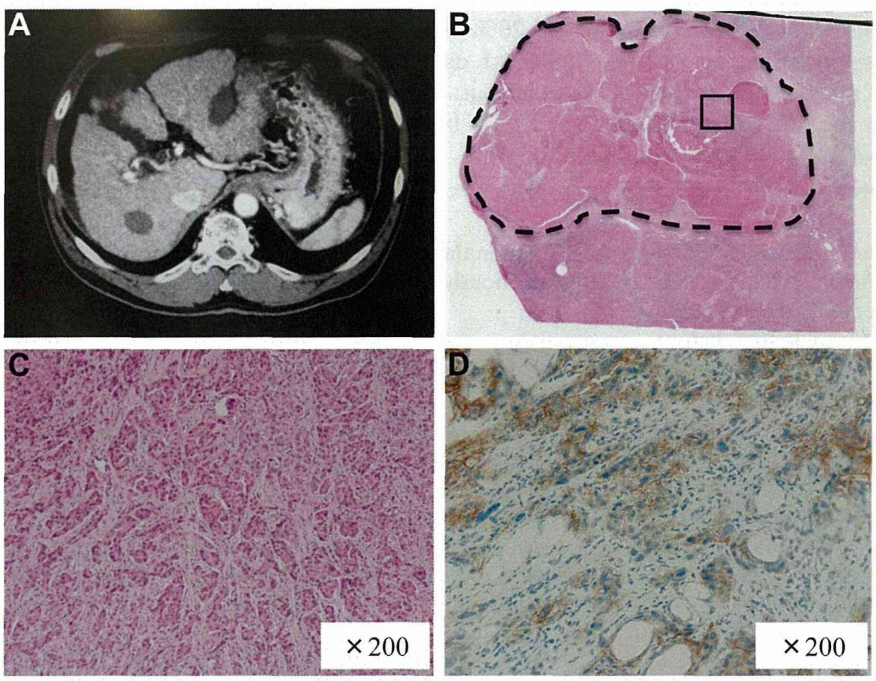

Fig 1. A 57-year-old man was diagnosed with multiple HCC associated with type C liver cirrhosis. The patient underwent transcatheter arterial embolization and radiofrequency ablation. The time to LDLT after the last treatment was 6 months. (**A**) Preoperative images: there were no viable lesions. (**B**) Gross inspection: the HCC lesions appeared to be entirely necrotic (major axis: 2.3 cm). (**C**) Histological examination: microscopically, a viable lesion (well-differentiated to moderately differentiated HCC) was observed. (**D**) E-cad expression was maintained in the histologically viable lesion.

22 histologically viable lesions measured 0.1 to 0.6 cm in diameter and were macroscopically indistinct, including 11 well-differentiated and 11 moderately differentiated lesions, with only 1 tumor exhibiting vascular invasion.

Immunohistochemically, the expression of E-cad was maintained in 17 histologically viable lesions (17/22, 77.3%). E-cad expression in the well-differentiated HCC lesions was almost as high as that observed in the normal liver and remained preserved in the moderately differentiated HCC lesions not treated with previous local therapy (Fig 1). Additionally, the expression of E-cad was maintained in 85% of the pathologically detectable HCC lesions, as well as the viable HCC lesions treated with local therapy. There were no signs of malignant transformation or sarcomatous changes in the HCCs treated with previous therapy. Two recipients received treatment for HCC before LDLT exhibited recurrence involving peritoneal dissemination after LDLT. The recipients with a maintained E-cad expression in the lesions treated with local therapy showed no evidence of recurrence or distant metastasis after LDLT.

DISCUSSION

We previously reported a case of the multicentric occurrence of HCC in a patient with advanced cirrhosis of the liver at the time of liver transplantation that was not detected on currently available modalities [3] and that half of necrotic lesions observed on preoperative imaging continue to have viable HCC cells [4]. Recent studies have demonstrated a correlation between the E-cad expression in tumor lesions and the recurrence of HCC. Furthermore, we previously reported that the serum soluble E-cad levels are elevated in patients with HCC and that a high serum soluble E-cad level is associated with early recurrence, despite performing curative resection of HCC, although the E-cad expression in HCC lesions is not related to the serum level of soluble E-cad [5].

In the current study, histological examinations performed according to the WLHE method showed that minute HCC cells continued to be present at a rate of approximately 24% in the patients who received previous treatment, even though preoperative imaging showed no viable lesions before LDLT. The viable HCCs were minute, well-differentiated, or moderately differentiated and macroscopically indistinct, without vascular invasion. Immunohistochemically, the expression of E-cad was maintained in most of the lesions. Therefore, this phenomenon may be related to the low recurrence rate after LDLT observed in our study, although the recurrence rate for HCC was 8% to 10% within 5 years after LDLT. We did not perform quantitative analysis of E-cad expression because these lesions were too small and sparse.

In conclusion, HCC cells persisted in approximately 20% of lesions evaluated in this study, even in those exhibiting necrosis on imaging of the explanted cirrhotic liver. The expression of E-cad was maintained in most of these lesions, and there were no significant differences in the rate of recurrence after LDLT between the patients who did and those who did not receive previous local treatment for HCC.

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Hybrid Procedure in Living Donor Liver Transplantation

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ABSTRACT

Background. We have previously reported a hybrid procedure that uses a combination of laparoscopic mobilization of the liver and subsequent hepatectomy under direct vision in living donor liver transplantation (LDLT). We present the details of this hybrid procedure and the outcomes of the procedure.

Methods. Between January 1997 and August 2014, 204 LDLTs were performed at Nagasaki University Hospital. Among them, 67 recent donors underwent hybrid donor hepatectomy. Forty-one donors underwent left hemihepatectomy, 25 underwent right hemihepatectomy, and 1 underwent posterior sectionectomy. First, an 8-cm subxiphoid midline incision was made; laparoscopic mobilization of the liver was then achieved with a hand-assist through the midline incision under the pneumoperitoneum. Thereafter, the incision was extended up to 12 cm for the right lobe and posterior sector graft and 10 cm left lobe graft procurement. Under direct vision, parenchymal transection was performed by means of the liver-hanging maneuver. The hybrid procedure for LDLT recipients was indicated only for selected cases with atrophic liver cirrhosis without a history of upper abdominal surgery, significant retroperitoneal collateral vessels, or hypertrophic change of the liver (n = 29). For total hepatectomy and splenectomy, the midline incision was sufficiently extended.

Results. All of the hybrid donor hepatectomies were completed without an extra subcostal incision. No significant differences were observed in the blood loss or length of the operation compared with conventional open procedures. All of the donors have returned to their preoperative activity level, with fewer wound-related complaints compared with those treated with the use of the conventional open procedure. In recipients treated with the hybrid procedure, no clinically relevant drawbacks were observed compared with the recipients treated with a regular Mercedes-Benz–type incision.

Conclusions. Our hybrid procedure was safely conducted with the same quality as the conventional open procedure in both LDLT donors and recipients.

APPLICATIONS of less invasive techniques, including laparoscopic procedures, have been reported in the field of living donor liver transplantation (LDLT) [1–3]. We have reported a hybrid procedure that uses a combination of hand-assisted laparoscopic mobilization of the liver and subsequent hilar dissection and parenchymal resection under direct vision in living donor hepatectomy [1,4]. In terms of appearance, sensation, and daily activities, our hybrid procedure had better donor self-assessment compared with those treated with a conventional incision, such as a right subcostal incision or Mercedes-Benz incision [5].

We also introduced the basic concept of the hybrid procedure into recipient surgery in selected cases [6]. We present the current practice of the hybrid procedure and the outcomes of the procedures in LDLT.

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