performed a national survey of pregnancy outcomes after LDLT in Japan. The society sent questionnaires to the institutes and retrospectively assessed data on pregnancy outcomes after LT until May 2012. The questionnaires included information about the LDLT, the clinical courses of pregnancy and delivery, and the neonates.

Pregnancy-induced hypertension was defined as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg after 20 weeks of gestation in a woman with previously normal blood pressure (26). Fetal growth restriction was defined as an estimated fetal weight <-1.5 the standard deviation of the normal reference range. The fetal weight was estimated with formulas from ultrasound measurements based on neonatal specific gravities and volumes (27). In 22 of 23 recipients (25 of 29 pregnancies) who received tacrolimus during pregnancy, consecutive serum trough levels of tacrolimus during pregnancy (at several times) were available, and the mean trough level was calculated. The pathological degree of acute rejection (rejection activity index) was assessed according to the Banff classification (28).

This study was approved by the ethics committee of Osaka City University Graduate School of Medicine (no. 1856) and conducted in accordance with the Declaration of Helsinki of 1996. Informed consent was obtained from the participants. No patient was excluded from the study because informed consent could not be obtained.

Statistics

To assess the relationships between the complication rates during pregnancy and pregnancy outcomes and the age at pregnancy and interval from LDLT to pregnancy, receiver operating characteristic (ROC) curves were constructed. In addition, areas under the ROC curve (AUC) with 95% confidence interval (CI) were calculated. The optimal cut-off values for the age and intervals were determined by Youden's index (sensitivity + specificity - 1). Categorical variables were compared using the chi-square or Fisher's exact tests where appropriate. The Student *t* test was used to analyze differences in ages. A P value <0.05 was considered significant. All statistical data were generated using JMP 9.0 (SAS Institute, Cary, NC, USA).

Results

Recipient characteristics

The study participants were 30 LT recipients who had 38 pregnancies (Fig. 1). The recipients underwent LDLT at 11 institutes. The indications for LDLT included congenital biliary atresia in 14 recipients, acute liver failure in 9, primary sclerosing cholangitis in 2, autoimmune hepatitis in 1, liver cirrhosis caused by hepatitis B virus

in 1, Budd-Chiari syndrome in 1, familial amyloid polyneuropathy in 1, and hepatocellular carcinoma in 1 (Table 1). The age of the recipients at the time of LDLT ranged from 4 to 38 years. The age at which pregnancy was diagnosed ranged from 22 to 41 years (mean, 30.3 years). The time from LDLT to pregnancy diagnosis ranged from 356 to 6798 days (median, 1751 days).

At pregnancy diagnosis, tacrolimus was administered in 27 pregnancies (23 recipients), cyclosporine in 2 pregnancies (2 recipients), a combination of tacrolimus and steroids in 2 pregnancies (2 recipients), a combination of cyclosporine and sirolimus in 1 pregnancy (1 recipient), and a combination of tacrolimus, steroids, and mycophenolate mofetil (MMF) in 1 pregnancy (1 recipient). The mean trough level of tacrolimus at pregnancy diagnosis was 4.5 ng/mL (range, 0.9–10.0 ng/mL) and that during pregnancy was 4.5 ng/mL (range, 1.5–10.0 ng/mL). No immunosuppressive drugs were administered for 3 pregnancies at pregnancy diagnosis because of auxiliary partial orthotopic LT in 1 pregnancy (1 recipient) and the discontinuation of drugs after LDLT in childhood in 2 pregnancies (1 recipient). The serum creatinine levels at pregnancy diagnosis were available for 32 pregnancies (24 recipients), which were within the reference range.

Pregnancy outcomes

Thirty-eight pregnancies in 30 recipients resulted in 31 (81.6%) live births (25 recipients), 3 artificial abortions (3 recipients), 1 spontaneous abortion (1 recipient), and 3 fetal deaths (3 recipients) (Fig. 1). Artificial abortion was performed in the first trimester because of MMF use in 1 pregnancy (1 recipient), sirolimus use in 1 pregnancy (1 recipient), and a short time after LDLT (356 days) in 1 pregnancy (1 recipient).

Obstetric complications

Among the 35 pregnancies in 27 recipients (other than the 3 artificial abortions in 3 recipients), spontaneous abortion occurred in 1 (2.9%) pregnancy (1 recipient) and fetal death occurred in 3 (8.6%) pregnancies (3 recipients) as previously described (Table 2). Pregnancy-induced hypertension developed in 6 (17.1%) pregnancies (5 recipients), fetal growth restriction in 7 (20.0%) pregnancies (6 recipients), and ileus in 1 pregnancy (1 recipient). Liver dysfunction (elevated serum activities of aspartate aminotransferase, alanine aminotransferase, and/or gamma-glutamyl transpeptidase) was detected in 4 pregnancies (4 recipients). Acute rejection, diagnosed by liver biopsy (rejection activity index, 2) and laboratory test results, occurred in 2 of these 4 recipients; an increased

dose of cyclosporine and steroid pulse therapy was given to 1 recipient, and an increased dose of tacrolimus was administered to 1 recipient. Other obstetric complications such as gestational diabetes, infection, placental abruption, and thromboembolic disorders did not occur in any recipient. In 1 (auxiliary partial orthotopic LT) of 2 recipients who did not receive immunosuppressive drugs, fetal death occurred because of umbilical cord coiling. In another patient (2 pregnancies), no complications developed during pregnancy.

In 1 of 8 recipients who were pregnant twice, the second pregnancy resulted in spontaneous abortion (at 7 weeks of gestation), although the first pregnancy was uneventful. Another recipient had pregnancy-induced hypertension in both the first and second pregnancies; fetal death occurred in the first pregnancy (25 weeks), and fetal growth restriction occurred in the second pregnancy.

Delivery outcomes

Among 31 pregnancies (27 recipients), preterm delivery (<37 weeks) occurred in 10 (32.3%) pregnancies (10 recipients). Cesarean delivery was performed in 12 (38.7%) pregnancies (12 recipients) because of pregnancy-induced hypertension in 6 pregnancies (6 recipients), hypotonic contraction during labor in 1 pregnancy (1 recipient), transient

bradycardia of the fetus in 1 pregnancy (1 recipient), ileus in 1 pregnancy (1 recipient), previous multiple abdominal operations in 1 pregnancy (1 recipient), previous cesarean delivery in 1 pregnancy (1 recipient), and the recipient's will in 1 pregnancy (1 recipient).

After delivery, liver dysfunction (elevated serum activities of aspartate aminotransferase, alanine aminotransferase, and/or gamma-glutamyl transpeptidase) occurred in 4 pregnancies (4 recipients), and acute rejection, diagnosed by liver biopsy (rejection activity index, 2 to 4), occurred within 4 months after LDLT in 3 of these 4 recipients. For acute rejection, steroid pulse therapy was administered in 2 recipients, and steroid and MMF were added to tacrolimus therapy in 1 recipient (Fig. 2). The recipients' liver function improved with these treatments. In 1 recipient, artificial respiration was necessary because of acute respiratory distress syndrome after delivery, and renal dysfunction persisted after recovery. Puerperal fever developed in 1 recipient. The pregnancy-induced hypertension improved after delivery in all recipients who had hypertension during pregnancy. In 1 recipient, re-transplantation was performed because of the recurrence of primary sclerosing cholangitis 5 years after delivery.

Among 31 live births, neonatal asphyxia occurred in 1 neonate. Twelve neonates

were born with low birth weights (<2500 g), and 4 of the 12 low birth weight neonates were born with extremely low birth weights (<1500 g). Although intracranial bleeding developed after delivery in 1 neonate with an extremely low birth weight, the condition improved without complications.

One neonate had tetralogy of Fallot, and 1 neonate had hypospadias.

Risk factors for obstetric complications, delivery outcomes, and birth defects

Relationships between the mean trough level of tacrolimus and obstetric complications, delivery outcomes, and birth defects were not found.

Relationships between age at pregnancy diagnosis and complications during pregnancy were studied using ROC curves. The AUC (95% CI) was 0.784 (0.613–0.905) for pregnancy-induced hypertension (Fig. 3A). The optimal cut-off value was 33 years (specificity, 83.3%; specificity, 69.0%). No significant relationship was found between age at pregnancy and other complications such as spontaneous abortion, fetal death, fetal growth restriction, and liver dysfunction. The incidence of pregnancy-induced hypertension in recipients aged ≥33 years at pregnancy diagnosis was significantly higher than that in recipients aged <33 years at pregnancy diagnosis ($P = 0.0278$ by Fisher's exact test).

Relationships between the interval from LDLT to pregnancy and delivery outcomes were studied using ROC curves. The AUC (95% CI) was 0.801 (0.632–0.916) for fetal growth restriction (Fig. 3B). The optimal cut-off value was 1096 days (sensitivity, 71.4%; specificity, 82.1%). The AUC (95% CI) was 0.822 (0.656–0.930) for pregnancy-induced hypertension (Fig. 3C). The optimal cut-off value was 1096 days (sensitivity, 83.3%; specificity, 82.8%). The AUC (95% CI) was 0.759 (0.573–0.893) for extremely low birth weight (Fig. 3D). The optimal cut-off value was 1096 days (sensitivity, 75.0; specificity, 81.5%). No significant relationship was found between the interval and other factors, including spontaneous abortion, fetal death, liver dysfunction, and preterm delivery.

The obstetric complications and delivery outcomes were compared in 10 pregnancies in which the interval from LT to pregnancy was <3 years (early group) and 25 pregnancies in which this interval was ≥3 years (late group) because the optimal cut-off value was 1096 days by the analysis using ROC curves (Table 2). The 3 pregnancies in which artificial abortion was performed in the first trimester were excluded from this comparison. The mean age at pregnancy was significantly higher in the early group than in the late group. The proportions of recipients with fetal growth restriction and pregnancy-induced hypertension were significantly higher in the early

group than in the late group. The proportion of neonates with extremely low birth weight was significantly higher in the early group than in the late group.

The incidence of pregnancy-induced hypertension in recipients aged ≥33 years at pregnancy diagnosis in the early group (5/8 pregnancies, 62.5%) was significantly higher than that in recipients aged <33 years at pregnancy diagnosis in the late group (1/19 pregnancies, 5.3%, P = 0.0037) and in recipients aged ≥33 years at pregnancy diagnosis in the late group (0/6 pregnancies, P = 0.031); the incidence of pregnancy-induced hypertension was the highest in recipients aged ≥33 years at pregnancy diagnosis in the early group (the interval from LDLT to pregnancy was <3 years).

Discussion

An increased risk of complications, including prematurity, low birth weight, pregnancy-induced hypertension, renal dysfunction, and cesarean delivery, has been reported by previous studies concerning pregnancy in LT recipients (most patients underwent cadaver LT) (1–24).

In this study, pregnancy-induced hypertension developed in 6 (17.1%) pregnancies (5 recipients). Shiozaki *et al.* (29) reported that pregnancy-induced hypertension was

present in 1.2% of pregnancies (2,802/241,292) in the Japan Society for Obstetrics and Genecology database. The incidence of pregnancy-induced hypertension seems to be higher in LDLT recipients than in the general population. Several studies reported that pregnancy-induced hypertension was common among LT recipients (11–43%) (1,3–6,10,11,13,17,20,23,24). The incidence of pregnancy-induced hypertension in LDLT recipients (17.1%) was similar to that in cadaver LT recipients. On the other hand, pregnancy-induced hypertension did not occur in 1 recipient (2 pregnancies) who did not receive immunosuppressive drugs during pregnancy. This complication has been shown to occur more frequently in LT recipients with renal dysfunction (11,12). Although no relationship between the mean trough levels of tacrolimus and pregnancy-induced hypertension was observed in this study, underlying renal dysfunction (11) and the vasoconstrictive effects of calcineurin inhibitors may affect hypertension. In addition, it is necessary to pay attention when the recipient's age at pregnancy diagnosis is ≥33 years.

In this study, spontaneous abortion occurred in 1 pregnancy (1 recipient), and fetal death occurred in 3 pregnancies (3 recipients). Coffin *et al.* (23) reported that infants of LT recipients had a 3-fold risk of complications, most notably fetal death (6% versus 2% in controls). Among 241 pregnancies in LT recipients described in the National

Transplantation Pregnancy Registry in 2008 (3), 19.2% and 2.1% ended in spontaneous abortions and stillbirths, respectively. The maternal and fetal conditions might affect the rates of spontaneous abortion and fetal death. Another adverse fetal outcome noted in this study was fetal growth restriction in 7 pregnancies (20.0%). The incidence of complications appears to be higher in these individuals than in the general population (23). However, the mechanisms underlying the high incidences of spontaneous abortion, fetal death, and fetal growth restriction are unclear.

Several previous studies have reported a high incidence of preterm delivery (14–53%) (1,3–6,8–10,13,14,17,18,20,23,24). In this study, preterm delivery (<37 weeks) occurred in 10 pregnancies (32.3%). The proportion of preterm delivery seemed to be high because the database of the Japan Society for Obstetrics and Genecology indicated that the rate of threatened premature delivery was 2.34% (30). Preterm delivery might be related to maternal conditions such as hypertension and fetal conditions, including fetal growth restriction.

Several previous studies have shown that cesarean delivery is more common among transplant recipients (4–6,10,13,15–17,20,23,24). In this study, cesarean delivery was performed in 12 (38.7%) of 31 pregnancies. The indications for cesarean delivery included pregnancy-induced hypertension, hypotonic contraction during labor, transient

bradycardia, ileus, multiple previous abdominal operations, previous cesarean delivery, and the recipient's will. Thus, it is likely that the high rate of cesarean delivery was attributable to pregnancy complications rather than to LT itself.

Acute rejection is an important problem during and after pregnancy because rejection may induce graft loss. In fact, the National Transplantation Pregnancy Registry (2006) (1) reported that 7% of pregnancies were complicated by acute rejection, and 8% of individuals lost their grafts within 2 years of delivery. Other studies have reported that rejection rates during pregnancy were 0–17% (2,3–6,9,10,13,15–17,20,23). It has been reported that rejection episodes up to 3 months after delivery are a risk factor for graft loss after delivery (5,7). Kainz *et al.* (31) reported that rejection was followed by preeclampsia, renal impairment, and infection. In this study, acute rejection occurred in 2 recipients during pregnancy and in 3 recipients after delivery (within 4 months after delivery), although these patients had no renal dysfunction. All recipients were successfully treated with an increased dose of tacrolimus and/or addition of corticosteroids or MMF, and graft loss did not occur. Thus, adequate treatment for acute rejection can prevent graft loss, although close follow-up of pregnant recipients is necessary even after delivery, especially when the recipients have renal dysfunction.

Congenital malformations in live-born neonates have been reported to occur in 3% of the non-transplant population (32). In transplant recipients, the incidence of congenital malformations has been reported to be 4% with corticosteroids (32), 7% with azathioprine (32), 3% with cyclosporine (33), and 4% with tacrolimus (14). Kainz *et al.* (31) reported that 4 neonates presented with malformations among 100 pregnancies in which the mother was treated with tacrolimus. In the present series, most recipients received tacrolimus-based therapy, and 2 (6.4%) of 31 neonates had congenital malformations (tetralogy of Fallot and hypospadia). A higher incidence of structural malformations was observed with MMF exposure during pregnancy (34). This agent is classified as Pregnancy Category D (with positive evidence of human fatal risk, but potential benefits may warrant use of the drug in pregnant women despite the potential risk; evidence of fetal risk) (35). No structural defects have been reported with early pregnancy sirolimus exposure to date. In this study, artificial abortion was performed in 2 recipients in whom MMF or sirolimus was administered. Calcineurin inhibitors are classified as Pregnancy Category C (animal reproductive studies have shown an adverse effect on the fetus or are lacking, and there are no adequate and well-controlled studies in humans, but the potential benefits may warrant the use of the drug in pregnant women despite the potential risks; fetal risk cannot be ruled out) (35). Thus,

calcineurin inhibitor-based therapy, including cyclosporine and tacrolimus, is favorable for pregnant recipients.

Although there is no established optimal interval between LT and pregnancy, a report from the National Transplantation Pregnancy Registry and the American Society of Transplantation recommended that LT recipients wait a minimum of 1 year before conception to stabilize graft function and immunosuppressant dosage. Christopher *et al.* (16) reported that pregnancies occurring within 1 year after LT had an increased incidence of prematurity, low birth weight, and acute rejection compared with those occurring >1 year after LT. Nagy *et al.* (15) reported that the risk of complications during pregnancy is low when liver LT recipients become pregnant >2 years after LT because recipients have stable and normal hepatic function and normal renal function, and immunosuppressive therapy is at a maintenance dosage. The results of the National Transplantation Pregnancy Registry (2008) (3) showed that the incidence of very low birth weight neonates in pregnancy within 2 years after LT was higher than that in pregnancy >5 years after LT. They also reported a higher incidence of rejection in pregnant recipients 1–2 years after LT. These results indicate better outcomes for recipients and infants with pregnancies occurring at least 2 years after LT. In this study, the incidences of fetal growth restriction, pregnancy-induced hypertension, and

neonates with extremely low birth weights were significantly higher in the early group (within 3 years after LDLT) than in the late group (≥3 after LDLT). In addition, the incidence of pregnancy-induced hypertension was higher in recipients aged ≥33 years at pregnancy diagnosis than in those aged <33 years at pregnancy diagnosis. Thus, it is necessary to pay careful attention to complications during pregnancy when the recipients become pregnant within 3 years after LDLT, particularly if the age at pregnancy diagnosis is ≥33 years.

The pregnancy outcomes of LDLT recipients were similar to those of cadaver LT recipients. Although most pregnancy outcomes are favorable, special attention should be given to obstetric complications such as pregnancy-induced hypertension, spontaneous abortion, fetal death, fetal growth restriction, preterm delivery, cesarean delivery, and acute rejection. It is difficult to draw definitive conclusions by this study because the number of recipients in this study was too small, and this survey might not reflect all pregnant recipients. Thus, it is necessary to analyze the outcomes after pregnancy in larger studies, with prospective registration, to establish and improve the clinical management of pregnancy in LT recipients.

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