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Original Article

Incidence of and risk factors for bile duct stones after living donor liver transplantation: An analysis of 100 patients

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Aim: Although bile duct stone (BDS) is one of the biliary complications of liver transplantation, analytical studies, particularly on living donor liver transplantation (LDLT) cases, are rare. This study aimed to clarify the incidence of and risk factors for BDS following LDLT.

Methods: We retrospectively reviewed the medical records of 100 patients who underwent LDLT at our institute from August 2000 to May 2012, and analyzed their clinical characteristics and risk factors for BDS.

Results: Of these, 10 patients (10.0%) developed BDS during the observation period. The median follow-up period to BDS diagnosis was 45.5 months (range, 5–84) after LDLT. Univariate analysis revealed male sex, right lobe graft and bile duct strictures as factors that significantly correlated with BDS formation. Multivariate analysis revealed bile duct strictures (odds ratio, 7.17; $P = 0.011$) and right lobe graft (odds ratio, 10.20; $P = 0.040$) to be independent risk factors for BDS formation. One patient with BDS and biliary strictures succumbed to sepsis from cholangitis.

Conclusion: In the present study, right lobe graft and bile duct strictures are independent risk factors for BDS formation after LDLT. More careful observation and monitoring are required in the patients with high-risk factors.

Key words: bile duct stone, complication, living donor liver transplantation, male sex, right lobe graft

INTRODUCTION

LIVER TRANSPLANTATION (LT) is a powerful therapy for patients with severe liver diseases, and its importance has been clearly recognized worldwide with the progression of surgical and perioperative care techniques. However, various complications still occur after LT, with biliary complications being relatively common. The reported incidence of biliary complications is approximately 5–25%.[1–3] Bile duct stone (BDS) is one of these biliary complications, often leading to severe cholangitis.[2,4,5] The reported incidence of BDS following LT is approximately 5%.[6,7] Moreover, several authors have reported the following risk factors for BDS after deceased donor LT: bile duct strictures, prolonged warm ischemia periods of grafts and increased total cholesterol levels.[8–11] However, few studies have analyzed BDS incidence after living donor liver transplantation (LDLT). In Japan, LDLT is predominantly performed because of the lack of deceased donor organs. This study aimed to review the clinical characteristics and outcomes of patients who developed BDS after LDLT and clarify the incidence of and risk factors for BDS after LDLT.

METHODS

WE ENROLLED 100 patients from a total of 157 patients who underwent LDLT at Nagasaki University Hospital from August 2000 to May 2012, excluding pediatric patients (aged <18 years) and patients who died in the early postoperative period (until 30 days). All of them were followed up for at least 5 months. We retrospectively reviewed their clinical course records, operative logs, blood examination, and radiology and endoscopic findings to analyze their clinical characteristics and risk factors for BDS.

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In our institute, biliary reconstruction was performed by duct-to-duct anastomosis with an interrupting suture over a retrograde transhepatic biliary drainage tube (tube diameter, 2 mm) whenever possible. However, for patients with biliary atresia, primary sclerosing cholangitis and intraoperative bile duct injuries we selected hepaticojejunostomy over internal stenting. To evaluate the association between biliary ischemic change and BDS formation, the total ischemia time (TIT), defined as the duration from clamping of donor vessels to reperfusion of the recipients' portal vein, was also recorded. In our institute, periodic examinations are regularly conducted after transplantation.

We usually check the liver function of patients by blood examination once a month and perform abdominal enhanced computed tomography (CT) every 6 months, even if recipients have no symptoms. When we detected clinically suspicious symptoms of cholangitis such as abdominal pain with fever or abnormal increase in liver enzymes, we performed either magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiography (ERC). When the cholangitis required drainage, our first choice was endoscopic treatment; therefore, percutaneous transhepatic cholangiography (PTC) was performed if ERC, including deep endoscopic procedures, failed because of bile duct deformity or hepaticojejunostomy. Bile duct stricture was defined as any narrowing of bile ducts identified by CT, MRCP or ERC that is associated with graft dysfunction and required any kind of interventional procedures. Hepatic artery complications and portal vein complications were diagnosed by enhanced CT and Doppler sonography.

Primary immunosuppression was induced after LDLT using standard dual therapy with tacrolimus (Tac) or cyclosporin (CyA) and steroids, although some patients with impaired renal function received basiliximab (BX) or mycophenolate mofetil (MMF).

Statistical analysis

Categorical variables were analyzed using the χ^2-test or Fisher's exact test, while continuous variables were analyzed using Student's *t*-test for normally distributed variables and the Mann–Whitney *U*-test for non-normally distributed variables. Logistic regression analysis was used to identify variables that independently predicted BDS incidence. A *P*-value of less than 0.05 was considered statistically significant in all analyses. All statistical analyses were performed using STATFLEX version 6 (Artech, Osaka, Japan).

RESULTS

Clinical characteristics of patients

IN TOTAL, 100 patients (42 men, 58 women; mean age, 52.9 ± 12.2 years [range, 22–72]) with LDLT were analyzed. The median observation period was 49.5 months (range, 5–143). The indications for liver transplantation are summarized in Table 1. The ABO blood type was incompatible in 15 (15%) patients. Of the 100 patients, 52 (52%) underwent right lobe transplantation and 48 (48%) underwent left lobe transplantation. For 92 (92%) patients, duct-to-duct anastomosis was selected to reconstruct the biliary system, while hepaticojejunostomy was selected for eight (8%). Multiple biliary reconstruction was performed in 13 patients, 12 of whom underwent right lobe grafting. Of the 13 patients, 12, including one who underwent left lobe grafting, required double anastomosis; the remaining one required triple anastomosis. The median TIT was 170 min (range, 106–555). Primary immunosuppression was induced after transplantation using Tac in 55 patients, Tac with MMF in 28 patients, CyA in nine, CyA with MMF in three, BX in one, BX with MMF in three and BX with Tac in one. With regard to other medications, ursodeoxycholic acid (UDCA) was used in 37 (37%) patients.

Incidence of BDS and other complications

Ten patients (10%) developed BDS during the observation period. Of the 10 patients, four developed BDS in the proximal bile duct above the anastomotic site, including intrahepatic duct. Composition of the stones

Table 1 Indications for liver transplantation

Primary disease	No. of patients
Hepatitis B virus-related cirrhosis (LCB)	28
Hepatocellular carcinoma (HCC) in LCB	13
Hepatitis C virus-related cirrhosis (LCC)	40
HCC in LCC	13
LCC with hepatitis B virus	2
Alcohol-induced cirrhosis (LCAL)	11
HCC in LCAL	3
Non-alcoholic steatohepatitis (NASH)	8
HCC in NASH	1
Primary biliary cirrhosis	4
Primary sclerosing cholangitis	1
Fulminant hepatitis	6
Biliary atresia	1
Caroli disease	1

was identified in six of 10 patients: one patients had a cholesterol stone and the rest had bilirubinate calcium stones. There was no bile duct filing defects diagnosed as biliary cast. The median duration from transplantation to BDS diagnosis was 45.5 months (range, 5–84). Twenty-two patients (14% of 157 patients) had bile duct strictures, six of whom also developed BDS. Bile duct stenting was performed for all patients with strictures, with 16 undergoing endoscopic stenting and six undergoing percutaneous stenting. Hepatic artery complications occurred in seven patients (7%): thrombosis ($n = 2$), endothelial dissection ($n = 1$), hemorrhage ($n = 2$) and blood flow decrease ($n = 2$). Patients with thrombosis, endothelial dissection and hemorrhage required surgical therapy, while blood flow decrease was treated with warfarin sodium.

Risk factors for BDS formation

To clarify the risk factors for BDS formation, we analyzed the relationships among some clinical variables and BDS formation (Table 2). BDS was significantly common in male patients ($P < 0.05$), right lobe graft cases ($P < 0.05$) and those with bile duct strictures ($P < 0.01$). There was no significant difference in age, body mass index, Model for End-Stage Liver Disease score, rate of ABO blood type incompatibility, biliary reconstruction method (duct-to-duct anastomosis vs hepaticojejunostomy, single anastomosis vs multiple anastomosis), hepatic artery complications and TIT between patients with BDS and those without. We also analyzed whether serum total cholesterol and serum triglyceride levels were elevated above 200 mg/dL and 150 mg/dL, respectively, during the observation period; however, there were no significant differences between groups. With regarding to medication use, we found that the use of CyA and UDCA did not influence BDS formation.

Univariate analysis revealed that male sex, right lobe graft and bile duct strictures significantly correlated with BDS formation. Multivariate analysis revealed that bile

Table 2 Multiple variables in living donor liver transplant patients with or without BDS ($n = 100$)

Variables	BDS (+)	BDS (–)	*P*
Age (mean ± SD)	58.3 ± 6.8	52.5 ± 12.6	0.146
Sex (*n*)			0.025
Male	9	49	
Female	1	41	
BMI (mean ± SD)	25.1 ± 3.3	23.9 ± 3.8	0.381
MELD score (mean ± SD)	17.4 ± 10.4	14.5 ± 8.1	0.344
Graft lobe (*n*)			0.011
Right	9	42	
Left	1	48	
Blood type compatibility (*n*)			0.720
Match and compatible	9	76	
Incompatible	1	14	
Reconstruction manner (*n*)			0.599
Duct-to-duct anastomosis	10	82	
Hepaticojejunostomy	0	8	
Multiple anastomosis (*n*)	1	11	0.657
Bile duct stricture (*n*)	6	16	0.002
Hepatic artery complications (number)	1	6	0.533
TIT (median, min)	178 (104–345)	169 (108–555)	0.381
Primary IS (*n*)			0.687
Cyclosporin	3	9	
Tacrolimus/others	7	81	
Use of UDCA (*n*)	4	33	0.920
TC elevation >200 mg/dL (*n*)	6	54	0.734
TG elevation >150 mg/dL (*n*)	5	42	0.539

P-value for age and MELD score based on Student's *t*-test, and for TIT based on Mann–Whitney *U*-test; all others based on Fisher's exact test.

BDS, bile duct stone; IS, immunosuppressant; MELD, Model for End-Stage Liver Disease; SD, standard deviation; TC, total cholesterol; TG, total triglyceride; TIT, total ischemic time; UDCA, ursodeoxycholic acid.

Table 3 Risk factors for bile duct stone formation after living donor liver transplantation: Multivariate analysis ($n = 100$)

Variables	OR	CI	*P*
Male sex	6.00	0.65–55.79	0.115
Right lobe graft	10.20	1.12–93.21	0.040
Bile duct stricture	7.17	1.58–32.60	0.011

P-value for all variables based on multiple logistic regression analysis.
CI, confidence interval; OR, odds ratio.

duct strictures (odds ratio [OR], 7.17; $P = 0.011$) and right lobe graft (OR, 10.20; $P = 0.040$) were independent risk factors for BDS formation (Table 3).

Treatment of BDS and clinical outcome

Four (40%) patients with BDS who were asymptomatic and showed no abnormalities in liver function test were carefully followed. On the other hand, six (60%) patients required admission and interventional procedures such as ERC and/or PTC because of cholangitis. The median number of admissions and length of hospitalization (days) were 2.67 (range, 1–4) and 37.8 (range, 8–125) respectively, for these patients. The treatments administrated to these patients and their clinical outcomes are shown in Table 4. In five of six treated patients, primary stone extraction was successful, and stone clearance had been confirmed using balloon cholangiography and intraductal ultrasonography. However, two patients developed recurrence of BDS and one had a residual intrahepatic stone. The patient who had a residual intrahepatic stone received stenting across stricture and stone, which stabilized their condition (Fig. 1).

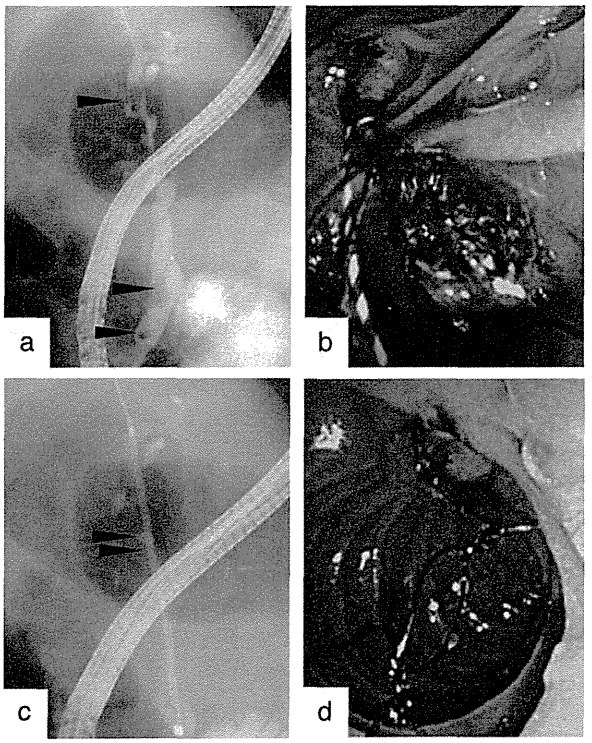

Figure 1 (a) A patient with multiple bile duct stones (BDS; arrowhead), including intrahepatic (IH) stones, with anastomotic biliary strictures after living donor liver transplantation. (b) A stone located in the common bile duct was extracted successfully by using a basket catheter, and it was a calcium bilirubinate stone. (c) To prevent cholangitis caused by the residual IH stone, an internal stent (7-Fr, 5-cm, plastic stent) was inserted over the anastomotic stricture (arrowhead). (d) A 2-0 nylon thread was attached to the distal side hole of the stent for easy removal.

Table 4 Summary of treatment and clinical outcome in bile duct stone cases

Case (age, sex)	Location and size of BDS	Treatment	Clinical outcome
63 years, F	CBD (10 mm)	These cases have been followed up with no symptoms	
59 years, M	IH (13 mm)		
57 years, M	CBD (10 mm)		
65 years, M	CBD (5 mm)		
56 years, M	CBD, IH (multiple)	ESWL + PTC	Death of sepsis
51 years, M	CBD (8.3 mm), IH (15.3 mm)	EST + stenting†	IH stone remained
63 years, M	CBD (20 mm)	EPBD + stenting†	No recurrence
54 years, M	CBD (5 mm)	EPBD + stenting†	No recurrence
44 years, M	CBD (10 mm)	EST + stenting†	Recurrence in CBD
66 years, M	IH (5 mm)	Stenting†	Recurrence in CBD

†"Stenting" means internal tube-stent insertion over biliary stricture.
BDS, bile duct stone; CBD, common bile duct; EPBD, endoscopic papillary balloon dilation; EST, endoscopic sphincterotomy; ESWL, extracorporeal shock wave lithotripsy; IH, intrahepatic duct.

To prevent ascending cholangitis, we inserted stents into the bile duct in all the patients that required drainage. The stents were placed across the stricture, and the distal edge was located above the sphincter of Oddi. In all patients, we used the stent delivering system (Flexima Biliary Stent System; Boston Scientific, Marlborough, MA, USA) and modified plastic tube stent (sizes 7.0 Fr, a 2-0 nylon thread attached to the distal side-hole for easy removal). No procedure-related complications occurred in these patients.

One patient succumbed to sepsis following severe cholangitis. This patient was a 56-year-old man who underwent LDLT with duct-to-duct anastomosis using right lobe graft. Four month after LDLT, he developed biliary duct strictures with cholangitis; therefore, PTC and balloon dilation were performed because endoscopic therapy was impossible due to bile duct deformity. However, the patients developed repeated cholangitis, and all our attempt to clear BDS and bile duct strictures using non-surgical techniques, including cholangioscopy or extracorporeal shock wave lithotripsy, failed. Although the necessity of retransplantation was recognized and the procedure was scheduled, it was not undertaken because the patients developed sepsis with acute respiratory distress syndrome.

DISCUSSION

IN OUR STUDY, BDS were developed in 10% of adult recipients who underwent LDLT. The reported incidence of post-transplant BDS varies widely among different study groups depending on the nature of the study population and manner of subject setting. In the study of Spier *et al.*, 49 of 1289 recipients (3.8%) developed BDS.[8] In the majority of the other studies, the incidence was reported to be approximately 5%,[6,7] whereas it was as high as 37% in another report.[12] In almost all previous studies, BDS was identified and diagnosed in patients who underwent examinations for clinically suspected cholangitis. However, some patients with BDS in our study had no symptoms and were incidentally diagnosed by protocol CT. Therefore, the incidence of BDS in the present study may be relatively higher than that in other reports, and our data may represent the actual state of BDS after LDLT.

According to multivariate analysis, bile duct strictures and right lobe graft were independent risk factors for BDS. The association between bile duct strictures and BDS has also been reported in previous studies.[8,10,11] We also speculate that bile duct strictures are likely to cause bile stasis and secondary infection, which results in the formation of bile duct sludge and stones. Nevertheless, eight patients developed common bile duct (CBD) stones, and of these, six had only CBD stones (Table 4). In addition, bile duct strictures were not observed in four of six patients with CBD stones. For this reason, it is suggested that some factors related to operation other than bile duct strictures, such as ischemic change or nerve disorder of the tissue surrounding CBD followed by biliary epithelial damage, influence BDS formation.

As shown above, right lobe graft was an independent risk factor of BDS. Some authors also indicated that the incidence of biliary complications was higher in patients who underwent LDLT with right lobe grafting than in those who underwent LDLT with left lobe grafting. In recent studies, the incidence of bile duct strictures in patients who underwent right lobe grafting was 8.3–32.8%,[13–15] while that in patients who underwent left lobe grafting was less than 15%.[16–18] We performed subgroup analysis to elucidate difference between patients who underwent right lobe grafting and those who underwent left lobe grafting and found no statistically significant difference in the incidence of bile duct strictures (Table 5). However, the number of men was significantly higher among the patients who underwent right lobe grafting. It is reasonable that the right lobe is selected to ensure appropriate size of grafts in male patients. With regard to the epidemiological survey of 1997 conducted by the Japanese Ministry of Health, Labor and Welfare, BDS was more common among males than among females. Although its cause is not clear, sex may have some relation to the development of BDS in patients who undergo right lobe grafting.

Several studies have reported that biliary ischemic change was a risk factor for the development of BDS after LDLT.[6,10] In patients with biliary cast syndrome in particular, identified as the hard, dark material taking the physical shape of the bile duct, biliary ischemia is believed to damage the bile duct mucosa and lead to cast formation.[10] However, ischemic factors such as TIT or hepatic artery complications were not detected as significant risk factors for BDS in the present study. We suggest that the characteristics of patients without cast formation contribute to this result.

Recently, endoscopic treatment is usually chosen as the primary approach for the management of biliary complications following LT.[19–21] Endoscopic procedure also makes it possible to shorten hospitalization of most post-transplant BDS patients with less invasiveness.[8,11,22] However, in some difficult situations, such as displacement of duodenal papilla or deformity of biliary tract,

Table 5 Comparison and univariate statistical analysis between right lobe graft and left lobe graft. ($n = 100$)

Variables	Right lobe graft	Left lobe graft	*P*
Bile duct stone (*n*)	9/52	1/48	0.011
Age (mean, years)	51.8	53.9	n.s.
Sex (number, male/female)	36/16	22/26	0.018
MELD score (mean, points)	15.1	14.7	n.s.
ABO incompatibility (*n*)	7/52	8/48	n.s.
TIT (median, min)	177 (104–555)	165 (109–250)	n.s.
Hepatic artery complication (*n*)	3/51	4/48	n.s.
Bile duct stricture (*n*)	13/51	9/48	n.s.
Cholangitis (*n*)	18/51	10/48	n.s.

P-value for age and MELD score based on Student's t-test, and for TIT based on Mann-Whitney *U*-test; all others based on Fisher's exact test.
MELD, Model for End-Stage Liver Disease; n.s., not significant; TIT, total ischemic time.

endoscopic intervention is somewhat complicated and challenging. In addition, duodenobiliary reflux and bacterial contamination of bile duct related to recurrence of BDS may occur after endoscopic intervention. Many authors reported that the incidence of biliary complications, such as cholangitis and recurrence of BDS, was higher in patients after EST than in those after endoscopic papillary balloon dilation (EPBD).[23–25] Moreover, Natsui *et al.* reported that EPBD has a possibility of suppressing bacterial contamination of the biliary tract compared with EST in patients with small stones.[26] Therefore, it is desirable to choose EPBD for treatment of BDS whenever possible, especially in patients treated with immunosuppressants after transplantation. Although we mainly treated patients who underwent right lobe grafting in the present study, it appears that there is no great difference between right lobe graft cases and left lobe graft cases regarding treatment of CBD stones. Nevertheless, in the case of BDS located in the proximal bile duct above the anastomotic site, endoscopic intervention may be more difficult in patients with right lobe grafting than in those with left lobe grafting because of multiple biliary reconstruction or acute angulation of the bile duct. As described in Table 4, we performed endoscopic therapy in six of 10 patients who developed BDS, with successful stone removal in five patients (83%). The success rate of stone extraction in previous studies ranged 71–100%.[8,11,22] We believe that endoscopic therapy for BDS can be successfully performed in most cases, even after transplantation. However, two patients (40%) developed recurrence of BDS in our study, and both also had biliary strictures. The recurrence rate of treated BDS developed after LT has rarely been reported. In the study of Rerknimitr *et al.*, eight of 46 patients (17%) developed recurrence of BDS after treatment.[2] In previous reports on not post-transplant populations, the BDS recurrence rate was 3.2–8.8%.[27–29] As mentioned above, biliary stricture is an independent risk factor, besides it can also be considered as a cause of high recurrence rate in the absence of drastic treatment, namely surgery, including retransplantation. One patient with biliary strictures and BDS in our study succumbed to biliary sepsis during the observation period. The optimal timing of retransplantation is difficult to determine because of the limited supply of organs available for LT. In Japan, the shortage of donors is a particularly serious problem because deceased organ donation is not well established owing to religious beliefs. Therefore, we have to rely on graft donation from family members in most patients. However, this is sometimes a restricting factor for retransplantation.

Several studies about post-transplantation BDS, including biliary cast syndrome, have been reported till date; however, none have centrally focused on BDS after LDLT. In the present study, we determined the risk factors for and clinical features and clinical outcomes of BDS following LDLT. We identified two independent risk factors, namely bile duct strictures and right lobe graft which were significantly related to BDS formation after LDLT. Furthermore, bile duct stricture may be a predictor of poor outcome in patients with BDS after LDLT. Therefore, we should pay special attention to LDLT patients who develop BDS accompanied by bile duct strictures and schedule timely retransplantation. We believe that it is important to shorten follow-up period of patients with bile duct stricture, especially in right lobe graft cases.

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Predictive value of the efficacy of tolvaptan in liver cirrhosis patients using free water clearance

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Abstract. Tolvaptan, an arginine vasopressin V2 antagonist, is available for patients with refractory ascites. Free water clearance was evaluated as a predictor of tolvaptan efficacy. Twenty-one patients with refractory ascites were enrolled in the present study. Liver function test, renal function test, urine volume, free water clearance and osmotic pressure were measured at baseline (day 0) and for each dose of tolvaptan (1.875, 3.75 and 7.5 mg), and compared for efficacy. Tolvaptan increased urine volume and free water clearance decreased osmotic pressure at each dose of tolvaptan, compared to pretreatment levels. Compared to baseline, an increased volume of free water clearance at 1.875 mg of tolvaptan showed a significant correlation with body weight reduction (r=0.480 and P=0.028). Any factors (age, liver function test and renal function test) at pretreatment showed no significant correlation with body weight reduction. An increased volume of urine and osmotic pressure at each dose was not significantly correlated with the tolvaptan effect. Compared to baseline, an increased volume of free water clearance at 1.875 mg of tolvaptan in responders was significantly increased, compared to non-responders (270±241 ml/day: 27±257 ml/day; P=0.042). In conclusion, an increased volume of free water clearance on day 1 was significantly associated with body weight reduction. Free water clearance could be a simple and useful marker for the prediction of tolvaptan efficacy.

Introduction

Ascites is one of the most frequent complications of liver cirrhosis. In total, 5-10% of patients with cirrhosis develop refractory ascites (1,2). Diuretic agents have been used for the treatment of refractory ascites. However, traditional diuretics often complicate electrolyte disorders, such as hyponatremia and hypokalemia. Therefore, solute-free water diuretics are preferable for the treatment of ascites. Recently, tolvaptan, an arginine vasopressin V2 receptor antagonist, was approved in Japan for the treatment of refractory ascites as an anticipated new treatment (3-5). There are controversial studies regarding the predictive factors for the efficacy of tolvaptan in patients with liver cirrhosis (5-7). The present study examined the predictive value of free water clearance for the efficacy of tolvaptan in patients with liver cirrhosis.

Materials and methods

Patients and study design. Twenty-one patients with refractory ascites were enrolled in the study. The characteristics of the patients are shown in Table I. Patients were treated with tolvaptan as follows: 1.875 mg on day 1, 3.75 mg on day 2 and 7.5 mg on day 3. After day 3, the tolvaptan dose was 3.75 or 7.5 mg. Urine volume and free water clearance were measured over 24 h, and urine osmotic pressure was measured at each dose of tolvaptan. The change in body weight from baseline to 2 weeks after initial tolvaptan administration was assessed. Patients who had a weight reduction of >3 kg in 2 weeks were defined as responders.

Measurement of free water clearance. The formula for free water clearance was as described previously (8):

$$\text{Free water clearance} = UV - \text{E-Cosm}$$

$$\text{E-Cosm} = (U_{Na} + U_K) \times UV/P_{Na}$$

where UV is the urine volume over 24 h, E-Cosm is the electrolyte clearance, U_{Na} is the urinary sodium concentration, U_K is the urinary potassium concentration and P_{Na} is the plasma sodium concentration.

Statistical analysis. SPSS version 20.0 software (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Comparisons between the two groups were performed using Student's t-test. Correlations were determined using Pearson's

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