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CLINICAL RESEARCH

Clostridium difficile-associated diarrhea after living donor liver transplantation

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Abstract

AIM: To assess the incidence and analyze the risk factors for Clostridium difficile-associated diarrhea (CDAD) after living donor liver transplantation (LDLT) in adult.

METHODS: The micobiological data and medical records of 242 adult recipients that underwent LDLT at the Tokyo University Hospital were analyzed retrospectively. The independent risk factors for postoperative CDAD were identified.

RESULTS: Postoperative CDAD occurred in 11 (5%) patients. Median onset of CDAD was postoperative d 19 (range, 5-54). In the multivariate analyses, male gender (odds ratio, 4.56) and serum creatinine ($\geqslant$ 1.5 mg/dL, odds ratio, 16.0) independently predicted postoperative CDAD.

CONCLUSION: CDAD should be considered in the differential diagnosis of patients with postoperative diarrhea after LDLT.

Key words: Living donor liver transplantation; Clostridium difficile; Diarrhea

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INTRODUCTION

Clostridium difficile (C. difficile) is a Gram-positive anaerobic spore-forming bacteria, first identified as the cause of antibiotic-associated diarrhea and colitis in 1978[1]. The pathogenesis of this infection involves several steps, including alteration of the normal colonic flora by antibiotic therapy, colonization of the gut by C. difficile, and finally, intestinal mucosal damage and inflammation due to enterotoxin production by this organism[2,3].

The incidence of infection with C. difficile is increasing in hospitals worldwide, and it is the leading identified cause of nosocomial diarrhea associated with antibiotic therapy, due to the widespread use of broad-spectrum antibiotics[4,5]. McFarland reported that C. difficile-associated diarrhea (CDAD) occurred in 31 of 126 (25%) patients that developed nosocomial diarrhea[6]. In a more recent prospective study, CDAD occurred in 47 of 271 (17%) patients admitted to the general medical wards[7]. Illness associated with C. difficile ranges from mild diarrhea to life-threatening colitis[3,8]. In one prospective study, 38 of 908 patients with CDAD died, among whom pseudomembranous colitis was the primary cause of death in 5[9]. Liver transplant recipients are high-risk candidates for CDAD, as multiple antibiotics are administered for perioperative prophylaxis or postoperative therapy[10]. Some studies have analyzed the prevalence of CDAD among deceased LDLT recipients[11,12]. Samore *et al* reported that liver transplantation was associated with the highest risk of C. difficile infection: transplantation recipients that acquired C. difficile were more likely to be symptomatic than were nonrecipients that acquired the infection (3 of 4 recipients *vs* 7 of 30 nonrecipients were symptomatic)[11]. Wong *et al*[12] reported that diarrhea occurred in 31 of 302 patients, and 10 of the 31 were infected with C. difficile.

To date, there have been no detailed studies on the prevalence and magnitude of CDAD among patients after LDLT. The aim of the present study was to assess the incidence and analyze the risk factors for CDAD after adult-to-adult LDLT.

MATERIALS AND METHODS

We retrospectively reviewed the data from 242 patients that underwent LDLT at the Tokyo University Hospital, a 1150-bed teaching hospital, between January 1996 and November 2004. The backgrounds of the 242 patients are shown in Table 1. The microbiological data, including the results of stool culture, immunochromatography assay (ICA) for toxin A (UNIQUICK, Kanto Chemical Co., Inc., Tokyo, Japan), and latex agglutination test for C. difficile protein (glutamate dehydrogenase, CD CHECK, Shionogi & Co., Ltd., Osaka, Japan), and medical records of the patients from hospital admission to 3 mo after LDLT were reviewed. Our surgical technique for recipient and donor surgery is described elsewhere[13].

Perioperative management protocol

Antibacterial prophylaxis consisted of intravenous cefotaxime (1 g immediately before surgery, followed by 1 g every 6 h intraoperatively and thereafter), ampicillin/sulbactam (1 g immediately before LDLT, followed by 1.5 g every 12 h intraoperatively and thereafter), and gentamicin, 60 mg every 12 h after surgery for 5 d.

To prevent fungal infection, fluconazole (200 mg every 24 h) was given intravenously for 7 d after LDLT. All the patients received the same immunosuppressive regimens using tacrolimus and methylprednisolone. The detailed regimen is reported elsewhere[14].

Indications for perioperative aphaeresis were as follows. Hemodialysis or continuous hemodiafiltration were indicated when the serum creatinine level exceeded 3.0 mg/dL or when there was a 10% increase in body weight compared to the preoperative baseline. Plasma exchange combined with hemodialysis was indicated for fulminant hepatitis, hyperbilirubinemia (serum bilirubin $\geqslant$ 20 mg/dL), and postoperative graft dysfunction with or without multi-organ failure.

Microbiologic surveillance and definition of C. difficile-associated diarrhea

Stool cultures were obtained at least once a week during the first month after LDLT, and additional samples were taken when diarrhea occurred. Stool samples were transferred to the laboratory, inoculated anaerobically onto cycloserine-cefoxitin-mannitol agar, and incubated at 37℃ for 48 h. ICA for toxin A (from October 2001 to November 2004) or latex agglutination test for C. difficile protein (glutamate dehydrogenase; from January 1996 to September 2001) were also added when CDAD was suspected. Cytotoxin assay for toxin B was not available during the study period.

Diarrhea was defined as the passage of three or more unformed stools on at least two consecutive days[15]. CDAD was defined as diarrhea that was not attributed to any other cause and when at least one of the following tests was positive: stool culture for C. difficile, ICA for toxin A, or latex agglutination test for C. difficile protein (glutamate dehydrogenase). Patients without diarrhea but positive for the aforementioned tests were regarded as asymptomatic carriers.

Table 1 Background of the 242 patients

Age (yr)	51 (18-67)[1]
Gender, male/female	132/110
Underlying Liver disease	
Hepatitis C	66
Primary biliary cirrhosis	52
Hepatitis B	35
Fulminant hepatitis	27
Biliary atresia	14
Autoimmune hepatitis	9
Primary sclerosing cholangitis	9
Metabolic disease	9
Cryptogenic cirrhosis	6
Alcoholic cirrhosis	4
Other	11
Hepatocellular carcinoma	68
Child-Pugh score	10 (5-10)[1]
MELD score	13 (-3-48)[1]

[1]Median and range. MELD: model for end stage liver diseases.

Table 2 Characteristic of patients with postoperative CDAD in 11 patients

Onset of CDAD (postoperative day)	19 (5-54)[1]
Symptoms other than diarrhea	
Fever	9
Abdominal pain	3
Duration	11 (5-43)[1]
Treatment	
Oral vancomycin	8
Conservative management	3
Recurrence	2
Use of antibiotics one month prior to LDLT	9

[1]Median and range. CDAD: clostridium difficile associated diarrhea.

Statistical analysis

Quantitative variables are expressed as median and range. Categorical variables are described by their absolute counts. Univariate analysis was used to identify associations between each of the variables recorded and postoperative CDAD. Fisher's exact test was used to compare categorical data. For multivariate analysis, only variables with a *P* value of less than 0.20 in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of *P* less than 0.1. The results of the logistic regression are reported as odds ratios with 95% confidence intervals. A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the JMP 5.1 software package (SAS institute Inc., Cary, NC).

RESULTS

Diarrhea occurred in 76 of 242 patients after LDLT, among whom 11 patients developed CDAD (Table 2). Median onset of CDAD was postoperative d 19 (range, 5-54). Nine patients received antibiotics during the month prior to the onset of diarrhea. As for symptoms other than

Table 3 Microbiological data of patients with postoperative CDAD and asymptomatic carriage of C. difficile

	Positive microbiological test			
	Culture	ICA for toxin A	Latex test[3]	Total
CDAD[1]	5	10	1	11
Asymptomatic carriage[2]	16	1	0	16
Total	21	11	1	27

[1]C. difficile was detected both by stool culture and ICA for toxin A in 4 patients, and both by stool culture and latex agglutination test for C. difficile protein (glutamate dehydrogenase) in one patient; [2]C. difficile was detected both by stool culture and ICA for toxin A in one patient; [3]Detection for C. difficile protein (glutamate dehydrogenase). ICA: immunochromatography assay; C. difficile: Clostridium difficile; CDAD: Clostridium difficile associated diarrhea.

Table 5 Association between postoperative CDAD and surgical and postoperative variables

Variables	CDAD (-) (n = 231)	CDAD (+) (n = 11)	P
Surgical variables			
Operation time (h)	15.3 (10.7-40.1)[1]	14.9 (12.5-19.8)[1]	
< 16	143	8	0.54
⩾ 16	88	3	
Blood loss (mL)	5150 (830-55 165)[1]	5155 (1555-17 475)[1]	
< 10 000	191	9	1
⩾ 10 000	40	2	
Blood transfusion (mL)	7000 (900-46 120)[1]	8640 (2860-14 800)[1]	
< 10 000	169	8	1
⩾ 10 000	62	3	
GV/SLV ratio (%)	46 (25-88)[1]	46 (36-67)[1]	0.75
< 50	152	8	
⩾ 50	79	3	
Duct to duct biliary reconstruction	160	9	0.51
Postoperative variables			
Postoperative ICU stay (d)	5 (3-242)[1]	6 (3-22)[1]	
< 10	205	10	1
⩾ 10	26	1	
Postoperative apheresis	28	3	0.15
Reoperation after LDLT	86	2	0.37
Acute rejection	66	2	0.73
Cytomegalovirus infection	105	3	0.35
Variation number of antibiotics administered after LDLT	5 (3-9)[1]	5 (3-8)[1]	1
< 6	159	72	
⩾ 6	72	3	

[1]Median and range. GV: graft volume; SLV: standard liver volume; ICU: intensive care unit; LDLT: living donor liver transplantation.

Table 4 Association between postoperative CDAD and preoperative variables

Variables	CDAD (-) (n = 231)	CDAD (+) (n = 11)	P
Age (yr)	51 (18-67)[1]	55 (26-64)[1]	
< 55	161	5	0.1
⩾ 55	70	6	
Gender, male/female	123/108	9/2	0.07
Underlying liver disease			
Hepatitis C	62	4	
PBC	49	3	
Hepatitis B	33	2	
Fulminant hepatitis	26	1	
Biliary atresia	14	0	
Autoimmune hepatitis	9	0	
PSC	9	0	
Metabolic disease	8	1	
Cryptogenic cirrhosis	6	0	
Alcoholic cirrhosis	4	0	
Other	11	0	
Hepatocellular carcinoma	63	5	0.3
Child-Pugh score	10 (5-14)[1]	9 (7-14)[1]	
< 10	107	6	0.76
⩾ 10	124	5	
MELD score	13.3 (-3.4-48.2)[1]	15.9 (5.3-33.9)[1]	
< 20	153	6	0.52
⩾ 20	78	5	
Ascites	110	6	0.76
Use of Diuretics	123	8	0.23
Encephalopathy	39	3	0.41
Preoperative apheresis	50	3	0.71
PT-INR	1.61 (0.89-7.48)[1]	1.56 (1.33-2.73)[1]	
< 2.0	187	8	0.45
⩾ 2.0	44	3	
Serum bilirubin (mg/dL)	4.4 (0.3-40.0)[1]	5.1 (0.8-17.9)[1]	
< 10.0	163	9	0.52
⩾ 10.0	68	2	
Serum albumin (mg/dL)	2.9 (1.5-4.4)[1]	2.6 (1.8-3.3)[1]	
< 2.5	37	194	0.03
⩾ 2.5	5	6	
Serum creatinine (mg/dL)	0.69 (0.2-7.7)[1]	1.02 (0.4-4.4)[1]	
< 1.5		6	0.0003
⩾ 1.5	219	5	
	12		
Use of steroid	28	1	1
Preoperative antibiotics use	58	3	1
Beta lactam	45	3	0.46
Second or third generation cepharosporins	17	0	1
Glycopeptide	3	2	0.02
Fluroquinolone	15	2	0.18
Aminoglycoside	5	1	0.25
Other	4	1	0.21
History of abdominal surgery	108	5	1
Diabetes mellitus	29	0	0.37
Preoperative ICU stay			0.35
Yes	25	2	
No	206	9	

[1]Median and range. PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; MELD: model for end stage liver diseases; PT-INR: the international normalized ratio of prothrombin time; ICU: intensive care unit.

diarrhea, fever was observed in 9 and abdominal pain in 3. Median duration of diarrhea was 11 d (range, 5-43). Oral vancomycin was used to treat 8 patients, and conservative management was used to treat the 3 other patients. Recurrence occurred in 3 patients.

Microbiological data of the patients with postoperative CDAD or asymptomatic carriers of C. difficile are shown in Table 3. C. difficile was detected in 27 patients during the 3 mo after LDLT. Among these 27 patients, C. difficile was detected both by stool culture and ICA for toxin A in 2 patients, stool culture and latex test for C. difficile protein (glutamate dehydrogenase) in 4, only stool culture in 15, only ICA for toxin A in 2, and only latex test for C. difficile protein (glutamate dehydrogenase) in 4.

Among preoperative covariates (Table 4), postoperative CDAD was significantly associated with serum albumin level (< 2.5 mg/dL) (P = 0.03) and serum creatinine level (⩾ 1.5 mg/dL, P = 0.0003). No variable was associated with

Table 6 Multivariate analysis of risk factors for CDAD after LDLT

Variable	Odds ratio (95% CI)	*P*
Age ⩾ 55	3.56 (0.92-15.0)	0.07
Male gender	4.56 (1.02-33.3)	0.05
Serum creatinine (mg/dL) ⩾ 1.5	15.99 (3.85-68.3)	0.0003

postoperative CDAD by univariate analysis (Table 5). In the multivariate analyses (Table 6), 7 risk factors with a *P* value less than 0.20 were entered into a logistic regression model by the backward-elimination procedure. In the final model, male gender (odds ratio, 4.56), and serum creatinine (⩾ 1.5 mg/dL; odds ratio, 16.0) independently predicted postoperative CDAD.

DISCUSSION

This is the first large series report on the incidence and risk factors of CDAD after LDLT. In the present retrospective study, CDAD occurred in 11 of 242 patients (5%). This finding is consistent with the previous report by Wong *et al*, in which the incidence of CDAD among liver transplant recipients was 3%[12]. Unlike in previous studies of surgical patients[16-19], all patients with CDAD were successfully treated with conventional modalities that prevented exacerbation of the disease to a fatal condition. CDAD occurred soon after LDLT. Of the 11 patients with CDAD, 7 (64%) developed CDAD within 1 mo after LDLT. A possible explanation for this finding is that many immunosuppressive drugs and prophylactic or therapeutic antibiotics are administered during the early period after LDLT, rendering patients more susceptible to C. difficile infection[20,21]. CDAD recurs after treatment in 8 % to 50% of cases[4]. In the present study, 2 of 8 (25%) patients who received oral vancomycin experienced CDAD recurrence. A similar result was reported in a recent prospective study, in which 7 of 34 (21%) patients relapsed during the 3 mo after successful treatment of CDAD with oral metronidazole or vancomycin[21]. In the current series, recurrent disease was treated without difficulty with repeated application of the previous treatment.

Intensity of antibiotic use, measured by the presence of preoperative antibiotic use or number of antibiotics used postoperatively, did not correlate with the incidence of CDAD in the present study. In general, recent antibiotic use is the most important risk factor for developing CDAD because the normal stable gut microbial flora must be disrupted before C. difficile can become established and produce toxins[22]. This discrepancy is probably due to the following reasons. First, only 11 patients were complicated with CDAD in the present study, which limited the power to examine risk factors specific for diarrhea. Second, all the patients received multiple antibiotics as perioperative prophylaxis in the present study, and it is difficult to detect the effect of antibiotics on the occurrence of CDAD after LDLT. Similar results were reported by Thibault *et al*[23], in which no specific antibiotic could be linked to CDAD in surgical patients because of uniform perioperative antibiotic use. Males were more susceptible than females to CDAD in the present study, although the reason for this finding is not known. In contrast, some previous studies reported more females than males among patients with CDAD[24-27]. Renal insufficiency increased the risk for CDAD after LDLT in the present study. The reason for the association between renal insufficiency and CDAD is unclear, but might be related to impaired host-defense mechanisms in patients with renal insufficiency[28].

One of the limitations of the present study is that a cytotoxin assay for toxin B was not examined. Tissue culture cytotoxin assay detecting the presence of toxin B in stool culture is considered the "gold standard" for the diagnosis of CDAD because of its accuracy[22]. In addition, we could not find toxin A-negative/toxin B-positive strains[29-31] and could not differentiate strains that produced binary toxins[32] from toxin A-positive/toxin B-negative strains in the present study. A prospective study is needed to examine the true incidence of CDAD, including strains such as toxin A-negative/toxin B-positive strains, among LDLT recipients.

The future direction of the current series is to study the clinical impact of asymptomatic carriage of C. difficile on postoperative CDAD after LDLT. Asymptomatic carriage of C. difficile appears to reduce the risk of subsequent development of CDAD[7,33]. In the present study, 16 of 27 (59%) patients with C. difficile were asymptomatic, but it is not known whether they were asymptomatic carriers on admission or colonized after admission.

In conclusion, CDAD should be considered in the differential diagnosis of patients with postoperative diarrhea. Male gender and renal insufficiency independently increased the risk for CDAD after LDLT.

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