ORIGINAL ARTICLE

Risk Factors and Outcomes in Post–Liver Transplantation Bile Duct Stones and Casts: A Case-Control Study

Bret J. Spier, Patrick R. Pfau, Katelin R. Lorenze, Stuart J. Knechtle, and Adnan Said
Section of Gastroenterology and Hepatology, Department of Medicine, University of Wisconsin Hospital and Clinics, Madison, WI

Bile duct stones and casts (BDS) after liver transplantation are associated with significant morbidity. Risk factors for BDS formation and the efficacy of treatment in liver transplant recipients have not been systematically studied. The aim of this study was to evaluate potential risk factors for the formation of BDS in patients post–liver transplant. A case-control study of consecutive liver transplant recipients at a university hospital from 1989 to 2007 was performed to identify risk factors for BDS formation. Cases included all liver transplant recipients with BDS, excluding those with concurrent t-tubes or biliary stents. Controls were chosen randomly from the total liver transplant population matched for year of transplantation. Pre- and post-OLT risk factors were analyzed with univariate and multivariate analyses. There were 49 cases and 101 controls over an 18-year-period (1289 liver transplants performed) with an incidence of 3.8% for BDS. In the cases, the median time to BDS diagnosis was 613 days from time of transplant. The controls had a median follow-up of 1530 days. Use of ursodeoxycholic acid was protective ($P = 0.005$), whereas bile duct pathology ($P = 0.003$), total cholesterol $\geq$ 200 mg/dL ($P = 0.008$), and triglyceride $\geq$ 150 mg/dL ($P = 0.008$) were significant risk factors for BDS formation. Endoscopic retrograde cholangiopancreatography (ERCP) was technically successful in all cases with resolution or improvement of liver chemistries in 59% (29) of patients. In conclusion, significant risk factors for forming BDS included bile duct pathology and elevated total cholesterol and triglyceride levels. Ursodeoxycholic acid had a significant effect in preventing the development of posttransplant BDS and should be used in those that are at increased risk. ERCP is a safe and effective diagnostic and therapeutic modality for these patients. ***Liver Transpl 14:1461-1465, 2008.*** © **2008 AASLD.**

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Bile duct stones and casts (BDS) are common after liver transplantation with a reported incidence of 5%-10% of all adult recipients.[1,2] Severe complications such as cholangitis and secondary biliary cirrhosis may occur as a result of BDS in transplant recipients.[3-5]

After liver transplantation, multiple risk factors have been suggested for BDS formation. Biliary strictures resulting from surgical or mechanical factors at transplant or following ischemic injury from hepatic artery thrombosis have been associated with stone and cast formation.[1,6] Medications commonly used in the transplant population may play a role as cyclosporine inhibits bile acid synthesis, thereby promoting stone formation.[7,8] Ursodeoxycholic acid has been shown to be effective in preventing gallstone formation and in the dissolution of gallstones in the nontransplant setting.[9] Its role in preventing or dissolving biliary stones in the transplant recipient has not been demonstrated.

The extent to which posttransplant BDS formation is influenced by hepatic/metabolic factors inherent to the liver is unknown. The relative roles of recipient risk factors and the risk that a donor liver might carry in influencing the formation of post-transplant biliary

Abbreviations: BDS, bile duct stones and casts; CI, confidence interval; ERCP, endoscopic retrograde cholangiopancreatography; OR, odds ratio.

Address reprint requests to Adnan Said, M.D., M.S.P.H., Section of Gastroenterology and Hepatology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, H6/516 CSC, 600 Highland Avenue, Madison, WI 53792. Telephone: (608) 263-4034; FAX: (608) 265-5677. E-mail: axs@medicine.wisc.edu

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stones have not been studied. Thus, the primary aim of our study was to evaluate potential risk factors for the formation of BDS in liver transplant recipients. We also examined the efficacy and safety of endoscopic retrograde cholangiopancreatography (ERCP) in the management of BDS post-transplant.

PATIENTS AND METHODS

We performed a case-control study collecting data from 1289 consecutive primary liver transplant recipients at the University of Wisconsin Hospital and Clinics from 1989 to 2007. Data from cases and controls were obtained from a prospectively constructed database, a review of medical records, radiography, and pathological specimens. There were 49 cases of BDS and 101 randomly selected controls identified following institutional review board approval.

Cases were adult (>17 years) liver transplant recipients who developed post–liver transplantation BDS confirmed by ERCP. Control subjects were adult (>17 years) liver transplant recipients who did not develop BDS after liver transplantation. Controls were chosen randomly from the total adult liver transplant population matched to cases by year of transplantation. The case-to-control ratio was 1:2. Recipients who developed stones and casts in association with foreign bodies such as t-tubes or stents in the bile duct were excluded.

Gallstone status was determined in both the recipient and the donor. In the recipient, a history of symptomatic gallstones with cholecystectomy, asymptomatic gallstones present on pretransplant abdominal imaging, or a pathological examination of the explants including the gallbladder was considered a positive result. In the donor, gallstone status was determined by a pathological examination of the transplanted organ including the donor gallbladder when present. When the donor gallbladder was absent, this was also considered a positive history of donor gallstones.

Data pertaining to the ERCP was collected, including indication and outcome data. Indications for ERCP were the presence of jaundice, abdominal pain, and elevated liver function tests along with abnormal radiography (computed tomography, magnetic resonance imaging, or ultrasonography). Outcomes were described by technical success, which was determined if successful biliary cannulation was achieved with BDS removal and sphincterotomy, or clinical success, which was determined by the resolution of patient symptoms and improvement of liver function tests by at least 25% after ERCP.

Other potential risk factors considered in the final analysis that were collected after liver transplantation included patient characteristics (age, gender, race, and body mass index), liver transplant indication, medication use (cyclosporine and/or ursodiol), evidence of bile duct pathology prior to BDS diagnosis (defined as the presence of either anastomotic or other strictures), diabetes, cold ischemia time [evaluated as both a continuous and categorical variable (<12 hours versus ≥12 hours)], warm ischemia time, ABO compatibility, ischemic reperfusion injury (defined as a total bilirubin peak within the first 10 days post-transplant), fasting total serum cholesterol ≥ 200 mg/dL and fasting triglyceride ≥ 150 mg/dL, evidence of hepatic artery stenosis or thrombosis, time (in days) to stone diagnosis, and follow-up days (in the control group).

Statistical Analysis

Categorical variables were compared with the chi-square test or Fisher exact test, and continuous variables were compared with the Wilcoxon sign rank test or unpaired *t* test. Variables that were significant in the univariate analysis ($P < 0.05$) were further analyzed in a multivariate logistic regression model.

RESULTS

Patient Characteristics

From 1989 to 2007, 1289 primary liver transplants were performed. In the cases and controls, all liver transplants were ABO-compatible, procured and preserved (University of Wisconsin Solution) in the same fashion, and performed with the conventional piggyback technique. Forty-nine recipients (3.8%) developed BDS at a median interval of 613 days from transplantation. The control group consisted of 101 recipients, and their average follow-up was 1530 days. The mean age was similar in both groups (Table 1). The most common indication for transplantation in both cases and controls was alcoholic cirrhosis (26.5% and 27.7%, respectively). Hepatitis C was the second most common single indication in both groups (Table 2). Baseline characteristics of the 2 groups are shown in Table 1. Of the 49 recipients with BDS, 22 presented with jaundice (44.9%), and 11 (22.4%) patients presented with right upper quadrant abdominal pain (shown later in Table 4). Radiographic abnormalities in noninvasive studies were seen in 18 (36.7%) of 49 cases. Most notably, abdominal ultrasound did not indicate biliary abnormalities in 47% of the cases.

Table 1 also depicts the frequency of potential risk factors among the case and control patients. Of the 49 recipients with posttransplant BDS (cases), 28 (57.1%) had pretransplant biliary stones, whereas in the control group, 48 of 101 recipients (47.5%) had pretransplant biliary stones ($P = 0.27$). In the cases, only 5 (10.2%) of the 49 corresponding donors had previous evidence of stones. In the control population, 10 (9.9%) of the 101 corresponding donors had stones present in the donor gallbladder ($P = 0.93$).

Analysis of Risk Factors

A univariate analysis of risk factors for BDS post-transplant is shown in Table 1. The presence of bile duct

TABLE 1. Baseline Patient Characteristics and Potential Risk Factors for Bile Duct Stone and Cast Formation Post–Liver Transplant and Results of Univariate Statistical Analysis

Characteristic	Cases (49)	Controls (101)	OR (95% CI)	*P* Value
Mean age (years)	51.9	49.6	—	0.25
Gender ratio (male:female)	35:14	67:34	—	0.58
Race (% white)	93.8	92.1	—	1.00
Body mass index (mean)	29.32	28.14	—	0.34
Cold ischemia time (mean hours)	14.72	11.16	—	0.28
Ischemic reperfusion injury [mean total bilirubin (mg/dL)]	6.8	7.9	—	0.26
Diabetes mellitus (% yes)	22 (44.8)	44 (43.5)	—	0.88
Recipient stone status pre–liver transplant (% yes)	28 (57.1)	48 (47.5)	—	0.27
Donor stone status (% yes)	5 (10.2)	10 (9.9)	—	0.93
Bile duct pathology (% yes)	14 (28.6)	10 (9.9)	3.64 (1.48–8.96)	0.003
Hepatic artery stenosis/thrombosis (% yes)	6 (12.2)	10 (9.9)	—	0.91
Cyclosporine use (% yes)	22 (44.8)	40 (39.6)	—	0.53
Ursodiol use (% yes)	28 (57.1)	78 (77.2)	0.39 (0.19–0.82)	0.01
Serum cholesterol (mg/dL)	201.5	161.5	—	<0.0001
Serum triglycerides (mg/dL)	212.7	138.8	—	0.001

Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 2. Indications for Liver Transplantation

Indication for Liver Transplant	Cases	Controls
Alcohol	13	28
Hepatitis C	7	18
Alcohol + hepatitis C	8	18
Primary sclerosing cholangitis	3	12
Other	18	25

TABLE 3. Risk Factors for Bile Duct Stones and Casts Post–Liver Transplant: Multivariate Analysis

Variable	OR	95% CI	*P* Value
Bile duct pathology	4.42	1.65–11.82	0.003
Total serum cholesterol ≥ 200 mg/dL	2.30	1.02–5.22	0.008
Triglyceride level ≥ 150 mg/dL	2.38	1.08–5.23	0.008
Ursodiol	0.31	0.14–0.70	0.005

Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 4. ERCP: Indications and Outcomes

Pre-ERCP Indication	Patients (%)
Abdominal pain	22.4
Jaundice	44.9
Liver test abnormality	98
Abnormal imaging	36.7

Post-ERCP Outcome	Patients (%)
Technical success	100
Clinical success	59

Abbreviation: ERCP, endoscopic retrograde cholangiopancreatography.

pathology was a significant predictor of posttransplant BDS ($P = 0.003$). Elevated levels of total serum cholesterol ($P < 0.0001$) and triglyceride levels ($P = 0.001$) post-transplantation were also significantly associated with BDS. Furthermore, the use of ursodeoxycholic acid (started from the time of transplantation) was shown to have a protective effect on the occurrence of posttransplant BDS ($P = 0.01$). In the multivariate analysis, bile duct pathology, total serum cholesterol ≥ 200 mg/dL, and triglyceride levels ≥ 150 mg/dL remained significant predictors of BDS ($P = 0.003$, $P = 0.008$, and $P = 0.008$, respectively). Ursodeoxycholic acid use also remained a significant protective factor ($P = 0.005$; Table 3). Cold ischemia time was not a significant predictor of BDS either as a dichotomous variable (≥ 12 hours, $P = 0.21$) or as a continuous variable ($P = 0.28$). The remaining factors examined, including warm ischemia time, were not statistically significant factors for the prediction of posttransplant BDS.

Procedure Data and Clinical Outcomes

ERCP was the eventual diagnostic modality in all cases. Derived data describing procedure indications and technical and clinical success from ERCP for the cases are shown in Table 4. Technical success with bile duct cannulation and BDS removal was 100%. The clinical success rate of ERCP for improvement or normalization of liver function tests was 59%.

DISCUSSION

After liver transplantation, BDS are common, occurring in 5%-10% of recipients. Despite this, there have been no systematic, controlled studies examining the contributing risk factors for BDS formation in this select group of patients. Common risk factors in the general population such as gender, age, and obesity did not prove to be associated with stone and cast formation in our population of transplant recipients.

There was a statistically significant association between total serum cholesterol and triglyceride levels for the development of BDS when cases and control subjects that were matched for year of transplantation were compared. Biliary stone formation can result from an imbalance between cholesterol, phospholipid, and bile acid secretion in bile. Cholesterol stones can precipitate as a result of cholesterol excess or a decrease in phospholipid or bile acid secretion in bile, resulting in supersaturation of bile with insoluble cholesterol.[10,11] Hyperlipidemia is considered to be a risk factor for gallstone formation.[12] Several studies have addressed this issue by measuring cholesterol precursors as predictors of increased biosynthesis of cholesterol. Bjorkhem et al. and Kempen et al. suggested using serum lathosterol levels as a predictor of increased biosynthesis of cholesterol, although this has not been used clinically.[10,11] We have shown that elevated cholesterol levels $\geq$ 200 mg/dL and triglyceride levels $\geq$ 150 mg/dL lead to a marked increase in stone formation post-transplant, thus allowing transplant physicians to identify patients at risk of developing biliary stones; more importantly, this suggests that hyperlipidemia should be aggressively treated in liver transplant recipients.

Although gallbladder supersaturation is necessary for gallstone formation, gallbladder bile from patients with gallstones nucleates more rapidly than equally saturated bile from control patients.[13] This suggests that other factors, in addition to saturated bile, must be present for cholesterol nucleation to occur. Proposed factors at the metabolic level include defects in pronucleation proteins such as α1-acid glycoprotein and genetic factors such as apolipoprotein E mutations.[14,15] Prolongation of the large bowel transit time has been shown to alter deoxycholic acid metabolism and contribute to stone formation.[16] Furthermore, bile salt defects can cause a higher propensity to form cholesterol crystals.[15] Recent literature has pointed to genetic point mutations and low biliary phospholipid concentrations as a further risk factor for cholelithiasis in adults.[17] Inherent metabolic propensities such as these and others suggest that the risk for posttransplant bile duct stones could be predicted by a pretransplant history of gallstones. However, we did not find the presence of cholelithiasis in the recipient prior to transplant to be a risk factor for post–liver transplant choledocholithiasis, and this suggests that factors leading to post–liver transplant biliary stone formation are somewhat different than those for the general population.

Liver transplantation involves potential ischemic damage to the liver during cold and warm preservation as well as ischemic reperfusion injury that can lead to imbalances between biliary concentrations of cholesterol, phospholipids, and bile acids. Our study did not show these factors to significantly increase the risk for BDS formation. Furthermore, although there is an increased risk of ischemic strictures after donation after cardiac death liver transplants, the strictures are primarily intrahepatic and diffuse in nature, can result in worse patient and graft survival, and do not appear to increase large bile duct lithogenesis.[18] Animal studies have demonstrated altered bile salt kinetics leading to increased lithogenic indices post–liver transplantation, so further evaluation needs to be done to identify the effects of these important lithogenic factors in humans.[15] Liver transplantation also introduces the potential for biliary pathology, primarily with respect to stricture formation at the anastomosis. Biliary duct stones frequently form proximal to strictures as the bile becomes deconjugated and insoluble secondary to stasis or a bacterial infection.[19-22] Our study has further validated prior work suggesting that bile duct pathology significantly increases the risk for BDS.

We have demonstrated that ursodiol is associated with a greatly reduced risk of bile duct stone development post-transplantation. The mechanism of ursodeoxycholic acid in the prevention of gallstones may involve the stimulation of bile flow[23,24] as well as facilitation of the transport of toxic bile acid into bile conjugation.[2] In hepatic disease, ursodiol is thought to protect cholangiocytes against the cytotoxicity of bile acids as well as bile acid–induced apoptosis.[25] Ursodiol is also thought to decrease hepatocyte immune-mediated destruction by decreasing human leukocyte antigen expression on hepatocytes or potentially decreasing eosinophilic destruction of the hepatocytes.[26,27] Although ursodiol has been shown to have a protective effect in preventing recurrent gallstones in pretransplant patients,[28] its routine use post-transplant for the prevention of biliary stones needs to be prospectively investigated. It may play an important role in preventing the development of BDS post-transplant in high-risk recipients, and our data show its use results in a significant decrease in posttransplant BDS formation.

Lastly, we have demonstrated ERCP to be an effective technique for retrieving BDS from the extrahepatic biliary tree. The success rate in the population was high, and complications were rare and no more frequent than those reported in the nontransplant population.[29,30] Our study reaffirms the diagnostic accuracy and therapeutic value of ERCP in treating choledocholithiasis post–liver transplant.[3,31,32] The majority of patients noted improvements in their symptoms along with resolution of liver function tests.

Our study was limited by a retrospective design, which has the inherent drawback of not accurately identifying all risk factors. However, our database is prospectively maintained, and we believe that we have minimized this complication. To account for selection bias, we matched cases and controls that were transplanted in the same year to minimize different

medication protocols and immunosuppressive regimens.

In conclusion, our study demonstrates that total cholesterol ≥ 200 mg/dL and triglyceride ≥ 150 mg/dL as well as bile duct pathology confer a significant risk of clinically significant BDS post-transplantation. Ursodeoxycholic acid, when taken at the standard dose of 300 mg twice daily, may be an effective therapy in this subgroup of patients and is recommended for up to 1 year post-transplant and possibly further if patients have persisting risk factors such as hyperlipidemia or biliary strictures. Lastly, these patients can be safely and effectively managed with ERCP.

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