

Biliary casts after liver transplantation: Morphology and biochemical analysis

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Abstract

AIM: To investigate the pathogenesis of biliary casts after liver transplantation relative to their morphology and biochemical markers.

METHODS: The microstructure of biliary casts was assessed using scanning electron microscopy and Hematoxylin and eosin staining assessed their histology. The expression levels of CD3, CD5, CD34, CD68 and CD79a in these biliary casts were evaluated immunohistochemically.

RESULTS: Biliary casts differed widely in their microstructure, with some containing blood vessels positive for CD34 and collagen fibers with positive Masson staining. Large numbers of neutrophils and other inflammatory cells were present, but only on the edge of the biliary casts; although the boundaries were clear without crossover. None of the biliary casts contained T-lymphocytes, B-lymphocytes, macrophages and other inflammatory cells.

CONCLUSION: The microcostructure of biliary casts

differed. Bacteria and acute rejection are not clearly related to their formation.

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Key words: Biliary cast; Biliary cast syndrome; Liver transplantation; Blood vessels; Acute rejection

Core tip: This experimental study employed scanning electron microscopy, Hematoxylin and eosin staining and immunohistochemistry to investigate biliary casts following liver transplantation. The results indicated that blood vessels and collagen fibers are present in biliary casts; however, bacteria and acute rejection are not clearly related to their formation, as evidenced by blood vessels positive for CD34 and collagen fibers with positive Masson staining, and no T-lymphocytes, B-lymphocytes, macrophages and other inflammatory cells. Thus, although bile duct injury after liver transplantation is significantly associated with biliary cast formation, their role in acute rejection is unclear.

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INTRODUCTION

Despite advances in the management of patients who have undergone cadaveric liver transplantation, 6%-34% patients experience biliary complications^[1]. Biliary cast syndrome (BCS), first described in 1975^[2], occurs less frequently than biliary sludge and stones, with an incidence of 2.5% after orthotopic liver transplantation^[3]. Multiple intrahepatic biliary strictures, ductal dilatation,

intrahepatic abscesses, and biliary anastomotic leakage characterize BCS. The clinical symptoms of BCS usually include high fever, jaundice and cholestatic liver enzyme elevation, similar to the symptoms observed in some patients with intrahepatic bile duct stones. Surgical management is the treatment of choice, and endoscopic techniques have been successful and safe in the removal of biliary casts^[4-6]. Morphologically, biliary casts are a similar shape to bile ducts, appearing as a hardened, dark material in the biliary ductal system. Biliary casts can prevent bile drainage, resulting in biliary obstruction and inducing biliary tract infection. Biliary casts can ultimately cause substantial injury to the liver, with some transplant recipients requiring retransplantation. Although the associations between biliary casts and clinical treatment have been assessed recently, less is known about the associations between biliary casts and biochemical markers. We therefore investigated the pathogenesis of biliary casts after liver transplantation relative to their morphology and biochemical markers.

MATERIALS AND METHODS

Isolation of biliary casts

We evaluated 15 patients with a history of orthotopic liver transplantation, who were treated in our department for jaundice, recurrent cholangitis and high fever. There were 10 males and 5 females, with a mean age of 52.1 years (range, 34-78 years). Of these patients, five underwent deceased donor liver transplantation for hepatitis B-induced cirrhosis and primary liver cancer, one for primary hepatocellular carcinoma and nine for cirrhosis during the decompensated period. Choledochoscopy and duodenoscopy have been used frequently to assess patients with biliary complications after liver transplantation^[7,8]. Patients with T-tube fistulae can be evaluated by insertion of a cholangioscope directly into the common hepatic duct, whereas patients without T-tube fistulae are evaluated preferably by percutaneous transhepatic cholangioscopy or endoscopic retrograde cholangiopancreatography^[9]. The distal aspect of the cast was secured using a basket, allowing each cast to be successfully removed as a single piece. All the casts were stored in liquid nitrogen.

Scanning electron microscopy

Following their isolation, biliary casts that were kept at room temperature were rinsed in sterile normal saline solution, fixed with 10% neutral formalin for 12 h at 4 °C, rinsed in 0.1 mol/L phosphate buffer (pH 7.0) and dehydrated through a graded series of ethanol (10 min each at 10%, 30%, 50%, 70% and 90%, and 15 min each three times at 100%). After critical point drying at 30 °C with CO₂ for 6 h, the samples were mounted, coated with 1-μm gold particles and evaluated using a Hitachi S 4800 field emission scanning electron microscope at 2 kV.

Histological and immunohistochemical examination

Biliary casts stored in liquid nitrogen were rinsed in sterile

normal saline solution, fixed with 10% neutral formalin for 12 h at 4 °C, embedded in paraffin, cross-sectioned into 10 mm slices and placed onto glass slides. Some of these histological sections were stained with hematoxylin and eosin (HE) and Masson trichrome, according to standard procedures. The remaining histological sections were deparaffinized, rehydrated, incubated in 3% hydrogen peroxide/absolute methanol for 5 min to block endogenous peroxidase activity and rinsed in distilled water. Nonspecific binding of antibodies was blocked by incubation with 5% normal goat serum for 10 min at room temperature. After washing, the sections were incubated with primary rabbit antibodies against human CD3, CD5, CD34, CD68 and CD79a, overnight at 4 °C. The sections were subsequently incubated with biotinylated secondary antibody for 30 min at 37 °C, with streptavidin biotin complex reagent for 30 min at 37 °C, and with DAB Plus reagent for 10 min, with the sections repeatedly washed with PBS, pH 7.4, between incubations. The sections were counterstained with hematoxylin, mounted and examined by optical microscopy. All antibodies and reagents for immunohistochemistry were purchased from the Beijing Zhongshan Golden Bridge Biotechnology Company, Beijing, China.

RESULTS

Biliary casts have a variety of morphological structures

Morphologically, biliary casts have a cordlike, columnar, dendritic shape within the biliary ductal system (Figure 1A). Scanning electron microscopy, however, showed that biliary casts were present in a variety of forms: irregular sheets composed of imbricated accumulations (Figure 1B); honeycombs with porous structures and adherent crystalline substances (Figure 1C); and filamentous structures (Figure 1D).

Biliary casts contain blood vessels and collagen fibers

HE staining revealed large numbers of lacunae containing bilirubin, tubiform (Figure 2A) and filamentous structures (Figure 2B). To determine the composition of the tubiform and filamentous structures, we incubated these sections with antibodies to cell markers and with Masson stain. We found that the tubiform structures were positive for CD34 (Figure 2C), whereas the filamentous structures were positive for Masson stain (Figure 2D). These findings indicated that the tubiform structures were blood vessels and the filamentous structures were collagen fibers.

Formation of biliary casts is not related to inflammatory response

HE staining showed large numbers of neutrophils and other inflammatory cells on the edge of the biliary casts; however, the boundaries were clear without crossover, and no inflammatory cells were present within the biliary casts (Figure 2E). Scanning electron microscopy showed no evidence of bacteria or bacterial debris on the surface of the biliary casts.

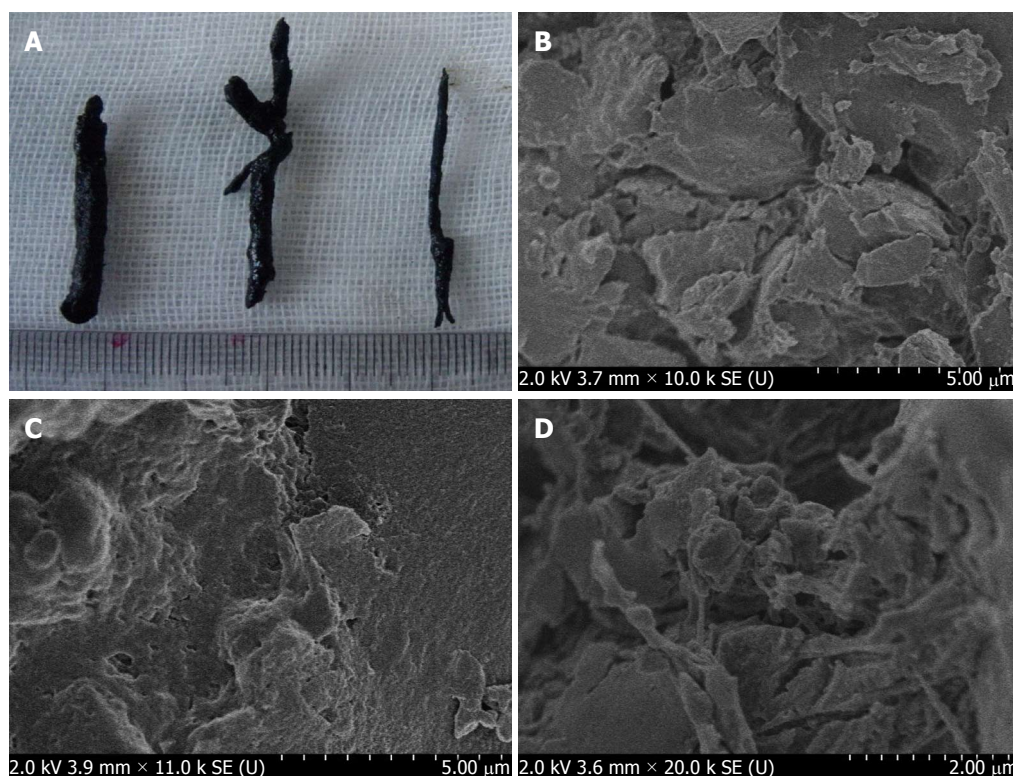


Figure 1 Morphology of biliary casts. A: Cordlike, columnar and dendritic shapes of biliary casts within the biliary ductal system; B: A biliary cast in the shape of an irregular sheet composed of imbricated accumulation ($\times 10000$); C: A biliary cast in the shape of a honeycombs with porous structures and adherent crystalline substances ($\times 10000$); D: A biliary cast in the shape of filamentous structures ($\times 10000$).

Immune rejection is not significantly related to biliary cast formation

Acute rejection generally occurs 1 to 3 wk after liver transplantation. To determine the relationship between immune rejection reactions and biliary cast formation, we incubated the biliary cast samples with antibodies to CD3, CD5, CD68, and CD79a. None of the biliary casts was positive for any of these markers, indicating that these biliary casts did not contain T-lymphocytes, B-lymphocytes and macrophages.

DISCUSSION

Physically and morphologically, biliary casts appear as dark, hardened material in the shape of bile ducts within the biliary ductal system, but differ from bile duct stones. Scanning electron microscopy showed that biliary casts appear in a variety of forms, including irregular sheets composed of imbricated accumulations; honeycombs with a porous structure and adherent crystalline substances; and filamentous structures. Although bile duct stones and biliary casts have a similar microstructure^[10], their mechanism of formation differs significantly. Biliary casts that form after liver transplantation are not caused by a single pathogenic factor, but may be associated with late functional rehabilitation, biliary strictures and obstruction, acute rejection, recurrent cholangitis, cold and warm ischemia times, hepatic ischemia and reperfusion injury^[3,11-13].

Bilirubin has been reported to be the primary component of biliary casts (approximately 10%-50%), followed by bile acid synthesis products and cholesterol, with protein comprising only 5%-10%^[14]. In comparison, we observed large amounts of bilirubin, as well as blood vessels and collagen fibers, consistent with our earlier findings. Choledochoscopy showed a large number of flocs in bile duct cellulose 5 mo after transplantation, with histopathological examination showing that these flocs were composed of cellulose, bile duct epithelial cells and necrotic inflammatory cells. These elements then become structureless, with biliary casts observed in the bile ducts 9 mo after transplantation^[15]. The presence of blood vessels and collagen fibers in the biliary casts was related to injury to the bile duct mucosa. The extent of bile duct injury during orthotopic liver transplantation differs, with cold preservation/reperfusion injury being the most important initiator of bile duct tree injuries and vessel plexus damage. Bile duct injury may, therefore, be associated with microcirculatory disturbances surrounding the bile ducts^[16]; however, the specific mechanisms underlying bile duct injury require further investigation.

Acute rejection after liver transplantation generally occurs 1 to 3 wk postoperatively. Typical clinical symptoms include unexplained fever, loss of appetite, poor spirit, liver pain, progressively deepening jaundice, and elevated bilirubin and transaminase. The diagnosis mainly depends on liver puncture biopsy and pathology. Biliary casts and acute rejection after transplantation have

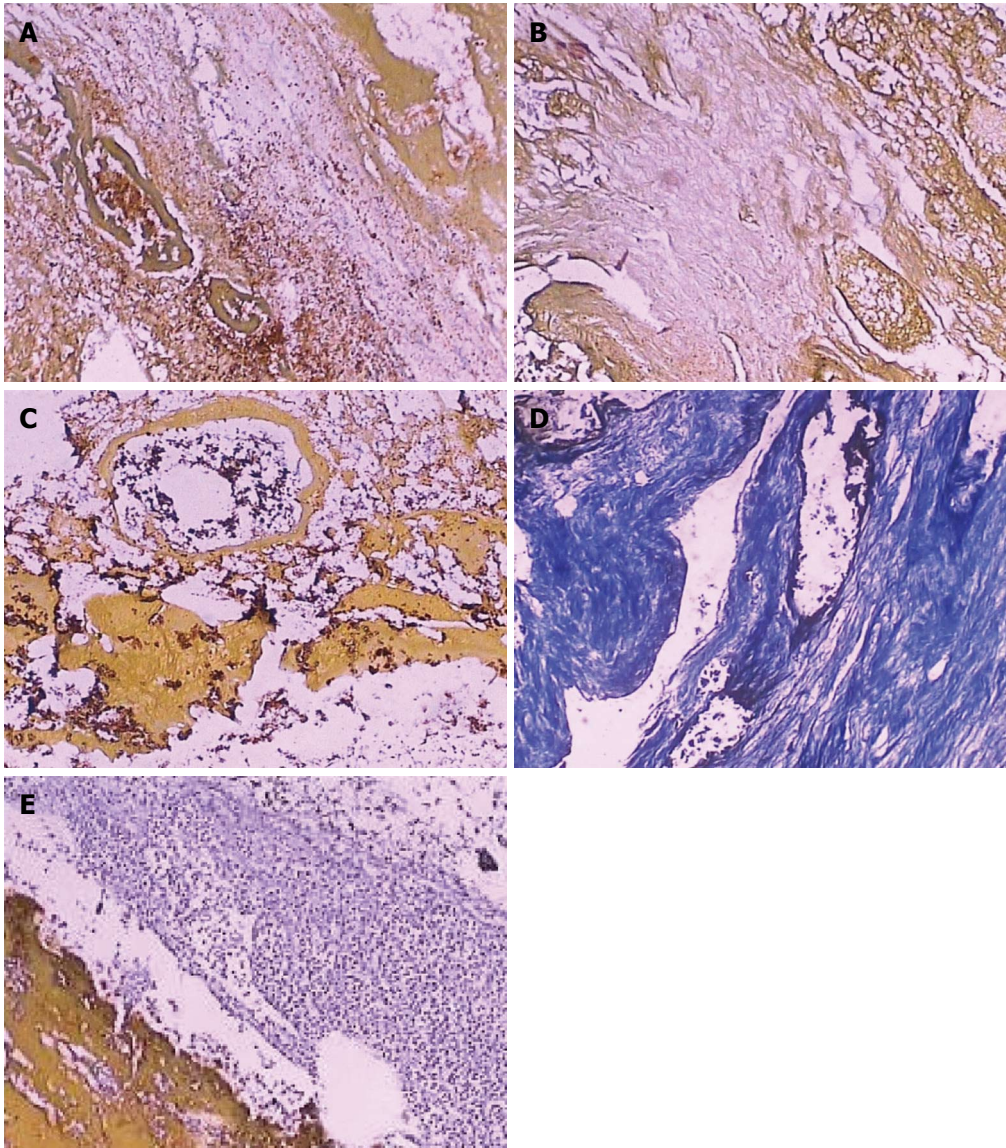


Figure 2 Histological and immunohistochemical examination of biliary casts. A: Histological examination of a biliary cast, showing tubiform structures (HE staining $\times 100$); B: Histological examination of a biliary cast, showing filamentous structures (HE staining $\times 100$); C: A biliary cast with tubiform structures positive for CD34 (brown color, $\times 100$); D: A biliary cast with filamentous structures positive for collagen fibers (Masson staining $\times 100$); E: A biliary cast showing peripheral positivity for neutrophils and other inflammatory cells (HE staining, $\times 100$).

a similar time of onset and similar clinical symptoms. However, biliary casts generally form at least 1 mo after transplantation^[17]. Liver recipients with high serum concentrations of soluble major histocompatibility complex class I related chain A (sMICA) tend to develop BCS more easily than recipients with normal post-transplant sMICA concentrations^[18]. We hypothesized that the formation of biliary casts was related to acute rejection and that T lymphocytes, B lymphocytes and macrophages would be present in biliary casts. However, we found that these cells were absent from biliary casts arising after liver transplantation, similar to the findings in patients who underwent non-liver transplantation^[19,20]. Therefore, our findings suggest that acute rejection after liver transplantation was not significantly associated with biliary cast formation.

Electron microscopic examination of cholesterol

calculi showed the presence of bacteria in the core and periphery of cholesterol stones, suggesting that bacteria may be involved in initiating the formation of cholesterol stones^[21,22]. Patients with biliary casts usually have recurrent episodes of cholangitis. *Escherichia coli*, which has glucuronidase activity and can grow in cultures of biliary casts, can degrade conjugated bile acids and conjugated bilirubin, yielding free bile acids and free bilirubin, respectively. Free bile acids and free bilirubin are relatively insoluble and are not present in the bile of patients. Damage to the bile duct mucosa can result in their precipitation into biliary casts, suggesting that a number of factors, including infection, supersaturation with cholesterol and mucosal damage, may be involved in bile cast formation after liver transplantation^[2]. To assess the relationship between bacteria and biliary casts, we evaluated biliary casts using scanning electron mi-

croscopy. However, neither bacteria nor bacterial debris was observed in the interior or surface of biliary casts. Large numbers of neutrophils were observed on the periphery of biliary casts, but the boundaries were clear and there were no neutrophils or similar cells within the mold. The multiplication of bacteria in an environment of poor bile drainage and cholestasis caused by biliary casts may therefore induce recurrent fever, obstructive jaundice and other complications. Biliary tract infections may be secondary pathological changes following biliary cast formation, rather than being the direct cause of mold formation. Therefore, when treating patients who experience complications after liver transplantation, anti-infectious agents may only alleviate the symptoms. The removal of the biliary casts may therefore be primary.

COMMENTS

Background

Biliary casts are infrequent complications after liver transplantation, resulting in various clinical symptoms. Although the associations between biliary casts and clinical treatment have been assessed recently, less is known about the associations between biliary casts and biochemical markers.

Research frontiers

The current pathogenesis study of biliary casts after liver transplantation mostly concentrated on clinical aspects. Biliary casts were not caused by a single pathogenic factor, but may be associated with late functional rehabilitation, biliary strictures and obstruction, acute rejection, recurrent cholangitis, cold and warm ischemia times, hepatic ischemia and reperfusion injury.

Innovations and breakthroughs

The results indicated that blood vessels and collagen fibers are present in biliary casts; however, bacteria and acute rejection are not clearly related to their formation, as evidenced by blood vessels positive for CD34 and collagen fibers with positive Masson staining, and the absence of T-lymphocytes, B-lymphocytes, macrophages and other inflammatory cells.

Applications

These findings indicate that bile duct injury is clearly associated with biliary cast formation after liver transplantation; however, bacteria and acute rejection were not significantly related to their formation.

Terminology

Biliary cast syndrome, first described in 1975, occurs less frequently than biliary sludge and stones, with an incidence of 2.5% after orthotopic liver transplantation. Orthotopic liver transplantation refers to a procedure in which a failed liver is removed from the patient's body and a healthy donor liver is transplanted into the same location. Biliary casts are infrequent complications after liver transplantation, resulting in various clinical symptoms.

Peer review

The authors analyzed the pathogenesis of biliary casts after liver transplantation relative to their morphology and biochemical markers. These findings indicate that bile duct injury was clearly associated with biliary cast formation after liver transplantation, but that bacteria and acute rejection are not clearly related to their process of bile duct injury. Therefore, it is an interesting study. The analytical approaches are described in detail, and the results are impressive.

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