post-vaccination antibody titers decline over the course of a year in both healthy children and adults (17–19). Otherwise, there are few studies concerning persistence of influenza vaccine-induced antibodies in solid organ transplant patients (20–22). Moran *et al.* reported that post-immunization antibody titers among healthy adult controls were higher than in adult lung transplant recipients; however, few differences in antibody titers were observed 11 months after vaccination in these adult recipients (20). In particular, about 70% of recipients maintained seroprotective antibody concentrations to most vaccine antigens. In contrast, Cordero *et al.* reported detection of long-term antibody titers to previous vaccination in 30% of recipients aged ≥15 years (21). In the present study, pre-vaccination seroprotection rates in adult patients were 45–65% and GMT 18.7–54.6 to the three antigens. Unexpectedly, GMT fold increases of all three antigens in recipients who received two consecutive vaccinations were significantly lower than in recipients who had not been vaccinated in the previous season. This result may be attributable to relatively high pre-vaccination titers to both H1 and H3 antigens; however, the reason for this finding is not clear. Moreover, there were no significant differences in general antigen responses between the adult and pediatric groups who had received preceding vaccinations. Long-term responses to preceding vaccinations were insufficient in our adult and pediatric liver transplantation recipients in that they did not contribute to boosted responses. Immune-enhancing vaccine strategies, such as high-dose vaccines (23, 24), booster dose re-vaccination (25) or adjuvanted vaccines (26, 27) should be considered.

We consider the incidence of influenza infection after vaccination the most important indicator of the effectiveness of the vaccine. In the present study, 5/15 and 3/21 pediatric liver transplant recipients were infected with influenza in the 2010–2011 2011–12 season, respectively, whereas no adult recipient was infected. Thus, there was a significant difference between pediatric and adult patients in the frequency of influenza. Madan *et al.* demonstrated that cell-mediated immune responses to influenza vaccination are diminished in pediatric liver transplant recipients, whereas seroprotection and seroconversion rates are similar to those in healthy siblings (12). Although cell-mediated immune responses to influenza vaccination have not been shown in adult recipients, it is possible that they are weaker in pediatric than in adult recipients. This may affect the morbidity rate of influenza after vaccination. Further studies are needed to clarify differences in incidence of influenza between pediatric and adult recipients.

In conclusion, in this single hospital study, adult and pediatric liver transplant recipients generated immune responses to inactivated seasonal influenza vaccine and no significant differences were seen between adult and pediatric liver transplant recipients in immunogenicity of influenza vaccination over two influenza seasons. Long-term persistence and contribution to a boosted response were insufficient in both recipient groups. The number of patients with severe adverse reactions did not differ significantly between the two recipient groups. Immune-enhancing vaccine strategies for liver transplant recipients should be considered in future.

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DISCLOSURE

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Endoscopic biliary drainage for children with persistent or exacerbated symptoms of choledochal cysts

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Abstract
Background Symptoms of choledochal cysts sometimes persist or become exacerbated. As preoperative management for patients with these cysts, we prospectively employed endoscopic drainage, based on the theory that protein plugs cause symptoms by obstructing the pancreatobiliary ducts.
Methods Children with choledochal cysts underwent endoscopic retrograde cholangiopancreatography (ERCP). When ERCP showed compaction with filling defects in patients with persistent or worsening symptoms (study patients), the placement of a short biliary stent tube was attempted for drainage. The clinical and ERCP findings of the study patients were compared with those of patients who were asymptomatic at ERCP (asymptomatic patients).
Results There were 13 study patients (median age 2.9 years) and 41 asymptomatic patients (4.7 years) enrolled in the study between August 2005 and February 2011. Study patients more frequently had jaundice and elevated transaminase levels. ERCP showed that all study patients had obstruction or compacted filling defects in the common channel or the narrow segment distal to the cyst. Insertion of a stent tube was successful in 11 patients. Symptoms were relieved soon after biliary drainage. Surgery revealed that the obstructing materials were protein plugs, except in one case, which involved fatty acid calcium stones.
Conclusions These results support the protein plug theory. Endoscopic short-tube stenting is adequate and effective as preoperative management.

Keywords Choledochal cyst · Pancreaticobiliary maljunction · Protein plug · Endoscopic management

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Introduction

Patients with choledochal cysts develop abdominal pain, vomiting, and/or jaundice during childhood. Usually, symptoms are mild and self-limited [1]. In some patients, however, symptoms become exacerbated or continue for weeks. The most severe sign of exacerbation is rupture of the cyst [2, 3]. For these patients, no definite management has been established. Some physicians perform emergent cyst excision, and others, biliary drainage [1, 3–5]. Emergent excision, however, may be dangerous, except when precise pancreatobiliary anatomy is obtained by magnetic resonance cholangiopancreatography. A lack of information leads to pancreatic duct injury and the overlooking of coexisting anomalies of the intrahepatic bile ducts [5, 6]. To treat persistent or exacerbated symptoms, we have tentatively been adopting external biliary drainage, including percutaneous transhepatic biliary drainage or open T-tube drainage [1, 2]. External drainage immediately relieves the symptoms, and has the advantage of facilitating thorough examinations of the pancreatobiliary system until definitive cyst excision can be undertaken [1, 2, 5, 7]. However, some cases of ineffective external drainage have been reported, and the procedure has the disadvantages of

creating an extra scar and the necessity for tube management in these children [8].

Previous reports have not clarified why the symptoms of choledochal cysts persist or worsen. We have proposed that protein plugs cause the symptoms (protein plug theory) [1]. Protein plugs consist of lithostathine, a protein secreted from the pancreas. Lithostathine and trypsinogen are regurgitated into the cyst through an abnormal pancreaticobiliary junction. Activated trypsin cleaves soluble lithostathine into insoluble forms that aggregate to form plugs [9]. Protein plugs are compacted in the common channel or the narrow segment distal to the cyst. The obstruction increases the intraductal pressure in the pancreatobiliary tract, producing symptoms. Most plugs are fragile and disappear spontaneously, which relieves the symptoms. This explains why symptoms are usually mild and self-limited. The repeated production of protein plugs explains the intermittent symptoms. However, some plugs do not disappear, which may exacerbate the symptoms. Based on this protein plug theory, we prospectively employed an alternative drainage procedure free of tube management, using an endoscope, for patients with persistent and/or exacerbated symptoms.

Patients and methods

Since August 2005 at our hospital, children with choledochal cysts have undergone endoscopic retrograde cholangiopancreatography (ERCP), performed with a side-viewing duodenoscope (PJF-7.5; Olympus, Tokyo, Japan, or JF-240; Olympus for older patients) under general anesthesia as early as the regular examination schedule permits after referral to our hospital. Symptomatic patients were managed with nil per os, and usually without antibiotics. For patients with persistent and/or exacerbated symptoms exhibited by the time of ERCP, when ERCP showed filling defects compacted in the common channel or in the narrow segment distal to the cyst, the placement of a short biliary drainage stent tube (GEPD; Cook Medical, Bloomington, IN, USA) was attempted. The criteria for persistence and exacerbation of the symptoms were duration for 2 weeks or more, and symptoms too serious to wait for the scheduled examination, respectively. The final decision on stent tube insertion was made by one of the authors (K.K.) at the time of ERCP. The stent tube has offset flaps to minimize stent migration, and spiral side holes for enhanced drainage. After the cholangiopancreatography, a guide wire was inserted into the biliary tract, through which the stent tube was advanced. All endoscopic procedures were performed by one of the authors (A.I.) after informed consent was obtained from the parents. The clinical features and ERCP findings of patients with persistent and/or exacerbated symptoms (study patients) were compared with those of patients who had been free of symptoms at the time of ERCP examination (asymptomatic patients). The success of endoscopic biliary drainage and the clinical course after drainage were evaluated.

Results

At our institution 55 patients with choledochal cysts were treated between August 2005 and February 2011. Their ages ranged from 2 weeks to 15 years (median 4.3 years). An 8-year-old girl showed perforation of a choledochal cyst, and underwent emergent T-tube drainage. Obstruction was shown by cholangiography through the T-tube. Among the other 54 patients, symptoms persisted or were exacerbated until the examination in 13 patients (study patients), 10 of whom had persistent symptoms: abdominal pain in 10, jaundice in 7, elevated transaminase levels in 10, hyperamylasemia in 7, and fever in 2. Three of these 13 patients had symptoms too serious to wait for the scheduled examination, and they underwent emergent ERCP. The remaining 41 patients were asymptomatic at the time of ERCP.

Study patients more frequently had jaundice and elevated transaminase levels compared with the asymptomatic patients. ERCP showed that all but one of the study patients had filling defects, showing compaction in the common channel or in the narrow segment distal to the cyst. The one patient without a filling defect showed an obstruction in the narrow segment connecting to the cyst, through which contrast material did not proceed. No obstruction or impacting filling defect was observed in any patients in the asymptomatic group.

Placement of a biliary stent tube was successful in 11 of the 13 study patients (Fig. 1). A 5-Fr 5-cm stent tube (GEPD 5-5; Cook) was used in 9 patients, and a 5-Fr 7-cm tube (GEPD 5-7; Cook) in 2. One patient in whom placement of the stent tube was unsuccessful was a 1-year-old girl who had an obstruction in the narrow segment, described above. The narrow segment was at a right angle to the common channel, and a guide wire could not be inserted. After ERCP, she continued to experience abdominal pain, vomiting, and fever until definitive surgery was performed. The other patient in whom placement of the stent tube was unsuccessful was also a 1-year-old girl; in this patient, the insertion of a guide wire was successful, but the stent tube did not proceed to the narrow segment. Repeated insertion of the guide wire crushed the plugs in the common channel. After this procedure, she experienced no symptoms. In all 11 patients with successful biliary drainage, the symptoms were relieved soon after the procedure (Table 1). The elevated amylase levels returned to normal significantly earlier than the elevated

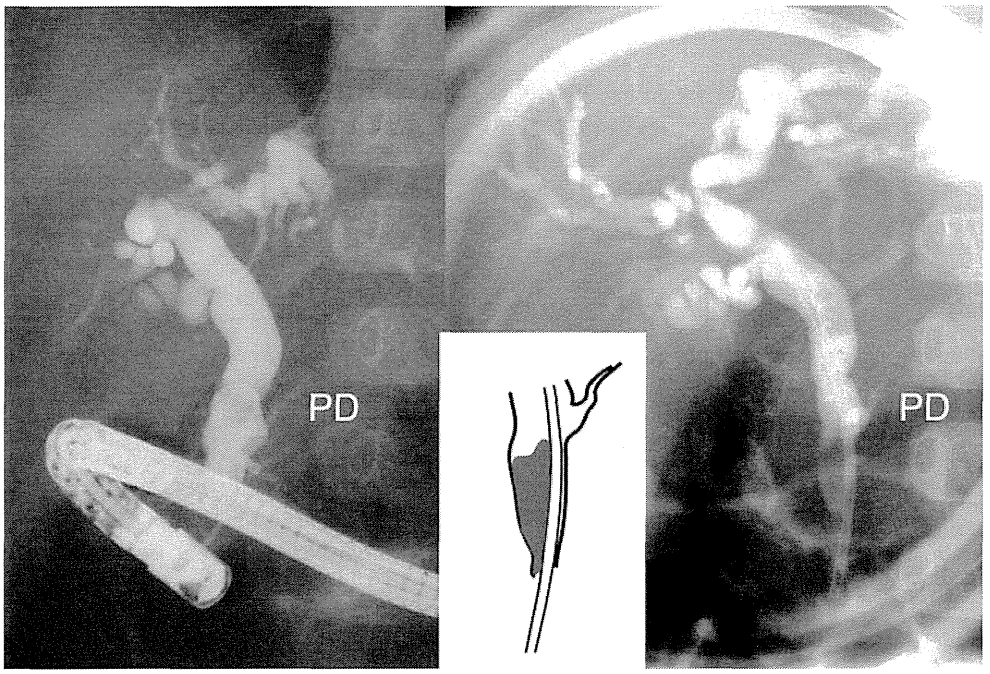

Fig. 1 Representative endoscopic retrograde cholangiopancreatography (ERCP) in a patient with stent placement. A 2-year-old girl underwent biliary drainage owing to exacerbated symptoms of jaundice. ERCP showed filling defects compacting the common channel. A 5-Fr 5-cm stent tube was inserted. The stent tube penetrated the filling defects. *PD* pancreatic duct

Table 1 The duration from biliary drainage to relief of symptoms in the 11 patients in whom the procedure was successful

Symptoms and laboratory data	Number of patients	Median duration (days)	Range
Abdominal pain	9	1	1–4
Jaundice	6	3	1–7
Hyperamylasemia	5	1	1–5
Elevated transaminase levels	8	6.5	1–15

transaminase levels ($P = 0.019$, Mann–Whitney U-tests). There were no complications, such as pancreatitis, related to endoscopic management. The 11 patients who received a stent underwent excision of the extrahepatic bile duct after a median period of 12 days (range 5–47). The stent tubes were removed at the time of bile duct resection, but the position of the stent tube was unknown in one patient until surgery. In another patient, abdominal pain recurred 18 days after endoscopic drainage. Surgery revealed hard fatty acid calcium stones obstructing the stent tube, a finding which has been reported previously [10]. In another 6 study patients and also in 8 control patients, materials were obtained during surgery. On infrared absorption spectrometry, the materials proved to be protein plugs. Severe inflammatory adhesions around the bile duct were significantly more frequent in the study group, but did not increase the surgical complication rate.

Discussion

The findings in the present study supported the protein plug theory. All patients with persistent and/or worsening symptoms showed obstructions in the common channel or the narrow segment distal to the cyst. The relief of obstruction obtained by stent placement or by crushing the blocking materials successfully resolved the symptoms. The obstructing materials were protein plugs. However, we found another, rare, cause of obstruction involving fatty acid calcium stones, a finding which has also been described elsewhere [10].

Endoscopic management for children with choledochal cysts is not new [4, 7, 8, 11–16]. However, the authors of these previous reports (excluding 2 studies [7, 8]) did not realize that protein plugs were the real cause of the choledochal cyst complications. Most authors, including those of the 2 studies noted above [7, 8] adopted endoscopic sphincterotomy, considering sphincter insufficiency [7, 8, 11–13, 15, 16]. However, other studies have reported that sphincterotomy or sphincteroplasty did not prevent the recurrence of protein plug formation after cyst excision [7, 17, 18]. Incomplete cyst excision is the cause of recurrence, because patients with recurrence always had a residual bile duct in the pancreas, and, conversely, complete excision prevented recurrence [6, 7, 17, 18]. After cyst excision, the sphincter function seems normal as far as pancreatic secretion is concerned. As protein plugs are

fragile, sphincterotomy is unnecessary for treatment, and may be harmful for children. A temporary stent is adequate for the relief of symptoms.

Endoscopic retrograde cholangiopancreatography (ERCP) is generally dangerous for patients with pancreatitis, but it may be beneficial in those with pancreaticobiliary maljunction, as it is in patients with gallstone pancreatitis [15]. However, true pancreatitis is rare. Most hyperamylasemia may be attributed to cholangiovenous reflux caused by regurgitated pancreatic juice with increased bile duct pressure, which was once called fictitious pancreatitis or pseudopancreatitis [19, 20]. In the present study, among 33 patients with hyperamylasemia, only 2 exhibited pancreatic enlargement on computed tomography. Hyperamylasemia diminished 1 day after drainage, while the elevated transaminase levels returned to normal after several days. These results support the pseudopancreatitis theory.

An endoscopic approach has some advantages over external drainage, including percutaneous transhepatic biliary drainage and T-tube use. External procedures have some limitations, including tube management in children, severe inflammation around the bile duct, and scarring at the insertion site. In 1997, Tagge et al. [4] were the first to use a short biliary stent, in three children with complicated choledochal cysts. Endoscopic short-tube stenting is beneficial for children, because it can avoid scar formation, and does not require tube management. Spontaneous dropout of the stent tube was seen in one of our patients, and X-ray check up may be necessary directly before the operation. ERCP provides not only an accurate diagnosis, but also useful anatomical details that help in the planning of appropriate surgical intervention [6, 14, 21]. The only drawback to short-tube stenting is adhesion around the choledochal cyst, as occurs in patients with external drainage. However, it was not known whether the adhesions were caused by stent placement or the accompanying cholangitis.

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Conflict of interest None.

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Liver Fibrosis in Prenatally Diagnosed Choledochal Cysts

To the Editor: The incidence of liver cirrhosis after choledochal cyst (CC) has been reported to be 2.1% to 11.8% (1). It is particularly important to prevent liver damage progressing toward cirrhosis in CC. It is unknown whether hepatic fibrosis in symptomatic patients after birth with prenatally diagnosed CC is present or absent, especially regarding the severity of hepatic fibrosis.

Of the 27 cases with CC, 8 were diagnosed prenatally (mean fetal age, 27 weeks [20–36 weeks]), and were categorized into 2 groups: a symptomatic infant group including 5 patients (mean fetal age, 27 weeks [20–31 weeks] and 10-year follow-up] and an asymptomatic infant group including 3 patients (mean fetal age, 28 weeks [20–36 weeks] and 4-year follow-up).

Histological findings of the hematoxylin and eosin–stained liver biopsy specimens, especially with regard to the developmental degree of liver fibrosis, were classified into 5 grades (Ohkuma classification) (2). The symptomatic CC group consisted of 1 case of grade 0 and 4 cases of grade 1. The asymptomatic CC group consisted of 1 case of grade 0 and 2 cases of grade 1. There was a histological difference between symptomatic and asymptomatic infants with prenatally diagnosed CC ($P = 0.0312$).

It is important to keep in mind that liver fibrosis is significantly positive in symptomatic infants with prenatally diagnosed CC, although it is mild, compared with that in asymptomatic infants. The conclusion drawn from this study is consistent with the hypothesis that timely surgical intervention can lead to the reversal of liver fibrosis.

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Colon Preparation for Children: The Quest for the Ideal Protocol

To the Editor: The publication of cleansing colonic protocols for colonoscopy procedure in children in the February 2013 issue of *JPGN* was a wake-up call to address this topic (1–4). These prospective studies confirmed the acceptability and safety of polyethylene glycol (PEG) 3350, but its low effectiveness (adequate preparation <90%) showed that we are yet a long way from the optimal protocol needed for our patients.

PEG 3350 has been proven to be safe, palatable, acceptable, and successful for constipation, fecal impaction, and colon cleansing protocols. It is time to conclude that PEG 3350 is the best solution we have for colon cleansing in children. Unfortunately, to date, there is no criterion standard protocol for colon cleansing, and each medical center uses its "in house" protocol, in many cases without carefully documenting the end result. Patel and Pashankar (4) stated that there are few head-to-head comparisons between protocols in children and even fewer comparisons between the PEG 3350 solutions, adjusting for timing, duration, dosing, and so on. To find the PEG 3350 criterion standard protocol for children, studies adjusting these variables and measuring outcomes need to be conducted. The various attempts to shorten the protocol, almost being absurd (even counting the hours!), are usually blocked by the limitation of human consumption, and is the wrong direction to follow. We recently completed the first head-to-head comparison between 2 PEG 3350–based protocols (5,6) to find the superior protocol and to examine protocol reproducibility (7). In the quest for the criterion standard, we need to concentrate on the simplicity, acceptability, reliability of grading, and, most important, the rate of successful preparation. So far, we are a long way from an adequate success rate.

In summary, the quest to find the best colon cleansing protocol for children needs to be pursued with a change of direction. I believe PEG 3350 should be the solution used in all protocols, with the aim of finding the best variables associated with those protocols. In addition, we should always apply head-to-head comparison between protocols. Only in this way will we be able to assess reproducibility and superiority of one protocol against the other. Without it, we will fail in our mission to provide the best available intervention for our children.

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LETTER FROM THE FRONTLINE

Hepatocyte Transplantation Using a Living Donor Reduced Graft in a Baby With Ornithine Transcarbamylase Deficiency: A Novel Source of Hepatocytes

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TO THE EDITORS:

We performed hepatocyte transplantation (HT) in an 11-day-old infant with ornithine transcarbamylase deficiency (OTCD). We used cryopreserved hepatocytes prepared from remnant liver tissue, a byproduct of a hyper-reduced left lateral segment from living donor liver transplantation (LDLT). The patient exhibited hypothermia, drowsiness, and apnea at 3 days of age; these symptoms were accompanied by hyperammonemia (1940 μg/dL at maximum), although there were no abnormalities at birth or an obvious family history (Fig. 1). Further examinations confirmed that the hyperammonemia was the result of OTCD. Multimodal treatments, including alimentotherapy, medications, and continuous hemodiafiltration (CHDF), did not improve the patient's clinical state, and severe hyperammonemia attacks recurred. Because of the patient's small body size (2550 g) and the lack of an available liver donor, HT was indicated. Hepatocytes of the same blood type were chosen from an institutional repository of cryopreserved hepatocytes prepared from the remnant tissue of segment III from unrelated living donors. Thawed hepatocytes were transplanted twice at 11 and 14 days of age with a double-lumen catheter inserted into the left portal vein via the umbilical vein (Fig. 2). The amounts of transplanted hepatocytes were 7.4×10^7 and 6.6×10^7 cells/body, and the viability rates were 89.1% and 82.6%, respectively. The portal flow was kept stable at greater than 10 mL/kg/minute, and the pressure was maintained at less than 20 mm Hg during and after HT. The immunosuppressive treatment followed the same protocol used for LDLT with tacrolimus and low-dose steroids.[1] The patient was weaned from CHDF and the ventilator at 26 and 30 days of age, respectively, with a stable serum ammonia level of 40 μg/dL. The patient was ultimately discharged 56 days after HT. During the 3 months of follow-up, the baby did well with protein restriction (2 g/kg/day), medication for OTCD, and immunosuppression. No neurological sequelae related to hyperammonemia have been observed so far (Fig. 1).

DISCUSSION

For children with metabolic liver disease, HT is indicated as an alternative or bridge to liver transplantation.[2] HT is less invasive than liver transplantation and can be performed repeatedly. Limitations to the widespread application of HT include the poor availability of hepatocytes. Therefore, it is important to find new sources of high-quality hepatocytes. We previously prepared a repository of hepatocytes obtained from remnant liver tissue, a byproduct of hyper-reduced left lateral segmentectomy in LDLT.[1]

The cell donor was an unrelated volunteer with the same blood type who had previously undergone hyper-reduced left lateral segmentectomy. The main unit of segment II was used as a monosegmental liver graft for the primary recipient with end-stage liver disease, and the remnant was used to isolate hepatocytes with fully informed consent. The hepatocytes were isolated according to the collagenase perfusion method, as described elsewhere,[3] with Liberase MTF C/T GMP grade (Roche). All procedures were performed at our cell processing center according to a strictly controlled protocol based on good manufacturing practices. The total number of transplanted live hepatocytes was 1.4×10^8 cells/body; the ammonia removal rate was more than 200 fmol/cell/hour (203.4 and 265.4 fmol/cell/hour with the first and second injections, respectively). The dose was judged to be sufficiently high to obtain therapeutic effectiveness according to our theoretical background.[4]

This work was supported by a grant-in-aid from the National Center for Child Health and Development and the Highway Program for the Realization of Regenerative Medicine (Japanese Science and Technology Agency). This study protocol was approved by institutional review board in National Center for Child Health and Development (reference number 433).

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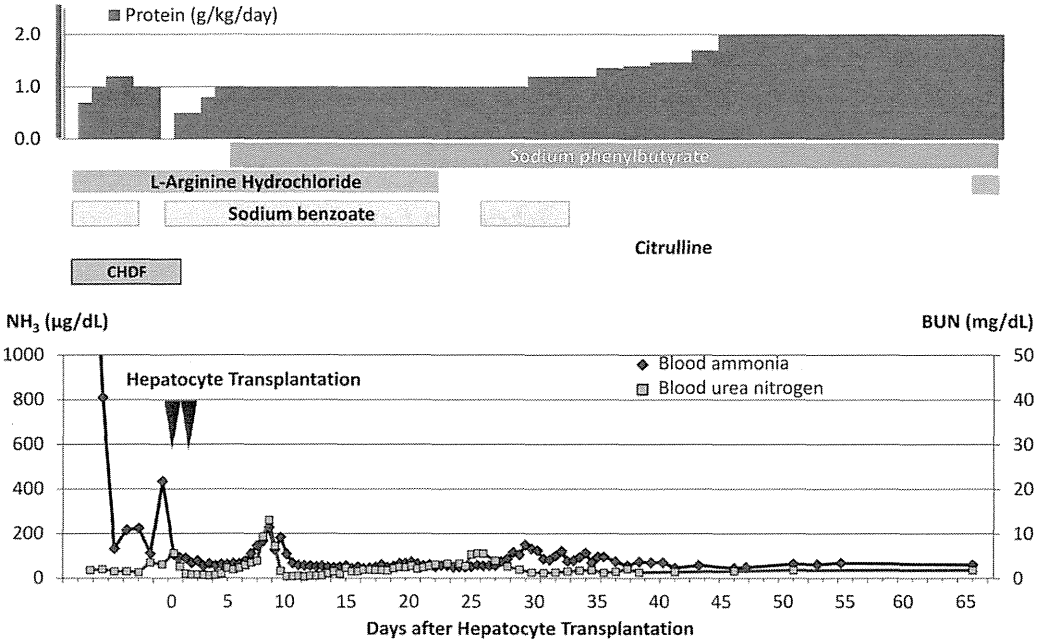

Figure. 1. Treatment schedule (top) and patient condition (bottom). The changes with time for blood ammonia and blood urea nitrogen are shown. The baby was delivered vaginally as a first child. At 3 days of age, hypothermia, low oxygen saturation, and, finally, respiratory arrest occurred. The patient was incubated and given artificial respiration. Concurrently, hyperammonemia (1940 μg/dL) was found, and continuous hemodiafiltration (CHDF) was started in addition to alimentotherapy (protein withdrawal) and medications. Whenever the administration of essential amino acids was restarted, the blood ammonia level became elevated, and at 9 days of age, despite the suspension of essential amino acid administration, the level increased up to 434 μg/dL. At 11 days of age, HT was performed for the first time, and it was performed for the second time at 14 days of age. After HT, amino acid intake was restarted along with the continuation of multimodal treatments, and blood ammonia was controlled well except for episodic increases. The patient was weaned from CHDF and the ventilator at 26 and 30 days of age, respectively, and the patient was ultimately discharged 56 days after HT.

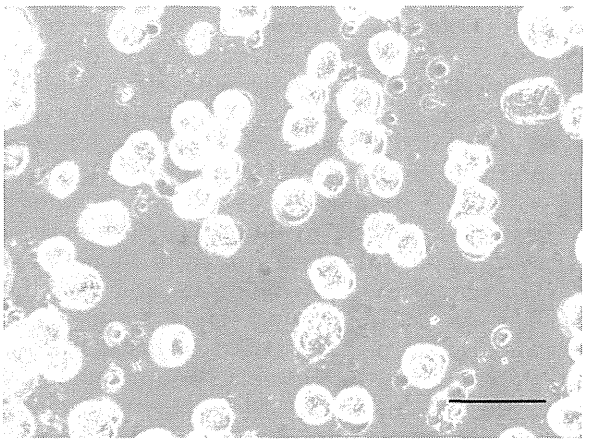

Figure. 2. Hepatocytes transplanted during the first injection. The cells showed a glazed and firm surface. The bar indicates 50 μm.

Because liver transplantation is approved as a treatment for end-stage hepatic failure, donor livers are preferentially allocated for organ transplantation and not for hepatocyte isolation. On rare occasions, the lack of appropriate donor-recipient matching (eg, infant donor livers) provides good-quality hepatocytes.[2] Fetal livers are also considered to be an alternative cell source, although ethical issues remain to be resolved. At present, we have little choice but to use marginal donor tissues, such as livers obtained from donors after cardiac death and organs with steatosis, fibrosis, or a long ischemia time. However, there are unfavorable issues related to the use of marginal donors, including low viability and vulnerability to cryopreservation. In this respect, the remnant liver tissue of hyper-reduction procedures used in LDLT has the same quality as that of left lateral segment grafts. As for availability, there are 5 cases of hyper-reduction per year at our institution on average.[5] The use of remnant liver tissues obtained from hyper-reduced LDLT procedures will, therefore, help to address the shortage of hepatocyte donors.

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Biliary atresia type I cyst and choledochal cust: can we differentiate or not?

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Abstract
Background/purpose It is difficult to discriminate between choledochal cust with obstructive jaundice and biliary atresia with a cyst at the porta hepatis in neonates or young infants. This review evaluates whether it is possible to differentiate between these two diseases. We here also provide an overview of our experience with type I cyst biliary atresia patients.
Methods Among all the biliary atresia infants who we treated, the infants who were diagnosed with type I cyst biliary atresia were identified and reviewed for their management and outcome. The clinical course and management in different reports were reviewed and compared to the cases presented to our institution.
Results Among the 220 biliary atresia cases, 11 (5 %; male/female: 4/7) were diagnosed to be type I cyst biliary atresia. Two received hepaticoenterostomy and nine received hepatic portoenteros. Three patients had severe late complications; overall, nine (81.8 %) were alive with their native liver and without jaundice.
Conclusions Patient with choledochal cust are likely to represent larger cysts and inversely, smaller, static, anechoic cysts are more likely to represent cystic biliary atresia. However, exceptional cases were yet presented, and a definitive diagnosis may not be reached. Thus a complete differentiation between choledochal cust from type I cyst biliary atresia is yet hard to reach.

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Keywords Biliary atresia · Choledochal cust · Cyst at the porta hepatis

Introduction

Biliary atresia (BA) is an obstructive condition in which all or parts of the extra hepatic bile ducts are absent [1]. Cystic BA is subtype of BA with a cyst at the porta hepatis. Type I BA is a case with atresia of the common bile duct and when a cystic dilatation of the porta hepatis connecting with intrahepatic bile ducts is accompanied of type I atresia, it is called a type I cyst BA (I cyst BA) [2]. Choledochal cyst (CC) is a rare medical condition with abnormal cystic dilatation of biliary tree in several parts and degrees. CC commonly present prior to the age of 2 years, although it can be diagnosed antenatally [1]. Both I cyst BA and CC are well-known causes of jaundice in neonates and young infants, with a cyst at the porta hepatis. However, cystic BA and CC are two entities with dramatically different management approaches and prognosis. Early surgical intervention should be required because age at surgery markedly affected outcome as judged by clearance of jaundice in the BA cases [3–5]. However, there are patients with cystic BA who are not relived from jaundice in spite receiving early hepatic portoenterostomy. In contrast, CC is a curable choledocal malformation that can have excellent prognosis after resection of the cyst and hepaticojejunostomy. Hence, it is very important to differentiate cystic BA from CC when a portal cyst is found in a fetus, neonate or young infant [6]. However, it is not easy to discriminate between CC and type I cyst BA in neonates or early infants in spite of the several diagnostic tests, and in many patients exploratory laparotomy with surgical cholangiography is required to establish a definitive diagnosis [7].

Yet the question still stands, is it possible to differentiate type I cyst BA from CC?

Here we present our experience in management of I-cyst BA cases with a review of literature that studied this disease.

Methods

Among the infants diagnosed with BA, infants further diagnosed as type I cyst BA during the period from January 1969 to December 2011 at Nagoya City University Hospital and Fujita Health University Hospital were included in the study. The diagnosis of BA was confirmed in all cases by perioperative clinical findings, and when available with cholangiography. Clinical records were reviewed for details on the operative biliary reconstruction, postoperative courses, and their outcomes. The cases which had interesting clinical courses were further expressed.

Statistical analysis

Kaplan–Meier survival curves were obtained and data analyses were performed with the commercially available statistical analysis software package SPSS 14 (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

Two hundred and twenty infants (male/female, 92/128) with BA were managed in our institutions.

Among these infants 11 (5 %; male/female, 4/7) were identified to be with type I cyst BA (Table 1). Three cases received an initial surgical tubal drainage of gallbladder to relieve their jaundice and a corrective surgery was performed for BA after 49–203 days. Hepaticoenterostomy was performed in 2 cases and the remaining 9 cases received hepatic portoenterostomy. The median follow-up period for infants with I cyst BA was 22.8 years (range 0.4–42.1 years). Overall, nine (81.8 %) survived with their native liver and were free from jaundice (Fig. 1).

A female infant failed to clear her jaundice postoperatively and second girl who received liver transplantation for relapse of her jaundice at the age of 12 died at the age of 6 months and 12 years, respectively.

Two female patients developed gastroesophageal variceal hemorrhage at the age of 19 and 17 years old, and required endoscopic sclerotherapy. A male patient developed liver dysfunction reviled during his medical checkup at the age of 24 years. He had multiple intrahepatic stones and required the removal of the calculus via percutaneous transhepatic cholangioscopy at the age of 24 (Fig. 2).

Table 1 The number of biliary atresia (BA) cases and their type classifications, treated in Nagoya City University Hospital and Fujita Health University Hospital, between January 1969 and December 2011

Type I	13 cases (5.9 %) (M/F: 5/8)	Type I cyst	11 cases (5.0 %) (M/F: 4/7)
		Type I without cyst	2 cases (0.9 %) (M/F: 1/1)
Type II	6 cases (5.9 %) (M/F: 2/4)		
Type III	201 cases (91.4 %) (M/F: 85/116)		
Total	220 cases (M/F: 92/128)		

M/F male/female

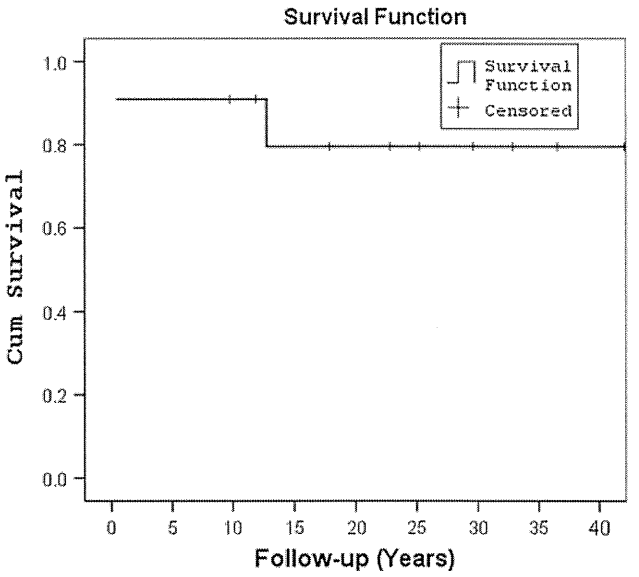

Fig. 1 Overall survival curve in 11 cases diagnosed as type I cyst BA. The median follow-up period for cases was 22.8 years (range 0.4–42.1 years), nine (81.8 %) survived

Case report

A 46-day-old female infant with persistent jaundice and acholic stools was referred to our hospital. Clinical jaundice and acholic stools were recognized around the end of the first month of life. Laboratory findings revealed a total bilirubin of 8.1 mg/dl, direct bilirubin of 7.1 mg/dl, AST of 184 IU/l, ALT of 208 IU/l, and γ-GTP of 1240 IU/l (normal: 2–45). Preoperative ultrasonography and CT scan revealed a cystic mass, 10 × 20 mm in diameter, at the porta hepatis and without intrahepatic bile duct dilatation. On exploratory laparotomy at 59 days of life, a cystic mass, 10 × 15 mm in diameter, was identified at the porta hepatis and contained bilious fluid without being identified

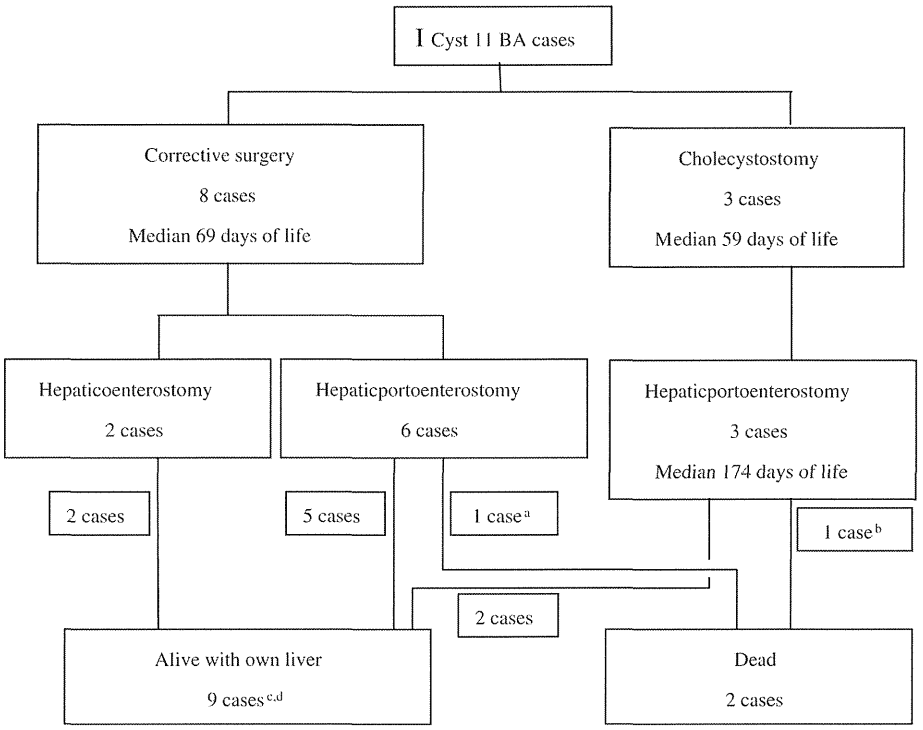

Fig. 2 A hierarchy figure presenting the selected operative procedures and outcomes of the 11 cases diagnosed as type I cyst BA. *a* A 6-month-old female infant failed to clear her jaundice and *b* a 12 year-old girl with post-liver transplantation for relapse of her jaundice; both cases died. *c* A case complicated from cholangitis and intrahepatic calculus (24-year-old male). *d* Two cases complicated from ruptured of esophageal varices (17- and 19-year-old females), and required endoscopic sclerotherapy

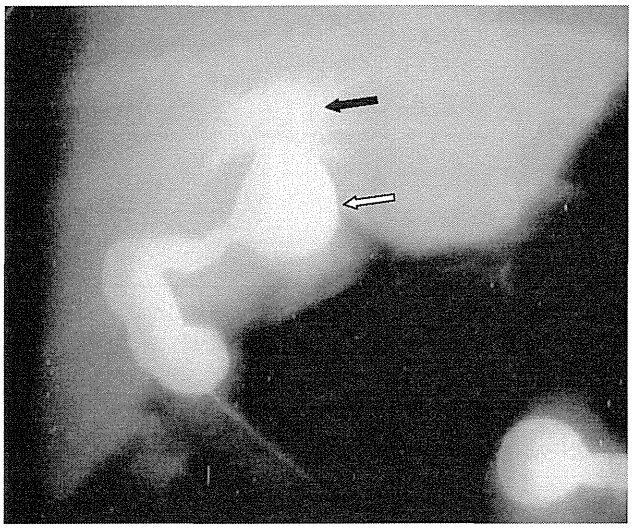

Fig. 3 Intraoperative cholangiogram. A cystic mass at the porta hepatis (*white arrow*) with connection to the cloudy intrahepatic bile ducts (*black arrows*) and no connection to the intestinal lumen

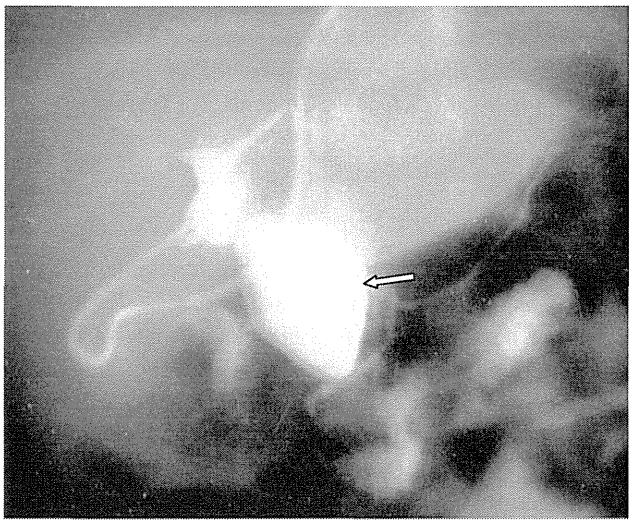

Fig. 4 Cholangiogram at the 39 postoperative days. Common bile duct with the abnormal pancreaticobiliary junction and dilatation in a fusiform shape (*arrow*) instead of the cystic mass at the primary operation

as the common bile duct at any part of hepatoduodenal ligament. Intraoperative cholangiography was carried out and it showed the cystic mass at the porta hepatis with connection to cloudy intrahepatic bile ducts and without identifying any connection with the intestinal lumen (Fig. 3). Tubal drainage of gallbladder was performed to relieve her jaundice and was diagnosed as I cyst BA. A radiographic image performed at the 39 postoperative days revealed the common bile duct with the abnormal pancreaticobiliary junction and dilatation with a fusiform shape instead of the cystic mass at the initial tubal drainage operation (Fig. 4). An average of 80 ml bile, containing low levels of amylase, was drained through the tube insured in her gallbladder and she became free from jaundice. At the 203 postoperative days hepatic portoenterostomy was performed, because the perioperative findings revealed an expanded common bile duct without apparent macroscopically structures of hepatic duct. The

histological findings showed that her cholestatic cirrhosis was somewhat severer than it was at the initial tubal drainage operation and no epithelial cell was found on the resected bile duct wall. She became free from jaundice and was doing well until the age of 17 years old, when she developed gastroesophageal variceal hemorrhage and required endoscopic sclerotherapy.

Discussion

Biliary atresia is an obstructive condition in which all or parts of the extra hepatic bile ducts are absent [1]. The etiology of BA is currently under investigation and two major forms of biliary atresia have been described: embryonic and prenatal [1]. The embryonic type accounts for 10–20 % and is associated with other congenital anomalies. The perinatal type accounts for the majority of cases [1]. According to the Japanese Biliary Atresia Registry, BA is classified into 3 types: type I, atresia of the common bile duct, type II, atresia of the hepatic duct, and type III, atresia of the porta hepatis and approximately 90 % of BA are type III [8]. Cystic dilatation of any part of the extrahepatic biliary tract may also occur, however, BA with a hilar cyst is an uncommon variant. The BA variant type I cyst constitutes <10 % [9] where a cystic dilatation of the common bile duct is accompanied of type I atresia.

On the other hand, choledochal cyst (CC) is a rare medical condition with abnormal dilatation of biliary tree in several parts and degrees. The etiology of CC is caused by an abnormal pancreaticobiliary junction proximal to the ampulla of Vater, resulting in an abnormal long common channel [1]. CC usually occurs in rather older children or adults [10], symptoms in CC patients appear in 22 % during the first year of life [11]. It is difficult to discriminate cystic BA from CC with obstructive jaundice and clay colored stools, although both are important causes of jaundice in neonates and young infants associated with a cyst at the porta hepatis.

Clinicians need to be aware of such cystic BA (including type I cyst) and how to distinguish it from CC to avoid inadequate initial surgical intervention [12].

Recently, improvements in perinatal diagnosis by advances in fetal ultrasonography (US) are leading to more frequent prenatal diagnosis of cystic BA or CC [13]. In these cases, the cyst could be identified at the right upper quadrant of the abdomen, which could be miss diagnosed, if confused with the gall bladder or the umbilical vein. Other common cystic lesions which appear similar to CC and cystic BA and could also cause misdiagnosis, include hydronephrosis, duodenal duplication, intestinal atresia, mesenteric cyst, and ovarian cyst. Recent development of ultrafast magnetic resonance imaging (MRI) techniques has allowed MRI to become an important further confirmatory investigation to US in fetal imaging. MRI is useful for the differentiation of biliary cystic malformation and other cystic lesions at the right upper quadrant in the fetal abdomen [14], but it is still not easy to discriminate between cystic BA and CC.

Saito et al. [15] showed that perinatal transition of the cyst size in biliary cystic malformation cases could be helpful in assessing the level of bile flow. Perinatal small cystic mass at the porta hepatis during gestation can include some cystic BA with poorer bile flow, on the other hand in CC cases, their cysts grew larger perinatally. Also, other previous reports showed that size of the cyst in fetus and neonates were different between cystic BA and CC, literatures stated several diameter for differentiation, as the smaller cystic diameter (<1.5 cm [16], <2.5 cm [17, 18], and <2.1 cm) [6] favor a cystic BA over CC (>4 cm) [18], or the mean width and length of the cysts in CC (62.2 ± 39.9 and 41.1 ± 30.7 mm, respectively) were significantly larger than those in cystic BA (16.2 ± 13.2 and 8.9 ± 6.5 mm) [6]. The small cysts in the hepatic hilum are highly suspicious for cystic BA [6, 12, 17, 19], however, there were clear exceptions to these rules [5, 6] and it is, in addition, hard to draw a clear line between a large cyst and a small cyst [5]. The ultrasonography finding of gallbladder abnormalities, triangular cord sign (an abnormal hyperechogenic triangular area in the porta hepatis), and dilatation of the hepatic artery, are useful in identifying BA. On the other hand, other ultrasonography findings are useful in identifying CC, such as intrahepatic bile duct dilatation, normal gallbladder, and sludge in the cyst [5], and hence, further specific and objective US features for differentiation and diagnosis of the two diseases are needed in neonates and early infants.

Huang et al. [20] reported that MR cholangiopancreatography (MRCP) yields a high degree of accuracy in the diagnosis of biliary atresia and CC. However, MRCP could only give information similar to US, unless the patency of the biliary tract is proved, in differentiating cystic BA and CC.

Then would invasive imaging studies such as endoscopic retrograde cholangiopancreatography (ERCP) and/or percutaneous transhepatic cholangiography (PTC) be useful for differentiating cystic BA and CC?

Several authors showed that ERCP is feasible and safe in the workup of neonatal cholestasis when other imaging modalities are inconclusive [21–23]. PTC is also an effective modality for diagnosis of cholestatic disorders in neonates, including BA patients [4, 24]. CC is differentiated from cystic BA by the patency of the biliary tract from intrahepatic bile duct to duodenum. These imaging studies are essentially useful for differentiating non-surgical and surgically correctable causes of cholestasis, such as

neonatal hepatitis and BA, respectively, which in turn prevents explorative laparotomy in the non-surgically correctable cases. On the other hand, ERCP and/or PTC are not essential in all cases of cystic malformation of the porta hepatis with obstructive jaundice and acholic stools, because early surgical intervention will be required after all and a definitive diagnosis would be given by intraoperative cholangiography.

Jiexiong et al. [19] showed that similar histological features such as inflammation of the liver and proliferation of the canalicular bile ducts could been found by the percutaneous liver biopsy in patients with both cystic BA and in some cases CC. Okada et al. [7, 13] have suggested that to assess fibrosis or biliary tract expression of neural cell adhesion molecule 1(CD56) may permit discrimination of cystic BA and CC. These reports are very interesting although included small number of patients.

Furthermore, the CD56-immunostaining is not widely performed. Thus the authors deny that the preoperative liver biopsy is essential for the diagnosis of these cases.

In conclusion, it is not easy to discriminate between cystic BA and CC with obstructive jaundice and gray colored stools in neonates or early infants in spite of the availability of several diagnostic tests including laboratory analyses [7, 19], and in these patients exploratory laparotomy with surgical cholangiography is essential to establish a final diagnosis.

Nio et al. [8], identified 3 characteristic bile duct images: namely, cloudy (numerous fine proliferative ductules demonstrated a cloudy pattern), treelike (interlobular bile ducts were clearly demonstrated like a tree structure) and mixed type (the cholangiogram consisted of both components of cloudy and treelike pattern).

In the case we fully presented, she was diagnosed as I cyst BA with the typical cloudy intrahepatic bile duct but her cholangiogram at 39 days after the initial operation showed patency between the intrahepatic bile duct and the duodenum. The radiological finding in this case could classify it to be CC, but the postoperative course and the findings of bile duct on the porta hepatis during her second operation confirm the diagnosis to be BA. Masumoto et al. [25] reported a case of cystic BA changing from I cyst BA to IIId (i.e., cystic BA without connection between the cyst at the porta hepatis and intrahepatic bile duct). By the current classification, we cannot fully differentiate cystic BA and CC by the patency of the extrahepatic bile duct without intraoperative cholangiography findings.

Early surgical intervention should be required in cases that cannot exclude BA from their diagnosis, because age at surgery has a marked influence on the outcome as judged by clearance of jaundice in these cases [3, 4, 6]. However, the optimal time to treat CC patients in early infants has not been established. Obstructive jaundice and increasing cyst size are indications for early surgery for definitive CC case [4, 18].

The management of CC at any age is by excision and hepaticojejunostomy which is similarly the optimal definitive procedure for CC in infants [4]. On the other hand the optimal operative procedure for the cystic BAs remains controversial. Takahashi et al. [26] showed excellent long-term outcome by hepaticojejunostomy in cystic BA. Other reports showed that hepatic portoenterostomy was effective after an unsuccessful hepaticojejunostomy for cystic BA [9, 12, 27]. Similar to other surgical groups, we also perform hepatic portentoerostomy for the treatment of cystic BA cases with insufficient diameter of hepatic duct for anastomosis [9, 12].

The postoperative course of CC in early infancy is usually satisfactory and long-term complications from complete cyst excision are rare [1]. The prognosis on type I BA (including I cyst) is usually much better than that of the other types [8]. However, Nio et al. [9] reported that the incidence of late cholangitis was considerably higher in type I patients. They also mentioned that patients with treelike pattern in their cholangiogram in corrective surgery showed excellent long-term prognosis [9]—they were more like patients with CC rather than BA.

In conclusion, cystic lesion of the porta hepatis in a fetus, newborn, or early infant might be the common finding of cystic BA and CC. It is difficult to differentiate between cystic BA and CC on the basis of clinical manifestation, even with using several imaging modalities in some cases. Early definitive surgery should be required in these patients when cystic BA is doubtful. It is a fact that in very rare cases a definitive diagnosis to differentiate between cystic BA and CC was not reached, and intraoperative cholangiography is effective in reaching the final diagnosis. Thus a preoperative definitive diagnosis between CC and I cyst BA is not essential.

Conflict of interest None.

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Children undergoing liver transplantation for treatment of inherited metabolic diseases are prone to higher oxidative stress, complement activity and transforming growth factor-β1

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B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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Summary

Background: Main indications for liver transplantation in the pediatric population include biliary atresia and inherited metabolic diseases. The present study evaluated whether there are differences between pediatric patients undergoing living-related liver transplantation due to the two diseases in terms of their oxidative and immunological status during their regular outpatient follow-up visits.

Material/Methods: A clinical outpatient study measuring serum oxidative stress index (calculated as serum oxidant/antioxidant ratio, in the form of serum total hydroperoxide/serum biological antioxidative potential), serum terminal complement component 5a, as an indicator of complement activity and immunological status, and transforming growth factor-β1, as a marker of liver fibrosis, in 16 patients (6 males and 10 females, 2.5–15 years old) who received living-related liver transplantation due to inherited metabolic diseases (n=6; in the form of propionic acidemia [n=1], methylmalonic acidemia [n=1], arginase deficiency [n=1], tyrosinemia [n=2], and glycogen storage disease type 1b [n=1], with an age range of 2.4–14.6 years old) and due to biliary atresia ([n=10], with an age range of 2.9–14.5 years old).

Results: Serum oxidative stress index, complement component-5a, and transforming growth factor-β1 were significantly higher in the inherited metabolic diseases group than in the biliary atresia group. In all patients, serum oxidative stress index correlated positively with complement component-5a and transforming growth factor-β1.

Conclusions: Patients who receive living-related liver transplantation due to inherited metabolic diseases are prone to higher oxidative stress, complement activity, and serum transforming growth factor-β1.

Key words: free radicals • cytokines • biliary atresia

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BACKGROUND

Liver transplantation (LTx) has evolved as the treatment of choice for patients with end-stage liver disease.

The main indications for liver transplantation in the pediatric population include extrahepatic cholestasis, biliary atresia (BA), and inherited metabolic diseases (IMD), e.g., Wilson's disease, α1-antitrypsin deficiency, Crigler-Najjar syndrome, inborn error of bile acid metabolism, tyrosinemia, disorders of the urea cycle, organic acidemia, acid lipase defects, and disorders of carbohydrate metabolism [1].

Little is known about the inflammatory mediators and oxidative markers in metabolic disease. However, it has been reported that fatal systemic inflammatory response syndrome occurred unexpectedly in an ornithine transcarbamylase-deficient patient following a trial of therapy using adenoviral gene transfer [2]. This suggests that IMD patients may respond vigorously to immunological stimuli.

Liver transplantation leads to activation of the complement cascade in association with the reperfusion of the transplanted liver [3]. Complement anaphylatoxin C5a activates leukocytes through the expression of adhesion molecules [4] and the release of arachidonic acid metabolites, interleukins (IL)s, reactive oxygen species (ROS), and lysosomal and proteolytic enzymes [5]. Transforming growth factor (TGF)-β1, which is a chief cause of liver fibrosis [6], has been proven to enhance the production of extracellular components [7] and the deposition of extracellular matrix by promoting synthesis and inhibiting degradation [8]. Mediators that suppressed TGF-β1 caused less hepatic fibrosis in an animal model [9].

No studies have compared pediatric post-LTx patients by examining the original disease for which they received LTx in terms of their serum oxidative stress markers and terminal complement component (C5a), as indicators of complement activity and immunological status, and TGF-β1, as a liver marker.

Unfortunately, most of the studies related to oxidative stress in liver transplantation in pediatric patients focused on the ROS generated during the liver transplantation procedure to evaluate the effect of the ischemia followed reperfusion (I/R) [10]. Only a few studies focused on oxidative stress in the long-term follow-up of pediatric patients who received LTx [11].

The total hydroperoxide (TH) represents a measure of overall oxidative injury because they are the intermediate oxidative product of lipids, peptide, and amino acids [12,13]. On the other hand, the biological antioxidant potentials (BAPs) represent the total antioxidative activity [11,14,15].

The homeostatic balance between the formation of ROS and their removal by endogenous antioxidant-scavenging compounds has been evaluated in previous studies using the oxidative stress index (OSI) calculated by the ratio TH/BAP [11,14,15]. Oxidative stress occurs when this balance is disrupted by any excessive production of TH or by inadequate antioxidant defenses by lower BAPs [12].

The aim of our previous study was to evaluate the differences in the serum oxidative stress markers,

Table 1. Clinical and laboratory data of the enrolled living-related liver transplantation recipients.

Disease diagnosis	Patient gender (M/F)	Patient age	Donor age	Post-LTx duration (Y.M)	BW (kg)	ABO compatible (Yes/No)	Patient-donor gender matched (Yes/No)	SGPT (IU/L) SGOT (IU/L)	γGTP (IU/L) ALP (IU/L)	LDH (mg/dL) BUN (U/L)
BA* (n=10)	2/8	8.6 (2.9–14.5)	36.1 (24.6–56)	6.6 (0.1–7.4)	20.5 ± 3 (6.7–30)	9/1	7/3	25.6±3 32.7±3.5	28.6±8.9 775±57	248±13.3 11.5±0.9
IMD** (n=6)	4/2	7.7 (2.4–14.6)	31.5 (27–40)	3.4 (0.9–7.1)	15.6 ± 4 (7.4–34)	4/2	3/3	29.7±6.2 42.3±4.4	30±8.7 1077±214	277.7±22.6 14.9±1.4
p value					NS***					

* BA – biliary atresia; ** IMD – inherited metabolic disease; *** NS – none significant. Data presented as mean (range) or mean ±SEM.

C5a, and TGF-β1 between pediatric patients who received living-related LTx (LRLTx) due to inherited IMD and due to biliary atresia (BA) during their regular outpatient follow-up visit.

Material and Methods

Design

This clinical outpatient study measured serum OSI, C5a, and TGF-β1 in pediatric patients who had undergone LRLTx due to inherited IMD and due to BA during their regular outpatient follow-up visit.

Patients

The study included 16 patients (6 males and 10 females), ranging in age from 2.4 to 14.6 years (median age at time of study: 8.7 years) who had undergone LRLT from 9 months to 13 years (median: 5.5 years) prior to the study, at ages ranging from one month to 12.7 years (median age at LRLT: 2 years). The patient clinical data are presented in Table 1.

Sample collection

Blood samples were obtained from all patients between November 2008 and March 2009, during their regular post-LRLT follow-up visits to the Outpatient Clinic for Pediatric Surgery and Liver Transplantation at Fujita Health University Hospital.

Measurements

Each patient's serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), gamma-glutamyl transpeptidase (ãGTP), alkaline phosphatase (ALP) lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) were measured; they were also measured at every follow-up visit. Informed consent for enrollment in the study and for our use of surplus serum samples for study measurements was obtained from the parents of the patients, and serum TH, BAP, C5a, and TGF-β1 levels were measured. The study protocol was approved by the ethical committee and the Fujita Health University review board and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

TH production was measured using a d-ROMs kit (Diacron srl, Proma, Italy) in the free radical analytic system (FRAS), as previously described [12,13]. Briefly, in the presence of iron (which is released from the proteins by an acidic buffer), free radicals are able to generate alkoxyl and peroxyl radicals, as a result of Fenton's reaction. Such radicals are, in turn, able to oxidize an alkyl-substituted aromatic amine (A-NH2, dissolved in a chromogenic mixture), which transforms them into a pink-colored derivative. Finally, this colored derivative was photometrically quantified. The intensity of the developed color is directly proportional to the concentration of TH.

Results were expressed in conventional arbitrary units, called Carr units, equal to a concentration of 0.08 mg/dl of hydrogen peroxide.

BAPs were measured using a commercial assay kit (Diacron srl, Proma, Italy) in the FRAS, as previously described [14,15]. The BAPs test is based on the ability of a colored solution containing a source of ferric (Fe^{3+}) ions adequately bound to a special chromogenic substrate to decolor when Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}), which occurs through the addition of a reducing/antioxidant system.

The ratio of TH to BAP gave the OSI, because the shift of the oxidative/antioxidative balance toward the oxidative side is considered to be oxidative stress [11,16].

Serum C5a was evaluated using the BD OptEIA human C5a ELISA kit II (BD Biosciences, San Jose, CA, USA), specific for human C5a-desArg. The quantitation of C5a-desArg in serum samples should yield a reliable measurement of the level of complement activation that has occurred therein.

Serum TGF-β1 was measured using a specific Quantikine human TGF-β1 ELISA kit (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

The distributions of data were tested using the Shapiro-Wilk test. To compare data between two groups, we used the Mann-Whitney test. Coefficients of relation were analyzed using the Spearman two-tailed test. Data are reported as mean ± standard error of the mean (SEM) unless otherwise noted. Probability values of less than 0.05 were considered significant. All data analyses were performed with the commercially available statistical analysis software package SPSS 14 (Statistical Package for Social Sciences, Chicago, Illinois, USA).

Results

There were no differences between the BA and IMD groups in terms of age, post-LTx duration, donor age, body weight, gender, or recipient-donor gender matching (Table 1).

The serum levels of TH levels showed a tendency to be higher and the serum levels of BAP showed a tendency to be lower in the patients of the IMD group compared to the BA group. The OSI was higher in the patients of the IMD group compared to that in the BA group, (0.054±0.01 *vs.* 0.021±0.002, $p<0.005$) (Figure 1A). The highest OSI were found in the PPA and MMA patients (data not shown).

Serum C5a and TGF-β1 were significantly higher in the patients of the IMD group than those in the BA group (76.42±7.27 pg/ml *vs.* 46.67±3.3 pg/ml and 8077.41±2831.34 pg/ml *vs.* 4179.88±950.33 pg/ml, $p<0.005$ and 0.05, respectively) (Figure 1B, 1C).

In all patients, serum BAP correlated negatively with C5a and TGF-β1 (r=–0.70 and –0.71, $p=0.002$ in both). Serum OSI correlated positively with C5a and TGF-β1 (r=0.73 and 0.60, $p=0.002$ and 0.019, respectively). There were no differences in the laboratory data or liver enzymes between the two groups (Table 1).

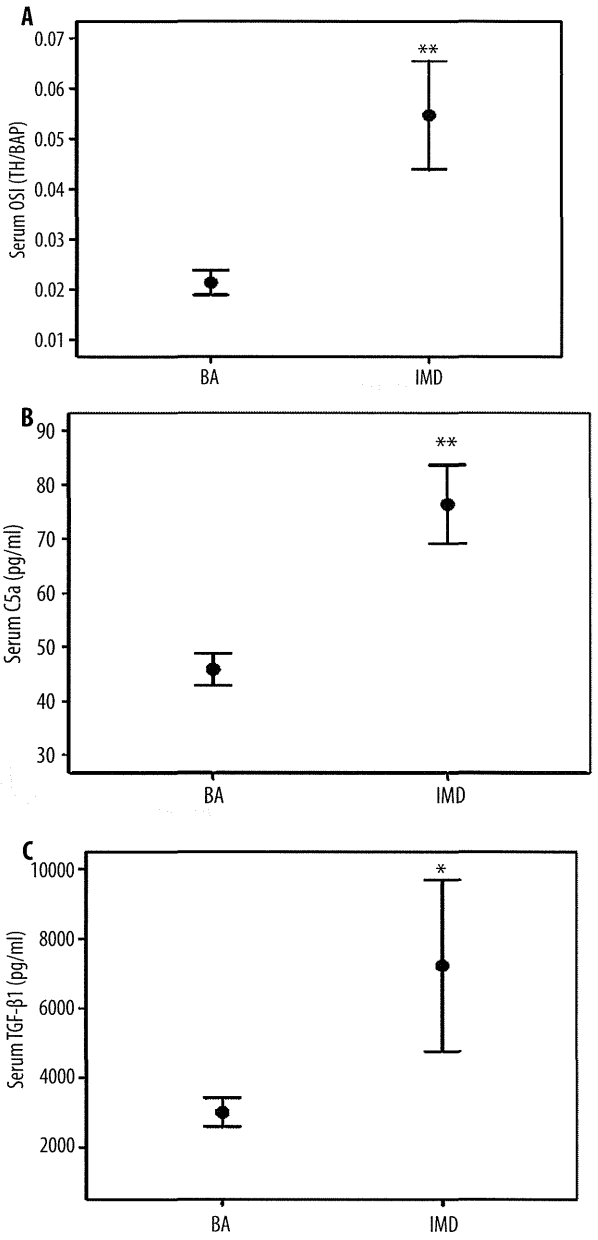

Figure 1. Serum levels of (**A**) OSI (TH/BAP), (**B**) C5a, and (**C**) TGF-1β in patients who underwent LRLTx during their childhood due to BA (n=10) and due to IMD (n=6). * and ** $p<0.05$ and 0.005, respectively.

Discussion

Oxidative stress results from the metabolic reactions that use oxygen; it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells [17–20]. This definition of oxidative stress implies that cells