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ORIGINAL ARTICLE

A morphological study of the removed livers from patients receiving living donor liver transplantation for adult biliary atresia

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Abstract
Background In liver transplantation (LT) for adult biliary atresia (BA), we often encounter a cirrhotic deformation of the native liver. We aimed to investigate a morphological study of the removed livers and the patient's clinical status.
Methods We examined 8 BA patients who had undergone LT in adulthood at our hospital. The presence of hypertrophic or atrophic areas of the removed liver was recorded macroscopically. We graded the microscopic findings in the porta hepatis area, a hypertrophic area, and an atrophic area, respectively. Moreover, we investigated the relationship between these morphological findings and the pre-transplant clinical status (MELD score).
Results Macroscopically, a hypertrophic area existed in central liver in all cases (8/8 cases), while an atrophic area was existed in peripheral liver (7/8 cases). Microscopically, an atrophic area was the most severely impaired, while the porta hepatis and hypertrophic area were relatively intact. The pathological score in a compensatory hypertrophic area was strongly correlated with the MELD score.
Conclusions This study suggests that the partial shrinking is not uncommon in BA cirrhotic liver. It may be due to the imbalance of bile drainage by the different segment. The patient's pre-transplant status depends on the compensatory hypertrophic liver.

Keywords Biliary atresia · Removed liver · Hypertrophy · Atrophy · MELD score

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Introduction

The prognosis of patients with biliary atresia (BA) has improved since the introduction of hepatic portoenterostomy by Kasai and his colleagues in 1959 [1]. Although the majority of patients have decreased or a complete lack of jaundice, and have a normalized liver function test transiently after Kasai's portoenterostomy (KP) [2], most patients unfortunately experience the progression of liver dysfunction related to ongoing liver fibrosis and postoperative complications such as cholangitis, portal hypertension, esophageal varices, and hepatic failure.

Liver transplantation (LT) is the only curative treatment for cirrhotic BA, although some patients are now reaching adulthood with their native liver after treatment with KP. However, most BA patients still have to eventually undergo LT. In LT for adult BA, we often encounter a cirrhotic deformed native liver. The pathogenesis of the deformity of the cirrhotic BA liver is still unclear. In this paper, we aimed to investigate the importance of a long-term biliary drainage by a morphological and clinicopathologic study of the livers removed during living donor liver transplantation for adult BA. Moreover, we also investigated the relationship between the patients' pre-transplant status and the histopathology of the removed livers.

Patients and methods

We examined 8 BA patients who had undergone living donor LT in adulthood (19–28 years old) at Department of Pediatric Surgery in Kyushu University Hospital between October 1996 and December 2009. All removed whole livers at LT were examined. The livers were macroscopically examined for the presence of hypertrophic or atrophic areas. Standard histology blocks were taken from the porta hepatis area, the hypertrophic area, and the atrophic area (about 10 blocks per case). Specimens were stained with hematoxylin and eosin, and the extent and distribution of fibrosis was assessed. The grade of liver damage based on the findings, including intrahepatic inflammation, fibrosis, ductular reaction, and bile congestion, was assessed and scored in a porta hepatis area, a hypertrophic area, and an atrophic area, respectively.

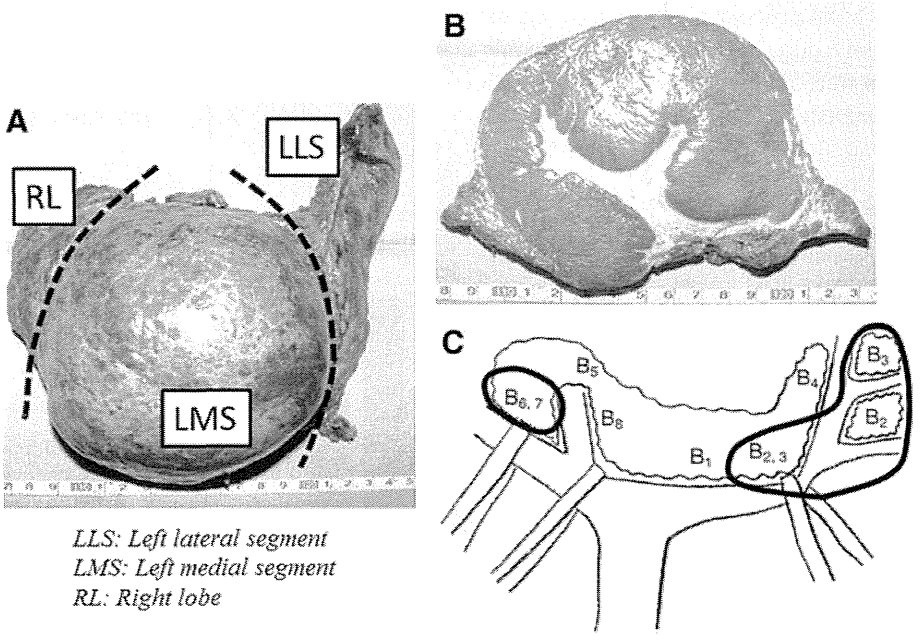
The histological scoring was determined as follows: (1) intrahepatic inflammatory cells proliferation [none: A0/score 0, mild: A1/+1, moderate: A2/+2, and severe: A3/+3], (2) fibrosis [none: F0/0, fibrous dilatation of periportal area: F1/+1, bridging fibrosis: F2/+2, fibrosis with deformed centrilobular construction: F3/+3, and cirrhosis: F4/+4], (3) ductular reaction [none: D0/0, mild: surrounding less than 1/3 of the portal area (D1/+1), moderate: surrounding 1/3–2/3 of the portal area (D2/+2), and severe: surrounding more than 2/3 of the portal area (D3/+3)], (4) bile congestion [none: B0/0, mild (in hepatocytes): B1/+1, moderate (in the ductular area): B2/+2, and severe (in the bile duct at the centrilobular area): B3/+3.] This pathological grading as for A and F is a standard classification described in the previous report [3], while the grading system as for D and B is our original classification. These histological features thought to be important in the evaluation of a cholestatic liver damage were selected according to the previous report [4]. These scoring were reviewed blindly by two pathologists.

Moreover, we investigated the relationship between these morphological findings and the patient's pre-transplant clinical status (model for end-stage liver disease, MELD score) which is calculated by serum creatinine, bilirubin and international normalized ratio (INR) of prothrombin time as described in the previous literature [5]. In this study, the MELD score was calculated in all cases by the blood test performed just before LT. This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan on July 30, 2003.

Results

Macroscopic findings in the removed livers

Macroscopically, all removed livers were able to be clearly divided into atrophic and compensatory hypertrophic areas as shown in Fig. 1. The macroscopic findings in all cases are summarized in Table 1. The atrophic areas exist in the right anterior segment (RAS) + right posterior segment (RPS) + left lateral segment (LLS) in three cases, only LLS in three cases, RAS + RPS in one case, and LLS + left medial segment (LMS) in one case. On the



LLS: Left lateral segment
LMS: Left medial segment
RL: Right lobe

Fig. 1 **a** A deformed liver removed at liver transplantation in adulthood. The liver shows markedly atrophic in the right lobe and left lateral segment, and remarkable hypertrophy in left medial segment. **b** A divided surface shows the two distinct areas. **c** The micro bile duct orifice from each area at the hilum. The orifice from the atrophic peripheral liver locates the bilateral sides at the hilum

Table 1

Case	Age at LT (year)	Sex	Atrophic area	Hypertrophic area
1	19	F	LLS, RAS, RPS	LMS
2	23	F	LLS, RAS, RPS	LMS
3	25	M	LLS, LMS	RAS, RPS
4	24	F	LLS	RAS, RPS
5	23	M	LLS	RAS
6	28	F	LLS	LMS
7	27	F	RAS, RPS	LLS, LMS
8	28	F	LLS, RAS, RPS	LMS

LLS left lateral segment, *LMS* left medical segment, *RAS* right anterior segment, *RPS* right posterior segment

other hand, the hypertrophic areas were present in LMS in four cases, RAS + RPS in two cases, RAS in one case, and LLS + LMS in one case. The atrophic area was generally located in peripheral liver like LLS (7/8 cases). The hypertrophic area was found in central liver such as LMS and RAS in all cases (8/8 cases).

Microscopic findings in the removed livers

Microscopically, the cirrhotic changes in the peripheral liver with various sizes of nodules separated by fibrous bands with a mild chronic inflammatory infiltrate in the stroma, and marked loss of the interlobular bile duct and slight cholestatic changes were also seen. On the other hand, the histology of the hypertrophic central liver indicates the diffuse regenerative hyperplasia of hepatocytes and a relatively normal appearance. The mean score in each area with regard to the four parameters is shown in Fig. 2. Microscopically, the atrophic areas were the most severely impaired, and the hypertrophic and porta hepatis areas were relatively normal grade with regard to all four parameters.

The relationship between the microscopic findings in the removed livers and the patients' pre-transplant status (MELD score)

The microscopic pathological scores in the hypertrophic area and MELD score in all cases are summarized in Fig. 3a. A significant positive correlation between the sum of the four pathological scores in the compensatory hypertrophic area and MELD scores was found (Fig. 3b).

Discussion

More than 50 years have passed since Kasai and his colleagues [1] introduced hepatic portoenterostomy as an option for biliary drainage in patients with non-correctable types of BA. Although KP has been accepted worldwide as the primary operation for treating non-correctable BA, long-term follow-up data have shown evidence of progressive liver disease in a high percentage of patients [6]. For example, Shinkai et al. [6] reported that at least half of the adult survivors who had received KP would require LT in the future because they already had liver cirrhosis at age 20. Therefore, some researchers do not consider KP to be a curative treatment by itself, but rather a bridge to LT [7].

In LT for adults with BA, we often encounter a cirrhotic deformed liver. In the present study, all removed livers were morphologically divided into two distinct areas: the atrophic area was located in the peripheral liver like LLS (7/8 cases) and the hypertrophic area was in the central liver such as LMS and RAS in all cases (8/8 cases). This result is supported by the previous published data [8–10]. Yeung et al. [8] reported that the hepatectomy specimens showed a central zone of relatively normal parenchyma and a peripheral zone of cirrhosis. Takahashi et al. [9] reviewed five livers from patients undergoing OLT for BA. All explants showed a hypertrophic perihilar region in segment 4 with near normal bile ducts and atrophic, fibrotic areas

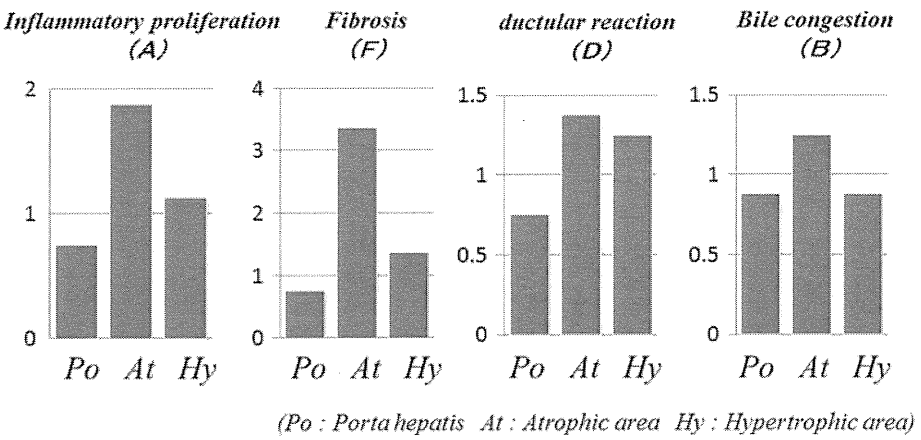
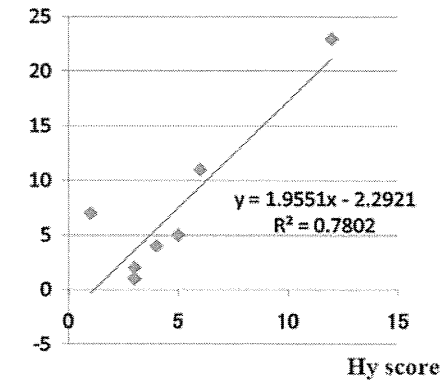


Fig. 2 The mean pathological scores of the removed livers. The atrophic area shows the highest score in all parameters [inflammatory cells proliferation (*A*), fibrosis (*F*), ductular reaction (*D*), bile congestion (*B*)], followed by hypertrophic area and porta hepatis. In hypertrophic and porta hepatis areas, the score demonstrated nearly normal compared to the atrophic area

A

case	Hy score	MELD score
1	3	1
2	3	2
3	5	5
4	3	1
5	4	4
6	1	7
7	12	23
8	6	11

B



(Hy : Hypertrophic area)

Fig. 3 The relationship between the histological scores and the MELD scores. **a** The sum of pathological scores (*A, F, D, B*) in the compensatory hypertrophic area and MELD score in each patients. **b** There is a strong correlation between the pathological scores in the compensatory hypertrophic area and MELD score

with ductopenia more peripherally. Ijiri et al. [11] reviewed post-Kasai hepatectomy specimens; a large central regenerative nodule with relatively normal liver architecture was found in three older children and a poorly defined central hypertrophic area in another two cases. Taken all together including our study, the long-term BA cirrhotic liver has two distinct areas in most cases. This partial shrinking pattern is different from the cirrhosis caused by the other etiology, which is mostly totally shrunken. This different shrinking pattern would come from the imbalance of bile drainage by the different areas.

In the present microscopic study, the pathological scores were worse in the atrophic area than in the hypertrophic and the porta hepatis areas. Interestingly, the bile congestion is not always diffusely occurred, the atrophic area showed the most severe. In other words, there is an imbalance of bile drainage. From this finding, the following two explanations would be discussed. The first is the inherent regional differences in the central versus peripheral BA liver and the second is the differences of bile drainage after KP from two areas. Ductopenia is well documented in liver biopsies from patients with BA after KP. Kasai et al. showed that intrahepatic ductopenia was progressive with increasing age, it is the character of BA liver and may progress inherently earlier in the peripheral liver. Regarding the bile drainage after KP, many pediatric surgeons have historically paid a lot of attentions to the technical aspects of KP procedure.

The extent of dissection at KP may be important for the biliary drainage. However, the optimal depth and extent of fibrous tissue transection and the site of suturing placement are still controversial. Many pediatric surgeons tried modifying the original KP, whose fibrous tissue dissection and anastomosis area were actually quite shallow and limited, to improve results, the anastomosis had been extended laterally in order to drain from the lateral micro bile ducts [12, 13]. However, recently, the extended lateral wide dissection has been thought to cause more injury to micro bile ducts and could in fact only worsen the risk for injury. Superina et al. [14] reported that the extent of hilar dissection had no effect on the KP outcome. Moreover, the recently reported outcome of laparoscopic KP with narrow hilar dissection shows good jaundice clearance [15]. Although we are not able to conclude the optimal extent of transection from only our results, the long-term bile drainage especially from the peripheral liver was thought to be a critical problem at KP.

In the cirrhotic liver, the hypertrophic segment must be a functional area, because this area had nearly normal histology. It is interesting to note that the pathological scores in the compensatory hypertrophic area demonstrated a significant correlation with the MELD scores in the present study. MELD score is generally used as a prognostic indicator for patients with advanced chronic liver disease waiting for LT on the United Network for Organ Sharing (UNOS) waiting list [5]. To our knowledge, this is the first report that demonstrated the significant correlation between the pathological findings in the compensatory hypertrophic area of the cirrhotic liver and the MELD score. The removed BA livers in infancy or early childhood are not always divided into two distinct areas such as the present adult cases and look diffusely shrunken. In other words, only the "successful" adult KP cases could have the compensatory area and show the lower MELD score.

In conclusion, the livers removed at LT for adult BA generally showed the atrophic area to be localized in peripheral liver. It has clinical implications that the morphological cirrhotic pattern after KP was similar in all the present cases. Although we are not able to conclude the optimal extent of transection from only our results, the long-term bile drainage especially from the peripheral liver might be a key factor. In addition, from our result of

MELD analysis, we could conclude that the histological status of the compensatory hypertrophic area in "successful" adult BA liver was a predictive factor of the necessity of liver transplantation.

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CASE REPORT

Hepatic osteodystrophy complicated with bone fracture in early infants with biliary atresia

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Abstract

Biliary atresia (BA) is one of the major hepatobiliary abnormalities in infants and one of the causes of hepatic osteodystrophy. Bone disease may be caused by the malabsorption of calcium and magnesium by vitamin D in hepatobiliary diseases in which bile flow into the intestines is deficient or absent. Bone fracture before Kasai hepatic portoenterostomy or within one month after the procedure in an infant with BA is very rare. We herein report two infants: one infant with BA who initially presented with a bone fracture before Kasai hepatic portoenterostomy, and the other at 4 wk after Kasai hepatic portoenterostomy, and also provide a review of the literature. Moreover, we conclude that clinicians should consider BA in infants with bone fracture during early infancy.

Key words: Biliary atresia; Bone fractur; Hepatic osteodystrophy; Kasai hepatic portoenterostomy; Vitamin D deficiency

Peer reviewers: Qiang Liu, PhD, Vaccine and Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road, Saskatoon, Saskatchewan, S7N 5E3, Canada; Pietro Invernizzi, MD, PhD, Division of Internal Medicine and Hepatobiliary Immunopathology Unit, IRCCS Istituto Clinico Humanitas, via A. Manzoni 113, 20089 Rozzano, Milan, Italy

Okada T, Honda S, Miyagi H, Minato M, Taketomi A. Hepatic osteodystrophy complicated with bone fracture in early infants with biliary atresia. *World J Hepatol* 2012; 4(10): 284-287 Available from: URL: http://www.wjgnet.com/1948-5182/full/v4/i10/284.htm DOI: http://dx.doi.org/10.4254/wjh.v4.i10.284

INTRODUCTION

Clinical findings in children with biliary atresia (BA) characteristically include jaundice and acholic stools at 1 or 2 mo after birth[1]. Osteodystrophy is a well-recognized complication of chronic liver disease. BA is one of the major hepatobiliary abnormalities in infants and one of the causes of hepatic osteodystrophy[1].

Vitamin D is hydroxylated at the carbon 25 position to form 25-hydroxy-vitamin D (25-OH-D)[2]. This occurs primarily in the liver[2]. Bile is important for the intestinal absorption of calcium and magnesium because it is necessary for the absorption of vitamin D[1].

In chronic liver disease, particularly where there is chronic cholestasis, generalized skeletal demineralization or rachitic change is seen[3]. Multiple spontaneous fractures of both the ribs and long bones have been reported in such infants. Furthermore, bone fractures are sometimes noted in patients with BA in the end-stage before liver transplantation[4]. However, bone fracture before Kasai hepatic portoenterostomy and within one month after the procedure in infants with BA is very rare.

We report two infants: firstly, a patient with BA who initially presented with bone fracture before Kasai he-

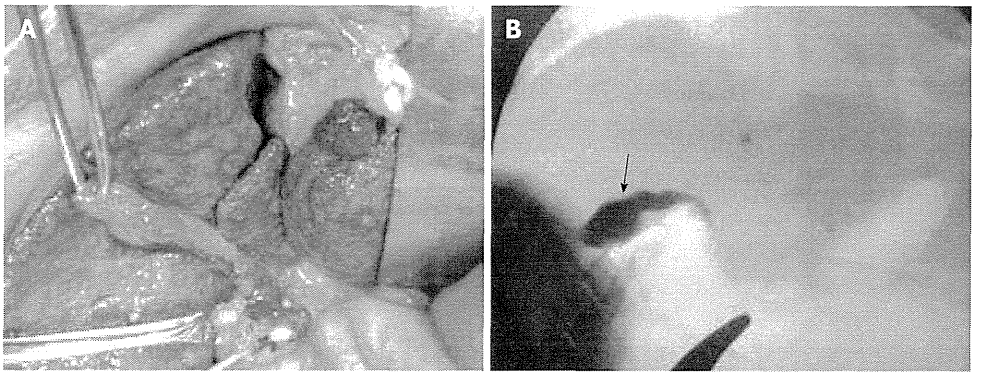

Figure 1 Intraoprative and imaging features. A: On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis; B: Intraoperative cholangiography revealed a patent gallbladder (arrow) and no patency of the extrahepatic bile duct.

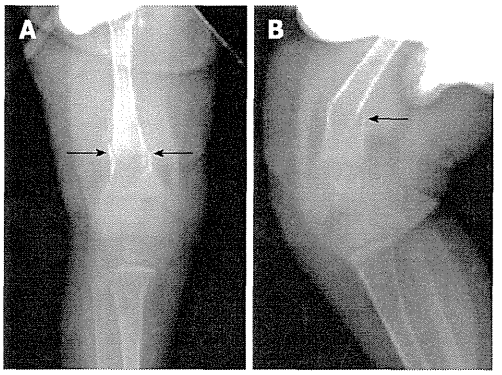

Figure 2 Plain skeletal radiographic features at the 7 d after hepaticojejunostomy in the case 1. Anteroposterior (A) and lateral (B) plain radiographs showing a displaced fracture (arrows) of the right distal femur.

patic portoenterostomy, and secondly, a patient with the onset of bone fracture within one month after Kasai hepatic portoenterostomy, and also provide a review of the literature.

CASE REPORT

Case 1

A girl was born vaginally at 39 wk gestation, weighing 2522 g. She presented with neither jaundice nor acholic stools. The infant was fed human milk. She was well nourished but was observed to have jaundice at a medical check-up at 1 mo of age. Abdominal ultrasonography (US) and computed tomography showed a sufficiently large gallbladder. Total and direct bilirubin (DB) decreased gradually at the follow-up checks. The patient presented with acholic stools and increased jaundice at the age of 5 mo, and was subsequently admitted to our institution for further examinations. Laboratory studies upon admission revealed the following: asparate aminotransferase (AST) 337 IU/L (normal range), alanine aminotransferase (ALT) 241 IU/L (normal range), total bilirubin (TB) 11.3 mg/dL, DB 7.4 mg/dL, alkaline phosphatase (ALP) 5,547 IU/L (normal range), γ-glutamyl transpeptidase (γ-GTP) 457 IU/L (normal range), choline esterase 192 IU/L (normal range), and serum calcium 8.1 mg/dL (normal range). There was severe jaundice noted at the conjunctiva. The findings on abdominal US were unevenness on the liver surface and an atrophic gallbladder which did not contract after the feeding of milk. Magnetic resonance cholangio-pancreatography (MRCP) revealed dilatation of neither the common bile duct nor intrahepatic bile duct. Therefore, BA was suspected based on these findings, and the infant underwent an exploratory laparotomy at 182 d of age. The patient started oral vitamin D at 173 d of age.

On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis (Figure 1A). Intraoperative cholangiography revealed a patent gallbladder and no patency of the extrahepatic bile duct (Figure 1B). The macroscopic findings showed that the bilateral hepatic ducts and extrahepatic bile duct consisted of only remnants. The infant was diagnosed as BA (IIb1γ)[5] based on cholangiographic and macroscopic findings. The remnants were totally removed en block and a Roux-en-Y hepaticojejunostomy was performed with a Roux loop of 60 cm applied antecolically. Microscopic findings of the liver biopsy specimen were pre-cirrhotic.

The patient could not move her right leg 1 d before the laparotomy, and a plain skeletal radiograph of the femur was performed 7 d after the HJ, when the general condition of the patient was stable. A displaced fracture of the right distal femur was shown by the plain radiograph (Figure 2A and B). Hepatic osteodystrophy was suspected based on the fact that there was no history of femur trauma and the patient suffered from chronic cholestasis. Child abuse by the family was not considered from the situation. Callus formation was seen 8 d after the application of an immobilizing plaster bandage (Figure 3A). The plaster bandage was removed after 20 d and the fracture of the right femur was cured at 6 mo post fracture (Figure 3B and C). The patient coughed up blood due to the perforation of esophageal varices and underwent a living-related liver transplantation at 10 mo of age. The postoperative course of living-related liver transplantation was uneventful and she is currently well at 4 years of age.

Case 2

A girl was born vaginally at 36 wk gestation, weighing 2310 g. She presented with neither jaundice nor acholic stools. She was well nourished but was observed to have

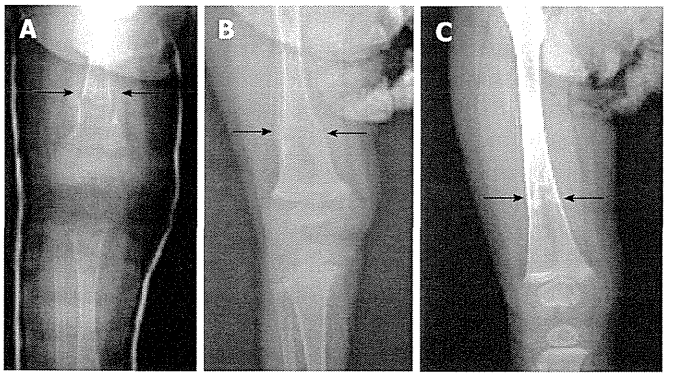

Figure 3 Plain skeletal radiographic features at the 8 d after the application of an immobilizing plaster bandage for the femur fracture in the case 1. Callus formation (arrows) was seen 8 d after the application of an immobilizing plaster bandage (A) in case 1. The plaster bandage was removed after 20 d (B) and the fracture of the right femur was cured 6 mo post-fracture (C).

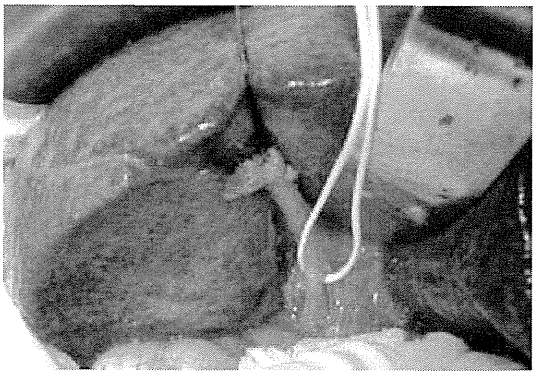

Figure 4 Intraoprative features. On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis.

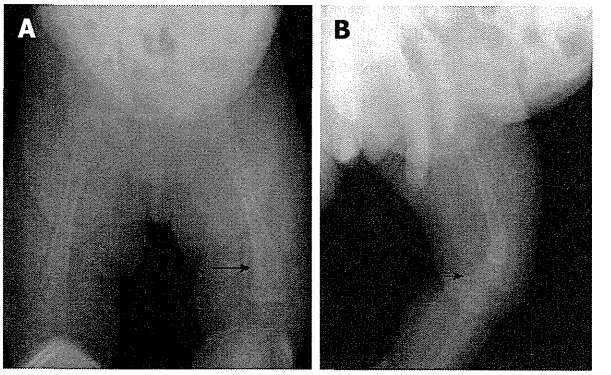

Figure 5 Plain skeletal radiographic features at the 28 d after hepaticojejunostomy in the case 2. Anteroposterior (A) and lateral (B) plain radiographs showing a displaced fracture (arrows) of the left distal femur.

jaundice at a medical check-up at 1 mo of age. The patient presented with acholic stools and increased jaundice at the age of 3 mo at a medical check-up, and was consequently admitted to our institution for further examinations. Laboratory studies upon admission revealed the following: AST 573 IU/L, ALT 377 IU/L, TB 6.6 mg/dL, DB 4.4 mg/dL, ALP 2248 IU/L, γ-GTP 666 IU/L, choline esterase 181 IU/L, and serum calcium 9.2 mg/dL. The findings on abdominal US and MRCP were just as same as those of the case 1. Therefore, BA was suspected, and the infant underwent an exploratory laparotomy at 113 d of age. The patient started oral vitamin D at 3 mo of age.

On laparotomy, the liver was brown and firm with a dull edge, suggesting cholestasis (Figure 4). Intraoperative cholangiography revealed a patent gallbladder and no patency of the extrahepatic bile duct. The infant was diagnosed as BA (II b1γ)[5] based on cholangiographic and macroscopic findings. The remnants were totally removed en block and a Roux-en-Y hepaticojejunostomy was performed with a Roux loop of 60 cm applied antecolically. Microscopic findings of the liver biopsy specimen were cirrhotic.

The patient could not move her left leg at 28 d post-laparotomy. A displaced fracture of the left distal femur was shown by plain skeletal radiograph (Figure 5A and B). Hepatic osteodystrophy was suspected based on the fact that there was no history of femur trauma and the patient suffered from chronic cholestasis. Child abuse by the family was not considered from the situation. Callus formation was seen 14 d after the application of an immobilizing plaster bandage. The plaster bandage was removed after 20 d and the fracture of the left femur was cured at 6 mo after post-fracture. Jaundice has been resolved and she is currently well at 11 mo of age.

DISCUSSION

BA is a rare disease with an incidence of approximately 1:10 000 live births in Japan and the Far East[6]. The most frequent symptom is prolonged jaundice. Several reports have shown that osteodystrophy was associated with severe chronic liver disease despite the administration of vitamin and mineral supplements[1]. Argao *et al*[7] suggested that the bone mineral content of patients with hepatic osteodystrophy did not improve despite successful normalization of the serum 25-OH vitamin D concentration by enhancing vitamin D absorption from the gastrointestinal tract. Chongsrisawat *et al*[8] reported that osteoporosis was recognized in up to 80% of a group of jaundiced BA patients in comparison with only 13.6% in a non-jaundiced group.

In BA, metabolic disturbance results from impairment of the passage of bile salts into the alimentary canal. As a consequence, the inadequate emulsification of fat results

in the incomplete absorption of vitamin D. Vitamin D is hydroxylated to 25-OH-D in the liver[2]. Additionally, over the course of the disease, liver cirrhosis develops and the hydroxylation of vitamin D is impaired. Vitamin D and hence calcium absorption are thus diminished. 25-OH-D is thought to be converted to more active forms, 125- or 2125-dehydro-OH-D. Rickets and osteoporosis were reported to be found in 23 of 39 patients (59%) with surgically unrepaired BA[1].

We herein report two infants: one infant with BA who initially presented with a bone fracture before Kasai hepatic portoenterostomy, and the other at 4 wk after Kasai hepatic portoenterostomy. There are a number of factors which may be important in the etiology of bone fractures in children, including trauma, metabolic bone disease, drugs, and immobilization[3]. However, the lack of significant trauma in the majority of cases (91%) is a notable feature in children with BA[3]. Hill *et al*[3] reported 12 (19%) children with fractures before and after transplantation out of 63 undergoing liver transplanatation. Eight of 12 children with fractures in BA had no identifiable trauma. The age at the time of fracture in BA ranged from 3 to 16 mo after birth, and the affected children suffered from osteopenia (generalized reduction in bone density). The fracture site was the ribs or long bones, and multiple fractures were seen in 2 children with BA (7 and 8 mo after birth). However, Hill *et al*[3] did not describe administering vitamin D supplements. BA patients with severe cholestasis have a risk of bone fracture despite the administration of essential vitamins and minerals such as our cases. In our cases, BA was diagnosed at 6 mo after birth in case 1 and at 3 mo after birth in case 2, with suspected severe cholestasis.

Conservative management such as immobilization using plaster bandages is generally effective for fractures in BA, and there were no complications related to fractures in our cases. In the literature, internal fixation was required in one case with oxalosis for a fractured neck of the femur[1]. The early diagnosis and treatment of BA before the occurrence of bone fracture is important. The measurement of reflected light from the surface of feces by near infrared reflectance spectroscopy was introduced by Akiyama *et al*[9] for the differential diagnosis of cholestatic diseases in infants. Another method, mass screening using color picture cards depicting normal and acholic stools, was carried out at 1 and 2 mo after birth in a Japanese prefecture[10]. Eight cases of BA were detected using this mass screening method during a 3-year period, with a specificity of 99.9% and a sensitivity of 80.0%. Such screening procedures could result in improved detection of BA in infants before bone disorders occur.

In summary, clinical awareness of BA should be maintained both in terms of careful handling to prevent possible bone fracturing and also in considering fractures as a possible diagnostic factor in children with reluctance to use a limb, even in the absence of previous trauma, before Kasai hepatic portoenterostomy. Radiological awareness is also important to avoid missing unsuspected fractures on radiographs.

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Risk factors affecting late-presenting liver failure in adult patients with biliary atresia

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Key words:
Biliary atresia;
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Liver failure;
Operative age;
Cholangitis

Abstract

Purpose: Following the Kasai operation, a number of patients have developed liver failure, even after long-term postoperative courses. We assessed the clinical parameters to clarify the early risk factors affecting late-presenting liver failure in biliary atresia.

Materials and Methods: From 1955 to 1991, 277 patients underwent a Kasai operation. Among those patients, 92 survived with their native liver for more than 20 years, and 72 continue to survive with their native liver in good condition (Group 1). In 20 patients, persistent jaundice recurred after the age of 20 years (Group 2). The postoperative courses of these patients were assessed retrospectively, and the clinical parameters, including age at the time of the Kasai operation (AGE, days), the period required for jaundice to disappear (PJD, days), and the association with early cholangitis (CG), were compared between the 2 groups.

Results: Of the 20 patients in Group 2, 8 survived after a liver transplantation (LTx). Eight patients had recurrent jaundice, including 4 on the waiting list for anLTx. Additionally, 2 patients died after anLTx at the ages of 22 and 39. Another patient died of liver failure at the age of 28. One patient died of massive esophageal variceal bleeding at the age of 29. Significant differences were confirmed with respect to AGE (Group 1 < Group 2, $p < .001$), PJD (Group 1 < Group 2, $p < .001$), and CG (Group 1: Group 2 = 47 %: 75 %, $p = .028$).

Conclusions: A considerable number of adult patients developed liver failure, even after the age of 20 years. AGE, PJD, and CG were found to be risk factors affecting late-presenting liver failure. Therefore, close patient follow-up is essential, especially for long-term survivors with a late operative age and early postoperative complications.

The Kasai operation has been a first-line modality of treatment for biliary atresia since its development in the 1950s. A considerable number of patients, however, have required liver transplant during the postoperative course. According to a recent report from the Japanese Biliary Atresia Registry, approximately 40% of patients required a liver transplant (LTx) within the 5 years following a Kasai operation [1]. The remaining patients survived for a longer period with their native livers. Among those patients, a number who were older than 20 years developed liver failure. Predicting the long-term prognosis is important to

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Table 1 Patient characteristics.

	Group 1, n = 72	Group 2, n = 20	*P*
Male:female	29:43	10:10	ns
Median age * (y/o)	31	30.5	ns
Type of obstruction			
I	19	6	
II	5	2	ns
III	48	12	
Reconstruction			
Suruga II	27	3	
Kasai's Double Y	19	9	ns
Original Kasai	10	5	
Others	16	3	

* Median of the current ages of native-liver survivors. In Group 2, the ages at the time of death or at the time of LTx were used for patients who died or underwent an LTx.

offer the appropriate management to patients who require lifelong follow-up. Thus, in this study, we assessed the clinical parameters to clarify the early risk factors affecting late-presenting liver failure in biliary atresia.

1. Materials and methods

This retrospective study was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine. From 1955 to 1991, 277 patients underwent a Kasai operation in the Tohoku University Hospital. Among these patients, 92 survived with their native liver for more than 20 years, and 72 (43 females and 29 males) continue to survive with their native liver in good condition (Group 1). However, 20 patients (10 females and 10 males) developed a recurrence of persistent jaundice after the age of 20 years (Group 2). Patients who transiently developed jaundice due to cholangitis followed by complete recovery were assigned to Group 1. The median values of the current ages of the native-liver survivors are 31 and 30.5 years in Groups 1 and 2, respectively. In Group 2, the ages of the native-liver survivors and the ages at the time of death or at the time of LTx in the patients who died or underwent an LTx were used to find the median age of this group. The patients in Group 1 were classified as follows: 19 patients were type I, atresia of the common bile duct; 5 were type II, atresia of the hepatic duct; and 48 were type III, atresia at the porta hepatis. In Group 2, 6, 2 and 12 patients were type I, type II and type III, respectively (Table 1).

Various modifications were employed for the biliary reconstruction in each group at the time of the Kasai operation. In Group 1, the Suruga II procedure (total external biliary conduit), Kasai's Double-Y procedure (partial external biliary conduit), and Original Kasai procedure (simple Roux-en-Y) were performed in 27, 19 and 10 patients, respectively (Fig. 1). Sixteen patients underwent other modifications, which included hepatic portocholecystostomy, hepatic portoduodenostomy and hepaticojejunostomy. In Group 2, Suruga II, Kasai's Double-Y procedure, the Original Kasai procedure and others were performed in 3, 9, 5 and 3 patients, respectively (Table 1).

The postoperative courses of these patients were assessed retrospectively, and the age at the time of the Kasai operation (AGE, days), the period required for jaundice to disappear (PJD, days), and the association of early cholangitis (CG) were compared between the 2 groups.

The PJD was defined as the number of days between the date of the Kasai operation and the date when the serum total bilirubin level fell below 2.0 mg/dl for the first time following the Kasai operation. The PDJ was available for 63 patients in Group 1 and 16 patients in Group 2. The significant difference between the PJD in both groups was assessed using the data from these 79 patients.

CG was defined as the number of episodes of cholangitis during the first postoperative year following the Kasai operation.

The incidences of portal hypertension that required treatment before the age of 20 years were compared between Groups 1 and 2.

The statistical analysis was performed using the chi-square test or the Mann–Whitney U test, as appropriate. $P < .05$ was considered to be significant.

Most patients were followed in Tohoku University Hospital or its affiliated hospitals in the Tohoku district (the northeastern area of Japan). For those patients who moved far from the Tohoku area during the postoperative period, the pediatric surgeons of the regional hospital where the patient lived were asked to perform the follow-up, and

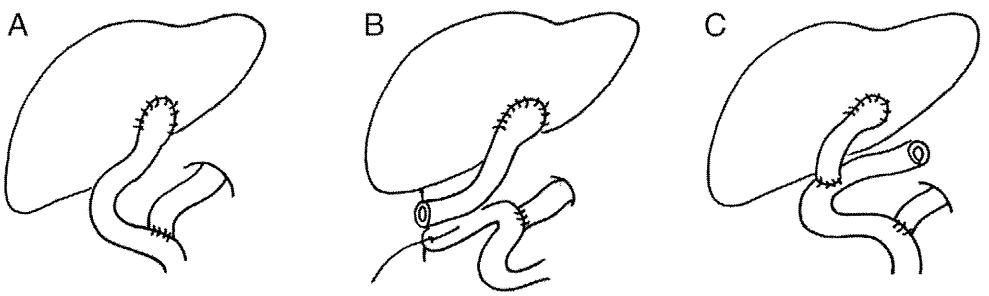

Fig. 1 Modifications of biliary reconstruction. Original Kasai Roux-en Y (A), Suruga II (B), and Kasai's double Roux-en Y (C).

Table 2 Patients in Group 2 who died or underwent an LTx.

Patient #	Sex	Current status	Current age (y/o)	Age at LTx or death (y/o)	Donor type	Cause of death
1	M	Dead	-	28	-	Liver failure
2	F	Alive	48	33	Living	-
3	F	Alive	46	44	Living	-
4	M	Alive	40	26	Living	-
5	M	Dead	-	39	Living	Portal thrombosis
6	F	Alive	39	37	Living	-
7	F	Alive	37	24	Living	-
8	F	Dead	-	21	Living	Pulmonary hypertension
9	M	Alive	36	28	Living	-
10	M	Alive	35	31	Cadaveric	-
11	F	Alive	35	28	Living	-
12	M	Dead	-	29	-	Variceal bleeding

the information was obtained from the patient or his/her family by mail or telephone in these cases.

2. Results

Of the 20 patients in Group 2, 8 survived after an LTx. Eight patients had recurrent jaundice, including four who were on the waiting list for an LTx. Two patients died after the LTx due to complications at the ages of 22 and 39. Another patient died of liver failure at the age of 28. The remaining patient died of massive esophageal variceal bleeding at the age of 29 (Table 2).

The statistical analysis using the Mann–Whitney U test revealed a significant difference in the AGE between Groups 1 and 2 ($P < .001$, Fig. 2). Of the 13 patients who underwent the Kasai operation before 7 weeks of age, only 1 patient (7.7%) belonged to Group 2, whereas 5 patients (33%) of the 15 patients operated at 80 days of age or later belonged to Group 2.

A significant difference in the PJD between the 2 groups was also confirmed ($P < .001$, Fig. 3). Although the 36 patients with a PJD of 60 days or less included 4 from Group 2 (11%), 24 patients with more than 100 days of PJD included 8 patients from Group 2 (33%).

CG developed in 34 (47%) and 15 (74%) patients in Groups 1 and 2, respectively ($P = .028$, Table 3).

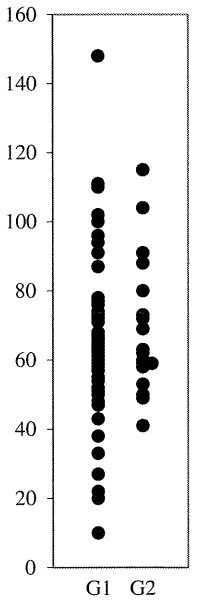

Fig. 2 Scatter diagram of the age at Kasai operation. The significant difference in the distribution of the age at Kasai operation between group 1 (G1) and group 2 (G2) was confirmed by the Mann–Whitney U test ($P < .001$).

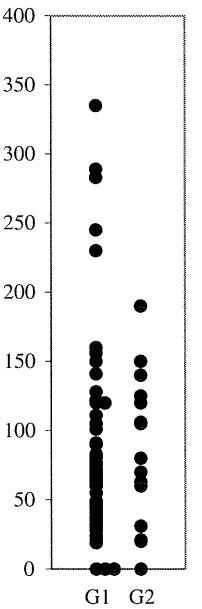

Fig. 3 Scatter diagram of PJD. The significant difference in the distribution of PJD between group 1 (G1) and group 2 (G2) was confirmed by the Mann–Whitney U test ($P < .001$).

Table 3 The impact of early cholangitis and portal hypertension on late-presenting liver failure.

	Group 1, n = 72	Group 2, n = 20	p
CG: Cholangitis in the 1st year (yes:no)	34:38	15:5	.028
Portal Hypertension * (yes:no)	12:60	8:12	.025

* The number of patients who required treatment for portal hypertension before 20 years of age.

Portal hypertension, such as esophageal varices and hypersplenism, was treated in 12 (17%) and 8 (40%) patients before the age of 20 years in Groups 1 and 2, respectively ($p = .025$, Table 3). In Group 1, a patient underwent endoscopic injection sclerotherapy (EIS) for esophageal varices, 7 underwent partial splenic embolization (PSE), and 4 underwent both procedures. In Group 2, 5 patients underwent EIS, and 3 underwent PSE.

3. Discussion

The outcome of the Kasai operation for biliary atresia has greatly improved in recent decades, and long-term survivors with their native livers are becoming more common throughout the world. In this study, the 20-year native-liver survival rate at Tohoku University Hospital was 33% (92/277). This rate increased to 44% (76/173, data not shown) in patients who underwent the procedure after 1972, when the importance of an early Kasai operation was established, and the operative technique was standardized at our institution.

Shinkai et al. [2] reported a 20-year native-liver survival rate of 44% (35/80) for patients treated at Kanagawa Children's Medical Center between 1971 and 1986. Outside Japan, de Vries et al. [3] and Lykavieris et al. [4] reported 20-year native-liver survival rates of 23% and 27% from the Netherlands and France, respectively. Long-term native-liver survivors over 20 years of age are currently increasing in number. However, various severe complications have been reported, even among long-term survivors (> 20 years) [2,5].

The most serious complication was late-presenting liver failure, and the last resort for this condition was liver transplantation. Although cadaveric-donor transplantation was legalized quite recently in Japan, it has been difficult to employ for mainly social reasons. As such, patients are still dependent on living-relative transplantation. In this series, of the 10 recipients who required an LTx, only 1 underwent a cadaveric-donor LTx. One of the problems related to living-donor LTx was the limited availability of donors, especially for adult recipients. For example, the advanced age of the parents, who were the main donor source, and the inappropriate size and/or condition of the graft were occasionally encountered problems that were notably difficult to manage. From these unique circumstances in Japan, the importance of predicting a patient's long-term outcome to provide the best management is evident. In this study, we analyzed the significance of several perioperative clinical parameters of the Kasai operation as predictors of long-term prognosis.

Only a few reports have examined the predictors of the long-term (> 20 years) prognosis of the Kasai operation. Lykavieris et al. [4] reported the importance of an early Kasai operation to achieve a long-term good outcome and demonstrated a similar result to the current study. Davenport et al. [6] reported the limited usefulness of age at the time of surgery as a predictor of the long-term outcome. Although the operative age might be a predictor of the long-term prognosis, its significance varied among researchers. The significance of this age is still an issue of debate, and further study is required to draw a conclusion.

The type of obstruction is hypothesized to be related to the prognosis. The rate of jaundice disappearance in the patients with type I biliary atresia (common bile duct) following the Kasai operation is much better than that in type III biliary atresia (porta hepatis) (type II is rare). Consequently, the overall survival rate of patients with type I biliary atresia is higher than patients with type III biliary atresia. However, an analysis of adult patients revealed that patients with both types of biliary atresia developed severe complications equally after surviving for 20 years or more [7]. Therefore, the type of obstruction is certainly a prognostic factor, but it does not predict the ultimate outcome for long-term survivors (> 20 years).

To the best of our knowledge, although the relationship between the early postoperative response to the Kasai operation and the short- to medium-term prognoses has been reported [8], our study is the first analysis in which only native-liver survivors of adult age were used as subjects.

In this study, the age at the time of surgery, the time required for jaundice to disappear, and the association with early cholangitis were all useful predictors of long-term survival. An uneventful postoperative course during the early phase following the Kasai operation had a significant effect on both the short-term prognosis and the long-term prognosis over 20 years. However, whether these factors are independent of each other is unknown. A multivariate analysis using a larger number of patients is still required because the sample size of the current study was too small to perform such an analysis appropriately.

In Group 2, 4 patients eventually died. One of the patients who died of liver failure at 28 years of age was reported by Kasai et al. [9] in 1988, when LTx was rarely available in Japan. One patient died of deterioration of pulmonary hypertension associated with sepsis after the LTx at the age of 21 years. Before the LTx, the patient's pulmonary hypertension, which had been associated with liver cirrhosis and portopulmonary hypertension, had already advanced without conspicuous pulmonary symptoms, such as dyspnea

or cyanosis. The patient's pulmonary condition should have been more carefully monitored.

Two patients died at the ages of 39 and 29 years. These patients had not participated in our follow-up regimen, including diagnostic imaging and intermittent endoscopy, because they had moved far from our hospital. The patients associated with a poor preoperative condition died after an LTx, due to multiple complications, and another patient who developed recurrent jaundice subsequently died of massive esophageal bleeding. These patients could have survived if an LTx had been indicated early and under better general conditions following the endoscopic treatment of the esophageal varices, if required.

Several reports have evaluated the risks related to pregnancy and delivery in female patients with biliary atresia [10,11]. In this series, one patient developed liver failure following her second delivery and ultimately required an LTx at the age of 44. All patients should be carefully followed during pregnancy and after delivery.

More patients in Group 2 required treatment for portal hypertension before the age of 20, such as EIS and PSE. Although portal hypertension is not an early risk factor, it is apparently an important prognostic factor for the long-term results of the Kasai operation.

Of the long-term survivors who developed persistent recurrent jaundice, most patients were associated with multiple cholangitis, due to intrahepatic biliary dilatation and/or intrahepatic stones. However, several patients unexpectedly developed severe cholangitis that led to liver failure without such abnormal findings in the intrahepatic biliary tree [12]. We emphasize the importance of close patient follow-up, paying special attention to the morphological/functional changes of the hepatobiliary system and the findings of portal hypertension [13,14].

Although many long-term survivors with their native livers were leading normal lives at the time of publication, some patients had developed liver failure even past the age of 20 years. Age, PDJ, and CG were found to be early risk factors affecting late-presenting liver failure. Therefore, close patient follow-up is essential, especially for long-term survivors with a late operative age and early postoperative complications.

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ORIGINAL ARTICLE

Post-transplantation lymphoproliferative disorder in living-donor liver transplantation: a single-center experience

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Abstract
Background Post-transplantation lymphoproliferative disorder (PTLD) is a group of life-threatening complications of organ transplantation, which occurs most frequently in pediatric patients. This retrospective study evaluates a single-institution experience of five cases of PTLD after living-donor liver transplantation (LDLT).
Patients and method We reviewed the records of 78 pediatric patients (<18 years old) and 54 adult patients, who underwent LDLT between July 1991 and December 2009.
Result PTLD was diagnosed in five pediatric patients, yielding an overall incidence of 3.8%. There were no significant differences between the pediatric patients with and those without PTLD in terms of their age, sex, reason for transplantation, calcineurin inhibitor, Epstein–Barr virus (EBV) serostatus, ABO compatibility, lymphocyte cross-matching, or episodes of biopsy proven rejection. Two patients with abdominal lymphadenopathy and one with gastrointestinal PTLD responded to a reduction in immunosuppression. Treatment with rituximab was necessary for another gastrointestinal PTLD patient. Diffuse large-B-cell lymphoma was diagnosed in one patient with mediastinal and lung masses. This patient was treated with chemotherapy and rituximab, followed by surgical resection. All patients survived and no evidence of recurrence has been found since.
Conclusion Although PTLD is potentially life-threatening, it can be managed by appropriate and prompt treatment, with a good outcome.

Keywords PTLD · EBV · Liver transplantation · Living donor · Rituximab

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Introduction

Post-transplantation lymphoproliferative disorder (PTLD) is a life-threatening complication of solid organ transplantation. The development of lymphoma after transplantation was first described in 1968, by Doak et al. [1], in a renal transplant recipient, but the term "PTLD" was introduced in 1984, by Starzl et al. [2]. The severe impairment of T-cell function as a result of the immunosuppression required to prevent allograft rejection also places transplant patients at risk of the development of PTLD. Epstein–Barr virus (EBV) plays an essential role in the development of these lymphomas, and impaired T-cell function allows the uncontrolled proliferation of B lymphocytes that are infected and transformed by the virus [3].

However, PTLD is not exclusively associated with EBV infection, because EBV-negative PTLD is also being increasingly recognized, usually developing late after transplantation, [4, 5, 6]. This entity appears to be predominantly restricted to adult recipients [7]. The risk factors for PTLD include the degree of immunosuppression and the development of a primary infection after transplantation, so a higher incidence is seen in EBV-seronegative pediatric patients receiving a transplant from an EBV-seropositive donor [8]. The incidence of PTLD ranges from 1 to 20% among solid organ transplant recipients [9, 10]. Among liver transplant recipients, the prevalence of PTLD ranges from 2 to 4% in adults, but is reported to be as high as 20% in pediatric recipients [7, 11, 12]. However, these studies predominantly analyzed deceased-donor liver transplantation recipients. Interestingly, a recent Japanese study found that the incidence of PTLD after adult-to-adult living-donor liver transplantation was relatively low (0.9%) [13]. Another study reported no PLTD patients among 81 children who underwent living-donor liver transplantation (LDLT) [14].

Histologically, PTLD comprises a heterogeneous group of lymphoproliferative disorders, ranging from reactive, polyclonal hyperplasia to aggressive non-Hodgkin's lymphoma [3]. PTLD characteristically involves extranodal sites, with frequent involvement of the allograft and the gastrointestinal tract [15, 16], but it may occur at virtually any site, including the skin [17] and central nervous system [18]. Although they are not equal to PTLD, primary extranodal non-Hodgkin's lymphomas arising from the common bile duct have been reported [19].

A reduction in immunosuppression is considered as the first-line therapy for PTLD [20, 21]. Monoclonal antibody therapy (rituximab) is also frequently used and now widely regarded as the first-line therapy [22, 23]. Chemotherapy with the regimens used for lymphoma, such as cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), is reserved for patients with more extensive high-grade disease or those in whom initial treatments have failed [3]. When PTLD is confined to one site, radiation and/or surgery can effectively control the local disease [5]. Surgery and radiation also play a role in the management of local complications in vital organ structures.

We report our institutional experience of PTLD in LDLT recipients, focusing on the diversity of PTLD manifestations and the different approaches to treatment.

Patients and methods

The records of 78 pediatric patients (<18 years old) and 54 adult patients who underwent LDLT between July 1991 and December 2009 were reviewed retrospectively. This study was approved by the Ethics Committee of Tohoku University Hospital. All patients had given their informed consent for all procedures and treatments. The primary immunosuppression regimen consisted of a calcineurin inhibitor (tacrolimus or cyclosporine) and steroids. In the early 1990s, we had no protocol for discontinuing steroid therapy, but in the late 1990s, steroid therapy was ceased 12 months after transplantation by design, and from 2000 onwards steroid therapy was discontinued around 6 months after transplantation. The majority of patients transplanted before 1998 and a few patients transplanted after 1999 received azathioprine, calcineurin inhibitor, and steroids. Mycophenolate mofetil was given to some patients. Forty-five adult patients and three pediatric patients transplanted after December 2002 received induction therapy with anti-interleukin 2 (IL2) receptor antibody. Rejection episodes were treated with methylprednisolone pulse therapy. Deoxyspergualin [24] or murine monoclonal anti-CD3 antibody (OKT3) was given when steroid treatment failed.

In patients with suspected EBV infection, we performed quantitative real-time PCR to detect EBV DNA in their peripheral-blood mononuclear cells and/or serum (cut-off value $>10^{2.5}$ copies/μg DNA) [25]. When an elevated EBV DNA load was detected, we performed a lymph-node biopsy. If there was no safely accessible lymph node, we made all decisions based on a comprehensive assessment of clinical symptoms, EBV DNA load, and images.

Statistical analysis

Comparative statistical analyses were performed using the Mann–Whitney U test. The χ^2 and Fisher's exact tests were used for categorical variables. $p < 0.05$ was considered significant.

Results

Incidence and presentation of PTLD

The overall incidence of PTLD in our institutional experience was 3.8% (5/132). All five patients in whom PTLD was diagnosed were children under 6 years old at the time of transplantation. Thus, the incidence of PTLD in the adult and pediatric patients was 0 and 6.4%, respectively. The overall median time to the development of PTLD was 14 months (4–31 months; Table 1).

The initial symptom of PTLD in all five patients was fever. Two patients suffered gastrointestinal symptoms, including diarrhea, and one patient with large pulmonary and mediastinal masses suffered respiratory symptoms, including cough, wheezing, and tachypnea (Fig. 1a). The lymph nodes were involved in four patients. The patient characteristics are summarized in Table 1.

Table 1 Clinical features of the patients with post-transplantation lymphoproliferative disorder

Patient	Sex[a]/ age[b]	Age at LTx[c]	Primary disease	EBV status[d] (donor/recipient	Immunosuppressive therapy at the onset of PTLD	EVB load (copies/μg DNA	PTLD: sign/ symptoms	PTLD: localization	PTLD: histology	Therapy	Outcome (after PTLD treatment)
1	F/94	63	BA	Positive/negative	Tacrolimus Dose 10 mg/day Trough 8.7 ng/mL	1,975	Fever Mediastinal adenopathy Abdominal adenopathy	Mediastinal LN Abdominal LN	Not accessible	RI	Alive (13 years)
2	F/59	45	BA	Positive/negative	Tacrolimus Dose 1.5 mg/day Trough 8.1 ng/mL Steroid	26,891	Fever Abdominal adenopathy	Abdominal LN	Not accessible	RI	Alive (12 years)
3	F/28	11	BA	Positive/negative	Tacrolimus Dose 2.4 mg/day Trough 11.0 ng/mL	122,539	Fever Diarrhea Gastrointestinal edema	Gut	Not accessible	RI	Alive (8 years)
4	M/10	6	BA	Positive/negative	Tacrolimus Dose 4.8 mg/day Trough 7.5 ng/mL Steroid	13,415	Fever Respiratory symptoms[e] Pulmonary mass Mediastinal mass	Lung Mediastinal LN	Diffuse large- B-cell lymphoma CD20+, EBER+ Recipient origin	RI R-CHOP Resection	Alive (10 months)
5	M/13	6	BA	Positive/positive	Tacrolimus Dose 0.8 mg/day Trough 11.4 ng/mL Steroid	39,090	Fever Diarrhea Abdominal adenopathy	Gut Abdominal LN	Not accessible	RI Rituximab	Alive (1.1 years)

PTLD posttransplantation lymphoproliferative disorder, *LTx* liver transplantation, *BA* biliary atresia, *EBV* Epstein–Barr virus, *AZA* azathioprine, *LN* lympho Nonde, *EBER* EBV-encoded small RNA, *RI* reduction in immunosuppression, *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine and predonisone

[a] M = male; F = female

[b] Age in months at time of diagnosis of PTLD

[c] Age in months at liver transplantation

[d] Serologocal status at liver transplantation

[e] Wet cough, wheeze and tachypnea

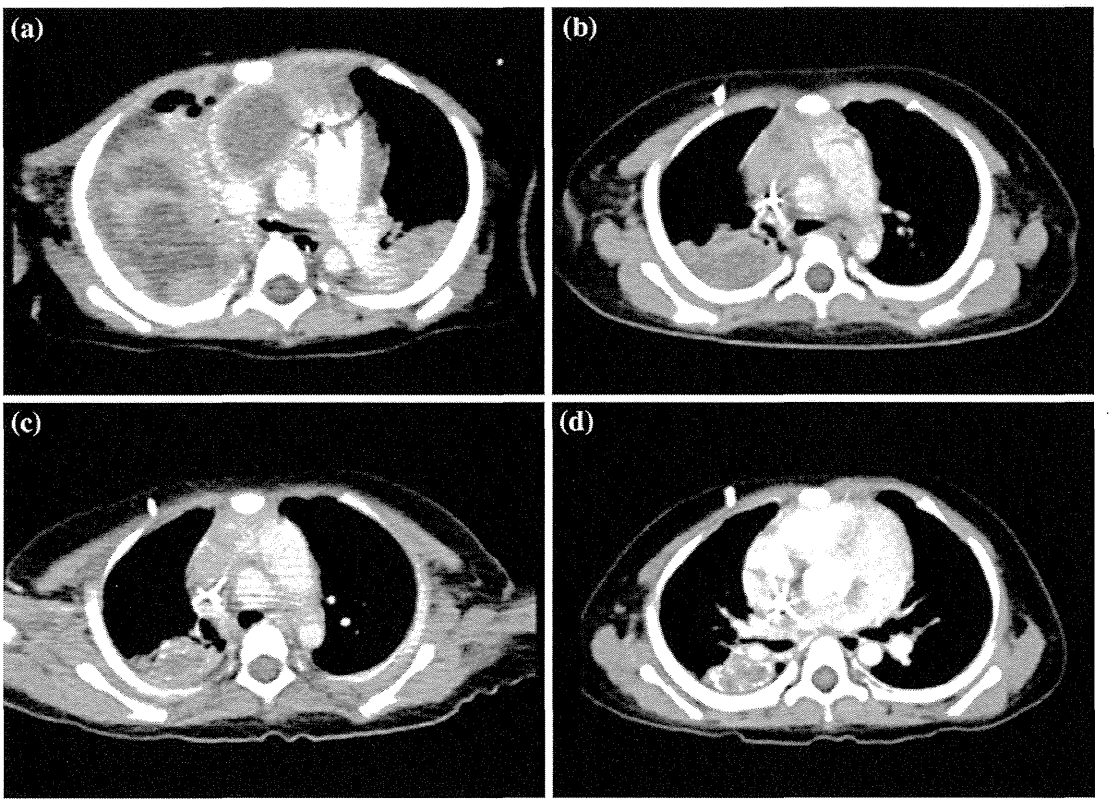

Fig. 1 Computed tomography (CT) scans of patient 4, showing **a** large pulmonary and mediastinal masses in a patient with respiratory symptoms, including cough, wheezing, and tachypnea. A CT-guided needle biopsy was performed, confirming the diagnosis of diffuse, large-B-cell lymphoma. **b** CT after six cycles of rituximab and three cycles of cyclophosphamide and prednisolone chemotherapy showed reduction in the sizes of the pulmonary and mediastinal masses. **c** CT after another two cycles of rituximab, cyclophosphamide, adriamycin, vincristine, and prednisolone (R-CHOP) chemotherapy revealed residual mediastinal and pulmonary tumors. **d** After another two cycles of CHOP chemotherapy following surgical resection of the residual mediastinal tumor, CT revealed a residual a lung mass, in which FDG-PET CT showed no abnormal uptake

Patient characteristics

The pediatric PTLD patients were compared with pediatric non-PTLD patients in terms of their age, sex, primary disease, primary immunosuppression, EBV serostatus before transplantation, ABO compatibility, lymphocyte cross-matching, and rejection (Table 2). All five PTLD patients underwent liver transplantation for biliary atresia and four were seronegative for EBV before transplantation. Lymphocyte cross-matching was negative in all the PTLD patients. However, there were no significant differences between the pediatric patients with and those without PTLD in terms of age, sex, reason for transplantation, calcineurin inhibitor, EBV serostatus, ABO compatibility, lymphocyte cross-matching, or episodes of biopsy-proven rejection.

Immunosuppression before PTLD onset

All five patients received tacrolimus, and three were still receiving steroids at the onset of PTLD (Table 1). Patient 2 had suffered from biopsy proven repeated rejection. PTLD developed only 4 months after transplantation in patient 4 and immediately before the scheduled discontinuation of steroid therapy in patient 5. Hence, these patients were still receiving steroids. Although patients 1 and 3 were not receiving steroids at the onset of PTLD, we could not discontinue their steroid therapy within 12 months because they were still suffering episodes suggesting rejection.

The primary immunosuppressive agents given to four patients were tacrolimus and steroids. Only patient 1 received tacrolimus, steroids, and azathioprine as the primary immunosuppression. Patient 2 was started on azathioprine 1 year after transplantation, but it was discontinued after drug-induced liver dysfunction before the onset of PTLD. Tacrolimus was changed to cyclosporine only for patient 2, followed by reconversion to tacrolimus before the onset of PTLD. None of the PTLD patients received OKT3, but one (patient 2) received deoxyspergualin for steroid-resistant rejection. No PTLD patient received induction therapy with anti-IL2 receptor antibody.

Table 2 Characteristics of post-transplantation lymphoproliferative disorder (PTLD) and non-PTLD pediatric patients

	PTLD ($n = 5$)	non-PTLD ($n = 73$)	p value
Age at LTx (months)	11 (6–63)	22 (5–213)	0.27[a]
Gender			
Male	2 (40%)	30 (41.1%)	
Female	3 (60%)	43 (58.9%)	0.67[b]
Primary disease			
Biliary atresia	5 (100%)	60 (82.2%)	
Others	0 (0%)	13 (17.8%)	0.39[b]
Primary immunosuppression			
Tacrolimus	5 (100%)	61 (83.5%)	
Cyclosporine	0 (0%)	12 (16.4%)	0.42[b]
EBV Serostatus at LTx (donor/recipient)			
Positive/positive	1 (20%)	30 (41.1%)	
Positive/negative	4 (80%)	36 (49.3%)	
Negative/positive	0 (0%)	2 (2.7%)	
Negative/negative	0 (0%)	2 (2.7%)	0.66[c]
Loss of data	0 (0%)	3 (4.1%)	
ABO compatibility			
Identical/compatible	4 (80%)	65 (89.0%)	
Incompatible	1 (20%)	8 (11.0%)	0.90[b]
Lymphocyte cross-matching			
Negative	5 (100%)	69 (94.5%)	
Positive	0 (0%)	4 (5.5%)	0.76[b]
Rejection <1 year	2/5 (40%)	29/73 (39.7%)	0.67[b]
OKT3 therapy	0/5 (0%)	3/73 (0.4%)	0.82[b]

PTLD posttransplantation lymphoproliferative disorder, *LTx* liver transplantation, *EBV* Epstein–Barr virus, *OKT3* anti-CD3 antibody

[a] Mann–Whitney *U* test

[b] Fisher's exact probability test

[c] Chi-square for independent test

Histopathological presentation

The disease of three of the five PTLD patients was not easily accessible for biopsy, but they had persistently high EBV loads and abdominal lymphadenopathy. One of the patients with gastrointestinal symptoms had no lymphadenopathy, but the diagnosis of gastrointestinal PTLD was based on a persistently high EBV load and gastrointestinal edema. A computed tomography (CT)-guided needle biopsy was performed only in one patient with large pulmonary and mediastinal masses. Histology showed EBV-related, CD20-positive, diffuse large-B-cell lymphoma, the recipient origin of which was confirmed by fluorescence in situ hybridization (FISH; Fig. 2).

Treatments and outcomes

Our initial three patients (patients 1–3) responded well to reduced immunosuppression and have shown no evidence of recurrence to this point. In patient 1, tacrolimus was discontinued altogether and steroid therapy was resumed; then, after the PTLD improved, cyclosporine was started. In patient 2, cellular rejection occurred after tacrolimus and steroid therapies were discontinued altogether, so low-dose tacrolimus was restarted, maintaining a trough at around 4.0 ng/mL, followed by steroids again. A study undertaken at our institution subsequently demonstrated that EBV infection can be kept asymptomatic with a tacrolimus trough level below 3.0 ng/mL [21]. Therefore, in patient 3, the tacrolimus dose was reduced, maintaining the trough below 3.0 ng/mL. A reduction in the dose of tacrolimus to maintain the trough below 3.0 ng/mL, together with the withdrawal of steroids, is now the initial therapeutic approach for patients with an increased EBV load or PTLD, at our institution, as was done for patients 4 and 5. When the trough is high, tacrolimus should be discontinued until it drops to about 3.0 ng/mL. However, reduced immunosuppression was insufficient to stabilize the PTLD in our two most recent patients (patients 4 and 5).

One patient (patient 4) with gastrointestinal symptoms, high fever, and abdominal lymphadenopathy, did not respond to a reduction in immunosuppression. Thus, he was commenced on rituximab at a dose of 375 mg/m^2 once a week, following which, his gastrointestinal symptoms and high fever improved quickly and his blood EBV load decreased to normal control levels. Although cellular