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ORIGINAL ARTICLE

Microvascular proliferation of the portal vein branches in the liver of biliary atresia patients at Kasai operation is associated with a better long-term clinical outcome

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Abstract

Aim of the study We previously showed an increased number of smaller portal vein (PV) branches in the portal areas of liver biopsy specimens of biliary atresia (BA) patients. We evaluated the correlation between this histopathological feature and the prognosis.

Patients and methods Twenty-five consecutive patients with BA encountered between 2000 and 2012 were classified into three prognostic groups based on their postoperative outcomes: Excellent ($n=11$) for native-liver survivors with a normal liver function, Good ($n=6$) for native-liver survivors with liver dysfunction, and Poor ($n=8$) for survivors after liver transplant or on a waiting list. Data from morphometrical analyses, including the fibrotic portal area, numbers of PVs, diameter and total area of PV branches, were statistically compared among the three groups.

Main results The number of PV branches per unit area of the whole-liver specimen in the poor prognostic group was significantly lower than that in the excellent group (3.1 ± 0.6 vs. $5.2 \pm 2.0/mm^2$, $p=0.03$). There were no significant differences in the other parameters.

Conclusions This is the first report on the relationships between morphometrically analyzed PV branches and the postoperative course in BA patients. The portal venous system is involved as the primary lesion in BA.

Keywords Biliary atresia · Portal vein · Hepatic artery · Morphometrical analysis · Postoperative course

Introduction

Biliary atresia (BA) is a disease of unknown etiology. Its pathological features include not only the fibrotic obliteration of the extrahepatic bile ducts presented in early infancy, but also the destruction of the intrahepatic bile ducts with progressive portal fibrosis leading to refractory obstructive jaundice. Although Kasai portoenterostomy (KPE) has been established as a standard surgical procedure, approximately half of BA patients undergo liver transplantation by 20 years of age because of hepatic failure, repeated cholangitis, progressive liver fibrosis or portal hypertension, even in jaundice-free survivors [1].

We previously showed an increased number of smaller portal vein (PV) branches, a decreased total luminal area of the PVs per fibrotic portal area and medial hypertrophy of the hepatic artery (HA) in the portal areas of liver biopsy specimens of BA patients [2].

The aim of the present study was to clarify the correlation between such morphological characteristics of the PVs at the time of Kasai operation and the clinical outcome.

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Materials and methods

Study population

A total of 25 patients with BA who underwent KPE at our institution from 2000 to 2014 were registered in the present study. The medical records of BA patients were retrospectively reviewed. Patients were classified into 3 prognostic groups based on their postoperative outcomes at their latest evaluation: excellent group ($n=11$), defined as native-liver survivors with a normal liver function; good group ($n=6$), defined as native-liver survivors with liver dysfunction; and poor group ($n=8$), defined as survivors after liver transplant or on a waiting list.

Liver tissue sampling and pathological parameters

During the initial KPE surgery, wedge biopsy samples were obtained from the edge of the right lobe of the liver. The liver biopsy specimens were fixed in formalin and embedded in paraffin. We morphometrically analyzed the PVs, their branches and HAs, excluding lymphatic vessels, by double chromogenic immunostaining for CD34+ podoplanin (D2-40), as described in the previous paper [2].

The parameters measured in this study were as follows: total area of the specimen, total area of the fibrotic portal area, luminal area of the PV, diameter of the PV, the length of the luminal circumference of the HA, area and length of the outer circumference of the HA and luminal diameter of the HA. We investigated the relationship between the morphological parameters and the long-term outcome.

Data collection

A retrospective chart review and data collection were performed after receiving institutional review board approval. The patients' characteristics, operative results and outcomes were reviewed based on their medical records. The data that were collected included the age at the time of KPE surgery and tissue sampling, follow-up period and patient condition and liver function test results. The patient condition and blood test results were examined within a few days before KPE and at their latest follow-up evaluation. The liver function tests included assessments of the serum levels of total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GT), albumin, prothrombin time international normalized ratios (PT-INR) and platelet counts. Previously reported non-invasive prognostic markers, such as the infant BA liver fibrosis (iBALF) score [3], the pediatric end-stage liver disease (PELD) score [4] and the aspartate aminotransferase-to-platelet ratio index (APRI) [5], which are all based on standard liver tests, were also evaluated in our patient's cohort and statistically compared with these histopathological parameters to determine the predictability of the prognosis.

Statistical analyses

The predictability of the postoperative outcome over 10 years based on the morphological parameters and other markers was assessed by area under the receiver operating characteristic (AUROC) curve analyses. Other statistical analyses were performed using Fisher's exact probability, the Mann–Whitney U test and a one-way analysis of variance. A probability value of less than 0.05 was considered to be statistically significant. Data are expressed as mean ± standard deviation.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [6].

Ethical approval

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All biopsy specimens and surgeries were performed after obtaining written informed consent. This study was approved by the Research Ethics Committee of Kagoshima University Hospital (registration number: 170347).

Results

Patients' background characteristics and clinical course

All patients underwent KPE, and none died during this study period. A total of 25 patients with BA (males, $n=13$; females, $n=12$) were included in this study. The median age at KPE was 62 days (range 44–143 days). The demographics of the patients in each group are shown in Table 1. There were no significant differences among the groups in the age at the time of KPE or the follow-up period. Regarding the liver function tests at their latest evaluation, there were significant differences between the excellent and good groups in the serum levels of AST, ALT, GT and PT-INR (Table 2). Among the eight patients in the poor group, three underwent living donor liver transplantation (LDLT) before one year of age, four patients underwent LDLT after one year of age, and

Table 1 Patients' background

	Excellent	Good	Poor	*p* value
Number of patients	11	6	8	
Days of age at KPE surgery	58.8 ± 13.7	73.7 ± 19.9	82.4 ± 30.0	0.11
Follow up period (years)	13.9 ± 4.5	10.0 ± 3.7	10.0 ± 2.9	0.11

KPE Kasai portoenterostomy

Table 2 Blood test results at their latest evaluation of the excellent group and good group

	Excellent	Good	*p* value
TB (mg/dl)	0.63 ± 0.36	0.68 ± 0.23	0.78
DB (mg/dl)	0.06 ± 0.04	0.05 ± 0.04	0.74
AST (IU/l)	26.3 ± 5.9	50.2 ± 24.2	0.009
ALT (IU/l)	24.2 ± 46.6	46.6 ± 25.0	0.05
GT (IU/l)	28.7 ± 23.5	88.0 ± 63.3	0.02
Albumin (g/dl)	4.3 ± 0.3	4.2 ± 0.3	0.24
PT-INR	1.01 ± 0.07	1.09 ± 0.04	0.03
Platelet count ($\times 10^9$/l)	181 ± 105	153 ± 41	0.57

TB total bilirubin, *DB* direct bilirubin, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GT* γ-glutamyltransferase, *PT-INR* prothrombin time international normalized ratios

one was on a waiting list for LDLT. No patients underwent primary LDLT.

Comparisons of the number of PV branches per unit area of the whole-liver specimen, iBALF score, PELD score and APRI

The number of PV branches per unit area of the whole-liver specimen in the poor prognostic group was significantly lower than that in the excellent group (3.1 ± 0.6 vs. 5.2 ± 2.0/mm^2, $p = 0.03$) (Fig. 1). There were no significant differences in the other parameters, including the fibrotic portal area, diameter of PV branches, total area of PV branches and medial thickness of the hepatic arteries.

Figure 2 shows the AUROC curve of the number of PV branches per unit area of the whole-liver specimen, and Table 3 shows the AUROC curve, 95% confidence interval, cut-off value, sensitivity and specificity of the number of PV branches per unit area of the whole-liver specimen and 3 non-invasive markers (iBALF score, PELD score and APRI) between the excellent and poor groups. The AUROC curve of each parameter was as follows: number of PVs per unit area, 0.88; iBALF score, 0.65; PELD score, 0.55; and APRI, 0.47. The sensitivity and specificity in the number of PV branches were highest compared with the other three scores. The number of PV branches per unit area of the whole-liver specimen had better predictability for the long-term outcome than the other three markers, and the cut-off value of the number of PV branches per unit area was 3.34 ($p < 0.005$). The number of PV branches per unit area of the whole-liver specimen provided good predictability for the long-term outcome over 10 years.

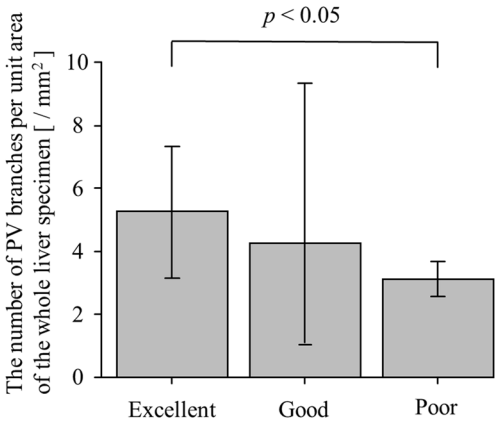

Fig. 1 The number of PV branches per unit area compared with the long-term outcome. *PV* portal vein

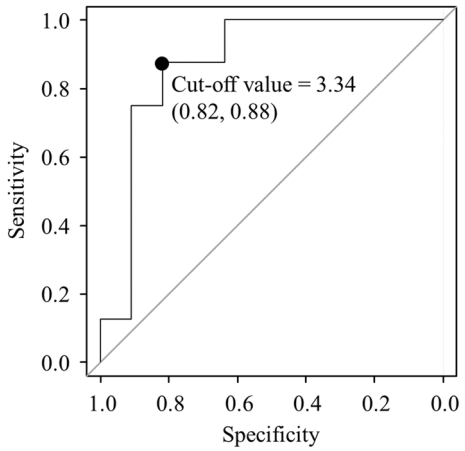

Fig. 2 The ROC curve of the number of PV branches per unit area compared with the long-term outcome. The black circle indicates the cut-off point. The gray oblique line indicates the reference line. *ROC* receiver-operating characteristic, *AUROC* area under the receiver-operating characteristic, *PV* portal vein

Discussion

In 1992, Desmet proposed the ductal plate malformation theory for the etiology of BA [7]. He reported that the "pollard willow" branching pattern of portal tracts in BA is due to a lack of remodeling of the ductal plates, leading to abnormalities in the ramification pattern of the PVs.

Table 3 Result of the ROC curves

Score	AUROC	95% CI	*p* value	Cut-off	Sensitivity	Specificity
the number of PV branches per unit area	0.88	0.703–1.00	0.005	3.34	88	82
iBALF score	0.65	0.382–0.914	0.295	3.63	63	64
PELD score	0.55	0.264–0.827	0.57	4.83	50	73
APRI	0.47	0.178–0.754	0.18	0.54	63	55

PVs portal vein, *iBALF* infant biliary atresia liver fibrosis, *PELD* the pediatric end-stage liver disease, *APRI* the aspartate aminotransferase-to-platelet ratio index, *AUROC* area under the receiver operating characteristic

Narrowing of PV branches was subsequently reported as a histopathological feature in BA liver [8–10]. Our previous paper also quantitatively revealed decreased numbers of PV branches and increased microvascular proliferation of the PV branches of the portal area at the time of KPE. The present analysis newly showed that an increase in the numbers of small branches of PVs is associated with a better long-term clinical outcome, and that the degree of microvascular proliferation showed a comparable predictability with the three markers (iBALF score, PELD score and APRI). Nio et al. recently reported that ^{99m}Tc-DTPA galactosyl human serum albumin (^{99m}Tc-GSA) liver scintigraphy at 1–2 years of age reflects the mid- and long-term prognoses, suggesting that the portal flow is a primary prognostic determinant [11]. This recent information is also quite compatible with our present findings on PV vascular changes.

The cause of the marked decrease in the number of PVs in BA liver tissue has not been fully discussed since Desmet's original description as described above (antenatal developmental abnormality theory). An alternative explanation is that the PV changes are the result of acquired degeneration and/or destruction of PVs that have been formed normally. We hypothesize that the PVs are a target of immunological attack through graft-versus-host disease mechanisms by maternal-derived microchimeric cells. This hypothesis appears to be supported by the fact that the endothelial cells of the PVs are positive for VCAM, B7 and C4d [12–19]. Given this assumption, the proliferation of small branches of PVs noted in the present study would function as collateral vessels to compensate for the damaged PVs. This increase in the number of vessels in liver tissue would deliver a greater blood supply to liver parenchymal cells, which would decrease the risk of progressing to liver cirrhosis with portal hypertension. Therefore, we may reasonably conclude that an increased number of PV branches is associated with a better clinical outcome in BA patients. Further analyses will be required to validate our hypothesis concerning the graft-versus-host disease mechanism.

This study is limited by the small number of patients and the results being derived from such a small population. We need to conduct a multicenter study in the future.

Conclusions

This is the first report on the relationship between the number of PV branches and the postoperative course in BA patients. Both the biliary and portal venous systems seem to be involved as the primary lesions in BA.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in association with the present study.

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