Oral administration of eicosapentaenoic acid suppresses liver fibrosis in postoperative patients with biliary atresia

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Abstract

Purpose: Biliary atresia (BA) is characterized by progressive inflammation of the biliary system. This inflammation persists and causes liver fibrosis, although jaundice disappears after Kasai portoenterostomy (KP). We aimed to confirm whether the oral administration of eicosapentaenoic acid (EPA) suppresses liver fibrosis in postoperative patients with BA.

Methods: We reviewed patients who underwent laparoscopic KP (lapKP) between January 2014 and September 2017. From December 2016, 30 mg/kg/day of EPA was orally administered to patients who opted to take medicine (EPA group). Patients who did not receive EPA were assigned to the non-EPA group. Mac-2 binding protein sugar chain modified isomer (M2BPGi) and hyaluronic acid (HA) levels were compared between the two groups in patients showing disappearance of jaundice at 6 months after the first lapKP.

Results: Seventeen patients in the non-EPA group and 11 in the EPA group were enrolled. At 6 months after the first lapKP, 10 patients in the non-EPA group and six in the EPA group were without jaundice. M2BPGi and HA levels were significantly lower in the EPA group.

Conclusions: Liver fibrosis was suppressed in patients without jaundice six months after lapKP, who were administered EPA. We believe that periductular inflammation was

alleviated by EPA supplementation.

Keywords: Biliary atresia, liver fibrosis, eicosapentaenoic acid, inflammation

Introduction

Biliary atresia (BA) is characterized by progressive periductular inflammation and fibrosis associated with the progressive obliteration of the bile ducts; however, the etiology this condition remains unknown[1]. Kasai portoenterostomy (KP), wherein a Roux-Y loop of the jejunum is anastomosed to the porta hepatis, facilitates bile drainage. However, when the disappearance of jaundice is not achieved by KP, liver cirrhosis progresses and the patient requires liver transplantation (LT)[2].

Even when jaundice disappears with KP, this inflammation in the liver persists and causes the slow progression of fibrosis into liver cirrhosis. Because of cirrhosis, a certain number of patients with BA without jaundice require LT due to uncontrollable gastrointestinal bleeding from portal hypertension[3].

Recently, the metabolic products of polyunsaturated fatty acids (PUFA) have been shown to play an immunoregulative role in the communication network of the immune system. PUFA contains essential fatty acids, which consist of two families: n-3 (or omega-3) and n-6 (or omega-6). The n-3 PUFA family is a precursor of cytokines having an anti-inflammatory effect (e.g., resolvins), whereas the n-6 PUFA family is a precursor of cytokines having a pro-inflammatory effect (e.g., leukotriene B4)[4].

We hypothesized that it was possible to suppress liver fibrosis by reducing this

inflammation in postoperative patients with BA via the administration of eicosapentaenoic acid (EPA), a type of n-3 PUFA. In December 2016, we initiated a clinical trial to investigate the effect of EPA administration on patients with BA.

This historical control study aims to confirm whether the oral administration of eicosapentaenoic acid (EPA) suppresses liver fibrosis by reducing inflammation in postoperative patients with BA.

Methods

We reviewed patients with BA who underwent laparoscopic Kasai portoenterostomy (lapKP) between January 2014 and September 2017 at our hospital.

This clinical research was conducted to administer EPA to postoperative patients with BA and was commenced in December 2016. The study was approved by the Ethics Committee (Ref No. 2016-0306). After receiving informed written consent, EPA was orally administered to patients with BA from the 7th postoperative day. Administration of EPA was continued until liver transplantation was planned, severe bleeding (e.g. esophageal varices) was experienced, or discontinuation of the study was requested. The dose of EPA was determined to be 30 mg/kg/day (an allowance of ±10 mg/kg/day). The administered drug was Epadel S (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan). The

decision to administer EPA was made by the parents of the patient after receiving an explanation about the trial and was not a randomized assignment.

The enrolled patients were divided into two groups: patients who did not receive EPA (non-EPA group) and patients who received EPA (EPA group). During the review period, there were no changes in the postoperative treatment except administration of EPA, which was as follows; Antibiotics for postoperative prophylaxis was administered for 2 days. Oral intake was started in 3rd operative day. As a cholagogue, 200 mg/day of dehydrocholate was administered intravenously for 4 days after surgery, and after that, 20mg/kg/day of ulsodeoxycholate was administered orally. Predonisolone was initiated in 5th postoperative day. Initial dosage was 4mg/kg/day, and thereafter, it was decreased to 2 mg, 1 mg, and 0.5 mg every 5 days and after that, administration of predonisolone was terminated. When the patient experienced high grade fever, administration of antibiotics was initiated under consideration of cholangitis. If jaundice rose, pulse therapy of steroid or re-operation was considered.

We reviewed the demographics and laboratory data of all enrolled patients. Further comparison was made between the two groups of patients on the disappearance of jaundice (serum total bilirubin $\leq$ 1.2 mg/dl) at 6 months after the first lapKP. In these instances, we compared laboratory data, the diameter of the portal vein, the flow velocity

of the portal vein, and the diameter of the spleen. We also compared Mac-2 binding protein sugar chain modified isomer (M2BPGi) and hyaluronic acid (HA) levels as indicators of liver fibrosis.

Statistical analysis was performed using Fisher's exact test, Mann–Whitney U test, and Wilcoxon signed rank test. $P < 0.05$ was considered to be statistically significant.

The authors have no conflict of interest to declare.

Results

In total, 31 patients underwent KP during the review period. Three patients underwent open surgery (social indication, two; medical indication, one). Consequently, 28 patients underwent lapKP and were enrolled in our study. Of the 28 patients, 17 who underwent lapKP between January 2014 and November 2016 were assigned to the non-EPA group. All the patients who were introduced to our trial involving EPA supplementation decided to take EPA. Therefore, all 11 of the patients who underwent lapKP between December 2016 and September 2017 were assigned to the EPA group.

The non-EPA group comprised four males and 13 females, whereas the EPA group comprised three males and eight females. The median age at the first lapKP was 55 days in the non-EPA group and 40 days in the EPA group. Jaundice disappeared in 12

(71%) patients in the non-EPA group and eight (73%) patients in the EPA group. Ten (59%) patients in the non-EPA group and six (55%) patients in the EPA group were without jaundice at 6 months after the first lapKP. No statistically significant differences were observed between the two groups with respect to any of these variables (Table 1).

A further comparison was done between the two groups of patients without jaundice at 6 months after the first lapKP. Regarding the levels of aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), total bilirubin, and direct bilirubin, no significant difference was observed between two groups (Table 2). No significant differences were observed between the two groups regarding the diameter of the portal vein, the flow velocity of the portal vein, and the diameter of the spleen (Table 2), although these data were not available for all cases (Table 2). While the data of HA level were available for all patients, the data of M2BPGi level of the earliest 5 patients were not available because M2BPGi had not been routinely measured yet in this period. HA and M2BPGi levels were significantly lower in the EPA group, ($p = 0.023$, $p = 0.018$, respectively) (Fig. 1).

The concentration of EPA in the EPA group was significantly higher 6 months after the first lapKP than that before surgery ($p = 0.028$; Fig. 2). We did not measure the concentration of EPA in the non-EPA group.

Discussion

BA is a disease characterized by progressive fibro-obliteration and obstruction of the biliary tract caused by inflammation. KP is the only effective therapy available, except for LT. KP offers the chance of survival to children with BA and remains a standard first line treatment for BA in Japan at present, despite the introduction of LT [5].

Failure to accurately treat BA results in cholestasis, leading to progressive cirrhosis and hepatic failure; few patients survive for >2 years [6]. The clearance of jaundice can be achieved in approximately 60% of cases undergoing KP [7]. However, for the patients in whom jaundice fails to disappear, LT remains the only option.

However, it is not the case that all cases in whom jaundice disappears can escape from LT. An earlier review reported that portal hypertension accounted for 50% of the indications of LT in jaundice-free patients with BA [3]. This fact indicated that liver fibrosis had been progressing and that portal hypertension continued to rise, even if jaundice disappeared. This appeared to cause inflammation around the Glisson's capsule, which is characteristic of BA. It is known that such inflammation continues even after the disappearance of jaundice and also that this inflammation leads to liver cirrhosis, although little is known of the mechanism involved.

A recent study reported that n-3 PUFA is metabolized to eicosanoids, which can suppress inflammation and inhibit inflammation[4]. In patients with BA, it has been reported that the ratio of n-3/n-6 PUFA was decreased, suggesting that the inflammation tended to progress, even in patients after the disappearance of jaundice. This decrease in n-3 PUFA was reported to be improved after LT [8]. It is still unclear whether this decrease in n-3 PUFA was due to the fundamental pathogenesis of BA or due to consumption as a result of inflammation.

In the case of present study, the values of EPA before administration were below the normal range in the half of cases. In the remaining half cases, these were only a little above the lower limit. The value of EPA at the 6 months after surgery obviously increased by the supplement. Considering that n-3 PUFA and n-6 PUFA were competitive substrates[9], the fact that the value of EPA increased by supplement would strengthen the anti-inflammatory function of n-3 PUFA.

It is known that the inflammation in the liver of BA patient persists even when jaundice disappears after KP. Recently, the role of interleukin (IL)-8 has attracted attention in hepatic disorders of various diseases such as alcoholic hepatitis, cirrhosis, viral hepatitis, or autoimmune hepatitis. Also in BA, IL-8 is overexpressed, which is suggested to have an effect on liver inflammation[10]. It has been reported that EPA

suppress the expression of IL-8 on stimulation[11].

We hypothesized that it was possible to suppress liver fibrosis in patients with BA by the reduction in the inflammatory response with EPA supplementation. Therefore, we planned a clinical trial involving the supplementation of EPA. We chose to make a comparison with past cases because after starting the trial, all patients with BA elected to take the EPA supplementation.

In the enrolled patients, the concentration of EPA was actually increased in patients in the EPA group. However, the concentration of EPA in the non-EPA group was not routinely measured when they underwent surgery. Consequently, it was not possible to compare the concentration of EPA during the same period because appropriate data was not available. However, analysis of previous studies indicates that the concentration of EPA in the non-EPA group would have been expected to be reduced [8].

Although histological examination of liver biopsies should be the gold standard for evaluating liver fibrosis, liver biopsy is hazardous in complicated patients with severe liver disease. Therefore, in this study, we evaluated liver fibrosis using two biochemical markers, M2BPGi and HA. HA is one of the components of proteoglycan in the extracellular matrix of hepatocytes. Following damage to the liver tissue, the hepatic stellate cells synthesize HA and the HA level begins to increase [12]. M2BPGi is a

juxtacrine-acting messenger sent by the hepatic stellate cells to the Kupffer cells during liver fibrosis and plays an important role in the progression of fibrosis. Thus, M2BPGi levels reflect the activation of hepatic stellate cells during the progression of liver fibrosis [13]. Both of these biochemical markers have been frequently used over recent times to reflect the degree of liver fibrosis. Regarding BA, these two markers have also been reported to reflect the degree of hepatic fibrosis confirmed by liver biopsy [14,15]. Therefore, these have been routinely measured in our institute and these were adopted as markers of liver fibrosis in this study.

We conducted a trial to supplement EPA in postoperative patients with BA. Liver fibrosis at 6 months after lapKP was definitely suppressed following EPA supplementation in patients without jaundice, although the probability of achieving the disappearance of jaundice was not increased. It was thought that inflammation of Grisson's capsule, which continued after the disappearance of jaundice in patients with BA, was alleviated by EPA supplementation. The alleviation of inflammation resulted in the suppression of liver fibrosis.

In the present trial, we only compared the patient's state 6 months after initial surgery. Furthermore, the number of enrolled patients in the present study was small. It is now necessary to investigate a larger number of patients over a longer time period.

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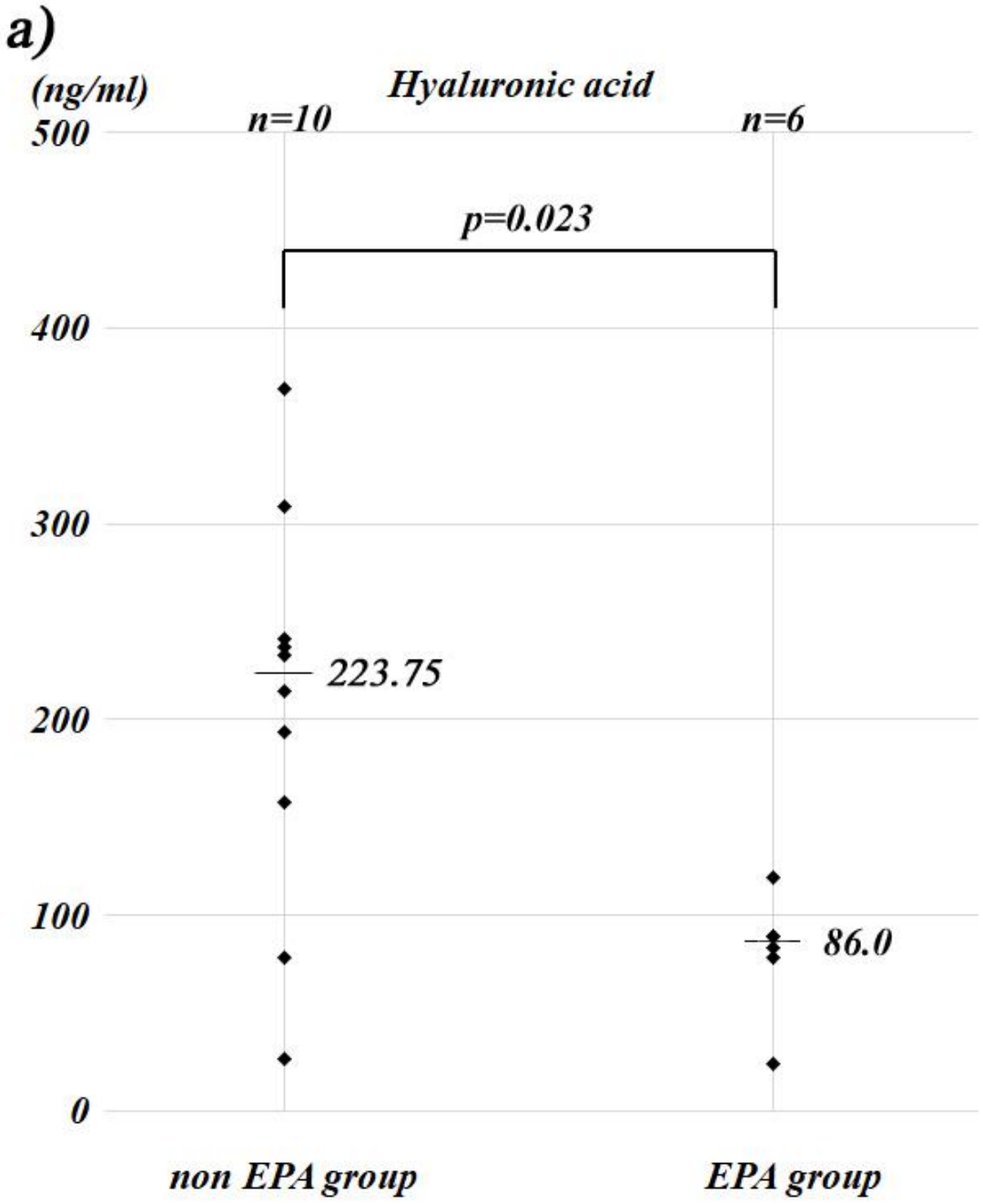
a)
(ng/ml)
Hyaluronic acid
n=10
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500
p=0.023
400
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223.75
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86.0
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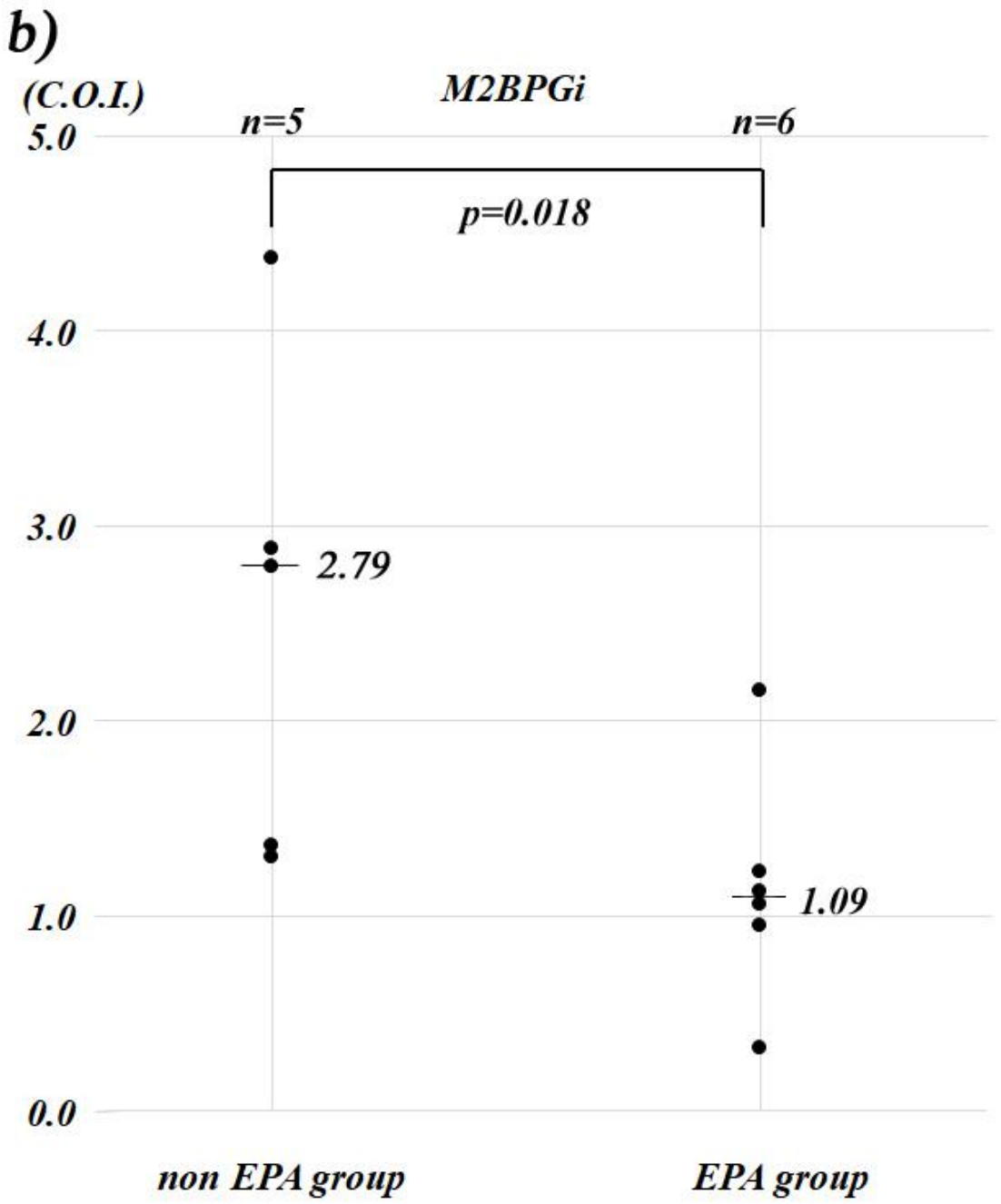

Figure 1. Comparison of biomarker for liver fibrosis in patients from the non-EPA group and the EPA group. The black dots indicate the levels of each case. The horizontal line and number indicate the median of each group.

a) Comparison of hyaluronic acid levels

b) Comparison of Mac-2 binding protein sugar chain modified isomer (M2BPGi) levels

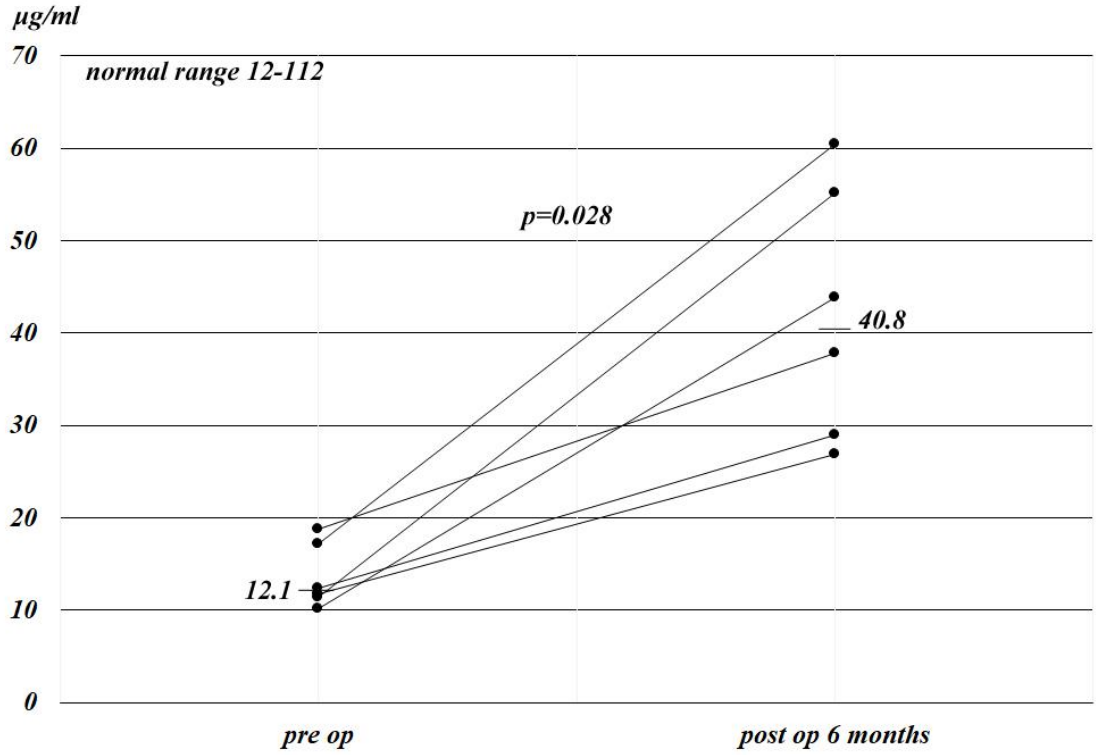

Figure 2. Change in the concentration of eicosapentaenoic acid (EPA) in patients in the EPA group before and after EPA supplementation. After EPA supplementation, the concentration of EPA was significantly higher. The horizontal line and number indicate the median of each group.

Table 1. Geographic data of the patients enrolled in this study.

	Non-EPA Group	**EPA Group**	**P value**
All patients	17	11	-
Male:Female	4:13	3:8	1
Age at first Kasai portoenterostomy (day) median (range)	55 (40–77)	40 (28–79)	0.33
Number of cases in whom jaundice disappeared	12	8	1
Number of cases who were jaundice-free 6 months after the first surgery	10	6	1

Table 2. Comparison between the two groups of patients without jaundice at 6 months after the first lapKP.

	Non-EPA Group	**Data available**	**EPA Group**	**Data available**	**P value**
Number of cases	10		6		-
Male : female	3:7		0:6		0.25
AST (IU/L)	111 (40–198)	10	71 (36–82)	6	0.07
ALT (IU/L)	97 (16–203)	10	43 (17–101)	6	0.21
Gamma-GT (IU/L)	346 (24–723)	10	236 (16–810)	6	0.52
Total bilirubin (mg/dl)	0.8 (0.4–1.2)	10	0.6 (0.2–1.0)	6	0.55
Direct bilirubin (mg/dl)	0.3 (0.1–0.8)	10	0.2 (0.1–0.5)	6	0.24

Diameter of PV (mm)	4.3 (3.2–6.0)	6	4.5 (3.4–4.8)	6	0.93
Flow velocity of PV (cm/s)	19 (13–30)	6	21 (12–23)	6	1
Diameter of spleen (mm)	73 (58–85)	8	64 (54–76)	6	0.14

Data are described as median (range).