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ORIGINAL ARTICLE

Serum retinol binding protein 4 and clinical outcome in postoperative biliary atresia

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Abstract
Background Biliary atresia (BA), a chronic inflammatory disease of bile ducts, is characterized by the obliteration of bile flow. The aim of the present study was to investigate serum retinol binding protein 4 (RBP4) in postoperative BA patients and the association of RBP4 with clinical parameters and liver stiffness scores.
Methods A number of forty-eight BA patients post Kasai operation and 24 controls were enrolled. None of the patients had undergone liver transplantation. BA patients were classified into two groups according to their serum total bilirubin (TB) levels (non-jaundice, TB < 2 mg/dl vs. jaundice, TB ≥ 2 mg/dl) and liver stiffness (insignificant fibrosis, liver stiffness <7 kPa vs. significant fibrosis, liver stiffness ≥7 kPa). Serum RBP4 levels were determined by enzyme-linked immunosorbent assay (ELISA). Liver stiffness scores were measured by FibroScan.
Results BA patients had lower RBP4 levels (14.9 ± 1.0 vs. 18.7 ± 1.0 ng/ml, $P = 0.02$), but higher liver stiffness than controls (29.5 ± 3.3 vs. 5.0 ± 0.5 kPa, $P < 0.001$). Serum RBP4 levels were significantly decreased in BA patients with jaundice, compared with those without jaundice (9.5 ± 0.9 vs. 18.2 ± 1.2 ng/ml, $P < 0.001$). Moreover, BA patients with significant liver fibrosis displayed lower serum RBP4 than those with insignificant fibrosis (14.1 ± 1.2 vs. 21.2 ± 1.4 ng/ml, $P = 0.02$). Further analysis showed that serum RBP4 was strongly correlated with liver stiffness and serum albumin ($r = -0.72$, $P < 0.001$, and $r = 0.65$, $P < 0.001$, respectively). BA patients with portal hypertension (PH) had lower serum RBP4 than those without PH (12.8 ± 1.2 vs. 19.2 ± 1.7 ng/ml, $P = 0.003$).
Conclusion Serum RBP4 levels decreased in BA patients compared with normal participants, and its levels declined significantly in patients with more severe disease. RBP4 may play a role in the pathogenesis of hepatic fibrosis and serve as a possible biomarker reflecting disease severity in postoperative BA patients.

Keywords Biliary atresia · Jaundice · Liver fibrosis · Portal hypertension · Retinol binding protein 4

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Introduction

Biliary atresia (BA) is a chronic inflammatory disease of extrahepatic bile ducts resulting in the obstruction of bile flow [1]. The overall incidence is approximately 1 per 8,000–12,000 live births worldwide [2]. Without proper management, most patients die within 2 years from cirrhosis and hepatic failure. Although hepatoportoenterostomy or Kasai operation can successfully establish bile flow in approximately 80% of cases, 65% of those progress to end-stage liver diseases, and finally require liver transplantation [3]. As a result, BA is one of the major indications for liver transplantation in children. To date, etiologies of BA have not been well established; however, many theories have been proposed to explain the pathogenesis of this disease including genetic factors, viral

infections, defects in bile duct morphogenesis, and immune response abnormalities [4, 5]. BA is a heterogeneous disorder with several pathogenic pathways culminating in one common phenotype, the obliteration of bile ducts [5].

Bile duct inflammation, cytokine response, and bile acid toxicity are the three major contributors to liver parenchyma destruction and hepatic fibrosis in patients with BA [2]. Upon activation, hepatic stellate cells (HSC), the key effecter cells in hepatic fibrogenesis, are transformed into extracellular matrix-producing myofibroblasts. This process results in the production of collagen and the accumulation of extracellular matrices in the liver parenchyma, thus initiating and perpetuating liver fibrosis [6, 7]. Recent studies have shown that obesity and insulin resistance might accelerate the pace of liver fibrosis in various chronic liver diseases, hence the role of adipokines in hepatic fibrogenesis [8]. The role of retinol binding protein 4 (RBP4) and the potential association with the disease severity in BA remain obscure. Thus, this study was aimed to evaluate the relationship between RBP4 and clinical outcome in BA patients.

RBP4, a 21 kDa protein of the lipocalin family, is mainly synthesized by hepatocytes and to a lesser extent by adipose tissues. RBP4 functions as a sole transport protein of retinol (vitamin A) to tissues [9]. Both animal and human studies have shown that RBP4 might be involved in the development of insulin resistance and diabetes [10, 11]. Serum levels of RBP4 have been previously shown to be associated with the degree of steatosis and fibrosis in liver diseases of various etiologies, such as non-alcoholic fatty liver disease and chronic hepatitis C [12–20]. However, the precise mechanism of RBP4 influence on liver fibrogenesis is not clearly understood.

Although it has been reported that a number of cytokines, adipokines, and growth factors play critical roles in the pathophysiology of BA [21–23], published data on serum RBP4 levels from various clinical stages of BA are currently not available. To our knowledge, this is the first study to evaluate the correlation of serum RBP4, liver stiffness, and clinical outcome in postoperative BA. We have postulated that serum RBP4 levels might be associated with clinical outcome and liver stiffness in BA patients after Kasai procedure. Therefore, the objective of the study was to investigate serum RBP4 in postoperative BA patients and the association of RBP4 with clinical outcome in BA patients after surgical treatment.

Patients and methods

Study population

BA patients (28 girls and 20 boys with a mean age of 10.1 ± 0.8 years) and healthy controls (15 girls and 9 boys with a mean age of 10.6 ± 0.6 years) were enrolled in this study. All BA patients had undergone hepatic porto jejunostomy with Roux-en-Y reconstruction (Kasai procedure), and they were generally in good health; no signs of suspected infection or bleeding abnormalities at the time of blood sampling. None of them had undergone liver transplantation. Healthy controls who attended the Well Baby Clinic of King Chulalongkorn Memorial Hospital had normal physical findings and no underlying disease. Written informed consents were obtained from the participants' parents upon informing them about the protocol and procedures involved in the research. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

BA patients were categorized into two groups according to serum total bilirubin (TB), serum alanine aminotransferase (ALT), and liver stiffness score. Based on their jaundice status, BA children were divided into a non-jaundice group (TB < 2 mg/dl, $n = 30$) and a persistent jaundice group (TB ≥ 2 mg/dl, $n = 18$). Further classification was achieved according to their levels of serum ALT status into a normal ALT group (ALT < 45 IU/l, $n = 11$) and a high ALT group (ALT ≥ 45 IU/l, $n = 37$). Portal hypertension (PH) was validated by the presence of esophageal varices as diagnosed by endoscopy. Sixteen of 30 BA patients without jaundice and 16 of 18 patients with persistent jaundice had evidence of PH. Subsequently, BA patients were classified into a non-significant fibrosis group (liver stiffness <7 kPa, $n = 8$) and a significant fibrosis group (liver stiffness ≥ 7 kPa, $n = 40$).

Laboratory methods

Samples of peripheral venous blood were collected from every participant, and were stored at −70°C for further analysis. Quantitative determination of RBP4 concentration in serum was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA). According to the manufacturer's instructions, recombinant human RBP4 standards, and serum samples were pipetted into each well, which had been pre-coated with specific antibody to RBP4. After incubating for 1 h at room temperature on a horizontal orbital microplate shaker set at 500 rpm, every well was thoroughly washed four times with wash buffer. Then, an RBP4 conjugate was added to each well, and the plate was incubated for 1 h at room temperature on the shaker. After four washes, substrate solution was pipetted into the wells and the microplate was incubated for 30 min at room temperature protected from light. Finally, the reaction was stopped by stop solution and the color intensity was measured with an automated microplate reader at 450 nm.

The RBP4 concentration was determined by a standard optical density–concentration curve. The liver function tests including serum albumin, TB, direct bilirubin (DB), aspartate aminotransferase (AST), ALT, and alkaline phosphatase (ALP) were performed using a Hitachi 912 automated machine at the central laboratory of our hospital. The AST to platelets ratio index (APRI) was calculated as follows: AST/upper limit of normal × 100/platelet count (10^9/l) [24].

Liver stiffness measurement

Transient elastography or FibroScan (Echosens, Paris, France) measured the liver stiffness between 25 and 65 mm from the skin surface, which is approximately equivalent to the volume of a cylinder of 1 cm diameter and 4 cm length. The measurements were performed by placing a transducer probe of FibroScan on the intercostal space at the area of the right lobe of the liver with patients lying in a dorsal decubitus position with maximum abduction of the right arm. The target location for the measurement was a liver portion that was at least 6 cm thick, and devoid of major vascular structures. The measurements were performed until 10 validated results had been obtained with a success rate of at least 80%. The median value of 10 validated scores was considered the elastic modulus of the liver, and it was expressed in kilopascals (kPa).

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 for Windows. Student's unpaired *t* test was used to compare the difference of RBP4 levels in serum between groups. Correlation between serum RBP4 levels and other serological markers, liver stiffness scores, and body mass index (BMI) was calculated using Pearson's correlation coefficient (*r*). Sensitivity, specificity, receiver–operating characteristic (ROC) curves and area under the curve (AUC) were also analyzed. Data were expressed as mean ± SEM. *P* values < 0.05 were considered statistically significant.

Results

Comparison between biliary atresia patients and healthy controls

A total of 48 postoperative BA patients and 24 healthy controls were enrolled in this study. The characteristics of participants in both groups are described in Table 1. Mean age and gender ratio in controls and BA patients were not different, while serum RBP4 levels in BA patients were significantly lower than those in controls (14.9 ± 1.0 vs. 18.7 ± 1.0 ng/ml, $P = 0.02$) (Fig. 1). Additionally, liver stiffness scores in BA patients were markedly higher than those in controls (29.5 ± 3.3 vs. 5.0 ± 0.5 kPa, $P < 0.001$).

Table 1 Demographic data, biochemical characteristics, and liver stiffness scores of controls and postoperative BA patients

Variables	Controls ($n = 24$)	BA patients ($n = 48$)	*P* value
Age (years)	10.6 ± 0.6	10.1 ± 0.8	0.6
Gender (Female:Male)	15:9	28:20	0.5
BMI (kg/m^2)	18.5 ± 0.5	18.1 ± 0.7	NA
Albumin (g/dl)	–	4.29 ± 0.1	NA
TB (mg/dl)	–	2.5 ± 0.6	NA
DB (mg/dl)	–	1.9 ± 0.6	NA
AST (IU/l)	–	122.2 ± 13.0	NA
ALT (IU/l)	–	107.8 ± 13.2	NA
ALP (IU/l)	–	415.4 ± 31.0	NA
Platelet count (10^3/mm^3)	–	156.1 ± 14.8	NA
APRI	–	1.6 ± 0.2	NA
RBP4 (ng/ml)	18.7 ± 1.0	14.9 ± 1.0	0.02
Liver stiffness (kPa)	5.0 ± 0.5	29.5 ± 3.3	<0.001

The data was expressed as mean ± SEM

BA Biliary atresia, *BMI* Body mass index; *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *APRI* Aspartate aminotransferase to platelets ratio index, *RBP4* Retinol binding protein 4, *NA* Not applicable

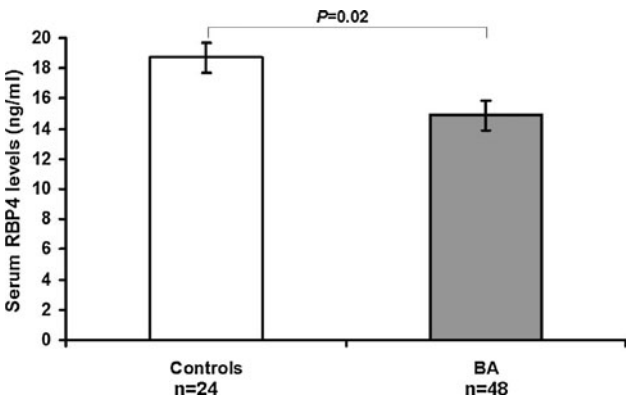

Fig. 1 Comparison of serum RBP4 levels in postoperative BA patients and healthy controls. The data was expressed as mean ± SEM

Comparison between biliary atresia patients with and without persistent jaundice

We further categorized BA patients into a jaundice ($n = 18$) and non-jaundice group ($n = 30$). As presented in Table 2, BA patients with jaundice had significantly higher

Table 2 Comparison between BA patients without and with jaundice

Variables	BA patients without jaundice ($n = 30$)	BA patients with jaundice ($n = 18$)	P value
Age (years)	10.0 ± 1.0	10.2 ± 1.2	0.9
Gender (Female:Male)	18:12	10:8	0.5
BMI (kg/m^2)	18.7 ± 0.6	17.0 ± 0.7	0.03
Albumin (g/dl)	4.5 ± 0.1	3.9 ± 0.1	<0.001
TB (mg/dl)	0.6 ± 0.1	5.6 ± 1.2	<0.001
DB (mg/dl)	0.3 ± 0.1	4.8 ± 1.2	<0.001
AST (IU/l)	80.7 ± 12.2	191.5 ± 19.4	<0.001
ALT (IU/l)	78.6 ± 12.0	156.3 ± 25.9	0.01
ALP (IU/l)	333.1 ± 36.0	552.5 ± 40.1	<0.001
Platelet count (10^3/mm^3)	178.0 ± 18.5	119.5 ± 22.7	<0.05
APRI	1.1 ± 0.2	3.2 ± 0.3	<0.001
RBP4 (ng/ml)	18.2 ± 1.2	9.5 ± 0.9	<0.001
Liver stiffness (kPa)	17.1 ± 2.7	50.1 ± 4.4	<0.001

The data was expressed as mean ± SEM

BA Biliary atresia, *BMI* Body mass index, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase, *APRI* Aspartate aminotransferase to platelets ratio index, *RBP4* Retinol binding protein 4

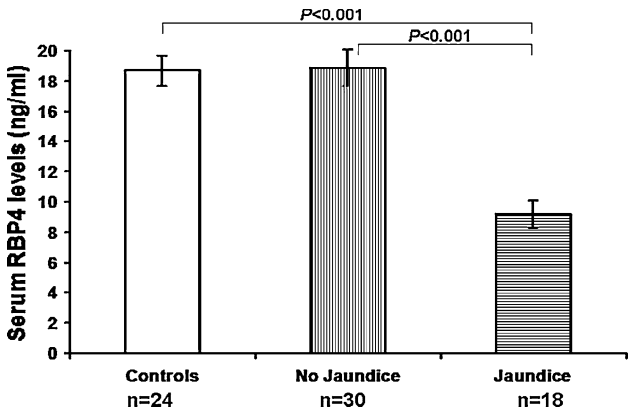

Fig. 2 Comparison of serum RBP4 levels in controls, BA patients without jaundice, and BA patients with jaundice. The data was expressed as mean ± SEM

serum TB, AST, ALT, and ALP, AST to APRI, and liver stiffness values compared to those without jaundice. Serum RBP4 levels in BA patients with persistent jaundice were, on the contrary, lower than in BA patients without jaundice (9.5 ± 0.9 vs. 18.2 ± 1.2 ng/ml, $P < 0.001$) (Fig. 2). We also found that BA patients with normal ALT ($n = 11$) had higher levels of serum RBP4 than those with elevated ALT ($n = 37$) (18.7 ± 1.7 vs. 13.8 ± 1.1 ng/ml, $P < 0.05$). Similarly, BA patients with insignificant fibrosis ($n = 8$) displayed higher serum RBP4 than those with significant fibrosis ($n = 40$) (21.2 ± 1.4 vs. 14.1 ± 1.2 ng/ml, $P = 0.02$).

Further analysis demonstrated that serum RBP4 levels were inversely correlated with serum TB ($r = -0.35$, $P = 0.02$), prothrombin time ($r = -0.40, P = 0.005$), ALT ($r = -0.40, P = 0.005$), ALP ($r = -0.46, P = 0.001$), and liver stiffness values ($r = -0.72$, $P < 0.001$). Conversely, serum levels of RBP4 were positively correlated with serum albumin ($r = 0.65, P < 0.001$) and platelet count ($r = 0.48$, $P = 0.001$). However, serum RBP4 was not correlated with either serum AST or BMI. Correlations between serum RBP4 and liver stiffness, and serum albumin are shown in Figs. 3 and 4.

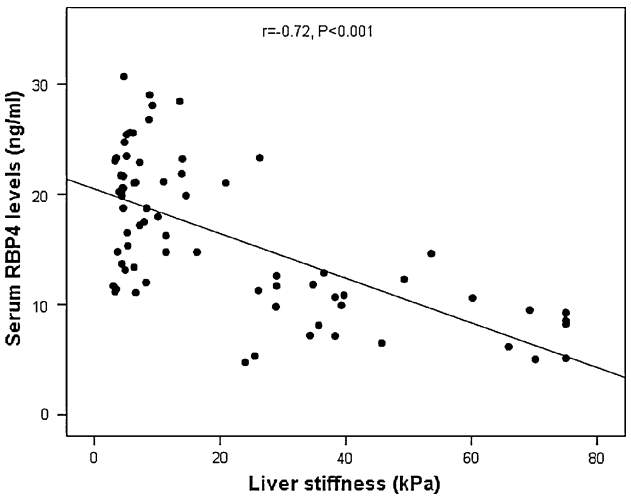

Fig. 3 Correlation between serum RBP4 levels and liver stiffness scores in postoperative BA patients ($r = -0.72$, $P < 0.001$)

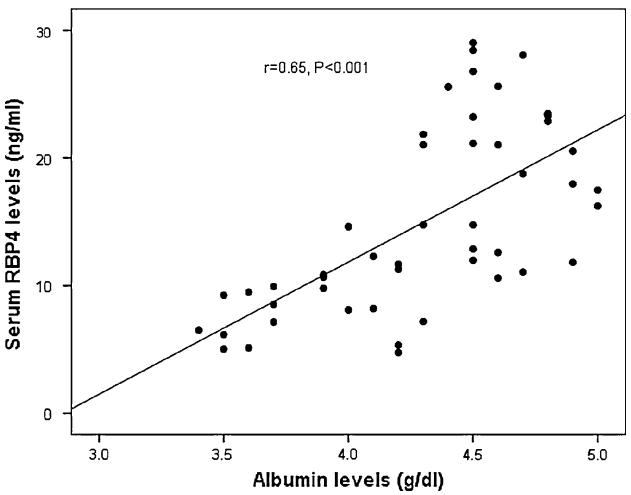

Fig. 4 Correlation between serum RBP4 levels and serum albumin levels in postoperative BA patients ($r = 0.65$, $P < 0.001$)

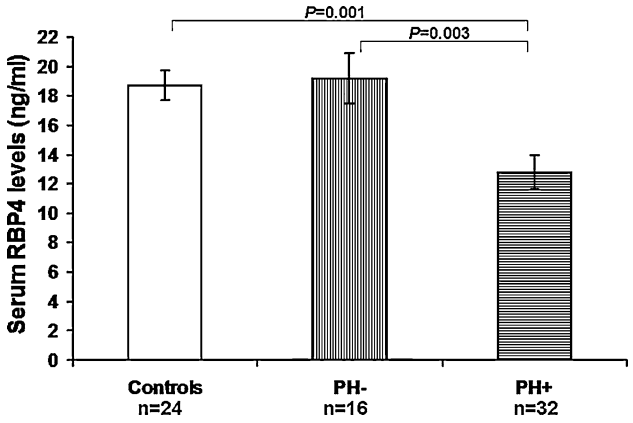

Fig. 5 Comparison of serum RBP4 levels in controls, BA patients without PH, and BA patients with PH. The data was expressed as mean ± SEM. PH, portal hypertension

Comparison between biliary atresia patients with and without portal hypertension

BA patients with PH ($n = 32$) displayed significantly lower serum RBP4 than those without PH ($n = 16$) and healthy controls as illustrated in Fig. 5 (12.8 ± 1.2 vs. 19.2 ± 1.7 vs. 18.7 ± 1.0 ng/ml, $P = 0.003$ and $P = 0.001$, respectively). There was no significant difference in serum RBP4 between the patients without PH and controls. Moreover, ROC curve analysis demonstrated a good discriminating power of serum RBP4 for PH/esophageal varices in postoperative BA patients, with a cut-off value of 11.5 ng/ml and an AUC of 0.766 (95% CI 0.628–0.903).

Discussion

Despite successful Kasai operation, a significant amount of BA patients developed liver fibrosis and cirrhosis. Thus, the evaluation of fibrogenic progression in BA is undeniably important. An ideal monitoring method would be one which is accurate, easily-performed, non-invasive, and cost effective. Although none of the currently available tests has met those requirements, transient elastography or FibroScan has emerged as the most promising one [25, 26].

Hepatic stellate cell activation followed by extracellular matrix production and accumulation was a main mechanism contributing to the development of liver fibrosis [6]. A number of cytokines are believed to play important roles in this process and they have become topics of research interest in an attempt to evaluate the use of serum cytokine as a biochemical marker of liver fibrosis. In the present study, we evaluated the role of serum RBP4 in determining liver fibrosis and liver function deterioration. RBP4 has been implicated in the development of insulin resistance and diabetes [10, 11], but its role in liver diseases is still a subject of debate. A previous study proposed that RBP4 might influence hepatic fibrogenesis and steatosis through the depletion of cellular retinol [27]. Ohata et al. [28] showed that the loss of cellular content of retinoic acid could prompt HSC activation and promote liver fibrosis, whereas retinoid supplement could reduce hepatic fibrosis. Similarly, Wang and colleagues [29] demonstrated that retinoic acid could decrease HSC proliferation and collagen production.

The present study illustrated that serum RBP4 levels were significantly lower in postoperative BA patients compared with healthy controls. In postoperative BA patients, serum RBP4 levels of patients without jaundice were greater than those of patients with persistent jaundice. Further analysis showed that BA patients with normal ALT had increased levels of serum RBP4 compared to those with high ALT. Moreover, BA patients with insignificant fibrosis had more elevated serum RBP4 than those with significant fibrosis. In the present study, we used the cut-off point of liver stiffness score for significant fibrosis at the elastic modulus of 7 kPa. This value was based on the study by Castera et al. [26] with 67% sensitivity and 89% specificity. These findings suggested that serum RBP4 levels decreased in direct relation to disease severity determined by elevated liver enzymes/parameters and liver stiffness scores.

It is notable that our findings were in accordance with observations from previous studies [14–20], which demonstrated that serum RBP4 levels were lower in patients with chronic liver diseases and the levels decreased as the diseases progressed to more severe stages. Nevertheless, recent investigations have shown that serum RBP4 levels are higher in patients with non-alcoholic fatty liver disease and chronic hepatitis C and these levels increase with the degree of liver steatosis [12, 13]. The exact reasons for these discrepancies among reports remain unclear and merit further investigations. However, the explanation for these conflicting results could be attributed to the difference in stages of disease among the patients recruited in these studies. The synthesis function of the liver greatly influences the levels of RBP4 since this protein is mainly secreted by hepatocytes. Therefore, serum RBP4 levels might be decreased or increased depending on the degree of liver injuries.

We also found that serum RBP4 was negatively correlated with TB, ALT, ALP, prothrombin time, and liver stiffness, but positively correlated with serum albumin and platelet counts. These results support that serum RBP4 is associated with the synthesis abilities of the liver and the degree of liver fibrosis. Its association with platelet count might imply that serum RBP4 is related to the severity of hypersplenism, which indicates the degree of PH. The

same correlation between serum RBP4 and ALT, albumin, and prothrombin time have been shown in other studies as well. Frey et al. [30] found that serum RBP4 was inversely correlated with AST, ALT, and ALP, while the studies by Bahr et al. [14] and Yagmur et al. [15] demonstrated that serum RBP4 was positively associated with serum albumin and coagulation factors. We hypothesized that the decrease in serum RBP4 levels could be due to the loss of liver mass resulting from the ongoing liver inflammation in BA patients post Kasai operation. The decrease in RBP4 resulted in the decrease of blood retinol levels [31], thus promoting further extracellular matrix accumulation and liver fibrosis [28, 29]. As this investigation was designed as a cross-sectional study, it is not possible to determine the causal relationship between RBP4 and liver fibrosis. Recently, we reported low serum leptin in jaundice BA patients [22]. Future studies on additional adipokines may help identify more pieces of the inflammatory jigsaw of BA; nonetheless, the challenge remains to piece them together to originate a rational solid hypothesis concerning their precise role.

In this study, we utilized FibroScan as a diagnostic method of liver fibrosis because it is a reproducible, rapid, and non-invasive technique for measuring the degree of liver fibrosis, which can be accomplished in the out-patient setting. FibroScan can measure liver stiffness in a volume 100 times larger than that obtained from liver biopsy and is, therefore, more representative for the whole liver parenchyma [26]. Despite being a gold standard in diagnosing liver fibrosis and determining its severity, liver biopsy is a painful and invasive procedure with infrequent but possible life-threatening complications [32]. Thus, liver biopsy might not be a suitable test for clinically stable children with biliary atresia. Moreover, there have been questions about the accuracy of liver biopsy, which is adversely affected by sampling errors, intra- and inter-observer variability. These ambiguities could lead to false staging of liver fibrosis [26].

Subsequently, the present study demonstrated that BA patients with PH displayed markedly lower serum RBP4 levels than those without PH. Therefore, these findings suggest that low levels of serum RBP4 are closely linked to the degree of hepatic fibrosis and perpetuation of PH in postoperative BA. Further analysis also revealed that there was no association between serum RBP4 and BMI in postoperative BA patients. A similar result was shown by other studies on liver diseases. No correlation between RBP4 and BMI was found in the studies by Alkhouri et al. [18] and Cengiz et al. [33]. Moreover, Schina and co-workers [16] demonstrated that serum RBP4 was not associated with metabolic parameters, such as serum triglyceride and cholesterol. The negative result in our study could, nevertheless, be due to the small number of obese participants, since the correlation between RBP4 and obesity or insulin resistance was usually found in patients with morbid obesity.

In conclusion, this study showed that BA patients had significantly lower serum RBP4 than healthy controls. Serum RBP4 was associated with clinical outcomes (status of jaundice, hepatic dysfunction, liver stiffness, and PH) in BA. Based on these findings, RBP4 may play important roles in the pathogenesis of hepatic fibrosis and PH in postoperative BA. Serum RBP4 could serve as a possible biomarker to determine the disease severity and the development of PH in the post Kasai BA patients. Further studies should address the relationship between serum and hepatic RBP4 levels among BA patients at the time of surgery and include the different groups of age-matched patients with other cholestatic liver diseases for comparison. The exact mechanisms resulting in decreased serum RBP4 in BA will require future investigation.

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References

1. Mckirnan PJ, Baker AJ, Kelly DA. The frequency and outcome of biliary atresia in the UK and Ireland. Lancet 2000;355:25–29
2. Balistreri WF, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, et al. Biliary atresia: current concepts and research directions. Summary of symphosium. Hepatology 1996;23:1682–1692
3. Altman RP, Lilly JR, Greenfield J, Weinburg A, van Leeuwen K, Flanigan L. A multivariable risk factor analysis of the portoenterostomy (Kasai) procedure for biliary atresia: twenty-five years of experience from two centers. Ann Surg 1997;226:348–353
4. A-Kader HH, Abdel-Hameed A, Al-Shabrawi M, Mohsen N, El-Karaksy H, Hassanein B, et al. Is biliary atresia an autoimmune disease? Eur J Gastroenterol Hepatol 2003;5:447
5. Mark CL, Sokol RJ. Unraveling the pathogenesis and etiology of biliary atresia. Pediatr Res 2005;57:87R–94R
6. Gressner OA, Gressner AM. Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. Liver Int 2008;28:1065–1079
7. Gressner AM, Weiskirchen R. Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. J Cell Mol Med 2006;10:76–99
8. Chuang JH, Wang PW, Tai MH. An adipocentric view of liver fibrosis and cirrhosis. Chang Gung Med J 2004;27:855–868
9. Blaner WS. Retinol-binding protein: the serum transport protein for vitamin A. Endocr Rev 1989;10:308–316
10. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin

resistance in obesity and type 2 diabetes. Nature 2005;436:356–362
11. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552–2563
12. Petta S, Camma C, Di Marco V, Alessi N, Barbaria F, Cabibi D, et al. Retinol-binding protein 4: a new marker of virus-induced steatosis in patients infected with hepatitis C virus genotype 1. Hepatology 2008;48:28–37
13. Seo JA, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, et al. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. Clin Endocrinol 2008;68:555–560
14. Bahr MJ, Boeker KHW, Manns MP, Tietge UJF. Decreased hepatic RBP4 secretion is correlated with reduced hepatic glucose production but is not associated with insulin resistance in patients with liver cirrhosis. Clin Endocrinol 2009;70:60–65
15. Yagmur E, Weiskirchen R, Gressner AM, Trautwein C, Tacke F. Insulin resistance in liver cirrhosis is not associated with the circulating retinol-binding protein 4. Diabetes Care 2007;30:1168–1172
16. Schina M, Koskinas J, Tiniakos D, Hadziyannis E, Savvas S, Karamanos B, et al. Circulating and liver tissue levels of retinol-binding protein 4 in non-alcoholic fatty liver disease. Hepatol Res 2009;39:972–978
17. Nobili V, Alkhouri N, Alisi A, Ottino S, Lopez R, Manco M, et al. Retinol-binding protein 4: promising circulating marker of liver damage in pediatric nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:575–579
18. Alkhouri N, Lopez R, Berk M, Feldstein AE. Serum retinol-binding protein 4 levels in patients with nonalcoholic fatty liver disease. J Clin Gastroenterol 2009;43:985–989
19. Tacke F, Weiskirchen R, Trautwein C. Liver function critically determines serum retinol-binding protein 4 (RBP4) levels in patients with chronic liver disease and cirrhosis. Hepatology 2008;48:1724–1725
20. Huang JF, Dai CY, Yu ML, Shin SJ, Hsieh MY, Huang CF, et al. Serum retinol-binding protein 4 is inversely correlated with disease severity of chronic hepatitis C. J Hepatol 2009;50:471–478
21. Honsawek S, Chayanupatkul M, Chongsrisawat V, Vejchapipat P, Poovorawan Y. Increased osteopontin and liver stiffness measurement by transient elastography in biliary atresia. World J Gastroenterol 2010;16:5467–547
22. Honsawek S, Chaiwatanarat T, Chongsrisawat V, Thawornsuk N, Vejchapipat P, Poovorawan Y. Circulating leptin levels and bone mineral density in children with biliary atresia. Acta Paediatr 2008;97:206–211
23. Honsawek S, Chongsrisawat V, Vejchapipat P, Thawornsuk N, Poovorawan Y. High levels of serum basic fibroblast growth factor in children with biliary atresia. Hepatogastroenterology 2008;55:1184–1188
24. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–526
25. Chang HK, Park YJ, Koh H, Kim SM, Chung KS, Oh JT, et al. Hepatic fibrosis scan for liver stiffness score measurement: a useful preendoscopic screening test for the detection of varices in postoperative patients with biliary atresia. J Pediatr Gastroenterol Nutr 2009;49:323–328
26. Castara L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver fibrosis for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343–350
27. Bluher M, Tonjes A, Stumvoll M. Dose retinol-binding protein 4 cause or reflect fatty liver disease? Hepatology 2008;48:4–6
28. Ohata M, Lin M, Satre M, Tsukamoto H. Diminished retinoic acid signaling in hepatic stellate cells in cholestatic liver fibrosis. Am J Physiol 1997;272:G589–G596
29. Wang L, Attard FA, Tankersley LR, Potter JJ, Mezey E. Effect of retinoic acid on the enhancing effect of acetaldehyde on mouse type I collagen expression. Arch Biochem Biophys 2000;376:191–198
30. Frey SK, Nagl B, Henze A, Raila J, Schlosser B, Berg T, et al. Isoforms of retinol binding protein 4 (RBP4) are increased in chronic diseases of the kidney but not of the liver. Lipids Health Dis 2008;7:29
31. Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantedosi R, Gouras P, et al. Impaired retinol function and vitamin A availability in mice lacking retinol-binding protein. EMBO J 1999;18:4444–4633
32. Foucher J, Chanteloup E, Vergniol J. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006;55:403–408
33. Cengiz C, Ardicoglu Y, Bulut S, Boyacioglu S. Serum retinol-binding protein 4 in patients with nonalcoholic fatty liver disease: dose it have a significant impact on pathogenesis. Eur J Gastroenterol Hepatol 2010;22:813–819