Elevation of Serum Galectin-3 and Liver Stiffness Measured by Transient Elastography in Biliary Atresia

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Key words
- biliary atresia
- galectin-3
- jaundice
- liver stiffness
- portal hypertension

Abstract

Background and aim: Biliary atresia (BA) is an intractable neonatal liver disease characterized by progressive fibrosclerotic obliteration of the extrahepatic biliary tree. The aim of this study was to evaluate serum galectin-3 in postoperative BA patients and the association between galectin-3, clinical outcome and liver stiffness score.

Methods: 58 BA patients post Kasai operation and 20 controls were enrolled. None of the patients had undergone liver transplantation. BA patients were classified into 2 groups according to their serum total bilirubin (TB) levels (TB < 2 mg/dL, no jaundice vs. TB ≥ 2 mg/dL, persistent jaundice) and alanine aminotransferase (ALT) levels (ALT < 45 IU/L, normal ALT vs. ALT ≥ 45 IU/L, elevated ALT). Serum galectin-3 levels were determined by enzyme-linked immunosorbent assay. Liver stiffness scores were measured by transient elastography (FibroScan).

Results: BA patients had higher serum galectin-3 levels (5.1 ± 0.3 vs. 3.8 ± 0.4 ng/mL, p = 0.01) and greater liver stiffness values than healthy controls (29.7 ± 3.0 vs. 5.1 ± 0.5 kPa, p < 0.001). Serum galectin-3 levels were markedly elevated in BA patients with jaundice compared to those without jaundice (6.4 ± 0.5 vs. 4.4 ± 0.3 ng/mL, p = 0.001). Furthermore, BA patients with elevated ALT displayed significantly higher levels of serum galectin-3 than those with normal ALT (5.9 ± 0.4 vs. 3.8 ± 0.3 ng/mL, p = 0.001). Additionally, BA patients with portal hypertension had considerably higher serum galectin-3 levels than those without portal hypertension (6.1 ± 0.4 vs. 3.7 ± 0.3 ng/mL, p < 0.001).

Conclusions: Increased serum galectin-3 is associated with a poor outcome in postoperative BA patients. Serum galectin-3 could be used as a biochemical parameter reflecting the deterioration of liver function and the severity of liver fibrosis in postoperative BA.

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Introduction

Biliary atresia (BA) remains one of the most common causes of pathologic jaundice in neonates and is characterized by progressive inflammatory cholangiopathy leading to obliteration or discontinuity of the bile ducts at any point from the porta hepatis to the duodenum. The obstruction manifests with the triad of jaundice, acholic stools, and hepatosplenomegaly. If untreated, BA patients develop severe hepatic fibrosis, hepatic failure, and finally die within a few years [1]. The standard surgical procedure for re-establishing bile flow in BA children is the Kasai portoenterostomy. Despite undergoing early Kasai procedure, the vast majority of patients eventually develop biliary cirrhosis, portal hypertension, and liver failure [2, 3]. At this stage, liver transplantation is an effective treatment modality when the Kasai operation has failed and severe end-stage liver disease develops. The etiology, pathogenesis, and mechanisms modifying disease progression have not been well established; however, several lines of evidence have been proposed, including genetic insults, viral infections, morphogenic abnormalities and immune-mediated bile duct injuries [4].

Galectins are members of a growing family of extensively distributed carbohydrate-binding proteins characterized by their affinity for β-galactoside-containing glycans. Currently, more than 15 galectins have been identified [5]. Among all these galectins, galectin-3 has been the most widely studied [6]. Galectin-3 is a 30-kD mammalian lectin which comprises a carboxyl-terminal carbohydrate recognition domain and an amino-terminal domain with multiple repeats of a sequence that is rich in glycine, proline, tyro-

sine, and glutamine [7]. Galectin-3 is a pleiotropic protein that is implicated in a variety of biological processes including cell proliferation, adhesion, and survival [8–10]. Galectin-3 is expressed in several cell types including hepatocellular cacinoma cells as well as in hepatic stellate cells [11]. In addition, upregulation of galectin-3 expression has been reported in tissue fibrosis and recombinant galectin-3 stimulates myofiboblast proliferation [12]. Previous studies showed that galectin-3 has been involved in the development of inflammation and fibrosis in various organs, giving rise to such conditions as chronic heart failure, chronic pancreatitis, pulmonary fibrosis, and renal fibrosis [13–16].

According to our knowledge, serum galectin-3 during various clinical stages of BA and its plausible role in BA patients have not been previously studied. We hypothesized that serum galectin-3 would be more elevated in BA patients compared to healthy controls, and to prove this hypothesis, we measured serum galectin-3 concentrations in BA patients and healthy controls. Thus, the aim of this study was to investigate serum galectin-3 levels in postoperative BA patients and to examine the possible association of serum galectin-3 with clinical outcome.

Patients and Methods

This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, and complied with the ethical guidelines of the 1975 Declaration of Helsinki. All parents of children were informed of the purpose of the study and of any interventions involved in this study. Written informed consents were obtained from the participants' parents on informing them about the protocol and procedures involved in the research.

Study population

58 BA patients (30 girls and 28 boys with mean age of 9.4 ± 0.7 years) and 20 healthy children (10 girls and 10 boys with mean age of 10.1 ± 0.7 years) were recruited for this study. All BA patients had undergone hepatic portojejunostomy with Roux-en-Y reconstruction (original Kasai procedure), and they were generally in good health with no signs of suspected infection or bleeding abnormalities at the time of blood sampling. None of them had undergone liver transplantation. Healthy controls attending the Well Baby Clinic at King Chulalongkorn Memorial hospital for vaccination had normal physical findings and no underlying disease. BA patients were classified into 2 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) and a persistent jaundice group (TB ≥ 2 mg/dl). Patients were further classified, according to their levels of serum ALT, into a normal ALT group (ALT < 45 IU/L, n = 22) and a high ALT group (ALT ≥ 45 IU/L, n = 36). Subsequently, portal hypertension (PH) was validated by the presence of ascites and/or esophageal varices as diagnosed by endoscopic screening. 15 of the 38 BA children without jaundice and 18 of 20 children with persistent jaundice had evidence of portal hypertension.

Laboratory methods

Samples of peripheral venous blood were collected from every participant, and were stored at −70 °C for further analysis. Galectin-3 concentrations were quantified in serum samples using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA). According to the manufacturer's protocol, recombinant human galectin-3 standards, and serum samples were pipetted into each well, which had been pre-coated with the specific antibody to galectin-3. After incubation for 2 h at room temperature, every well was thoroughly washed 4 times with wash buffer. Then, a galectin-3 conjugate was added to each well, and the plate was incubated for 2 h at room temperature. After 4 washes, a 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 galectin-3 concentrations were determined using a standard optical density-concentration curve. Serum hyaluronic acid (HA) levels were measured using a competitive inhibition based-ELISA as described previously [17]. Liver function tests including serum albumin, total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were performed using a Hitachi 912 automated machine in the central laboratory of our hospital. The aspartate aminotransferase to platelets ratio index (APRI) was calculated as follows: (AST/upper limit of normal) × 100/platelet count (10^9/L) [18].

Liver stiffness measurement

Transient elastography measured the liver stiffness between 25 to 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 FibroScan transducer probe on the intercostal space at the area of the right lobe of the liver with the patients lying in a dorsal decubitus position with maximum abduction of the right arm. The target location for measurement was a liver portion that was at least 6 cm thick and without 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 for Windows, version 16.0. Comparisons of demographic and clinical parameters between groups were performed using chi-square and Student's unpaired *t*-test. Data were expressed as mean ± SEM. *p*-values < 0.05 were considered statistically significant.

Results

A total of 58 BA patients and 20 healthy controls were enrolled in this study. The characteristics of the participants in both groups are shown in **Table 1**. Mean age and gender ratio in controls and BA patients did not differ, while serum galectin-3 levels were significantly elevated in BA patients compared to healthy controls (5.1 ± 0.3 vs. 3.8 ± 0.4 ng/mL, $p = 0.01$; **Fig. 1**). BA patients had significantly higher serum hyaluronic acid levels than controls (50.1 ± 7.2 vs. 23.9 ± 1.5 ng/mL, $p = 0.001$). In addition, liver stiffness scores in BA patients were considerably higher than those in controls (29.7 ± 3.0 vs. 5.1 ± 0.5 kPa, $p < 0.001$).

Table 1 Demographic data, biochemical characteristics, and liver stiffness scores of controls and biliary atresia patients. Data are expressed as mean ± SEM.

Variables	Controls (n = 20)	BA patients (n = 58)	*p*-value
age (years)	10.1 ± 0.7	9.4 ± 0.7	0.1
gender (female:male)	10:10	30:28	0.5
albumin (g/dL)	–	4.3 ± 0.1	NA
total bilirubin (mg/dL)	–	2.6 ± 0.5	NA
direct bilirubin (mg/dL)	–	2.1 ± 0.5	NA
AST (IU/L)	–	128.4 ± 11.3	NA
ALT (IU/L)	–	111.8 ± 10.9	NA
ALP (IU/L)	–	437.3 ± 28.3	NA
platelet count (10^3/mm^3)	–	164.7 ± 13.2	NA
APRI	–	3.0 ± 0.4	NA
galectin-3 (ng/mL)	3.8 ± 0.4	5.1 ± 0.3	0.01
hyaluronic acid (ng/mL)	23.9 ± 1.5	50.1 ± 7.2	0.001
liver stiffness (kPa)	5.1 ± 0.5	29.7 ± 3.0	<0.001

BA: biliary atresia; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; APRI: aspartate aminotransferase to platelets ratio index; NA: not applicable

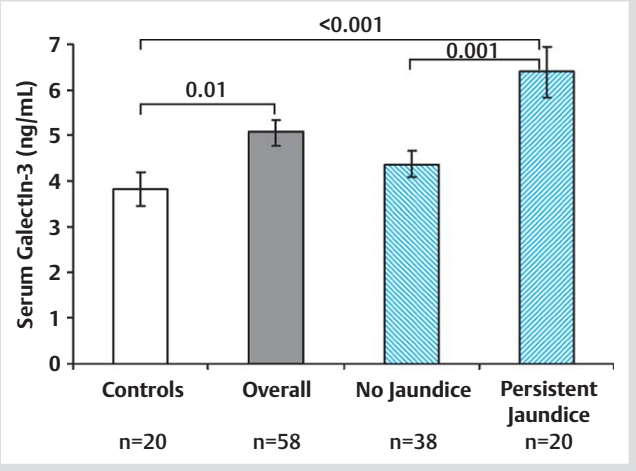

Fig. 1 Comparison of serum galectin-3 levels based on total bilirubin in biliary atresia patients and controls. The data are expressed as mean ± SEM.

Table 2 Comparison of biliary atresia patients without and with jaundice. Data are expressed as mean ± SEM.

Variables	BA patients without jaundice (n = 38)	BA patients with jaundice (n = 20)	*p*-value
age (years)	8.9 ± 0.8	10.1 ± 1.2	0.4
gender (female:male)	20:18	10:10	0.5
albumin (g/dL)	4.5 ± 0.1	3.9 ± 0.1	<0.001
total bilirubin (mg/dL)	0.7 ± 0.1	6.2 ± 1.2	<0.001
direct bilirubin (mg/dL)	0.4 ± 0.1	5.4 ± 1.2	<0.001
AST (IU/L)	107.8 ± 14.2	167.6 ± 15.6	0.01
ALT (IU/L)	105.6 ± 15.7	123.7 ± 10.0	0.4
ALP (IU/L)	387.8 ± 36.7	531.5 ± 35.5	0.01
platelet count (10^3/mm^3)	190.3 ± 16.2	116.2 ± 18.7	0.01
APRI	2.1 ± 0.4	4.8 ± 0.6	<0.001
galectin-3 (ng/mL)	4.4 ± 0.3	6.4 ± 0.5	0.001
hyaluronic acid (ng/mL)	29.3 ± 3.5	89.6 ± 16.9	<0.001
liver stiffness (kPa)	18.9 ± 2.5	50.1 ± 4.5	<0.001

BA: biliary atresia; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; APRI: aspartate aminotransferase to platelets ratio index

We further categorized BA patients into jaundice (n = 20) and non-jaundice groups (n = 38). As presented in **Table 2**, BA patients with jaundice had a significantly higher aspartate aminotransferase to platelets ratio index (APRI), and higher hyaluronic acid, and liver stiffness values compared to those without jaundice. In BA patients, serum galectin-3 levels of patients with persistent jaundice were markedly elevated compared to those of patients without jaundice (6.4 ± 0.5 vs. 4.4 ± 0.3 ng/mL, $p = 0.001$; **Fig. 1**). We also found that BA patients with elevated ALT (n = 36) had significantly higher levels of serum galectin-3 than those with normal ALT levels (n = 22) (5.9 ± 0.4 vs. 3.8 ± 0.3 ng/mL, $p = 0.001$; **Fig. 2**). Moreover, BA patients with portal hypertension (n = 33) displayed remarkably higher serum galectin-3 levels than those without portal hypertension (n = 25) as shown in **Fig. 3** (6.1 ± 0.4 vs. 3.7 ± 0.3 ng/mL, $p < 0.001$).

Discussion

Biliary atresia is one of the most common cholestatic liver diseases in neonate. The etiology and pathogenesis of BA are still incompletely understood. Irrespective of the initiating disorder, all BA patients share a unique progressive inflammatory and fibrosis obstruction of the biliary system [1–3]. The progression

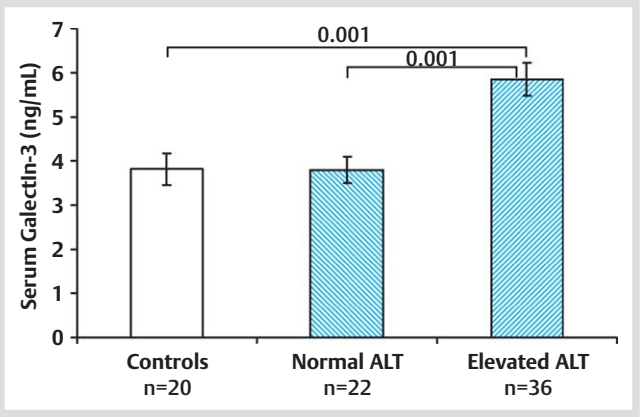

Fig. 2 Comparison of serum galectin-3 levels based on serum ALT levels in biliary atresia patients and controls. The data are expressed as mean ± SEM. ALT, alanine aminotransferase.

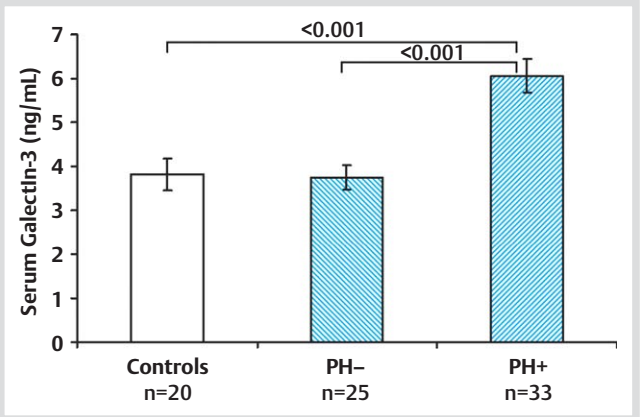

Fig. 3 Comparison of serum galectin-3 levels based on the presence or absence of portal hypertension in biliary atresia patients and controls. The data are expressed as mean ± SEM. PH, portal hypertension.

of BA leads to liver fibrosis and portal hypertension, with severe complications including gastroesophageal varices, splenomegaly, and progressive ascites. Although several cytokines and growth factors have been previously reported as playing a pathophysiological role in biliary atresia [19–22], published data on serum galectin-3 levels at different clinical stages of BA are not currently available. The present study aimed to investigate serum galectin-3 concentrations, liver stiffness scores, and clinical parameters in BA patients after surgical treatment.

The present study revealed that serum galectin-3 levels in BA patients were markedly higher than those in healthy controls. Among BA patients, serum galectin-3 levels were substantially greater in the BA patients with persistent jaundice compared to those without jaundice. Subsequent analysis revealed that BA patients with high ALT had more higher concentrations of serum galectin-3 compared to those with normal ALT levels. These findings suggest that serum galectin-3 is associated with jaundice status and hepatocellular damage in postoperative BA. Furthermore, this study showed more elevated galectin-3 levels in BA patients with portal hypertension than in those without portal hypertension. Therefore, serum galectin-3 might be a useful biochemical marker in determining hepatic dysfunction and the development of varices in BA patients.

To our knowledge, the present study is the first to show that serum galectin-3 is elevated in BA patients compared with healthy controls, and that serum galectin-3 is associated with clinical outcome (status of jaundice, hepatic dysfunction, and portal hypertension) in BA patients. Recently, galectin-3 has been found to play a potential role in a number of liver diseases, including liver cirrhosis, hepatocellular carcinoma, and intrahepatic cholangiocarcinoma [23–28]. In accordance with our findings, Matsuda et al. reported that serum galectin-3 in patients with hepatocellular carcinoma was significantly higher than in controls [23]. Hsu and coworkers demonstrated that galectin-3 was absent in normal hepatocytes, but was predominantly expressed in cirrhotic liver and extensively in hepatocellular carcinoma [24]. Moreover, Henderson et al. revealed that hepatic expression of galectin-3 was elevated in carbon tetrachloride-induced liver injury and that disruption of the galectin-3 gene inhibited hepatic stellate cell activation and liver fibrosis [25]. In addition, Butscheid et al. showed an association between galectin-3 expression and different forms of functional liver impairment [26]. More prospective studies on hepatic galectin-3 expression are needed to elucidate the mechanisms of galectin-3 in BA.

It is important to note that several potential mechanisms may be responsible for the significant elevation of serum galectin-3 in BA patients, particularly in patients with a poor outcome. Firstly, the production of galectin-3 in the damaged liver may result in high serum galectin-3. Secondly, the increased galectin-3 levels could be attributed to the imbalance between galectin-3 production and galectin-3 clearance. In advanced stage BA, reduced biliary clearance of galectin-3 may possibly contribute to elevated serum galectin-3 levels. Furthermore, extrahepatic organs can synthesize and secrete galectin-3 in the systemic circulation. The higher galectin-3 levels could be regarded as indicating hepatic damage and cholestasis in BA patients. Further studies will provide more valuable information on the pathophysiological roles of galectin-3 in BA patients.

It should be noted, however, that there are some limitations to our study. The sample size of BA patients enrolled in this study was relatively small. Additional studies with random samples from a larger population will be warranted to make a more definite conclusion. Furthermore, the study was limited to those patients who attended our hospital. Consequently, the results may not be directly applicable to subjects from other ethnic groups. In addition, we analyzed galectin-3 at a single time point and therefore can only speculate on its importance over time. Because of these limitations, we regard our study principally as a hypothesis-generating study. However, with the supporting evidence from other studies based on the role of galectin-3 in liver cirrhosis and hepatic fibrosis [23–26], it is plausible that the elevated serum galectin-3 observed in postoperative BA patients could be involved in the pathophysiology of hepatocellular injury and perpetuation of portal hypertension.

Conclusion

To summarize, this study showed that BA patients had significantly elevated serum galectin-3 levels compared with healthy controls. Serum galectin-3 was more pronounced in BA patients with persistent jaundice compared to BA patients without jaundice. Furthermore, serum galectin-3 in BA patients with high ALT values was significantly elevated compared to BA patients with normal ALT levels. Subsequent analysis revealed that BA patients with portal hypertension had considerably higher serum galectin-3 levels than BA patients without portal hypertension. These findings suggest that high serum galectin-3 is associated with hepatic injury and hence reflects the magnitude of liver deterioration in the BA patient. Galectin-3 could become a novel biomarker for progression to hepatic dysfunction and might be predictive with respect to the development of PH in the follow-up of postoperative BA patients.

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Conflict of Interest: None

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