Association of Serum Levels of Tissue Inhibitors of Metalloproteinase-1 with Clinical Outcome in Children with Biliary Atresia

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SUMMARY The purpose of this study was to determine the possible role of serum levels of tissue inhibitors of metalloproteinase-1 (TIMP-1) in the pathogenesis of the progressive inflammation and fibrosis in biliary atresia (BA). Serum concentrations of TIMP-1 were measured in 57 BA patients and 15 healthy controls using commercially available enzyme-linked immunosorbent assays. The mean ages of the BA patients and the controls were 6.1 ± 0.6 and 6.7 ± 1.1 years, respectively. The patients were categorized into two groups according to their clinical outcomes: patients with jaundice (total bilirubin ≥ 2 mg/dl) and patients without jaundice (total bilirubin < 2 mg/dl). In our study, serum levels of TIMP-1 were significantly higher in the BA patients than in healthy subjects (4.8 ± 0.4 *vs.* 3.5 ± 0.3 ng/ml, respectively; $p < 0.05$). Additionally, serum levels of TIMP-1 significantly increased in the BA patients with jaundice in comparison to those without jaundice (6.3 ± 0.7 *vs.* 3.1 ± 0.3 ng/ml, respectively; $p = 0.001$). Patients with persistent jaundice had lower levels of albumin but had greater levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transpeptidase compared with patients without jaundice. Furthermore, patients with portal hypertension (PH) had higher TIMP-1 levels than those without PH (5.3 ± 0.4 *vs.* 1.9 ± 0.3 ng/ml, respectively; $p < 0.001$). It is concluded that serum levels of TIMP-1 increased in patients with BA. The significant increase in TIMP-1 levels is related to the presence of PH and the severity of jaundice. The elevated TIMP-1 levels may reflect the degree of hepatic fibrosis and development of PH. The data suggest that TIMP-1 may play a role in the pathophysiology of post-Kasai BA.

Biliary atresia (BA) is one of the most common causes of obstructive jaundice in neonates and remains the leading indication for liver transplantation in children. It is characterized by a fibrosclerotic obliteration of the extrahepatic and intrahepatic biliary system in the first few months of life.[1] Without medical and surgical intervention, BA leads to progressive deterioration with severe bile retention, cholestatic liver damage, liver failure, and death. Although biliary obstruction may be surgically relieved by hepatic portoenterostomy or Kasai procedure, a number of patients ultimately develop various complications such as biliary cirrhosis, portal hypertension, and gastroesophageal varices.[2] Potential etiopathogenic mechanisms of BA include perinatal viral infections, genetic defects, immune-mediated bile duct injury, and autoimmune disorders involving the bile ducts.[3]

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Hepatic fibrosis is characterized by a progressive pathological process involving multiple cellular and molecular events that lead ultimately to deposition of excess extracellular matrix proteins.[4] When this process is combined with ineffective regeneration and repair, there is an increasing disruption of the normal liver architecture resulting in cirrhosis. In the extracellular space, matrix degradation occurs predominantly as a consequence of the action of a family of enzymes called the matrix metalloproteinases (MMPs).[5] These enzymes are in turn inhibited by tissue inhibitors of metalloproteinases (TIMPs). The imbalance between MMPs and TIMPs is considered to be an important determination for deposition and breakdown of the extracellular matrix. Thus far the TIMPs family consists of TIMP-1, TIMP-2, TIMP-3, and TIMP-4.[6] Of the four known TIMPs, TIMP-1 has strong inhibitory effects on various types of MMPs and is an essential regulator of fibrogenic events in the liver.[7]

Although TIMP-1 can be readily measured in serum samples from patients, there is only limited data on the clinical significance of measuring serum levels of TIMP-1 in BA patients.[8] Thus, the objectives of the study were to investigate the serum levels of TIMP-1 in patients with BA and to determine whether there is an association between serum TIMP-1 and clinical outcome.

MATERIALS AND METHODS

The protocol of this study has been approved by the Ethics Committee for Human Research of the Faculty of Medicine, Chulalongkorn University, Thailand. All parents of children with BA and healthy controls had been informed of the objectives and the protocol of the study. The written informed consents were obtained from the parents before commencing the study.

Population

Fifty-seven pediatric patients with BA undergoing hepatic portojejunostomy with Roux-en-Y (original Kasai operation) (25 boys and 32 girls; mean age 6.1 ± 0.6 years) who attended the pediatric liver clinic were recruited. Fifteen healthy children (7 boys and 8 girls; mean age 6.7 ± 1.1 years) who attended the well-baby clinic at King Chulalongkorn

Memorial Hospital for immunization and had normal physical examinations and no underlying diseases served as controls.

Portal hypertension (PH) was validated by the presence of ascites and/or esophageal varices demonstrated by endoscopy. The BA patients were classified into two groups according to their serum total bilirubin (TB) levels: patients without jaundice (TB < 2 mg/dl, n = 34) and patients with persistent jaundice (TB $\geq$ 2 mg/dl, n = 23). Among the 57 BA patients, 20 patients had no PH, whereas the rest 37 of them suffered from it. None of the patients received liver transplantation or exhibited symptoms and signs of fever or ascending cholangitis or clotting abnormalities at the time of blood sampling.

Laboratory methods

The serum samples were collected and stored at -70ºC until assayed. Serum levels of TIMP-1 were determined by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R & D Systems, Minneapolis, MN) according to the manufacturer's instruction. Recombinant human TIMP-1 was used to generate a standard curve. Liver function tests including serum albumin, total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and gamma glutamyl transpeptidase (GGT) were measured by automation using the Hitachi 912 machine.

Statistical analysis

Comparisons of serum levels of TIMP-1 between those of the BA patients and the controls were performed using unpaired t-test. The levels of significance were set at p-values < 0.05. Data are expressed as mean $\pm$ SEM.

RESULTS

None had serial measurements of serum TIMP-1. There were no significant differences regarding age and gender between the 57 BA patients and the 15 normal healthy controls. Table 1 illustrates demographic data between BA patients without jaundice and BA patients with persistent jaundice. The BA patients with jaundice had lower albumin

Table 1 Demographic data, liver function test, and serum levels of TIMP-1 of all studied patients, BA patients with jaundice, and patients without jaundice. Data are expressed as mean ± SEM. *P*-values for differences between the jaundice and jaundice-free patients

BA patients	All patients	Patients with jaundice	Patients without jaundice	*p*
No. of patients	57	23	34	
Gender (M/F)	25/32	10/13	15/19	NS
Age (years)	6.1 ± 0.6	5.1 ± 1.0	6.8 ± 0.7	NS
Albumin (3.4-5.5 g/dl)*	4.0 ± 0.1	3.6 ± 0.2	4.4 ± 0.1	NS
Total bilirubin (0-1 mg/dl)*	6.5 ± 1.4	15.0 ± 2.5	0.8 ± 0.1	< 0.0005
Direct bilirubin (0-0.25 mg/dl)*	4.9 ± 1.1	11.8 ± 1.8	0.2 ± 0.1	< 0.0005
AST (0-38 U/l)*	150.0 ± 17.1	243.3 ± 29.4	86.9 ± 11.7	< 0.0005
ALT (0-38 U/l)*	124.0 ± 13.2	158.2 ± 20.0	101.6 ± 16.4	0.02
ALP (39-117 U/l)*	473.5 ± 35.6	576.4 ± 50.9	404.0 ± 45.1	0.01
GGT (7-50 U/l)*	256.0 ± 30.5	359.4 ± 54.0	186.1 ± 30.4	0.002
TIMP-1 (ng/ml)*	4.8 ± 0.4	6.3 ± 0.7	3.1 ± 0.3	< 0.001

*Normal values; NS, not significant; U/l, units per liter.

levels than those without jaundice, but the difference was not statistically significant. However, the patients with jaundice had higher levels of AST, ALT, ALP, and GGT compared with those without jaundice.

Serum levels of TIMP-1 in the jaundice-free group, jaundice group, all patients, and healthy controls were 3.1 ± 0.3, 6.3 ± 0.7, 4.8 ± 0.4, and 3.5 ± 0.3 ng/ml, respectively. Serum levels of TIMP-1 were significantly higher in patients with BA than in controls ($p < 0.05$). In the BA patients, serum TIMP-1 levels were significantly elevated in the jaundice group compared with the jaundice-free group ($p = 0.001$). There was no significant differences in serum levels of TIMP-1 between the jaundice-free patients and the controls (Fig. 1). Patients with PH (n = 37) had higher serum levels of TIMP-1 than those without PH (n = 20) (5.3 ± 0.4 *vs.* 1.9 ± 0.3 ng/ml, $p < 0.001$) as demonstrated in Fig. 2.

DISCUSSION

Biliary atresia, a fibrotic hepatic disease, is a congenital obliterative cholangiopathy of unknown aetiology, affecting hepatic bile ducts. Without proper treatment, the infants succumb to hepatic failure within two years. Currently, the Kasai portoenterostomy is accepted as the first line of treatment for establishing bile flow to the gastrointestinal tract in BA children. Even when the bile flow is established, hepatic fibrosis, cirrhosis, and hepatic failure may still occur.[1-3] Hepatic fibrosis can be particularly detrimental, leading to portal hypertension and other complications such as ascites, splenomegaly, or gastroesophageal varices. Although there have been several studies concerning the pathophysiology of progressive liver fibrosis in post-operative BA patients including serum levels of cytokines[9-11] and serum growth factors,[12-14] the exact mechanism is still unknown.

Hepatic fibrosis results from an increase in liver collagen and other matrix proteins and represents a continuous wound healing process in the liver in response to chronic hepatic insults.[15] This process is characterized by activation of hepatic stellate cells, which involve the synthesis of matrix proteins and the regulation of matrix degradation.[16] The result of an increase in synthesis or a decrease in degradation ultimately leads to accumulation of excess matrix proteins that distorts the liver architecture and impairs liver functions.[17] MMPs play a predominant role in the degradation of extracellular matrix proteins and are secreted in an inactive proenzyme that is then stimulated by a number of specific cleavage mechanisms. These active enzymes are principally regulated by specific tissue inhibitors of TIMPs.[5,6] Among the four known TIMPs, the universal MMP-
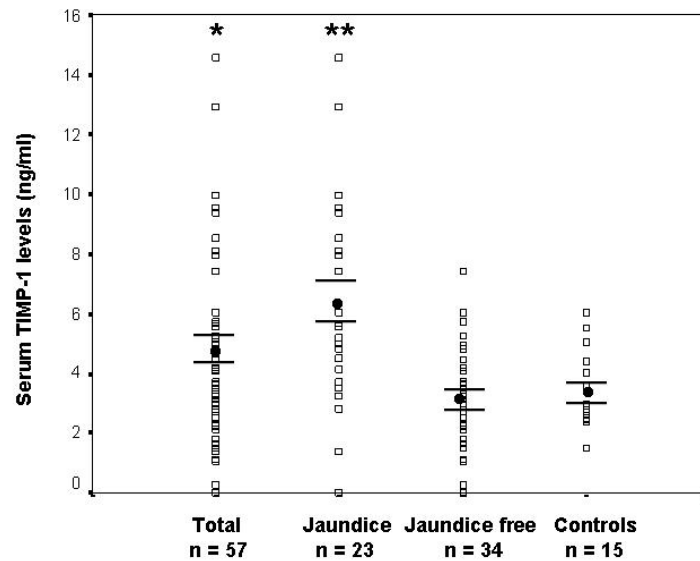
Fig. 1 Serum levels of TIMP-1 in BA patients (based on total bilirubin) and controls. Data are expressed as mean ± SEM. *$p < 0.05$ compared with controls. **$p = 0.001$ compared with controls.

inhibitor TIMP-1 is a substantial regulator of fibrogenic events in the liver.[18] The primary cellular source of TIMP-1 in the injured liver is the activated hepatic stellate cells. Recently, high levels of TIMP-1 being associated with poor prognosis have been reported in various cancer diseases including gastric cancer,[19] colorectal cancer,[20] breast cancer,[21] and head and neck cancer.[22] Moreover, elevated serum levels of TIMP-1 reflects hepatic fibrogenesis in cystic fibrosis liver disease,[23] hepatitis C,[24] and alcoholic liver disease.[25] However, only limited amounts of data are available at present regarding the clinical significance of TIMP-1 in BA.

In the present study, our data demonstrated that the serum levels of TIMP-1 were elevated in the BA patients compared with those of the controls. We have shown that TIMP-1 concentrations were associated with serum aminotransferase activities (AST, ALT, and GGT). Nevertheless, we cannot exclude the possibility that this association might be attributable to advanced liver fibrosis in the BA patients. In accordance with our findings, numerous reports have previously demonstrated that patients with liver diseases had elevated serum levels of TIMP-1 compared with those of healthy individuals.[23-27] These suggest that high serum levels of TIMP-1 are associated with liver damage, and hence

reflect hepatic fibrosis. Furthermore, recent investigations have reported a significant difference in levels of TIMP-1 expression between BA patients and normal controls.[28,29] Expression of TIMP-1 increased in the livers of BA patients using DNA microarray, quantitative reverse transcription-polymerase chain reaction (QRT-PCR),[28] and differentially expressed sequence tags screening.[29] However, in a recent study by Kobayashi *et al.*[8] which included a limited number of patients, serum levels of TIMP-1 were found to be no significant difference between BA patients and controls. The discrepancy of the previous report includes: (1) different age groups with older children were studied; (2) a limited number of patients were recruited.

It is of particular interest that the highest levels of TIMP-1 were revealed in BA patients with PH. Recent studies have also demonstrated that serum levels of TIMP-1 and its hepatic expression were closely correlated with certain degree of portal inflammation and hepatic fibrosis in patients with cirrhosis, suggesting that TIMP-1 may contribute to the development and perpetuation of PH.[26,27] Although further prospective studies are warranted to fully evaluate the importance of TIMP-1 in portal inflammation, our present results suggest that increased levels of serum TIMP-1 could play an essen-
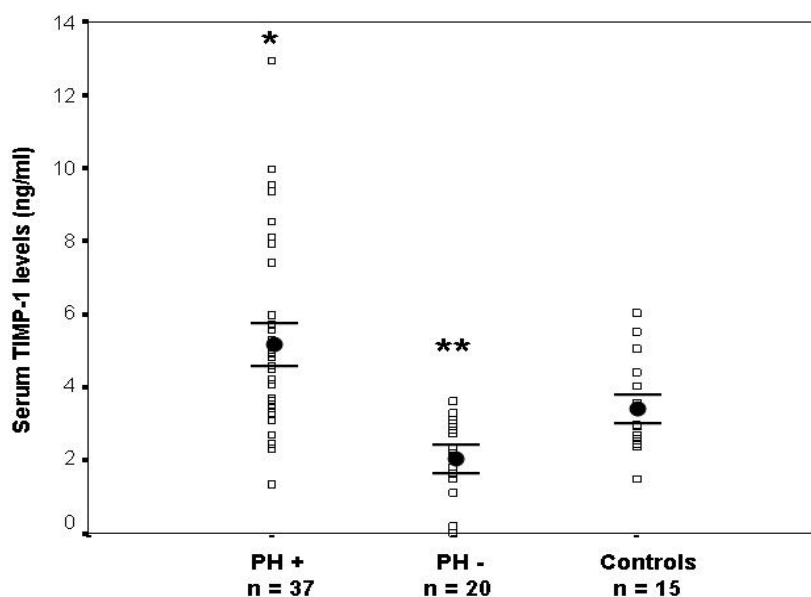
Fig. 2 Serum levels of TIMP-1 in BA patients (based on portal hypertension) and controls. Data are expressed as mean ± SEM. *$p < 0.05$ compared with controls. **$p < 0.001$ compared with PH+. PH, portal hypertension.

tial role in the progressive hepatic fibrosis and PH in patients with BA.

In conclusion, BA patients had significantly increased serum levels of TIMP-1 compared with controls. Additionally, serum levels of TIMP-1 were higher in BA patients with PH than those without PH. It is likely that TIMP-1 increment would impair the metalloproteinase activities and thus results in an abnormality of matrix degradation leading to hepatic fibrosis. The results obtained from the present study show that the elevation of serum levels of TIMP-1 is associated with liver dysfunction in BA patients, and that serum levels of TIMP-1 may be utilized as a clinical parameter of progression to hepatic fibrosis and development of PH in the follow-up of post-Kasai BA patients.

ACKNOWLEDGEMENTS

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