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Original article

Elevated adiponectin is associated with poor outcome in children with biliary atresia

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Background: Biliary atresia (BA) is a severe neonatal liver disease characterized by progressive fibrosclerotic obliteration of the extrahepatic biliary tree.

Objective: We compared serum adiponectin in post Kasai BA patients with healthy controls and associate adiponectin with clinical outcomes of BA patients.

Methods: One hundred and six postoperative BA patients and 40 controls were recruited in this study. BA patients were categorized into two groups based on 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 adiponectin levels were determined by enzyme-linked immunosorbent assay.

Results: BA patients had higher serum adiponectin levels than healthy controls (172.8±90.9 vs. 93.9±53.5 ng/mL, p <0.001). Serum adiponectin levels were elevated in BA patients with jaundice compared to those without jaundice (229.6±89.0 vs. 139.7±74.5 ng/mL, p <0.001). Furthermore, BA patients with elevated ALT displayed significantly higher levels of serum adiponectin than those with normal ALT (187.2±91.8 vs. 117.6±62.8 ng/mL, p <0.001). Additionally, BA patients with portal hypertension had substantially higher serum adiponectin than those without portal hypertension and a poorer clinical outcome (207.0±90.2 vs. 118.5±62.1 ng/mL, p <0.001).

Conclusions: Increased serum adiponectin was associated with a poor outcome in postoperative BA patients. Serum adiponectin might be utilized as a biochemical indicator reflecting the deterioration of liver function and poorer outcome in BA after Kasai operation.

Keywords: Adiponectin, biliary atresia, jaundice, liver fibrosis, portal hypertension

Biliary atresia (BA) is a chronic cholestatic liver disease of children and is characterized by progressive inflammatory cholangiopathy and periportal fibrosis. Obstructive cholestasis can be surgically relieved by Kasai portoenterostomy, which re-establishes ductal continuity for bile drainage [1]. Despite early diagnosis and Kasai procedure, the great majority eventually develop heptic fibrosis, biliary cirrhosis, portal hypertension, and end-stage liver disease [2, 3]. Liver transplantation is then necessary when the Kasai operation has failed. Although several etiologies of BA have been postulated including perinatal and neonatal viral infections, congenital malformations, vascular abnormalities and autoimmune defects, the exact pathogenesis of BA remains as yet obscure [4].

Adiponectin is encoded by the ADIPOQ gene located on the chromosomal region 3q27 and is one of adipokines exclusively secreted by adipocytes. Adiponectin is also known as complement-related protein 30 (Acrp30), adipose most abundant gene transcript (apM1) and adipoQ [5]. A number of animal models and clinical investigations demonstrated that adiponectin mediated anti-obesity, anti-atherosclerotic, and anti-inflammatory effects [6]. Direct effects on hepatocytes via a specific receptor (AdipoR2 receptor) and anti-inflammatory properties are partially mediated by its antagonism against TNF-α [7]. This raises the hypothesis of a potential hepatoprotective role of adiponectin against liver

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fibrosis and cirrhosis. However, the precise role of adiponectin in the pathogenesis of BA remains poorly understood. Adiponectin is associated with liver fibrosis and inflammation [8, 9], suggesting that it might be associated with the pathogenesis of liver dysfunction in biliary atresia

Although circulating levels of several growth factors and cytokines have been extensively studied in patients with BA, published data of serum adiponectin levels from various clinical stages of BA are still limited [10-13]. We have previously reported liver stiffness measurement and the relationship of liver stiffness with adiponectin in BA [14]. However, the relationships between serum adiponectin levels and biochemical parameters and clinical outcomes in a large number of BA children have not been documented recently. We hypothesized that serum adiponectin would be increased in BA patients. The objective of the present study was to investigate serum concentrations of adiponectin in post Kasai BA patients and to determine the possible association of serum adiponectin and clinical outcome of BA patients after Kasai operation.

Patients and methods

Study population

One hundred and six pediatric patients with BA (44 boys and 62 girls; mean age = 5.8±4.7 years) who experienced hepatic portojejunostomy with Roux-en-Y reconstruction (Kasai procedure) and never received liver transplantation were enrolled in the study. The control group comprised 40 healthy children (19 boys and 21 girls; mean age = 5.9±2.4 years) who attended the Well Baby Clinic at King Chulalongkorn Memorial Hospital for vaccination. The children in the control group had normal physical findings and no previous medical illness. During blood collection, none of the BA patients displayed fever, ascending cholangitis or clotting disorders.

In order to associate biochemical parameters with clinical outcomes among BA patients, they were placed in two groups according to serum total bilirubin (TB) and serum alanine aminotransferase levels (ALT). According to the jaundice status, BA patients were categorized as no jaundice group (TB<2 mg/dL; good outcome, n = 67) and persistent jaundice group (TB≥ 2 mg/dL; poor outcome, n = 39). Further analysis was based on serum ALT and they were classified into a normal ALT group (ALT<45 IU/L, n = 22) and a high ALT group (ALT≥45 IU/L, n = 84). Subsequently, portal hypertension (PH) was validated by the presence of ascites and/or esophageal varices diagnosed by endoscopy. Thirty of 67 BA children without jaundice and 36 of 39 children with persistent jaundice had evidence of portal hypertension. The study protocol was approved by the Institutional Ethical Review Board of the Faculty of Medicine, Chulalongkorn University. The present study was conducted in agreement with the guidelines of the Declaration of Helsinki. All parents of children were counseled and informed of the study's purposes and any intervention involved in this study. Written informed consent was obtained before enrolment.

Laboratory methods

Specimens of peripheral venous blood were collected from each patient and healthy control, centrifuged, and then stored at -80^{O}C until subsequent analysis. Quantitative determination of adiponectin concentration in serum was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA). According to the manufacturer's protocol, 50 µl of recombinant human adiponectin standards and serum samples were pipetted into each well, which has been pre-coated with specific antibody for adiponectin. After incubating for 2 hours at room temperature, every well was washed thoroughly with wash buffer 4 times. 200 µl of a horseradish peroxidase-conjugated monoclonal antibody specific for adiponectin was then added to each well and incubated for a further 2 hours at room temperature. After 4 washes, substrate solution was pipetted into the wells and the microplate was incubated for 30 min at room temperature with protection from light. Lastly, the reaction was stopped by the stop solution and the color intensity was measured with an automated microplate reader at 450 nm. The adiponectin concentration was determined using a standard optical density-concentration curve. Twofold serial dilutions of recombinant human adiponectin with a concentration of 3.9 to 250 ng/ml were used as standards. In addition, liver function tests including serum albumin, total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were determined using an automated chemical analyzer (Hitachi 911) at the central laboratory of our hospital.

Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. All values are expressed as a mean±standard deviation (SD) unless otherwise specified. Comparison of demographic data and biochemical parameters between groups were analyzed by unpaired *t*-test. Correlation between numerical data was acquired using the Pearson correlation coefficient (r). A *p*-value<0.05 indicated statistical significance.

Results

One hundred and six serum samples from BA patients and 40 from healthy controls were measured for adiponectin levels. Demographic data and biochemical parameters including liver function tests and serum adiponectin levels based on jaundice status are presented in **Table 1**. BA patients with persistent jaundice had significantly lower albumin levels than those without jaundice. Serum levels of AST, ALT, ALP, and GGT were markedly elevated in BA patients with jaundice compared to those without jaundice.

As shown in **Figure 1**, serum adiponectin concentrations measured in all of the BA group including the persistent jaundice, no jaundice, and healthy control groups were 172.8±90.9, 229.6±89.0, 139.7±74.5, and 93.9±53.4 ng/mL, respectively. BA patients had significantly higher serum adiponectin concentrations than healthy controls (p <0.001). In BA patients with persistent jaundice, serum adiponectin levels were significantly elevated compared to those in patients without jaundice (p <0.001). BA patients without jaundice still had higher serum adiponectin levels than healthy controls (p = 0.007).

BA patients were later re-evaluated regarding serum ALT levels, as shown in **Table 2**. Subgroup analysis of BA children displayed that the patients with high ALT had more elevated serum adiponectin than those with normal ALT and healthy controls (187.2±91.8 vs. 117.6±62.8 vs. 93.9±53.4 ng/mL, respectively, p <0.001) (**Figure 2**). In addition, BA patients with portal hypertension (PH) had significantly higher levels of serum adiponectin with respect to those without PH (207.0±90.2 vs. 118.5±62.1 ng/mL, p <0.001), as illustrated in **Figure 3**. Especially in BA patients without jaundice, serum adiponectin levels were significantly higher in the patients with PH compared to those without PH (170.7±80.3 vs. 116.9±63.3 ng/mL, p = 0.003). Furthermore, serum adiponectin positively correlated with serum AST (r = 0.626, p <0.001), ALT (r = 0.344, p <0.001), ALP (r = 0.335, p = 0.001), and TB (r = 0.626, p <0.001) as shown in **Figure 4**.

Table 1. Demographic data, liver function test and serum adiponectin levels of biliary atresia patients categorized according to their jaundice status. The data are expressed as mean±SD.

	Total	Persistent jaundice	No jaundice	*p*-value
No. patients	106	39	67	
Gender (M/F)	44/62	16/23	28/39	NS
Age (years)	5.8±4.7	5.9±5.7	5.7± 3.4	NS
Albumin (g/L)	4.2±0.7	3.8± 0.8	4.5±0.4	<0.001
Total bilirubin (mg/dL)	4.5±7.2	11.1±8.4	0.7± 0.4	<0.001
Direct bilirubin (mg/dL)	3.7±6.4	8.9±7.0	0.6±3.3	<0.001
AST (IU/L)	137.2±103.5	213.7±106.9	92.7±70.7	<0.001
ALT (IU/L)	121.9±96.4	159.4±101.4	100.0±86.84	0.009
ALP (IU/L)	468.3±275.0	586.7±274.9	400.9±253.3	0.004
GGT (IU/L)	286.5±323.9	424.3±407.6	184.9±195.4	0.011
Adiponectin (ng/mL)	172.7±90.8	229.6±89.0	139.7±74.5	<0.001

NS: not significant

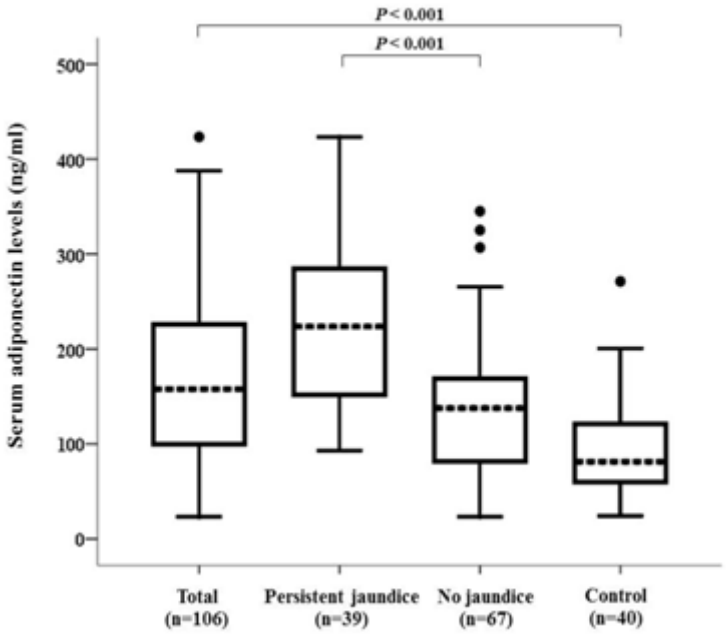

Figure 1. Serum adiponectin levels between biliary atresia patients based on total bilirubin and controls.

Table 2. Demographic data, liver function test, and serum adiponectin levels of biliary atresia patients categorized according to serum ALT status. The data are expressed as mean□SD.

	Normal ALT	**High ALT**	**p-value**
No. patients	22	84	
Gender (M/F)	4/18	40/44	NS
Age (years)	6.5±3.5	5.6±5.0	NS
Albumin (g/L)	4.7±0.4	4.1±0.7	<0.001
Total bilirubin (mg/dL)	1.3±3.4	5.4±7.7	<0.001
Direct bilirubin (mg/dL)	1.8±5.9	4.2±6.4	NS
AST (IU/L)	37.7±9.8	163.2±101.1	<0.001
ALT (IU/L)	27.9±8.2	146.5±93.7	<0.001
ALP (IU/L)	238.5±150.9	524.4±269.7	<0.001
GGT (IU/L)	51.0±48.8	355.7±337.9	<0.001
Adiponectin (ng/mL)	117.6±62.8	187.2±91.8	<0.001

NS: not significant

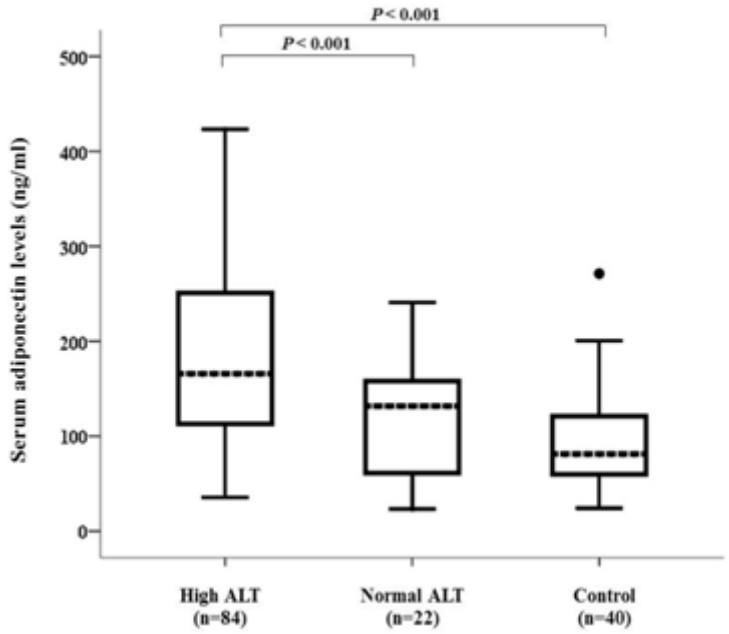

Figure 2. Serum adiponectin levels between biliary atresia patients based on serum ALT and controls. ALT, alanine aminotransferase.

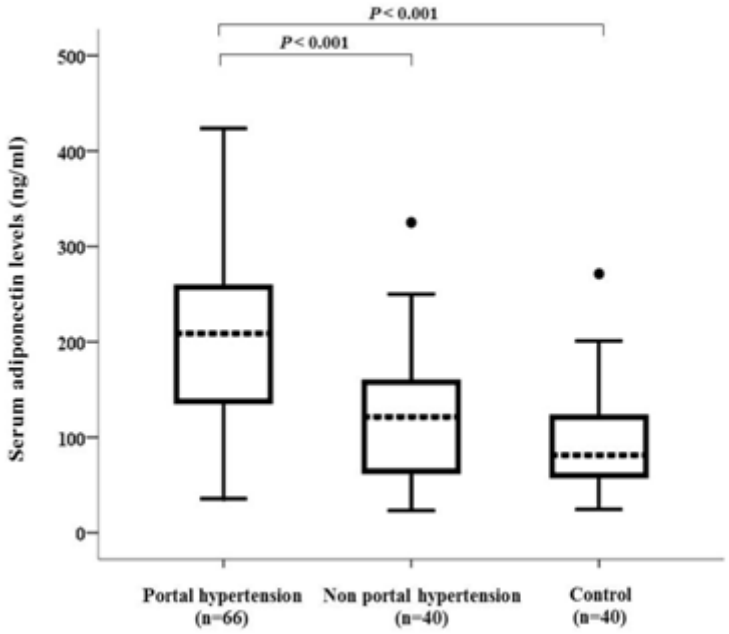

Figure 3. Serum adiponectin levels between biliary atresia patients based on the presence of portal hypertension and controls.

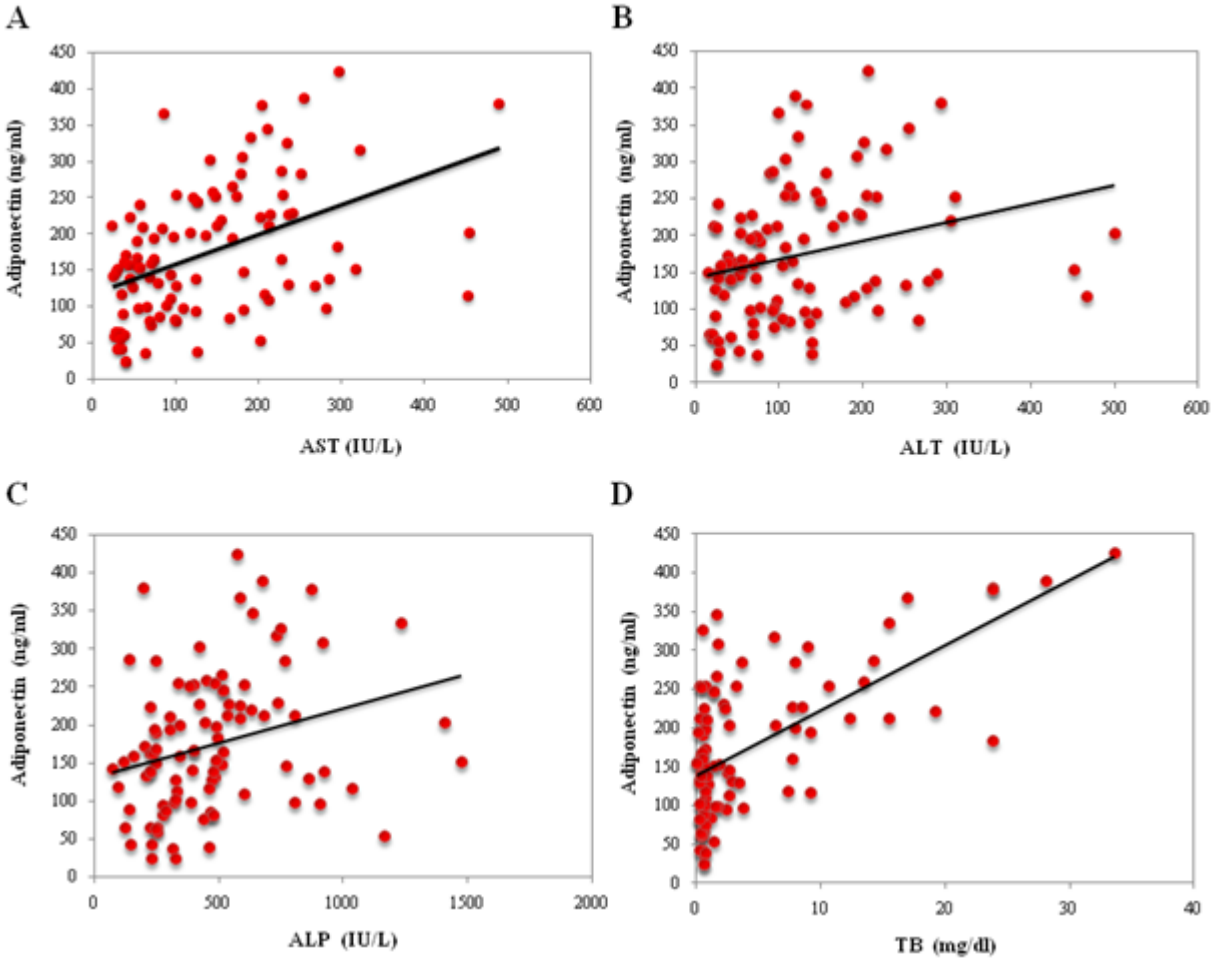

Figure 4. Correlation analysis between adiponectin levels and biochemical data. Serum adiponectin levels were positively correlated with (A) aspartate aminotransferase (AST, r = 0.626, p <0.001), (B) alanine aminotransferase (ALT, r = 0.344, p <0.001), (C) alkaline phosphatase (ALP, r = 0.335, p = 0.001) and (D) total bilirubin (TB, r = 0.626, p <0.001) in patients with BA.

Discussion

The present study showed that serum adiponectin levels in BA patients were significantly higher than in healthy controls. In the BA patients, serum adiponectin levels were more elevated in BA patients with persistent jaundice than in those without jaundice. Subsequent analysis revealed that the BA patients with high ALT had increased concentrations of serum adiponectin compared to those with normal ALT. Elevated serum adiponectin positively correlated with serum AST, ALT, ALP and with serum total bilirubin in postoperative BA patients. Serum AST and ALT routinely serves as a specific biochemical parameter of liver dysfunction reflecting hepatocellular damage. In addition, serum ALP is likely to be an indicator for the severity of biliary obstruction. Therefore, these findings suggest that serum adiponectin can be used as an indicator of ongoing liver injury and biliary obstruction in postoperative BA patients.

This study clearly demonstrated the relationship between serum adiponectin and outcome parameters in biliary atresia patients. Serum adiponectin level was associated with clinical outcomes (status of jaundice, hepatic dysfunction, and portal hypertension). Recently, an increase of adiponectin expression in circulation and/or liver tissues has been documented in a number of liver diseases, including chronic hepatitis, hepatic steatosis, hepatic fibrosis, liver cirrhosis, and liver cancer [14-19]. More prospective studies on hepatic adiponectin expression are needed to understand mechanisms.

Findings from this study are in agreement with our previous publication showing that serum galectin-3, matrix metalloproteinase-3, osteopontin and bone

morphogenetic protein 7 were more elevated in BA patients than in healthy controls [10-13]. These cytokines were significantly associated with clinical outcomes in BA and have been implicated in progressive hepatic fibrosis. Adiponectin was found to correlate with the progression of liver dysfunction and portal hypertension. Further studies on additional cytokines and growth factors may help identify more pieces of the inflammatory jigsaw in BA.

Several possible mechanisms could be responsible for the elevation of serum adiponectin in BA patients. Increased adiponectin production in the damaged liver may result in high levels. The high adiponectin concentrations could be from the imbalance between adiponectin synthesis and adiponectin clearance. In advanced stage of BA, reduced adiponectin clearance could contribute to elevated serum adiponectin levels. Furthermore, other organs apart from liver can synthesize and secrete adiponectin and the elevated serum adiponectin could be of extrahepatic origin including from adipose tissues.

The present study was limited to patients who attended our hospital. In addition, incomplete assessment of potential confounders such as age, gender, medical comorbidities need to be taken into account. Moreover, this study was cross-sectional in its design, definite cause and effect relationship may not be drawn. The results showed that serum adiponectin levels were more pronounced in BA patients with high ALT than in those with normal ALT. It appears that elevated serum adiponectin levels found in our post Kasai BA patients are associated with poor outcome in BA patients after surgical correction, possibly due to surgery or other factors. Future investigation on pre-operative BA patients and/or non-BA children with cholestatic liver diseases may answer this question.

To sum up, we showed that BA patients had significantly elevated serum adiponectin levels compared with healthy controls. Serum adiponectin was more pronounced in BA patients with persistent jaundice compared to those without jaundice. Serum adiponectin in BA patients with high ALT was significantly elevated compared to those with normal ALT. Further analysis showed that BA patients with PH had markedly higher serum adiponectin levels than those without PH. These findings suggest that high serum adiponectin is associated with hepatic injury and hence reflects the degree of liver dysfunction in BA. There was a positive correlation between serum adiponectin, serum AST, ALT, ALP, and TB. These findings suggest that adiponectin may play a possible role in the pathogenesis of hepatic fibrosis in post Kasai BA patients.

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