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Title: Impact of serum autotaxin level correlating with histological findings in biliary atresia

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Abstract

Purpose: Portoenterostomy is the standard treatment for biliary atresia (BA) that reduces jaundice in two thirds of cases. However, progressive liver fibrosis is common, leading to cirrhosis in most patients. Autotaxin is a new marker for the progression of hepatic fibrosis. We examined the relationship between serum autotaxin levels and liver histological findings in patients with BA. Methods: BA patients with native livers were identified in our hospital. Patients underwent protocol liver biopsies every 1 to 5 years, and liver fibrosis was evaluated based on the METAVIR score. Serum autotaxin levels were compared with the last available pathological findings. Results: Thirty-five patients were included and the median age was 10.6 years. Serum autotaxin levels was median 1.6 mg/L. The mean autotaxin level was 1.08 mg/L in F0, 1.07 mg/L in F1, 0.95 mg/L in F2, 2.17 mg/L in F3, and 2.50 mg/L in F4; it was significantly higher in F4 than in F0–F2 ($P<0.0024$). For predicting cirrhosis (F4) and advanced liver fibrosis (≥F3), autotaxin had the almost same areas under the curve (AUCs 0.78 and 0.90, respectively) as well as M2BPGi. Conclusion: Autotaxin levels could be used to evaluate the status of native liver fibrosis.

Keywords: liver fibrosis, cirrhosis, liver biopsy, liver transplantation, fibrosis marker

Type of study: Study of Diagnostic Test

Level of evidence: Level II

Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase; AUC: area under the curve; BA :biliary atresia ; FIB-4: Fibrosis-4; LPA: lysophosphatidic acid; LPC: lysophosphatidylcholine

M2BPGi: Mac-2 binding protein glycosylation isomer; PCR: polymerase chain reaction; ROC: Receiver operating characteristic

Manuscript Text

Introduction

Biliary atresia (BA) is characterized by complete obliteration of the extrahepatic bile ducts. Portoenterostomy is the standard treatment for BA that reduces jaundice in two thirds of cased. However, fibrosis of the liver generally progresses, even in jaundice free patients. Patients who develop end-stage cirrhosis may require liver transplantation.

Liver biopsy, an invasive technique requiring general anesthesia in children, is the gold standard for assessing the degree of liver fibrosis. However, the evaluation of fibrosis can be uncertain due to sampling error and variations among observers. Several noninvasive biomarkers to predict fibrosis in BA have been identified, for instance Fibrosis(FIB)-4 index, type IV collagen 7s domain and Mac-2 binding protein glycosylation isomer (M2BPGi)[1].

Autotaxin is a 125-kDa secreted glycoprotein belonging to the ENPP family. Autotaxin catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA), which functions as a phospholipase [2]. It regulates a variety of cellular processes, including proliferation, migration, angiogenesis, fibrogenesis, and cancer progression [3]. Autotaxin is synthesized by a variety of normal cells and tissues, secreted into the circulation as a glycoprotein, and later degraded by liver sinusoidal endothelial cells [4]. Recently, elevated levels of serum autotaxin were implicated in fibrosis progression in chronic hepatitis C[5]; a main mechanism was considered to be the retarded degradation of circulating autotaxin due to liver sinusoidal endothelial cell dysfunction secondary to liver fibrosis [6].

The aims of this study were to examine the relationship between serum autotaxin levels and liver histological findings in patients with BA, and to compare both with other laboratory parameters.

Methods

Patients

Thirty-five patients who were diagnosed with BA and followed at our hospital between June and December 2019 were included in this study. Serum autotaxin and other laboratory data were evaluated during this period. All patients received blood tests at least every 6 months and routine biopsies per-protocol every year by 6-year-old, every two years by 12-year-old, and every 3 to 5 years thereafter. Once patients reached stage F4 fibrosis, further biopsies were not performed. We examined the relationship between autotaxin levels and the last available histological findings of liver fibrosis, and compared both with other laboratory markers of liver fibrosis, including FIB-4 index, platelet count, aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, type IV collagen 7s domain, and M2BPGi.

Measurement of autotaxin levels

Serum levels of autotaxin were measured using a two-site enzyme immunoassay. The assay reagent, which is commercially available in Japan, was provided by Tosho Corp. (Tokyo, Japan) and was used with the automated immunoassay analyzer AIA-2000 System (Tosho Corp.). This system includes automated dispensation of 10 μL of specimen, incubation of the reaction cup, bound/free washing, 4-methylumbelliferyl phosphate substrate dispensation, and fluorometric detection [7].

Histological assessment

Liver biopsy samples were assessed with hematoxylin–eosin and Masson's trichrome stains. Liver biopsies were performed percutaneously with a 16-gauge biopsy needle, using ultrasound guidance and with patients under either general anesthesia or intravenous sedation. After staining, Fibrosis was staged based on METAVIR score. No serious procedure-related complications occurred.

Statistical analysis

Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve (AUC) for laboratory markers of liver fibrosis for the presence of liver cirrhosis (F4) and advanced fibrosis (≥F3) on histological examination. For continuous variables, comparisons among groups were carried out using Student's t-test. Data are expressed as medians (range). $P<0.05$ was considered to be statistically significant. Statistical analyses were carried out with JMP 11 software (SAS Institute, Cary, NC, USA). This study was approved by our hospital institutional review board (approval number 17482).

Results

Demographic characteristics of the study patients

The characteristics of the study patients (n=35) are shown in Table 1. 8 were male and 27 were female, with a median age of 10.6 years (range, 1–39). Serum autotaxin levels ranged from 0.6 to 4.0 mg/L (median, 1.6 mg/L). Child-Pugh scores ranged from 5 to 9. Only 1 patient, with a Child-Pugh score of 9, was a candidate for liver transplantation. Regarding the degree of liver fibrosis–based histological

examination, F0 was observed in 4 patients, F1 in 6, F2 in 5, F3 in 7, and F4 in 13.

Autotaxin and liver fibrosis

Stratified by fibrosis stage (F0–F4), the mean serum autotaxin levels (SD) were as follows: F0, 1.08 mg/L (0.38 mg/L); F1, 1.07 mg/L (0.30 mg/L); F2, 0.95 mg/L (0.34 mg/L); F3, 2.17 mg/L (0.28 mg/L); and F4, 2.50 mg/L (0.38 mg/L). The mean autotaxin level was significantly higher in F4 than in F0–F2 ($P<0.01$) (Fig. 1).

Stratified by fibrosis stage (F0–F4), the mean M2BPGi values were plotted in Fig.2. The mean autotaxin level was significantly higher in F4 than in F0–F2 ($P<0.01$) in M2BPGi as well, but there were no stastically significance in type IV collagen 7s domain and FIB-4 between each fibrosis stages.

ROC analysis

For predicting liver cirrhosis (F4), an autotaxin level of 2.07 mg/L yielded a high AUC of 0.80 with sensitivity 71% and specificity 85%. For predicting advanced liver fibrosis (≥F3) (Fig. 3a), an autotaxin level of 1.64 mg/L yielded a high AUC of 0.93 with a sensitivity of 85% and specificity of 93% (Fig.3b). The AUC, optimal cutoff point, sensitivity, and specificity for each fibrotic marker are summarized in Table 2. For predicting cirrhosis (F4) and advanced liver fibrosis (≥F3), both autotaxin and another new fibrosis marker, M2BPGi, had higher AUCs (0.79 and 0.95, respectively) than the four conventional fibrosis markers including AST to ALT ratio, platelet count, FIB-4 index and type IV collagen 7s domain.

Discussion

BA patients with successful Kasai portoenterostomy will have some hepatic fibrosis or cirrhosis. BA leads to various complications, such as portal hypertension and liver failure, which depend on the stage of liver fibrosis. Assessment of liver fibrosis should be part of long-term surveillance, even in stable patients. Early detection and staging of liver fibrosis are beneficial for predicting complications. Therefore, an indicator of liver fibrosis avoiding invasive liver biopsy was explored.

Liver fibrosis is initiated by the activation of hepatic stellate cells, which results in the production and accumulation of collagen and other extracellular matrices in the liver parenchyma. LPA, produced by autotaxin, inhibited apoptosis and stimulated hepatic stellate cells in an animal model [8]. Watanabe et al. demonstrated that serum autotaxin levels were elevated in patients with chronic hepatitis C, and found that autotaxin was a key enzyme for converting LPC to LPA, and that plasma LPA levels were correlated with those of serum autotaxin in patients with chronic liver disease. [9]. Yamazaki et al. reported that the diagnostic accuracy of autotaxin was comparable to that of M2BPGi [10].

Udomsinprasert et al. reported that quantitative polymerase chain reaction (PCR) showed overexpression of autotaxin mRNA in the livers of patients with BA [11]; in addition, these individuals demonstrated positive staining for autotaxin in the hepatic parenchyma and biliary epithelium, as well as a positive correlation between hepatic autotaxin expression and fibrosis stage. Serum autotaxin levels could be a biochemical marker for activation of hepatic stellate cells and liver fibrosis for BA.

In this study, we demonstrated that serum autotaxin levels were correlated with pathological

fibrosis grade in BA. ROC analysis demonstrated that serum autotaxin levels in patients with BA had diagnostic ability for detecting cirrhosis (F=4) and advanced fibrosis (≥F3) of the native liver as well as M2BPGi.

Heterogeneous atrophy is often encountered in BA native liver. The explanted BA livers at the liver transplant time have central regenerative nodules encased in thick fibrous tissue, whereas the left lateral segment is degenerative and atrophic[12] . A pathological finding of liver biopsy might overestimate the stage of liver fibrosis because the biopsy specimen might be from the atrophied area. Since the atrophied part of the liver is highly fibrotic and diagnosed as F4, patients with stage F4 in this study might include cases with less fibrosis in the residual liver without biopsy. Therefore, autotaxin levels in patients with F4 had more variation than F3, and the sensitivity and specificity were lowered. On the other hand, by referring to the serum autotaxin level, it is possible to prevent overestimating a liver biopsy pathological result. It can support the interpretation of liver biopsy findings.

We acknowledge several limitations of this study. One was that measurement of autotaxin was not the same time of liver biopsy. The reason was that liver biopsy had been performed a maximum every 5 years or once cirrhosis was proven, no further liver biopsy was performed because liver biopsy cannot be done frequently, especially for patients who reached cirrhosis due to risk of complication. In older BA patients, who were the majority of this study, fibrosis did not progress rapidly due to the stable course. This study was a pilot study to show a tendency of autotaxin level. Therefore, further study is required to

evaluate autotaxin level at the time of biopsy. And, there were not enough patients to produce statistically significant results for mild liver fibrosis. Finally, we enrolled too few decompensated cirrhotic patients. Future studies should confirm whether serum autotaxin levels are correlated with the severity of chronic liver disease and its complications. Moreover, autotaxin itself may be associated with these complications.

In conclusion, serum autotaxin level is a novel marker for liver fibrosis in patients with BA. It may be useful for follow-up in patients with BA who have a native liver and can support the interpretation of liver biopsy findings. Further study is required to prove its usefulness.

Acknowledgments

We gratefully acknowledge Nobuaki Hata and Akie Iehara of the laboratory for clinical investigation, Osaka University Hospital, for their support in measuring autotaxin.

Declarations of interest: None

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Sources of support: None.

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Figure Legends

Figure 1. Autotaxin and fibrosis stages: Autotaxin level in F4 fibrosis was higher than F0-2 fibrosis stastically. Fibrosis stages were descried METVIR score.

Figure2. Relationship between M2BPGi and the stage of fibrosis: Fibrosis stages were descried METVIR score. M2BPGi: Mac-2 binding protein glycosylation-modified isomer

Figure3. ROC curve analysis of autotaxin and the stages of fibrosis: Fibrosis stages were described METVIR score (a) fibrosis F4, (b) fibrosis ≥ F3.

Table 1. Patient demographics: Diagnosis indicates type of BA. Age of PE indicates age (days) of portoenterostomy. Data were expressed as median with ranges. Historogical findings were based on METAVIR score. AST: aspartate aminotransferase; ALT: alanine transaminase; M2BPGi: Mac‐2 binding protein glycosylation isomer; FIB-4: Fibrosis-4

Table 2. Summary of AUC, optimal cutoff point, sensitivity, and specificity for each fibrotic marker: Histological findings were described by METAVIR score. M2BPGi: Mac-2 binding protein glycosylation-modified isomer; AST: aspartate aminotransferase; ALT: alanine aminotransferase; FIB-4: Fibrosis-4 index