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Title: Clinical implications of serum Mac-2 binding protein (M2BPGi) during regular follow-up of patients with biliary atresia

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Abstract

Purpose: The Mac-2 binding protein glycosylation-modified isomer (M2BPGi) is a new marker for progression of hepatic fibrosis. We examined the relationship between serum M2BPGi levels and liver histological findings in patients with biliary atresia (BA) who were not transplant candidates.

Methods: Patients with BA who were not transplant candidates with good liver function were included. We examined M2BPGi levels and histological findings in relation to other laboratory markers of liver fibrosis, including aspartate aminotransferase (AST) to platelet ratio index, fibrosis-4 index, and type IV collagen 7s domain. Liver fibrosis was evaluated based on the METVIR score.

Results: Thirty-seven patients were included. The median age was 18 years (range, 3–38 years). M2BPGi values ranged from 0.3 to 6.9 cutoff index (COI) (median, 1.6). The degree of liver fibrosis varied with M2BPGi level. For predicting cirrhosis (F4) and advanced liver fibrosis (≥F3), M2BPGi had higher areas under the curve (AUCs; 0.93, respectively) with cutoff COIs of 1.84 and 1.67, respectively, than for the four conventional markers for fibrosis.

Conclusion: M2BPGi is a novel marker for liver fibrosis in patients with BA. It is especially useful for following patients with BA with a native liver and supporting liver biopsy interpretation findings.

Key words: 1. Biliary atresia 2.liver fibrosis 3. FIB-4 4. APRI 5. Liver transplantation

TEXT

Introduction

Biliary atresia (BA) is characterized by complete obliteration of the extrahepatic bile ducts. Kasai portoenterostomy is the standard treatment for BA; however, progressive liver fibrosis usually continues to develop, leading to cirrhosis in most patients [1]. Most patients with decompensated cirrhosis may require a liver transplant. BA leads to various complications, such as portal hypertension and liver failure, which depend on the grade of liver fibrosis. Early detection and staging of liver fibrosis is beneficial for predicting complications resulting from progression of liver fibrosis.

Liver biopsy, an invasive technique, is the golden standard for assessing the degree of liver fibrosis. However, it may cause complications such as organ injury, hemorrhage, and pain. In pediatric patients, general anesthesia is needed for liver biopsy. The evaluation of fibrosis is uncertain due to sampling error and variations among observers [2]. Noninvasive biomarkers such as aspartate aminotransferase (AST) to platelet ratio index (APRI), type IV collagen 7s domain, and hyaluronic acid to predict fibrosis in BA have been identified [3-5]. These biomarkers were initially derived for the evaluation of liver fibrosis in hepatitis C, and their accuracy among patients with BA is under constant debate.

The Mac-2 binding protein glycosylation isomer (M2BPGi), which is also known as Wisteria floribunda agglutinin-positive human Mac-2 binding protein, was recently established as a glycol-

biomarker of liver fibrosis in patients with chronic hepatitis C with a unique fibrosis-related glycoalteration [6]. M2BPGi has been shown to be a useful predictor in various chronic liver diseases. M2BPGi is a marker of liver fibrosis and prognosis in patients with primary biliary cirrhosis (PBC) and autoimmune hepatitis, as well as a predictor of hepatocellular carcinoma development in hepatitis C and liver fibrosis stage in patients with nonalcoholic fatty liver disease [7-10]. However, there have been no available data regarding the relationship between serum M2BPGi levels and histological findings of liver fibrosis in regular follow-up of patients with BA with normal liver function, although Yamada et al. showed that M2BPGi values in patients with BA who are liver transplant candidates[11].

Thus, the aims of this study were to examine the relationship between serum M2BPGi levels and liver histological findings during regular follow-up in patients with BA and to compare them with other laboratory parameters.

Methods

Patients

A total of 37 patients diagnosed with BA who were not liver transplant candidates because of good liver function and followed at our hospital between January 2015 and February 2018 were included in this study. All patients received routine biopsy per protocol every 1 to 5 years. We examined the relationship between M2BPGi levels and histological findings of liver fibrosis and compared them with

other laboratory markers of liver fibrosis, including APRI, FIB-4 index, platelet count, AST to alanine aminotransferase (ALT) ratio, and type IV collagen 7s domain.

The APRI score was calculated using Wai's formula: (AST/upper limit of normal)/platelet count ($\times 10^9$/L) × 100 [12]. The FIB-4 index was calculated using Sterling's formula: age (years) × AST (IU/L) / platelet count ($\times 10^9$/L) × √ALT (IU/ L) [13].

Measurement of M2BPGi

Serum M2BPGi levels were measured by SRL (Tokyo, Japan). Blood was taken regardless of time. Quantification of M2BPGi was based on a lectin-antibody sandwich immunoassay using a fully automatic immune analyzer. M2BPGi measurements were indexed with values obtained using the following equation: cutoff index (COI) = ([M2BPGi] sample [M2BPGi] negative control (NC))/ ([M2BPGi] positive control (PC) + [M2BPGi] negative control (NC)). The PC was supplied as a calibration solution preliminarily standardized to yield a COI value of 1.0. M2BPGi COIs were graded as negative if COI < 1.00, 1+ if the COI was between 1.00 and 3.00, and 2+ if COI > 3.00 [6].

Histological assessment

Liver biopsy samples were assessed with hematoxylin-eosin and Masson's trichrome stains. All biopsies were performed under either general anesthesia for children or intravenous sedation for adult. Liver biopsies were taken percutaneously with a 16-gauge biopsy needle from avascular area under ultrasound. The specimens were fixed in 4% phosphate-buffered formaldehyde and embedded in paraffin.

After hematoxylin-eosin and Masson's trichrome staining, the liver biopsy specimens were examined microscopically. Well-experienced pathologists in our hospital evaluated the samples. Fibrosis stages were staged as F0–F4: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, liver cirrhosis [14].

No serious procedure-related complications were observed. In this study, advanced fibrosis was defined as ≥F3 and significant liver fibrosis was defined as ≥F2.

Statistical analysis

Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve (AUC) for serum M2BPGi level, platelet count, APRI, FIB-4 index, and type IV collagen 7s domain and select the optimal cutoff value that maximized the sum of sensitivity and specificity for the presence of liver cirrhosis (F4), advanced fibrosis (≥F3), and significant fibrosis (≥F2) 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 = 37) are shown in Table 1. There were 12 men and 25 women with a median age of 18 years (range, 3–38). Regarding the degree of liver fibrosis–based histological examination, F4 was observed in 11 patients, F3 in 4, F2 in 6, F1 in 10, and F0 in 6. The M2BPGi value in this study ranged from 0.3 to 6.9 COI (median, 1.6). Child-Pugh scores ranged from 5 to 6. All patients had good liver function, with Child-Pugh class A disease.

M2BPGi and liver fibrosis

By fibrosis stage (F0–F4), the median M2BPGi value (range) was: F0, 0.8 COI (0.5–1.1); F1, 0.9 COI (0.5–1.4); F2, 0.9 COI (0.5–1.4); F3, 1.5 COI (1.1–2.0); and F4, 3.1 COI (0.8–6.9). The median M2BPGi value was significantly higher for F4 than F0–F3 ($P< 0.001$) (Fig. 1a).

M2BPGi grade and liver fibrosis

By fibrosis stage (F0–F4), the M2BPGi grade was: F0, grade 0 (n=5) and grade 1 (n=1); F1, grade 0 (n=6) and grade 1(n=4); F2, grade 0 (n=4) and grade 1 (n=2); F3, grade 1 (n=4); and F4, grade 0 (n=1), grade 1 (n=5), and grade 2 (n=5).

Laboratory markers and M2BPGi

By fibrosis stage (F0–F4), the median type IV collagen 7s domain value (range) was: F0, 4.8 ng/mL (3.8–6.9 ng/mL); F1, 5.1 ng/mL (3.7-7.1 ng/mL); F2, 4.6 ng/mL (3.0–6.8 ng/mL); F3, 5.6 ng/mL (4.4–6.7 ng/mL); and F4, 8.2 ng/mL (4.4–12 ng/mL). The median type IV collagen 7s domain value was significantly higher for F4 than F0–F3 ($P< 0.05$) (Fig. 1b).

By fibrosis stage (F0–F4), the median APRI value (range) was: F0, 0.41 (0.15-0.68); F1, 0.87 (0.15-2.4); F2, 0.48 (0.15-1.38); F3, 1.24 (0.32-2.26); and F4, 1.68 (0.18-3.32). The median APRI value was significantly higher for F4 than F0–F2 ($P< 0.05$) (Fig. 1c).

By fibrosis stage (F0–F4), the median FIB-4 value (range) was: F0, 0.93 (0.41-2.41); F1, 0.94 (0.25-1.99); F2, 0.84 (0.12-2.26); F3, 0.73 (0.53-0.95); and F4, 0.75 (0.13-1.78). There was no stastically significance between each groups (Fig. 1d).

ROC analysis

For predicting liver cirrhosis (F4), an M2BPGi level of 1.84 COI yielded a high AUC (0.93). For predicting advanced liver fibrosis (≥F3), an M2BPGi level of 1.67 COI yielded a high AUC (0.93). For predicting significant liver fibrosis (≥F2), an M2BPGi level of 1.59 COI yielded a moderate AUC (0.81) (Fig. 2a–c). 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), M2BPGi had higher areas under the curve (AUCs; 0.93, respectively) with cutoff COIs of 1.84 and 1.67, respectively, than for the four conventional markers for fibrosis.

Discussion

BA, a cholestatic liver disease, is the main cause of liver fibrosis in early infancy and the leading cause of liver transplantation in children. Even children with an apparently successful Kasai

portoenterostomy will have some hepatic fibrosis or cirrhosis [15]. Liver biopsy may assist in the diagnosis and assessment of liver fibrosis status in BA. Liver biopsy should be part of long-term surveillance, even in stable patients, but it is invasive.

Kuno et al. [6] reported that M2BPGi is a predictor of liver fibrosis that performed better than other markers such as the FIB-4 index and hyaluronic acid. They also suggested that M2BPGi might be the most reliable serum biomarker. M2BPGi as a marker for assessing liver fibrosis in viral hepatitis has been studied in patients with chronic hepatitis C [16,10,6] or B virus infection [7]. In addition, M2BPGi is useful for assessing liver fibrosis in patients with PBC [17], autoimmune hepatitis [9], and nonalcoholic fatty liver disease [18]. Although M2BPGi is a novel marker for assessing liver fibrosis as described above, M2BPGi COI values may differ among patients stratified by cause of liver fibrosis, even for those with the same stage of fibrosis [19]. Yamada et al. reported a correlation between M2BPGi levels and histological findings in patients with BA who were liver transplant candidates [11]. However, as liver transplant candidates, the patients in their study had advanced fibrosis. They did not collect M2BPGi data with histological examination data from patients with a native liver who do not need liver transplantation; thus, their data did not reflect the entire spectrum of patients with BA.

In light of the uncertain utility of M2BPGi in patients with BA, our study results may be of clinical importance. We found that the degree of liver fibrosis differed significantly by M2BPGi level in patients with F0–3 versus F4 fibrosis. For predicting liver cirrhosis (F4) and advanced liver fibrosis (≥F3),

M2BPGi levels yielded the highest AUC (0.93). Yamada et al. reported that the AUC of serum M2BPGi values for diagnosing liver cirrhosis in patients with BA was 0.917 (cutoff value, 3.53) [11].Our study suggests that M2BPGi is useful for predicting F4 with a cutoff of 1.84 COI, which is lower than previously reported by Yamada et al[11]. The difference in cutoff values between our study (1.84) and that of Yamada et al. may be attributed to differences in the extent of fibrosis in the F4 stage. Grading system of M2BPGi is more convenient in clinical setting. Grade 1 and 2 supposed to be advance fibrosis so that further exploration for complications should be performed.

We acknowledge several limitations of this study. Liver biopsy for assessing the degree of liver fibrosis is associated with sampling error. There were enough patients to produce statistically significant results for mild liver fibrosis. Ultrasound-based transient elastography is another less invasive method to evaluate liver fibrosis that has recently been reported as highly useful [20,21]. We did not perform elastography and could not compare it with the M2BPGi data. Further analysis will be needed.

ROC analysis demonstrates that serum M2BPGi levels in patients with BA have better diagnostic ability for detecting fibrosis of the native liver with grade F4 fibrosis than other conventional serum biomarkers such as FIB-4, APRI, and type IV collagen 7s. M2BPGi had a wide AUC and extremely high sensitivity (91%) and specificity (96%) when the cutoff value was defined as 1.84. However, in grade F4 fibrosis, patients have the time to progress from compensated to decompensated cirrhosis. Our results clearly showed that M2BPGi could evaluate not only the status of the native liver, but also the degree of

liver function without liver biopsy. Additionally the measurement of M2BPGi is only about 1ml blood sampling regardless of time, and its cost is around 10-20 US$ per sample. This is convenient and cost-effective option for liver fibrosis check.

In conclusion, serum M2BPGi level is a novel marker for liver fibrosis in patients with BA. It is especially useful for follow-up in patients with BA who have a native liver, and can support the interpretation of liver biopsy findings.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required

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Figure legends

Fig. 1 Relationship between fibrosis markers and the stage of fibrosis: Fibrosis stages were descried METVIR score. (a) M2BPGi, (b) Type IV collagen 7s domain, (c) APRI, (d) FIB-4 index. M2BPGi: Mac-2 binding protein glycosylation-modified isomer; APRI: aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis-4 index

Fig. 2 ROC curve analysis of M2BPGi and the stages of fibrosis: Fibrosis stages were described METVIR score (a) F4, (b) ≥F3, (c) ≥F2.