Clinical usefulness of Mac-2 binding protein glycosylation isomer for diagnosing liver cirrhosis and significant fibrosis in patients with chronic liver disease

A retrospective single-center study

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Abstract

Accurate diagnosis of liver cirrhosis (LC) and significant fibrosis in patients with chronic liver disease (CLD) is important. The Mac-2 binding protein glycosylation isomer (M2BPGi) has emerged as a novel serum biomarker for liver fibrosis; however, insufficient clinical data of M2BPGi are available in patients with CLD. Therefore, we performed a retrospective cohort study to investigate the clinical usefulness of serum M2BPGi for assessing LC and significant fibrosis in CLD patients. We retrospectively reviewed the CLD patients with measured serum M2BPGi at Kosin University Gospel Hospital between January 2016 and December 2019. Multivariate logistic regression analyses were conducted to identify the independent factors associated with LC. The diagnostic power of serum M2BPGi for LC and significant fibrosis (≥F2) was evaluated and compared to that of other serum biomarkers using receiver operating characteristic curve and area under the curve (AUC). A total of 454 patients enrolled in this study. M2BPGi (adjusted odds ratio [aOR], 1.77; 95% confidence interval [CI], 1.52–2.07) and fibrosis index based on four factors (aOR, 1.23; 95% CI, 1.11–1.37) were identified as significant independent factors for LC. The AUC of M2BPGi for LC (0.866) and significant fibrosis (0.816) were comparable to those of fibrosis index based on four factors (0.860, 0.773), aspartate aminotransferase-to-platelet ratio index (0.806, 0.752), and gamma-glutamyl transpeptidase-to-platelet ratio (0.759, 0.710). The optimal cut-off values for M2BPGi for LC and significant fibrosis were 1.37 and 0.89, respectively. Serum M2BPGi levels were significantly correlated with liver stiffness measurements ($\rho = 0.778$). Serum M2BPGi is a reliable noninvasive method for the assessment of LC and significant fibrosis in patients with CLD.

Abbreviations: ALD = alcoholic liver disease, ALT = alanine aminotransferase, aOR = adjusted odds ratio, APRI = aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, AUC = area under the curve, CLD = chronic liver disease, COI = cut-off index, FIB-4 = fibrosis index based on four factors, GPR = gamma-glutamyl transpeptidase-to-platelet ratio, HBV = hepatitis B virus, HCV = hepatitis C virus, HSCs = hepatic stellate cells, INR = international normalized ratio, LC = liver cirrhosis, LSM = liver stiffness measurement, M2BPGi = Mac-2 binding protein glycosylation isomer, NAFLD = nonalcoholic fatty liver disease.

Keywords: biomarkers, chronic hepatitis, fibrosis, liver cirrhosis, Mac-2 binding protein glycosylation isomer

1. Introduction

Chronic liver disease (CLD) is a serious health issue worldwide because it can lead to liver fibrosis and cirrhosis. Causes of CLD include hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), and autoimmune hepatitis (AIH). Liver cirrhosis (LC), the end stage of fibrosis, leads to increased
morbidly and mortality due to various complications such as gastrointestinal bleeding, encephalopathy, ascites, hepatorenal syndrome, or development of hepatocellular carcinoma.[3,4] Accurate diagnosis of LC and liver fibrosis in patients with CLD is clinically important, since management and prognosis are mainly dependent on it.[5–7] However, diagnosing LC and liver fibrosis is still challenging.

Liver biopsy is considered as the gold-standard for diagnosing LC and liver fibrosis[8]; however, it is invasive and painful, with potential complications, thereby making it difficult to repeat the procedure multiple times in clinical practice.[8,9] Moreover, liver biopsy has several limitations, such as interobserver variability and sampling errors.[10,11] Therefore, many noninvasive methods have been developed to assess LC and liver fibrosis.[12–14] Conventional serum markers using routine laboratory parameters include the fibrosis index based on four factors (FIB-4), aspartate aminotransferase-to-platelet ratio index (APRI), and gamma-glutamyl transeptidase-to-platelet ratio (GPR). Furthermore, these serum markers have been extensively reported to accurately predict LC; however, it is inconvenient to calculate their values using formulas.[12–16] Transient elastography (FibroScan®; Echosens, Paris, France) is a validated noninvasive ultrasonography-based method and is the most widely used for diagnosing LC and fibrosis in patients with various CLD etiologies.[17–21] Nevertheless, FibroScan® (Echosens) cannot be performed on certain patients with morbid obesity or ascites, and the results may not be reliable in cases of acute hepatitis,[22] extrahepatic cholestasis,[23] or congestive heart failure.[24]

Recently, the Mac-2 binding protein glycosylation isomer (M2BPGi, Wisteria floribunda agglutinin-positive Mac-2 binding protein, WFA+ -M2BP) has emerged as a novel serum biomarker for the assessment of LC and liver fibrosis. M2BPGi, also known as WFA+ -M2BP, is a non-enzymatic glycosylation isomer of the carbohydrate side chain of Mac-2-binding protein, which has been shown to be upregulated in various liver diseases.[25,26] The Mac-2 binding protein has been extensively studied as a potential biomarker for LC and liver fibrosis.[27–29] M2BPGi has been found to be a powerful tool for predicting LC and liver fibrosis in patients with CLD.[17–21]

In the present study, we aimed to evaluate the diagnostic power of M2BPGi and to identify the cut-off values of M2BPGi for LC and significant fibrosis in patients with CLD presenting various etiologies.

2. Materials and methods

2.1. Study design and setting

This study is a retrospective cohort study. We retrospectively reviewed the patients diagnosed with CLD whose serum M2BPGi was measured at Kosin University Gospel Hospital between January 2016 and December 2019. The inclusion criteria:

(1) The diagnosis of CLD defined as decreased hepatic function which lasts over a period of 6 months, including chronic hepatitis to LC based on clinical features, histologic, laboratory, and radiological findings.

(2) The causes of CLD consisted of HBV, HCV, ALD, NAFLD, AIH, and primary biliary cirrhosis[30]; however, limited real-life clinical data on M2BPGi are available for assessing LC and liver fibrosis in patients with CLD.

In the present study, we evaluated M2BPGi levels in patients with CLD presenting various etiologies.

(1) Patients with conditions that may elevate serum M2BPGi (i.e., metastatic liver cancer, pancreatic cancer, chronic pancreatitis, idiopathic pulmonary fibrosis),[31]

(2) Insufficient clinical data for statistical analysis.

2.2. Patients

A total of 512 patients were initially reviewed, 58 patients were excluded. Among the 58 patients who were excluded, there were 19 patients with metastatic liver cancer, 11 patients with pancreatic cancer, 9 patients with chronic pancreatitis, 2 patients with idiopathic pulmonary fibrosis, and 20 patients with insufficient clinical data. Finally, 454 patients enrolled in this study. Laboratory data including age, sex, height/weight, HBV and HCV infection status, cirrhosis status, and alcohol consumption were collected from patients’ medical records. Laboratory data included platelet count, international normalized ratio (INR), bilirubin (total, direct), sodium, albumin, ammonia, gamma-glutamyltransferase, aspartate aminotransferase, and FibroScan® (Echosens) were obtained.

2.3. Main outcomes

Main outcomes of this study were LC and significant fibrosis. LC was diagnosed by histology and/or radiological findings (ultrasonography, CT, MRI) with clinical features such as gastrointestinal varices and ascites.[32] Significant fibrosis (≥F2) was defined by histology and/or FibroScan® (Echosens) with clinical features.

2.4. Variables

Patients with HBV were defined as those positive for HBsAg, and patients with HCV were defined as positive for both anti-HCV Ab and HCV RNA. ALD referred to consumption of at least 40 g of alcohol per day for men and 20 g alcohol per day for women without other causes of CLD.[33] AIH was diagnosed by the revised international AIH group modified scoring system.[34] The values of conventional serum biomarkers were evaluated using the following equations: FIB-4 = [age (years) × AST (U/L)]/[platelet count (10^9/L) × ALT (U/L)]0.5; APRI = [AST (U/L)/upper limit normal (ULN)]/platelet count (10^9/L) × 100; GPR = [GGT (U/L)/ULN]/platelet count (10^9/L) × 100.[13,14] Serum M2BPGi levels were measured using an automated HISCL-5000 immunoanalyzer (Sysmex Co., Kobe, Japan) via 2-step sandwich chemiluminescent enzyme immunoassay.[25,26] The values of M2BPGi were expressed as the cut-off index (COI) and were calculated using the following equation:

\[
\text{COI} = \frac{[\text{M2BPGi}]_{\text{sample}} - [\text{M2BPGi}]_{\text{control}}}{[\text{M2BPGi}]_{\text{PC}}}
\]

where \([\text{M2BPGi}]_{\text{sample}}\) is the M2BPGi value of the serum sample, \([\text{M2BPGi}]_{\text{NC}}\) is the value of the negative control, and \([\text{M2BPGi}]_{\text{PC}}\) is the positive control value. The positive control was provided as a calibration solution preliminarily standardized to yield a COI of 1.0. M2BPGi values >1.0 were considered positive (1+, 1.0 ≤ COI < 3.0; 2+, 3.0 ≤ COI).

Liver stiffness measurement (LSM) was performed using a FibroScan® (Echosens) by certified operators. At least 10 valid measurements were taken for each patient, and the median value was used for statistical analysis.

Figure 1. Flowchart of study population. CLD = chronic liver disease, M2BPGi = Mac-2 binding protein glycosylation isomer.
measurements were obtained for each patient. The median value of valid measurements was achieved as a representative of liver stiffness. Results were considered reliable when the interquartile range/median for LSM was ≤ 30%. The results were expressed in kPa. Liver stiffness cut-off values for stages F2 and F4 were set by FibroScan (Echosens) as 7.2 and 14.6 kPa, respectively.\[17,18\]

2.5. Ethical statement
This study was approved by the Kosin University Gospel Hospital Institutional Review Board prior to the study (date: 24/09/2020, No. 2020-09-005). The requirement for written informed consent was waived for this retrospective study.

2.6. Statistical analysis
Continuous variables are presented as mean ± standard deviation or medians with ranges. Student’s t test was used to compare mean values between the LC and non-LC groups. The Mann–Whitney U test and Kruskal–Wallis test were used to compare median values between the 2 groups and multiple groups, respectively. Categorical variables were expressed as frequencies and compared using the chi-square test or Fisher exact test, as appropriate. Univariate and multivariate logistic regression analyses were conducted to identify the independent factors associated with LC. The degree of collinearity among the independent variables was identified using variance inflation factors. The receiver operating characteristic curve analysis was performed to evaluate the serum M2BPGi levels for assessing LC were determined using receiver operating characteristic curve analysis. Spearman rank correlation analysis was performed to evaluate the serum M2BPGi levels with LSM (expressed in \( \rho \)). Statistical analysis was conducted using SPSS (version 23.0, SPSS Inc., Chicago, IL). Statistical significance was set at \( P < .05 \).

3. Results
3.1. Baseline characteristics
Baseline characteristics of the 454 patients are summarized in Table 1. The mean (±SD) age was 57.6 (±13.4) years, and the majority of the patients were male (69.8%). Among the study population, 144 (31.7%) patients were positive for HBV and 64 (14.1%) were positive for HCV. Eight (1.8%) patients were positive for both HBV and HCV. Moreover, 140 (30.8%) patients presented ALD. The mean (±SD) values of M2BPgi, FIB-4, APRI, and GPR were 3.77 (±4.12), 6.65 (±7.96), 1.85 (±2.78), and 1.51 (±3.11), respectively. Of the 454 patients, 285 (62.8%) were diagnosed with LC. The mean value of M2BPgi in the LC group was 5.31 (±4.29), which was significantly higher than that of M2BPgi in the non-LC group (\( P < .001 \) (Table 1). The FIB-4, APRI, and GPR values were also significantly higher in the LC group than those in the non-LC group (all \( P < .001 \)).

3.2. Univariate and multivariate analyses for cirrhosis
Univariate analyses revealed that M2BPgi, FIB-4, APRI, and GPR were significantly associated with LC (Table 2). Multivariate logistic regression analysis was performed for age, sex, HBV, HCV, ALD, platelet count, albumin, INR, total bilirubin, ALT, sodium, M2BPgi, FIB-4, APRI, and GPR to identify the independent factors for LC. Four different multivariate analysis models for M2BPgi, FIB-4, APRI, and GPR were used to avoid multicollinearity. In the M2BPgi model, M2BPgi was significantly associated with LC (adjusted odds ratio [aOR], 1.77;
Table 2
Univariate and multivariate analyses for cirrhosis.

| Variables                  | Univariate analysis |          |          |          | Multivariate analysis |          |          |          |
|----------------------------|---------------------|----------|----------|----------|-----------------------|----------|----------|----------|
|                            | OR (95% CI)         | P value  | OR (95% CI) | P value  | OR (95% CI) | P value  |
| Age, yr                    | 1.04 (1.02–1.05)    | <.001    | 1.03 (1.01–1.05) | .015    | 1.01 (0.98–1.03) | .726    |
| Male sex                   | 0.40 (0.26–0.61)    | <.001    | 0.48 (0.25–0.89) | .021    | 0.52 (0.27–0.97) | .04     |
| Positive HBsAg             | 2.62 (1.68–4.12)    | <.001    | 3.73 (2.05–6.84) | <.001   | 4.59 (2.45–8.58) | <.001   |
| Positive anti-HCV          | 2.62 (1.38–4.97)    | .003     | 1.99 (0.79–5.02) | .144    | 3.75 (1.58–8.93) | .003    |
| ALD                        | 2.16 (1.46–3.20)    | <.001    | 2.83 (1.51–5.32) | .001    | 2.72 (1.48–5.01) | .001    |
| Platelet count, ×10^9/L    | 0.99 (0.98–0.99)    | <.001    | 0.99 (0.99–1.00) | <.001   | 1.00 (0.99–1.00) | .021    |
| Albumin, g/L               | 0.17 (0.11–0.25)    | <.001    | 0.62 (0.33–1.15) | .129    | 0.43 (0.25–0.75) | .003    |
| INR                        | 1599.37 (285.51–8959.55) | <.001 | 2.73 (0.32–23.36) | .358    | 9.69 (1.30–72.12) | .027    |
| Total bilirubin, μmol/L    | 1.70 (1.40–2.06)    | <.001    | 0.93 (0.80–1.08) | .343    | 0.94 (0.79–1.11) | .435    |
| r-GTP, U/L                 | 1.00 (1.00–1.00)    | .05      | -         | -       | -         | -       |
| AST, U/L                   | 1.00 (1.00–1.00)    | .065     | -         | -       | -         | -       |
| ALT, U/L                   | 1.00 (1.00–1.00)    | .038     | 1.00 (0.99–1.00) | .004    | 1.00 (0.99–1.00) | .003    |
| Sodium, mmol/L             | 0.91 (0.86–0.96)    | <.001    | 1.03 (0.95–1.12) | .452    | 1.03 (0.95–1.11) | .516    |
| Ammonia, μmol/L            | 1.02 (1.01–1.03)    | .002     | -         | -       | -         | -       |
| M2BPGi                     | 1.87 (1.63–2.15)    | <.001    | 1.77 (1.52–2.07) | <.001   | -         | -       |
| FIB-4                      | 1.47 (1.34–1.60)    | <.001    | -         | -       | 1.23 (1.11–1.37) | <.001   |
| APRI                       | 1.44 (1.23–1.68)    | <.001    | -         | -       | -         | -       |
| GPR                        | 1.44 (1.21–1.71)    | <.001    | -         | -       | -         | -       |

ALD = alcoholic liver disease, ALT = alanine aminotransferase, APRI = aspartate aminotransferase-to-platelet ratio index, AST = aspartate aminotransferase, FIB-4 = fibrosis index based on four factors, GPR = gamma-glutamyl transpeptidase-to-platelet ratio, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus, INR = international normalized ratio, M2BPGi = Mac-2 binding protein glycosylation isomer, r-GTP = r-glutamyltranspeptidase.

95% CI, 1.52–2.07). Furthermore, other independent variables, such as age, sex, HBV, ALD, and platelet count, were significantly associated with LC. FIB-4 was significantly associated with LC in the FIB-4 model (aOR, 1.23; 95% CI, 1.11–1.37). In addition, other independent variables, such as sex, HBV, HCV, ALD, albumin, and INR, were significantly associated with LC (Table 2). In contrast, APRI (aOR, 1.18; 95% CI, 0.95–1.46) and GPR (aOR, 1.15; 95% CI, 0.99–1.34) were not significantly associated with LC.

3.3. Diagnostic power of M2BPGi for cirrhosis and significant fibrosis compared to other fibrosis markers

The AUC of M2BPGi (AUC, 0.866; 95% CI, 0.830–0.902) for diagnosing LC (F4) was comparable to that of FIB-4 (AUC, 0.860; 95% CI, 0.823–0.896), APRI (AUC, 0.806; 95% CI, 0.762–0.850), and GPR (AUC, 0.759; 95% CI, 0.711–0.808) (Fig. 2). The optimal cut-off value of M2BPGi for LC was 1.37, with a sensitivity of 86.7% and specificity of 78.7%. Moreover, the AUC of M2BPGi (AUC, 0.816; 95% CI, 0.773–0.858) for diagnosing significant fibrosis (≥F2) was comparable to that of FIB-4 (AUC, 0.778; 95% CI, 0.726–0.819), APRI (AUC, 0.752; 95% CI, 0.700–0.804), and GPR (AUC, 0.710; 95% CI, 0.634–0.766) (Fig. 3). Furthermore, the optimal cut-off value for M2BPGi for significant fibrosis was 0.89, with a sensitivity and specificity of 84.1% and 62.4%, respectively.

3.4. Correlation between M2BPGi and LSM

LSM was performed in 151 of the 454 patients. The fibrosis stage was categorized as F0/F1, F2/F3, and F4. Forty-five (29.8%) patients were F0/F1, 43 (28.5%) patients were F2/F3, and 63 (41.7%) patients were F4. The median levels of serum M2BPGi were 0.44 (F0/F1), 0.66 (F2/F3), and 4.03 (F4), respectively (Fig. 4). The Kruskal–Wallis test revealed a significant difference between the fibrosis stages (P < .001). Moreover, significant differences were observed between the F0/F1 and F2/F3 stages (P = .038), between the F2/F3 and F4 stages (P < .001), and between the F0/F1 and F4 stages (P < .001). Furthermore, LSM was significantly correlated with M2BPGi (ρ = 0.778, P < .001), FIB-4 (ρ = 0.704, P < .001), APRI (ρ = 0.702, P < .001), and GPR (ρ = 0.685, P < .001), respectively.

4. Discussion

Noninvasive methods to assess LC and liver fibrosis need to be accurate, convenient, safe, and repeatable; however, conventional serum markers and transient elastography do not fully satisfy these requirements. This study confirmed that serum M2BPGi is a reliable method for assessing LC and significant fibrosis in patients with CLD. Furthermore, serum M2BPGi levels were found to be independently associated with LC. This finding was in accordance with that of previous clinical studies in patients with CLD.

In this study, serum M2BPGi increased as fibrosis progressed. This is supported by the fact that M2BPGi is crucial in the process of liver fibrosis. In an in vitro study, Bekki et al.[19] showed that M2BPGi, which is secreted by hepatic stellate cells (HSCs), promotes Kupffer cells to express Mac-2 and induces the activation of HSCs. Activated HSCs are known to secrete inflammatory cytokines and produce substantial amounts of extracellular matrix, thereby resulting in liver fibrosis.[20] Therefore, serum M2BPGi would be useful for assessing significant fibrosis in clinical setting.

In the present study, we identified the strong diagnostic power of serum M2BPGi for LC (AUC: 0.866) and significant fibrosis (AUC: 0.816), which was comparable to that of other serum markers (FIB-4, APRI, and GPR). The cut-off value of M2BPGi for LC and significant fibrosis was 1.37 and 0.89, respectively. These results were consistent with those of previous studies.
Based on a study conducted against 680 HCV patients and 164 healthy controls in China, the AUCs of serum M2BPGi for LC and significant fibrosis were 0.892 and 0.774, respectively.\textsuperscript{27} And the cut-off values of M2BPGi for LC and significant fibrosis were 1.36 (sensitivity 86.8%, specificity 78.6%) and 0.95 (sensitivity 74.5%, specificity 69.4%), respectively. In a study comprising 327 HBV patients, the AUC of serum M2BPGi for F4 was 0.914 and the cut-off value of M2BPGi for F4 was 0.96, with a sensitivity of 83.3% and a specificity of 92.7\%.\textsuperscript{29} A study of 220 patients with NAFLD showed that the AUC and cut-off value of serum M2BPGi for F4 were 0.840 and 0.70, respectively, with a sensitivity of 85.7% and a specificity of 72.8\%.\textsuperscript{41} Although this study could not demonstrate that the diagnostic power of serum M2BPGi for LC and significant fibrosis was superior to that of other serum biomarkers, it was found that the diagnostic power of serum M2BPGi was comparable to that of other serum markers. Therefore, diagnostic performance for LC and significant fibrosis may be improved by using serum M2BPGi with conventional serum biomarkers in clinical practice.

Serum M2BPGi levels were found to be significantly correlated with LSM in this study. These findings were consistent with those of previous studies.\textsuperscript{27-30,42} Fibroscan\textsuperscript{®} (Echosens) is an already validated noninvasive method for diagnosing LC and fibrosis in patients with various CLD etiologies. Therefore, combination of serum M2BPGi and Fibroscan\textsuperscript{®} (Echosens) would improve the diagnostic performance for LC and liver fibrosis.

4.1. Limitations
This study also has several limitations. First, because this study was a single-center retrospective study, careful interpretation is required due to selection and information bias. Second, we were unable to identify a correlation between M2BPGi and the results of liver biopsy, because of lack of patients who underwent liver biopsy. Third, Fibroscan\textsuperscript{®} (Echosens) was conducted on only 151 patients out of total 454 patients. Thus, we could not separate the F0/F1 and F2/F3 stages due to the relatively small sample size of the F1 and F3 stages. Therefore, further large-scale prospective studies are required.

4.2. Conclusions
In this study, we confirmed that serum M2BPGi is a reliable noninvasive method for the assessment of LC and significant fibrosis in patients with CLD. Serum M2BPGi would help the physicians in clinical diagnosis of LC and significant fibrosis in patients with CLD.

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Figure 3. Receiver operating characteristic curves of serum M2BPGi, FIB-4, APRI, and GPR for assessing significant fibrosis (≥F2). APRI = aspartate aminotransferase-to-platelet ratio index, AUC = area under the curve, FIB-4 = fibrosis index based on four factors, GPR = gamma-glutamyl transpeptidase-to-platelet ratio, M2BPGi = Mac-2 binding protein glycosylation isomer.
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