Correlation of Mac-2 Binding Protein Glycosylation Isomer (M2BPGi) with Liver Transient Elastography Results: An Evaluating Liver Fibrosis in Chronic Hepatitis B Patients

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ABSTRACT

Background: *Transient elastography (TE) is widely recognized as a reliable surrogate marker for grading the severity of liver fibrosis in chronic hepatitis B (CHB) patients. Mac-2 binding protein of glycosylation isomer (M2BPGi) is a novel non-invasive serum biomarker for liver fibrosis staging in various liver diseases, including CHB. This study aimed to evaluate the correlation of M2BPGi and liver stiffness (LS), as measured through TE, in predicting the degree of liver fibrosis in CHB patients.*

Methods: *A cross-sectional study was conducted at Dr. Hasan Sadikin General Hospital, Bandung, between September 2021 and January 2022. The study included patients diagnosed with chronic hepatitis B (CHB) based on clinical and biochemical assessments. All subjects underwent TE examination using Fibroscan®, and the M2BPGi levels were determined with an automated immunoassay analyzer HISCL-800, Sysmex, Japan. Statistical analysis was conducted using the Spearman rank correlation method, with a significance value of $p<0.05$.*

Results: *A total of 119 patients with chronic hepatitis B (CHB) were consecutively recruited (Male:Female = 66:53; median age: 43 years). The median M2BPGi level was 1.04 COI (range: 0.74–1.59), while the median liver stiffness (LS) was 7.3 kPa (range: 5.6–12.5). A moderate and statistically significant correlation was observed between M2BPGi and LS ($r = 0.525$; $p < 0.001$). Median M2BPGi values by fibrosis stage were 0.89 COI for F0–F1, 0.88 for F2, 1.61 for F3, and 2.24 for F4 ($p < 0.001$).*

Conclusion: *This study revealed a moderate positive correlation between serum M2BPGi level and LS in CHB patients.*

Keywords: *Chronic hepatitis B, Liver fibrosis, Transient elastography, Liver stiffness, M2BPGi.*

ABSTRAK

Latar belakang: *Hepatitis B merupakan masalah kesehatan masyarakat di dunia, termasuk di Indonesia. Pemeriksaan elastografi hati transien yang non invasif saat ini telah teruji dan digunakan untuk menilai fibrosis hati. Mac-2 binding protein glycosylation isomer (M2BPGi) adalah biomarker baru noninvasif untuk menilai derajat fibrosis hati yang disebabkan oleh berbagai penyakit hati termasuk hepatitis B. Tujuan penelitian ini*

adalah untuk membuktikan adanya korelasi antara M2BPGi dengan kekakuan jaringan hati hasil pemeriksaan elastografi transien hati dalam menilai fibrosis hati pada pasien-pasien hepatitis B kronik.

Metode: *Penelitian potong lintang dilakukan di Rumah Sakit Dr. Hasan Sadikin Bandung antara September 2021 sampai Januari 2022 untuk pasien-pasien dengan hepatitis B kronik tanpa penyakit penyerta berdasarkan pemeriksaan klinis dan biokimia. Subjek penelitian dilakukan pemeriksaan elastografi hati transien dengan alat Fibroscan® dan pemeriksaan M2BPGi diukur dengan alat analisis immunoassay (HISCL-800, Sysmex, Japan). Analisis statistik dengan metode korelasi rank Spearman dan nilai kemaknaan dengan p < 0,05.*

Hasil: *Subjek penelitian adalah 119 pasien hepatitis B kronik (Pria:Wanita = 66:53, median umur 43 tahun). Nilai median M2BPGi 1,04 COI (0,74–1,59) dan nilai median kekakuan jaringan hati 7,3 (5,6–12,5). M2BPGi berkorelasi sedang dan bermakna secara statistik dengan kekakuan jaringan hati (r = 0,525; p < 0,001). Nilai median M2BPGi pada derajat fibrosis hati sesuai skor Metavir F0-F1 0,89; F2 0,88; F3 1,61; dan F4 2,24 (p < 0,001).*

Kesimpulan: *Penelitian ini membuktikan adanya korelasi positif sedang antara M2BPGi dan kekakuan jaringan hati hasil pemeriksaan elastografi hati transien pada pasien-pasien hepatitis B kronik.*

Kata kunci: *hepatitis B kronik, fibrosis hati, elastografi hati transien, kekakuan jaringan hati, M2BPGi*

INTRODUCTION

Hepatitis B remains a major global public health concern, including in Indonesia. Worldwide, the hepatitis B virus (HBV) has infected approximately 1.5 billion people, with an estimated 296 million living with chronic hepatitis B. In 2019 alone, HBV-related complications led to around 820,000 deaths.[1,2] Indonesia ranks second after India in terms of countries with the highest number of HBV infections in the Asia Pacific Region and accounts for up to 74% of deaths from liver cancer globally.[3] The prevalence of hepatitis B in Indonesia is 7.1%.[4,5] Morbidity and mortality of hepatitis B patients are caused by advanced fibrosis, including cirrhosis, decompensated and/or hepatocellular carcinoma, in approximately 15–40% of chronic hepatitis B patients.[6]

Monitoring the progression and treatment of chronic hepatitis B requires periodic assessment of liver fibrosis. Identifying patients with significant fibrosis or cirrhosis is crucial to prevent disease progression and the onset of decompensated liver conditions.[9–11] Liver fibrosis can be evaluated invasively through liver biopsy or non-invasively using imaging-based techniques such as transient elastography, ultrasonography, and magnetic resonance imaging (MRI). Additionally, fibrosis staging can be estimated through serum-based indices, including APRI, FIB-4, Hui index, and Mac-2 binding protein glycosylation isomer (M2BPGi).[12–14] Currently, liver biopsy is the gold standard in assessing the degree of liver fibrosis in chronic hepatitis B. However, it has some limitations, such as invasive, sampling error, inter-observer variability, and may cause complications, including pain and bleeding.[15–19] As a substitute for liver biopsy, liver transient elastography (TE) can determine fibrosis accurately and non-invasively. Vibration-controlled transient elastography (VCTE) is an ultrasound-based tool to evaluate liver elasticity related to liver fibrosis. The VCTE was first introduced in 2003 by Echosens, Paris, France with the registered brand Fibroscan®.[13] Despite its advantages, VCTE has limitations: it is not universally available across healthcare facilities, requires trained operators, may struggle to detect early-stage fibrosis, is less effective in obese patients or those with ascites, and can be costly.[20,21]

Recent studies have shown that M2BPGi is a promising test for predicting liver fibrosis. M2BPGi is a glycoprotein produced by liver stellate cells and functions as an intermediary between Kupffer cells and stellate cells in the process of fibrogenesis.[22] M2BPGi is reported to have better precision in predicting severe fibrosis than non-invasive tests of liver fibrosis using serum formulas, such as FIB-4 score, APRI, measurements of hyaluronic acid, and collagen type-4.[22,23] M2BPGi has also been studied in various chronic liver diseases, including chronic hepatitis C, chronic hepatitis B, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and primary biliary cirrhosis (PBC). Its compatibility with various liver disease and the degree of fibrosis allows it to serve as a valuable reference such as in the use of liver transient elastography.[24,25] M2BPGi can also be used to predict the severity degree of histopathological abnormalities in chronic hepatitis B patients.[26,27] Compared to transient elastography, the use of M2BPGi as a serum biomarker is more accessible, as it does not require specialized equipment or operator expertise.. Thus, it can be used by all medical personnel and is not

disturbed by several conditions such as obesity and ascites. Practically, any health center can instruct a laboratory to measure M2BPGi at various degrees of fibrosis qualitatively. This M2BPGi examination is useful in monitoring patients after or in the treatment programmed; hence, it is useful to assess therapeutic response.[26–29] Recent studies on M2BPGi in Indonesia focused on the accuracy of M2BPGi in chronic hepatitis C patients and screening of high-risk esophageal varices in patients with liver cirrhosis. In contrast, there were only evidence-based case reports in chronic hepatitis B patients.[28,30,31] These reports concluded that M2BPGi could not be used as a diagnostic modality to detect liver fibrosis in chronic hepatitis B patients, as the sensitivity and specificity from these four studies showed that the M2BPGi are still insufficient to detect and rule out liver fibrosis in chronic hepatitis B patients. Additionally, the difference in the cut-off value of M2BPGi to determine the stage of fibrosis in each study means that this value cannot be directly used as an accurate standard for determining advanced ($F \geq 3$) liver fibrosis.[31] This is different from the results of studies in Thailand, Vietnam, Korea, and Japan, which showed the role of M2BPGi in assessing the progression of liver fibrosis and its cut-off value can be used as a reference to detect the presence of significant fibrosis.[26,32,33]

Therefore, preliminary research is needed to determine the correlation of serum M2BPGi with the results of liver transient elastography in assessing liver fibrosis among chronic hepatitis B patients in Indonesia. Additionally, it is expected that further studies will be carried out to find the cut-off value of M2BPGi as a significant reference for fibrosis to guide the initiation of therapy in chronic hepatitis B patients.

METHODS

This analytical study employed a cross-sectional method. The study sample consisted of chronic hepatitis B patients receiving outpatient care at the Gastroenterology Hepatology Clinic of Dr. Hasan Sadikin General Hospital between September 2021 and January 2022. Inclusion criteria were as follows: (1) age 18 years or older; (2) seropositive for HBsAg for more than six months; (3) diagnosed with chronic hepatitis B based on medical history, physical examination, and supporting laboratory tests (serum HBV DNA > 20,000 IU/mL in HBeAg-positive patients or 2,000-20,000 IU/mL in HBeAg-negative patients, with persistent or intermittent ALT elevation of 1–2× the upper limit of normal, but not exceeding 5× the upper limit); and (4) willingness to participate with written informed consent. Exclusion criteria included: (1) acute hepatitis; (2) acute exacerbation on chronic hepatitis; (3) hepatitis C; (4) autoimmune liver disease; (5) hepatitis B co-infection with hepatitis C or HIV; (6) hepatitis B with co-morbidities (type 2 diabetes mellitus, heart disease, chronic kidney disease, pulmonary tuberculosis, cancer); (7) habit of drinking alcohol; (8) pregnant or breastfeeding; (9) severe obesity (BMI > 27 kg/m^2); (10) severe anemia (Hb < 5 g/dL); (11) pulmonary fibrosis, chronic pancreatitis, liver cancer, and pancreatic cancer.

In this study, sampling was performed using a consecutive sampling technique, whereby patients who met the inclusion and exclusion criteria were enrolled in the order of their arrival until the minimum required sample size was achieved. The calculation of study samples was adjusted to the purpose of the research, particularly the correlation analysis using the formula as follows:

$$n = \frac{(Z_\alpha + Z_\beta)^2}{\left\{0{,}5 \ln\left(\frac{1+r}{1-r}\right)\right\}^2} + 3$$

Notes:

n = minimal sampel size
Zα = type I error (α) = 5%, so Zα = 1.96
Zβ = type II error (β) = type II error = 95%, so Zβ = 1.64
r = magnitude of correlation coefficient

To investigate the magnitude of the correlation coefficient of the relationship between the results of serum M2BPGi and the results of transient elastography, data were obtained from the study results of Yuki Tsuji et al. (r = 0.61).[25] Based on the sample size formula, n = 29 was obtained. From the above sample size calculation, the minimum sample size in this study was 29 patients.

There were 2 types of variables in this study: categorical and numerical variables. Numerical variables included the value of liver stiffness. Categorical variables consisted of the degree of liver fibrosis, which were further classified into 4 groups (F0-F1, F2, F3, F4) **(Table 1).** Numerical variables included serum M2BPGi.

Table 1. Grouping of the median value of liver tissue stiffness into the degree of liver fibrosis based on the METAVIR score

Median value of liver tissue stiffness (kPa)	Degree of Liver Fibrosis based on METAVIR score
≤ 6	F0-F1
6.1–8.9	F2
9–11.9	F3
≥ 12	F4

The research was carried out after obtaining ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran and Dr. Hasan Sadikin Hospital Research Ethics Committee (Certificate Number: LB.02.01/X.6.5/299/2021). Data collection from medical records was carried out after patients provided written informed consent.

Statistical Analysis

Data in this study were analyzed statistically using SPSS version 25.0. Correlation testing was carried out using the Pearson correlation test for normally distributed data, and the Spearman rank correlation test for non-normally distributed.[81] Comparison of categorical variables, expressed as proportions, was performed using the chi-square test and Fisher's exact test if necessary.[84] To evaluate the relationship between serum M2BPGi and liver fibrosis degree (expressed in F0-F1, F2, F3, F4), one-way ANOVA was used for normally distributed data, while the Kruskal–Wallis test was applied for non-normally distributed data.[85] A p-value of < 0.05 was considered statistically significant.74

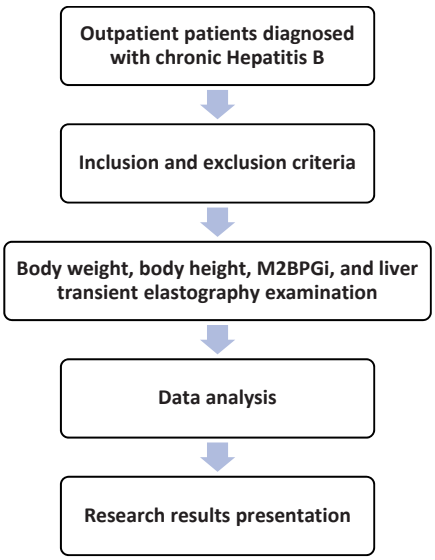

Figure 1. Research outline

RESULTS

A study to determine the correlation between M2BPGi and the results of liver transient elastography in assessing liver fibrosis in chronic hepatitis B patients has been carried out at the Gastroenterology Hepatology Polyclinic at Dr. Hasan Sadikin General Hospital from September 2021 to January 2022. The research subjects consisted of 119 patients who visited the outpatient ward during this period.

Information related to subjects' characteristics was obtained from medical records, history taking, and examination of the patients **(Table 2).** Each subject received information about the study to be carried out and signed an informed consent. The subjects underwent M2BPGi blood collection and examination in the laboratory of Dr. Hasan Sadikin General Hospital. A transient liver elastography was performed on the 3rd floor of the Diagnostic and Cardiac Center Building at the same hospital.

Table 2. Basic characteristic of study subjects (n = 119)

Characteristics	Statistical measures
Sex, n (%)	
Male	66 (55.5)
Female	53 (44.5)
Age (years), median (IQR)	43 (31–52)
Patients who have received antiviral treatment, n (%)	71 (59.7)
Body mass index (kg/m^2), mean ± SD	22.7 ± 2.8
Platelet count (thousands/uL), mean ± SD	233 ± 78
ALT (U/L), median (IQR)	35 (28– 50)
AST (U/L), median (IQR)	27 (22–34)
Bilirubin (mg/dL), median (IQR)	0.59 (0.416–0.81)
Albumin (g/dL), median (IQR)	4.07 (3.5–4.24)
HBV DNA (log IU/mL)	1.05 (0.00–3.35)
HBeAg, n (%)	
Reactive	34 (28.6)
Non-reactive	85 (71.4)
M2BPGi (COI), median (IQR)	1.04 (0.74–1.59)
M2BPGi (COI), n (%)	
COI < 1	54 (45.4)
1≤ COI < 3	46 (38.7)
COI ≥ 3	19 (16.0)
Liver stiffness value (kPA), median (IQR)	7.3 (5.6–12.5)
Degree of fibrosis, n (%)	
F0-F1	36 (30.3)
F2	42 (35.3)
F3	9 (7.6)
F4	32 (26.9)

n: frequency; SD: standard deviation; IQR: interquartile range; ALT: alanine aminotransferase; AST: aspartate aminotransferase; HBV DNA: hepatitis b virus deoxyribo nucleid acid; HBeAg: hepatitis B e-antigen; M2BPGi: mac-2 binding protein glycan isomer

In this study, a total of 119 subjects were enrolled, with a median age of 43 years and 55.5% of them being male. Most of the study subjects, particularly 71 patients (59.7%), had received antiviral treatment. The mean body mass index was 22.7 kg/m^2 (SD = 2.8 kg/m^2).

Laboratory results showed a mean platelet count of 233,000/uL (SD = 78,000/uL). The median ALT and AST were 35 U/L (IQR = 28–50 U/L) and 27 U/L (IQR = 22–34 U/L), respectively. The median bilirubin was 0.59 mg/dL (IQR = 0.416–0.81), albumin was 4.07 g/dL (IQR = 3.75–4.24 g/dL), and the median of HBV DNA was 1.05 log IU/mL (IQR = 0.00–3.35). HBeAg examination was reactive in 28.6% and non-reactive in 71.4%.

Results of this study revealed that the median of M2BPGi was 1.04 (IQR = 0.74–1.59), while the median liver tissue stiffness was 7.3 kPa (IQR = 5.6–12.5 kPa). The degree of liver fibrosis in chronic hepatitis B patients according to METAVIR score F0-F1 was as follows: F0-F1 in 36 patients (30.3%), F2 in 42 patients (35.3%), F3 in 9 patients (7.6%), and F4 in 36 patients (26.8%).

Table 4. Factors associated with M2BPGi in identified chronic hepatitis B based on correlation analysis

Variable	M2BPGi r coefficient	*p*-value
Age (years)	0.386[a]	< 0.001*
Sex (Female)	0.173[b]	0.030*
BMI (kg/m^2)	0.066[a]	0.239
HBeAg (reactive)	0.047[b]	0.307
Platelet (thousands/uL)	-0.361[a]	< 0.001*
AST (U/L)	0.420[a]	< 0.001*
ALT (U/L)	0.207[a]	0.012*
Bilirubin Total (mg/dL)	0.130[a]	0.080
Albumin (mg/dL)	-0.447[a]	< 0.001*
HBV DNA (log IU/mL)	-0.073[a]	0.215

Note: [a]Rank Spearman correlation; [b]Poin-biserial correlation

chronic To test the hypothesis regarding the correlation between M2BPGi and the results of liver elastography transient examination, particularly the median value of liver tissue stiffness, the Spearman rank correlation test was performed, as the data distribution was not normal. The results of the correlation test are presented in **Table 4.** At a 95% confidence interval, the test revealed a moderate and statistically significant correlation between M2BPGi and liver stiffness, with a correlation coefficient (r) of 0.525 and a p-value of < 0.001 ($p < 0.05$).

Table 4. Correlation of M2BPGi and liver stiffness value

	Value of liver tissue stiffness (kPa)	
	r coefficient	*p*-value
M2BPGi (COI)	0.525	< 0.001*

Figure 2 shows a scatter diagram of the relationship between M2BPGi and the median value of liver stiffness.

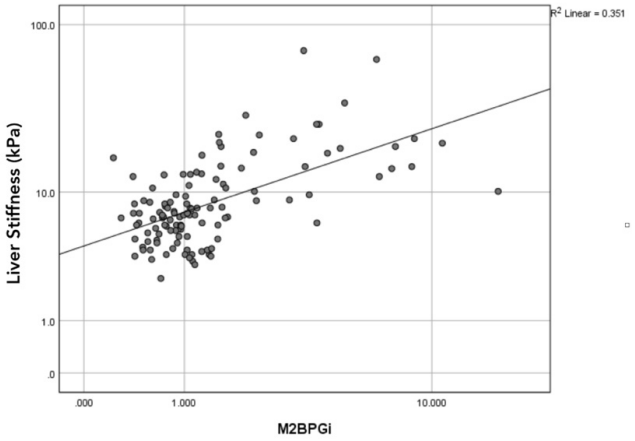

Figure 2. Scatter diagram showing the correlation of M2BPGi and median value of liver stiffness

The scatter diagram in **Figure 2** illustrates that the M2BPGi has a positive trend with respect to the median of liver tissue stiffness. Particularly the trend of M2BPGi increased with the increasing median of liver tissue stiffness, with a *p*-value < 0.05 (meaning that there was a significant relationship between the two).

Distribution of M2BPGi Based on the Degree of Liver Fibrosis

Liver transient elastography is a non-invasive examination to determine the degree of liver fibrosis, resulting in liver stiffness (LS). This examination has been tested and used as a substitute for liver biopsy. The median value of liver tissue stiffness can be grouped based on Metavir scores into F0-F1, F2, F3 and F4. The distribution of M2BPGi based on the degree of liver fibrosis can be seen in **Table 5.**

As shown in **Table 5**, the median of M2BPGi lebels varied across fibrosis stages: 0.89 (IQR: 0.64-1.10) for F0-F1, 0.88 (IQR: 0.71-1.20) for F2, 1.61 (IQR: 0.95-2.98) for F3, and the highest value was observed in F4 at 2.28 (IQR: 1.31-4.99). This distribution is illustrated by the boxplot image below **(Figure 3).**

Table 5. Distribution of M2BPGi based on the degree of liver fibrosis

M2BPGi	Degree of liver fibrosis				*p* value
	F0-F1 n = 36	F2 n = 42	F3 n = 9	F4 n = 32	
Median (IQR)	0.89 (0.64–1.10)	0.88 (0.71–1.20)	1.61 (0.95–2.98)	2.28 (1.31–4.99)	< 0.001
Min–Max	0.42–1.52	0.29–3.98	0.61–16.37	0.23–10.82	

Analysis was performed using Kruskall Wallis test; IQR: Inter quartile range

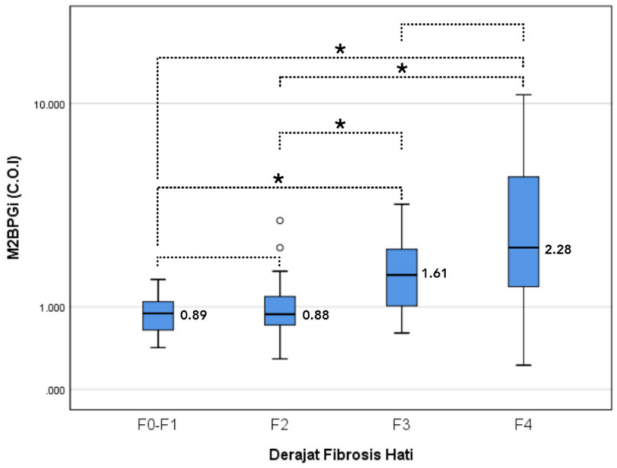

Figure 3. Boxplot showing distribution of M2BPGi based on the degree of liver fibrosis

The Kruskall-Wallis test revealed that the M2BPGi cut-off index (COI) values were stratified by the degree of fibrosis which had a significant difference ($p < 0.05$). There were significant differences between F0-F1 and F3 ($p < 0.05$), F0-F1 and F4 ($p < 0.05$), F2 and F3 ($p < 0.05$) and F2 and F4 ($p < 0.05$).

DISCUSSION

The subjects of this study consisted of 55.4% male and 44.5% female who had chronic hepatitis B. These findings align with those reported by Mak et al., who observed a higher prevalence of chronic hepatitis B among males (70%).[34] This was also similar to other studies by Zou et al, Wei et al, and Yeh et al. which also stated that chronic hepatitis B was more common in males than in females.[35,41,42] This was due to the global prevalence of positive hepatitis B surface antigen (HBsAg) which was higher in males than in females and males were more often exposed to the risk factors of hepatitis B transmission than females.[2,43,44] Male sex appears to accelerate the progression of hepatitis B virus-associated liver disease. The male-to-female ratio increased from 3.2 in asymptomatic carriers to 6.3 in chronic liver disease and finally to 9.8 in hepatocellular carcinoma.[45] Gender differences have been experimentally confirmed in a transgenic rat model of the hepatitis B virus. The study by Wang et al. showed that androgen hormones could enhance the transcription of the hepatitis B virus through direct binding to the androgen-responsive element site in viral enhancer I which resulted in male rats having higher serum DNA and HBsAg than female mice. This explains the increased risk of liver disease in males compared to females.[46] The ages of the study subjects ranged from 20 to 76 years, with a median of 43 years. This was in accordance with previous studies which found that the age range of chronic hepatitis B patients was 15 to 75 years.[34,36,41,42]

In this study, the majority of chronic hepatitis B patients were HBeAg-negative (85%), compared to those who were HBeAg-positive. This trend is likely due to the majority (59.7%) of chronic hepatitis B patients in the study had previously received antiviral therapy. However, HBeAg-negative status in untreated patients may also result from naturally occurring mutations in the hepatitis B virus that prevent HBeAg production. These mutations occur in the pre-core or core promoter region of the hepatitis B genome. The most common pre-core mutations are the change of G A in nucleoside 1896 (G1896A), which produces a stop codon and stop HBeAg synthesis. The most frequent pre-core promoter mutations involve 2 nucleotide substitutions at nucleotides 1762 and 1764.[47,48] The results of this study are in accordance with the results of the study by Widita et al. which stated that in chronic hepatitis B patients, more HBeAg negative was found, particularly 83.33%.[49]

Liver biopsy, which is the gold standard examination for assessing liver fibrosis, has many drawbacks because of the often-occurring sampling error, inter-observer variability and may cause complications such as pain (in 30-50% of patients), bleeding (0.6%), organ injury (0.08%), and death (up to 0.1%). Due to these risks, liver biopsy was not performed in this study.[15–19,50] As a substitute, an ideal non-invasive, comfortable, accurate, reproducible, and safe examination is needed. In this study, we performed two non-invasive examinations to assess liver fibrosis: transient liver elastography which is an imaging-based examination and M2BPGi which is a biochemical-based examination.

More than 90% of protein in human body is in the form of glycoprotein. Changes in the glycan structure of glycoproteins are associated with cellular inflammation and neoplastic transformation. The development of cancer-related glycoprotein-based biomarkers is currently an important area of research.[51] Mac-2 binding protein (M2BP) is a type of secretory glycoprotein secreted by many cell types including hepatocyte cells. M2BP can modulate several processes especially those related to cell adhesion. In addition, M2BP interacts with several extracellular proteins related to fibrosis, including collagen IV–VI, fibronectin, and nidogen. Fibrosis occurs as a result of specific modifications of the glycosylation and sugar structure of M2BP. Changes in the glycan structure (N-acetygalactosamine residues of N-glycans and O-glycans) in M2BP were detected using a specific lectin Wisteria floribunda agglutinin (WFA). Consequently, the modified form of M2BP is referred to as hyperglycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA^+-M2BP).[22–24]

The study by Bekki et al. showed that M2BPGi is produced exclusively by activated hepatic stellate cells and plays an important role in the progression of liver fibrosis caused by various liver diseases, including chronic hepatitis B.[52]

This study found that M2BPGi was positively correlated with age, female sex, AST, ALT, and negatively correlated with platelet count and albumin levels (Table 3). Similar results were reported by Mak et al. and Wei et al., although in their studies, M2BPGi was not correlated with sex.[34,41] This can be explained by a meta-analysis study by Cai et al., showing that age, ratio of AST, and ALT were other influential factors causing liver fibrosis in chronic hepatitis B patients in addition to other factors, such as male sex, family history of hepatitis B, increased duration of hepatitis B, alcohol drinking habit, smoking, and increased total bilirubin levels.[53] Yang YT et al. also reported that platelet count can be used as a marker of the severity of liver injury and liver fibrosis in chronic hepatitis B infection.[54] In patients with advanced liver disease and decompensated cirrhosis, low serum albumin is caused by impaired liver function and albumin synthesis. This condition of hypoalbuminemia indicates an advanced degree of liver fibrosis.[55] The combination of age, platelets, and M2BPGi improves the accuracy in identifying patients with advanced fibrosis, yielding a sensitivity of 51% and a specificity of 95.4%.[42]

The main results of this study showed that M2BPGi as a new marker of liver fibrosis in chronic hepatitis B had a moderate correlation with liver tissue stiffness from Fibroscan® examination ($r = 0.525$; $p < 0.001$). The results of this study were consistent with those of Zou et al., reporting a good correlation between M2BPGi and liver tissue stiffness ($r = 0.614$; $p < 0.0001$).[36] A similar finding was reported by Mak et al. with a correlation coefficient of (r) = 0.611; $p < 0.001$ and Wei et al. with a correlation coefficient of (r) = 0.574; $p < 0.01$. All studies showed a positive direction of correlation, which means that the greater the M2BPGi value, the greater the liver tissue stiffness.[41,56]

Other studies using liver biopsies to assess liver fibrosis in chronic Hepatitis B patients and comparing them with M2BPGi showed a positive correlation between M2BPGi and the degree of liver fibrosis. The results of this study were reported by Tsuji et al. ($r = 0.61$; $p < 0.001$) and Zou et al. ($r = 0.451$; $p < 0.001$).[26,36]

In this study, the median of liver tissue stiffness was 7.3 kPa (IQR = 5.6–12.5). The results of transient liver elastography in this study were classified by the degree of liver fibrosis according to the Metavir score, with the following distribution: F0–F1 (30.3%), F2 (35.3%), F3 (7.6%), and F4 (26.9%). The corresponding median M2BPGi values for each fibrosis stage were 0.89 for F0–F1, 0.88 for F2, 1.61 for F3, and 2.28 for F4.

The results of this study showed that the more severe the degree of liver fibrosis, the higher the M2BPGi, and this was statistically significant ($p < 0.001$). Similar results were also reported by other studies as summarized in **Table 6**.

Table 6. Distribution of M2BPGi based on the degree of liver fibrosis in chronic hepatitis B patients from various studies

Study	Value	Median/mean	M2BPGi	Cut off index
	F0-F1	F2	F3	F4
Ishii et al[35]	0.9	1.4	1.6	3.1
Ichikawa et al[57]	0.75	1.14	1.03	1.64
Yeh et al[42]	0.64	1.36	1.65	2.7
Jekarl et al[58]	0.68	0.87	1.65	
Mak et al[34]	0.26	0.34	0.57	1.21
Wei et al[41]	0.88	1.17 (F2-F3)		1.92
Jun et al[59]		0.80 (F1-F3)		2.67
This study	0.89	0.88	1.61	2.28

The difference in M2BPGi values in each study was due to the different number of study subjects in each group of degrees of fibrosis, differences in the basic characteristics of study subjects, and differences in the distribution of groups of degrees of liver fibrosis.[24] n this study, the distribution of M2BPGi levels showed a statistically significant difference between patients with fibrosis degrees of ≥ F3 and F4 and those with fibrosis degree of < F2, particularly when compared to the F0-F1 and F2 groups (**Table 6**). Similar findings were reported by Wei et al. and Yamada et al., both of whom concluded that M2BPGi has strong predictive value for identifying liver cirrhosis.[41,60]

However, Zou et al. reported that M2BPGi was useful for assessing liver fibrosis in the early stages of liver fibrosis.[36] Similarly, a study by Ura et al. found that M2BPGi was more accurate in diagnosing significant liver fibrosis than advanced liver fibrosis.[61] In the M2BPGi meta-analysis study from Feng et al. involving 36 studies with 7362 study subjects, the overall AUROC of M2BPGi in the identification of mild fibrosis, significant fibrosis, and advanced fibrosis and cirrhosis of the liver was 0.75, 0.79, 0.82, and 0.88, respectively. The accuracy of the M2BPGi is strongly influenced by the etiology of liver disease and is compatible with other non-invasive tests in predicting early liver fibrosis. This meta-analysis study concluded that M2BPGi is sufficiently effective for diagnosing end-stage liver fibrosis, particularly liver cirrhosis.[62]

M2BPGi predicts different degrees of liver fibrosis based on the etiology of the liver disease. In this study, the median M2BPGi value in chronic hepatitis B patients was lower than in chronic hepatitis C patients. Nishikawa et al. found that the median levels for F2, F3, and F4 in hepatitis B were 1.49, 1.79, and 2.83, while those in chronic hepatitis C were 3.19, 3.79, and 5.03. This also applies to the cut-off value of transient liver elastography for diagnosing advanced liver fibrosis or cirrhosis was higher in patients with chronic hepatitis C than in chronic hepatitis B.[64] It appears that this difference is due to the difference in hepatic connective tissue material burden between the two hepatotropic virus infections. Differences in the pathomorphology of liver fibrosis can explain this condition. Compared to chronic hepatitis C, cirrhosis caused by chronic hepatitis B has the characteristics of larger regenerative nodules, thinner fibrous septum, lower inflammatory reaction and better hepatocyte regeneration in cleaning hyaluronic acid. Therefore, even though the degree of fibrosis is similar, for example in F3, the liver condition in both viral infections does not contain similar amounts of connective tissue material.[65]

Additionally, in patients with chronic hepatitis B infection, M2BPGi has lower accuracy than in patients with chronic hepatitis C infection, non-alcoholic fatty liver disease (NAFLD), or non-alcoholic steatohepatitis (NASH). This can be explained by the role of hepatic stellate cells (HSCs), which are the source of M2BPGi and are closely associated with the expression of alpha-smooth muscle actin. In patients with chronic hepatitis B, liver inflammation tends to be more subtle, and cirrhosis is characterized by larger regenerative nodules and thinner fibrous septa. These histological features contribute to the lower M2BPGi accuracy observed in this group.[62,65,66] The study of Sturm et al. concluded that the amount of fibrosis in chronic hepatitis C was higher than in chronic hepatitis B, which was primarily caused by the increased perisinusoidal fibrosis in chronic hepatitis C. This study also demonstrated the difficulty of differentiating minimal and significant fibrosis in chronic hepatitis B and chronic hepatitis C. This explains the difficulty of non-invasive methods to assess liver fibrosis at an early stage of the disease, especially between F1 and F2.[67]

In this study, most of the subjects, 71 patients (59.7%), had received antiviral drugs, which may have affected the results of the M2BPGi examination. The long-term use (more than 1 year) of nucleoside analogue drugs may cause a significant decrease in serum M2BPGi, as reported by Hsu et al.[68] The study of Hsu et al. showed the decreased M2BPGi serum in liver cirrhosis patients from 3.02 COI before treatment to 1.52 COI after 1 year treatment, and further to 1.47 COI after 2 years of treatment. In non-cirrhotic hepatitis B patients, there was a decrease in M2BPGi from 1.11 COI before treatment to 0.71 COI after the first and second year of treatment.[68] Research by Mak et al. reported that long-term use of nucleoside analogue drugs was associated with the decrease in serum M2BPGi, which was correlated with histological regression of fibrosis in patients who underwent repeated liver biopsies.[29]

Nucleoside analogue drugs with higher potency, such as tenofovir and entecavir, have been shown to induce significant regression of fibrosis and decreased liver tissue stiffness compared to other nucleoside drugs, such as lamivudine, telbivudine, and adenofovir.[69] Although several studies reported that long-term use of nucleoside analogue drugs was associated with regression of fibrosis, there was still a proportion of patients who experienced progressing liver fibrosis and decompensated cirrhosis.[70,71]

Regression of fibrosis in the study by Mak et al. occurred in 24.4% of patients, and this was shown in the changes of M2BPGi serum.[29] Therefore, M2BPGi can be used to evaluate the therapeutic response in chronic hepatitis B patients.[72]

This study has several limitations that may affect its results. First, liver biopsy, the gold standard for assessing liver fibrosis, was not performed, which may introduce bias in evaluating fibrosis stages based on transient elastography and M2BPGi alone. Additionally, other factors such as fatty liver disease, medication use, and co-infection with hepatitis D virus could have influenced the fibrosis measurements. The study was also conducted at a single center with a limited sample size, particularly in the advanced fibrosis group (Metavir score > F3), which may impact the generalizability and robustness of the findings.

CONCLUSION

There was a moderate and statistically significant positive correlation between M2BPGi levels and liver tissue stiffness, as measured by transient elastography, in patients with chronic hepatitis B.

Conflict of Interest

No conflict of interest was reported by the authors.

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Author Contribution

Each author has made substantial contributions to the work and approved the final manuscript for publication.

Data Availability

All data generated or analyzed during this study are available in the manuscript.

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