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

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ABSTRACT

Background: *Hepatitis B virus (HBV) is a serious health problem in the world, including in Indonesia. Transient elastography (TE) is now regarded as a reliable surrogate marker for grading the severity of liver fibrosis in chronic hepatitis B (CHB) patients. The 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 aims to evaluate the correlation of M2BPGi and liver stiffness (LS), measured through TE, in predicting liver fibrosis among CHB patients.*

Method: *A cross-sectional study was conducted at Dr. Hasan Sadikin General Hospital Bandung between September 2021–January 2022 on patients diagnosed with CHB based on clinical and biochemical examination. The subjects underwent TE examination using Fibroscan® and 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 CHB (M:F = 66:53, median age 43 years) patients were consecutively recruited. The median M2BPGi level was 1.04 COI (0.74–1.59) and the median of LS was 7.3 (5.6–12.5). M2BPGi had a moderate and significant correlation with LS ($r = 0.525$; $p < 0.001$). Median M2BPGi values in each fibrosis stage were 0.89 COI in F0-F1, 0.88 in F2, 1.61 in F3, and 2.24 in 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 Umum 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, umur median 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).*

Simpulan: *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 is a worldwide public health problem, including in Indonesia. Hepatitis B virus (HBV) has infected a total of 1.5 billion people in the world and around 296 million people suffer from chronic hepatitis B infection. It is estimated that as many as 820,000 people in 2019 died from causes related to hepatitis B infection.[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 such as 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 disease requires periodic assessment of liver fibrosis, so identification of patients with significant liver fibrosis or cirrhosis is essential to prevent the progression and decompensation of the disease.[9–11] Assessment of liver fibrosis can be performed invasively by liver biopsy, or non-invasively by imaging-based assays, such as liver transient elastography, ultrasonography, magnetic resonance imaging (MRI), or calculation of serum formulas such as 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, but it has some limitations, such as invasive, sampling error, inter-observer variability, and may cause complications, such as 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] However, limitations of this tool are not all healthcare facilities have this tool, requires operator training, difficulties in detecting early liver fibrosis, difficult to assess in obese or patients with ascites, and expensive.[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 such as chronic hepatitis C, chronic hepatitis B, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and primary biliary cirrhosis (PBC) so that those that are compatible with liver disease and the degree of fibrosis can be determined as a 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] The use of the M2BPGi seromarker

is easier than transient elastography because it does not require additional tools and expertise such as in liver elastography. Thus, it can be used by all medical personnel and is not disturbed by several conditions such as obesity and ascites. Practically, any health centre 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 programme; hence, it is useful to assess threaupetic 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 oesophagal varices in patients with liver cirrhosis, whereas in chronic hepatitis B patients, there were only evidence-based case reports.[28,30,31] This report concluded that M2BPGi could not be used as a diagnostic modality to detect liver fibrosis in chronic hepatitis B patients, because 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. 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 in 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 study is an analytical study with a cross-sectional method. The study sample was chronic hepatitis B patients who were on an outpatient basis at the Gastroenterology Hepatology Clinic, Dr. Hasan Sadikin General Hospital in the period September 2021–January 2022. The inclusion criteria for this study included: (1) Age 18 years and over; (2) Seropositive HBsAg for more than 6 months; (3) Diagnosed with chronic hepatitis B from history taking, physical examination, and supporting examinations (serum HBV DNA > 20,000 IU/mL on positive HBeAg or < 2,000–20,000 IU/mL on negative HBeAg, persistent or intermittent increase in ALT (1–2x the upper limit of normal and should not be more than 5x the upper limit of normal); (4) Willing to participate as a research subject in writing. 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 anaemia (Hb < 5 g/dL); (11) Pulmonary fibrosis, chronic pancreatitis, liver cancer, and pancreatic cancer.

In this study, sampling was performed using the consecutive sampling technique, namely, the order of arrival of patients who met the inclusion, and exclusion criteria were included as study subjects until the minimum sample size was met. 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 know 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, obtained n = 29. 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, namely categorical and numerical variables. Numerical variables included the value of liver stiffness and categorical variables included the degree of liver fibrosis which were further classified into 4 groups (F0-F1, F2, F3, F4). Numerical variables included serum M2BPGi.

Table 1. Operational definition of study variables

Variable	Operational definition	Measuring instrument	Measurement results	Scale
Chronic hepatitis B	Hepatitis B with seropositive HBsAg more than 6 months or results of serum HBV DNA > 20,000 IU/mL in positive HBeAg or > 2000 IU/mL in negative HBeAg. Persistent of intermittent elevation of ALT.[10]	Rapid diagnostic test (RDT)	HBsAg +/-	Categorical
		Immunology instrument siemens advia series	Reactive/non-reactive HBeAg	Categorical
		Cobas 4800 system (roche)	HBV DNA IU/mL and Log IU/mL	Numeric
		Chemistry instrument siemens dimension series	ALT U/L	Numeric
M2BPGi	Glycoprotein is produced by liver stellate cells and functions as an intermediary between Kuppfer and stellate cells in the fibrogenesis process.	HISCL M2BPGi reagent kit (Sysmex, Hyogo, Japan) with automatic immune analyzer HISCL 800 (Sysmex, Hyogo, Japan)	Cut-off Index (COI) 0.1–20.00 COI < 1 : negative 1.0 ≤ COI < 3 : 1 positive COI ≥ 3 : 2 positive	Numeric
Liver stiffness	Results of liver transient elastography or transient elastography (TE)	FibroScan® 502 serial F00734 (Echosen, Paris, France) using M probe	The median value from ≥ 10 valid examinations. Measurement results can be trusted if the success rate was ≥ 60% with an interquartile range (IQR)/ median liver stiffness (MLS) ≤ 30% Examination results were stated in kPa	Numeric
			The median value of liver stiffness was grouped based on the degree of liver fibrosis based on the Metavir score as follows: < 6 kPa : F0-F1 6.1–8.9 kPa : F2 9–11.9 kPa : F3 > 12 kPa : F4	Categorical

HBsAg: hepatitis B surface antigen; HBV DNA: hepatitis b virus deoxyribo nucleic acid; ALT: alanin aminotransferase; kPa: kilopascal

The results of liver elastography examination were expressed in kPa units and the median value of liver tissue stiffness was grouped as follows.[37]

Table 2. 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 Padjajaran and Dr. Hasan Sadikin Hospital Research Ethics Committee. Data collection from medical records was carried out after the patient gave informed consent.

Chronic hepatitis B patients who were the subjects of the study were examined for weight, height, transient elastography, and serum M2BPGi. All examinations were carried out by implementing the COVID-19 health protocol for patients in the form of wearing surgical masks and examiners using level 2 PPE.

Data in this study were analyzed statistically using SPSS version 25.0. Correlation testing was carried out using the Pearson correlation test if the data were normally distributed and rank Spearman correlation if the data were not 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 test was performed if the data were normally distributed and the Kruskall Wallis test if the data were not normally distributed.[85] The result is declared statistically significant if the *p* value < 0.05.

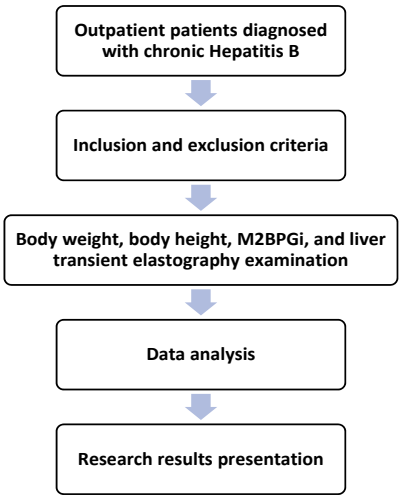

Figure 1. Research outline

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–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 patient. Each subject was given information about the study to be carried out and signed an informed consent. The subject underwent M2BPGi blood collection and examination in the laboratory of Dr. Hasan Sadikin General Hospital. A transient liver elastography examination was performed on the 3rd floor of the Diagnostic and Cardiac Center Building, Dr. Hasan Sadikin Hospital.

Table 3. 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 (thousand/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, there were 119 subjects with a median age of 43 years with 55.5% of subjects 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-reative 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 36 patients (30.3%), F2 42 patients (35.3%), F3 9 patients (7.6%) and F4 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

To test the hypothesis whether there is a correlation between M2BPGi and the results of liver elastography transient examination, particularly the median value of liver tissue stiffness, Rank Spearman's correlation test was performed as the data distribution was not normal. The results of the correlation test were described in table 5. Based on the correlation test at 95% confidence interval, there was a moderate and significant correlation between M2BPGi and the median value of liver tissue stiffness with a correlation coefficient (r) of 0.525 and a significance value of < 0.001 ($p < 0.05$).

Table 5. Correlation of M2BPGi and liver stiffness value

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

The following was a scatter diagram of the relationship between M2BPGi and the median value of liver stiffness.

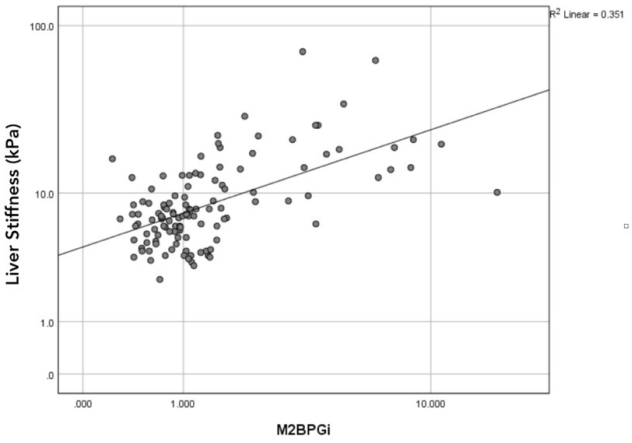

Figure 2. Scatter diagram showing the correlation of M2BPGi and median value of liver stiffness

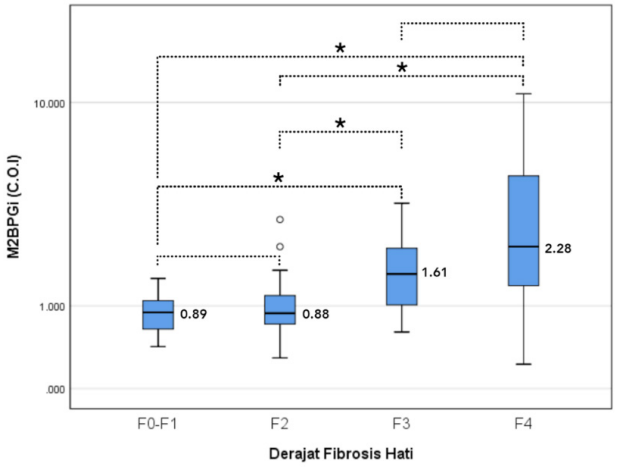

Figure 3. Boxplot showing distribution of M2BPGi based on the degree of liver fibrosis

The scatter diagram in Figure 2 showed 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 examination 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 6.

The results of Table 6 showed that the median of M2BPGi in the degree of fibrosis F0-F1 was 0.89 (IQR = 0.64–1.10), F2 0.88 (IQR = 0.71–1.20), F3 1.61 (IQR = 0.95–2.98), and the highest in F4 was 2.28 (IQR = 1.31–4.99). This distribution is illustrated by the boxplot image below (Figure 3).

Based on the Kruskall-Wallis test, 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. This was the same as the study by Mak et al who found that the number of chronic hepatitis B patients was higher in 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 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]

Table 6. 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

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, there were a higher number of chronic hepatitis B patients with negative HBeAg (85%) than positive HBeAg. This was because the majority (59.7%) of chronic hepatitis B patients in the study had received antiviral therapy. Hepatitis B patients who have not received therapy and were HBeAg negative may be caused by the naturally occurring mutant forms of hepatitis B that do not produce HBeAg. 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%); therefore, this was not conducted in this study.[15–19,50] As a substitute for liver biopsy, 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, namely transient liver elastography which is an imaging-based examination and M2BPGi which is a biochemical-based examination.

More than 90% of the protein in the 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 a number of processes especially those related to cell adhesion. In addition, M2BP interacts with a number of extracellular proteins related to fibrosis such as 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), so it is also known 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]

In this study, it was found that M2BPGi was positively correlated with age, female sex, AST, ALT, and negatively correlated with platelets and albumin (Table 4). The same results were found in Mak et al and Wei et al, except that M2BPGi was not correlated with gender.[34,41] This can be explained by a meta-analysis study by Cai et al who found 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] A study by Yang YT et al 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 will increase the accuracy in identifying patients with advanced fibrosis (sensitivity 51%, specificity 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 who reported 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 (r) = 0.611; $p < 0.001$ and Wei et al with a correlation coefficient (r) = 0.574; $p < 0.01$. All of these 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, which were F0-F1 30.3%, F2 35.3%, F3 7.6% and F4 26.9%. The median M2BPGi values in each group were 0.89 (F0-F1), 0.88 (F2), 1.61 (F3), and 2.28 (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.

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]

The distribution of M2BPGi to the degree of liver fibrosis in this study had a significant difference in the degree of liver fibrosis in the group ≥ F3 and F4 & degree of fibrosis < F2 compared to the group F0-F1 & F2 (see figure 6). A similar study was reported by Wei et al and Yamada et al which stated that M2BPGi had the best ability to predict 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 aetiology of liver disease and is compatible with other non-invasive tests in predicting early liver fibrosis. This meta-analysis study concluded that M2BPGi is good enough to diagnose end-stage liver fibrosis, especially liver cirrhosis.[62]

M2BPGi predicts different degrees of liver fibrosis based on the aetiology 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 with 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]

In addition, 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 is explained by hepatic stellate cells (HSC) which are the source of M2BPGi to be closely related to the expression of alpha-smooth-muscle actin in chronic hepatitis B patients who are more likely to experience a more subtle liver inflammation and liver cirrhosis associated with hepatitis B have larger regenerative nodules with thinner fibrosis septa.[62,65,66] The study of Sturm et al concluded that the amount of fibrosis in chronic hepatitis C was greater than in chronic hepatitis B, which was primarily caused by the increased perisinusoidal fibrosis in chronic

Table 7. 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

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]

Most of the subjects in this study (71 patients (59.7%)) had received antiviral drugs and this could affect the results of the M2BPGi examination. The long-term use (more than 1 year) of nucleoside analogue drugs can 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 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 can cause a decrease in serum M2BPGi which is associated with histological regression of fibrosis in patients who underwent repeated liver biopsies.[29]

Nucleoside analogue drugs with higher potency, such as tenofovir and entecavir show 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 experience 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 the results of the study. The limitations of this study include: (1) This study did not perform a liver biopsy as a gold standard examination so there may still be a bias in assessing the degree of liver fibrosis from the results of transient liver elastography and M2BPGi; (2) There were other factors that influence liver fibrosis test results such as fatty liver, drugs and co-infection with the hepatitis D virus; (3) This study was limited to one hospital (single centre) and the number of samples was limited, especially for the liver fibrosis degree group according to the metavir score > F3.

CONCLUSION

There was a moderate and significant correlation between M2BPGi and liver tissue stiffness on transient liver elastography examination results in chronic hepatitis B patients and there was a positive direction correlation between M2BPGi and liver tissue stiffness results on transient liver elastography examinations in chronic hepatitis B patients.

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