MANSOURA JOURNAL OF Chemistry
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Assessment of prognostic Significance of some Blood proteins in Diagnosing Liver Cirrhosis Among Patients with Chronic Liver Disease

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Received:25/4/2025
Accepted: 17/5/2025

Abstract: Chronic liver disease (CLD) and liver cirrhosis are two critical worldwide health problems that raise morbidity and death rates. The last stage of liver disease, cirrhosis is characterized by the replacement of healthy liver tissue with fibrotic scar tissue, which impairs liver function and causes complications like variceal bleeding, portal hypertension, ascites, and hepatic encephalopathy. The primary causes of cirrhosis are autoimmune liver diseases, nonalcoholic fatty liver disease (NAFLD), chronic viral hepatitis, and excessive alcohol use. The current study has a number of advantages and impacts It examined the most recent developments in the clinical, laboratory, and diagnostic features of liver cirrhosis patients, affording insights into the course of the medical condition and possible biomarkers for its detection and treatment. Usually, this disease progresses gradually over several months or years. The entire body is responding to the damaged liver, including the skin, brain, kidneys, gastrointestinal tract, immune system, bone marrow, heart, etc. In order to help with early diagnosis and save more lives, the current study concentrated on the most recent developments in blood-based diagnostic biomarkers to assess the diagnostic power of Mac-2 binding protein glycosylation isomer (M2BPGi) and determine the cut-off values of M2BPGi for liver cirrhosis in patients with chronic liver disease.

keywords: chronic liver disease (CLD), hepatocellular carcinoma (HCC), nonalcoholic fatty liver disease (NAFLD), Mac-2 binding protein glycosylation isomer **(M2BPGi).**

1. Introduction

One major health issue is liver cirrhosis (LC) (1). Transmissible infectious diseases such viral hepatitis, alcohol use, metabolic syndrome, autoimmune disorders, storage diseases, toxic chemicals, and pharmaceuticals are typically linked to it (2). The final stage of several chronic liver diseases that have characteristics in common is cirrhosis. fibrosis, necroinflammation, and regenerative nodules, which change the vascular architecture and eliminate the liver's functional mass (2).

In a Physicians Administration study of 68,673 patients from a national population of patients (2020–2021), cirrhosis was caused by hepatitis C (24.0%), alcohol (27.9%), hepatitis C and alcohol (17.4%), nonalcoholic fatty liver disease (NAFLD) (25.9%), and other causes (3.7%). (3)
Men account for 54% to 60% of all cirrhosis cases (5) (4). LC is a remarkable problem in Egypt with a higher percentages comparing with other regions in the world. Age-standardized prevalence rates (ASPR) for liver cirrhosis, which is mostly caused by non-alcoholic fatty liver disease (NAFLD), were highest in Egypt in 2019 (6).

Moreover, about 8–10 million Egyptians undergo viral hepatitis, which is a respectable burden in Egypt. The previous studies showed that, hepatitis B, hepatitis C, and hepatitis D viruses are the principal causes of chronic hepatitis, liver cirrhosis, and liver cancer. Interestingly, Egypt had a high death rates from 1990 to 2017 with cirrhosis (7). Since management and prognosis mostly depend on an accurate diagnosis of LC and liver fibrosis, it is clinically significant in patients with CLD. [7–9] It is still difficult to diagnose liver

fibrosis and LC, nevertheless.

Although liver biopsies are the gold standard for identifying hepatic fibrosis and LC [10], they are invasive, unpleasant, and may cause problems, which makes it challenging to do the technique repeatedly in clinical practice [10,11]. Additionally, there are a number of drawbacks to liver biopsies, including sample mistakes and interobserver variability [12,13]. As a result, numerous noninvasive techniques have been created to evaluate hepatic fibrosis and LC [14–15]. The fibrosis index based on four components (FIB-4), aspartate aminotransferase-to-platelet ratio index (APRI), and gamma-glutamyl transpeptidase-to-platelet ratio (GPR) are examples of conventional blood markers that use standard laboratory parameters.

Additionally, it has been widely documented that these serum indicators can reliably predict LC; however, employing formulas to determine their values is inconvenient [14–18]. A new serum biomarker for the evaluation of liver fibrosis and LC has recently surfaced: the glycosylation isomer of the Mac-2 binding protein (M2BPGi, Wisteria floribunda agglutinin-positive Mac-2 binding protein, WFA+-M2BP) [19,20]. Few real-life clinical data on M2BPGi are available for evaluating LC and liver fibrosis in patients with CLD, despite several recent studies reporting that serum M2BPGi is a predictive biomarker of LC and liver fibrosis in patients with HCV [21,22], HBV [23,24], alcoholic liver disease (ALD) [25], nonalcoholic fatty liver disease (NAFLD) [26], autoimmune hepatitis (AIH) [27], and primary biliary cirrhosis [28].

2. Materials and methods

18 were normal control (I) included 14 males and 4 females and 44 were Liver Cirrhosis patients (II), the patients diagnosed with LC In the gastrointestinal surgery center at MansouraUniversity, the 44 Liver Cirrhosis patients included 32 male and 12 female.

The proposal was submitted to the Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB) for approval (ethical code: MS.21.08.1603).

Sample collection:

Subject (patient and controls) had 3 ml of whole blood collected and left at room temperature for clotting, serum separated by centrifugation at 2000 rpm for 5 min, then refrigerated at -40C till serum NO was measured.

Procedures:

In gastrointestinal surgery center lab, all sample taken to measure CEA and CA19.9.

3. Results and Discussion

This study was performed on 44 patients with clinically and laboratory confirmed to have liver cirrhosis and 18 healthy people as control group.

1.Demographic Data:

1.1. Age:

The median age of liver cirrhosis patients was 51.0 (36.0-60.0), and control was 25.0 (22.5-33.75) years. There were statistically significant differences between liver cirrhosis group and those of control group regarding age (p=0.0001) as shown in **table 1**.

1.2. Gender:

Liver Cirrhosis group included 32 (72.7%) males and 12 (27.3%) Female.

There was no statistically significant difference between the sex of two groups (p = 0.76) as shown in **table 1**.

Table 1. Age and Gender of Liver Cirrhosis patients and control group:

Variable	Control group (N = 18)	cirrhosis group (N = 44)	P value
Age (yr) Median (IQR)	25 (22.5-33.75)	51 (36.0-60.0)	<0.0001
Gender Male n (%) Female n (%)	 14 (77.8%) 4 (22.2%)	 32 (72.7%) 12 (27.3%)	0.76

Variable were expressed as Median (IQR) and gender was expressed as number (percentage). (IQR): Interquartile range.

2. Laboratory Data for Studied Groups: as shown in Table 2

2.1. Viral Hepatitis

2.1.1. Hepatitis B surface Ag:

The number of positive cases of patient with HBsAg of liver cirrhosis patients was 1(2.3%), with no positive cases of control group. there were not statistically significant differences between liver cirrhosis group and those of control group (p = 0.329).

2.1.2. HCV:

The number of positive HCV of LC patients was 14 (31.8%), and control was 0 (0%). there were statistically significant differences between liver cirrhosis group and those of control group (p=0.006).

Table 2. Number and percent of positive cases HBsAg and HCV Abs.

Variable	Control group (N=18)	cirrhosis patients (N=40)	P value
HBsAg **Negative n (%)** **Positive n (%)**	18 (100%) 0 (0%)	39 (88.6%) 1 (2.3%)	0.329
HCV ab **Negative n (%)** **Positive n (%)**	18 (100%) 0(0%)	26 (59.1%) 14(31.8%)	0.006

P value > 0.05 is considered not significant;

P value< 0.05 is considered significant.

HCV: Hepatitis C virus antibodies, HBs: Hepatitis B virus antigen.

2.2. Liver pannel of cirrhosis patient groups:

Albumin (ALB):

The mean serum Alb level of liver cirrhosis patients was 3.24 ± 0.63 g/dL, and control was 4.58 ± 0.33 g/dL. There was statistically significant change in serum albumin when compared to the value of control group (p<0.0001).

2.2.2. Total bilirubin level:

The median total bilirubin level in liver cirrhosis patients was 1.6 (1.0-4.6) mg/dl, and the median total bilirubin level in control was 0.6 (0.5-0.7) mg/dl. the statistical analysis of these results showed a significant increase in that marker when compared to the value of control group (p< 0.0001).

2.2.3. Direct bilirubin level:

The median Direct bilirubin level in liver cirrhosis patients was 1.0 (0.5-2.1) mg/dl, and control was 0.1 (0.1-0.2) mg/dl. There was statistically significant increase in this marker when compared to the value of control group (p < 0.0001).

2.2.4. Alkaline Phosphatase (ALP):

The median activity of Alkaline Phosphatase level in liver cirrhosis patients was 5.0 (5.0-7.0) mg/dl, and control was 5.0 (5.0-5.0) IU/L. There were statistically significant differences between liver cirrhosis group and those of control group (p

< 0.002).

2.2.5. Aspartate Amino Transferase (AST):

The median activity of Aspartate Amino Transferase levels in liver cirrhosis patients was 40.0 (26.0-70.0) mg/dl, and that of the control was 21.0 (20.0-21.0) mg/dl.

The statistical analysis of these results showed a significant increase in that marker when compared to the value of control group (p<0.0001).

2.2.6. Alanine Amino Transferase (ALT):

The median Alanine Amino Transferase level in liver cirrhosis patients was 26.0 (21.0-43.0) mg/dl, but the control was 22.5 (21.0-26.25) mg/dl. There was no statistically significant change in this marker when compared to the value of control group (p=0.218).

2.2.7. Gama Glutamyl Transferase (GGT):

The median activity of Gama Glutamyl Transferase level in liver cirrhosis patients was 44.5 (28.5-91.75) mg/dl, and control was 17.0 (12.25-26.25) mg/dl. The statistical analysis of these results shown a significant increase in that marker when compared to the value of control group (p< 0.0001).

Table 3. Liver Pannel of liver cirrhosis patients:

Variables	Group(control) N = 18	Group(patients) N= 44	P. value
AST (U/L) Median (IQR)	21.0 (20.0-21.0)	40.0 (26.0-70.0)	< 0.0001
ALT (U/L) Median (IQR)	22.5 (21.0-26.25)	26.0 (21.0-43.0)	0.218
GGT (IU/L) Median (IQR)	17.0 (12.25-26.25)	44.5 (28.5-91.75)	< 0.0001
Alb (g/dl) (Mean±SD)	4.580 ± 0.339	3.248 ± 0.632	< 0.0001
T. Bili (mg/dl) Median (IQR)	0.6 (0.5-0.7)	1.6 (1.0-4.6)	< 0.0001
D. Bili (mg/dl) Median (IQR)	0.1 (0.1-0.2)	1.0 (0.5-2.1)	< 0.0001
ALP (U/L) Median (IQR)	5.0 (5.0-5.0)	5.0 (5.0-7.0)	0.002
INR Median (IQR)	1.0 (1.0– 1.07)	1.4 (1.2– 1.6)	< 0.0001

Non parametric variables were expressed as Median Interquartile range (IQR), while parametric variables were expressed as mean±standard deviation SD. Albumin (Alb); Aspartate aminotransferase (AST); Alanine amino transferase (ALT); Gama glutamyl transferase (GGT); Alkaline phosphatase (ALP); International normalized ratio (INR). P value > 0.05 is considered not significant; P value < 0.05 is considered significant.

2.2.8. International Normalization Ratio (INR):

The median international normalization ratio of liver cirrhosis patients was 1.4 (1.2– 1.6) mg/dl, but the control was 1.0 (1.0– 1.07) mg/dl. There was statistically significant increase in this marker when compared to the value of control group (p< 0.0001).

Serum levels of Potassium, Sodium, Creatinine, and Uric Acid of liver cirrhosis patients: as shown in table 4

Potassium (k):

The mean value of Potassium in liver cirrhosis patients was 4.01 ± 0.62, and control was 4.28 ± 0.43. There were no statistically significant differences between liver cirrhosis group and those of control group (p= 0.097).

2.3.2. Sodium (Na):

The mean value of Sodium in liver cirrhosis patients was 137(132.7-139.2), and control was 139.0(137.0-141.0). There were statistically significant differences between liver cirrhosis group and those of control group (p= 0.021).

2.3.3. Creatinine (Cr):

The mean value of Creatinine in liver cirrhosis patients was 0.7 (0.6-0.92), but that of the control was 0.8 (0.7-0.9). The statistical analysis of these results revealed a non-significant difference in such serum marker when compared to its corresponding value of the control group (p=0.596).

2.3.4. Uric Acid (UA):

The mean value of Uric Acid in liver cirrhosis patients was 4.83 ± 1.75, and control was 4.97±0.93. There were no statistically significant differences between both of liver cirrhosis group and control group (p=0.707).

Table 4. Serum levels of K, Na, Cr, and UA of liver cirrhosis patients:

Variables	Group (control) N = 18	Group (patient) N = 44	P. Value
UA (mg/dl) (Mean ± SD)	4.97 ± 0.93	4.83 ± 1.75	0.707
Cr (mg/dl) Median (IQR)	0.8 (0.7-0.9)	0.7 (0.6-0.92)	0.596
K (m Eq/L) (Mean ± SD)	4.28 ± 0.43	4.019 ± 0.627	0.097
Na (m Eq/L) Median (IQR)	139.0 (137.0-141.0)	137 (132.7-139.2)	0.021

Non-Parametric variables were expressed as Median Interquartile range (IQR). Parametric Data were expressed as Mean ± standard deviation SD. P value > 0.05 is considered not significant; P value < 0.05 is considered significant.

Hematology parameters:

Red Blood Cells Count:

The mean Red Blood Cells Count in liver cirrhosis patients was 3.69 ± 0.86, but the control was 5.004 ± 0.606. the statistical analysis of these results showed lowered count of these marker when compared to its corresponding value of the control group (p< 0.0001).

2. Hemoglobin (HB):

The mean Hemoglobin level in the whole blood of LC patients was 10.40 ±2.22 g/dl, but the control was 14.25 ± 1.47 g/dl. There was statistically significant decrease in this marker when compared to the value of control group (p< 0.0001).

3. White Blood Cells (WBCs):

The median of white blood cells count in liver cirrhosis patients was 4.06 (2.9-5.5), but the control was 5.85 (4.2-6.87). There was statistically significant difference between liver cirrhosis group and those of control group (p=0.006).

4. Platelet count (PLT)

The mean platelets count in the whole blood of liver cirrhosis patients was 111.17 ±70.48, but of the control was 219.15 ± 47.36. the statistical analysis of these results shown decrease count when compared to control group (P<0.0001).

Table 5. RBCS, WBCs and Platelet count of the two studied groups:

Variable	Group (control) N = 18	Group (patient) N = 44	P-Value
RBCS (m/UL) (Mean ± SD)	5.004 ± 0.606	3.69 ± 0.86	< 0.0001
HB (g/dl) (Mean ± SD)	14.25 ± 1.47	10.40 ± 2.22	< 0.0001
WBCs (k/U l) Median (IQR)	5.85 (4.2-6.87)	4.06 (2.9-5.5)	0.006
PLT (k/µl) Mean ± SD	219.15 ± 47.36	111.178 ± 70.485	<0.0001

Variable were expressed as Median Interquartile range (IQR), and mean ± standard deviation SD.

P value > 0.05 is considered not significant. P-value < 0.05 is considered significant.

3. The classification of the studied groups according to Child Pugh, APRI, FIB4 score:

3.1. Use of the child pugh score:

The Child A score in LC patients was 7 (15.9%), and control was 18 (100%). while Child B in LC patients was 18 (40.9%), and Child C was 16 (36.4%). there were statistically significant differences between liver cirrhosis group and those of control group (p< 0.0001)**.**

3.2. APRI score:

The APRI score Grade 1 in LC patients was7(18.4%) and control was 17(94.4%). while Grade 2 in LC patients was 13(34.2%) and control was 1(5.6%), also Grade 3 was 18(47.4%). there were statistically significant differences between liver cirrhosis group and those of control group (p<0.0001)**.**

3.3. FIB4 score:

The FIB4 score Grade 1 in liver cirrhosis patients was 4(10.8%) and control was 18(100%). while Grade 2 in LC patients was 10(27%) and control was 0(0%), also Grade 3 was 23 (62.2%). there were statistically significant differences between liver cirrhosis group and those of control group (p<0.0001)**.**

Table 6. The classification of the studied groups according to Child Pugh, APRI, FIB4 score:

Variables	Group (control) N = 18	Group (patients) N= 44	P. value
Child N=41			
Child A	18 (100%)	7 (15.9%)	
Child B	0 (0%)	18 (40.9%)	< 0.0001
Child C	0 (0%)	16 (36.4%)	
APRI N=38			
1< 0.5	17 (94.4%)	7(18.4%)	
2 (0.5-1.5)	1 (5.6%)	13(34.2%)	< 0.0001
3> 1.5	0 (0%)	18 (47.4%)	
FIB4 N=37			
1	18 (100%)	4 (10.8%)	
2	0 (0%)	10 (27%)	
3	0 (0%)	23 (62.2%)	< 0.0001

Child A (5 to 6 points); Child B (7 to 9); Child C (10 to 15). Low cirrhosis (APRI score<0.5); Moderate cirrhosis (APRI score 0.5 – 1.5); Cirrhosis (APRI score > 1.5); minimal or no fibrosis (FIB4 score < 1.45); intermediate range (FIB4score1.45-3.39); Cirrhosis (FIB4 score ≥3.4)

Conclusions:

The present study has several strengths and implications, explored the latest advancements in the clinical, laboratory, and diagnostic characteristics of patients with liver cirrhosis (LC) providing insights into the disease's progression and potential biomarkers for its diagnosis and management. The present study focuses on the diagnostic potential of blood-based biomarkers, specifically Mac- 2 binding protein glycosylation isomer (**M2BPGi**) as a non-invasive biomarker. aiming to enhance early-stage detection and diagnosis of the disease.

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