Iranian Journal of Pathology | ISSN: 2345-3656

Circulating HBcrAg, miRNA-122, and M2BPGi as Predictive Biomarkers for Hepatocellular Carcinoma in Chronic Hepatitis B Patients: A Case-Control Study

Amgad Ahmed Ezzat Othman[1], Mohamed S. Hemeda[2]*, Khaled Mohamed Hassanein Mohamed[3], Mohammed Saad Ahmed[1], Ayman S Yassin[1], Mohamed Nabil Mohamed[1], Abd Elmoaty Arafat Abd Elmoaty[4], Mahmoud M. Khafagi[4], Mohammed Mostafa Mohammed Abdo[1], Mohamed Abdel Basit Ibrahim Mohamed[1]

1. *Department of Medical Microbiology and Immunology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*
2. *Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Port Said University, Port Said, Egypt*
3. *Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt*
4. *Hepatology and Infectious Diseases Department, Faculty of Medicine, Al-Azhar University, Assiut, Egypt*

KEYWORDS

miRNA-122, HBcrAg, M2BPGi, Hepatocellular carcinoma, Chronic hepatitis B, Forensic biomarkers, Toxicological relevance

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Main Subjects:
Microbiology

Received 22 Nov 2025;
Accepted 20 Jan 2026;
Published Online 20 Feb 2026;

10.22034/ijp.2026.2075531.3567

ABSTRACT

Background & Objective: Hepatitis B Virus (HBV) infection has highly negative consequences for the global population, with chronic hepatitis B (CHB) infection and the resulting hepatocellular carcinoma (HCC) representing one of the most concerning long-term outcomes. This study focuses on the use of several biomarkers (miRNA-122, HBcrAg, and M2BPGi) in liquid biopsies to evaluate their efficacy in detecting early-stage HCC and assessing risk in patients with CHB.

Methods: This study included 90 participants. Of these participants, 30 had chronic hepatitis B (CHB), 30 had hepatocellular carcinoma with HBV (HCC), and 30 were healthy controls. Serum miRNA-122 was quantified with real-time PCR, and HBcrAg and M2BPGi were measured using ELISA.

Results: MiRNA-122 had an AUC of 0.801 and a sensitivity of 85.2%, indicating the highest diagnostic performance among the studies evaluated. M2BPGi demonstrated strong performance, with an AUC of 0.84. By contrast, HBcrAg had a very weak discriminative value. These previously stated biomarkers can improve the peripheral non-invasive screening.

Conclusion: In this initial cohort study, circulating miRNA-122 and M2BPGi demonstrate an unsuspected capacity to predict HCC differentiation in CHB patients. However, these are preliminary and have yet to be tested for robustness across multicenter studies with appropriately estimated sample sizes.

Corresponding Information: **Mohamed S. Hemeda**, Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Port Said University, Port Said, Egypt. Email: mohamudsadawy@med.psu.edu.eg

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Introduction

Despite achieving advanced degrees at the estimated level of impact, viral hepatitis infection continues to devastate the global health community. People continue to die as a result of the chronic form of virus infection. Six hundred thousand deaths die annually of complications. First, new cases are reported every day in regions worldwide that are endemic for the virus. Complications of the virus include chronic hepatitis B infection. Reports of new infections of the hepatitis B virus are daily in China, Southeast Asia, and sub-Saharan Africa.

Liver virus infections are curtailed or alleviated by endogenous ribonucleic (RNA) micro molecules, some of which are called regulators of post-transcriptional control of protein synthesis (3, 4) called micro ribonucleic acids (micro RNAs). These microRNAs mainly regulate sorbin, a major hepatocyte (liver cell) protein, and play a significant role in healthy liver function.

Diagnostics of primary liver cancer HCC traditional imaging uses some significant tumor markers such as alpha-fetoprotein (AFP), des-γ-carboxy prothrombin DCP), and vitamin K-dependent protein induced by vitamin K absence, two prothrombin PIVKA 2. Like any imaging modality, disease stage is a critical factor; in HCC, stage 1 (early) is a relatively large (and suspicious) tumor. The lack of sensitivity (minimal harmful or abnormal tissue) and specificity (without a precise target) makes ethical confidence almost impossible (5, 6).

In recent years, the clinical markers hepatitis B surface antigen (HBsAg), HBV DNA, and hepatitis B

core-related antigen (HBcrAg) have been studied to estimate chronic hepatitis B virus (HBV) infection and the degree of viral replication and infection (5,6). HBcrAg functions as a surrogate biomarker of intrahepatic cccDNA and HBV replication, thereby providing some prognostic information on disease severity and treatment response (6).

Moreover, M2BPGi, which was initially aimed at fibrosis quantification in patients with chronic hepatitis C, has shown potential for liver fibrosis evaluation in HBV, NAFLD, and biliary diseases, and for HCC risk prediction (7).

This study aims to analyse the clinical significance of serum levels of miRNA-122, HBcrAg, and M2BPGi in patients with chronic hepatitis B infection and those with HBV-associated hepatocellular carcinoma. The goals of the study are to define the expression profiles and diagnostic accuracy of these biomarkers, and to determine their applicability as non-invasive methods for early detection of HCC and for more accurate risk stratification.

Materials and Methods

Ethical Approval and Informed Consent

This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (Males), Al-Azhar University, Assiut, Egypt. The committee operates in accordance with the Declaration of Helsinki, the Islamic Organisation for Medical Sciences, the World Health Organisation (WHO), and the International Council for Harmonisation of Good Clinical Practice (ICH-GCP). The approval was granted under protocol number MD/AZ.AST/MIC0071/200/10/2021, on October 12, 2021. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee. Informed consent was obtained from each participant before the study began.

Study Design and Participants

The present cross-sectional case–control study with prospective recruitment was conducted from June to December 2023 at Al-Azhar University Hospital, Assiut, in conjunction with the Departments of Medical Microbiology & Immunology and Tropical Medicine & Gastroenterology. A total of 90 participants were recruited and divided into three equal groups:

• Group I: comprised thirty patients with clinically diagnosed, biochemically confirmed (elevated liver enzymes AST, ALT; HBsAg and HBeAg positive) CHB, and verified through quantitative PCR.

- Group II: comprised thirty participants with HCC secondary to chronic infection with HBV, based on clinical imaging (US, CT, MRI) and confirmed by histopathological examination.
- Group III: comprised thirty age and sex matched controls with no significant medical history, including regular liver function tests and negative for HBV and HCV markers.

Study subjects were enrolled through a non-probability sampling method from among the inpatient and outpatient departments. Participants were enrolled during the study period, and blood samples were collected at enrolment; no longitudinal follow-up was performed.

Sample Size Calculation

Using pilot data comparing miRNA-122 levels between the CHB and HCC groups, an a priori sample size estimation was performed. Assuming an effect size (Cohen's d) of 0.6, 80% power, and α=0.05, the minimum required sample size was 45 participants per group. Because recruitment was limited to a fixed study period (June–December 2023) and depended on the availability of eligible cases, we enrolled 30 participants per group. Accordingly, this study should be considered exploratory and hypothesis-generating; the reduced sample size may limit power to detect modest effects and yield less precise estimates, which require confirmation in larger multicentre cohorts.

Inclusion Criteria

- Adults aged 18 years and older.
- Confirmed diagnosis of chronic hepatitis B for more than 6 months (for Groups I and II).
- Histologically and/or radiologically confirmed HCC associated with HBV (Group II).
- Healthy individuals with normal liver function and negative viral markers (Group III).

Exclusion Criteria

- Co-infection with other hepatitis viruses (e.g., HCV).
- Presence of autoimmune hepatitis, alcoholic liver disease, or other chronic systemic diseases.
- Any history of non-HBV-related malignancies.

Sample Collection and Laboratory Investigations

Blood samples from participants were collected through peripheral venous access and processed under standard sterile techniques. The following tests were conducted:

• Liver Function Tests (LFTs): AST, ALT, total and direct bilirubin, albumin, and ALP were measured with the Roche Integra 400 autoanalyser (Roche Diagnostics, Japan).

• HBV Serological Markers: HBsAg, HBeAg, HBsAb, and HBcAb were screened using ELISA kits from DiaSorin Biomedica Co., Spain.

• Alpha-fetoprotein (AFP): Tested on Elecsys 2010 (Roche Diagnostics, Germany) via electrochemiluminescence immunoassay.

Trained laboratory personnel, who were blinded to the participants' groupings to minimise detection bias, performed all laboratory analyses.

Quantification of Biomarkers

miRNA-122 Expression:

The miRNeasy Mini Kit (Qiagen, Germany) was used for total RNA extraction. Reverse transcription was conducted with the miScript II RT Kit, followed by quantitative real-time PCR on the Rotor-Gene Q system (Qiagen). The expression levels of miRNA-122 were normalised to U6 RNA and quantified using the 2^−ΔΔCt technique. Relative expression values are reported in arbitrary fold-change units (2^−ΔΔCt) after normalisation to U6; therefore, group means are not expected to be centred on 1.0 unless the control group mean itself is explicitly used as the calibrator. The primer sequences and heat cycling parameters are available upon request.

HBcrAg Levels:

Serum HBcrAg was quantified using a double-antibody sandwich ELISA kit (SunRed Bio, Shanghai, China) according to the manufacturer's instructions.

M2BPGi Levels:

Serum levels of Mac-2 binding protein glycosylation isomer (M2BPGi) were measured using a sandwich ELISA kit (IBL International, Japan). Absorbance was measured at 450 nm with a microplate reader.

Statistical Analysis

We used SPSS version 25.0 (IBM Corp., Armonk, NY, USA) to perform the statistical analyses. One-way analysis of variance (ANOVA), accompanied by Tukey's post hoc test for pairwise comparison, was used for continuous variables presented as mean ± SD. The chi-square (χ^2) test was used to analyze the frequency distribution of the categorical variables.

The diagnostic accuracy of miRNA-122, HBcrAg, and M2BPGi was assessed using ROC curves. Measurement of the area under the curve (AUC), sensitivity and/or specificity of the test, positive and/or negative predictive value, and overall test accuracy were included. The statistical significance level was set at $p < 0.05$.

Table 1. Demographic Characteristics of the Studied Groups

Parameter	Group I (CHB)	Group II (HCC)	Group III (Control)	P-value
Age (Mean ± SD)	43.4 ± 5.2	55.0 ± 4.1	36.9 ± 7.6	< 0.001
Male (%)	70%	76.6%	60%	0.928
Female (%)	30%	23.4%	40%	
Rural Residence (%)	73.3%	63.3%	70%	0.495
Urban Residence (%)	26.7%	36.7%	30%	

Results

Demographic and Clinical Characteristics

Ninety individuals were enrolled in three groups: 30 individuals in each group (Group I: patients with chronic HBV (n=30), Group II: patients with HBV-related HCC (n=30), and Group III: healthy controls (n=30)). Thus, three equally constructed groups were formed.

There was a significant difference in mean age across the three groups (P < 0.001), with the HCC group older than the CHB and control groups.

The proportion of participants' gender was also similar across the cohorts, with roughly 70% of Group I, 76.6% of Group II, and 60% of Group III male (P = 0.928).

Participants from rural areas were predominant in all groups, though the difference is not significant (P = 0.495). These demographic characteristics are shown in Table 1.

Clinical Manifestations and Haematological Parameters

Ascites was noted in 80% of patients with chronic CHB and 66% with HCC (P = 0.399). Hematemesis was recorded as more frequent in the HCC group (78%) compared to the CHB group (50%) (P = 0.002). Furthermore, it was noted that hepatic encephalopathy is substantially more prevalent among HCC patients (63%) in comparison to CHB patients (19%) (P = 0.001).

Both CHB and HCC patients had statistically, and clinically significantly lower haemoglobin and red blood cell counts than the controls (P = 0.001). The HCC group also demonstrated a significant decline in platelet counts (59.01 ± 7.90 ×10³/cm³) when compared to CHB patients (172.45 ± 67.16 ×10³/cm³) and controls (362.8 ± 53.05 ×10³/cm³) (P = 0.001).

As with other parameters, serum AST and ALT levels were elevated in both patient groups relative to the controls (P = 0.001) and were higher in the HCC group. Albumin was significantly lower in the HCC group compared to the CHB group and controls (2.62 ± 0.29 g/dL) (P = 0.001). Total bilirubin and AFP were highest in group II, with AFP reaching 1478.96 ± 1085.30 ng/mL, while group I and III had 47.83 ± 54.41 ng/mL and 3.3 ± 2.3 ng/mL, respectively (P = 0.001). Comprehensive results are provided in Table 2.

AFP values in the HCC group showed wide dispersion (large SD). While markedly elevated AFP supports HCC when present, normal or mildly elevated AFP does not exclude HCC; thus, AFP should be

interpreted in conjunction with imaging and clinical context. (8)

Table 2. Comparison of Clinical Manifestations, Laboratory Findings, and Statistical Significance Among HBV, HCC, and Control Groups

Parameter	Group I (HBV)	Group II (HCC)	Group III (Control)	P-Value	Rating Scale
Ascites (%)	80%	66%	-	0.399	None (0) - Mild (1) - Moderate (2) - Severe (3)
Hematemesis (%)	50%	78%	-	0.002	None (0) - Occasional (1) - Frequent (2) - Severe (3)
Lower Limb Oedema (%)	74.07%	79.2%	-	0.92	None (0) - Mild (1) - Moderate (2) - Severe (3)
Encephalopathy (%)	19%	63%	-	0.001	None (0) - Grade I (1) - Grade II (2) - Grade III (3) - Grade IV (4)
Haemoglobin (g/dL)	10.37 ± 1.08	10.05 ± 0.91	13.25 ± 1.18	0.001	Severe (<9) - Moderate (9-10) - Normal (>10)
RBCs (x10⁶/cm³)	3.2 ± 0.9	3.1 ± 0.9	4.6 ± 0.65	0.001	Severe (<3) - Moderate (3-4) - Normal (>4)
TLC (x10³/cm³)	4.78 ± 1.17	4.91 ± 1.52	7.34 ± 1.88	0.001	Severe (<4) - Moderate (4-5) - Normal (>5)
Platelets (x10³/cm³)	172.45 ± 67.16	59.01 ± 7.90	362.8 ± 53.05	0.001	Severe (<100) - Moderate (100-200) - Normal (>200)
AST (IU/L)	45.60 ± 16.32	60.13 ± 16.96	25.8 ± 6.59	0.001	Normal (<40) - Elevated (>40)
ALT (IU/L)	55.33 ± 19.53	68.13 ± 21.18	28.16 ± 6.77	0.001	Normal (<40) - Elevated (>40)
Albumin (g/dL)	3.17 ± 0.42	2.62 ± 0.29	4.90 ± 0.41	0.001	Severe (<2.5) - Moderate (2.5-3.5) - Normal (>3.5)
T. Bilirubin (mg/dL)	1.25 ± 0.43	3.06 ± 0.80	0.71 ± 0.18	0.001	Normal (<1) - Elevated (>1)
AFP (ng/mL)	47.83 ± 54.41	1478.96 ± 1085.30	3.3 ± 2.3	0.001	Normal (<10) - Elevated (>10) - High (>400)

Table 3. Serum miRNA-122 Expression Levels in the Studied Groups

Group	miRNA-122 Expression (Mean ± SD)
Group I (CHB)	0.452 ± 0.176
Group II (HCC)	0.350 ± 0.300
Group III (Control)	1.90 ± 0.10

Expression Levels of miRNA-122

Patients with CHB and HCC demonstrated a significant decrease in miRNA-122 expression levels (relative 2^−ΔΔCt units) compared with healthy controls (P = 0.0001). The average values for CHB, HCC, and the control group were 0.452 ± 0.176, 0.350 ± 0.30, and 1.90 ± 0.10, respectively (Table 3).

Values are relative miRNA-122 expression units (2^−ΔΔCt) normalised to U6; values are not absolute concentrations and are dependent on the chosen calibrator/reference sample.

ROC Curve Analysis for miRNA-122

The ROC curve study for miRNA-122 demonstrated an AUROC of 0.801. At a threshold of <0.88, it had a sensitivity of 85.2%, specificity of 73.7%, positive predictive value of 60.5%, negative predictive value of 91.3%, and an overall accuracy of 77.38%. (Table 4 and Figure 1)

The ROC curve utilising serum miRNA-122 for differentiating HCC and CHB infections yielded an AUROC of 0.801. The red marker indicates the optimal cutoff values for sensitivity (>85.2%) and specificity (73.7%), which are <0.88. The grey dashed line represents no discrimination (AUROC = 0.5)

Table 4. Diagnostic Performance of miRNA-122 in Predicting HCC

Parameter	Value
AUROC	0.801
Cut-off Value	< 0.88
Sensitivity (%)	85.2
Specificity (%)	73.7
Positive Predictive Value (PPV) (%)	60.5
Negative Predictive Value (NPV) (%)	91.3
Diagnostic Accuracy (%)	77.38

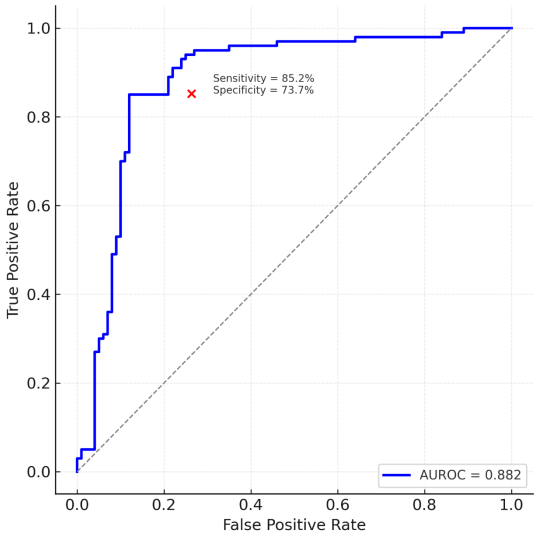


Fig 1. ROC curve for miRNA-122 in predicting HCC among chronic hepatitis B patients.

HBcrAg Levels and Diagnostic Performance

Patients with CHB and HCC exhibited markedly elevated blood HBcrAg levels compared to healthy controls (P = 0.039). Despite a slight reduction in the HCC group compared with CHB, the disparity between the illness groups was not statistically significant. HBcrAg ROC curve analysis yielded an AUROC of 0.367, indicating poor discriminative ability for differentiating HCC from CHB in our dataset. Accordingly, HBcrAg should not be interpreted as a standalone diagnostic marker for HCC; instead, it may reflect HBV activity/status, and its diagnostic utility for HCC requires confirmation in larger cohorts. These results are shown in Figures 2–4.

The mean serum HBcrAg levels in patients with CHB, those with HBV-related HCC, and healthy controls are displayed in a bar chart. The results reveal that CHB patients had the highest levels of HBcrAg (4.3 ± 0.7 log IU/mL), while HCC patients with HBV-related HCC had slightly lower levels (3.9 ± 0.4 log IU/mL); controls showed the lowest levels (2.8 ± 0.6 log IU/mL). Standard deviations are indicated by the error bars.

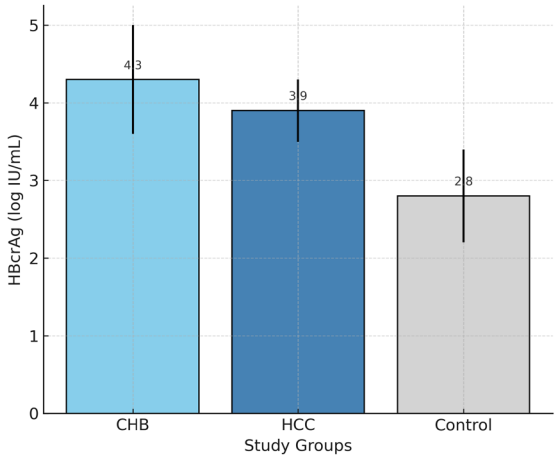


Fig. 2. Mean Serum HBcrAg Levels Across Study Groups

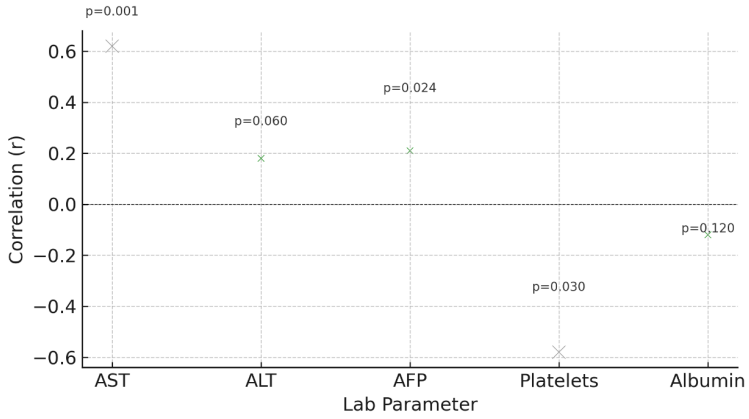


Fig. 3. Correlation of Serum HBcrAg with Laboratory Parameters

Scatter plots showing the correlation between serum HBcrAg levels and key laboratory tests in the studied population. A single point represents each participant. The colour intensity and point size represent the significance level (P-value) of the correlation. Positive correlations were observed only with AST and platelet count, and no significant correlations were found with ALT, AFP, or albumin.

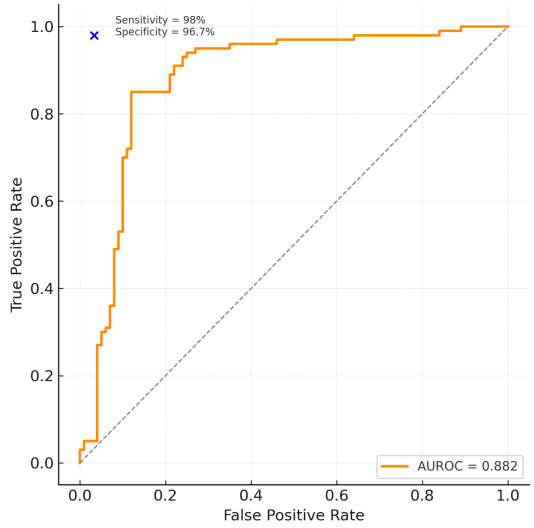


Fig. 4. ROC Curve for HBcrAg in Predicting Hepatocellular Carcinoma

ROC curve analysis of HBcrAg for differentiating HCC from CHB demonstrated deplorable discrimination (AUROC = 0.367), indicating very low diagnostic accuracy in this cohort. No clinically optimised cut-off can be inferred; any threshold shown should be considered exploratory and dataset derived.

M2BPGi Levels and Diagnostic Performance

Levels of M2BPGi were significantly altered from the control group in both the CHB and HCC groups (P = 0.039). In CHB patients, however, M2BPGi levels were higher than in the HCC cohort.

ROC curve analysis showed good discriminative ability of M2BPGi for differentiating HCC from CHB (AUROC = 0.84). At a cut-off value of >0.68 ng/mL, sensitivity and specificity were 90% and 80%, respectively. The observed PPV (25.8%) and NPV (99.3%) were calculated in this case–control sample and should be interpreted cautiously, as predictive values are prevalence-dependent and may not reflect real-world clinical performance. Prospective external validation in representative cohorts is required before considering any rule-out ("exclusion") use. (Figures 5–8.)

Correlation Analyses

- miRNA-122 had significant positive correlations with ALT (P = 0.041) and direct bilirubin (P = 0.002), but not with AST, AFP, or platelets.
- HBcrAg levels showed significant correlation with AST (P = 0.002) as well as with platelet count (P = 0.010), but not with ALT, albumin, or AFP.
- M2BPGi positively correlated with AST (P = 0.001), AFP (P = 0.024), and platelet count (P = 0.030), showing its importance in liver inflammation and tumour burden.

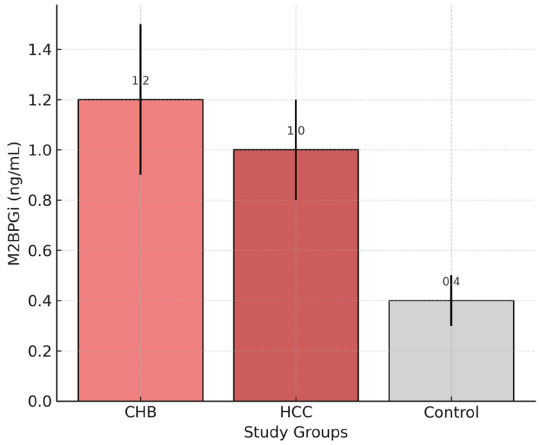


Fig. 5. Mean Serum M2BPGi Levels Across Study Groups

Bar graph depicting average values of serum M2BPGi levels across the groups studied. The M2BPGi levels were the highest in the CHB group at (1.2 ± 0.3 ng/mL), as well as the second highest group, which was the HCC group, which had (1 ± 0.2 ng/mL), and the lowest group was the control group (0.4 ± 0.1 ng/mL). The standard deviations are represented by the error bars shown.

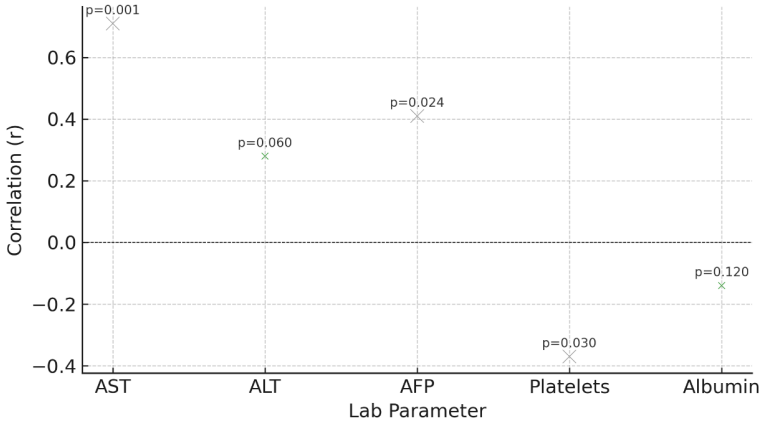


Fig. 6. Correlation Between Serum M2BPGi and Liver-Related Markers in the Studied Population

Scatter plots identify the relationship between M2BPGi level and essential markers in the liver. M2BPGi was positively correlated with nearly all M2BPGi levels and liver markers, including levels of AST (P = 0.001), AFP (P = 0.024), and total platelet count (P = 0.030). These findings suggest that M2BPGi may reflect hepatic inflammatory activity and may be associated with tumour burden; however, these correlations should be interpreted cautiously and validated in larger cohorts.

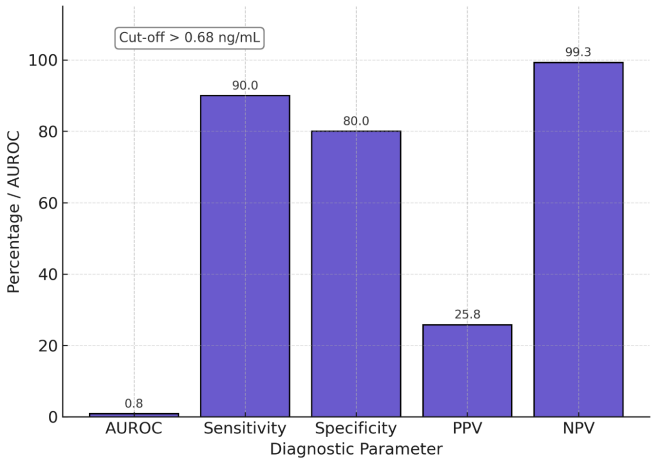


Fig. 7: Diagnostic Performance of M2BPGi in Predicting Hepatocellular Carcinoma

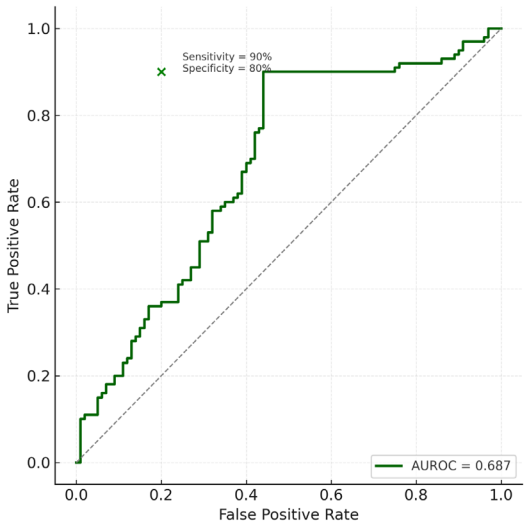


Fig. 8. ROC Curve for M2BPGi in Predicting Hepatocellular Carcinoma

The bar chart summarises the diagnostic indices of M2BPGi for differentiating HCC from CHB at a cut-off value of >0.68 ng/mL (AUROC = 0.84; sensitivity 90%; specificity 80%). The PPV was low (25.8%), and predictive values are prevalence-dependent; therefore, these estimates should be interpreted cautiously and require prospective external validation in representative cohorts.

Analysing the ROC curve for M2BPGi in differentiating HCC from CHB disease states. M2BPGi showed good discrimination (AUROC = 0.84) at a cut-off value of >0.68 ng/mL, with sensitivity of 90% and specificity of 80%. Predictive values are prevalence-dependent and require prospective validation in representative cohorts.

Discussion

HCC continues to cause the highest number of cancer deaths worldwide, and it commonly occurs with chronic liver disease, such as cirrhosis and CHB (9). HBV infections lead to ongoing inflammation in the hepatic system alongside regeneration, both factors integral to the development of liver cancer. As a result, reliable non-invasive indicators for the preliminary evaluation and monitoring of the advancement of the ailment must be sought.

In this study, the expression levels of miRNA-122, HBcrAg, and M2BPGi were measured in patients with CHB and patients with CHB-associated HCC and in healthy subjects.

The results showed that both patient groups exhibited a marked decrease in miRNA-122, with the lowest levels detected in HCC patients. These results corroborate previous findings that associate miRNA-122 downregulation with increased HBV replication and hepatic inflammation (10). Furthermore, miRNA-122 has been shown to inhibit liver cell proliferation and tumour growth by regulating the expression of PBF, a target oncogenic miRNA.

The ROC curve analysis performed in this study demonstrated that the area of the curve for sensitivity and specificity of the test was 85.2% and 73.7%, respectively, at a <0.88 cutoff value for HCC, predicting Piri's diagnostic accuracy to be 77.38%.

These findings are consistent with published evidence suggesting that circulating miR-122 has **moderate** diagnostic accuracy for HCC, including when compared with chronic viral hepatitis, and should be interpreted as preliminary pending validation in larger cohorts. (11)

Serum HBcrAg levels remained consistently elevated in CHB but were moderately lower in the HCC group, suggesting active replication of the Hepatitis B virus (12).

Serum HBcrAg levels remained consistently elevated in CHB but were moderately lower in the HCC group, suggesting active HBV replication. The AUROC value for HBcrAg was 0.367, indicating poor discrimination between HCC and CHB. Therefore, HBcrAg may be better interpreted as a marker of HBV activity/cccDNA-related transcription rather than a standalone marker for HCC diagnosis.

Although these values may indicate high predictive capacity at first glance, M2 HBcrAg's low AUROC and moderate positive predictive value of 93.7% reinforce the need to incorporate additional markers alongside HBcrAg to strengthen diagnostic certainty.

Given HBcrAg's correlation with AST and platelet count, but not ALT or AFP, this suggests a stronger association with hepatic inflammation than with tumour burden. This is in line with the literature, which claims that HBcrAg levels are related to intrahepatically present cccDNA and that viral transcription levels are more indicative of oncogenic activity. (13)

In addition, higher HBcrAg levels have been associated with an increased long-term risk of hepatocellular carcinoma in cohort studies, supporting its role in risk stratification rather than direct tumor burden assessment. (14)

Both disease cohorts showed significantly higher levels of M2BPGi than controls, with a slight decline in HCC patients compared with those with CHB. The ROC analysis yielded an AUROC of 0.84, with a sensitivity of 90% and a specificity of 80%, suggesting good discrimination between HCC and CHB in this exploratory dataset. However, the PPV was low (25.8%), indicating limited rule-in capability, and predictive values are strongly influenced by disease prevalence—particularly in case–control sampling. Although the NPV was high (99.3%), this finding is preliminary and requires prospective external validation in clinically representative cohorts before any rule-out ("exclusion") claims can be made.

Its relationship to AST, AFP, and platelet count, which in turn are known to increase with hepatic injury, indicates that M2BPGi also shows a strong association with injury and cancer risk. These findings are comparable to Mak et al. (2019), who described similar performance characteristics burdened in M2BPGi in CHB cohorts (12).

The demographic pattern observed is broadly consistent with the known epidemiology of HCC, where HBV-related HCC is more frequent in older patients and shows a male predominance in many cohorts. In Egypt, environmental exposures (including pesticides and aflatoxin) have also been reported among relevant risk factors. (15,16)

The evaluation of CHB and HCC patients in comparison to controls showed increasing liver enzymes AST and ALT, which coincides with previously reported data regarding these enzymes and hepatic necroinflammation (17).

AFP was markedly higher in the HCC group in our cohort, which may reflect more advanced tumour burden in these patients. Nevertheless, AFP is an imperfect biomarker for HCC detection and surveillance: AFP may remain normal in a substantial proportion of early-stage tumours and can be elevated in active hepatitis or cirrhosis, limiting its sensitivity and specificity in at-risk populations. Therefore, AFP should not be used as a stand-alone test and should be interpreted alongside imaging and the clinical context. (18)

Although the study offers promising outcomes, some limitations still need to be addressed. The first limitation is the small sample size, which is restricted to one region, and its external validity. Besides, the cross-sectional design of the study does not enable exploring the causal relationships between the concentration of biomarkers and the disease

advancement. Additionally, there are some other important unconsidered factors that have greater potential for bias, such as genetic background, lifestyle, coexisting medical conditions, and environmental factors. Moreover, the study lacked the continuous monitoring of changes in the biomarkers, which diminishes the understanding of the prognostic value of the studied biomarkers. In order to improve the clinical relevance of miRNA-122, HBcrAg, and M2BPGi in clinical practice, the study suggests conducting multicenter longitudinal studies with larger sample sizes.

To conclude, this exploratory case-control study suggests the feasibility of using miRNA-122, HBcrAg, and M2BPGi as non-invasive markers of HCC in patients with CHB. However, given the limited sample size, these findings should be interpreted cautiously and validated in adequately powered, multicentre prospective studies.

These findings need deeper, well-structured research with larger populations to validate that they meet the research objectives and the scope of their implications.

As with miRNA-122, M2BPGi, and HBcrAg, further research is needed. However, they could aid risk profiling and identify cancer in those with chronic hepatitis B. M2BPGi and the others, as stated earlier, are non-invasive and economical, enhancing diagnostic methods by eliminating the need for expensive equipment while improving existing procedures.

Forensic and Toxicological Relevance

The serum biomarkers identified in this study — miRNA-122, HBcrAg, and M2BPGi — suggest applications that extend beyond clinical hepatology into forensics and toxicology. Forensic pathologists frequently deal with cases of sudden or unexplained death in which there is suspected hepatic pathology, but no clinical documentation exists. In life, abnormal levels of these biomarkers may assist in the diagnosis of occult liver disease, such as hepatocellular carcinoma or advanced fibrosis from chronic hepatitis B. In toxicological contexts, miRNA-122 is well documented as an early marker of hepatocellular injury, for example, from acetaminophen overdose or exposure to aflatoxins. Furthermore, the involvement of these biomarkers in diagnosis could improve not only early clinical HCC detection in patients with chronic HBV but also forensic testing by providing molecular markers of necrosis resulting from drug or toxin exposure. Their shared importance highlights converging paradigms that demand collaborative efforts across the scope of primary preventive interventions and the framework of medicolegal investigation.

Strengths and Limitations

The present study has several strengths. To begin with, the case-control design features an indisputable comparison between the cases with hepatocellular carcinoma and the controls with chronic hepatitis B without cancer. Furthermore, the study strategically utilized a trio of non-invasive biomarkers, namely miRNA-122, HBcrAg, and M2BPGi, which addresses a critical diagnostic need and aligns with other studies focused on non-invasive tests. The study also employed molecular amplification by PCR in combination with ELISA, which certainly strengthened the reliability of the findings. The study also included a control group of healthy volunteers, as well as advanced statistical methods, particularly ROC analysis, which further strengthened the conclusions. The research particularly with miRNA-122, has opened a new borderline area of research which is, forensic and toxicological research, something the hepatology literature has not focused on. The findings are also unfortunately subject to some important limitations, the most important being the sample size of the study which included only ninety participants. It is a problem of the sample size is very small particularly for preliminary studies as this will compromise the statistical power of the findings and the extent to which the results can be generalized.

Furthermore, the actual sample size was lower than the target; the current study may be underpowered for specific comparisons, and the assessment of diagnostic performance may be more uncertain. As such, external validation remains necessary for larger cohorts.

The fact that the research is located at only one center in Upper Egypt is criticized for its potential to introduce selection bias and regional specificity. Also, the study design is cross-sectional, which does not allow one to make any claims about causation or the temporal development of biomarkers in relation to the progression of the disease. Furthermore, the research did not account for many potential variables, ranging from genetic polymorphisms and environmental toxins (aflatoxins) to basic lifestyle activities. For this reason, longitudinal studies as well as studies conducted across multiple sites will need to be conducted to broaden the scope of the present research.

Abbreviations

CHB: Chronic Hepatitis B

HCC: Hepatocellular carcinoma

HBcrAg: Hepatitis B core-related antigen

miRNA: Micro-ribonucleic acid

M2BPGi: Mac-2 binding protein glycosylation isomer

AFP: Alpha-fetoprotein

AST: Aspartate transaminase

ALT: Alanine transaminase

PCR: Polymerase chain reaction

ELISA: Enzyme-linked immunosorbent assay

ROC: Receiver operating characteristic

AUROC: Area under the ROC curve

PPV: Positive predictive value

NPV: Negative predictive value

cccDNA: Covalently closed circular DNA

Acknowledgments

The authors would like to thank the staff of the Medical Microbiology and Immunology and Tropical Medicine & Gastroenterology Departments at Al-Azhar University, Assiut, for their support during the study.

Authors' Contributors

KMHM, MSH, and MMA designed the study. MSH and AAAO contributed to data collection and laboratory analyses. MSA and AY participated in the statistical analysis and interpretation of data. MABIM and KMHM drafted the manuscript. All authors read and approved the final manuscript.

Data Availability

The data supporting the results of this study are available upon request from the corresponding author.

Funding

No specific funding was received for conducting this study.

Ethics Approval

This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (Males), Al-Azhar University, Assiut, Egypt. The committee operates by the Declaration of Helsinki, the Islamic Organisation for Medical Sciences, the World Health Organisation (WHO), and the International Council for Harmonisation and Good Clinical Practice (ICH-GCP). The approval was granted under protocol number MD/AZ.AST/MIC0071/200/10/2021, on October 12, 2021. Informed consent was obtained from all participants before their inclusion in the study.

Conflict of Interest

The authors declared no conflict of interest.

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