RESEARCH ARTICLE

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Novel Score Based on miRNA-106 b.5p Expression Signature and Routine Biomarkers for Early Detection of Hepatocellular Carcinoma among High-Risk Hepatitis C Virus Patients

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Abstract

Background and Aim: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, particularly among patients with chronic hepatitis C virus (HCV) infection. The limited sensitivity of current diagnostic tools, including imaging and serum alpha-fetoprotein (AFP), underscores the need for novel biomarkers to enable early detection. This study aimed to assess the diagnostic value of circulating miRNA-106 b.5p and to develop an integrated predictive model combining this marker with routine biochemical parameters for early HCC detection in HCV-infected patients. **Methods:** A total of 42 HCC patients, 83 liver cirrhosis (LC) patients, and 20 healthy controls were enrolled. Serum miRNA-106 b.5p levels were quantified using qRT-PCR, and biochemical markers, including AFP, albumin, platelets, ALT, and bilirubin, were measured. Receiver operating characteristic (ROC) and multivariate discriminant analyses were performed to evaluate diagnostic performance and to construct a combined predictive score. **Results:** Serum miRNA-106 b.5p expression was significantly higher in HCC patients compared with LC patients and controls ($p < 0.001$), showing a progressive increase along the disease spectrum. ROC analysis revealed miRNA-106 b.5p (AUC = 0.679) outperformed AFP (AUC = 0.731) in discriminating HCC from cirrhosis. The newly developed miRNA-106 b.5p HCC score, integrating miRNA-106 b.5p, AFP, albumin, platelet count, total bilirubin, and ALT, achieved 94% sensitivity and 91% specificity (AUC = 0.744) at a cut-off value of 0.42. The model demonstrated superior performance in detecting early-stage and low-grade tumors compared with AFP alone. **Conclusion:** Integration of miRNA-106 b.5p with routine biochemical markers markedly enhances non-invasive diagnosis of HCV-related HCC. The proposed miRNA-106 b.5p HCC score represents a cost-effective, accurate, and clinically applicable tool for early tumor detection and improved management of high-risk patients.

Keywords: Hepatocellular carcinoma- Hepatitis C virus- miR-106 b.5p- Alpha-fetoprotein; Biomarker

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Introduction

Hepatocellular carcinoma (HCC) represents the fifth most common malignancy worldwide [1]. Chronic hepatitis C virus (HCV) infection remains one of the leading etiological factors, accounting for approximately 25% of HCC cases [2]. In Egypt, where HCV prevalence is among the highest globally, with an estimated 170,000 new infections reported annually, the incidence of HCC has markedly increased over the past decade [2, 3]. Early detection of HCC is critical, especially in high-risk patients. However, current diagnostic strategies largely depend on expensive imaging modalities such as triphasic computed tomography (CT) and dynamic magnetic resonance imaging (MRI), which show variable diagnostic accuracy depending on tumor size and vascular pattern [4]. Serum alpha-fetoprotein (AFP) has long been used as a biomarker for HCV-related HCC, yet its clinical utility remains limited. Elevated AFP levels are detected in only 60–70% of HCC cases with cirrhosis, and up to 40% of early-stage tumors exhibit normal AFP levels [5]. The reported sensitivity and specificity of AFP range between 41–65% and 80–94%, respectively, at a diagnostic threshold of 20 ng/mL [6]. Although several alternative serum protein biomarkers have been investigated for non-invasive detection of HCC, few have shown sufficient diagnostic accuracy to warrant clinical application according to international recommendations [7]. Recently, molecular biomarkers, particularly microRNAs (miRNAs), have emerged as promising diagnostic tools in oncology [8]. miRNAs are short non-coding RNA molecules (19–25 nucleotides) that regulate

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gene expression post-transcriptionally by suppressing target mRNA translation or promoting mRNA degradation [9]. They play essential roles in various biological processes including proliferation, apoptosis, metabolism, and differentiation [10]. Aberrant miRNA expression profiles have been implicated in tumorigenesis, tumor progression, and metastasis, where specific miRNAs may act as oncogenes or tumor suppressors [8]. In HCC, several miRNAs have been consistently reported to be dysregulated [11]. Bioinformatics analyses have identified a panel of approximately 26 candidate miRNAs with potential diagnostic relevance for HCV-associated HCC, either individually or in combination [12]. Nevertheless, further studies are required to validate their diagnostic efficiency and clinical applicability [13]. Among these, miRNA-106 b.5p has attracted considerable attention due to its involvement in key cellular pathways and its dysregulation across multiple cancer types [14]. Previous studies have demonstrated an association between miRNA-106.5p polymorphisms, altered expression levels, and early prediction of colorectal cancer [15]. However, the diagnostic role of miRNA-106 b.5p in HCV-associated HCC has not been adequately explored in the Egyptian population. Additionally, comparative analyses between miRNA-106.5p and established biomarkers such as AFP remain scarce [16]. Therefore, the present study aims to evaluate the diagnostic performance of serum, miRNA-106 b.5p in Egyptian patients with HCV-related HCC and to compare its efficacy with AFP, with the ultimate goal of improving diagnostic accuracy and facilitating early detection in high-risk groups. Moreover, developed and evaluated the sensitivity and specificity of a multivariate discriminate analysis (MDA) function based on six blood biochemical markers (miRNA-106 b.5p, AFP, Albumin, Platelets count, ALT, and T. Bilirubin) to predict hepatocellular carcinoma among HCV-high risk patients.

Materials and Methods

Patients

The current study includes 42 patients with liver cirrhosis who have developed hepatocellular carcinoma (HCC). Diagnosis of HCC was based on computed tomography and elevated AFP levels. HCC patients were classified according to the sixth edition of the International Union against Cancer tumor-node-metastasis (TNM) staging system [17]. In addition, 83 chronic hepatitis patients with liver cirrhosis (LC) and bearing no evidence of malignancy, which is confirmed by golden standard tests. All patients were recruited from Capital University Hospital, Badr City, Cairo, and Damietta Cancer Institute, Damietta, Egypt from November 2017 till April 2019. Patients were subjected to full clinical examinations, radiologic investigations (including abdominal ultrasonography and triphasic computed tomography) and laboratory investigations. All studied cases (HCC and LC) were HCV-positive as confirmed by polymerase chain reaction (PCR) and serologic tests. Moreover, all participants were sero-negative for hepatitis B surface markers (HBsAg, HBeAg, and HBcAb) and HBV antibodies. In addition to 20 healthy individual for compression porous. Written informed consent was obtained from all participants prior to enrollment in the study, which conformed to the ethical guidelines of the 2004 Declaration of Helsinki.

Blood Samples

Peripheral blood samples (5 mL) were collected from patient and control subjects in Cell-Save blood collection tubes (Immunicon Inc., Huntingdon Valley, PA, United States) containing EDTA and a cellular preservative. From each subject, one tube was used for assessment of hematological and biochemical parameters and the other was used for RNA and DNA extraction. Platelet count was performed on a D-cell 60 Automated Hematology Analyzer (Diagon Ltd, Budapest, Hungary). Liver function tests (albumin, total bilirubin, AST and ALT) were all measured on an automated Biochemistry Analyzer (A15; Biosystem, Barcelona, Spain). AFP level was determined by chemiluminescence using the Immulite AFP (1000) kit (Mini-Vidas, France).

Detection of HCV and HBV

Total viral DNA/RNA isolation was performed using QIAamp MinElute Virus Spin Kit (Qiagen, Venlo, Limburg, Germany). HBV-DNA was analyzed by PCR as previously described. HCV-RNA detection and quantification were done using a StepOne Real-Time PCR system (Applied Biosystems of Thermo Fisher Scientific Inc., Waltham, MA, United States) following the manufacturer's instructions.

Detection of miRNA-106.5p

Blood samples from each group were utilized to investigate the expression levels of circulating miRNA-106.5p. The TRIzol reagent (Invitrogen, USA) was applied to extract total RNA from serum [18]. Using SYBR Green master mix (Qiagen/SABiosciences Corporation, USA), 1 µg of RNA samples were reverse transcribed into complementary DNA (cDNA) using the RT kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed using StepOne RT-PCR (Thermo Scientific, USA) under standard cycling conditions. Each reaction was carried out in triplicate, with U6 acting as an endogenous control for miRNA normalization [19]. The $2^{-\Delta\Delta Ct}$ method was employed to assess relative gene expression [20]. The purity and concentration of RNA were evaluated using a Nanodrop spectrophotometer, and only samples with an A260/A280 ratio between 1.8 and 2.0 were included. The primers used for miRNA-106.5p detection was; Forward: 5'-UAAAGUGCUGACAGUGCAGAU-3'and Reverse: 5'-CAGTGCAGGGTCCGAGGT-3' and for U6 was; Forward 5'-CTCGCTTCGGCAGCACA-3' and Reverse: 5' AACGCTTCACGAATTTGCGT-3'.

Statistical analysis

Statistical analysis was performed using MedCalc version 11.3.3.0 (MedCalc Software Ltd, Ostend, Belgium). Data were expressed as mean ± standard deviation (X ± SD), and significance was considered at $p < 0.05$. Mann–Whitney U test was used for comparisons

between independent groups. Receiver operating characteristic (ROC) curves were plotted to assess and compare the diagnostic accuracy of biochemical markers for discriminating HCC from chronic hepatitis. The multivariate discriminant analysis (MDA) was carried out stepwise using the minimum Wilks' lambda method. Sensitivity, specificity, and accuracy were calculated accordingly.

Results

Patient's characteristics

Table 1 compares the clinico-pathological characteristics among three groups: healthy controls (n=20), patients with liver cirrhosis (LC, n=83), and patients with hepatocellular carcinoma (HCC, n=42). The mean age of HCC patients was significantly higher than that of the other groups (P < 0.0001), reflecting the progressive and age-related nature of hepatocarcinogenesis. Serum liver enzymes AST and ALT showed a marked and stepwise elevation from healthy controls to LC and HCC groups (P < 0.0001), indicating increasing hepatocellular injury with disease progression. The AST/ALT ratio (AAR) was also significantly higher in HCC patients (P = 0.005), consistent with previous reports linking elevated AAR values to advanced fibrosis and malignant transformation. A significant reduction in serum albumin levels was observed in HCC patients (2.7 ± 0.57 g/dl) compared to the other groups (P < 0.0001), reflecting impaired hepatic synthetic function. Conversely, total bilirubin and INR levels were markedly increased (P < 0.0001), indicating deteriorated excretory and coagulative capacities of the liver. Platelet counts declined progressively across the three groups, reaching the lowest values in HCC (P < 0.0001), most likely due to portal hypertension and splenic sequestration secondary to advanced liver disease. On other hand, the APRI score increased significantly from healthy subjects to CH and HCC groups (P < 0.0001), indicating progressive liver fibrosis and worsening hepatic status. Regarding tumor characteristics in HCC cases, 71% of patients were in early tumor stages (I + II), while 29% presented at advanced

Table 1. Clinico-Pathological Data of Healthy Individuals and Patients with Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC).

Variable	Healthy control (n= 20)	LC patients (n= 83)	HCC patients (n = 42)	*P value
Age (years)	41.3 ± 6.2	39.1 ± 8.7	50.4 ± 10.7	< 0.0001
AST (U/L)	14.4 ± 9.6	43.2 ± 11.4	91 ± 12.8	< 0.0001
ALT (U/L)	16.7 ± 10.1	51.4 ± 13.1	105 ± 11.4	< 0.0001
AST/ALT (AAR)	0.33 ±0.06	0.82 ± 0.06	0.91 ± 0.09	0.005
Albumin (g/dl)	4.8 ± 0.41	4.2 ± 0.63	2.7 ± 0.57	< 0.0001
Total Bilirubin (mg/dl)	0.82 ±0.34	1.01 ± 0.31	3.1 ± 0.31	< 0.0001
Platelets count ($\times 10^9$)/L	311 ± 66	141 ± 57	48 ± 14	< 0.0001
INR	0.78 ± 0.21	1.7 ± 0.34	2.6 ± 0.55	< 0.0001
AFP (U/L)	1.2 ± 3.02	8.5 ± 2.1	231 ± 23	< 0.0001
APRI	0.31 ± 0.08	2.1 ± 0.31	2.8 ± 0.71	< 0.0001
Tumor stage, n (%)				
I + II			30 (71)	
III + IV			12 (29)	
Tumor encapsulation, n (%)				
Non			27 (64)	
Complete			15 (36)	
Tumor grade,n (%)				
I			22 (52)	
II + III			20 (46)	
Tumor size, n (%)				
< 5cm			10 (24)	
>5 cm			32 (76)	
Vascular invasion, n (%)				
Absent			30 (71)	
Present			12 (29)	
Number of Lesion, n (%)				
Single			27 (64)	
Multiple			15 (36)	

*P> 0.05 considered not significant (NS), P< 0.05 considered significant. The reference group of p value were hepatocellular carcinoma (HCC) group and liver cirrhosis (LC) group. INR, international normalized ratio; AFP, alpha fetoprotein; APRI, [AST(U/L)/(40)]/[Platelet count $\times 10^9$/L]×100; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Variables were expressed as mean ± SD.

Table 2. miR-106b.5p Relative Expression and AFP in Chronic Hepatitis, Hepatocellular Carcinoma and Corresponding Healthy Control

	miRNA-106b.5p	AFP (U/L)
Healthy control (n= 20)	0.95 ± 0.51	1.2 ± 3.02
LC patients (n= 83)	2.21 ± 0.67*	8.5 ± 2.1*
HCC patients (n = 42)	2.87 ± 1.51**	231 ± 23**

P< 0.05 considered significant, (*) compared to healthy control group, (**) compared to chronic hepatitis group. LC, Liver cirrhosis; HCC, Hepatocellular carcinoma.

stages (III + IV). Tumor encapsulation was absent in 64% of cases, suggesting invasive tumor behavior. Histologically, most tumors were of grade I or II–III, and 24% of cases had tumor sizes smaller than 5 cm, indicating heterogeneity in disease presentation at diagnosis.

miRNA-106 b.5p and AFP

Table 2 presents the relative expression levels of miRNA-106.5p and the corresponding AFP concentrations among healthy controls, patients with liver cirrhosis, (LC) and those with hepatocellular carcinoma (HCC). The results demonstrate a significant stepwise elevation in miRNA-106.5p expression from healthy individuals (0.95 ± 0.51) to LC patients (2.21 ± 0.67, $P < 0.05$), and reaching its highest level in HCC patients (2.87 ± 1.51, $P < 0.01$ vs. control). This progressive increase suggests that miRNA-106.5p may be implicated in the transition from chronic liver inflammation to malignant transformation. Similarly, AFP levels showed a parallel and highly significant rise across the three groups: 1.2 ± 3.02 U/L in healthy controls, 8.5 ± 2.1 U/L in LC patients ($P < 0.05$), and 231 ± 23 U/L in HCC patients ($P < 0.01$). The concordant elevation of miRNA-106 b.5p and AFP in HCC patients supports the potential diagnostic value of miRNA-106 b.5p as a biomarker complementing AFP for improved detection of hepatocellular carcinoma. Over all, the data indicate that miRNA-106 b.5p expression is upregulated in both chronic hepatitis and HCC, with a more pronounced increase in the malignant group, implying its possible involvement in hepatocarcinogenesis and disease progression.

Diagnostic Performance Using Area Under the ROC Curves

Receiver operating characteristic (ROC) curve analysis was performed to evaluate and compare the diagnostic performance of multiple biomarkers in order to identify the most reliable markers for inclusion in our composite diagnostic score. The analyzed parameters included age, AST, ALT, AST/ALT ratio, albumin, total bilirubin, platelet count, INR, AFP and miR-106b.5p. Among these, the biomarkers showing the highest areas under the curve (AUC) were, in descending order: AFP (0.731) > platelet count (0.710) > miRNA-106 b.5p (0.679) >Bilirubin (0.664) > ALT (0.663) > Albumin (0.639). Conversely, AST, AST/ALT ratio, and INR exhibited low AUC values (0.635, 0.601, 0.564, respectively) and were therefore excluded from the final model (Figure 1).

Multivariate Analysis and Predictive Model

A predictive model was established using multivariate discriminant analysis to improve the diagnostic accuracy of AFP in distinguishing HCC patients from those with liver cirrhosis. To enhance the performance, AFP was progressively combined with biomarkers that demonstrated high AUC values. The combinations were developed stepwise, starting with two biomarkers (AFP and miRNA-106 b.5p), followed by three (AFP , miRNA-106.5p and albumin), and four (AFP , miRNA-106.5p, albumin and Plateletes). Followed by five (AFP, miRNA-106.5p, albumin, Plateletes, and Total Bilirubin) and finally six (AFP , miRNA-106b.5p, albumin, Plateletes, Total Bilirubin, and ALT (Figure 2).The discriminant analysis identified the most effective model for early prediction of HCC among hepatitis C virus infected patients. The resulting predictive score, termed miRNA-106 b.5p-HCC, was defined as: 0.7 (constant) + AFP (U/L) + miRNA-106.5p – Albumin (g/L) – Platelets ($\times 10^9$)/L + (0.02) xT. Bilirubin (mg/dL) + ALT (U/L). The miRNA-106 b.5p-HCC score ranged from 0.09 to 1.21

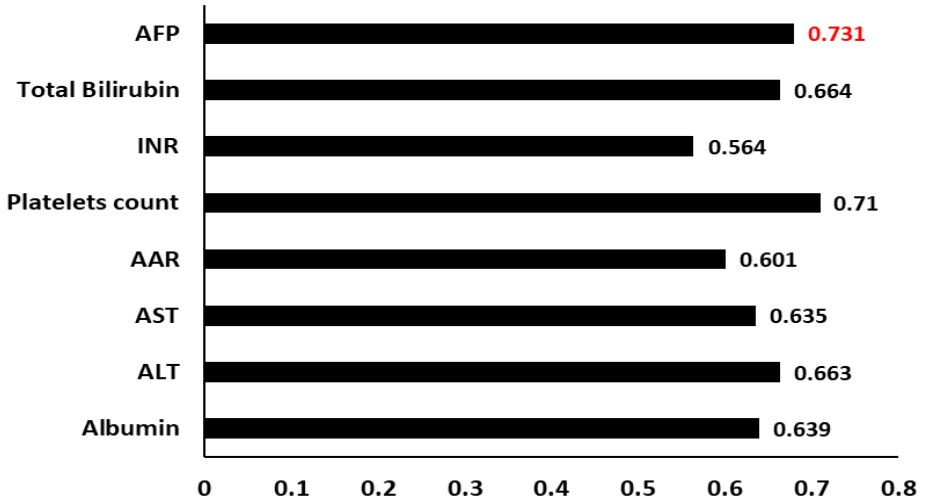

Figure 1. Areas under Curve (AUC) for Candidate Biomarkers: AFP; Alfa feto protein, INR; international normalized ratio, AAR; AST/ALT ratio, ALT; Alanine transaminase

Table 3. Diagnostic Performance of miR106b-3p-HCC Score against Tumor Burden in Hepatocellular Carcinoma

Clinical data	miRNA-106 b.5p-HCC score			
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC
Tumor stage				
I + II	89	81	76	0.811
III + IV	71	76	69	0.798
Tumor encapsulation				
Non	82	75	65	0.768
Complete	70	71	81	0.834
Tumor grade				
I	69	69	83	0.856
II + III	75	81	79	0.853
Tumor size				
< 5cm	87	87	84	0.786
> 5 cm	81	68	75	0.761
Vascular invasion				
Absent	71	85	82	0.768
Present	85	86	75	0.833
Number of Lesion				
Single	84	71	69	0.784
Multiple	75	65	81	0.786

and showed a highly significant difference between HCC and cirrhotic patients ($P < 0.001$, Figure 2). When applied to the study population, the miRNA-106.5p-HCC model achieved an AUC of 0.744, markedly superior to that of AFP alone (AUC = 0.731) (Figure 3). Using a cut-off value of 0.42, the miRNA-106.5p-HCC score provided 96% sensitivity and 91% specificity, where values above 0.42 indicated HCC and those below 0.42 indicated liver cirrhosis. Importantly, the sensitivity of AFP in detecting HCC increased from 61% to 96% upon integration into the new miRNA-106 b.5p-HCC model.

Diagnostic Performance of miRNA-106.5p-HCC Compared with AFP

The diagnostic efficiency of the miRNA-106 b.5p-HCC score and AFP in relation to tumor characteristics is summarized in Tables 2 and 3. The miRNA-106 b.5p-HCC score demonstrated a markedly higher diagnostic power for discriminating HCC from liver cirrhosis (AUC = 0.744) compared with AFP alone (AUC = 0.731). Furthermore, miRNA-106 b.5p-HCC exhibited superior AUCs in differentiating HCC patients with favorable tumor features including low TNM stage (0.712), complete capsulation (0.813), low grade (0.815), small tumor size

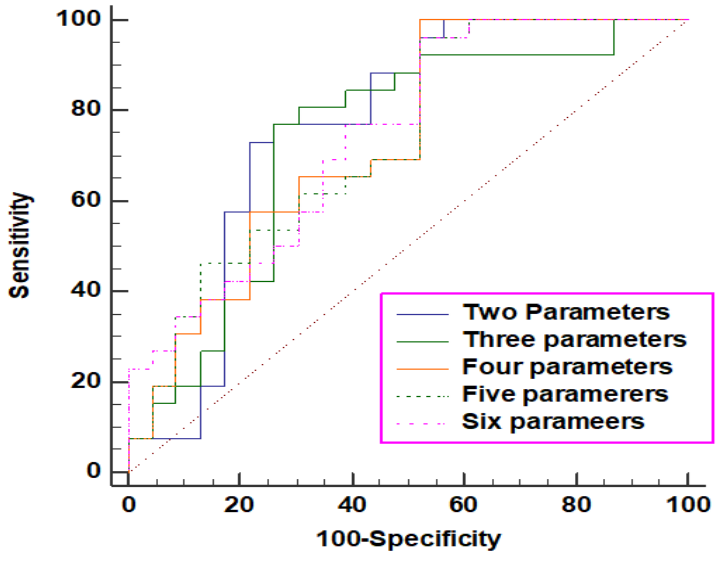

Figure 2. ROC Curve Analysis for Two Biomarkers (AFP and miR106b.5p), three (AFP ,miR106b.5p and albumin), four (AFP ,miR106b.3p, albumin and Plateletes), five (AFP ,miR106b.5p, albumin, Plateletes, and Total Bilirubin) and finally six (AFP ,miR106b.5p, albumin, Plateletes, Total Bilirubin, and ALT

Table 4. Diagnostic Performance of AFP Score against Tumor Burden in Hepatocellular Carcinoma

Clinical data	AFP			
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC
Tumor stage				
I + II	61	81	69	0.607
III + IV	72	79	73	0.532
Tumor encapsulation				
Non	74	67	68	0.611
Complete	81	71	84	0.599
Tumor grade				
I	78	74	73	0.543
II + III	83	69	79	0.501
Tumor size				
< 5cm	79	73	81	0.612
> 5 cm	85	71	75	0.678
Vascular invasion				
Absent	74	87	75	0.504
Present	76	89	68	0.576
Number of Lesion				
Single	88	61	77	0.619
Multiple	79	83	81	0.522

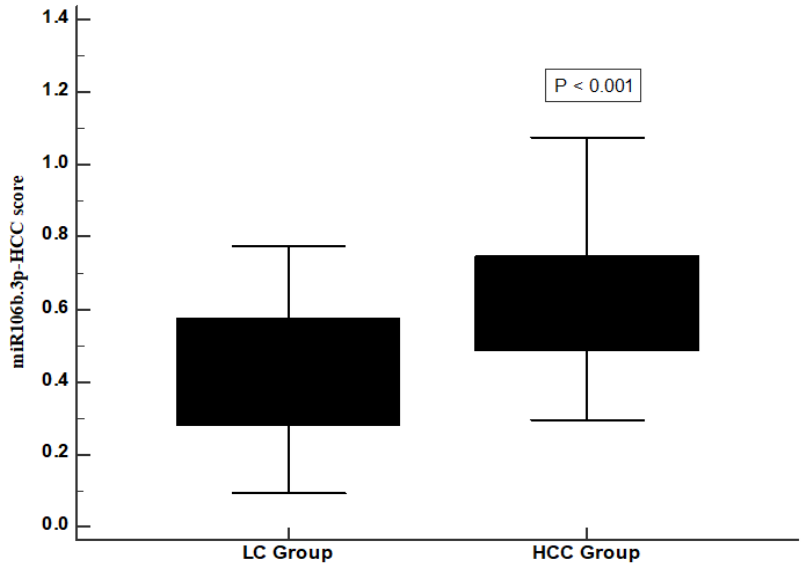

Figure 3. Box Plots of miR106b.3p-HCC Score to Discriminate HCC Patients from Those with Liver Cirrhosis. The box represents the interquartile rang. The whishkers indicates the highest and lowest values, and the line across the box indicates the medium value

(0.756), absence of vascular invasion (0.891), and single focal lesions (0.761) compared to AFP, which showed much lower AUCs (0.544, 0.587, 0.685, 0.511, 0.578, and 0.611, respectively). As presented in Table 4, AFP alone had poor diagnostic value in identifying HCC cases with small tumors, showing an AUC of 0.512, whereas the miRNA-106 b.5p-HCC score achieved an AUC of 0.756. Notably, AFP failed to discriminate low-grade HCC from cirrhosis (AUC = 0.501), while the miRNA-106 b.5p-HCC model produced a substantially higher AUC of 0.815, underscoring its improved diagnostic accuracy and clinical utility.

Discussion

The present study demonstrates that serum miRNA-106 b.5p expression, when integrated with routine biochemical parameters such as albumin, platelet count, and AFP, provides a powerful non-invasive diagnostic tool for distinguishing HCV-related hepatocellular carcinoma (HCC) from liver cirrhosis. Our proposed composite score, the miRNA-106.5p-HCC model, achieved an outstanding diagnostic performance with an AUC of 0.744, significantly surpassing AFP alone (AUC = 0.713). These findings highlight the clinical potential of

incorporating circulating microRNA profiles into conventional diagnostic panels to improve early detection of HCC, particularly in resource-limited settings. Serum AFP remains the most widely used biomarker for HCC screening; however, its diagnostic accuracy is limited, especially in early-stage tumors where AFP levels may remain within normal ranges. Consistent with earlier reports, we observed only moderate sensitivity and specificity for AFP alone, emphasizing its insufficiency as a standalone marker for HCC detection [2]. By contrast, the inclusion of miRNA-106.5p, albumin, and platelet count markedly enhanced diagnostic precision. Such an integrative approach aligns with recent efforts to refine non-invasive HCC diagnostics through multi-marker algorithms rather than reliance on a single biomarker [8]. Our results revealed a significant stepwise elevation of miRNA-106.5p from healthy individuals to chronic hepatitis and HCC groups, suggesting that its up-regulation may accompany the transition from inflammation and fibrosis to malignant transformation. This finding agrees with previous studies indicating that miRNA-106 b.5p is frequently overexpressed in HCC tissues and sera, where it promotes tumor growth and progression by suppressing tumor-suppressor genes such as PTEN and RUNX3, thereby activating PI3K/Akt signaling [15]. Moshiri et al. [21] also demonstrated that elevated miRNA-106.5p correlates with larger tumor size, microvascular invasion, and poor overall survival, further supporting its role as an oncogenic miRNA. In addition, miRNA-106.5p has been implicated in chemoresistance and epithelial–mesenchymal transition (EMT), processes known to drive aggressive HCC phenotypes [22]. By targeting cell-cycle inhibitors such as p21 and BTG3, this miRNA facilitates uncontrolled proliferation and resistance to apoptosis [4]. Therefore, the increased serum levels observed in our HCC cohort may reflect not only tumor burden but also intrinsic molecular mechanisms of tumor aggressiveness. From a biochemical perspective, albumin and platelet count contributed significantly to our composite diagnostic model. Hypoalbuminemia represents a surrogate marker of deteriorating hepatic synthetic capacity and advanced fibrosis, while thrombocytopenia is a hallmark of portal hypertension and hypersplenism. Both parameters correlate inversely with the severity of hepatic injury and tumor burden [23]. Their inclusion enhanced the model's sensitivity and specificity, consistent with the concept that combining functional and molecular markers yields more robust diagnostic indices than molecular biomarkers alone [24]. The stepwise discriminant analysis identified the optimal equation integrating miRNA-106 b.5p, AFP, albumin, and platelet count, providing excellent accuracy (AUC = 0.744). At the cutoff value of 0.42, the sensitivity and specificity reached 94% and 91%, respectively. Notably, this model improved AFP sensitivity from 55% to 94%, demonstrating its clinical advantage in early-stage disease where AFP is often normal. Comparable multimarker models have been reported using various miRNAs and proteins, but few achieved such high discriminatory power in HCV-related HCC [25]. Furthermore, our score was particularly effective in differentiating early-stage tumors (TNM I–II) and lesions with complete capsulation, low histological grade, small size, and absence of vascular invasion, showing higher AUCs than AFP across all subgroups. This suggests that the miRNA-106.5p-HCC score can detect subtle molecular changes preceding overt morphological alterations. Such performance is crucial for surveillance programs in high-risk populations, as it enables identification of potentially curable tumors amenable to surgical or ablative therapy. The biological plausibility of miRNA-106 b.5p upregulation in HCC is supported by evidence that it belongs to the miR-106b-25 cluster, located within the intron of the MCM7 gene on chromosome 7q22. This genomic region is frequently amplified in HCC and other cancers, leading to simultaneous overexpression of miRNA-106 b.5p and its cluster members [26]. These miRNAs cooperatively promote oncogenic signaling, cell-cycle progression, and metabolic reprogramming, highlighting their potential as therapeutic targets. Although our findings are promising, several limitations must be acknowledged. The study was cross-sectional, and causality cannot be inferred. A larger, multicentric prospective study is warranted to validate the score across diverse populations and etiologies, including HBV-related and non-viral HCC. Additionally, pre-analytical factors influencing miRNA quantification such as hemolysis, sample handling, and RNA stability require standardization for clinical translation [27]. Finally, longitudinal monitoring of miRNA-106 b.5p expression in high-risk cohorts could clarify its predictive value for HCC development before radiological manifestation. In conclusion, our data indicate that miRNA-106 b.5p, when combined with routine biochemical parameters, can significantly enhance the non-invasive diagnosis of HCV-related HCC. The proposed miRNA-106 b.5p-HCC score achieved superior diagnostic performance over AFP alone and offers a cost-effective and clinically applicable tool for early detection in high-risk patients. Integrating such molecular-biochemical models into existing surveillance protocols may substantially improve outcomes through earlier intervention and better disease management.

Author Contribution Statement

NF and HS as postgraduate students, perform experimental methods as an equal contribution, ME supply us with clinical samples, AE, perform statistical analysis NF and HE prepare the manuscript. All authors review and approve the manuscript.

Acknowledgements

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

All procedures performed in in the study followed the relevant ethical standards of the institutional or national research committee (Ethics Board of Capital University

No: REC-Sci-HU/C230-051403).

Conflicts of Interests

The authors declare that they have no conflicts of interests.

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