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Hepatocellular carcinoma associated with the Hepatitis B virus and microRNA-155 expression

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ABSTRACT

MicroRNA-155 (miR-155), identified as an oncogenic regulator in certain malignancies, is linked to tumor progression and aggressiveness. This cross-sectional observational study investigated miR-155 expression levels in plasma samples from hepatocellular carcinoma (HCC) patients with and without concurrent hepatitis B virus (HBV) infection. A cohort of 51 patients (aged 18–65 years) with histologically confirmed HCC was analyzed, stratified by HBV serostatus. Results demonstrated a 2.55-fold elevation in miR-155 expression in HBV-positive HCC patients compared to HBV-negative counterparts ($p \leq 0.001$). These findings suggest miR-155 may serve as a prognostic biomarker and therapeutic target in HBV-associated HCC. Mechanistically, miR-155 exhibits oncogenic activity by suppressing tumor suppressor pathways. The HBV-encoded HBx protein is implicated in upregulating miR-155, potentially driving its sustained expression during hepatocarcinogenesis. Additionally, biochemical analyses revealed significantly elevated levels of hepatic injury markers including GPT, GOT, ALP, and indirect bilirubin in HBV-infected patients relative to healthy controls ($p \leq 0.001$), further underscoring the interplay between viral infection, hepatic dysfunction, and miR-155 dysregulation.

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Introduction

Hepatitis B virus (HBV) is a DNA virus that infects both animals and birds. Epidemiological studies have found a clear association among chronic HBV infection and the development or occurrence of hepatocellular carcinoma (HCC).

Cirrhosis caused by persistent HBV infection can alter the assembly and function of liver cells. Liver cancer may occur if gambling therapy is not received (Han et al., 2012). Multiple mechanisms have been identified to contribute to this phenomenon, including the buildup of hereditary alterations due to immune-mediated hepatitis, escalation of oxidative stress, and various viral apparatuses that link viral proteins like HBx and HBs.

These viral proteins enhance mutagenesis, which modifies the endogenous gene expression or leads to chromosomal instability by assimilating HBV DNA into the host genome. Additionally, they affect genome methylation, leading to epigenetic changes governed by miRNAs (Tarocchi et al., 2014). HCC is a leading malignant liver tumor that poses a significant threat to global health. HCC is a type of liver cancer that poses significant challenges for early detection and is associated with a declining survival rate., represents approximately 70–80% of liver cancer cases. HCC is classified as seventh

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in women and sixth in malignant cancer, which frequently affects males. The mortality rate for male patients is the second highest, while that for female patients was the sixth highest. Approximately ¾ of all instances of HBV infection arise in Asian nations due to the elevated prevalence of chronic infections (Mohsen et al., 2025; Torresi et al., 2019).

Vagaries in essential gene guidelines that control cellular processes are said to occur as HCC develops. Numerous studies have focused on the proteins and inheritable variables that support the growth of HCC. miRNAs are crucial regulators of protein expression. These small non-coding RNAs, typically comprising approximately 22 nucleotides, are frequently involved in carcinogenesis because of their capacity to modulate the production of essential proteins complicated in cancer-related pathways (Mohsen, et al., 2024). As vital post-transcriptional regulators, miRNAs play a significant role in gene expression. miRNAs interact with the three untranslated regions (UTRs) of mRNAs (Cao et al., 2018; Cione et al., 2022).

B cells, T cells, monocytes, and macrophages all express microRNA-155, which is involved in the processing of B cell integration clusters (BIC). In chromosome 21q21, the BIC gene is located. Several hematological and solid cancers frequently express microRNA-155. Since microRNA-155 has been consistently associated with the expansion of leukemia, lung cancer, and gastric cancer, it is often utilized as a focal point in evaluation, prognosis, and therapy (Hussein & Mohsen, 2024; Khudhair et al., 2023).

MicroRNA-155 is recognized as an oncomir because of its augmented production in tumor cells and because it targets numerous cancer suppressor genetic factors in HCC, according to previous research. According to (Han et al., 2012), the levels of microRNA-155 are 1.5–6 times greater in HCC tissue than in typical tissue of liver. A study found that microRNA-155 was positively associated with HCC cell invasion and was significantly upregulated in HCC tissues(Guan et al., 2016). Additionally, some studies have suggested that higher levels of microRNA-155 in HCC contributes to cancer development by promoting cell division, influencing tumors stem cells, and aiding the continual proliferation of HCC cells (. Hussein & Mohsen, 2018).

The fact that several routes contribute to HCC cell development must be clarified through regular education. One of the main reasons for this is hepatocarcinogenesis, caused by prolonged HBV infection. Additionally, microRNAs, particularly microRNA-155, plays a crucial regulatory role in hepatocarcinogenesis. Therefore, the purpose of this study was to examine microRNA-155 expression in individuals with HCC who also had HBV infection.

Materials and Methods

Patients and samples

This analytical cross-sectional study used 51 blood plasma samples from individuals diagnosed with HCC. The participants were recruited from standard hospitals. Eligibility criteria required participants to be aged between 18 and 65 years, have a primary HCC diagnosis verified through histological examination, and successfully complete two imaging assessments. Individuals with malignancies in other organs, serious infectious diseases, or cardiovascular conditions were excluded. Prior to study initiation, all eligible participants reviewed and signed an informed consent form.

MicroRNA Analysis

RNA was isolated by centrifuging 6 ml of venous blood treated with EDTA at 15,000 × g for 10 min. was used to remotely measure total RNA. Total RNA specific to miRNA was obtained using the miRCURY RNA Isolation Kit (Biological Fluids). Then, microRNA-specific cDNA was created using a PCR warmer (Biorad C 1000) and the Universal cDNA Synthesis Kit II, which is adequate for 8–64 processes. miRNA qRT-PCR performed using a Bio-Rad CFX 96 system.

Data analysis and statistics

To evaluate the expression of miR 155-5p and compute the tender curvature, melt peak curve, and Cq, Bio-Rad CFX Manager 96 software was utilized. miRNA expression was compared between HBV-related HCC patients and HBV-deprived HCC patients using the the Mann-Whitney U test; statistical significance was defined as a p-value lower than 0.05.

Results

Up-regulation of MicroRNA-155 in HBV-related HCC

This research examined the level of expression of MicroRNA-155 in blood plasma specimens from 51 individuals diagnosed with HCC. Among these, 20 individuals were infected with HBV, as indicated by positive HBsAg results, whereas 11 patients tested negative for HBsAg. Notably, 80% (24 of 30) of the blood plasma samples associated with HBV and HCC exhibited elevated levels of microRNA-155 expression. In contrast, only 23% (5 of 21) of HCC patients without HBV infection demonstrated an increase in microRNA-155 levels, show figure 1

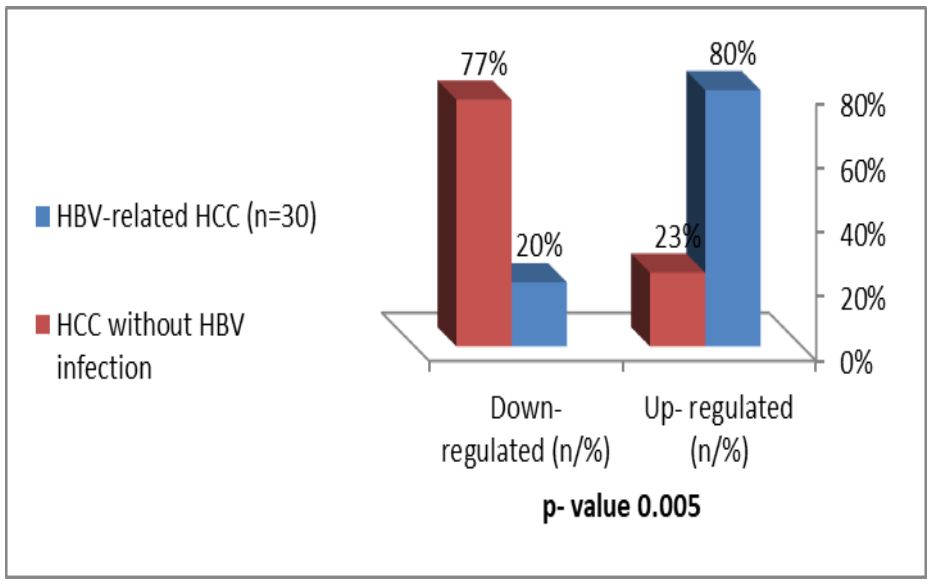

Fig 1. Quantitative analysis of microRNA-155 expression in HCC patients with and without HBV infection.

The level of microRNA-155 was 2.33 times greater in those with HBV-associated liver cancer than in research participants without HBV infection, according to the Livak approach. Additionally, the Mann-Whitney test revealed that persons with HBV-associated liver cancer had significantly higher levels of microRNA-155 in their blood plasma than those who were not infected (p=0.005) with the virus. These results suggest that microRNA-155 might donate to the expansion of liver cancer in patients with HBV-associated liver cancer.

Biochemistry Parameters in Patients with HCC

The distributions of the normal (1) and aberrant (24) indirect bilirubin levels in patients with HCC are shown in Table (1). P=0.003 indicates that these differences were statistically significant. There was also a statistically significant relationship between the percentages of normal and abnormal creatinine and GOT levels. The biochemical parameters in HBV patients had greater levels of GPT, GOT, Alkaline Phosphatase (ALP), and Indirect Bilirubin than normal individuals, as shown in Table (2). All these levels had significant values (p≤0.001).

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Table1 Correlation analysis of biochemical variables in HCC.

Biochemical test	Normal	Abnormal	Total	P value
Blood Sugar	17	8	25	0.7222
Urea	21	4	25	0.58
Creatinine	14	11	25	0.0333*
GPT (ALT)	21	4	25	0.9
GOT(AST)	15	10	25	0.0399*
Alkaline Phosphatase	20	5	25	0.9
Total Bilirubin	18	7	25	0.8
Indirect Bilirubin	1	24	25	0.003***

AST is both normal (3) and abnormal (7), whereas GPT (ALT), ALP, and Indirect Bilirubin are both normal (2) and abnormal (8).

However, the other elements showed no discernible correlations with each other. Each of these tests measured the liver test function.

Table 2 Relationship between biochemical parameters and HBV patients

Biochemical test	Normal	Abnormal	Total	P value
Blood Sugar	8(10)	2(10)	10	0.0888
Urea	7(10)	3(10)	10	0.077
Creatinine	3(10)	7(10)	10	0.05*
GPT (ALT)	2(10)	8(10)	10	0.0021***
GOT(AST)	3(10)	7(10)	10	0.0033**
Alkaline Phosphatase	2(10)	8(10)	10	0.0022***
Total Bilirubin	6(10)	4(10)	10	0.7
Indirect Bilirubin	3(10)	7(10)	10	0.0023***

Examination of the biochemistry of HCV patients revealed that GOT, Total Bilirubin, and Indirect Bilirubin were all greater in HCV patients, according to Table (3). An abnormal (90%) to normal ratio (10.00%) and a corresponding significant P value of (P=0.002) were observed. The normal proportion of GPT was 30.00%, while the abnormal proportion was 70.00%. The normal proportion of ALP was 30.00%; however, the pathological number was 70.00%. There was no significant correlation observed between the two measurements.

Table 3 The association coefficient among biochemistry variables associated with HCV

Biochemical test	Normal	Abnormal	Total	P value
Blood Sugar	8(10)	2(10)	10	0.9
Urea	9(10)	1(10)	10	0.9
Creatinine	3(10)	7(10)	10	0.02*
GPT (ALT)	4(10)	6(10)	10	0.009**
GOT(AST)	2(10)	8(10)	10	0.0013***
Alkaline Phosphatase	3(10)	7(10)	10	0.004**
Total Bilirubin	1(10)	90(10)	10	0.002***
Indirect Bilirubin	0(10)	10(10)	10	0.001***

However, no significant correlation was observed with any other component. Our study supports the findings of Mikamori et a(2017)., who reported that blood bilirubin levels (total, direct, and indirect) The presence of amyloid particles is higher in males with HCC than in females. Their research suggested that these tests may help identify the location of liver damage and, based on the observed patterns, assist in formulating a differential diagnosis. Elevated ALT and AST levels that did not correspond to Increases in bilirubin and ALP levels indicate a hepatocellular disease. In contrast, a cholestasis pattern is marked by increased bilirubin and ALP levels in comparison to ALT and AST levels. When comparing the HCV positive group to the control group, the serum ALT and AST levels in the HCV positive group were found to be statistically significantly higher (P<0.001).

Nevertheless, the prothrombin level was markedly reduced in the HC +ve group (P=0.001). One important measure of liver health is the amount of clotting factor produced.

Discussion

HCC expansion is a multifaceted procedure that is influenced by various factors. Recent research has increasingly concentrated on molecular alterations in cell signaling and genomic variations to elucidate the fundamental causes of this malignancy. To date, only a limited amount of training has highlighted the involvement of microRNA-155 in viral infections. This study aimed to assess whether the adjacent non-tumor tissues of patients positive for HBV exhibited higher levels of microRNA-155 expression compared to those who were negative for HBV(Ekiz et al., 2019). This finding is primarily attributed to the sustained presence of HBx proteins during infection with HBV. Certain research has indicated that HBx induces the overexpression of microRNA-155 (Mohsen et al., 2019). Su et al. found expression to be a critical post-transcriptional

regulator of the development of HBV-related HCC, which was consistent with the current findings. Numerous studies have connected these miRNAs to immunity and inflammatory conditions associated with the immune system(Ambros, 2004).

Furthermore, microRNA-155 has been implicated in the pathophysiology of several cancers, including breast and pancreatic cancers (Wang et al., 2009), colon cancer (Cao et al., 2018; Mikamori et al., 2017), and other cancers. Furthermore, Wang et al. found that microRNA-155 was markedly overexpressed (Xie et al., 2012). By targeting several suppressor tumors, up-regulation of microRNA-155 encourages the development of HCC carcinogenesis. It is commonly recognized that elevated microRNA-155 regulation inhibits the production of the SOX6 transcription factor, which is a member of the SOX family, hence reducing the propagation of carcinoma cells.

MicroRNA-155 affects the cell cycle and apoptosis by enhancing the stability of AT-rich interaction domain 2 (ARID2), which is linked to the activation of the Akt signaling pathway. The development of cancerous tumors is accelerated by these factors. Furthermore, studies indicate that microRNA-155 can initiate HCC by influencing the transcriptional regulator sex-determining region Y box 6 (SOX6), which, in turn, activates p21 in a p53-dependent manner to inhibit cell proliferation(Hassan et al., 2024 ;Mohsen et al., 2020 Mahmoud;et al.,2020).Analysis of plasma samples revealed elevated miRNA expression levels in patients diagnosed with HBV-associated HCC relative to those with HBV-negative HCC cases. Notably, microRNA-155 expression was markedly upregulated in HBV-positive HCC patients compared to uninfected controls. Mechanistic investigations indicate that the HBx viral protein promotes microRNA-155 upregulation in HBV-associated HCC, subsequently suppressing suppressor of cytokine signaling-1 (SOCS1) expression (Wang et al., 2009). Therapeutic strategies targeting HBV infection prevention appear to involve augmentation of IFN signaling pathways and activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) cascade (Mohsen et al., 2019).

Alternatively, interaction with the CCAAT/enhancer-binding protein (C/EBP) facilitates transcriptional activation of the HBV enhancer II/core promoter (EnhII/Cp), HBx increases microRNA-155 levels, which can prevent HBV replication (Xie et al., 2012). Directing the action of phosphatase and tensin homolog 3 UTR (PTEN) may additionally inhibit HCC cells from experiencing apoptosis (Fu et al., 2017; Mohsen, 2020; Su et al., 2011). According to Fu et al. (2017), HBx dysregulation enhances the production of microRNA-155, thereby facilitating cell invasion, migration, and proliferation (Fu et al., 2017).

Ormancey et al. (2023) indicated that, microRNA-155 is known to improve antiviral immunity against HBV . As stated by Wang et al. (2009), extended exposure to inflammatory responses may worsen liver damage and HCC cell growth, because it increases the production of microRNA-155 (Wang et al., 2009). Regulation of the immune response is affected by the abnormal synthesis of microRNA-155 during HBV infection, which may result in immune-mediated liver damage and, eventually, HCC(Mohsen et al., 2022).

However, there were no discernible correlations with any of the other components. The present study corroborates the findings of Liu et al (2016), who demonstrated elevated serum bilirubin and amyloid concentrations in male patients with HCC compared to females (Liu et al., 2016). Their research suggested that these biomarkers may assist in localizing hepatic injury and facilitate differential diagnosis based on distinct biomarker patterns. Hepatocellular pathology is characterized by disproportionate elevations in ALT and AST levels without concurrent increases in bilirubin and ALP levels. Conversely, concurrent proportional increases in bilirubin and ALP levels relative to transaminase levels characterize a cholestatic pattern (Mohsen et al., 2024). These findings underscore the diagnostic utility of biomarker profiling for distinguishing hepatic disease etiology.

Conclusion

The current investigation revealed markedly increased microRNA-155 expression in patients diagnosed with HBV-related HCC. As a well-characterized oncogenic miRNA, microRNA-155 facilitates carcinogenesis via simultaneous downregulation of tumor suppressor pathways. Mechanistic analyses identified HBV-encoded HBx protein as a critical regulator of microRNA-155 overexpression and sustained activity during hepatic malignant transformation. These findings emphasize the need to elucidate the precise molecular interactions involved in HBV-driven HCC pathogenesis, which may inform the rational development of miRNA-directed therapeutic interventions focusing on microRNA-155.

The contribution of the authors

Each author played an equal role in the experimental design, data acquisition and interpretation, as well as in the preparation of the manuscript.

Ethics statement

The scientific research Ethics Board of the University of Anbar approved this investigation, Ministry of Higher Education, and Scientific Research (No. 233, 6/3/2023). All experimental protocols involving human subjects adhered to institutional regulatory standards with rigorously maintained participant anonymity. No identifiable patient data or imagery were used.

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