



The Role of p53 in Hepatocellular Carcinoma: Implications for Chronic Hepatitis B Virus Infection

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Abstract

Background: Hepatocellular carcinoma (HCC) remains a major global health challenge, with chronic HBV infection accounting for a substantial proportion of cases, particularly in regions where HBV prevalence is high. **Aim:** This study aimed to assess the relationship between the levels of p53 and the risk of viral hepatitis B-related HCC. **Methods:** The study included 120 samples clustered into three groups. Patients with viral hepatitis B-derived liver cancer (40) and chronic viral hepatitis (40) and 40 healthy controls (HC). These samples were collected from the Medical City/Digestive System Hospital, and the time period was from January 2023 to June 2023. Serum samples were analyzed for ALT and AST levels, as well as p53 protein concentration using ELISA. **Results:** This shows Significant p53 expression levels higher in the HCC group (321.56 ± 76.71) compared to the HBV group (264.67 ± 60.21) and the control group (192.15 ± 77.64) with highly significantly of p. value (0.001), indicating the potential diagnostic relevance of p53 measurement in these patient groups. **Conclusion:** The study highlights the significant role of p53 in the progression of HCC linked to chronic hepatitis B virus infection. The elevated p53 levels in HCC patients suggest its potential role as a valuable biomarker for HCC diagnosis and prognosis.

Keywords: HCC, HBV, p53

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Introduction:

Hepatocellular carcinoma (HCC) remains a major global health challenge, with chronic HBV infection accounting for a substantial proportion of cases, particularly in regions where HBV prevalence is high. The virus contributes to carcinogenesis through multifaceted mechanisms, including direct viral integration into host DNA, chronic inflammation, and deregulations of host cell signaling pathways. These processes disrupt cellular homeostasis and promote oncogenic transformation of hepatocytes (1). Globally, HCC ranks as the fifth most frequently diagnosed cancer worldwide and is the second leading cause of death among cancer patients. Between 1990 and 2015, approximately 854,000 new

cases of HCC were reported each year, resulting in around 810,000 annual deaths (2).

Hepatocellular carcinoma ranks as the seventh most frequently diagnosed cancer globally and is the second most common cause of mortality related to cancer. Prevalence rates are highest in Asia and Africa. The highest incidence rate is in Mongolia at 93.7 per 100,000, while China has the most cases, due to its higher rate of 18.3 per 100,000 and the biggest population of the world, with 1.4 billion people (3). The HCC poses a significant global health challenge, ranking as the 6th most common cancer worldwide with 866,136 new cases and the 3rd leading cause of cancer death with 758,725 fatalities in 2022, according to

GLOBOCAN data (4). HBV is a principal causative agent of HCC globally, accounting for approximately 50-55% of cases (5,6).

The relationship between CHB, HCC, and p53 centers on the role of hepatitis B virus (HBV) in driving genetic and molecular alterations that promote hepatocarcinogenesis. Chronic HBV infection induces persistent liver inflammation and oxidative stress, which contribute to DNA damage and somatic mutations, particularly in the *TP53* tumor suppressor gene (7), maintenance of genomic stability, apoptosis, autophagy, immune response, and regulation of the tumor microenvironment (TME) (8)(9). Over 75% of TP53 gene mutations lead to the loss of wild type p53 functionality. Mutated p53 proteins may behave as dominant negatives to wild-type p53 or acquire new tumorigenic features that undermine the protective role of wild-type p53 (10). The presence of chronic HBV infection exacerbates these disruptions, as the HBx protein can bind to p53, leading to its cytoplasmic retention and further impairing its tumor-suppressive functions(11,12). This interaction not only diminishes p53's ability to induce apoptosis but also promotes oncogenic pathways, facilitating the progression of HCC (13). This study aimed to assess the relationship between the levels of p53 and the risk of viral hepatitis B-related HCC.

Materials & Methods:

Subjects

In this study, 120 samples were taken, divided into 3 groups. 40 samples were from patients with liver cancer caused by viral hepatitis B, 40 samples were from patients with chronic viral hepatitis, and 40 samples were from healthy people. These samples were collected from the Medical City. / Digestive System Hospital, and the time period was from January 2023 to June 2023. Patients eligible for inclusion in the study of chronic HBV and HCC must meet specific criteria: positive HBV infection lasting

over 6 months, PCR viral load exceeding 2000, confirmed HCC diagnosis. Conversely, exclusion criteria encompass new or acute HBV infections, ongoing treatment for chronic diseases (e.g., diabetes, hypertension), current antiviral therapy, alcoholism, and cigarette smoking.

Sample Collection & Analytical Processing:

Venous blood samples were obtained from subjects, with 5 mL collected in gel tubes for serum separation and 2 mL collected in K3-EDTA tubes for complete blood count (CBC) analysis. The remaining 3 mL of blood was transferred to a 5 mL gel tube and centrifuged at 2500 rpm for 5 minutes to separate serum for subsequent biochemical assays. Serum samples were collected for the assessment of ALT and AST concentrations in colorimetric method, Hematological indices such as white blood cell count (WBC) and hemoglobin (Hb) levels were derived from EDTA-anticoagulated blood samples via automated hematology analyzer. Furthermore, all analytical data, i.e. for p53 detection using ELISA (sunlong™), were collected, tabulated, and stored for pursuit of statistical interrogation.

Ethical approval

The study followed the ethical principles of human subject research in medicine, according to the Declaration of Helsinki (1964). The study protocol, including participant details and consent procedures, was reviewed and approved by the Hepatology and Gastroenterology Teaching Hospital in Baghdad's Medical City (approval number: 45704, date of approval: 30 November 2023). The study also has been approved by two additional committees: The Committee of the Institute for Genetic Engineering and Biotechnology for Postgraduate Studies for the University of Baghdad and the Ethics Committee of Iraq's Ministry of Health and Environment.

Statistical analysis

The study utilized various statistical tools and methods for data analysis. GraphPad Prism® and Microsoft Excel 2019 were employed for comprehensive data analysis. One-way ANOVA (Tukey's multiple comparisons test) was used to

compare means across multiple groups and examine normally distributed numerical variables. Data was presented using mean values, while nominal variables were graphically depicted using frequencies and percentages. The level of statistical significance was established at a threshold of $P < 0.05$.

Results:

This study compared the demographic characteristics of patients with HCC, those infected with HBV, and a control group. Participants were categorized into different age groups, and their distribution was compared across three categories: patients with HCC, patients with

HBV, and a control group, each comprising 40 individuals. The data are presented as the number and percentage of individuals within each age group, followed by the mean age. The age group (50-59) has the highest percentage of HCC patients (37.5%). HBV patients are predominantly found in the (30-39) age group (30%). **Table (1)** shows further breaks down the age groups by sex across all participants, presenting a total for each combination. This distribution aids in understanding how age and sex intersect in the context of HCC, HBV, and control populations. The (40-49) age group has a slightly higher representation among males (17) compared to females (15).

Table (1): Age Distribution by Sex Among Patients and Controls.

		Age groups					Total
		20-29	30-39	40-49	50-59	≥ 60	
Sex	Female	5	15	17	12	5	54
	Male	7	17	16	14	12	66
Total		12	32	33	26	17	120

Presents a comparative analysis of Hb, WBC, and Platelet count across three groups: HCC patients, HBV patients, and a control group are presented in table (2). The mean Hb levels were 12.99 g/dL for the HCC group, 15.40 g/dL for the HBV group,

and 14.73 g/dL for the control group. The mean WBC counts were $5.74 \times 10^3/\mu\text{L}$ for the HCC group, $7.88 \times 10^3/\mu\text{L}$ for the HBV group, and $7.13 \times 10^3/\mu\text{L}$ for the control group.

Table (2): Comparison between patients and control groups in Hb and WBC.

Groups		Haemoglobin (g/dl)	WBC $\times 10^9/\text{L}$
HCC	Mean	12.99 a	5.74 a
	Std. Deviation	1.34	1.15
HBV	Mean	15.40 b	7.88 b
	Std. Deviation	1.31	1.312
Control	Mean	14.73 b	7.13 b
	Std. Deviation	1.92	1.36
<i>P</i> -value		0.001**	0.001**

The presence of statistical symbols ("a," "b," "c") and significant *P*-values (**) indicate significant differences

The study compared ALT and AST levels between patients with HCC, HBV, and a control group. The HCC group had significantly higher mean ALT levels (151.63 ± 17.76) than the HBV group and

the control group. Additionally, AST levels were elevated in the HCC group (134.74 ± 41.39) compared to the HBV group and the control group show in **Table (3):**

Table (3): Comparison between Patients and Control Groups in ALT and AST.

Groups		ALT	AST
HCC	Mean	151.63 a	134.73 a
	Std. Deviation	17.76	41.38
HBV	Mean	44.35 b	55.97 b
	Std. Deviation	19.80	22.15
Control	Mean	32.97 c	29.94 c
	Std. Deviation	6.08	4.85
<i>P</i> -value		<0.001**	<0.001**

The presence of statistical symbols ("a," "b," "c") and significant *P*-values (**) indicate significant differences

In **table (4)** the meaning p53 expression levels across three groups: HCC, HBV, and Control. were significantly higher in the HCC group (321.56 ± 76.71) compared to the HBV group (264.67 ± 60.21) and the control group ($192.15 \pm$

77.64). The p-value of 0.001 indicates a statistically significant difference between the groups. The provided p-values strongly support these findings, underscoring the potential diagnostic relevance of p53 measurement in these patient groups.

Table (4): Comparison of p53 Levels Among HCC, HBV, and Control Groups.

Groups	Mean	Std. Deviation	p-value
HCC	321.56 a	76.71	<0.001**
HBV	264.67 b	60.21	
Control	192.15 c	77.64	

The presence of statistical symbols ("a," "b," "c") and p-values (**), significant difference.

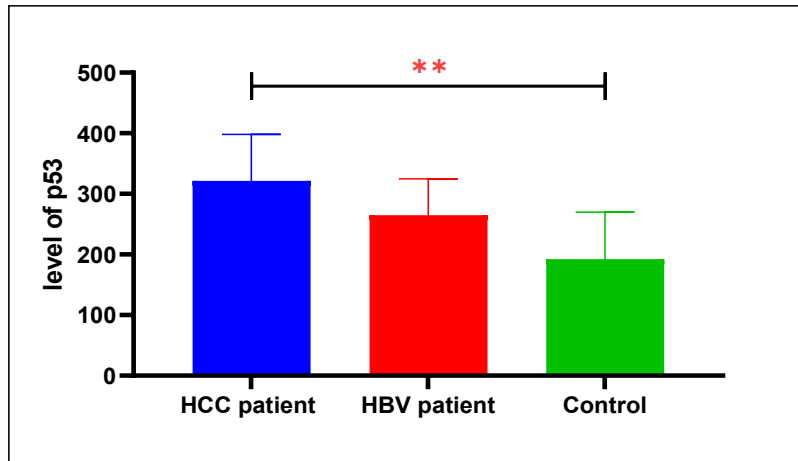


Figure (1): A bar chart comparing p53 levels in HCC, HBV, and control groups.

The ROC data for the HCC group, indicating excellent ability to distinguish between cases and controls as shown in table (5) . The AUC is 0.914, with a significant difference from 0.5, indicating predictive

power. The 95% confidence interval ranges from 0.810 to 1.018, emphasizing test robustness. The best cut-off value is 205.00, with 100% sensitivity and 85% specificity.

Table (5): ROC for the HCC group

Area	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval		The best Cut off	Sensitivity %	Specificity%
		Lower Bound	Upper Bound			
0.914	0.000	0.810	1.018	205.00	100	85

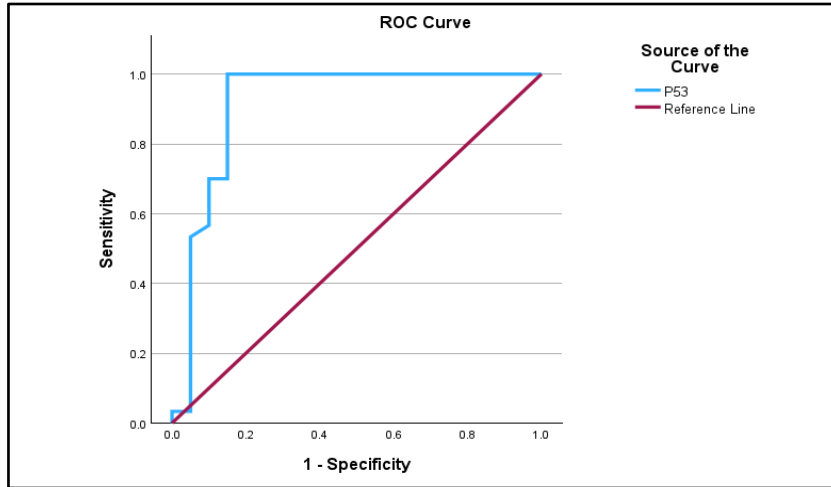


Figure (2): Receiver Operating Characteristic curve of the p53 (HCC Group).

Discussion:

This study investigates the levels of Hb and WBC count, across three groups: HCC patients, HBV patients, and a control group. The findings reveal significant differences among these groups. A study conducted by Fatma *et al.* in Tanta, Egypt, compared hemoglobin, WBC count, and platelet count among HCC patients, CHB patients, and a control group. The results indicated that both HCC and CHB patients exhibited significantly lower Hb and platelet counts compared to the control group. Additionally, WBC counts were also lower in these patient groups relative to the controls (14). This aligns with another study that examined hepatitis B as a marker for HCC in chronic hepatitis B patients, which also found lower hemoglobin and WBC, in HCC and CHB patients compared to the control group (15)

Several studies have reported that the ALT and AST levels in HCC patients were higher than those in patients with HBV

infection or HC. The study showed an increase in liver enzyme levels (such as ALT and AST) in liver cancer patients resulting from viral hepatitis (B), reflecting damage or inflammation in liver tissue. Clinician provided information is key important to manage liver condition, for example liver enzyme. Higher serum ALT and AST levels were observed in the study of CHB patients, where serum liver enzymes are identified as disease markers in chronic HBV infection (16). Another study found that serum transaminases (ALT, AST) levels were higher in HCC patients compared to non-HCC patients with CHB virus infection (17). A new diagnostic model to detect early-stage HBV-related HCC that utilizes AST, ALT, and total bilirubin has been proposed, suggesting a new approach to diagnosis, in a recently published study. This model exhibited enhanced diagnostic efficacy compared to single markers (18). Another research explored enzyme levels liver

otolaryngologist enzymes, including ALT and AST, in patients with Hepatitis B in Kirkouk, Iraq, and identified that ALT and AST were significantly increased in HBV patients, so do ALT, AST in HCC patients (19).

The study also showed highly significant differences in the protein levels of p53 between patients with HCC and HBV infection and controls. The levels of the p53 protein have been examined in multiple studies involving HCC, in HBV-infected patients, and in control groups, and significant differences have been observed in p53 levels across studies. For instance, Wungu *et al.* (2021). investigated the levels of p53 in HBV-infected patients with advanced liver disease. They observed a negative association between advanced liver disease and high p53 serum levels, whilst demonstrating markedly elevated levels of p53 in HCC tissues relative to controls (20). Similarly, Yu *et al.* (2021) reported a marked increase in p53 protein levels in HCC tissues when compared to both HBV-infected and control groups (21).

Adding to these results, Qawy *et al.* (2020) studied the impact of AFP and p53 in diagnosing chronic HBV patients and patients with early-stage HCC. In this study we examined 56 subjects; healthy controls, Patients who have chronic HBV infection and recent diagnosis stage II HCC patients. The findings demonstrated that p53 levels in the HCC group exhibited significant more elevated than what was observed in the control group, thus giving it a potential for being explored as an early biomarker for diagnosing HCC in patients diagnosed with HBV (22). Additionally, Le-qu (2010) analyzed p53 and p21 protein expression levels in HCC occasions with both HBV infections. The expression levels of p53 were markedly elevated in HCC tissues compared to normal liver tissues, with a positivity rate of 79.1% in HCC and no detectable expression in normal tissues.

Notably, the experimental group (HCC with HBV) showed a 95.2% positive rate of p53, whereas control groups exhibited much lower rates (20% and 73.3% for different control groups), suggesting a significant overexpression of p53 in HCC tissues (23).

Additionally, Zhu *et al.* (2020) assessed the immunohistochemical expression of p53 and p21 in HBV-related HCC patients. Although the study found that the expression of p53 and p21 in cancer tissues did not significantly affect clinical outcomes over 2 years, 5 years, or overall, it underscores the complex role of p53 in the prognosis of HBV-related HCC (24). Rahadiani *et al.* (2023) investigate the potential of p53 as a prognostic indicator in HCC, specifically focusing on the Indonesian population. The study found that 85.4% of the patients were positive for p53 expression. Male patients over 60 years old with solitary HCC nodules larger than 5 cm in diameter and vascular invasion exhibited a higher p53 expression (25). Heidari *et al.* (2021) investigated the expression patterns of p53 in liver tissues from patients with HBV-related HCC and healthy controls. The research revealed that individuals with both HBV and HCC exhibited markedly elevated levels of p53 expression when compared to those with HBV alone. The p53 protein demonstrated strong diagnostic potential for HCC, with a sensitivity of 77.3% and a specificity of 76.4%. The analysis of p53 expression provided a more reliable diagnostic tool for HBV-related HCC, with higher specificity and sensitivity (26). Sun *et al.* (2021) examined the expression levels of p53 and p21 proteins in HCC tissues with HBV infection. The positive expression rate of p53 in HCC tissues was significantly higher (79.1%) compared to normal liver tissues (0%). The experimental group (HCC with HBV) had a 95.2% positive rate for p53, suggesting a significant overexpression of p53 in HCC tissues compared to controls. This study underscores the potential of p53 as a

biomarker for diagnosing HCC in the context of HBV infection (27).

Conclusion: These findings provide compelling evidence that underscores the pivotal role for p53 in HCC associated with chronic hepatitis B virus infection. The results contribute to a growing body of evidence that supports the potential of p53 as a biomarker for diagnosing HBV-associated HCC. Measurement of p53 can be added to a currently used diagnostic protocol to further improve sensitivity and specificity.

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