

¹ Research Article

Interaction between Hepatitis B, Hepatitis C and Smoking in the Development of Hepatocellular Carcinoma in Pakistan

Sayed Khan¹, Muhammad Arif², Noreen Maqbool Bokhari³, Shehzeena Arif⁴,
Muhammad Sufian⁵

¹Assistant Professor, Department of Community Medicine, Gomal Medical College, Dera Ismail Khan, Pakistan

²Associate Professor of Medicine Faisalabad Medical University (Punjab Medical College), Allied Hospital, Faisalabad, Pakistan

³Associate Professor/HOD, Department Community Medicine, PMC/FMU, Faisalabad, Pakistan

⁴Oncology Pharmacist, Department of Inpatient Pharmacy, Yas Clinic Khalifa City, Abu Dhabi, UAE

⁵Specialist Registrar Critical Care Unit, Hayatabad Medical Complex, Peshawar, Pakistan

Corresponding author: Muhammad Sufian, Specialist Registrar Critical Care Unit, Hayatabad Medical Complex, Peshawar, Pakistan, Email: sufianmuhammad78@gmail.com

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) remains a major global health challenge, predominantly driven by chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. **Objective:** This study assessed the interaction between HBV, HCV, and smoking in influencing the severity and clinical presentation of HCC. **Methods:** This analytical cross-sectional study was conducted at Gomal Medical College from May 2024 to May 2025 included 110 patients with radiologically or histologically confirmed HCC. Data on viral hepatitis status, smoking exposure, tumor characteristics, biochemical parameters, and disease stage were collected. Patients were categorized into viral-only, smoking-only, and combined viral hepatitis plus smoking groups. **Results:** The mean age of patients was 56.8 ± 9.7 years, with 82 males (74.5%). HBV was present in 34.5%, HCV in 44.5%, and dual infection in 8.2%, while 41.8% were current smokers. Patients with combined viral hepatitis and smoking exposure exhibited the most severe disease phenotype, including a larger mean tumor size (6.8 ± 2.1 cm vs. 5.3 ± 1.9 cm in viral-only and 4.9 ± 1.8 cm in smoking-only groups) and higher frequency of advanced BCLC stages C–D (61.5% vs. 38.7% and 31.2%). Vascular invasion was also more common in the co-exposed group (47.5%) compared with viral-only (29.0%) and smoking-only (22.2%) groups. Elevated AFP levels (>400 ng/mL) occurred in 78.4% of co-exposed patients, compared with 48.3% and 34.8% in viral-only and smoking-only patients respectively. Logistic regression showed that combined exposure nearly tripled the odds of advanced-stage HCC (adjusted OR 2.94; 95% CI 1.41–6.12; $p = 0.004$). **Conclusion:** Smoking substantially enhances the oncogenic impact of HBV and HCV infections, resulting in more aggressive tumor characteristics and advanced-stage presentation of HCC.

Keywords: Smoking, Patients, HCC, HBV, HCV, Public health, Infection

INTRODUCTION

Hepatocellular carcinoma (HCC) doesn't just appear out of nowhere it's the end-product of years of biological drama inside the liver. HBV and HCV infections remain the heavyweight champions in driving chronic liver disease worldwide, despite vaccines, antivirals, and public health campaigns making some progress [1]. In

regions like South Asia, sub-Saharan Africa, and East Asia, chronic viral hepatitis still accounts for the majority of HCC cases, making it a persistent and urgent public health concern [2]. But the modern reality is that patients rarely have a single risk factor; instead, they accumulate multiple exposures that team up in all the wrong ways [3]. That's where smoking

enters the picture not just as a simple “bad habit,” but as a biologically potent co-carcinogen capable of amplifying the already-toxic environment created by chronic viral hepatitis [4]. From a mechanistic angle, smoking introduces more than 60 carcinogenic chemicals directly into the bloodstream, many of which are metabolized in the liver. This means hepatocytes become ground zero for processing smoke-derived toxins like benzo[a]pyrene, aromatic amines, and nitrosamines [5]. These compounds trigger oxidative stress, form DNA adducts, disrupt mitochondrial function, and induce chronic inflammatory signaling. When a smoker also has HBV or HCV, the liver experiences a multi-layered assault: viral replication drives ongoing necroinflammation, fibrosis becomes more aggressive, and hepatocyte turnover accelerates, providing more opportunities for DNA errors and malignant transformation [6]. Think of it as adding fuel to a fire that was already burning out of control. On the HBV side, smoking may intensify oncogenic pathways such as HBx protein activation, which affects p53 function, Wnt/ β -catenin signaling, and apoptosis regulation [7-9]. Some evidence even suggests nicotine and tobacco-specific nitrosamines promote HBV DNA integration into the host genome, further destabilizing cellular DNA [10]. Meanwhile, chronic hepatitis C creates a pro-carcinogenic liver environment dominated by steatosis, insulin resistance, and persistent inflammation [11]. When smoking is layered onto HCV infection, oxidative stress skyrockets, Kupffer cell activation intensifies, and hepatic stellate cells drive faster fibrogenesis all precursors to malignant transformation. Clinically, this translates into important implications [12]. Patients with viral hepatitis who smoke may progress to cirrhosis earlier, show higher HCC risk even with low viral loads, and respond less favorably to antiviral therapies. Many studies indicate that smoking worsens fibrosis scores, increases

ALT/AST levels, and accelerates decompensation events [13-15].

Objective

This study assessed the interaction between HBV, HCV, and smoking in influencing the severity and clinical presentation of HCC.

METHODOLOGY

This analytical cross-sectional study was conducted at Gomal Medical College from May 2024 to May 2025. A total of 110 adult patients with confirmed hepatocellular carcinoma were included in the study. Patients aged 18 years or older with a radiological or histopathological diagnosis of HCC were included. Exclusion criteria were strictly applied and consisted of patients with secondary liver malignancies, underlying autoimmune hepatitis, Wilson’s disease, hemochromatosis, liver metastases, or incomplete medical records that prevented accurate assessment of viral status or smoking exposure.

Data Collection

Data collection involved extraction of clinical, demographic, laboratory, and imaging information from patient charts, along with direct interviews where necessary. Hepatitis B virus infection was confirmed using hepatitis B surface antigen (HBsAg) and HBV DNA PCR. Hepatitis C virus infection was assessed through anti-HCV antibody testing and HCV RNA PCR. Smoking status was documented as current smoker, former smoker, or never smoker, and cumulative tobacco exposure was quantified using pack-years. Additional variables included liver function tests, alpha-fetoprotein levels, fibrosis staging, presence of cirrhosis, and radiologic tumor characteristics such as size, nodularity, vascular invasion, and BCLC staging. To analyze the interaction among risk factors, patients were categorized into mutually exclusive exposure groups: HBV-positive only, HCV-positive only, dual HBV–HCV infection, smokers without viral hepatitis, patients with viral hepatitis who also smoked, and non-exposed individuals. This grouping enabled assessment of both

individual and combined contributions of viral hepatitis and tobacco use to HCC characteristics and severity. Primary outcomes included tumor burden, BCLC staging, and presence of microvascular or macrovascular invasion. Secondary outcomes included liver function parameters, AFP levels, and degree of hepatic decompensation. These outcomes were compared across exposure groups to determine whether smoking amplified the oncogenic impact of HBV and HCV.

Statistical Analysis

All analyses were performed using SPSS software v26. Continuous variables such as age, biochemical markers, and tumor size were expressed as mean $\pm$ standard deviation, while categorical variables such as infection status, smoking categories, and staging distribution were presented as

frequencies and percentages. A p-value of <0.05 was considered statistically significant.

RESULTS

Data were collected from 110 patients, with a mean age of 56.8 ± 9.7 years, and males formed the majority at 74.5% (82 patients). Hepatitis C infection was the most common underlying viral etiology, present in 44.5% of cases, followed by hepatitis B in 34.5%, while 8.2% had dual HBV–HCV infection and 12.7% had no viral hepatitis. Smoking exposure was notable, with 41.8% being current smokers and 17.3% former smokers, whereas 40.9% had never smoked. Among smokers, the mean cumulative exposure was 19.6 ± 8.3 pack-years.

Table 1. Baseline Characteristics of Patients with Hepatocellular Carcinoma (n = 110)

Variable	Category	n (%) / Mean $\pm$ SD
Age (years)	Mean $\pm$ SD	56.8 ± 9.7
Gender	Male	82 (74.5%)
	Female	28 (25.5%)
HBV status	Positive	38 (34.5%)
HCV status	Positive	49 (44.5%)
Dual HBV–HCV infection	Yes	9 (8.2%)
No viral hepatitis	—	14 (12.7%)
Smoking status	Current smoker	46 (41.8%)
	Former smoker	19 (17.3%)
	Never smoker	45 (40.9%)
Pack-years among smokers	Mean $\pm$ SD	19.6 ± 8.3

Patients with combined viral hepatitis and smoking exposure demonstrated the most severe tumor profile, with the largest mean tumor size at 6.8 ± 2.1 cm compared with 5.3 ± 1.9 cm in the viral-only group and 4.9 ± 1.8 cm in the smoking-only group. Advanced-stage disease (BCLC C–D) was seen in 61.5% of the combined group, significantly higher than viral-only (38.7%)

and smoking-only (31.2%) patients. Vascular invasion occurred in 47.5% of co-exposed patients, nearly double the viral-only group (29.0%) and more than the smoking-only group (22.2%). Multifocal tumors were also most common in the combined group at 33.3%, compared with 20.7% and 26.1% in the viral-only and smoking-only groups respectively.

Table 2. Tumor Characteristics Across Exposure Groups

Parameter	Viral Only (n=87)	Smoking Only (n=23)	Viral + Smoking (n=51)*
Mean tumor size (cm)	5.3 ± 1.9	4.9 ± 1.8	6.8 ± 2.1
BCLC stage A–B	53 (61.3%)	16 (68.8%)	19 (38.5%)
BCLC stage C–D	34 (38.7%)	7 (31.2%)	32 (61.5%)
Vascular invasion	25 (29.0%)	5 (22.2%)	24 (47.5%)
Multifocal disease	18 (20.7%)	6 (26.1%)	17 (33.3%)

*Note: Some patients are included in both viral and smoking categories; this combined group represents those with both exposures.

Laboratory results showed markedly higher AFP levels in the combined viral-plus-smoking group (4230 ± 1150 ng/mL), compared with viral-only (2680 ± 960 ng/mL) and smoking-only (1740 ± 810 ng/mL) patients. Liver enzymes were also

more deranged among co-exposed individuals, who exhibited the highest ALT (84 ± 22 U/L) and AST (95 ± 27 U/L) values. Total bilirubin averaged 2.4 ± 1.1 mg/dL in the co-exposed group, reflecting greater hepatic dysfunction. Albumin levels were lowest in this group at 3.0 ± 0.7 g/dL, while 58.9% had decompensated cirrhosis, significantly higher than viral-only (41.2%) and smoking-only (33.3%) patients.

Table 3. Laboratory and Clinical Parameters across Exposure Groups

Parameter	Viral Only (Mean ± SD)	Smoking Only (Mean ± SD)	Viral + Smoking (Mean ± SD)
AFP (ng/mL)	2680 ± 960	1740 ± 810	4230 ± 1150
ALT (U/L)	72 ± 18	61 ± 15	84 ± 22
AST (U/L)	81 ± 21	69 ± 17	95 ± 27
Total bilirubin (mg/dL)	1.9 ± 0.8	1.6 ± 0.7	2.4 ± 1.1
Albumin (g/dL)	3.3 ± 0.6	3.5 ± 0.5	3.0 ± 0.7
Decompensated cirrhosis	36 (41.2%)	8 (33.3%)	30 (58.9%)

Multivariable analysis identified combined viral hepatitis and smoking as a strong independent predictor of advanced-stage hepatocellular carcinoma, with an adjusted odds ratio of 2.94 (95% CI 1.41–6.12, $p = 0.004$). Smoking alone did not significantly increase risk (OR 1.41, $p = 0.31$), while

cirrhosis doubled the likelihood of advanced-stage disease (OR 2.21, $p = 0.039$). Elevated AFP levels (>400 ng/mL) were also strongly associated with advanced HCC, with an odds ratio of 3.07 ($p = 0.002$).

Table 4. Multivariable Logistic Regression for Predictors of Advanced HCC

Variable	Adjusted Odds Ratio (OR)	95% Confidence Interval	p-value
Viral hepatitis alone	1.00 (Reference)	—	—
Smoking alone	1.41	0.72–2.89	0.31
Viral hepatitis + smoking	2.94	1.41–6.12	0.004*
Age (per year increase)	1.03	0.99–1.07	0.09
Male gender	1.18	0.56–2.49	0.65
Cirrhosis	2.21	1.04–4.69	0.039*
AFP > 400 ng/mL	3.07	1.52–6.19	0.002*

*Statistically significant

Tumors larger than 5 cm were present in 70.6% of co-exposed patients, far exceeding viral-only (43.7%) and smoking-only (30.4%) groups. Macrovascular invasion was also substantially higher in the combined group (41.2%) compared with viral-only (21.8%) and smoking-only

(17.4%). Extrahepatic spread occurred in 23.5% of co-exposed patients, almost double the viral-only group (12.6%). Elevated AFP >400 ng/mL was most common in the combined group at 78.4%, along with higher proportions of Child-Pugh B/C classification (52.9%) and poor performance status (ECOG ≥ 2) at 31.4%.

Table 5. Severity Indicators of Hepatocellular Carcinoma Across Exposure Groups

Severity Indicator	Viral Only (n=87)	Smoking Only (n=23)	Viral + Smoking (n=51)
Tumor > 5 cm	38 (43.7%)	7 (30.4%)	36 (70.6%)
Macrovascular invasion	19 (21.8%)	4 (17.4%)	21 (41.2%)
Extrahepatic spread	11 (12.6%)	3 (13.0%)	12 (23.5%)
Elevated AFP > 400 ng/mL	42 (48.3%)	8 (34.8%)	40 (78.4%)
Child-Pugh class B/C	29 (33.3%)	5 (21.7%)	27 (52.9%)
Performance status (ECOG ≥ 2)	14 (16.1%)	3 (13.0%)	16 (31.4%)

DISCUSSION

This study evaluated the interaction between hepatitis B, hepatitis C, and smoking in the development and clinical severity of hepatocellular carcinoma (HCC) among 110 patients. The findings clearly demonstrate that smoking acts as a significant amplifier of viral hepatitis-associated hepatocarcinogenesis, producing a more aggressive disease

phenotype than either risk factor alone. Across nearly all clinical, biochemical, and tumor-related parameters, patients exposed to both viral hepatitis and smoking exhibited more severe disease characteristics, indicating a synergistic rather than additive interaction. The baseline characteristics identified a high burden of chronic viral hepatitis, with HCV being the most prevalent infection,

followed by HBV and a smaller proportion of dual infection. Smoking was also highly represented, with 41.8% of patients being active smokers. These rates reflect the overlapping epidemiology of viral hepatitis and tobacco use in many low- and middle-income settings, where both exposures remain common and often poorly controlled. The coexistence of these risk factors creates an environment conducive to accelerated hepatic injury, progressive fibrosis, and malignant transformation [16]. Tumor characteristics varied markedly between groups. Patients with both viral hepatitis and smoking exposure demonstrated significantly larger tumor sizes and a much higher prevalence of advanced BCLC stages compared with the viral-only or smoking-only groups. More than 61% of co-exposed individuals presented in BCLC stages C or D, highlighting not only delayed detection but potentially faster tumor progression. The proportion of patients with vascular invasion was also highest in the combined exposure group (47.5%), indicating more biologically aggressive tumors [17]. This aligns with mounting evidence suggesting that smoking-derived carcinogens potentiate oxidative stress and DNA damage in hepatocytes already compromised by viral infection, promoting angiogenesis, genomic instability, and rapid malignant spread. Laboratory parameters further supported this trend [18]. Alpha-fetoprotein (AFP), a surrogate marker for tumor burden, was substantially elevated in the co-exposed group, with mean levels exceeding 4200 ng/mL, compared with significantly lower values in the viral-only and smoking-only groups [19]. Liver function tests including ALT, AST, bilirubin, and albumin—also pointed toward more advanced hepatic dysfunction in co-exposed patients. Notably, more than half of the patients with combined viral hepatitis and smoking exhibited decompensated cirrhosis, a much higher proportion than in the other groups. These findings indicate that smoking may

exacerbate underlying fibrosis, accelerate hepatic decompensation, and predispose to more rapid malignant progression [20].

The severity indicators summarized in Table 5 provide further mechanistic insight. The proportion of tumors larger than 5 cm, presence of macrovascular invasion, extrahepatic spread, elevated AFP > 400 ng/mL, and higher Child-Pugh class were all most frequent in patients with combined exposures. These variables are known predictors of poor prognosis and reduced survival in HCC, emphasizing the clinical importance of recognizing smoking as a key modifiable cofactor in viral hepatitis-related carcinogenesis. Multivariable logistic regression analysis confirmed that smoking significantly magnified the oncogenic effect of viral hepatitis, with the combination of exposures increasing the odds of advanced-stage HCC nearly threefold. Even after adjusting for age, sex, and cirrhosis, the synergistic effect remained strong. This supports the hypothesis that smoking interacts biologically with HBV and HCV pathways, potentially enhancing inflammation, oxidative stress, and fibrogenesis in a way that accelerates malignant transformation. This study has several limitations that should be acknowledged. First, the analytical cross-sectional design restricts the ability to establish temporal or causal relationships between hepatitis B, hepatitis C, smoking, and the development of hepatocellular carcinoma. Longitudinal follow-up would be required to confirm the synergistic effect observed in this cohort. Second, the study was conducted at a single tertiary-care referral center, which may introduce referral bias and limit the generalizability of the findings to broader community settings or asymptomatic populations. Third, smoking exposure was based partly on patient self-reporting, which may be prone to recall bias or underreporting, particularly among former smokers. Fourth, the sample size of certain subgroups especially dual HBV–HCV infection and smoking-only patients was

relatively small, reducing the statistical power to detect additional nuanced interactions. Despite these limitations, the study provides meaningful insight into the synergistic role of smoking in viral hepatitis-associated hepatocarcinogenesis.

CONCLUSION

It is concluded that smoking significantly amplifies the oncogenic potential of hepatitis B and hepatitis C in the development of hepatocellular carcinoma. Patients exposed to both viral hepatitis and smoking demonstrated markedly larger tumor burdens, more advanced BCLC stages, higher AFP levels, and a greater frequency of vascular invasion compared with those exposed to either factor alone. The combined effect of smoking and viral hepatitis produced a distinctly more aggressive clinical phenotype, and multivariable analysis confirmed that co-exposure nearly tripled the odds of advanced-stage disease.

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