

# Hepatitis B virus and hepatitis C virus dual infection among patients with chronic liver disease

Shanmugam Saravanan<sup>1,2</sup>, Vijayakumar Velu<sup>1</sup>, Subhadra Nandakumar<sup>1</sup>, Vidya Madhavan<sup>2</sup>, Uma Shanmugasundaram<sup>2</sup>, Kailapuri G. Murugavel<sup>2</sup>, Pachamuthu Balakrishnan<sup>2</sup>, Nagalingeswaran Kumarasamy<sup>2</sup>, Suniti Solomon<sup>2</sup>, Sadras Panchatcharam Thyagarajan<sup>1,2</sup>

<sup>1</sup>Department of Microbiology, Faculty of Medicine, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras; and <sup>2</sup>YR Gaitonde Centre for AIDS Research and Education, Voluntary Health Services Hospital Campus, Chennai, India

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**Background and purpose:** Hepatitis B virus (HBV) and hepatitis C virus (HCV) dual infection accounts for a substantial proportion of liver diseases worldwide. Although the exact prevalence is not known, these viral infections are common among patients with chronic liver disease (CLD). This study was performed to determine the prevalence of HBV and HCV dual infection among patients with CLD in Chennai, India.

**Methods:** 251 patients were tested for the presence of hepatitis B surface antigen (HBsAg), immunoglobulin (Ig)-M/IgG antibody to hepatitis B core antigen (anti-HBc) and anti-HCV antibodies, and HBV-DNA and HCV-RNA by qualitative polymerase chain reaction.

**Results:** Coinfection with HCV and HBV was detected in 15 patients (5.9%), 12 of whom (80.0%) were positive for HCV-RNA and IgG anti-HBc with no evidence of HBV-DNA, while 3 HBsAg-negative patients (20.0%) were positive for HBV-DNA in addition to HCV-RNA. Liver function test profiles were significantly altered for HCV-positive patients compared with HBV-positive and HBV/HCV coinfecting patients ( $p = 0.001$ ). Bilirubin and alanine aminotransferase levels were significantly raised in coinfecting patients compared with non-HBV, non-HCV patients ( $p = 0.001$ ).

**Conclusions:** The results demonstrated that HBV was predominantly associated with underlying CLD among this group of patients in India and suggest that HBV coinfection in HCV-infected patients should not be excluded by negative HBsAg status alone.

**Key words:** Hepacivirus; Hepatitis B virus; India; Infection; Liver diseases

## Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common causes of chronic liver disease (CLD) worldwide, and can lead to cirrhosis and hepatocellular carcinoma (HCC) [1]. Coinfection with both HBV and HCV can occur because of shared routes of infection [2-4]. Approximately 400 million people are reported to be infected with HBV worldwide [5] and the Centers for Disease Control and Prevention

estimates that approximately 170 million people are infected with HCV [6]. Frequent coinfection of hepatitis B surface antigen (HBsAg)-negative HBV (silent HBV) in HCV-associated CLD has also been reported [7]. Coinfecting patients represent a diverse group with various viral replication and immunity profiles that could lead to more aggressive liver disease [8]. Owing to their distinct clinical course and heterogeneity, identification of patients who are candidates for therapy and selection of the optimal antiviral therapy is a challenge for clinicians.

The prevalence of coinfection is approximately 10% to 20% of patients with chronic HBV infection [9-13], and 2% to 10% of anti-HCV-positive patients

Corresponding author: Dr. Shanmugam Saravanan, YR Gaitonde Centre for AIDS Research and Education, Voluntary Health Services Hospital Campus, Taramani, Chennai 600113, India.  
E-mail: saravanan@yrgcare.org or vvjai2000@yahoo.com

are reported to have markers of HBV infection. In addition to CLD, coinfection with HBV and HCV is frequently found among injection drug users (IDUs; 42.5%) [14], patients undergoing hemodialysis (3.7%) [15] or organ transplantation (8%) [16], human immunodeficiency virus–positive individuals (66%) [17], and people with  $\beta$ -thalassemic traits (10%) [18], which represents the high-risk population. In India, the prevalence of HBV and HCV coinfection among patients not undergoing hemodialysis has been reported to range from 3% to 56% [8,19,20]. Seroprevalence studies have shown that HBV and HCV coinfection status might vary with geographical location [11] and the exact number of patients infected with both HCV and HBV is ambiguous in the South Indian setting. Therefore, this study was performed to investigate the seroprevalence of HCV and HBV dual infection among South Indian patients with underlying CLD manifestations.

## Methods

Serum samples from 251 patients with CLD who were referred to the National Reference Center for Viral Hepatitis, Department of Microbiology, Faculty of Medicine, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, India, were retrospectively studied. Ethical approval was given by the Dr. ALM Post Graduate Institute of Basic Medical Sciences.

The available biochemical, clinical, and liver histopathological information was used to correlate with the virological profile. The diagnosis of CLD, including mild chronic hepatitis, moderate or severe chronic hepatitis, liver cirrhosis, and HCC, were assessed based on the histological activity index as per the standard guidelines [21]. HCC was assessed by needle aspiration cytology/biopsy collected from the liver lesions of the study participants by experienced gastroenterologists. Non-invasive abdominal ultrasonography (USG) with Doppler-based information regarding the gross appearance of the liver and blood flow in the portal and hepatic veins of patients suspected to have cirrhosis were noted from the patient's medical records. Although computed tomography/ultrasonography (CT/USG) and magnetic resonance imaging are generally poor at detecting morphologic changes associated with early cirrhosis, visualized nodularity and lobar atrophic and hypertrophic changes, as well as evidence of ascites and varices were included in the diagnosis of liver disease.

All patients answered a predesigned questionnaire regarding occupation, past history of jaundice (its nature, etiology, and outcome), blood transfusion, surgery, dental/gynecological procedures, radiologic intervention, exposure to syringes and needles, ear piercing, and sharing of razors and/or blades, as well as mother being HBsAg- and/or anti-HCV–positive and information on high-risk behavior such as IDU, sexual promiscuity, and tattoos. None of the patients had a history of hemodialysis or organ transplantation.

Patients with clinically and histopathologically proven CLD fulfilled the criteria for inclusion, defined as inflammation of the liver continuing without improvement for 6 months or longer. Accordingly, 5 mL of blood was collected aseptically, and the serum was separated, aliquoted, and stored at  $-70^{\circ}\text{C}$  until tested. Markers of HBV and HCV, including antibody to hepatitis B core antigen (anti-HBc), HBsAg, hepatitis B e antigen (HBeAg)/antibody to HBeAg (anti-HBe) [Bio-Rad Laboratories, Salt lake City, UT, USA] and anti-HCV (Murex Diagnostics Ltd, Dartford, UK) were detected by using commercially available enzyme-linked immunosorbent assay kits with known positive and negative controls. HBV DNA and HCV RNA were detected by amplifying the surface region and 5' non-coding region (5'NCR), respectively, by polymerase chain reaction (PCR) using the methods previously described [22,23].

## Statistical analysis

The median values of alanine aminotransferase (ALT) levels according to age, HBV-/HCV-positivity, with or without DNA/RNA detection, and liver status were analyzed by the non-parametric Mann-Whitney *U* test. Whenever variables were continuous, the results were analyzed by the *t* test. All *p* values were 2-sided hypothesis. The evaluation was performed by using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 13.0; SPSS Inc, Chicago, IL, USA).

## Results

The study group comprised 186 men (74%) and 65 (26%) women. The median  $\pm$  standard deviation age of the patients was  $55.8 \pm 7.6$  years (range, 39–76 years). 157 patients (62.5%) had a risk factor of history of blood transfusion, hospital admission, or surgery; 26 (10.4%) had a history of sexual promiscuity, 53 (21.0%) were IDUs, 97 (38.6%) had tattoos or ear piercing, 32 (12.7%) had been exposed to unsterilized syringes, and 22 (8.7%)

had no history of risk factors. Of 251 patients, 67 (26.7%) were positive for anti-HCV, 112 (44.6%) were positive for HBV, 15 (5.9%) had dual infection, and 57 (22.7%) were non-HBV/non-HCV (Table 1). Among the 15 patients with dual infection, none were positive for anti-HBc immunoglobulin (Ig)-M.

112 patients (44.6%) had HBsAg, anti IgG-HBc, and/or HBV-DNA. Sixty seven patients (26.7%) were positive for HCV by detection of anti-HCV or HCV-RNA. Coinfection of HCV and HBV was detected in 15 patients (5.9%). Fifty seven patients (22.7%) had cryptogenic CLD, as none of the markers for HBV or HCV infection could be identified. Of the 251 patients with underlying liver disease, the majority had cirrhosis ( $n = 95$ ; 38%), followed by chronic hepatitis ( $n = 91$ ; 36%), HCC ( $n = 35$ ; 14%), and CT/USG-confirmed liver ailments ( $n = 30$ ; 12%) [Table 1].

Of the 15 patients with HCV/HBV coinfection, HBsAg was detected in 12 (80%), HBV-DNA by PCR alone in 3 (20%), anti-HCV alone in 13 (87%), and both anti-HBc and HCV-RNA PCR in 15 (100%) [Table 2]. A statistically significant difference was noted between patients with chronic hepatitis (47%), cirrhosis (40%), and HCC (13%) [ $p = 0.001$ ]. Occult

hepatitis B was evident among 3 patients in whom the conventional HBsAg marker was not detected, whereas both anti-HBc (IgG) and HBV-DNA were demonstrable by enzyme immunoassay and PCR, respectively. HCV-RNA was significantly higher than HBV-DNA for PCR-positivity (20% vs 100%;  $p = 0.001$ ).

Comparison of the diagnostic groups showed that the liver function test profiles were altered and statistically significant for HCV-positive patients compared with coinfecting patients (bilirubin,  $p = 0.001$ ; ALT,  $p = 0.001$ ), and significantly higher than in HBV-positive patients and non-HBV, non-HCV patients (bilirubin,  $p = 0.001$ ; ALT,  $p = 0.001$ ) [Table 1].

## Discussion

Approximately 6% of patients with CLD had dual infection with HBV and HCV, although there was minimal HBV-DNA positivity. In concordance with previous studies, HCV infection had a suppressive effect on the replication of HBV, shown by the loss of replicative markers such as HBV-DNA by qualitative PCR [24,25]. This impact of HCV on HBV replication needs confirmation by longitudinal follow-up. The

**Table 1.** Characteristics of patients with chronic liver disease ( $n = 251$ ).

Characteristic	HBV ( $n = 112$ ) No. (%)	HCV ( $n = 67$ ) No. (%)	HBV and HCV ( $n = 15$ ) No. (%)	Non-HBV/ non-HCV ( $n = 57$ ) No. (%)	$p$
Age (years; mean $\pm$ SD)	55.9 $\pm$ 7.37	55.6 $\pm$ 7.91	51.8 $\pm$ 6.8	56.8 $\pm$ 7.7	0.157
Sex					
Male	81 (72)	50 (75)	11 (73)	44 (77)	0.923
Female	31 (28)	17 (25)	4 (27)	13 (23)	
Risk factors					
Sexual promiscuity	10 (8.9)	7 (10.4)	3 (20)	6 (10.5)	0.940
Blood transfusion/ hospital admission/surgery	69 (61.6)	43 (64.1)	10 (66.7)	35 (61.4)	
Intravenous drug use	26 (23.2)	13 (19.4)	3 (20)	11 (19.3)	
Tattoo/ear piercing	37 (37.5)	29 (43.3)	8 (53.3)	23 (40.4)	
Exposure to unsterilized syringes and needles	30 (26.7)	17 (25.3)	2 (13.3)	16 (28)	
Unknown	11 (9.8)	6 (8.9)	-	5 (8.8)	
Liver biochemistry marker					
Total bilirubin ( $\mu$ mol/L)	42.75 $\pm$ 10.94	79.2 $\pm$ 21.03	55.06 $\pm$ 9.92	43.95 $\pm$ 12.14	0.001
Alanine aminotransferase (U/L)	127.8 $\pm$ 82.4	319.2 $\pm$ 167.4	260.6 $\pm$ 147.0	98.8 $\pm$ 66.6	0.001
Liver disease					
Chronic hepatitis	32 (28.6)	29 (43.3)	7 (46.7)	23 (40.3)	0.001
Cirrhosis	58 (51.8)	19 (28.3)	6 (40.0)	12 (21.0)	
Hepatocellular carcinoma	22 (19.6)	9 (13.4)	2 (13.3)	2 (3.5)	
Liver disease by CT/USG	0 (0)	10 (14.9)	0 (0)	20 (35.1)	

Abbreviations: HBV = hepatitis B virus; HCV = hepatitis C virus; SD = standard deviation; CT = computed tomography; USG = ultrasonography.

**Table 2.** Virological profile in patients with chronic liver disease and hepatitis B and C virus dual infection.

No. (%)	Anti-HBc IgM/IgG	HBsAg	HBeAg/anti-HBe	HBV-DNA	anti-HCV	HCV-RNA
2 (13.3)	±	+	±	-	+	+
8 (53.3)	±	+	±	-	+	+
3 (20.0)	±	-	±	+	+	+
2 (13.3)	±	+	±	-	-	+

Abbreviations: Anti-HBc = antibody to hepatitis B core antigen; Ig = immunoglobulin; HBsAg = hepatitis B surface antigen; HBeAg = hepatitis B e antigen; anti-HBe = antibody to HBeAg; HBV = hepatitis B virus; anti-HCV = antibody to hepatitis C virus.

available evidence demonstrates that both viruses can inhibit each other simultaneously; either virus can play a dominant role; both viruses have the ability to induce seroconversion of the other; the chronology of infection has a role in determining the dominant virus; and HBV and HCV can alternate their dominance [24-26]. However, the overall dominant effect appears to be HCV suppression of HBV. Different scenarios have been described for dual infection with HBV and HCV, including acute dual viral hepatitis, occult HBV coinfection of chronic hepatitis C, and superinfection by either virus in patients with pre-existing chronic hepatitis due to the other virus. In addition, coinfecting patients are often found to have evidence of both HBV and HCV infection without a clear chronology of infection. In India, which has intermediate to high rates of HBV infection due to vertical transmission, although several studies have shown that HBV and HCV interact with each other and affect immune responses, coinfection can generally be assumed to be due to HCV superinfection. In other geographic areas, the sequence of infection is less clear. HCV suppression of HBV replication has been demonstrated by many studies that have shown that patients with chronic HBV coinfecting with HCV have lower HBV-DNA levels, decreased HBV-DNA polymerase activity, and decreased expression of HBsAg in the blood [13,27,28].

In India, the literature consensually states that HBV and HCV dual infection is not rare, but the incidence varies in different clinical groups, with various risk factors involved. The prevalence of HBV and HCV coinfection in non-hemodialysis patients was reported to range from 3% and 56% [8,15,19,29]. Recently, a high incidence of HBV and HCV dual infection has been reported in patients with HCC (50%), CLD (37.5%), and renal failure (16-37%) [30,31]. Although HBV infection is conventionally diagnosed by circulating HBsAg, a unique persistent state of infection, namely occult HBV infection, characterized by the occurrence of HBV DNA in serum (detectable by qualitative diagnostic PCR assays), has been

identified in HBsAg-negative patients with or without serological markers of previous HBV infection (antibody to HBsAg or anti-HBc) [32-34]. The high prevalence of occult HBV infection in such patients has been suggested to have clinical implications for the pathogenesis of HCV-induced CLD. In this study, there was a lower prevalence of occult HBV infection among the HCV-infected patients with CLD (20%) than in other studies that have estimated a prevalence of 50% to 87% [35-40]. However, these findings might be anticipated because most adults in India are reported to contract HBV infection during childhood, and superinfection with other viruses including HCV may occur thereafter [6,8,20,41].

Occult HBV infection in individuals without detectable levels of HBsAg can occur and are usually found among patients with chronic HCV. Therefore, the clinical relevance remains controversial and previous data suggest that occult HBV infection does not have clinical significance in patients with chronic HCV living in areas where HBV infection is endemic [42]. This study found that 3 of 15 HCV-infected patients with CLD (20%) had occult HBV infection, 2 of whom had HCC. Recent cloning experiments have shown the accumulation of highly divergent HBV strains in the liver of patients with occult HBV, and have also confirmed the association of host factors rather than viral factors for cryptic HBV infection [43]. Although dual infection with HBV and HCV leads to mutual suppression of both viruses, several studies have suggested that dual HBV and HCV infection may be associated with a more severe clinical presentation [13,44]. Dual infection of HBV and HCV enhancing the severity of hepatitis has also been supported by histological evidence from studies comparing the histological characteristics of patients with chronic HBV and HCV with those of chronic HCV alone [45]. The findings of this study also suggest that severe chronic hepatitis was significantly more common among patients with HBV and HCV dual infection (47%) than among other groups ( $p = 0.001$ ).

Several studies have reported detectable levels of HBV-DNA from individuals with chronic HCV, but negative HBsAg. This so-called 'serologically silent' HBV infection or 'unapparent coinfection' has been correlated with impaired response to interferon (IFN) treatment. Other studies have reported significantly worse results for 14 chronically infected HCV patients with unapparent HBV coinfection (anti-HCV-positive, HBV-DNA-positive, HBsAg-negative) [44]. Silent HBV infection has been shown to be associated with higher ALT levels, greater histological activity scores, and poor efficacy of IFN treatment [38]. Some authors have proposed that the impaired response to IFN in such patients may be due to HBV-mediated downregulation of intrahepatic IFN receptor gene expression [46]. This study found that 20% of patients were serologically silent (occult HBV) with HCV coinfection, 2 of whom had associated HCC with significant ALT elevation ( $p = 0.05$ ). Hence, the regular serological test (HBsAg) might fail to identify HBV infection as a cause of liver HCC in a proportion of patients, which could be important for diagnostic, blood banking, and transplantation services. However, further studies, of a longer duration, are required to clarify the role of silent HBV infection in HCV-infected patients by the correlation of quantitative viral load (HBV-DNA and HCV-RNA). In tropical countries such as India, with intermediate HBV endemicity, occult infection may be diagnosed by incremental testing for IgG anti-HBc, serum HBV-DNA, and HBV-DNA in liver tissue [41].

This study has several limitations. Bias may have been introduced by the retrospective design of the study and the lack of uniform interpretation of data. Not all 251 patients were interviewed, and data such as liver function tests and imaging were not collected in a routine fashion. Due to under-reporting and missing information, it is possible that the study underestimates the prevalence of many population characteristics. No biopsies, USG, or CT were performed at the study center, and physicians in the referring hospitals did not routinely order imaging studies, biopsies, or liver function tests. This could also lead to an underestimation of the prevalence of HBV and HCV in this population of patients with CLD.

HBV and HCV continue to be major causes of CLD in southern India, with a substantial number of patients with HCV being coinfecting with HBV (5.9%); this rate may be underestimated in this study. HBV and HCV dual infection is not unusual, especially in areas of intermediate HBV prevalence. HCV

infection appears to have a suppressive effect on the replication of HBV, as shown by the loss of replicative markers such as HBV-DNA, although this needs to be confirmed with longitudinal follow-up study. It is postulated that HBV coinfection in HCV-infected patients cannot be excluded by negative HBsAg status alone. Repeat PCR analysis might be required for the detection of HBV infection, especially in patients with HCV-related CLD. In addition to the clinical complications of occult HBV infection in HCV-infected patients with CLD, dual infection presents unique management challenges given the complex interaction of HBV and HCV, and the propensity for developing more severe liver disease. Furthermore, no standard of care has been established for the treatment of coinfecting patients, so larger randomized controlled trials might be reasonable to clarify the optimal treatment policies and the role of newer antiviral agents.

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