The prevalence of hepatitis B virus and hepatitis C virus infection among patients with chronic liver disease in South India

Shanmugam Saravanana\textsuperscript{a,b}, Vijayakumar Velu\textsuperscript{a}, Nagalingeswaran Kumarasamy\textsuperscript{b}, Esaki Muthu Shankar\textsuperscript{b}, Subhadra Nandakumar\textsuperscript{a}, Kailapuri G. Murugavel\textsuperscript{b}, Pachamuthu Balakrishnan\textsuperscript{b}, Sunil Suhas Solomon\textsuperscript{b}, Suniti Solomon\textsuperscript{b}, Sadras Panchatcharam Thyagarajan\textsuperscript{a,b,c,*}

\textsuperscript{a}Department of Microbiology, Faculty of Medicine, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai 600 113, India
\textsuperscript{b}YR Gaitonde Centre for AIDS Research and Education, Voluntary Health Services, Chennai 600 113, India
\textsuperscript{c}Sri Ramachandra Medical University, Porur, Chennai 600 116, India

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KEYWORDS
Chronic liver disease; Hepatitis B virus; Hepatitis C virus; Genotype 1b; India

Summary
Objective: Determining the identity of hepatitis C virus (HCV) genotypes in liver disease has key implications for ascertaining the duration of antiviral therapy and disease prognosis. We investigated the presence of various genotypes of HCV among 69 chronic liver diseased (CLD) patients with chronic HCV infection.

Methods: Sixty-nine consecutive subjects with underlying chronic hepatitis (n = 28), cirrhosis (n = 35), and hepatocellular carcinoma (n = 6), diagnosed by clinical, biochemical, and histological means, were studied. Hepatitis B virus (HBV) and HCV diagnostic markers were used. HCV-RNA was extracted from sera of HCV-infected subjects and subsequently the HCV genotypes were determined using a commercial line probe assay (Inno-LiPA HCV II).

Results: Of the 69 CLD cases screened for possible markers of HBV and HCV infection, 39 (57%) were positive for HBV and 30 (43%) were HCV infected. The overall HCV-RNA positivity was 77% (23/30). Of these, the majority were genotype 1b (13/23, 57%), followed by 1a (6/23, 26%), mixed genotypes 3 and 4 (3/23, 13%), and mixed pattern of 1a, 1b, and 4 (1/23, 4.3%). The genotype 1b infected subjects demonstrated significantly elevated transaminase (ALT) levels ($p < 0.05$) as compared with the other non-1b HCV genotypes.

* Corresponding author. Tel.: +91 44 22542929; fax: +91 44 22542939.
E-mail address: saravanan@yrgcare.org (S.P. Thyagarajan).

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Introduction

Hepatitis C virus (HCV) infection is estimated to affect approximately 180 million people worldwide, i.e., about 3% of the world's population. Six major HCV genotypes have been described and these genotypes can differ up to 30% from each other in nucleotide sequence.³ Globally, 3—4 million persons are newly infected each year, with genotype 1 being the most common in terms of prevalence, followed by genotypes 2 and 3. The other genotypes, namely 4, 5, and 6, are reported to have specific geographical distributions. While the acute phase of HCV infection is reported to be asymptomatic in the majority of cases, this could however progress to chronic stage in approximately 70—80% of affected individuals.²,³ This consequently could lead to progressive liver damage and cirrhosis, with increased risk of hepatocellular carcinoma (HCC).⁴ HCV infection is reported to be responsible for 15—20% of all chronic liver disease (CLD) cases and approximately 5—10% of all HCC cases in India.⁵ Liver disease in HCV does not occur in a linear and predictable fashion, but is instead influenced by factors such as genotype, viral load, duration and age of infection, body weight, alcohol consumption, and concurrent infection by hepatitis B virus (HBV) or human immunodeficiency virus (HIV). While each of the viral and host factors influence the disease, HCV genotype has emerged as an independent factor, which largely influences the duration and treatment outcome.⁶ Genetic variability is increasingly recognized as an important factor to be considered in the prognosis, monitoring, and outcome of HCV mediated CLD.⁷ A recent Indian study reported steatosis in 70% of liver biopsy samples from chronic HCV cases.⁸ Furthermore, another study documented the close association of HCV genotype 3 with steatosis and fibrosis.⁹ Genotype 1b is less responsive to interferon-α (IFN-α) therapy compared to genotypes 2 and 3, which necessitates measures to track down the different HCV genotypes.¹⁰,¹¹ The assessment of HCV genotypes has become a pivotal determinant in deciding the treatment duration and dosage. Although there are several reports describing the prevalence of HCV genotypes in India, most of them have reported on the preponderance of genotype 3 in the north,⁹,¹²—¹⁹ whereas a few others from the south have reported genotype 1 to be the most common.²⁰,²¹ However, none have correlated the association of HCV genotypes with liver disease severity in the Indian sub-continent. Therefore, we studied the prevalence of various genotypes of HCV among subjects with CLD and chronic HCV infection.

Patients and methods

Study design

Sixty-nine consecutive histopathologically proven CLD cases referred from various gastroenterology departments of Government hospitals to the National Reference Center for Viral Hepatitis, Department of Microbiology, Dr ALM PG IMMS, University of Madras, Chennai, India, were studied. The study was approved by the Institutional Review Board (IRB) of the University of Madras.

Specimens and methods

Five milliliters of blood were collected, and serum was separated, aliquoted, and stored at −70 °C until further testing. The following markers of HBV and HCV using commercial ELISA kits viz. HBsAg, anti-HBs, HBeAg/anti-HBe (Biorad Laboratories, USA), and anti-HCV (Murex Diagnostics, UK) with known positive and negative controls were used. Qualitative HBV-DNA polymerase chain reactions (PCR)²³ and qualitative HCV-RNA reverse transcriptase-polymerase chain reactions (RT-PCR)²⁴ were performed with appropriate positive and negative controls. The sensitivities of PCR assays reached 10 copies of HBV-DNA and 50 copies of HCV-RNA per specimen, as determined by testing 10-fold serial dilutions of HBV-DNA/HCV-RNA at known concentrations. HCV genotyping was carried out using a commercial type-specific detection system targeting the PCR-amplified 5′ non-coding region (5′ NCR) (Inno-LiPA, Innogenetics, Belgium) according to the manufacturer’s instructions.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (SPSS for Windows, version 13.0, Chicago, IL, USA). The median values of alanine transaminase (ALT) levels according to age, HBV/HCV, with or without DNA/RNA, and liver status, were analyzed by non-parametric Mann—Whitney U-tests. In addition, whenever variables were continuous, the results were analyzed using the t-test. All p values were two-sided.

Conclusions: The predominance of HCV genotype 1b among CLD patients could pose a major challenge for the efficient management of HCV disease and the development of effective therapeutic interventions in peninsular India.
Results

The clinical and demographic characteristics of 69 CLD patients, consisting of 91% (63/69) with chronic hepatitis (with cirrhosis) and 9% (6/69) with HCC are shown in Table 1. The age range of the patients was 40 to 65 years. Figure 1 shows the risk factors associated with HBV and HCV etiology among the 69 CLD cases studied. Of the 69 CLD cases screened for serological and molecular markers of HBV and HCV, 39 (57%) were HBsAg positive and 30 (43%) were positive for HCV infection. Among the HBV infected CLD cases, 26% (10/39) were HBeAg positive and 74% (29/39) were anti-HBe positive, and none of them were anti-HBs positive. Twenty (51%) cases were positive for HBV-DNA and 23 (77%) were positive for HCV-RNA among the HBV and HCV infected cases respectively. None of these cases had dual infection of both HBV and HCV. All our study cases were tested and found to be negative for HIV-1/HIV-2 infection.

Among the 63 chronic hepatitis patients with cirrhosis, 59% (37/63) were HBV infected and 41% (26/63) were HCV infected. Furthermore, among the six cases with HCC, 67% (4/6) were HCV infected and 33% (2/6) were HBV infected. Among the CLD cases studied based on HBV and HCV etiologies, a significantly ($p < 0.05$) higher level of liver disorders were observed among viremic cases by qualitative PCR as compared to non-viremic HBV and/or HCV infected cases (Table 2). All six HCC patients were positive by diagnostic PCR (two HBV-DNA and four HCV RT-PCR) (Figure 2).

Four types of HCV genotypes (1a, 1b, 3 and 4, and 1a, 1b, with 4) were detectable among HCV RT-PCR positive samples studied by the Inno-LiPA method (Figure 3). When all 23 HCV-RNA positive cases were subjected to HCV genotyping, the majority of these were found to be infected with genotype 1b ($n = 13, 57%$), followed by 1a ($n = 6, 26%$), mixed genotypes of 3 and 4 ($n = 3, 13%$), and one mixed pattern of 1a, 1b, 4 (4%) (Figure 3). The level of transaminase (ALT) was significantly

Table 1 Demographic and clinical characteristics of liver disease patients according to hepatitis infection status ($N = 69$)

<table>
<thead>
<tr>
<th>Finding</th>
<th>HBV alone ($n = 39$)</th>
<th>HCV alone ($n = 30$)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55 (7)</td>
<td>56 (9)</td>
<td>0.822</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.310</td>
</tr>
<tr>
<td>Male</td>
<td>28 (72%)</td>
<td>24 (80%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11 (28%)</td>
<td>6 (20%)</td>
<td></td>
</tr>
<tr>
<td>HBV-DNA/HCV-RNA by PCR</td>
<td>20 (51%)</td>
<td>23 (77%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Hepatic enzyme (ALT)</td>
<td></td>
<td></td>
<td>0.741</td>
</tr>
<tr>
<td>$&lt; 50$ IU/l</td>
<td>70 (56—120)</td>
<td>75 (50—120)</td>
<td></td>
</tr>
<tr>
<td>$51—100$ IU/l</td>
<td>19 (49%)</td>
<td>12 (40%)</td>
<td></td>
</tr>
<tr>
<td>$&gt; 100$ IU/l</td>
<td>12 (31%)</td>
<td>10 (33%)</td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td></td>
<td></td>
<td>0.468</td>
</tr>
<tr>
<td>Chronic hepatitis with cirrhosis ($n = 63$)</td>
<td>37 (95%)</td>
<td>26 (87%)</td>
<td></td>
</tr>
<tr>
<td>HCC ($n = 6$)</td>
<td>2 (5%)</td>
<td>4 (13%)</td>
<td></td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction; ALT, alanine transaminase; HCC, hepatocellular carcinoma.

Figure 1 (a) Risk factors involved in hepatitis B virus (HBV) infected liver disease patients ($n = 39$). (b) Risk factors involved in hepatitis C virus (HCV) infected liver disease patients ($n = 30$).

Figure 2 Hepatitis B virus (HBV) and hepatitis C virus (HCV) in liver disease patients ($N = 69$).
(p < 0.05) elevated among 1b-only infected patients as compared to non-1b HCV genotypes.

**Discussion**

HCV genetic variability is increasingly recognized as a key factor in the prognosis and outcome of CLD. Current guidelines recommend its inclusion in HCV treatment algorithms as a means of optimizing efficacy and maximizing the chances for successful treatment outcome. Different HCV isolates from across the world show substantial nucleotide sequence variability throughout the viral genome. The distributions of HCV genotypes vary according to the geographic region and seem to be related to their time of divergence. It is important to note that this distribution is not static; as the frequency of infection increases, as populations migrate, and as modes of transmission change from transfusion to IVD use, the prevalence of the different genotypes and subtypes within a country or region changes.

Several studies have shown that epidemiological parameters such as age, risk factors, and infection duration may be associated with genotype. In Latin America, Europe, the USA, and Japan, HCV genotypes 1, 2, and 3 account for the majority of infections, with subtype 1b being the most prevalent. Fascinatingly, while most of the north Indian data suggest a high prevalence of genotype 3, studies from the south show a high proportion of genotype 1. In one report, HCV genotypes were studied in a high-risk population, while in the other, a tribal population was studied. From the available data, it is almost consensus that genotype 1 is the more common in this part of India, but it is more likely that the variability in other parts of India is due to the choice of special populations with small numbers of study subjects. The difference between our results and those from other Indian investigators suggests the lower

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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics according to viremic and non-viremic hepatitis infection status (HBV-DNA and HCV-RNA by qualitative PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Findings</td>
<td>Viremic (n = 43)</td>
</tr>
<tr>
<td>Age</td>
<td>57 (8)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (72%)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (28%)</td>
</tr>
<tr>
<td>Virology</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>20 (47%)</td>
</tr>
<tr>
<td>HCV</td>
<td>23 (53%)</td>
</tr>
<tr>
<td>LFT (ALT)</td>
<td></td>
</tr>
<tr>
<td>&lt;50 IU/l</td>
<td>90 (60–140)</td>
</tr>
<tr>
<td>51–100 IU/l</td>
<td>8 (19%)</td>
</tr>
<tr>
<td>&gt;100 IU/l</td>
<td>16 (37%)</td>
</tr>
<tr>
<td>Liver status</td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis with cirrhosis</td>
<td>37 (86%)</td>
</tr>
<tr>
<td>HCC</td>
<td>6 (14%)</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction; LFT, liver function test; ALT, alanine transaminase; HCC, hepatocellular carcinoma.

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![Figure 3](image-url) Prevalence of hepatitis C virus (HCV) genotypes among HCV infected liver disease patients (n = 23).
prevalence (n = 3) of subtype 3. We have clearly demonstrated that genotype 1 (83%) remains the most common genotype with a preponderance of genotype 1b (57%) to 1a in southern India. Furthermore, the prevalence of HCV genotype 1 concords with other earlier reports from this part of South India. Conversely, we suggest that the low prevalence of genotype 3 and very high proportion of genotype 1b among CLD cases may pose a major challenge for the efficient management and development of effective therapeutic strategies in this part of the country. Studies have suggested that some HCV genotypes, especially genotype 1b, lead to a more severe course and appear to be associated with distinct liver manifestations.

While evaluating the association of the different genotypes with demographic data, we could not find any notable difference in genotype distribution among genders. However, investigations in relation to age indicated that older age was strongly associated with genotype distribution. Genotype 1 was common among older patients, while others (viz. genotypes 3 and 4, and 1a, 1b and 4) were common among younger subjects. Therefore, conceptually we can assume that infection with genotype 1b in South India occurred much further in the past than with other genotypes, subtypes, and mixed inter-genotype viruses. Early investigations from the USA failed to find an association between HCV genotypes and transmission modes. However, investigators from Europe reported that patients with a history of blood transfusion were most often infected with genotype 1b and IVDJ abusers with genotype 3a. Our results have shown that genotype 1b is more predictive of blood transfusion and tattooing, in agreement with the above reports. Although we did not confirm the correlation of genotype 3 and 4 with any particular mode of HCV transmission, the presence of this genotype as the independent negative risk factor for blood transfusion may suggest certain other routes of transmission. Additionally, it is possible to conceptualize that the relatively high prevalence of mixed-inter-genotypes 3 and 4 (13%) among our cases reflects a possible re-infection in the same subjects, although we were unable to associate these genotypes with the transmission mode. Moreover, the association of genotype 1 (mostly subtype 1b) with advanced HCV-related liver disease has been proven repeatedly in this part of India. Therefore, we assume that this association could be paradigmatic of earlier infections with a similar subtype in comparison with subtype 3 and mixed infection with subtype 4. Earlier reports also suggested that subtype 1b could be associated with a more advanced stage of liver disease.

Steatosis is reported to be an important cofactor of liver necro-inflammation and is believed to be central for progression to fibrosis among chronic HCV cases. This has recently been confirmed among subtype 3 infected north Indian subjects. However, we failed to notice this correlate among our study subjects.

Despite the increasing understanding of the biologic and clinical aspects of HCV infection, this virus continues to be a major challenge to both virologists and physicians in the area of treatment and clinical management. Thus, we conclude that HCV is the second most prevalent virus among the CLD cases in southern India. It is clinically intriguing that while HCV genotype 3 is more prevalent in northern India with significant hepatic steatosis and fibrosis, the intrinsically INF-α resistant HCV genotype 1b remains highly prevalent in southern India. The role of subtype 1b that reportedly correlates with higher histological activity reflects the potentially pathogenic feature of this subtype, with consequences such as longer persistence and more aggressive influence on disease outcome that requires careful analysis in prospective settings. Hence, there is an urgent need for controlled multicentric clinical trials in India to clarify the potential risk factors, including specific HCV genotypes and treatment outcome. Alternative management and treatment strategies are also to be designed for managing HCV mediated CLD in India.

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Conflict of interest: No conflict of interest to declare.

References


