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Original Article

Seroprevalence of Hepatitis C Virus in Liver Disease Patients and Blood Donors from Northern India

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ABSTRACT

Background: The molecular epidemiology of HCV and its association with liver diseases in North India is not well understood.
Aim: To assess the incidence of HCV infection in blood donors and liver disease patients and the influence of HCV genotype on the severity of the liver disease.
Methods: We screened 487 patients with acute viral hepatitis (AVH), 141 patients of fulminant hepatic failure(FHF), 1058 patients of chronic liver disease (CLD) (chronic hepatitis-468, cirrhosis-527, HCC-63), and 3504 voluntary blood donors for anti-HCV. Anti HCV positive patients were further subjected to HCV RNA testing followed by genotyping.
Results: HCV infection was observed in 1.6%, 12.94%, 10.64%, 27.13%, 21.25% and 49.2% of blood donors, AVH,FHF, chronic hepatitis, cirrhosis and HCC patients respectively.
Conclusion: Genotype 3 was found to be the major genotype. HCV genotype one infection was associated with advanced liver disease.

KEYWORDS: Hepatitis C Virus, Chronic liver disease, Acute liver failure, North India, Epidemiology.

Introduction

Hepatitis C Virus (HCV) infection is the leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide and is emerging as a key infection among the developing countries of Asia and Africa.[1] The HCV infection is transmitted through various modes like transfusion of blood and blood products, intravenous drug abuse, tattooing, sexual transmission, and occupational exposure. However, the transmission of HCV related to blood products has decreased in most developed countries by universal HCV screening.

The global prevalence of HCV has increased from 122 million to185 million from 1990 to 2005.[2] The prevalence of HCV infection varies among the general population in different geographical regions of India.

The HCV prevalence varies from 1.4 to 2.02% in the South, 0.71% in the East, 0.09% in the West, and 7.89% in the North East in the general population.[3] Another study from India reported the 20.6% of HCV infection in acute hepatitis patients and 10.8% to 48.5% in chronic liver disease patients.[3,4] HCV endures as a chronic infection in ~75-85% of cases and is a major risk factor for the development of liver cirrhosis, extra-hepatic complications, and hepatocellular carcinoma.[5]

HCV isolates have been classified into six major genotypes and one new sequence, provisionally assigned as subtype 7a, which has been found with new recombinant forms of HCV having different crossover points.[6-10]

Age, duration of infection, co-infections with HIV or other hepatotropic viruses, and the virus intrinsic factor-like genotypes/quasispecies are the important parameters that may influence the outcome of the infection. HCV genotype one is associated with a longer therapy requirement and an increased risk of advanced-stage liver diseases (liver cirrhosis and hepatocellular carcinoma) compared to other genotypes.[6] Higher risks of HCC and mortality related to HCV genotype three infections have also been reported in some cohorts.[7,8,9] HCV genotype has been recognized as a strong independent risk factor in achieving sustained virological response.[11]

Hepatitis C virus genotype 3a is particularly prevalent in intravenous drug abusers in Europe and the United States.[12,13] There is a high prevalence of anti-HCV (86%) and both HBsAg and anti-HCV (9.2%) among the drug abusers in India.[14] Hepatitis C virus genotyping is an essential tool for epidemiological studies and tracing the source of contamination.

This study has been designed to determine the transmission mode of HCV infection, the incidence of HCV infection in blood donors and liver disease (ALD and CLD), and the association of HCV genotypes with disease severity in blood donors and liver disease patients from North India.

Methods

Patient selection and recruitment

The study included 1686 patients with liver disease and 3,504 consecutive, voluntary blood donors. Among the liver disease patients, 628 were of acute liver disease (ALD) (AVH-487, FHF- 141), and 1058 were of chronic liver disease (CLD) (CAH-468, Cirrhosis-527, HCC-68). These patients were recruited from the medical wards and Out-Patients Departments (OPD) of Maulana Azad Medical College and associated Lok Nayak hospital during the period from Dec 2008 to Jan 2015. 3,504 blood donors were included in the study from various blood banks in Delhi. The information of possible risk factors of the transmission of infection, history, and clinical examination details were recorded on the pre-designed proforma.

The study was conducted as per the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional ethical committee of Maulana Azad Medical College, New Delhi. Informed consent was taken from all the participants.

5 ml of blood samples were drawn from the patients, and the serum was separated. All the ALD patients were screened for liver function test, serology of hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), and HCV RNA. All CLD patients were screened for liver function test, serology of HBV, HCV. HCV RNA was screened in anti-HCV-positive CLD patients. HCV genotyping was done in HCV RNA-positive CLD patients. Three ml of blood samples were drawn from the blood donors, and serum was separated. The blood donors were screened for anti-HCV.

Diagnostic criteria

Acute Viral Hepatitis (AVH): AVH was defined in the cases which had increased levels of alanine transaminases to >10 times the upper limit of normal, with or without an increase in total bilirubin level. In the patients with acute hepatitis C, positivity for HCV RNA and exposure to the Hepatitis C virus during the preceding two-twelve weeks was actively asked for.

Fulminant hepatic failure (FHF): FHF was considered to exist when, after a typically acute onset, the patient became deeply jaundiced and went into hepatic encephalopathy within eight weeks of the onset of the disease, with no history of chronic hepatitis.[15]

Chronic Active Hepatitis (CAH): The patients with chronic active hepatitis and cirrhosis of the liver

were diagnosed by histopathological criteria laid down by the International Study Group on Chronic Hepatitis.[16] Chronic active hepatitis group included those patients who had a persistent elevation of transaminases levels (at least twice the upper limit of normal range) for more than six months and histologic evidence of chronic hepatitis on liver biopsy at the beginning of follow-up.

Liver Cirrhosis (LC): Diagnosis of cirrhosis was considered when the patient had features of decompensation in the form of (i) ascites, having evidence of shrunken liver, and the presence of splenomegaly. (ii) endoscopy showing the presence of esophageal varices and (iii) ultrasound findings suggesting altered echotexture, small-sized liver, and caudate lobe hypertrophy.

Hepatocellular carcinoma (HCC): HCC was diagnosed based on either pathological or cytological examination or an elevated -fetoprotein level (≥400 ng/ml) combined with at least one positive image on angiography, Sonography, or Computerized Tomography. Diagnosis of Hepatitis C virus infection was based on the criteria as described earlier.[17]

Serology of HAV, HBV, HCV, and HEV

The viral serology of HAV, HBV, HCV, and HEV was done using an ELISA kit. Anti-HAV IgM (Biokit, Barcelona, Spain) for HAV, HBsAg (Biokit, Barcelona, Spain) for hepatitis B, Anti-HCV (Innogenetics NV, Ghent, Belgium) for HCV, and HEV IgM (Genelabs Diagnostics, Singapore) for HEV as per the manufacturer's instructions.

Reverse Transcription – Polymerase Chain Reaction (RT-PCR) for HCV RNA: HCV RNA was extracted using a viral RNA extraction kit (Qiagen, GmBH, Hilden, Germany) according to the manufacturer's instructions. HCV RNA was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primers for the conserved 5' UTR region as described earlier by our group. 18 Two sets of primers were used to amplify the HCV RNA. The primers used were : Outer primers HC-1 (+): 5'-CTGTGAGGAACTACTGCTT-3', HC-2(-): 5'- GTGCTCATRGGTGCACGGTCTACGAGACCT CCGG-3' and inner primers HC-3 (+): 5'-TTCACGCAGAAAGCGTCTAG-3', HC-4 (-): 5'-CACTCGCAASGCACCCTATCAGGCATGCA-3'. The size of the PCR product observed on the agarose gel electrophoresis was 251 bp. Amplification of the specific product was carried out by PCR Thermal cycler (PTC-100.MJ Research, Watertown, Massachusetts, USA).

HCV genotyping by INNO-LiPA HCV II: HCV genotype was screened in all CLD patients positive for HCV RNA (n=251). Out of the total 251 HCV RNA positive CLD cases, 52 HCV-RNA positive cases were genotyped using the second generation INNO-LiPA HCV II method using INNO-LiPA HCV II kit (Belgium Innogenetics) per the manufacturer's instructions, and the probe reactivity patterns were interpreted by using the chart provided by the manufacturer. Another research group has also used this method.[19]

HCV genotyping by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP):199 HCV-RNA positive cases were genotyped by PCR-RFLP. HCV genotyping was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously by our research group.[20,21]

Statistical Methods: The data were tabulated and analyzed using SPSS software (Version 19.0). Quantitative parameters were expressed as mean ± S.D. Differences in the means between the two groups were analyzed using the Student's t-test. The differences in proportion between the different groups were analyzed by using the Chi-square test of significance. P-value ≤ 0.05 was considered significant.

Results

Demographic, biochemistry, and transmission factors of the study population

The demographic and biochemical parameters of the liver disease patients have been depicted in **table 1**. Female patients predominated in the AVH and FHF clinical categories, while male patients predominated in the CAH, liver cirrhosis, and HCC clinical categories. Transaminase level in AVH and FHF patients was very high compared to the CAH, liver cirrhosis, and HCC patients. However, the biochemical parameters between HCV positive and HCV negative patients in all the clinical categories (AVH, FHF, CAH, liver cirrhosis, and HCC) were comparable,

and the difference was not statistically significant within the clinical category.

The prevalence of HCV infection was detected in 1.6 % (56/3504) of voluntary blood donors and 20.64% (348/1686) of the patients with liver diseases. The difference in the prevalence of HCV infection was found to be statistically significant in liver disease patients ($p<0.0001$) compared to the voluntary blood donors.

The mode of transmission of HCV infection was identified based on the response of the liver disease patients in the questionnaire (**table-2**). Blood and blood products transfusion was observed as the most frequent source/ risk factor, followed by intravenous drug abuse, tattooing, and sexual transmission.

Hepatitis C Virus infection in acute liver disease patients:

Among the AVH patients, HCV RNA was detected in 12.94% (63/487) of patients.(**Table 3**). HCV co-infection with HBV and HEV was detected in 14.28% (9/63)and 46.04% (29/63) of the patients, respectively, while no co-infection of HAV and HCV was observed. Exclusive HCV infection was detected in 32.68% (25/63) of these patients (**Table 4**).

Among the FHF patients, HCV RNA was detected in 10.64% (15/141) of patients, and no HCV antibody was observed (Table-3). HCV co-infection with HEV was detected in 61.67% (10/15), while no co-infection of HAV and HBV with HCV was detected in these patients.

Table 1: Demographic and biochemical parameters of the liver disease patients.

Parameters Mean +/- S.D	Acute Viral Hepatitis (N = 487)	Fulminant Hepatic Failure (N = 141)	Chronic Hepatitis (N = 468)	Liver Cirrhosis (N = 527)	Hepatocellular Carcinoma (N=63)
Age range	44.5 +/- 17.2 (19-63 years)	33.2+/-8.6 (16-50 years)	42.12+/-16.7 (24-61 years)	44.34 +/- 18.16 (34-69 years)	49.34 +/- 16.78 (39-71 years)
Sex (M:F)	1.5:1	1:04	2:01	7:01	8:01
ALT (IU/L)	293.41+/-436.71	106.57+/-898.61	63.56+/-57.18	65.46+/-56.32	70.54+/-62.42
AST (IU/L)	274.67+/-323.47	1125.38+/-1026.43	60.37+/-59.28	72.62+/-70.34	69.14+/-54.62
ALP (IU/L)	76.26+/-104.73	212.83+/-172.34	38.42+/-43.34	73.43+/-95.32	86.63+/-76.12
T. Bil (mg/dl)	2.96+/-1.52	4.97+/-2.87	1.1+/-0.32	1.3+/-0.53	1.3+/-0.26

ALT : Alanine Transaminases, AST: Aspartate Transaminases, ALP: Alkaline Phosphatases, T. Bil: Total Bilirubin

Table 2: Mode of transmission of HCV infection in this study (n=348).

Mode of transmission	Number of patient (%)
Transfusion of blood and blood products	84 (24.14%)
Intravenous drug abuse	76 (21.84%)
Tattoo	49 (14.08%)
Sexual transmission	37 (10.63%)
Occupational exposure	6 (1.72%)
Unknown	96 (27.59%)

Table 3: Prevalence of HCV infection in the patients of acute liver disease (n=628).

Vira Infection	AVH, N=487 (%)	FHF, N=141 (%)
Anti HCV	5 (1.02%)	0 (0%)
HCV RNA	63 (12.94%)	15 (10.64%)
Anti HCV + HCV RNA	5 (1.02%)	0 (0%)

Exclusive HCV infection was detected in 33.33% (5/15) of these patients (**Table 4**).

Hepatitis C Virus infection in chronic liver disease patients

Among the chronic active hepatitis patients, anti-HCV and HCV RNA was detected in 27.13% (127/468) and 22.57% (115/468) of patients, respectively (**Table 5**). HCV co-infection with HBV was detected in 11.02% (14/127) of the patients. Exclusive HCV infection was detected in 88.98% (113/127) of these patients (**Table 6**).

Among cirrhotics, anti-HCV and HCV RNA was detected in 21.25% (112/527) and 19.92% (105/527) of patients, respectively (**Table 5**). HCV co-infection with HBV was detected in 11.6 % (13/112) of the patients. Exclusive HCV infection was detected in 88.4% (99/112) of these patients (**Table 6**).

Among HCC patients, anti-HCV and HCV RNA were detected in 49.2% (31/63) of these patients (Table-V). HCV co-infection with HBV was detected in 9.67 % (3/31) of the patients. Exclusive HCV infection was detected in 90.33% (28/31) of these patients (**Table 6**).

Distribution of HCV genotypes

HCV genotype was screened in the HCV RNA (N=251) positive CLD patients. Genotype three was the most frequent genotype, followed by genotypes one and two. Genotype three was detected in 52.98% (133/251) of the patients. The subtypes 3a, 3b was detected in 43.43% (109/251), 3.98% (10/251) of the patients, respectively. 5.57% (14/251) patients with genotype three could not be subtyped. Genotype 1 was detected in 41.44% (104/251) of the cases with its subtypes 1a and 1b in 7.97% (20/251) and 33.47% (84/251) of the cases, respectively. Genotype two was detected in 5.58% (14/251) of the cases, and these cases were of subtype 2b (**Table 7**). Interestingly, genotype 2b was detected only in the patients of the 40-50 years age group.

Table 4: Co-infections in hepatitis C associated acute liver diseases (n=78).

Infection/Disease	AVHN = 63(%)	FHFN = 15(%)
HCV + HAV	0 (%)	0 (0%)
HCV + HBV	9 (14.28%)	0 (0%)
HCV + HEV	29 (46.04%)	10 (66.67%)
Exclusive HCV	25 (39.68%)	5 (33.33%)

Table 5: Profile of serology and PCR in chronic liver disease cases (n=1058).

Virus Infection	Chronic Hepatitis N = 168 (%)	Cirrhosis N = 527 (%)	HCC = 63(%)
Anti HCV	127 (27.13%)	112 (21.25%)	31 (49.2%)
HCV RNA	115 (22.57%)	105 (19.92%)	31 (49.2%)

Table 6: Co-infections in hepatitis C associated chronic liver diseases (n=270).

Virus Infection	Chronic Hepatitis N = 127 (%)	Cirrhosis N = 112 (%)	HCC = 31 (%)
HCV + HBV	14 (11.02%)	13 (11.6%)	3 (9.67%)
Exclusive HCV	113 (88.98%)	99 (88.4%)	28 (90.33%)

Table 7: Incidence of HCV genotype and its association with the severity of liver diseases (n=251).

Genotype/ Subtube	Patients N=251 (%)	CAH (N = 115)	Cirrhosis (N=105)	HCC (N=31)	P Value < 0.05
1	**104 (41.44%)**	**29 (25.22%)**	**58 (55.24%)**	**17 (54.84%)**	Cirrhosis vs CAH HCC vs CAH
1a	20 (7.97%)	6 (5.22%)	10 (9.52%)	4 (12.9%)	
1b	84 (33.47%)	23 (20%)	48 (45.71%)	13 (41.94%)	
2	**14 (5.58%)**	**7 (6.08%)**	**5 (4.76%)**	**2 (6.45%)**	N.S. in all
2b	14 (5.58%)	7 (6.08%)	5 (4.76%)	2 (6.45%)	
3	**133 (52.98%)**	**79 (68.70%)**	**42 (40%)**	**12 (38.71%)**	CAH vs Cirrhosis CAH vs HCC
3a	109 (43.43%)	65 (56.52%)	35 (33.34%)	9 (29.04%)	
3b	10 (3.98%)	6 (5.22%)	3 (2.85%)	1 (3.22%)	
3*	14 (5.57%)	8 (6.96%)	4 (3.81%)	2 (6.45%)	

*Non subtypable, N.S. - Non significant.

Association of HCV genotype with disease severity

25.22% (29/115) of CAH, 55.24% (58/105) of cirrhosis, and 54.84% (17/31) of HCC cases were found to be infected with HCV genotype 1. HCV genotype 1 infection was statistically significantly higher in cirrhosis and HCC patients in comparison to CAH patients. However, infection of HCV genotype 1 was statistically non-significant between cirrhosis and HCC patients (**Table 7**). 68.7% (79/115) of CAH, 40% (42/105) of cirrhosis, and 38.71% (12/31) of HCC cases were infected with HCV genotype 3. HCV genotype 3 infection was statistically significantly higher in CAH patients in comparison to cirrhosis and HCC patients. However, HCV genotype 3 was statistically non-significant between cirrhosis and HCC patients (**Table 6**). 6.08% (7/115) of CAH, 4.76% (5/105) of cirrhosis, and 6.45% (2/31) of HCC cases were found to be infected with HCV genotype 2. There was no significant difference in the prevalence of genotype 2 among CAH, cirrhosis, and HCC (**Table 7**).

Discussion

The prevalence of anti-HCV among voluntary blood donors in this study was 1.6% (56/3504) which is in concordance with another study published previously by our group.[22] It is well-known that more than 60% (55-85%) of the patients with HCV-related acute liver disease progress to chronicity, which is established by elevated transaminases and the presence of HCV-RNA after six months of infection.[23]

The association of transaminase levels with HCV infection has been reported earlier.[24] The correlation between alanine transaminase (ALT) and liver damage histological parameters has also been reported.[25]

HCV infection was detected in 12.94% (63/487) of the AVH patients in the present study. These results are quite at par with the earlier reported prevalence of 12% of HCV infection in AVH patients.[26] Comparable rates of HCV infection of 11.98%, and 20.6% have been reported in AVH patients from Northern India.[27] Likewise,HCV infection was detected in 10.64% (15/141) of FHF patients in this study. This is in concordance with our previous study reporting 15.5% of HCV infections.[26] In another report, the rarity of HCV in FHF has also been reported.[28] It is important to note that more than 60% of AVH and FHF patients had a co-infection with other hepatotropic viruses in this study. Therefore, the prospect of the potential association of these viruses with the causation of AVH and FHF in the patients cannot be ruled out, and the HCV infection may be considered to play a minor role in the wide spectrum of acute liver diseases.

The prevalence of 27.13%, 21.25%, and 49.2 of HCV infections was detected in CAH, liver cirrhosis, and HCC patients, respectively, in this study. Thus, the HCV infection contributes notably to the causation of liver disease in this study. These observations agree with the previous reports from India, where the prevalence of

hepatitis C in India has ranged from 10.8% to 48.5% in patients with chronic liver disease.[3]

The HCV genotype has been established as a major viral factor influencing the outcome of liver diseases. Therefore, it is important to screen the genotypes in chronic liver disease patients. Genotype 3 was the most common HCV genotype in our group, followed by genotype 1 in this study and found in 52.98% (133/251) and 41.4% (104/251) of the patients. Genotype 2 was detected in 5.58% of the cases. These results are in accordance with the previous studies from North India, where HCV genotype 3 has been detected as the most common genotype. 29, 30, 31 HCV 3a was the most common genotype detected in 50-60%, followed by genotype 1a, 1b, and 6 in Thailand.[32] The association of HCV genotype with the disease severity is another significant aspect of the present study. The reports on the association between HCV genotype and their risk for causing the advanced stage of liver disease are inconsistent. A review based on the meta-analysis of 21 research papers calculated age-adjusted risk and reported that HCV genotype 1b infection had an about two-fold greater risk (a pooled relative risk of 1.78) of developing HCC as compared to other HCV genotypes.[6] Our results were also in accordance with that report. In our study, the HCV genotype 1 was significantly higher statistically in HCC compared to CAH patients. The HCV genotype 1 was also significantly higher statistically in cirrhosis compared to CAH patients. However, the prevalence of HCV genotype 3 was significantly higher statistically in CAH patients compared to cirrhosis and HCC patients. Therefore, these findings suggest that HCV genotype 1 may be a major factor for the prognosis or the development of the HCC, liver cirrhosis compared to genotype 3.

Conclusion

The seroprevalence of 1.6% in the voluntary blood donors may approximate the rate of HCV infection in the population. HCV appears to be a major etiological agent in CLD (CAH, cirrhosis & HCC) but not in ALD (AVH & FHF). HCV genotype 3 followed by 1 are the most common genotypes in our study and together contribute to ~95% of the study participants. Genotype 1 is associated with advanced stage of chronic liver disease (HCC, cirrhosis) compared to genotype three.

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