

## Seroprevalence of HCV and its co-infection with HBV and HIV among liver disease patients of South Tamil Nadu

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### Abstract

**AIM:** To determine the seroprevalence of hepatitis C virus (HCV) and its co-infection with hepatitis B virus (HBV), hepatitis delta agent (HDV) and human immunodeficiency virus (HIV) among liver disease patients of south Tamil Nadu.

**METHODS:** A total of 1012 samples comprising 512 clinically diagnosed cases of liver disease patients and 500 apparently healthy age and sex matched individuals were screened for Hepatitis C virus (anti HCV and HCV RNA), Hepatitis B virus (HBsAg), Hepatitis delta agent (anti HDV) and Human immuno virus (antibodies to HIV-1 and HIV-2) using commercially available enzyme linked immunosorbent assay kits. HCV RNA was

detected by RT-PCR. Liver function tests like ALT, AST, GGT, ALP, bilirubin and albumin were also studied.

**RESULTS:** The seroprevalence of HCV was found to be 5.6% among liver disease patients by ELISA. 27/512, 49/512 and 12/512 patients were positive for HIV, HBV & HDV respectively. Co-infection of HCV & HBV was found in 8 patients, with 6 for HCV & HIV and 4 for HCV, HBV & HIV co-infections. Sex-wise analysis showed that HIV, HCV & HBV and HCV & HIV co-infection was high among females whereas for HBV it was high in males. The mean ALT and AST in HCV positive cases were  $42.1 \pm 8.3$  and  $49 \pm 10.1$ . In people co-infected with HCV & HBV or HCV & HIV or HCV, HBV & HIV the mean ALT of  $58.0 \pm 03.16$ ,  $56.78 \pm 4.401$  and  $64.37 \pm 4.01$  respectively.

**CONCLUSION:** We strongly recommend routine test of the blood for HCV in addition to HBV and HIV. We also recommend individualized counseling to identify those at risk and testing for those who want it. Improved surveillance and periodic epidemiological studies will have to be undertaken to monitor and prevent these blood-borne viruses.

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**Key words:** Hepatitis C virus; Hepatitis B virus; Human immunodeficiency virus; Co-infection; Liver function test

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## INTRODUCTION

Hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the world<sup>[1]</sup>. HCV belongs to the family *flaviviridae* and the genus *hepacivirus*. It often causes lifelong persistent infection<sup>[2]</sup>. The prevalence of HCV infection worldwide has been estimated to be about 3% with 170 million people affected by HCV<sup>[3]</sup>. Meanwhile two billion people have been infected with hepatitis B virus (HBV). Of these, 360 million have chronic infection and 600 000 die each year due to HBV infection and related liver diseases<sup>[4]</sup>. HBV and HCV infections account for a substantial proportion of liver diseases worldwide. The majority of those with chronic HBV and/or HCV infection will develop complications i.e. 15%-40% may develop cirrhosis, liver failure and or hepatocellular carcinoma<sup>[5]</sup>. The exact number of patients infected with both HCV and HBV world wide is unknown<sup>[6]</sup>. It has been estimated that over the next 20 years, the proportion of HBV/HCV infected patients with cirrhosis will increase from 16% to 32% and that other complications will also increase dramatically including hepatic decompensation (increasing by 106%), HCC (increasing by 81%) and liver related deaths (increasing by 180%)<sup>[7]</sup>. In addition, prevalence of human immunodeficiency virus (HIV) is increasing everyday and it has become a disaster for humankind in certain areas. HIV accounted for 38.6 million infections world wide by the end of 2005<sup>[8]</sup>. These three viruses (HCV, HBV and HIV) have similar routes of transmission, namely through blood and blood products. Sharing of needles to inject drugs and sexual activity enables the co-infection of these viruses and thereby makes co-infection or super infection a common event<sup>[9]</sup>. End stage liver disease is currently a major concern among HIV positive individuals due to co-infection with hepatotropic viruses<sup>[10,11]</sup>. HIV infected patients with multiple hepatitis virus infections have a higher rate of liver related morbidity and mortality than patients with HIV infection alone or with a single hepatitis virus infection. The degree of immunodepression is an important factor in liver disease progression<sup>[12]</sup>. Current estimations indicate that approximately 1.8% to 2.5% of Indian population is presently infected by HCV<sup>[13]</sup>. The prevalence as well as the significance of HCV infection varies considerably from country to country, probably because of cultural factors and social habits that influence HCV transmission. A community based Indian study on HCV indicated a seroprevalence of 0.87% and that the rate reportedly increased from children < 10 years to 1.85% among subjects > 60 years of age<sup>[14]</sup>. Knowledge and awareness of HCV infection have been obtained from seroprevalence studies carried out in blood donors and hemodialysis patients from large cities<sup>[15-17]</sup>. Reports on the prevalence of HCV infection in the Indian subcontinent is scarce. Hence, this study was conducted to investigate the seroprevalence of HCV and its co-

infection with HBV and HIV in liver disease patients in South Tamil Nadu.

## MATERIALS AND METHODS

### Study samples

The study was performed in 512 clinically diagnosed cases of liver disease patients and 500 apparently healthy age and sex matched controls. All the liver diseases and control samples were diagnosed and given by the Gastroenterologist based on signs, symptoms and examination. Blood samples were collected from them, serum was separated and stored at -20°C until use. Among the liver disease patients clinical conditions including acute liver disease, chronic liver disease and cirrhosis were also diagnosed by the Gastroenterologist.

### Serology

The blood samples were screened for markers of various hepatitis viruses and HIV by using third generation ELISA kits. Hepatitis B surface antigen (HBsAg) was screened by Hepalisa, supplied by M/s. J. Mitra and Co Pvt. Ltd, India; Hepatitis B envelop antigen (HBeAg) and antibody to envelop antigen (anti-HBe) using ELISA kits supplied by M/s. Biorad laboratories, USA.; Antibodies to HCV, HDV and HIV were screened by using Microlisa kits supplied by M/s. J. Mitra and Co Pvt. Ltd, India. All reactive analyses were repeated twice.

### Biochemistry

Liver function tests studied were alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), serum alkaline phosphatase (SAP), bilirubin and albumin. The results were correlated with serological findings. The upper limits of normal for various tests were ALT 0-65 IU/L, AST 5-40 IU/L, GGT 0-61 IU/L, SAP 20-140 KA, Bilirubin Total 0.3 to 1.9 mg%, Direct *bilirubin*: 0 to 0.3 mg%, Indirect bilirubin 0.1-1.0 mg% and Albumin 35-50 gms%.

### Molecular diagnosis of HCV

For the detection of the HCV genome, RNA was extracted from the samples and was subjected to reverse transcriptase polymerase chain reaction (RT-PCR). This was carried out for the constant HCV 5' untranslated region (5' UTR).

### Statistical analysis

Statistical package for social sciences (SPSS, version 17.0) software was used for analyzing the data. Statistical tests used to find the significance were  $\chi^2$  for association of attributes and *t*-test for difference in the mean for independent samples.

## RESULTS

Among the 512 liver disease patients investigated, 281 were male and 231 were female. Overall 29 (5.6%) were

**Table 1** Incidence of blood borne viral infection (HCV, HBV, HDV and HIV) among liver disease patients *n* (%)

Category	Total number tested	Gender		HCV, HBV, HDV and HIV positive			
		Male	Female	HCV	HBV	HDV	HIV
Liver disease	512	281	231	29 (5.6)	49 (9.5)	12 (2.3)	27 (5.2)
Control	500	250	250	-	7 (1.4)	-	-
Total	1012	531	481	29	56	12	27

**Table 2** Incidence of blood borne viral infection and co-infection among liver disease patients

Category	Clinical condition	Gender						Total
		Male			Female			
		Virus positive	Virus negative	Total	Virus positive	Virus negative	Total	
Liver disease	Acute liver disease	22	55	77	12	38	50	127
	Chronic liver disease	45	34	79	26	51	77	156
	Cirrhosis	14	31	45	7	33	40	85
	Others	16	64	80	2	62	64	144
	Total	97	184	281	47	184	231	512
Control	Without any signs or symptoms	4	246	250	3	247	250	500
Total		101	430	531	50	431	481	1012

$\chi^2 = 12.598$  for *df* = 1, *P* < 0.01 for gender *vs* viral infection.  $\chi^2 = 41.33$  for *df* = 3, *P* < 0.01 for clinical condition *vs* viral infection.

**Table 3** Clinical condition *vs* blood-borne viral infection

Clinical condition	Different viral infection positive									Total virus negative	Total number tested
	HCV	HBV	HIV	HDV	HCV and HBV	HCV and HIV	HBV and HIV	HCV, HBV and HIV	Total virus positive		
Acute liver disease	-	19	10	4	-	-	2	-	35	92	127
Chronic liver disease except cirrhosis	13	11	17	8	6	6	6	4	71	85	156
Cirrhosis	12	5	-	-	2	-	1	-	20	65	85
Others	4	14	-	-	-	-	-	-	18	126	144
Total	29	49	27	12	8	6	9	4	144	368	512

$\chi^2 = 41.631$  for *df* = 3, *P* < 0.01.  $\chi^2$  is calculated for clinical condition against total viral infection.

positive for HCV. This positivity was either for anti HCV by ELISA, for HCV RNA by RT-PCR or for both. Results based on other viral infection revealed 27 (5.2%) for HIV, 49 (9.5%) for HBsAg, and 12 (23%) for HDV (Table 1). Table 2 shows the incidence of blood borne viral infection and co-infection among liver disease patients. Co-infection of HCV & HBV was found in 8 patients, with 6 for HCV & HIV and 4 for HCV, HBV & HIV co-infections. In addition, clinical conditions of all viral infections and co-infections were significantly (*P* < 0.01) associated with liver disease (Table 3). Table 4 gives the age-wise distribution of patients with different kinds of viral infections. The total viral positivity was insignificant in various age groups (*P* > 0.05). Among the viral positive cases, HBV was most prevalent followed by other viral infections and co-infections whilst the rest were insignificant (*P* < 0.05) (Tables 5 and 6). The results of liver function tests in HCV infected and co-infected patients are presented in Table 7. All the parameters tested were found to increase significantly (*P* < 0.05) when compared to controls. Data collected

based on risk factor for HCV seroconversion reveals, 47.6% of the patients had blood transfusion, 6 had surgical intervention (14.2%), 5 were intravenous drug users (11.9%) and 11 (26%) had unknown causes. Blood transfusion (65%) was observed as the predominant risk factors in the co-infected patients.

## DISCUSSION

HIV, HBV and HCV are the three most common chronic blood borne viral infections documented worldwide<sup>[18]</sup>. Epidemiological studies of blood-borne viral disease such as HCV, HBV and HIV are important for revealing the risk groups and risk factors for these infections. Screening these groups of viruses helps us to solve difficulties in collecting information among healthy populations<sup>[19]</sup>.

India has the second highest number of people living with HIV infection<sup>[20]</sup>. Co-infection of hepatotropic viruses with HIV infection reportedly leads to massive impairment of cell mediated responses and enhances

Table 4 Different viral infection *vs* age groups

Viral infection	Age group in years						Total
	11-20	21-30	31-40	41-50	51-60	61 and above	
HCV	2	6	7	7	4	3	29
HBV	5	19	6	10	7	2	49
HIV	-	5	4	8	7	3	27
HDV	-	1	5	6	-	-	12
HCV and HBV	1	2	1	1	2	1	8
HCV and HIV	1	1	2	1	-	1	6
HBV and HIV	1	1	4	2	-	1	9
HCV, HBV and HIV	1	-	1	1	-	1	4
All virus positive	11	35	30	36	20	12	144
All virus negative	42	83	82	94	51	16	369
Total	53	118	112	130	71	28	512

$\chi^2 = 4.689$  for  $df = 5$ ,  $P > 0.05$ ,  $\chi^2$  is calculated for age against total viral infection.

Table 5 Different viral infection *vs* sex

Viral infection	Gender		P value
	Male	Female	
HCV positive	18	11	0.423
HCV negative	263	220	
HBV positive	41	8	0
HBV negative	240	223	
HIV positive	14	13	0.745
HIV negative	267	218	
HDV positive	8	4	0.406
HDV negative	273	227	
HCV and HBV positive	5	3	0.663
HCV and HBV negative	276	228	
HCV and HIV positive	3	3	0.809
HCV and HIV negative	278	228	
HBV and HIV positive	6	3	0.474
HBV and HIV negative	275	228	
HCV, HBV and HIV positive	2	2	0.844
HCV, HBV and HIV negative	279	229	

$\chi^2$  is calculated for gender *vs* different category of viral infections;  $P < 0.05$  is significant, while  $P > 0.05$  is insignificant.

the kinetics of hepatotropic viral replication<sup>[21-24]</sup>. The prevalence of HCV in this study is 8.2% in liver disease patients. A study conducted by Chowdhury *et al.*<sup>[25]</sup> (2003) from eastern India showed a prevalence of 0.87%, which is ten times less than that observed in the present study. That study predominantly comprised blood-transfusion-acquired HCV infections rather than other modes of transmission. Age-wise analysis in the present study found HCV to be high among individuals belonging to the 41-50 years (28.5%) age group. HCV infections usually progress slowly to terminal liver disease<sup>[26]</sup>. It is, therefore, possible to recognize the impact that the disease may have in the future based on the knowledge of its previous incidence. It is also feasible to estimate the burden of late complications associated directly with the presence of chronic liver disease<sup>[27]</sup>.

It was observed that males were more susceptible to HCV than female among the study population. This concurs with a previous report that male subjects were at a higher risk of developing HBV infection than

females<sup>[28-29]</sup>.

Out of the 512 liver disease patients tested 49 (9.5%) were positive for HBsAg. Similar results were obtained in an earlier study conducted on chronic liver disease in India<sup>[30]</sup>. A multicenter study in Italy showed that the subjects with dual HBV and HCV infection were more likely to be older than 42 years<sup>[31]</sup>. Similar results (1.5%) were found in the current study.

Sud *et al.*<sup>[28]</sup> (2001) have reported 33.8% prevalence of HBV co-infection in HIV positive patients. Although the effect of HBV infection on HIV is uncertain, HIV appears to have marked influence on the natural history of HBV infection. Although HIV shares a common route of infection with HBV and HCV, its sexual transmission is known to be relatively efficient whereas the sexual transmission of HCV appears to be significantly less efficient than for HIV. Although detailed reports have documented HCV, HBV and HIV co-infection worldwide, only a few reports have been published regarding co-infection in India. Kumar *et al.*<sup>[32]</sup> (2003) reported a 2.9% co-infection of HBV and HIV in patients with liver diseases. The increased viral replication of HBV in AIDS patients indicates that HIV significantly affects the HBV life cycle and the host ability to clear HBV infection. If this is true, more HBV infection and more chronic carriers would be expected as the AIDS epidemic expands in this part of the country. Such a profile would have worrying public health implications<sup>[33]</sup>. The frequency of HCV & HIV co-infection in this study (1.9%) is much lower than that reported previously among HCV & HIV co-infections in India<sup>[29,34-38]</sup> and higher than from the general Indian population<sup>[39]</sup>. In the HIV & HCV co-infected patients, the HCV RNA positivity was found to be higher. These observations were in agreement with previous reports of increased hepatotropic viral replication in immunocompromised subjects<sup>[40,41]</sup>. Moderate or severe chronic hepatitis or cirrhosis was more frequent in patients with HBV and HCV co-infections than in patients infected with HBV or with HCV alone. Generally, HCV superinfection can cause a much more severe liver disease in patients with

**Table 6 Clinical condition vs different viral infection vs sex**

Sex	Clinical condition	Different viral infection positive								Total positive	Total negative
		HCV	HBV	HIV	HDV	HCV and HBV	HCV and HIV	HBV and HIV	HCV, HBV and HIV		
Male	Acute liver disease	-	14	5	3	-	-	-	-	22	55
	Chronic liver disease except cirrhosis	8	9	9	5	4	3	5	2	45	34
	Cirrhosis	7	5	-	-	1	-	1	-	14	31
	Others	3	13	-	-	-	-	-	-	16	64
	Total	18	41	14	8	5	3	6	2	97	184
Female	Acute liver disease	-	4	5	1	-	-	2	-	12	38
	Chronic liver disease except cirrhosis	5	2	8	3	2	3	1	2	26	51
	Cirrhosis	5	1	-	-	1	-	-	-	7	33
	Others	1	1	-	-	-	-	-	-	2	62
	Total	11	8	13	4	3	3	3	2	47	184
Total	29	49	27	12	8	6	9	4	144	368	
P value	0.211	0	0.853	0.256	0.484	1	0.323	1	-	-	

$\chi^2$  is calculated for gender vs different viral infections positive cases;  $P < 0.05$  is significant, while  $P > 0.05$  is insignificant.

**Table 7 Liver function test in HCV infected and co-infected patients (mean  $\pm$  SD,  $n = 29$ )**

Category	Liver function test							
	Bilirubin (mg%)			SAP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gms%)
	Total	Direct	Indirect					
Control	0.5340 $\pm$ 0.219	0.210 $\pm$ 0.055	0.386 $\pm$ 0.015	73.227 $\pm$ 21.622	37.76 $\pm$ 9.35	24.77 $\pm$ 07.65	69.70 $\pm$ 11.03	2.78 $\pm$ 0.219
HCV positive patients	3.461 $\pm$ 0.874	0.643 $\pm$ 0.1736	0.936 $\pm$ 0.016	144.630 $\pm$ 12.976	42.1 $\pm$ 8.3	49.00 $\pm$ 10.1	98.446 $\pm$ 09.57	3.87 $\pm$ 0.24
P value	0	0	0	0.005	0	0	0	0
HCV and HBV coinfectd patients	3.70 $\pm$ 0.96	1.59 $\pm$ 0.06	1.641 $\pm$ 0.052	140.887 $\pm$ 10.922	58.0 $\pm$ 03.16	40.412 $\pm$ 02.008	99.362 $\pm$ 06.150	3.14 $\pm$ 0.66
P value	0	0	0	0	0	0	0	0
HCV and HIV coinfectd patients	2.920 $\pm$ 1.032	1.975 $\pm$ 0.941	1.997 $\pm$ 0.078	134.53 $\pm$ 10.651	56.78 $\pm$ 4.401	39.65 $\pm$ 2.24	97.60 $\pm$ 5.94	3.12 $\pm$ 0.97
P value	0	0.001	0.001	0	0.001	0.001	0	0
HCV, HBV and HIV coinfectd patients	3.295 $\pm$ 1.424	3.202 $\pm$ 0.488	0.265 $\pm$ 0.052	141 $\pm$ 17.301	64.37 $\pm$ 4.01	42.87 $\pm$ 2.84	99.95 $\pm$ 03.039	3.14 $\pm$ 0.02
P value	0.009	0	0	0	0.005	0.006	0.003	0.004

HCV positive patients:  $P$  values are based on students  $t$  test;  $P < 0.05$  significant.

chronic HBV infection. This lends support to the notion that HBV superinfection may also aggravate the disease severity and increase the risk of fulminant hepatitis. In both HIV positive and negative cohorts, the presence of HBV & HCV, HBV & HDV or triple hepatitis infection was strongly associated with intravenous drug use (IDU). Overall, from 0.4% to more than 50% of HIV patients may carry more than one hepatitis virus<sup>[42-47]</sup>. The reported co-infection rates of HBV and HCV in HIV patients worldwide have varied, depending on the geographic regions, risk groups and the type of exposure involved<sup>[48,49]</sup>. Tankhiwale *et al*<sup>[50]</sup> (2003) reported 5.6% seroprevalence of HCV and 25.8% HBV in HIV infected patients. These co-infection rates were much higher than that of our findings. Four individuals co-infected with HCV, HBV & HIV had neither surgical intervention, blood transfusion or intravenous drug use, suggesting

that sexual intercourse could have been the route of infection.

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## COMMENTS

### Background

Hepatitis C virus (HCV) infection creates a significant burden on health care systems. HCV infection has probably been endemic in many populations for centuries. Despite a declining incidence of new infections, the burden of disease in terms of mortality is expected to increase over the next decade.

The complexity and uncertainty related to the geographic distribution of HCV infection and chronic HCV, determination of the associated risk factors and evaluation of cofactors that accelerate its progression, underscore the difficulties in global prevention and control of HCV. Thus, this study set out to determine the seroprevalence of HCV and its co-infection with other blood borne hepatitis group of viruses and human immunodeficiency virus.

### Research frontiers

As HCV is a silent killer virus, diagnosis, treatment and prevention are very important. Hence the results of this study will, if further explored, benefit the public, health care workers, voluntary blood donors and liver disease patients.

### Innovations and breakthroughs

Most earlier workers have studied the seroprevalence of HCV either by enzyme linked immunosorbent assay (Antigen or Antibody) specific for the virus or by RT-PCR (viral genome) alone. In this study the seroprevalence of HCV was evaluated by both the methods. This should help to both modify treatment and prevent infection.

### Applications

The identification of a high prevalence of HCV in this study indicates the significance of screening HBV, HCV and HDV in addition to HIV, among liver disease patients. Hence we strongly recommend routine testing for health care workers, voluntary blood donors and others to include HBV, HCV, HDV and HIV. We also recommend individualized counseling to identify those at risk as well as testing for those who request it. Improved surveillance and periodic epidemiologic studies will have to be undertaken to monitor and prevent the spread of virulent and interferon-resistant strains. Given the current incidence and prevalence data cited above, HCV infection and its co-infection is expected to remain a problem. Further research in the virology, epidemiology, treatment and prevention of blood-borne viral infection is essential if better outcomes to be achieved.

### Peer review

The present manuscript deals with an interesting epidemiologic data, and the topic is also interesting.

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