



## Seroprevalence of HBV and HCV among chronic liver disease patients

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### General Note

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### ABSTRACT

**Background:** Serological investigation and exact identification procedures are very important to impart the hepatitis infection control in the initial stage itself in order to avoid the major complications including chronic liver damage to successive organ failure. **Objective:** The main objective of the study is to determine the sero-prevalence of HBsAg, anti HCV antibodies, co-infections of HBV and HCV among clinically diagnosed CLD patients. **Methods:** A total of 56 subjects were included and all the serum samples were screened for markers of various hepatitis B and C viruses by using ELISA. The observation made out of the study further helped to know the probable mode of transmission and co-infection, so it was guided to follow the proper preventive measures. **Results:** Out

of 56 CLD patients, 21.42% of samples were reactive for HBsAg and 8.92% were reactive for antibodies against HCV. One sample showed reactive for both HBsAg and Anti HCV antibodies. The knowledge of HBV, HCV status of patients provided better status for optimal clinical managements of Chronic liver disease patients to improve the efficacy of therapy, to implement of additional supportive therapy and new drugs, new therapeutic strategies especially vaccination strategies of Hepatitis B. *Conclusion:* It is a single centre study, limited to 56 subjects, indirect evidence of serology by ELISA using biomarkers only attempted, difficulty in establishing relationships of patient and factors and the transversal studies tend to overestimate the long duration of infections and under estimate the short duration infection.

**Keywords:** HBV, HCV, CLD, seroprevalence

## 1. INTRODUCTION

Hepatitis is a general term that is defined as inflammation of the liver and can be caused by a variety of different viruses such as hepatitis A, B, C, D and E (WHO, 2002). Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the most common viral infections in humans and represent a major global public health problem (Saravanan *et al.*, 2009; Sharma *et al.*, 2014). Both of these viruses are transmitted mainly through the parenteral route and therefore a dual infection of these viruses can occur and even persists in the same patient (Sharma *et al.*, 2014). Hepatitis B and C viruses are hepatotropic viruses causing viral hepatitis, chronic liver disease (CLD) and hepatocellular carcinoma (Saravanan *et al.*, 2009).

CLD is responsible for over 1.4 million deaths annually (WHO, 2002) and is characterized by permanent inflammatory processes that predispose to liver cancer and in particular hepatocellular carcinoma (Jelic *et al.*, 2010). Cirrhosis is characterised by diffuse hepatic fibrosis and nodule formation. It can occur at any age, has significant morbidity and is an important cause of premature death and cirrhosis is present in 75-90% of individuals with HCC and is an important risk factor for the disease (Walker, 2014).

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and eighth most common cancer in women worldwide, resulting in at least 500 000 deaths per year. It accounts for 90% of all liver cancers. Chronic infection with HBV in the setting of cirrhosis increases the risk of HCC 100-fold. Some 5%–30% of individuals with HCV infection develop chronic liver disease, 30% progress to cirrhosis, and in these, 1%–2% per year develop HCC. Co-infection with HBV, alcohol abuse further increases the risk (Jelic *et al.*, 2010).

The seroprevalence data is essential to estimate the true burden and distribution of viral hepatitis in chronic liver disease patients. So this study aims to evaluate the presence of HBV surface antigen and antibodies to HCV in patients of clinically diagnosed chronic liver disease participants in tertiary care hospitals. Association of age, gender, marital status and associated risk factors with prevalence of these viruses in clinically diagnosed for CLD patients is investigated. The study outcome was vital for taking appropriate preventive measures and to implement the drug strategies, vaccination against hepatitis B. The major objectives of the study are to determine the seroprevalence of HBsAg, anti HCV antibodies, co-infections of HBV and HCV among clinically diagnosed CLD patients

## 2. MATERIALS AND METHODS

This is a prospective study performed from June to August 2016 after getting approval from institutional ethical committee (CMCH&RC/ IEC-No: 15 dt. 15.04.2016), informed consent from the participants and the proforma from case sheets were collected. In the present study, serological aspects were restricted to CLD only. The study was conducted in a tertiary care teaching hospital, Tiruchirapalli, Tamilnadu. After explaining the study methodology to the participants, 56 patients attended in Gastroenterology OP with clinically diagnosed CLD were included in the study. Socio-demographic status (age, sex and occupation), clinical complications data, laboratory parameters (serology) were collected and entered in the proforma.

All voluntary adult patients (age 18 and above) with clinically recognizable chronic CLD in hospitals during the study period were eligible and patients who were not willing to participate in the study, age below 18, unable to communicate and under coma were excluded.

Five millilitre of blood sample was collected from the patient and left for 30 minutes to facilitate clotting. Then the clotted blood was centrifuged to separate the serum from blood. The serum was stored at -20°C until used. The samples were screened for markers of various hepatitis B and C viruses by using ELISA. Hepatitis B antigen (HBsAg) was detected by HEPALISA and Hepatitis C Virus (anti HCV antibody) was detected by HCV Micro ELISA, manufactured by J. Mitra and Co. PVT. Ltd, NewDelhi (India). Blood from 10 healthy, age matched health staff without any evidence of disease were collected as control for this study.

### 3. RESULTS

As a result, Out of 56 CLD patients, 21.42% of samples were reactive for HBsAg and 8.92% were reactive for antibodies against HCV. One sample showed reactive for both HBsAg and Anti HCV antibodies (Table 1). Among the control group (n=10), all the subjects showed non reactive to HBsAg and Anti HCV antibodies by ELISA method. For control, 5 males and 5 females were included whose age groups belong to 21-30 years.

**Table 1** Prevalence of HBsAg positive, Anti-HCV Ab and Co-infection in Chronic Liver Disease patients

Features	Total Number	Percentage
Total No. of Chronic Liver Disease patients	56	100
No. of HBsAg positive	12	21.42
No. of Anti-HCV Ab positive	5	8.92
No of both HBsAg and Anti -HCVAb positive	1	1.78
Total no. of positive cases	18	32.14

Among 56 CLD patients, the commonest age group was 41- 50 years with 22 patients (39.28%). The second commonest group was 31-40 years with 13 patients (23.21%). The highest prevalence of HBsAg positive was 41.66% in the age group of 41-50 years. The highest anti HCV antibodies positive was 40% in the age group of 51-60 years. Single case of co-infection with 2 viruses was reported in the age group 31-40 (Table 2).

**Table 2** Age wise distribution of Chronic liver disease showing seropositivity

Age (in Years)	Chronic liver Disease patient's n (%)	HBsAg positive n (%)	Anti-HCVAb positive n (%)	Co infection (HBsAg & Anti-HCVAb) n (%)
21 - 30	4 (7.14)	2 (16.66)	-	-
31 - 40	13 (23.21)	3 (25)	1 (20)	1 (100)
41 - 50	22 (39.28)	5 (41.66)	1 (20)	-
51 - 60	13 (23.21)	2 (16.66)	2 (40)	-
61 - 70	1 (5.35)	-	-	-
71 - 80	3 (5.35)	-	1 (20)	-
Total	56 (100)	12 (21.42)	5 (8.92)	1 (1.78)

[Figure in parenthesis denoted percentages]

Female patients were lesser than male CLD patients; male being 47 (83.92%) & females 9 (16.07%). The prevalence of HBsAg positive is higher in males (83.33%) than females (16.66%). Of the male patients 60% were anti HCV antibodies positive which showed higher prevalence than females 40%. Single case of male out of 47 male CLD patients were reported as co-infection with 2 viruses in the present study (Table 3).

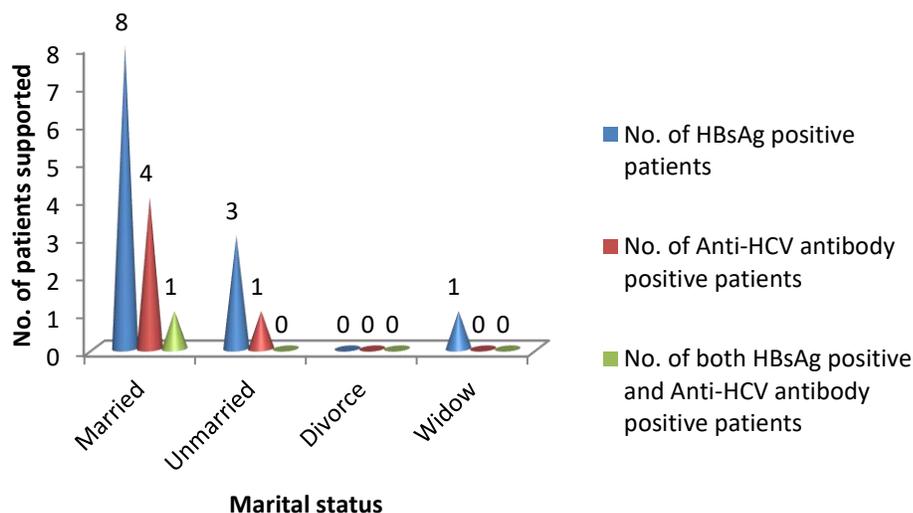
**Table 3** Sex wise distribution of Chronic Liver Disease showing seropositivity

Sex	Chronic liver Disease patients (n=56)	HBsAg positive (n=12)	Anti-HCVAb positive (n=5)	Co infection (n=1)
Male	47 (83.92)	10 (83.33)	3 (60)	1(100)
Female	9 (16.07)	2 (16.66)	2 (40)	-

[Figure in parenthesis denoted percentages]

Out of 56 Chronic liver disease patients, 45 (80.35%) were married, 8 (14.28%) unmarried, 2 (3.57%) widow & 1 (1.78%) divorce. The prevalence of HBsAg positive is higher in married (66.66%) than unmarried (25%). The highest anti HCV antibodies prevalence

was married (80%) than unmarried (20%). Single case of married patient was reported as co-infection with 2 viruses in this study (Figure 1).



**Figure 1** Marital status of the serological positive subjects included

Maximum CLD patients were farmer (23%) in occupation, followed by daily labourer (25%), Government/private employee (14.28%), house wife (8.92%), driver (7.14%) & unemployment (3.57%). The farmer (50% & 40%) had highest seroprevalence for HBsAg & anti-HCV antibodies positive, followed by daily labourer (25% & 40%) respectively. Single case from daily labourer occupation was reported as co-infection with 2 viruses in this study (Table 4).

**Table 4** Occupation distribution of Chronic Liver Disease showing seropositivity

Occupation	Chronic liver Disease patients (n=56)	HBsAg positive (n=12)	Anti-HCVAb positive (n=5)	Co infection (n=1)
Driver	4 (7.14)	-	-	-
Daily laborer	14 (25)	3(25)	2 (40)	1(100)
Farmer	23 (41.09)	6 (50)	2 (40)	-
Govt/Privt. Employee	8 (14.28)	1(8.33)	-	-
House wife	5 (8.92)	2(16.66)	1 (20)	-
Unemployment	2 (3.57)	-	-	-
Student	-	-	-	-

[Figure in parenthesis denoted percentages]

In relation to residence area, 39 patients (69.64%) of the CLD were from rural area and 17 patients (30.85%) were from urban area. Among 56 of total CLD patients, the prevalence of HBsAg and anti HCV antibodies was higher in rural areas (83.33% and 80%) than the urban areas (16.66% and 20%). Single case from rural area was reported as co-infection with 2 viruses in the present study (Table 5).

**Table 5** Residence Wise Distribution of Chronic Liver Disease showing seropositivity

Residence	Chronic liver Disease patients (n=56)	HBsAg positive (n=12)	Anti-HCVAb positive (n=5)	Co infection (n=1)
Urban	17 (30.85)	2 (16.66)	1 (20)	-
Rural	39 (69.64)	10 (83.33)	4(80)	1(2.56)

[Figure in parenthesis denoted percentages]

The predominant symptoms of HBsAg positive patients was fatigue, anorexia, nausea (91.6%) followed by headache, myalgia, pruritis, fever (75%) respectively and also shows pain in abdomen, vomiting and yellow discoloration of skin (53.84%) respectively. The anti HCV antibodies positive patients had most common symptoms of fatigue, anorexia, nausea, myalgia (80%), followed by headache, fever, pain and abdominal discomfort, abdomen swelling, yellow discoloration of skin (60%) respectively (Table 6).

**Table 6** Clinical features of HBs Ag, Anti-HCV Ab and Co infection patients

Clinical features	HBsAg positive (n=12)	Anti-HCV Ab positive (n=5)	Co infection( HBsAg & Anti-HCV Ab ) (n=1)
Anorexia	11 (91.6)	4 (80)	1 (100)
Nausea	11 (91.6)	4 (80)	1 (100)
Vomiting	7 (58.33)	3 (60)	1 (100)
Headache	9 (75)	3 (60)	1 (100)
Arthralgia	4 (33.33)	1 (20)	-
Myalgia	9 (75)	4 (80)	1 (100)
Chronic Diarrhoea	-	-	-
Loss of weight	2 (16.66)	1 (20)	-
Pruritis	9 (75)	2 (40)	1 (100)
Fever	9 (75)	3 (60)	1 (100)
Pain in Rt. Hypochondrium/ Abdominal discomfort	7 (58.33)	3 (60)	1 (100)
Abdominal Swelling	5 (41.66)	3 (60)	1 (100)
Fatigue	11 (91.6)	4 (80)	1 (100)
FT.Edema	4 (33.33)	2 (40)	-
Yellow discoloration of sclera and skin	7 (58.33)	3 (60)	1 (100)
Ascitis	3 (25)	2 (40)	-
Hepatomegaly	5 (41.66)	2 (40)	-
Splenomegaly	-	-	-
Portal hypertension	2 (16.66)	1(20)	-
Hepatic encephalopathy	1 (8.33)	-	-

[Figure in parenthesis denoted percentages]

Probe in to various risk factors to which study subjects were exposed are listed in (Table 7). The most common probable mode of acquiring infection was found to be Ear/Nose piercing, tattooing (58.33% and 40%), exposure to unsterilized needles and syringes (33.33% and 40%) in cases of HBV and HCV respectively, other causes include contact with sex workers, jaundice patient and others. In the patient of co infection with HBV and HCV the probable mode of infection may be through contact with sexual workers. History of alcohol consumption taken in this study to assess the severity of patient condition, prompt treatment and prognosis purpose only.

**Table 7** Possible Risk factors (probable mode of acquisition) in HBsAg, Anti-HCV Ab and Co infection patients

Possible Risk factors	HBsAg positive (n=12)	Anti-HCVAb positive (n=5)	Co infection (n=1)
Ear/ Nose piecing, Tatoo	7 (58.33)	2(40)	-
Exposure to unsterilized needles	4 (33.33)	2 (40)	-

and syringes			
Blood Transfusion	-	-	-
Contact with jaundice patient	2 (16.66)	1(20)	-
Hospital admission/ surgery and Venepuncture on Treatment	6 (50)	2(20)	-
Alcohol consumption	8 (66.66)	3(60)	1 (100)
Drug addiction	1 (8.33)	-	-
Contact with sexual workers	1 (8.33)	1 (20)	1 (100)
Unknown case	2 (16.66)	-	-

[Figure in parenthesis denoted percentages]

#### 4. DISCUSSION

HBV and HCV infections are among the most prevalent infectious diseases in humans worldwide. Both infections are associated with a broad range of clinical presentations ranging from acute or fulminant hepatitis to chronic infection that may be clinically asymptomatic or may progress to chronic hepatitis and liver cirrhosis. HBV infection has several modes of transmission of which perinatal transmission and transfusion of infected blood and blood products are most important. Other important modes include sexual transmission, tattooing, needle stick exposure etc. (Kondo *et al.*, 2015; Arora *et al.*, 2005; Sebastian *et al.*, 1990).

In the present study conducted in 56 clinically diagnosed cases of chronic liver disease, 21.42% cases were positive for HBsAg. In a study, 26% of cases were positive for HBsAg (Kondo *et al.*, 2015) which almost correlates with our study. Whereas in other studies reported to 40% and 43.7% (Arora *et al.*, 2005; Shanta *et al.*, 2002). It might be because in these studies various viral markers such as HBsAg, HBeAg and antibodies to were detected. Anti HCV antibodies positivity in the present study was 8.92%. Similar results were observed in other studies (Arora *et al.*, 2005; Chatterjee *et al.*, 2001), where anti HCV antibodies positive showed 8.33% and 4.26% respectively. It has been reported to be 40.80% which is quite high in a study conducted in Pakistan (Jelic *et al.*, 2010; Khan *et al.*, 2003). It might be because the results vary from place to place and with the type of test used. Dual infection of HBV and HCV was 1.78% in the present study. In a study, dual infection of HBV and HCV showed 0.3% (Sharma *et al.*, 2014) which is concurs with our study. It has been reported to be 5.9%, 5% and more than 30% by other authors (Saravanan *et al.*, 2009; Devi *et al.*, 2004; Chakravarti *et al.*, 2005) who are not associated with our study. 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 (Devi *et al.*, 2004; Reddy *et al.*, 2005).

In the present study, 39.28% of CLD cases were 41-50 yrs of age group. In a study from Ethiopia and Pakistan (Ayele *et al.*, 2013; Bukhtiarra *et al.*, 2003), it were reported 75.8% and 42% respectively of CLD cases <50 yrs which is associates with our study. The highest prevalence of HBsAg and Anti HCV antibodies positive patients in this study were 41.66% and 40% in the age group of 41-50 years and 51-60 years respectively. An another study showed 38.46% of HBsAg positive patients in the age group of 41-50 yrs and 42.86% of anti HCV antibodies positive patients in the age of 51-60 yrs (Sharma *et al.*, 2014) which correlates with our study. The age-related prevalence of HBsAg showed a significantly decreased after 50 years, so that among participants who were older than 60 years, the prevalence of anti-HCV was higher than that of HBsAg. The reason could be an early childhood infection with the hepatitis viruses. The low prevalence of hepatitis virus infection in the older age group may reflect loss of carrier status due to seroconversion with age (Ayele *et al.*, 2013).

In this study, the prevalence of HBsAg positive is higher in males (83.33%) than females (16.66%). In a study, 76.92% are males and 23.07% are females (Sharma *et al.*, 2014) which is associates with our study. It was observed that males (60%) were more susceptible to HCV than females (40%) among the study population. This concurs with previous report from India (Sharma *et al.*, 2014) that male (63%) were at high risk of HCV infection than females (36.36%). High prevalence of Hepatitis in males might be due to fact that males make more common visits to barber and they may be more probably to get wounded and may share equipment. Moreover, men also are more likely to have many sex partners and follow unprotected sex (Anbazhagan *et al.*, 2010).

In this study, the prevalence of HBsAg and anti HCV antibodies positive patients were high in married (66.7% and 80%) than unmarried (25% and 20%).The result of the present study was in agreement with the results of other studies (Sharma *et al.*, 2014; Ayele *et al.*, 2013), reporting the prevalence of Hepatitis B and C higher in married patients. Similarly a Nigerian study (Adekeye *et al.*, 2013) also reported the higher prevalence of Hepatitis B and C in married patients compared to unmarried. It might be due to lack of awareness or education in people that has pre-exposure prior to marriage which is then transmitted to her or his spouse.

In the present study, a higher prevalence of HBV was observed in farmer (50%) followed by daily labourer (25%) and prevalence anti HCV antibodies positive was seen in farmer (40%), daily labourer (40%). The high prevalence of HBsAg positive was seen in farmer followed by daily labourer (Sharma *et al.*, 2014) which is similar to our study. The higher seropositivity was recorded among lab technician even though it was not significantly associated with none of the occupational groups (Sharma *et al.*, 2014). Because of the small numbers statistically conclusive inferences may be difficult to make. Yet, this higher rate of HBV infection in farmer/ daily labourer/ house servant and office workers might raise concern regarding need for implementation of more effective public education.

In this study, the prevalence of HBV and HCV was higher in rural area than urban area. A comparable study from Sindh area (Aziz *et al.*, 2010) also reported higher prevalence of HCV in rural area than urban area. Because it may be the mode of transmission of these viruses, would be the unsafe injection practice prevailing in the vast rural areas of the country. In the present study jaundice was the common sign in HBV (58.33%) and HCV (60%) cases which coincides with the findings of other authors (Sharma *et al.*, 2014). Jaundice was also found in HCV positive cases, which is in contrast to the findings of authors (Arora *et al.*, 2005) who have not reported jaundice in any HCV case. These findings correlate with bilirubin levels recorded. Bilirubin levels among hepatitis B and hepatitis C in the present study coincided with the literature (Arora *et al.*, 2005). It is reported in the literature that patients with HCV infection may present in anicteric, icteric, or fulminant form. So, it cannot be distinguished solely by clinical features or by biochemical markers, and therefore serology is a must.

A study reported the prevalence of HCV from southern India as 2.5% in AVH, 35.2% in CAH patients, 23.9% in CLD and 19% in cirrhosis patients (Sumathy *et al.*, 1993). An Indian study in Calcutta and reported 84 patients with chronic hepatic illness (62 of cirrhosis, 22 of chronic hepatitis) and further explained that 7 (8.33%) patients with chronic hepatitis, 5 (8.06%) patients with cirrhosis and 2 (9.09%) with chronic active hepatitis were HCV positive among 22 chronic hepatitis (Chatterjee *et al.*, 2001). Other study from north-India has reported the prevalence of HCV in various liver disease patients as, 43% in fulminant hepatitis and 42% in CAH (Tandon *et al.*, 1991).

Patients with HCV infection may present in anicteric, icteric or fulminant form. So, it cannot be distinguished solely by clinical features or by biochemical markers, and therefore serology is a must. HBV is still the major cause of chronic liver disease, followed by HCV in this part of the country. Co-infection has been observed, and there is a danger of the devastating trio infecting a large proportion of cases. So, importance must be laid on proper preventive measures such as screening of blood, safe sexual practices, proper sterilisation of instruments, proper disposal of contaminated material, and immunisation of people at risk particularly health care workers.

## 5. CONCLUSION

Seroprevalence of HBV, HCV and co infection among CLD patients in our study was 21.42%, 8.92% and 1.78% respectively. To prevent the spread of HBV and HCV, awareness to the public should be provided about these infections and their mode of transmission especially regarding the proper sterilization of the instruments even in the routine activity like sharps used in barber shop, tattooing on the body etc. The knowledge of HBV, HCV status of patients will help to choose the optimal clinical managements of CLD patient i.e to improve the efficacy of therapy, to implement of additional supportive therapy and new drugs, vaccination against Hepatitis B. All the hospitals should be implemented with proper infection control strategies and policies. Furthermore all clinically diagnosed patients should be tested for HBV and HCV serostatus. Strength of the study is rigid criteria adapted to select subjects and controls and good laboratory practice adopted to process the samples.

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### Authors Contributions

Prasant HA – Principal Investigator (PI) who collected the data, samples and processed microbiologically and documented  
Lalithambigai J – Research Supervisor who guided and supported the PI for the completion and confirming the results  
Prabhusaran N – Guided partially and supported the PI for documentation, reviewing, data evaluation and pooling  
Uma A – Overall supervision, appropriate guidance, data evaluation and final corrections

**Conflicts of Interest:**

The authors declare no conflict of interest.

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**Abbreviations**

HBsAg – hepatitis B surface antigen; HBV – hepatitis B virus; HCV – hepatitis C virus; CLD – chronic liver diseases; ELISA – enzyme linked immunosorbent assay; WHO – World health organization; HCC – hepatocellular carcinoma; AVH – acute viral hepatitis; CAH – chronic active hepatitis.

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