Hepatitis B and C Viruses, Their Coinfection and Correlations in Chronic Liver Disease Patients: A Tertiary Care Hospital Study

Abstract

Introduction: There has been a rapid increase in the cases of viral hepatitis in Malwa region of Punjab. Quantification of seroprevalence of hepatitis B virus (HBV)/hepatitis C virus (HCV) and their coinfection among liver disease patients in tertiary care settings is needed to know the associated disease burden. Aim: The aim of this study is to analyze the seroprevalence of HBV, HCV, their coinfection, and implications in liver disease patients. Materials and Methods: This prospective study was conducted from June 2015 to August 2015 on a total of 100 chronic liver disease (CLD) patients. Venous blood samples were tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies by performing required serological tests using sandwich ELISA technique and solid-phase immunochromatography. Results: Out of 100 cases, 80 (80%) were male and 20 (20%) were female with mean age of 47.44 ± 14.56 years. Out of 100 cases of hepatic disorders, 26 were HBsAg positive and 40 were anti-HCV positive. Majority of the HBsAg-positive cases had alcohol as a risk factor (27%) and were diagnosed with cirrhosis (38.5%). Maximum number of anti-HCV-positive cases had blood transfusion as risk factor (30%) and were diagnosed with cirrhosis (45%). Of total 62 seropositive cases, 4 had coinfection of HBV and HCV. Coinfected patients did not demonstrate greater risk of developing cirrhosis or progressing to hepatocellular carcinoma than mono-infected patients. Conclusion: HBV and HCV are the major causes of CLD at the place of study. Patients with dual HBV and HCV infection do not have greater risk of developing cirrhosis or progressing to HCC than mono-infected patients.

Keywords: Chronic liver disease, hepatitis B, hepatitis C

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are endemic in India and have an etiological role in acute hepatitis, 50%–70%, of which leads to chronic liver disease (CLD).[1] In India, there are estimated 43–45 million hepatitis B surface antigen (HBsAg) carriers and 3%–4% of the Indian population is infected with HBV (HBsAg positive).[2] HBV infection can manifest as acute, subacute, and chronic hepatitis; liver cirrhosis; and primary hepatocellular carcinoma (HCC).[3] Current estimations indicate that approximately 1.8%–2.5% of Indian population is presently infected by HCV.[4] HCV has been recognized as a major cause of posttransfusion hepatitis and an important cause of CLD, particularly cirrhosis and hepatocellular carcinoma.[5] The rampant use of injections (unsafe), unscreened blood transfusion, and dental procedures are playing a significant role in increasing the reservoir of HCV infection in Malwa region of Punjab.[6]

Because of the shared modes of transmission, HBV and HCV coinfection is not uncommon in highly endemic areas and among participants with a high risk of parenteral infections. Some studies have shown that as compared to mono-infected patients, HBV and HCV coinfected persons tend to have more severe liver injury, a higher probability of liver cirrhosis and hepatic decompensation and a higher incidence of hepatocellular carcinoma.[7,8] Some others propose that coinfection with these two viruses puts the patients at higher risk for cirrhosis but not hepatocellular carcinoma.[9]

The present research was done to analyze the seroprevalence of HBV and HCV viral markers, their coinfection and relevant implications in CLD patients, which is crucial in light of rapidly rising cases of viral hepatitis in Punjab, particularly in...
Malwa region. Knowledge about the major risk factors and risk behaviors among seropositive individuals will encourage implementation of proper preventive measures such as routine screening and counseling, especially of those who are at a relatively higher risk.

Materials and Methods

The present study is a prospective study that was conducted at Department of Microbiology, Government Medical College, Patiala, under the Indian Council of Medical Research, New Delhi, from June 2015 to August 2015 on a total of 100 patients of CLD admitted in the wards of Department of Medicine at Government Medical College & Rajindra Hospital, Patiala.

The study group comprised males and females and belonging to different social strata. These patients presenting with a long history (>6 months) of symptoms of liver disease, had deranged liver function tests, showed evidence of liver parenchymal disease radiologically (either or both on ultrasound and CT scan) and also included cytologically proven cases of HCC with markedly raised AFP levels.

All the relevant patient history and information was obtained through a pro forma. Due consent from these patients was obtained through a consent form.

About 5 ml of venous blood samples were collected in clean vials and blood was allowed to clot for 45 min at room temperature and the serum separated after centrifugation at low speed. These samples were tested for HBsAg and anti-HCV antibodies by performing required tests.

Tests done for the detection of HBsAg include ErbaLisa® hepatitis B which uses the sandwich ELISA technique allowing quantitative estimation of HBsAg in patient serum/plasma and Hepacard Test (J. Mitra and Co. Ltd.) which uses solid-phase immunochromatographic technology for the qualitative detection of HBsAg in serum or plasma.

Tests conducted for detection of anti-HCV antibodies includes ErbaLisa® hepatitis C which is based on indirect ELISA using a solid phase prepared with the mixture of synthetic peptides and recombinant proteins of HCV, that is, CORE, NS3, NS4, and NS5 with detection being carried out using antihuman IgG antibodies conjugated with horseradish peroxidase; and HCV Tridot-Rapid Visual Test (J. Mitra and Co. Ltd.) based on solid-phase immunochromatography. The results of these tests were interpreted as per the instructions from the manufacturers. Ethical norms were strictly adhered to. Approval of the institutional ethics committee was obtained before beginning with the study. Statistical analysis was done using SPSS software (IBM version 20, IBM Corp., Armonk, NY).

Results

The present study consisted of 100 patients of CLD who were admitted in medicine wards of Rajindra Hospital, Patiala. HBsAg and anti-HCV antibodies were tested in these patients and the following observations were made.

Out of 100 cases, 27 were in age group of 21–40, 40 were in age group of 41–60, and 25 were in age group of above 60 years while 8 were in age group of 0–20 years.

Overall, the age ranged from 17 to 70 years with mean 47.44 ± 14.56. Out of 100 cases, 80 (80%) were male and 20 (20%) were female with male:female ratio 4:1. There was no statistically significant difference seen among number of males and females in various age groups (P = 0.611). Out of 100 cases, 58 were from rural area and 42 were from urban area.

Table 1 shows age-wise distribution of cases according to hepatic disorders. Table 2 shows the sex-wise distribution of cases according to hepatic disorders.

Analysis of the data for the history of risk factors in relation to sex of the patients has been shown in Table 3. Out of 100 cases of CLD, 26 were HBsAg positive. Figure 1 shows the percentage of HBsAg positivity in relation to hepatic disorders.

In 0–20 years, 21–40 years, 41–60 years, and above 60 years age groups, 2 (2 males and 0 females), 16 (14 males and 2 females), 4 (3 males and 1 female), and 4 (4 males and 1 female) cases were HBsAg positive, respectively. Table 4 shows HBsAg positivity in relation to sex and risk factors.

Out of 100 cases of hepatic disorders, 40 were anti-HCV positive. There was no statistically significant difference among various CLDs in relation to anti-HCV positivity observed by us as per the findings of the present study (P > 0.05). Figure 2 shows anti-HCV seropositivity in various hepatic disorders. Figure 3 shows anti-HCV seropositivity in relation to age and sex. Figure 4 shows anti-HCV seropositivity in relation to sex and risk factor.

| Table 1: Age-wise distribution of cases according to hepatic disorders |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|
| Diagnosis                  | 0-20 years (%)  | 21-40 years (%) | 41-60 years (%) | Above 60 years (%) | Total | P       |
| Viral hepatitis            | 6 (75.0)        | 9 (33.3)        | 5 (12.5)        | 6 (24.0)         | 26    | 0.014*  |
| Alcoholic hepatitis        | 1 (12.5)        | 9 (33.3)        | 14 (35.0)       | 6 (24.0)         | 30    |         |
| Cirrhosis                  | 1 (12.5)        | 9 (33.3)        | 20 (50.0)       | 10 (40.0)        | 40    |         |
| HCC                        | 0               | 0               | 1 (2.5)         | 3 (12.0)         | 4     |         |
| Total                      | 8               | 27              | 40              | 25               | 100   |         |

HCC: Hepatocellular carcinoma. Significant at alpha= 0.05 (*)
Out of total of 100 CLD patients, 4 (4%) had coinfection of HBsAg and anti-HCV. All the coinfected patients were male (100%) and 2 (50%) of them belonged to the age group 61–70 years, 3 (75%) coinfected patients belonged to the rural area, and 1 to the urban area. Three (75%) out of the 4 coinfected cases had cirrhosis, whereas 1 had chronic viral hepatitis. Blood transfusion (75%) was the only risk factor identified in the coinfected patients. The odds ratio of progression to cirrhosis in coinfected patients versus the mono-infected patients in our study was found to be 4.50 (95% CI = 0.45–45.48) and the statistical significance of the same was not established ($P = 0.020$). The odds ratio of progression to HCC in coinfected patients versus the mono-infected patients in our study was found to be 1.64 (95% CI = 0.08–35.51) and the statistical significance of the same was not established ($P = 0.75$).

**Discussion**

The present study included 100 cases with CLD and maximum cases in our study were of cirrhosis of liver (40%) as the various complications of cirrhosis usually necessitate hospital admission. The mean age of the CLD patients studied was 47.44 ± 14.56 years, which is comparable with the findings of Singh et al.$^{[10]}$ (mean = 46.5 years). The higher incidence of CLD in the age group 41–60 years recorded by us could be explained partly by the well-known effects of aging on hepatic dysfunction, either alone or in synergism with other factors like alcoholism. Nearly 50% of patients in 41–60 years age group had cirrhosis ($P = 0.014$), which is a severe and irreversible form of liver disease. We reported the male-to-female ratio as 4:1. Singh et al.$^{[10]}$ and Chakravarti et al.$^{[11]}$ also reported higher incidence in males, that is, 2.3:1 and 3.7:1, respectively.

In the present study, we found that there is a significant effect of age in relation to hepatic disorders ($P = 0.014$). In chronic viral hepatitis, maximum numbers of patients were in the age group of 21–40 years. The reason for the same could be an early childhood infection with hepatitis viruses. Both in chronic alcoholic hepatitis and cirrhosis, maximum numbers of patients belonged to the age group of 41–60 years.

| Table 2: Sex-wise distribution of cases according to hepatic disorders |
|-----------------------------------------------|---------------|---------|-------|
| Diagnosis          | Sex         | Total | $P$ |
|                   | Female (%)  | Male (%) |      |
| Alcoholic hepatitis | 0           | 30 (37.5) | <0.001** |
| Cirrhosis liver    | 8 (40)      | 32 (40)   | 40    |
| HCC                | 0           | 4 (5)     | 4     |
| Viral hepatitis    | 12 (60)     | 14 (17.5) | 26    |
| Total              | 20          | 80       | 100   |

**$P$<0.001. HCC: Hepatocellular carcinoma**

| Table 3: History of risk factors in relation to sex of the patients |
|---------------------------------------------------------------|-----------------|-----------------|------|
| Risk factors                      | Number of cases | Male (%)         | Female (%)    | $P$  |
| Alcohol addiction/drug addiction | 30              | 30 (37.5)        | 0              | 0.003** |
| Blood transfusion                | 25              | 20 (23.8)        | 5 (30.0)      | 0.564 |
| Sexual contact                   | 4               | 3 (3.8)          | 1 (5.0)       | 0.2500 |
| Prenatal transmission            | 1               | 1 (1.3)          | 0              | 0.615 |
| IDUs                             | 11              | 11 (13.8)        | 0              | 0.114 |
| Unknown risk factor              | 29              | 15              | 14             |      |
| Total                            | 100             | 80              | 20             |      |

**$P$<0.001. IDUs: Injecting drug users**

Figure 1: Percentage of hepatitis B surface antigen positivity in relation to hepatic disorders. HCC: Hepatocellular carcinoma
either had a history of alcohol/drug addiction (42.2%) or blood transfusion (35.2%). About 15.5% of patients exclusively had a history of injecting drug use (IDU). Rampant increase in IDU and unsafe blood transfusion in Malwa region of Punjab are already documented and have become a grave concern over the recent past.\[6\] In other studies such as Devi et al., the major risk factors were reported to be blood transfusion (35.3%) and history of multiple sexual contacts (29.4%). In their study, 14.7% cases reported IDU as a risk factor.\[12\] Similarly, Singh et al. reported blood transfusion (30%) and alcohol addiction (15%) as the major risk factors.\[10\] Therefore, the results of the present study are comparable to other studies.

Out of 100 patients in our study, 26 (26%) were HBsAg positive. Devi et al., Mathur et al., Kumar et al., and Singh et al. reported 17.3%, 5.89%, 17.34%, and 4% HBsAg seropositivity in CLD, respectively. The disparity in the findings of various studies may be due to difference in types of serological techniques used for diagnosis or differences in study population, risk factors, and risk behaviors among them.

Majority of the HBsAg-positive patients (61.54%) in the present study were in the age group of 21–40 years. Similarly, Devi et al. found HBsAg positivity to be higher in the age group of 22–32 years.\[12\] Out of 26 HBsAg-positive patients in our study, 23 were male and 3 were female (7.6:1). In their study, Kumar et al. also showed a higher prevalence of HBsAg in males than females with male:female ratio being 2.5:1.\[14\]

In our study, major risk factors for HBV infection in CLD were found to be alcohol/drug addiction (7/26, 26.9%) and unsafe blood transfusion (4/26, 15.4%). Out of 30 patients who were drug addicts, 7 were positive for HBsAg (23.3%). The results of the present study corroborate well with the study of Tiwari et al. who reported 23.6% prevalence of HBsAg seropositivity in drug addicts in their study.\[15\] In the present study, out of 100 cases, 25 gave a history of blood transfusion. Out of these 25 patients, 4 (16%) were positive for HBsAg, parallel to the study of Nandi et al., which reported 10.58% prevalence of HBsAg positivity in relation to blood transfusion in their study.\[16\]

Out of 100 cases of CLD in our study, 71 had history risk factors. Out of these 71 patients, majority of the patients

### Table 4: Hepatitis B surface antigen positivity in relation to sex and risk factors

| Risk factors          | Total cases | Male | Female | HBsAg positive | Male positive (%) | Female positive (%) | P   |
|-----------------------|-------------|------|--------|----------------|------------------|---------------------|-----|
| Alcohol/drug addiction| 30          | 30   | -      | 7              | 7 (100)          | -                   | 0.234|
| Blood transfusion     | 25          | 20   | 5      | 4              | 3 (75)           | 1 (25)              | 1.000|
| Sexual contact        | 4           | 3    | 1      | 1              | 1 (50)           | 1 (50)              | 0.142|
| Prenatal transmission | 1           | 1    | -      | 1              | 1 (100)          | -                   | 0.387|
| IDUs                  | 11          | 11   | -      | 1              | 1 (100)          | -                   | 0.0168|
| Unknown risk factor   | 29          | 15   | 14     | 11             | 10 (91)          | 1 (9.1)             | 0.006**|
| Total                 | 100         | 80   | 20     | 26             | 23               | 3                   |     |

**P<0.001. IDUs: Injecting drug users. HBsAg: Hepatitis B surface antigen

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Singh et al., HBsAg was found to be positive in 10.8% of the IDUs of Manipur.[10]

A total of 40 out of 100 cases of CLD (40%) in the present study were found to be anti-HCV positive. Similarly, Seyed-Moayed et al.,[17] Devi et al.,[12] and Singh et al.[18] and Chakravarti et al.[11] reported 40.7%, 30%, and 30% prevalence, respectively, of anti-HCV in their studies. Against the results of most studies like Sarin et al.[18] that have shown HBV as dominant cause, our study found HCV to be the major cause of CLD.

Out of 40 anti-HCV positive patients, majority were male, with male:female ratio of 7:1. Maximum anti-HCV patients belonged to the age group 41–60 years (20/40, 50%), corroborating well with the study of Singh et al.[10] where HCV was reported to more prevalent in patients above 40 years of age. The major risk factors for acquiring HCV infection in our study were found to be unsafe blood transfusion (12/40, 30%) and alcohol/drug addiction (10/40, 25%).

A total of 25 cases of CLD in our study had a history of blood transfusion. Out of these, 12 (48%) patients were anti-HCV positive. These results are comparable to Mathur et al.[13] who recorded 43.65% anti-HCV positivity in relation to unsafe blood transfusion.

Out of the 11 cases of CLD in our study who were reported to be IDUs, 9 (81.8%) were anti-HCV positive, comparable to the study of Devi et al. who reported 90.4% prevalence of HCV in IDUs of Manipur, India.[13] Injecting drug abuse is a significant concern in Punjab and a higher than national average prevalence of HIV among IDUs of Patiala, Punjab, has previously been reported by the authors of the present study.[19]

We reported the seroprevalence of HBV in patients with HCC to be 25% as compared to Paul et al. (51%).[20] Khan and Rizvi (33.35%),[21] and Han et al. (64.2%).[22] Seroprevalence of HCV in patients with HCC in the present study was 50%; slightly higher compared to Paul et al. (12%)[20] and Han et al. (12.7%).[22] Therefore, HCV is the dominant virus as compared to HBV in causing hepatocellular carcinoma; however, a vast study is needed to find out the exact prevalence of HCV and HBV in HCC patients of the region.

In the present study, 4 (4/100, 4%) cases of CLD had coinfection of HBV and HCV, as they were found to be seropositive for both HBsAg and anti-HCV. The results obtained by the present study are comparable to Xess et al. (3%).[11] Devi et al. (5%),[12] and Singh et al. (6%).[10]

The odds ratio of progression to cirrhosis in coinfected patients versus the mono-infected patients in our study was found to be 4.50 (95% CI = 0.45–45.48) and the statistical significance of the same was not established (P = 0.20), probably owing to the limited sample size of our study. Kruse et al. documented a significantly risk of developing cirrhosis of liver among coinfected patients as compared to mono-infected patients.[23] Interestingly, it has been found that liver disease activity and prognosis are generally more serious in the presence of double infection; although an inverse relationship in the replicative levels of the two agents has been noted, suggesting viral interference. Thus, the two viruses seem to inhibit each other at the molecular level, while cytopathic effects appear to be enhanced.[9] None of the patients having coinfection in our study was diagnosed with HCC, which could be explained by a subadditive effect of HBV and HCV coinfection. The odds ratio of progression to HCC in coinfected patients versus the mono-infected patients in our study was found to be 1.64 (95% CI = 0.08–35.51) and the statistical significance of the same was not established (P = 0.75). This finding could be explained by a subadditive effect of HBV and HCV coinfection. Therefore, our study does not show that coinfection of HBV and HCV warrants a poorer outcome in CLD patients, however, further studies with larger sample sizes are needed to document and confirm the effect of HBV and HCV coinfection on clinical outcome.

Conclusion

It can be concluded from this study that HBV and HCV contribute to majority of the CLD burden in the region, with HCV being the dominant cause as compared to HBV. Effective management and prevention of transmission of these viruses may, therefore, help reduce the burden of CLD and subsequent hospital admissions significantly. The main risk factors identified for acquiring HBV and HCV infection are unsafe blood transfusion and drug addiction. According to our study, coinfection of HBV and HCV is not uncommon. Patients with dual infection in the present study were not found to be more likely to develop cirrhosis and HCC than mono-infected patients, but further studies are needed to confirm these findings.

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Conflicts of interest

There are no conflicts of interest.

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