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Study of Hepatitis B, Hepatitis C and their Coinfection in Patients with Chronic Liver Disease in a Tertiary Care Hospital in Central India: A Cross-Sectional Study

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Abstract: ***Introduction: Among the various causes of Chronic Liver Disease(CLD), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are the most important due to their chronicity, complications, and potential to progress to cirrhosis and Hepatocellular carcinoma (HCC). Hence, the present cross-sectional study was conducted in a tertiary care hospital in Central India to determine the prevalence of Hepatitis B, Hepatitis C, and their coinfection among patients with chronic liver disease. It also aimed to assess their demographic and clinical profiles. The findings will help strengthen regional data on viral hepatitis and support targeted strategies for better management of CLD patients. Aim and Objectives: To determine the prevalence of Hepatitis B, Hepatitis C, and their coinfection among patients with chronic liver disease (CLD). To assess the viral load of Hepatitis B and Hepatitis C in patients diagnosed with chronic liver disease. Materials and methods: This cross-sectional study was conducted from June 2024 to May 2025, on a total of 300 CLD patients. Venous samples were tested for Hepatitis B surface antigen (HbsAg) and Anti-HCV antibodies by performing sandwich ELISA. Results: Out of 300 chronic liver disease (CLD) cases included in the study, 259 (86.3%) were males and 41 (13.7%) were females, with a mean age of 45.27 ± 12.36 years. Among these patients, Hepatitis B accounted for 20.7% (n = 62) of cases, while Hepatitis C contributed to 7% (n = 21). A small proportion of patients, 0.7% (n = 2), were found to have Hepatitis B and C coinfection. The majority of the HBsAg-positive cases and anti-HCV antibody-positive cases had blood transfusion as a risk factor. Out of 300 cases, 3(1%) cases were developed hepatocellular carcinoma, and all 3 cases were HBsAg positive. Conclusion: Hepatitis B and Hepatitis C are the most common causes of Chronic Liver Disease in India. Patients with dual infection do not have a greater risk of developing Hepatocellular carcinoma than mono-infection. Risk of Hepatocellular carcinoma in Hepatitis B infection is higher than Hepatitis C infection.***

Keywords: Chronic Liver Disease, Hepatitis B, Hepatitis C, Co-infection, Hepatocellular carcinoma, Viral load

Ethical approval

Ethical clearance for the study was obtained from the Institutional Ethics Committee. Patient confidentiality was maintained throughout the study by anonymising data, and all procedures were conducted in accordance with the ethical principles.

1.Introduction

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are major public health concerns worldwide. In India, their prevalence is higher in the northern and southern regions compared to the eastern and western parts of the country. Viral hepatitis remains one of the most significant global health challenges, alongside other major communicable diseases such as HIV, malaria, and tuberculosis [(1)]. Both hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that share similar modes of transmission. Consequently, co-infection with both viruses is relatively common, particularly in regions with a high prevalence of HCV infection and among individuals at increased risk for parenteral exposure [(2)]. According to the World Health Organisation (WHO), an estimated 254 million people were living with chronic hepatitis B infection and 50 million people with chronic hepatitis C infection in 2022. Hepatitis B accounted for approximately 1.1 million deaths, while hepatitis C caused around 242,000 deaths, primarily due to cirrhosis and hepatocellular carcinoma[(5)]. Chronic Liver Disease represents a series of liver disorders of varying causes and severity in which hepatic inflammation and necrosis continue for at least six months. Chronic liver disease (CLD) encompasses a wide spectrum of conditions, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [(3)]. Among these, HCC is a major global health concern, ranking as the sixth most common cause of cancer-related deaths worldwide and the second leading cause of overall cancer mortality. Notably, 80–90% of HCC cases develop in the setting of liver cirrhosis, irrespective of the underlying etiology [(4)] Prevalence of HBV among CLD patients is 17.6-47.9% in India and prevalence of HCV among CLD patients is 5.2-44.9% in India[(6)]. Hepatitis B virus (HBV) is the leading cause of chronic liver disease (CLD) in India, including cirrhosis and hepatocellular carcinoma (HCC)[(7)]. Although alcohol-related liver disease is rising, HBV remains the predominant cause of CLD. Patients with HBV and hepatitis C virus (HCV) co-infection are at an even greater risk, as they show accelerated progression to cirrhosis, decompensated liver disease, and a significantly increased likelihood of developing HCC[(8)]. The epidemiology of HBV and HCV infections varies across different regions of India, influenced by socioeconomic, cultural, and healthcare factors. Understanding the prevalence and clinical profile of

these viral infections in patients with CLD is crucial for planning effective preventive and therapeutic strategies, especially in regions with limited healthcare resources. This study aims to contribute to the regional database on viral hepatitis and support the formulation of targeted interventions for better management of CLD patients.

2.Materials and Methods

The present study is a cross-sectional study conducted in the Department of Microbiology, Government Medical College and Super Speciality Hospital, Nagpur, from June 2024 to May 2025. A total of 300 patients admitted in the wards of the Department of Medicine and the Department of Gastroenterology, Government Medical College, Nagpur, were enrolled in the study.

The study population consisted of both male and female patients representing different social strata. Patients were eligible for inclusion if they met one or more of the following criteria:

- A clinical history of liver disease symptoms persisting for more than six months.
- Radiological findings suggestive of parenchymal liver disease on ultrasound and/or CT scan.

Patients with Acute hepatitis, patients who were infected with other Hepatitis viruses like Hepatitis A, Hepatitis E and patients who were not willing to be a part of this study were excluded. All relevant patient history and clinical information were collected using a structured pro forma. Informed consent was obtained from each participant through a standardised consent form before inclusion in the study.

Sample collection

Approximately 5 mL of venous blood was collected from each patient under strict aseptic precautions in clean, sterile vials. The samples were allowed to clot at room temperature for 45 minutes.

Sample processing and testing

Serum was separated by centrifugation at low speed and stored under appropriate conditions until testing. The separated serum was tested for hepatitis B surface antigen (HBsAg) using an enzyme-linked immunosorbent assay (ELISA) kit (OSCAR, INDIA), a sandwich ELISA that enables quantitative estimation of HBsAg in patient serum or plasma. Then serum was tested for anti-HCV antibodies using an ELISA kit (OSCAR, INDIA), a sandwich ELISA utilising a solid phase coated with synthetic peptides and recombinant proteins representing HCV antigens (CORE, NS3, NS4, and NS5). Both tests were done according to the manufacturer's instructions. Appropriate positive and negative controls were included with each run to ensure test accuracy detection was achieved with anti-human IgG antibodies conjugated to horseradish peroxidase.

Samples that tested positive by ELISA were further subjected to viral load estimation using quantitative assays, as per standard protocols. Seropositive patients also underwent liver function testing. Parameters including serum bilirubin, ALT, AST, alkaline phosphatase, albumin, and prothrombin time were measured. Abnormal patterns consistent with hepatic dysfunction were noted.

3.Results

The present study included 300 patients of chronic liver disease (CLD) admitted to the Medicine and Gastroenterology wards of Government Medical College, Nagpur. All patients were tested for HBsAg and anti-HCV antibodies. Those who tested positive were further subjected to quantitative viral assays and liver function tests (LFTs). The results obtained are summarised below.

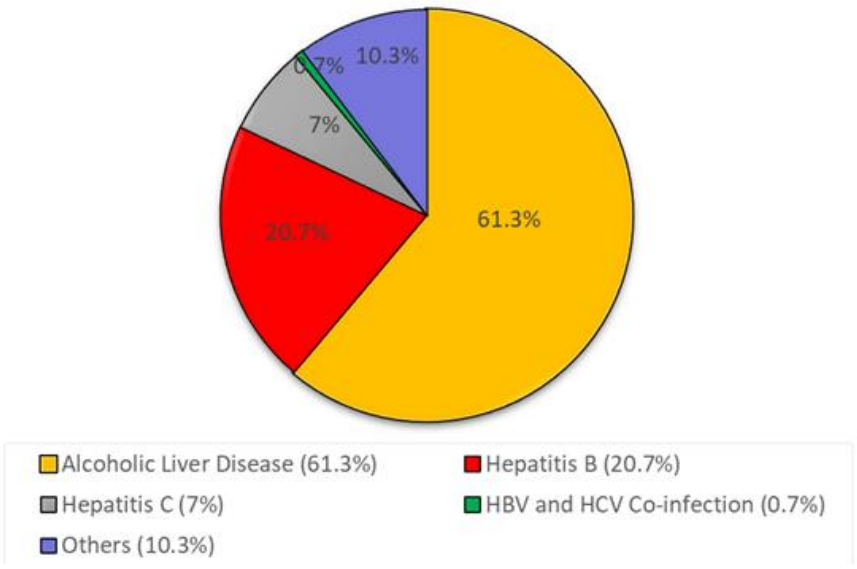

Figure 1: Causes of Chronic Liver Disease

Figure 1 shows the causes of Chronic Liver Disease. Among the 300 cases of chronic liver disease (CLD) included in the study, alcoholic hepatitis was the leading cause, accounting for 61.3% (n=184). Hepatitis B contributed to 20.7% (n=62),

while 7% (n=21) were due to Hepatitis C. A small proportion (0.7%, n=2) had Hepatitis B and C co-infection. The remaining 10.3% (n=33) were attributed to other causes, such as non-alcoholic steatohepatitis (NASH).

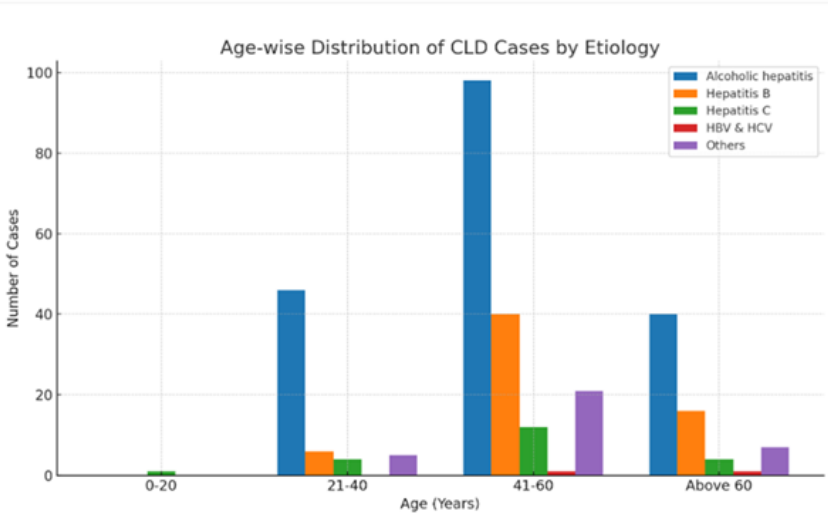

Figure 2: Age-wise distribution of CLD cases

Figure 2 shows the age-wise distribution of CLD cases. Out of 300 cases, the majority (58.3%, n=175) were in the age group of 41–60 years, followed by 21.4% (n=64) above 60 years and 20% (n=60) between 21–40 years. Only a single case was reported in the <20 years age group.

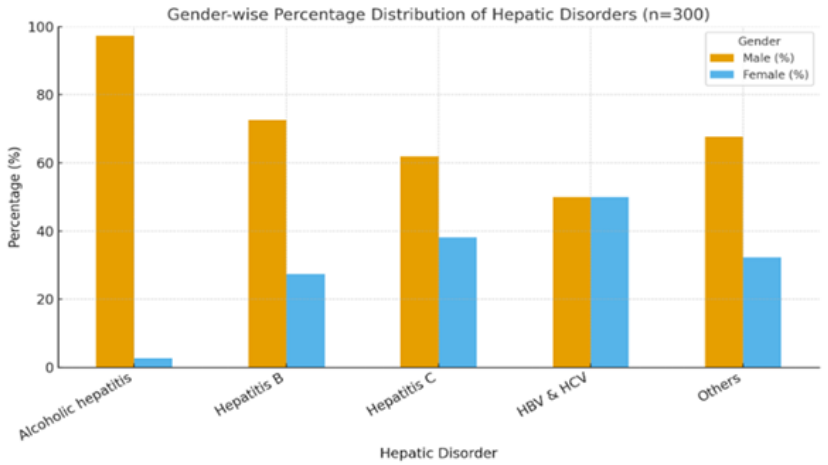

Figure 3: Gender-wise distribution of CLD cases

Figure 3 depicts the gender-wise distribution of CLD cases. Out of 300 cases, 259 (86.3%) were male and 41 (13.7%) were female. Among the 184 cases of alcoholic hepatitis, 179 (97.3%) were male and 5 (2.7%) were female. Of the 62 HBsAg-positive cases, 45 (72.6%) were male and 17 (27.4%) were female. Among the 21 anti-HCV–positive cases, 13 (61.9%) were male and 8 (38.1%) were female. One male and one female had co-infection with both Hepatitis B and C. In the remaining 31 cases attributed to other causes, 21 (67.7%) were male and 10 (32.3%) were female.

Out of 41 female CLD cases 17 were positive for HbsAg and 8 were positive for Anti-HCV antibodies. Eventhough the prevalence is more in males viral hepatitis due to HBV and HCV is more evident in females than alcoholic hepatitis.

Table 1: Risk factors for Hepatitis B infection

Risk factors	Total cases	HBsAg positive	Male	Female	P value
Alcohol addiction	210	38(18.1%)	37(97.4%)	1(2.6%)	1.872
Blood transfusion	52	**27(51.9%)**	13(48.1%)	14(51.9%)	0.0001
Multiple Sexual contact	23	4(17.3%)	4(100%)	0	0.685
Prenatal transmission	0	0	0	0	-
Injectable drug users	6	2(33.3%)	2(100%)	0	0.706
Tattooing	47	6(12.7%)	4(66.7%)	2(33.3%)	0.964
Unknown risk factors	24	4(16.6%)	2(33.3%)	2(66.7%)	1.502

Table 1 summarizes the multiple risk factors associated with Hepatitis B infection. Among 210 CLD cases with a history of alcohol addiction, 38 (18.1%) were HBsAg positive. Of the 52 cases with a history of blood transfusion, 27 (51.9%) were HBsAg positive. Among 23 cases with a history of multiple sexual partners, 4 (17.3%) tested positive for HBsAg. Out of 6 cases with a history of injectable drug abuse, 2 (33.3%) were positive. Similarly, among 47 cases with a history of tattooing, 6 (12.7%) were HBsAg positive, while 4 (16.6%) out of 24 cases with unknown risk factors were positive. None of the CLD cases had a history suggestive of prenatal transmission of Hepatitis B virus.

Table 2: Risk factors for Hepatitis C infection

Risk factors	Total cases	HCV IgM positive	Male	Female	P value
Alcohol addiction	210	4(2%)	4(100%)	0	0.845
Blood transfusion	52	**18(34.6%)**	12(66.7%)	6(33.3%)	0.003
Multiple Sexual contact	23	0	0	0	-
Prenatal transmission	0	0	0	0	-
Injectable drug users	6	**2(33.3%)**	2(100%)	0	0.724
Tattooing	47	0	0	0	-
Unknown risk factors	24	1(4.1%)	1(33.3%)	0	1.904

Table 2 presents the multiple risk factors associated with Hepatitis C infection. Among 210 CLD cases with alcohol addiction, 4 (2%) were HCV IgM positive. Of the 52 cases with a history of blood transfusion, 18 (34.6%) tested positive. None of the 23 cases with a history of multiple sexual partners were HCV IgM positive. Out of 6 cases with a history of injectable drug abuse, 2 (33.3%) were positive. Similarly, none of the 47 cases with a history of tattooing tested positive, while 1 (4.1%) out of 24 cases with unknown risk factors was positive. No CLD cases had a history suggestive of prenatal transmission of Hepatitis C virus.

Table 3: Risk Factors for Hepatitis B and Hepatitis C Co-infection

Risk factors	Total cases	HBsAg and HCV IgM positive	Male	Female
Blood transfusion	52	**2**	1(3.8%)	1(3.8%)
Multiple Sexual contact	23	0	0	0
Prenatal transmission	0	0	0	0
Injectable drug users	6	**0**	0	0
Tattooing	47	1	1(2.12%)	0

Table 3 shows the multiple risk factors associated with Hepatitis B and Hepatitis C co-infection. Among 52 CLD cases with a history of blood transfusion, 2 patients (1 male and 1 female) tested positive for both HBsAg and anti-HCV antibodies. The male patient also reported a history of tattooing as an additional risk factor.

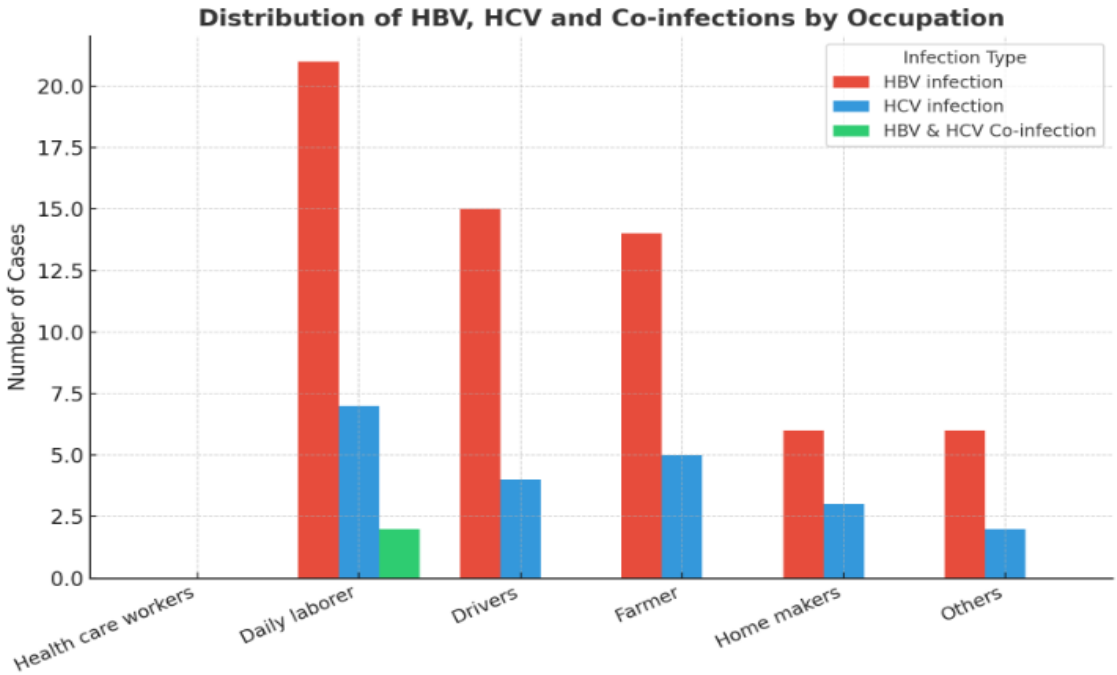

Figure 4: Distribution of Hepatitis B and Hepatitis C infected CLD cases based on Occupation

Figure 4 shows occupational distribution of Hepatitis B and Hepatitis C infection. Daily labourers are more commonly affected by HBV and HCV followed by drivers and farmer.

Table 5: Elevation of liver enzymes in CLD cases (Hepatitis B and Hepatitis C and their Co-infection)

Liver enzymes	Hepatitis B(%)	Hepatitic C(%)	Co-infection(%)
1. Alanine transferase (ALT/SGPT) (>100IU/L)	46.7	**47.6**	**100**
2. Aspartate aminotransferase (AST/SGOT) (>100IU/L)	**50**	42.8	**100**
3. Alkaline phosphatase (ALP) (>320IU/L)	24.2	38.1	**50**

Table 5 shows the elevation of liver enzymes in CLD patients. Among CLD cases with Hepatitis B infection, 46.7% had elevated ALT levels (>100 IU/L), while 50% and 24.2% showed elevated AST and ALP levels, respectively. In CLD cases with Hepatitis C infection, 47.6% had elevated ALT levels (>100 IU/L), and 42.8% and 38.1% had elevated AST and ALP levels, respectively. Both patients with HBV and HCV co-infection showed elevated ALT and AST levels, with one patient also showing elevated ALP levels.

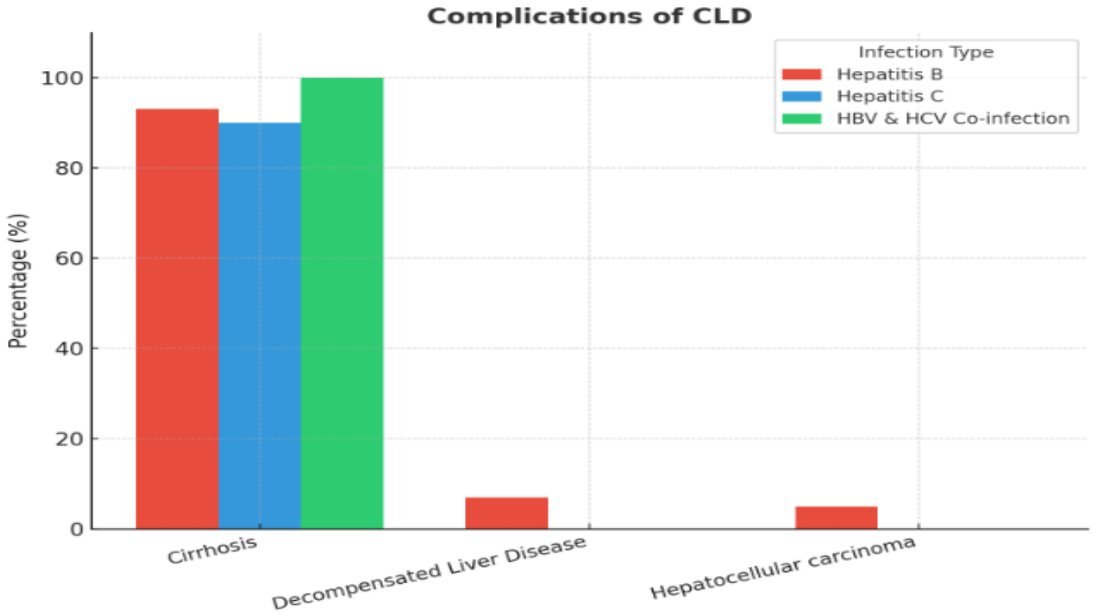

Figure 5: Complications of CLD cases associated with Hepatitis B, Hepatitis C, and their co-infection

Figure 5 depicts the complications of CLD cases associated with Hepatitis B, Hepatitis C, and HBV–HCV co-infection. Among CLD patients with Hepatitis B infection, 93.5% developed cirrhosis, 8.1% developed decompensated liver disease, and 4.8% developed hepatocellular carcinoma. In contrast, 90.4% of Hepatitis C infected CLD cases developed cirrhosis, while no one developed decompensated liver disease or hepatocellular carcinoma. Both patients with HBV–HCV co-infection developed cirrhosis, with no cases of decompensated liver disease or hepatocellular carcinoma.

Table 5: Viral load estimation in CLD cases infected with Hepatitis B and Hepatitis C and their co-infection

Viral load IU/ml	HBV (%)	HCV (%)	HBV&HCV Co-infection(%)	
			HBV	HCV
ND	6.5	4.7	0	0
<2000	9.7	23.8	0	0
2000-10^5	56.4	57.2	50	100
>10^5	27.4	14.3	50	0

Table 5 presents viral load estimation in CLD cases infected with Hepatitis B and Hepatitis C. Among Hepatitis B–infected CLD cases, viral load was undetectable in 6.5%, <2000 IU/L in 9.7%, 2000–10^5 IU/L in 56.4%, and >10^5 IU/L in 27.4% of cases. In Hepatitis C–infected CLD cases, viral load was undetectable in 4.7%, <2000 IU/L in 23.8%, 2000–10^5 IU/L in 57.2%, and >10^5 IU/L in 14.3% of cases. Among co-infected cases, one patient had viral loads of 2000–10^5 IU/L for both HBV and HCV, while another had an HBV viral load of >10^5 IU/L and an HCV viral load of 2000–10^5 IU/L.

4.Discussion

The present study included 300 cases of Chronic Liver Disease (CLD), with a mean age of 45.27 ± 12.36 years. This finding is comparable to the observations of Grewal *et al*(47.44 ± 14.56 years) [9], Singh *et al*(46.5 years)[10], and Alberts *et al*(47years)[11]. Male predominance is observed in our study, consistent with the reports of Grewal *et al* [9], Singh *et al* [10] and Alberts *et al*. [11]

Out of 300 patients, 62 (20.7%) were HBsAg positive. Comparable studies by Grewal *et al*.[9], Singh *et al*.[10], Devi *et al*.[12], Mathur *et al*.[13], and Kumar *et al*.[14] reported HbsAg seropositivity rates of 26%, 4%, 17.3%, 5.89%, and 17.34%, respectively. The variation in seropositivity rates

among these studies may be attributed to differences in diagnostic serological techniques, study populations, and associated risk factors.

Majority of the HbsAg positive cases are in the age group of 40-60 years. Similarly in Grewal *et al* [9] Devi *et al*.[12] and Ayele *et al* [4] HbsAg positive cases fell under the age group of 20-40 years, 22-32 years and 28-37 years respectively. Out of 62 HbsAg cases 45 (72.6%) were male and 17 (27.4%) were female with male to female ratio of 2.6:1 which is comparable with Kumar *et al*.[14] and Grewal *et al* [9] and Alberts *et al* [11]in which the male to female ratio is 2.5:1 and 7.6:1 and 3.7:1 respectively. Scientists in China have reported the discovery of unusual liver-specific proteins expressed exclusively in males, which may help explain the long-standing observation that Hepatitis B virus (HBV) infection exhibits a higher prevalence and severity in males than in females. [17]

In this study major risk factor for Hepatitis B infection is blood transfusion(51.9%) which is comparable with Ayele *et al* [4] Grewal *et al* [9], Devi *et al*.[12], and Nandi *et al* [15] , in which the proportion of CLD cases with HBV infection who have the history of blood transfusion were 22.2%, 15.4%, 35.3% and 10.5% respectively. In the current study 33.3% CLD cases have the history of injectable drug abuse, which is comparable with Grewal *et al* [9], Tiwari *et al* [16], Devi *et al*.[12] and Singh *et al*.[10]in which the prevalence of HBsAg seropositivity in injectable drug abusers are 9.1%, 23.7%, 14.7% and 10.8% respectively. In this study 17.3% of CLD cases with the history of having multiple sexual partners which is comparable with
Devi *et al*.[12] in which 29.4% CLD cases with HbsAg positive has the history of multiple sex partners in the past.

Out of 300 patients, 21 (7%) were anti-HCV Abs positive. Comparable studies by Similarly, Grewal *et al* [9], Seyed-Moayed *et al*.[18] Devi *et al*.[12]and Singh *et al*.[10] reported 40%, 40.7%, 30%, and 30% prevalence, respectively, of anti-HCV in their studies. The prevalence of HCV in CLD cases in this study is very low may be due to the effective treatment and timely management.

Majority of the anti-HCV positive cases are in the age group of 41-60 years. Similarly, in Grewal *et al* [10] and Ayele *et al* [4], anti-HCV positive cases fell under the age group of 41-60 years and 48-57 years respectively. Out of 21 anti-HCV positive cases 13 (61.9%) were male and 8 (38.1%) were female with male to female ratio of 1.6:1 which is comparable with Grewal *et al* [10] and Alberts *et al* [12] in which the male to female ratio is 7:1 and 1.07:1 respectively.

In this study major risk factor for Hepatitis C infection is blood transfusion(34.6%) which is comparable with Grewal *et al* [9], Ayele *et al* [4] and Mathur *et al* [19] in which the proportion of CLD cases with HCV infection who have the history of blood transfusion were 30% 29.6% and 43.65% respectively. In the current study 33.3% CLD cases with HCV infection were injectable drug abuser, which is comparable with Grewal *et al* [9], Devi *et al*.[12] and in which the prevalence of anti-HCV positivity in injectable drug abusers are 81.8% and 90.4% respectively.

Prevalence of hepatitis B and hepatitis C Co-infection is 0.7% in this study. Simlarly Vilas BN *et al* [20] shows 3.5% prevalence rate of HBV and HCV Co-infection in a study done in Karnataka, India. It is also comparable with Grewal *et al* [9] Xess *et al* [21], Anbazhagan *et al* [22] and Chawla *et al* [23] where the prevalence rate of HBV and HCV Co-infection rate are 4%, 3%, 1.5% and 0.4% respectively.

Elevated levels of liver enzymes such as ALT, AST, ALP are observed in CLD cases due to HBV and HCV infection. Other studies done by Mastoi *et al* [24] and Megha *et al* [25] also show the same results.

Daily labourers are more commonly affected by HBV and HCV followed by drivers and farmer in this study. Similarly in Tessema *et al*[26] farmers are more commonly affected by HBV and HCV followed by daily laborers.

In this study among 62 CLD patients with Hepatitis B infection, 93.5% developed cirrhosis which is comparable with Saravanan *et al*[27] in which HBV infection shows 95% of cirrhosis in a study conducted in south India. In this study 4.8% of CLD cases infected with HBV developed hepatocellular carcinoma which is comparable with Saravanan *et al* (5%)[27]

Among 33 CLD patients with Hepatitis C infection 90.4% cases developed cirrhosis, which is comparable with Saravanan *et al* (87%)[27]. No HCV infected CLD cases were developed Hepato cellular carcinoma in this study.

Both patients with HBV–HCV co-infection developed cirrhosis(100%). A study done by Xess *et al* [21] shows 53.3% of the cases with HBV and HCV Co-infection developed liver cirrosis.

5.Conclusion

The present cross-sectional study conducted among patients with chronic liver disease (CLD) in a tertiary care hospital in Central India highlights the significant burden of viral hepatitis as an etiological factor. Hepatitis B infection was found to be the most common viral cause, followed by Hepatitis C, while HBV–HCV coinfection was relatively rare. Most patients were middle-aged males, indicating a gender and age predisposition.

The findings emphasize the need for routine screening of all CLD patients for both HBV and HCV to ensure early detection and appropriate management. Strengthening preventive strategies such as hepatitis B vaccination, public awareness programs, and ensuring safe transfusion and injection practices remain crucial to reduce the transmission and burden of these infections. Hepatitis B is preventable and Hepatitis C is treatable. So timely vaccination and early diagnosis and treatment can reduce the CLD load. Future studies with larger sample sizes and molecular profiling could further elucidate the epidemiological trends and clinical outcomes of HBV and HCV coinfections in the Indian population.

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Conflict of interest: None

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