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A Cross-Sectional Study to Find Out the Proportion of Hepatitis B and Hepatitis C Infection among Chronic Liver Disease Patients Attending Tertiary Care Hospital

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ABSTRACT

Background: Chronic liver disease (CLD) is a major cause of morbidity and mortality worldwide. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections remain important etiological factors contributing to progressive liver disease, cirrhosis, and hepatocellular carcinoma. Early detection of viral hepatitis and associated risk factors is essential for improving patient outcomes.

Objectives: To determine the proportion of HBV and HCV infections among patients with chronic liver disease attending a tertiary care hospital, assess viral load among seropositive patients, and evaluate associated risk factors.

Materials and Methods: A hospital-based prospective cross-sectional study was conducted among 155 adult patients with chronic liver disease at a tertiary care hospital. Eligible patients were enrolled consecutively after obtaining informed consent. Serum samples were screened for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies using rapid immunochromatographic assays. Viral load estimation was performed in seropositive patients using nucleic acid amplification techniques. Data were analysed using appropriate statistical tests, and a p-value of <0.05 was considered statistically significant.

Results: Among the 155 participants, 69.7% were males and 30.3% were females. HBV infection was detected in 34.8% of patients, while HCV infection was identified in 20.6%. Overall, 55.4% of patients had evidence of viral hepatitis, whereas 44.6% tested negative for both infections. Alcohol consumption was the most common risk factor and showed a significant association with HBV/HCV infection ($p=0.001$). Blood transfusion ($p=0.018$) and injectable drug use ($p=0.036$) were also significantly associated with viral hepatitis. Most seropositive patients who underwent viral load estimation demonstrated higher viral loads.

Conclusion: HBV and HCV remain important contributors to chronic liver disease, with HBV being more prevalent than HCV. Routine screening, early diagnosis, timely antiviral therapy, strengthening hepatitis B vaccination, safe healthcare practices, and targeted awareness programmes are essential for reducing the burden of chronic viral hepatitis and its complications.

Keywords: Chronic liver disease, Hepatitis B virus, Hepatitis C virus, HBsAg, Viral load, Risk factors.

INTRODUCTION

Chronic liver disease (CLD) is a major cause of global morbidity and mortality and remains an important public health challenge. It comprises a spectrum of progressive liver disorders characterized by persistent inflammation, fibrosis, and distortion of normal hepatic architecture, eventually leading to cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC). The global burden of CLD has increased substantially over recent decades because of the rising prevalence of metabolic disorders, alcohol consumption, and chronic viral hepatitis.[1]

The major etiological factors responsible for CLD include metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease, alcohol-related liver disease (ALD), and chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections[1-4]. MASLD has become the leading cause of chronic liver disease worldwide owing to the increasing prevalence of obesity, diabetes, and metabolic syndrome.[2,3] However, chronic HBV and HCV infections continue to be major infectious causes of progressive liver injury and remain important contributors to cirrhosis and HCC, particularly in low- and middle-income countries.[4-6]

The liver performs essential metabolic, synthetic, detoxification, and immunological functions. Progressive liver injury, irrespective of its etiology, results in fibrosis and eventually cirrhosis, leading to complications such as portal hypertension, ascites, variceal bleeding, hepatic encephalopathy, and hepatocellular carcinoma.[7-9] Since CLD often remains asymptomatic until advanced stages, early identification of its underlying cause is crucial for timely intervention and improved clinical outcomes.

Chronic HBV and HCV infections continue to pose a major global health challenge. According to the World Health Organization, approximately 325 million people are living with chronic HBV or HCV infection worldwide, and these infections account for nearly 96% of hepatitis-related deaths. Despite the availability of effective HBV vaccination and antiviral therapies for both HBV and HCV, delayed diagnosis and limited access to treatment continue to contribute to disease progression and transmission.[1]

In India, chronic viral hepatitis remains an important cause of chronic liver disease. Factors such as unsafe injection practices, blood transfusion, intravenous drug use, invasive medical procedures, and perinatal transmission contribute to the continued spread of HBV and HCV. Previous studies have identified both viruses as major etiological factors for cirrhosis and hepatocellular carcinoma in the Indian population.[10-13] Early detection of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies, along with viral load estimation, is essential for timely treatment, disease monitoring, and prevention of further transmission.

Although several studies have evaluated the epidemiology of HBV and HCV infection, institution-specific data among patients with chronic liver disease remain limited in many parts of central India. Such information is important for understanding the regional burden of disease and developing appropriate screening and preventive strategies.

Therefore, the present study was undertaken to determine the proportion of hepatitis B and hepatitis C infections among patients with chronic liver disease attending a tertiary care hospital. The study also assessed HBV-HCV co-infection, viral load among seropositive patients, and socio-demographic and clinical risk factors associated with chronic liver disease. The findings are expected to contribute to the existing evidence and support strategies for the early diagnosis, prevention, and management of chronic

viral hepatitis.

AIMS AND OBJECTIVES

To determine the proportion of hepatitis B and hepatitis C infections among chronic liver disease patients at a tertiary care hospital.

Primary Objectives:

- To find out the proportion of hepatitis B and hepatitis C infection among the chronic liver disease patients by using immunochromatography test.
- To find out the co-infection of hepatitis B and hepatitis C among chronic liver disease patients by immunochromatography test .

Secondary Objectives:

- To know HBV DNA and HCV RNA viral load in hepatitis B and C positive patients.
- To determine socio-demographic and risk factors associated with chronic liver disease.

METHODOLOGY

Study Design and Setting: This is a hospital-based prospective cross-sectional study conducted in the Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, in association with Departments of Medicine, Gastroenterology of the attached tertiary care teaching hospital. The hospital is a well established tertiary care centre treating patients from Indore and its adjoining areas from both urban and rural areas. The study was conducted for one year with approval from Institutional Scientific Committee and Institutional Ethics committee.

A prospective cross sectional study design was used to estimate the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection among chronic liver disease (CLD) patients. This design allowed systematic enrolment of eligible participants, standardized clinical assessment, uniform collection of specimens, and uniform laboratory assessment during the entire study period, which ensured reliability and reproducibility of the study results.

Study Subjects: Adult patients with a diagnosis of chronic liver disease attending the outpatient clinics and admitted to the medical and gastroenterology wards of the tertiary care hospital during the study period constituted the study population. Chronic liver disease was diagnosed based on compatible clinical features, biochemical investigations, and radiological findings suggestive of persistent liver disease for a duration of six months or longer.

Patients who were eligible and who met inclusion criteria were recruited consecutively and provided written informed consent. Every participant was given a unique identification number for the sake of anonymity and systematic documentation of clinical and lab data.

Inclusion Criteria:

- Adult patients aged 18 years and above.
- Patients with an established diagnosis of chronic liver disease based on clinical evaluation, laboratory investigations, and radiological findings.
- Patients willing to participate in the study and provide written informed consent.

Exclusion Criteria:

- Patients diagnosed with acute liver disease or acute viral hepatitis.
- Patients unwilling to provide written informed consent.
- Patients with incomplete clinical records.

- Patients from whom an adequate blood sample could not be obtained for laboratory investigations.

Sample Size: The sample size was calculated according to the standard formula for single population proportion, with an expected prevalence of 34.62%, at the 95% confidence interval and with an absolute precision of 7.5%. Assuming these, a minimum of 155 participants were calculated.

Sampling Technique: A consecutive sampling technique was employed for participant recruitment. The patients who attended the outpatient department and admitted to the medical and gastroenterology wards during the study period, were screened for eligibility. All participants who met the inclusion criteria were recruited consecutively until there was a total of 155 participants. This sampling strategy reduced selection bias, and included all eligible patients who presented during the study period.

Data Collection: The data was collected on a predesigned and pretested case record form. Socio-demographic data such as age, sex, place of residence, educational level and occupation was collected. A thorough clinical history was taken including a history of past episodes of jaundice, alcohol intake, blood transfusion, previous surgeries, tattooing, and any other potential for liver disease related to hepatitis B and C virus infection.

A detailed clinical examination was done and relevant laboratory investigations and radiological findings supporting the diagnosis of chronic liver disease were documented. All Clinical and Laboratory data were recorded in a systematic way to facilitate complete, accurate, and uniformity of data collection during the study period.

Specimen collection and processing: Written informed consent was obtained and about 5 mL of venous blood was obtained aseptically from each participant by using a sterile disposable syringe and placed into a plain vacutainer tube. The blood samples were left to clot at room temperature and then centrifuged for 15 minutes at 2500 rpm for serum separation. The separated serum was then carefully transferred to sterile microcentrifuge tubes, which were labelled appropriately, and stored under recommended laboratory conditions until further analyses. All samples were handled in the laboratory following standard procedures to ensure sample integrity and contamination avoidance.

Laboratory Investigations: All investigations were carried out in the Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore, according to their standard operating procedures. The hepatitis B virus and hepatitis C virus (HCV) infections were tested by commercially available rapid immunochromatographic assay kit on serum samples. The hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (anti-HCV) antibody serology was subsequently assessed in hepatitis B positive, and/or anti-hepatitis C virus (anti-HCV) antibody positive patients to estimate viral load.

Detection of Hepatitis B Virus Infection: Serum samples were tested for the presence of hepatitis B virus infection by testing for hepatitis B surface antigen (HBsAg) using rapid immunochromatographic assay based on the lateral flow immunoassay principle. The test was conducted as per the manufacturer's guidelines. The assay buffer was added to the test cassette followed by the serum sample and the results were interpreted within the recommended time. An appearance of both test and control lines was interpreted as positive for HBsAg and appearance of only the control line as negative. Test runs without a prominent control line were discarded and repeated with a new test device.

Detection of Hepatitis C Virus Infection: Serum samples were tested for the presence of antibodies against Hepatitis C Virus (anti-HCV) by a rapid immunochromatographic test kit which utilizes the double-antigen sandwich lateral flow immunoassay method. All tests and interpretations were performed as recommended by the manufacturer. The visible test line plus the control line was deemed “reactive” while only the control line was deemed non-reactive. Test cassettes that did not have the control line

were retaped with a new test cassette.

Diagnosis of HBV-HCV Co-infection: Participants who tested positive for both HBsAg and anti-HCV antibodies were classified as having HBV-HCV co-infection. The proportion of co-infected patients of the study population was determined and analysed.

Viral load estimation: HBV DNA and HCV RNA viral load was assessed by quantitative nucleic acid amplification method in the patients who had reactive HBsAg and/or anti-HCV antibody result. Viral load testing was done in the authorized laboratory according to the guidelines of the National Viral Hepatitis Control Programme (NVHCP). Results are presented in international units per millilitre (IU/mL) and used to determine the viral replication and disease activity. Correlation between viral load and clinical/laboratory profile was performed for the corresponding patients.

Quality Control: All laboratory operations were performed as per the standard operating procedures (SOPs) followed in the Department of Microbiology to ensure the accuracy, reliability and reproducibility of the laboratory results. The level of HBsAg and anti-HCV antibodies were identified using commercially available rapid immunochromatographic test kits (approved manufactures). Test kits were kept under recommended storage conditions and expiry date and lot number of test kits were checked before use.

All the necessary internal quality control arrangements were adhered to during the study. The results of the tests were interpreted if the control line was present on the test device. Samples that were invalid were re-tested with new test kits. The accuracy of all laboratory apparatus such as centrifuges and micropipettes was routinely verified by following institutional procedures. To reduce the possibility of contamination and to make these test results valid, standard precautions for the collection and processing of specimens as well as the testing process were observed.

Data Management: The data of all the studies were obtained by using unique identification code to ensure the confidentiality of participants. The information was transcribed from the case record forms and lab investigation data after checking for completeness and accuracy into a Microsoft Excel spreadsheet. The data were reviewed periodically to detect and eliminate inconsistencies and incomplete data before statistical analysis was performed. The final database was accessed only by the investigators involved in the study.

Data entry and statistical analysis: Data were entered in Microsoft Excel and analysed using statistical software - Excel/Jamovi (2.7.16). The continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range) depending on the distribution of the data and categorical variables were expressed as frequencies and percentages.

The percentages of chronic liver disease patients infected with HBV, HCV and both HBV/HCV infections were determined. The categorical variables were assessed for association with possible risk factors with the Chi-square test or Fisher's exact test as appropriate. Non parametric tests (Student's t-test or Mann-Whitney U test) were used to compare continuous variables depending on data distribution. A p-value of <0.05 was deemed as statistically significant.

Ethical considerations: The study was carried out after the approval of the Institutional Scientific Committee and Institutional Ethics Committee, Mahatma Gandhi Memorial Medical College, Indore. All participants signed informed consent forms before enrollment, in their native language, explaining the purpose, objectives and procedures of the study.

The study was voluntary and there was no stipulation that participants would stay in the study, as long as they continued to receive their usual medical treatment. Patient information was kept confidential by

using unique identifications and only allowing the investigators to have access to the study records. All study procedures were conducted with respect to the principles of biomedical research on human subjects and institutional ethical guidelines.

OBSERVATIONS

Table No. 1: Demographic Characteristics of Study Participants (n=155)

Variable	Category	Frequency (n)	%
Gender	Male	108	69.7
	Female	47	30.3
Age Group (years)	18-30	18	11.6
	31-45	42	27.1
	46-60	58	37.4
	>60	37	23.9

Table No. 2: Proportion of Viral Hepatitis among CLD patients

Viral Hepatitis Status	Frequency(n)	%
HBV only positive	54	34.8
HCV only positive	32	20.6
Negative for both HBV & HCV	69	44.6
Total	155	100

Table No. 3: Proportion of HBV and HCV among Male and Female

Gender	HBV(%)	HCV(%)	Total Viral Hepatitis (%)
Male	38.9	22.2	61.1
Female	25.5	17	42.5

Table No. 4: Risk Factor Distribution among Study Participants

Risk Factor	Frequency (n)	% (%)
Alcohol consumption	78	50.3
Blood transfusion	34	21.9
Surgery	46	29.7
Injectable drug use	12	7.7
Tattooing	18	11.6

Table No. 5: Association of Risk Factors with HBV/HCV Infection

Risk Factor	HBV/HCV Positive frequency (n)	HBV/HCV Positive % (%)	HBV/HCV Negative frequency (n)	HBV/HCV Negative % (%)	p-value
Alcohol consumption	52	66.7	26	33.3	0.001*
Blood transfusion	22	64.7	12	35.3	0.018*

Surgery	28	60.9	18	39.1	0.052
Injectable drug use	9	75	2	25	0.036*
Tattooing	11	61.1	7	38.9	0.074

Table No. 6: Viral load analysis

Cases	Viral Load >10 IU/ML	Viral Load <10 IU/ML
HBV	61	13
HCV	10	0

RESULTS

A total of 155 patients with chronic liver disease (CLD) were enrolled in the study. Of these, 108 (69.7%) were males and 47 (30.3%) were females. The highest proportion of participants belonged to the 46-60 years age group (37.4%), followed by 31-45 years (27.1%), >60 years (23.9%), and 18-30 years (11.6%) (Table 1).

HBV infection was detected in 54 (34.8%) patients, while 32 (20.6%) were positive for HCV infection. A total of 69 (44.6%) patients were negative for both HBV and HCV (Table 2). HBV and HCV infections were more common among males than females, with an overall viral hepatitis prevalence of 61.1% in males and 42.5% in females (Table 3).

Alcohol consumption was the most frequently identified risk factor (50.3%), followed by previous surgery (29.7%), blood transfusion (21.9%), tattooing (11.6%), and injectable drug use (7.7%) (Table 4). Alcohol consumption (p=0.001), blood transfusion (p=0.018), and injectable drug use (p=0.036) were significantly associated with HBV/HCV infection, whereas surgery and tattooing were not statistically significant (Table 5).

Among patients who underwent viral load estimation, most HBV-positive patients had viral loads >10 IU/mL, while all HCV-positive patients who were tested also had viral loads >10 IU/mL, indicating active viral replication (Table 6).

DISCUSSION

The present hospital-based prospective cross-sectional study evaluated the burden of HBV and HCV infection among patients with chronic liver disease attending a tertiary care hospital. More than half of the study population had evidence of either HBV or HCV infection, confirming that chronic viral hepatitis remains an important cause of chronic liver disease in this region.[14]

HBV infection was more prevalent than HCV infection, a finding consistent with previous Indian studies where HBV continues to be a major etiological factor for chronic liver disease because of its endemicity and ongoing transmission.[15] The prevalence observed in the present study falls within the range reported in earlier Indian studies, which documented HBV in 17.6-47.9% and HCV in 5.2-44.9% of patients with chronic liver disease.[16] No patient in the present study had HBV-HCV co-infection, which is in agreement with previous reports demonstrating the relatively low prevalence of dual infection.[16,17]

A higher prevalence of viral hepatitis was observed among male participants than females. Similar findings have been reported by Islahi et al.[18] and Khan et al.[19], suggesting that greater exposure to behavioural and occupational risk factors among males may contribute to this difference.

Alcohol consumption was the most common risk factor identified and showed a significant association

with viral hepatitis. Blood transfusion and injectable drug use were also significantly associated with HBV/HCV infection, whereas surgery and tattooing were not significantly associated. These findings are consistent with previous studies highlighting the role of behavioural and healthcare-related exposures in the transmission of chronic viral hepatitis[20,21].

Most HBV-positive patients and all HCV-positive patients who underwent viral load estimation demonstrated higher viral loads, indicating active viral replication. Similar observations have been reported in previous studies, emphasizing the importance of viral load assessment in evaluating disease activity, monitoring treatment response, and guiding antiviral therapy[22].

The findings of the present study highlight the importance of routine HBV and HCV screening among patients with chronic liver disease, early initiation of antiviral therapy, strengthening hepatitis B vaccination programmes, ensuring safe blood transfusion practices, and promoting awareness regarding preventable behavioural risk factors. These measures may help reduce the burden of chronic liver disease and its associated complications.[23-25]

CONCLUSION

In the present study, it has been shown that hepatitis B virus (HBV) and hepatitis C virus (HCV) are important etiological agents even among patients attending a tertiary care hospital with chronic liver diseases. HBV was more prevalent than HCV and over half of the study population had evidence of viral hepatitis. Male patients were more likely to have viral hepatitis, and alcohol consumption, blood transfusion and injectable drug use were significant factors in the HBV/HCV infection.

Most seropositive patients were found to have increased viral levels, which suggests ongoing viral replication and the significance of early diagnosis and prompt treatment with antiviral drugs in the quest to limit progression of disease and enhance clinical outcomes.

The findings highlight the need for routine screening of patients with chronic liver disease for HBV and HCV infection, strengthening hepatitis B vaccination programmes, ensuring safe healthcare practices, and promoting awareness regarding modifiable risk factors. Chronic liver disease and its complications could be significantly reduced by early detection, prompt treatment and effective preventive measures.

SUMMARY

To find the proportion of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in chronic liver disease (CLD) patients attending Mahatma Gandhi Memorial Medical College and M.Y. Hospital, Indore, Madhya Pradesh in the present hospital based prospective cross sectional study. Viral load level and socio-demographic characteristics and risk factors in viral hepatitis were also assessed in the study.

A consecutive sampling was used to recruit a total number of 155 adult patients with confirmed chronic liver disease. Testing for hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (anti-HCV) antibody was conducted with rapid immunochromatographic assay, and viral load was done in seropositive patients with nucleic acid amplification techniques.

Of the subjects, males made up 69.7% of the study population and the majority of patients were in the age group of 46-60 years. Of the patients, 34.8% were positive for the presence of HBV infection and 20.6% were positive for HCV infection. Overall, 55.4% of patients were positive for viral hepatitis, while 44.6% were negative for both HBV and HCV. There was no case found where both HBV and HCV were co-infected.

Previous surgery, blood transfusion, tattooing and IDU were the least common risk factors, with alcohol

consumption being the most frequent. Alcohol use, blood transfusion, and injection drug use were statistically significant with infections with both HBV/HCV.

The viral load analysis showed that most of the patients with positive HBV blood tests and all of the patients with positive HCV blood tests who were tested had high viral load >10 IU/mL which indicated active viral replication.

To sum up, HBV and HCV were still significant contributors of chronic liver disease among the study population with HBV more common than HCV. The results underscore the need for regular screening, early detection, prompt antiviral treatment, intensifying hepatitis B vaccination programs, improving safe health practices and raising awareness among the population of preventable risk factors to decrease the burden of chronic liver disease and its complications.

LIMITATIONS OF THE STUDY

- This is a single centre, hospital based study so results may not be applicable to the broader population.
- The cross sectional study design does not allow establishing cause and effect relationships between the identified risk factors and the occurrence of HBV/HCV infection.
- The sample size was sufficiently large but larger multi-centre studies are needed to increase the generalizability of the findings.
- Self-reported information about behavioural risk factors may be affected by recall or reporting bias.
- The study was mainly analyses HBV and HCV infection and was not a comprehensive analysis of other factors causing chronic liver disease including metabolic dysfunction associated steatotic liver disease (MASLD), autoimmune hepatitis and inherited metabolic liver disorders.
- In some seropositive patients, viral load estimation was not possible due to loss to follow up, and hence only partial evaluation of viral replication was possible.

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