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Original Article

Microbiology Section

Prevalence of Hepatitis D Virus among the Patients at a Tertiary Care Hospital in Gujarat, India- A Cross-sectional Study

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ABSTRACT

Introduction: Hepatitis D (Delta) Virus (HDV) infection is present worldwide, with an estimated 18 million people being infected. HDV can infect individuals who are already infected with the Hepatitis B virus (HBV), leading to severe liver disease. The global prevalence of HDV varies by geographical region. In India, there is a lack of data on HDV infection among individuals exposed to HBV. Detecting HDV infection in Hepatitis B surface Antigen (HBsAg)-positive patients can improve patient outcomes.

Aim: To determine the seroprevalence of HDV in HBsAg-positive patients.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, Shri M.P. Shah Government Medical College and Guru Gobind Singh Government Hospital in Jamnagar, Gujarat, India, over a period of one year from September 2022 to August 2023. A total of 350 HBsAg Enzyme-Linked Immunosorbent Assay (ELISA)-positive cases were included in the study. Serum samples from patients in various inpatient and outpatient departments of the hospital were tested for HBsAg infection using ELISA. After confirming positive results for HBsAg, all samples were tested for anti-HDV IgM antibodies by ELISA. Demographic and clinical findings were recorded for all patients. Statistical analysis was performed using means and percentages, and p-values were calculated using the Chi-square test to determine the seroprevalence of HDV.

Results: Among the 350 HBsAg-positive cases, a higher prevalence was observed in middle-aged and older females. Ten (2.9%) cases were positive for anti-HDV antibodies. Of the 10 anti-HDV antibody-positive patients, 5 (50%) reported a history of blood transfusion.

Conclusion: The seroprevalence of HDV was found to be 2.9% in HBsAg-positive patients in this study, with a predominance in middle-aged and older females. Additionally, the most common route of transmission identified was blood transfusion.

Keywords: Blood transfusion, Hepatitis B virus, HDV, Viral hepatitis

INTRODUCTION

The HDV was first identified in a HBV infected patient with severe hepatitis by Rizzetto M et al., in 1977 [1]. HDV is a small defective Ribonucleic acid (RNA) virus belonging to the Delta viridiae family and the genus Delta virus, which requires HBsAg for its life cycle in host cells. HDV is a formalin-sensitive virus that measures 35-37 nm and has a hybrid structure. HDV has a unique antigen, the HDV antigen, which is closely associated with the HDV RNA genome; however, it is not secreted after affecting the host cell because it is located beneath the outer HBsAg layer [2]. Consequently, hepatitis D infection results from either an acute co-infection with HBV and HDV or HDV superinfection in patients who are chronically infected with HBV [3]. Thus, the production and transmission of HDV are entirely dependent on HBV.

The transmission routes of HDV are similar to those of HBV, including percutaneous routes such as blood transfusion, intravenous drug use, sexual contact, and vertical transmission. HDV, in association with HBV, causes significantly more severe disease than HBV alone, rapidly progressing to cirrhosis with liver failure and hepatocellular carcinoma. Persistent replication of HDV is the most important predictor of mortality [4]. HDV has eight phylogenetically distinct genotypes, each associated with different outcomes of infection. Genotype 1 is the most frequent and is found in Europe, the Middle East, North America, and North Africa; Genotype 2 occurs mainly in East Asia; Genotype 3 has been reported in the northern region of South America; Genotype 4 has been isolated in Taiwan and Japan; the remaining HDV genotypes are uncommon [5].

Approximately, 5% of HBV carriers worldwide are co-infected with HDV. Out of the 350 million HBV carriers globally, 18 million people are infected with HDV [6]. The global prevalence of HDV may vary depending on the geographical region; however, the worldwide prevalence of HDV has substantially declined over the years. This decline was achieved by increasing awareness among the general public and implementing vaccination measures against hepatitis B in various countries. Serological techniques form the basis for determining HDV prevalence in India. Considering the scarcity of data and the impact of the HDV on HBV infection, present study was planned to find the prevalence of HDV. This study aims to update current knowledge on HDV prevalence. The objective of the study was to determine the seroprevalence of HDV in HBsAg-positive patients attending a tertiary care hospital and to support the need for HDV infection detection in HBsAg-positive patients to improve patient outcomes. Additionally, the study aims to assess the association of age with the presence of anti-HDV antibodies.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology at Shri MP Shah Government Medical College and Guru Gobind Singh Government Hospital in Jamnagar, Gujarat, India, over a period of one year, from September 2022 to August 2023. Approval for this study was obtained from the Institutional Ethical Committee (IEC) (169/04/2022), and informed consent was obtained from the patients. This was a time-bound study, and only the samples available during the study period were included.

Inclusion criteria: The patient samples from various indoor and outdoor departments of the hospital, sent for routine serological HBsAg investigations, received at the Microbiology department during the study period, were included in the study.

Exclusion criteria: Patients with HIV infection and dual infections with hepatitis A, C, and E were excluded from the study.

Study Procedure

A total of 350 HBsAg positive samples were enrolled in the study. These samples were collected from a total of 30,000 samples received during the study period of one year. Approximately, 3-5 mL of venous blood was collected aseptically by venepuncture and transferred to a plain tube with a clot activator before being received in the laboratory. The samples were tested for HBsAg infection using the Merilisa HBsAg ELISA kit, a Direct Sandwich ELISA (Meril Diagnostic Pvt., Ltd., ICMR approved) [7]. Those who tested positive for HBsAg were subsequently tested for the detection of anti-HDV IgM antibodies using a capture ELISA kit [8]. The assay cut-off was calculated using the mean value of the Negative Control (NC) with the following formula: Cut-off = NC+0.250. Results were interpreted as a ratio of the sample's Optical Density (OD) value to the cut-off value. Ratios for IgM antibodies <0.9 were considered negative, 0.9-1.1 were considered equivocal, and >1.1 were considered positive, as specified by the manufacturer (HDV IgM Dia.Pro Diagnostic Bioprobes Srl, ISO approved) [9]. Demographic and clinical findings such as age, gender, and risk factors- including blood transfusion and surgery- were recorded for all patients, as the transmission routes of HDV are similar to those of HBV [10]. Patients for whom no risk factors were identified were included in the "others" category. Subsequently, all data were analysed to determine the seroprevalence of HDV.

STATISTICAL ANALYSIS

The statistical analysis was conducted using means and percentages. Differences between proportions were analysed using p-values calculated from Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

Out of 350 HBsAg-positive patients, 10 (2.9%) were found to be positive for anti-HDV antibodies [Table/Fig-1]. So the seroprevalence of Hepatitis D among the HBsAg reactive patients was 2.9%.

Total no. of HBsAg positive samples	Total no. of anti-HDV positive samples	Seroprevalence
350	10	2.9%

[Table/Fig-1]: Seroprevalence of HDV infection among HBsAg positive patients.

Age-wise analysis in the present study showed a higher seropositivity among individuals in the age group of 51-60 years, followed by the age group of 21-30 years [Table/Fig-2].

Age group (years)	n (%)
0-10	0
11-20	0
21-30	3 (30)
31-40	0
41-50	1 (10)
51-60	4 (40)
>60	2 (20)
Total	10 (100)

[Table/Fig-2]: Age wise distribution of anti- HDV positive cases.

Association with age and anti-HDV antibodies was not significant (p-value=0.08) [Table/Fig-3]. The gender-wise distribution of Hepatitis D antibody cases is shown in [Table/Fig-4].

Anti-HDV antibodies	Mean age±SD (in years)	p-value
Positive	48.4±18.11	0.08
Negative	40.64±19.13	

[Table/Fig-3]: Association of age with presence of anti-HDV antibodies.
*Data are expressed as mean±SD; A p-value <0.05 was considered to be statistically significant, here not significant

Gender	No. of cases (n=350)	Positive cases
Male	201 (57.43%)	3 (1.5%)
Female	149 (42.57%)	7 (4.7%)

[Table/Fig-4]: Gender-wise distribution of Hepatitis D antibody cases.

There was no significant association between the presence of anti-HDV antibodies and gender of the cases in the study (p-value=0.07), as shown in [Tables/Fig-5], respectively.

Gender	Positive cases	Negative cases	Chi-square value	p-value
Male	3 (1.5%)	198 (98.50%)	3.168	0.07
Female	7 (4.7%)	142 (95.30%)		

[Table/Fig-5]: Association of gender with presence of anti-HDV antibodies.
* -Chi-square test; - p-value – If <0.05 consider as statistically significant

The [Table/Fig-6] shows that the rate of positive cases was higher among individuals with a history of blood transfusion, which accounted for five cases (50%).

Risk factor	n (%)
Blood transfusion	5 (50)
Surgery	1 (10)
Others	4 (40)
Total	10 (100)

[Table/Fig-6]: Distribution of Anti-HDV positive cases according to risk groups.

DISCUSSION

The HDV infection is associated with an increased risk of cirrhosis and hepatocellular carcinoma compared to those who only have chronic HBV infection [10]. Therefore, early diagnostic intervention in HDV-infected patients may be the right way to prevent end-stage liver disease and the development of cirrhosis.

Present study showed a prevalence of 2.9% of HDV antibodies among HBsAg-positive patients. This finding was consistent with similar studies conducted by Sonkar A et al., and Ajayi BB et al., which reported HDV seroprevalences of 2.1% and 3.3%, respectively [11,12]. Many factors can influence the prevalence of HDV infection, such as drug abuse, multiple sexual partners, HIV infection, geographical location, and a high incidence of HBV infection [13]. The seroprevalence of HDV was highest in the age group of 51-60 years, at 40%. In a study conducted by Tahaei SME et al., HDV seropositivity was observed in older patients [10]. Another study by Joseph K et al., also reported a high prevalence in older individuals [14]. The study revealed a female predominance over males. Present study finding was in agreement with the study carried out in Brazil by Lago BV et al., where only women were found to be HDV-infected, and the study by Gheorghe L et al., also showed a female predominance [15,16]. However, there was no association between HDV and variables such as age and sex, as the p-value was >0.05. In this study, hepatitis D antibodies were detected in 50% of cases with a history of blood transfusion, followed by surgery in 10%. According to a study conducted by Gheorghe L et al., showed 31% of a patients had a history of blood transfusion [16]. Another study by Shah L et al., also identified blood transfusion as the most common risk factor [17]. This could be attributed to the fact that multiple blood transfusions allow a large quantity of infective virions to enter a susceptible patient, increasing the chances of infection. However, there are differences

in risk factors as well as lifestyle in different areas, so it is important to know the prevalence in specific regions.

HDV is the most severe form of chronic hepatitis. In resource-limited countries, it is challenging to conduct clinical trials, and consequently, HDV infection is endemic in these regions. However, due to significant advances in the characterisation of the viral life cycle, several host-targeting molecules are currently undergoing clinical evaluation with promising results. The seroprevalence of HDV infection was notably high in present study. Therefore, employing an HDV ELISA, which is a simple, easy, and less time-consuming procedure compared to molecular tests, can help screen patients for HDV infection. This approach may help reduce transmission and complications such as early hepatic decompensation, liver failure, and an increased risk of hepatocellular carcinoma compared to HBV monoinfection.

Limitation(s)

Virological replication markers such as HDV RNA and HBeAg were not measured for a more precise estimation of individual viral replication in the disease process due to cost constraints.

CONCLUSION(S)

The seroprevalence of HDV was found to be 2.9% among HBsAg-positive patients in a study that predominantly involved middle-aged and older females. The most common route of transmission identified was blood transfusion. Co-infection with Hepatitis B aggravates the progression of liver diseases. Given that one of the primary routes of HDV transmission is blood transfusion, there is a clear need for blood screening for HDV, particularly in patients who have received multiple blood transfusions.

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