Section: Microbiology

Original Research Article

SEROPREVALENCE OF VIRAL HEPATITIS B & D HIGH RISKS GROUPS

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ABSTRACT

Background: Hepatitis B virus (HBV) infection remains a notable public health challenge worldwide, particularly in developing nations. However, efforts to reduce its prevalence have shown promise through increased awareness, vaccination campaigns, and improved healthcare infrastructure. The aim is to study Prevalence of HBV, HDV in high-risk groups. o study the epidemiology, risk factors, clinico-biochemical correlation of HBV and HDV infections in high-risk groups. to determine the seroprevalence of HBV and HDV infection in high-risk groups to determine risk factors significance and a justification for prevention.

Materials and Methods: A prospective observational study was conducted among hospital-based populations and high-risk patients. Screening for HBsAg was performed, followed by confirmation using ELISA. Samples positive for HBsAg were further tested for type-specific HDV antibodies. Demographic data, including education, occupation, marital status, and HIV status, were collected and analyzed for associations with HBV infection.

Results: The study identified a seroprevalence of 7.5% for HBV, with higher rates observed among HIV-positive patients (9%) and Thalassemia patients (7%). HDV seroprevalence was found to be 2.22%, with one case identified among HIV-positive patients in the high-risk group. Analysis of demographic and behavioral factors revealed important insights into the epidemiology of HBV infection.

Conclusion: This study underscores the ongoing importance of surveillance and intervention efforts to control HBV infection. Increasing awareness, promoting vaccination, improving healthcare facilities, and addressing associated risk factors are crucial strategies for reducing the burden of HBV and HDV infections and improving public health outcomes.

Keywords: HBV, HDV, seroprevalence, high-risk populations, HIV, Thalassemia, risk factors.

INTRODUCTION

Hepatitis refers to the inflammation of the liver, with viral hepatitis representing a significant public health issue impacting billions worldwide.[1] Hepatitis B (HBV) and Hepatitis D (HDV) are blood-borne viruses primarily transmitted through breaches in the skin via percutaneous or mucosal routes, as well as parenterally. While all viral hepatitis infections present acutely, both HBV and HDV have the potential to progress to chronic infections, which can lead to hepatic failure and hepatocellular carcinoma.[2]

Hepatitis D virus (HDV) is a defective virus requiring Hepatitis B virus (HBV) as a helper virus for replication. It is estimated that approximately 5% of global carriers of hepatitis B surface antigen (HBsAg) are co-infected with HDV, resulting in a worldwide prevalence of 10-15 million HDV carriers.[3] HDV infection poses a significant risk to chronic HBV carriers, as co-infection with HDV accelerates the progression of chronic HBV infection to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The transmission of both HBV and HDV occurs through blood-borne, sexual, and percutaneous exposures, with percutaneous

exposures, such as intravenous drug use, being the most efficient mode of transmission.[4]

HDV infection induces both acute and chronic liver inflammation and is associated with the most severe form of chronic viral hepatitis. The exact pathogenesis of HDV-induced disease remains unclear. Hepatocellular damage may result from the direct cytotoxic effects of HDV or from a host-mediated immune response. Clinically, HDV infection can present with either acute or chronic manifestations.[5,6]

Acute HDV infection can occur in two distinct ways. The first type, termed acute HBV and HDV co-infection, arises from simultaneous acquisition of both viruses. This type can manifest with severe acute hepatitis and potentially follow a fulminant course.[7] The second type, known as HDV superinfection, occurs when an individual with chronic HBV infection acquires an acute HDV infection.[8,9] Research indicates that chronic HDV infection leads to more severe liver disease than chronic HBV Mono infection, and is associated with accelerated fibrosis progression, an increased risk of hepatocellular carcinoma (HCC), and early decompensation in cases of cirrhosis.[10-12]

To reduce the morbidity and mortality associated with HBV and HDV infections, this study presents data on hospital-based patients, emphasizing the importance of these infections and providing a rationale for prevention strategies. The study advocates for early detection through routine screening for anti-HDV in individuals who are HBsAg positive, as well as timely intervention measures.

Aim & Objective

- To study Prevalence of HBV, HDV in high-risk groups.
- To study the epidemiology, risk factors, clinico-biochemical correlation of HBV and HDV infections in high-risk groups.

To determine the seroprevalence of HBV and HDV infection in high-risk groups to determine risk factors significance and a justification for prevention.

MATERIALS AND METHODS

Study design: This is a prospective and observational study.

Study Area: It will be conducted at Department of Microbiology at B.J.G.M.C and SGH, Pune.

Study Period: 1 year.

Sample size: 1200 patients were screened, out of which 600 was general population & 600 patients in high-risk groups. We will be studying only high-risk group.

Inclusion Criteria

- Patients with clinical features suggestive of viral hepatitis
- Patients with abnormal LFT suggestive of viral Hepatitis
- Patients of all age groups of both sexes.

Exclusion Criteria

- Patients with alcoholic liver diseases
- Patients with other known infective cause of hepatitis like Leptospirosis, dengue, CMV, etc.
- Patients with known non-infectious hepatitis.

Data collection: The study procedure involves obtaining detailed history, physical examination, and recording biochemical parameters. Data on socio-demographic, sexual, obstetric, and transfusion history are collected. Ethical clearance and consent are obtained. Venous blood (5-10 ml) is drawn from each patient and serum is stored at -20 to -80 degrees Celsius. Serum samples are analyzed for hepatitis B and D markers using ELISA. Tests include HbsAg and Anti-HBc IgM for hepatitis B differentiation, and Anti-HDV for hepatitis D. This comprehensive screening aims to identify viral hepatitis prevalence and differentiate acute from chronic cases.

Risk Factors: Not more than minimal risk of sample collection.

Statistical analysis: Appropriate statistical tests will be applied.

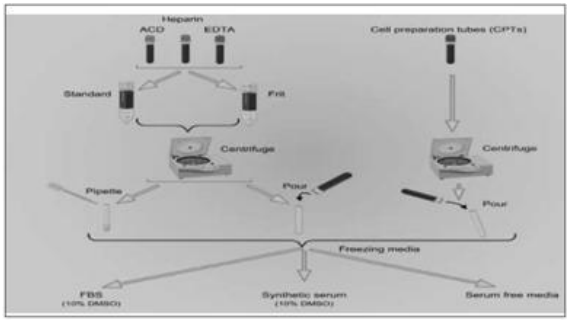

Figure 1: Isolation of peripheral blood mononuclear cells from blood

Table 1: Summary of the procedure using ELISA

Step	Method	Observation
Dilution	Prepare wash solution (1x)	Dissolve salt crystals
Incubation	Cover the wells and incubate for 60 ± 2 minutes at 37± 1^0C	In incubator
Dilution	Conjugate preparation (1x)	During incubation with the sample, dilute the concentrated conjugate (51x)
Conjugate	Add 100ul Conjugate (1x)	
Incubation	Cover the wells and incubate for 30 ±1 minutes at 37 ± 1^0C	In incubator
Dilution	Substrate preparation (1x)	During incubation with the conjugate dilute TMB (100x)
Substrate	Add 100ul Substrate	Avoid contact with oxidizing agents avoid light exposure
Incubation	30 ±2 minutes at 18-25^{0}C	Keep the wells protected form light
Reading	Read in spectrophotometer	Read within 5 to 30 minutes

RESULTS

Out of the total 1200 patients screened, comprising both general and high-risk populations, the focus of this study centers solely on the high-risk group. Specifically, 600 individuals were identified from the high-risk category, with 300 being HIV-positive individuals and 300 patients with thalassemia. This targeted approach allows for a detailed examination of hepatitis B and D prevalence and associated factors within this specific at-risk population subset.

Table 2: Distribution of HBsAg-positive patients in high-risk patients

Population group	Tested (N)	Total positive patients	% of total positive patients
HIV + ve	300	27	9
Thalassemia	300	21	7

[Table 2] illustrates the outcomes of hepatitis B and D screening conducted within two distinct high-risk population cohorts. Among the 300 HIV-positive individuals screened, 27 were found to be positive for hepatitis, accounting for 9% of the total positive cases detected across the study. Similarly, within the thalassemia patient group comprising 300 individuals, 21 patients tested positive for hepatitis, constituting 7% of the overall positive cases identified.

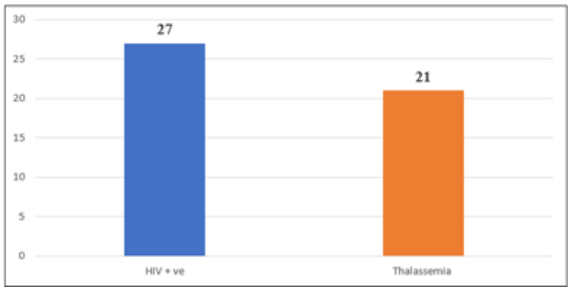

Figure 2: Distribution of HBsAg-positive patients in high-risk patients

Table 3: Distribution of HDV positive patients in high-risk patients

Population group	Total HBV positive tested for HDV	HDV positive
HIV + ve	27	1(3.70)
Thalassemia	21	0

Table 4: Distribution of age & gender in high-risk patients.

Age	Male	Female	Total	P -value
<= 20	3	2	5	0.975
21-30	4	1	5	
31-40	7	2	9	
41-50	7	2	9	
51-60	8	3	11	
61-70	4	2	6	
> 70	2	1	3	
Total	35	13	48	

[Table 3] illustrates the distribution of Hepatitis D Virus (HDV) positive patients among high-risk population. Among the HBV-positive individuals within the HIV-positive group, consisting of 27 patients, one individual was found to be positive for HDV upon screening, indicating a prevalence rate of 3.70% within this subgroup. None of the HBV-positive patients within the thalassemia group, totalling 21 individuals, tested positive for HDV.

[Table 4] provides a breakdown of hepatitis B and D positive patients based on age and gender distribution in high-risk patients. the distribution appears relatively consistent across different age brackets, with no statistically significant difference observed between genders (p = 0.975).

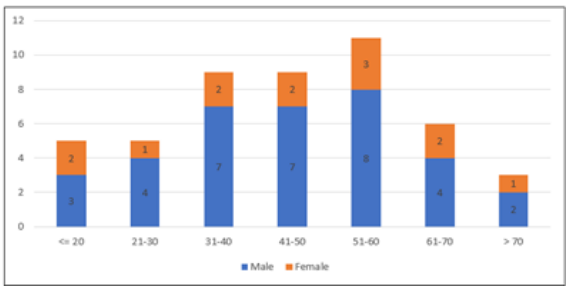

Figure 4: Distribution of age & Gender in high-risk patients.

Table 5: Distribution of socio – demographic parameters in high-risk patients.

Education	Frequency (N)	Percentage (%)
Graduate	20	41.67
Undergraduate	28	58.33
Occupation		
Business	7	14.58
Farmer	10	20.83
Housewife	6	12.50
Service	14	29.17

Student	6	12.50
Worker	5	10.42
Marital Status		
Married	30	62.5
Separated	6	12.5
Unmarried	12	25

Table 6: Study of Comorbidities in high-risk patients

Comorbidities	Yes (N)	Percentage (%)	No (N)	Percentage (%)
Abdominal Pain	7	14.58	41	85.42
Nausea	7	14.58	41	85.42
Vomiting	4	8.33	44	91.67
Body ache	10	20.83	38	79.17
Fatigue	10	20.83	38	79.17
Loss Of Appetite	3	6.25	45	93.75
Dark Urine	5	10.42	43	89.58
Jaundice	3	6.25	45	93.75
Clay Coloured Stool	0	0.00	48	100.00
Diabetes	7	14.58	41	85.42
Hypertension	4	8.33	44	91.67

[Table 5] presents socio - demographic data on education, occupation, and marital status. It shows that out of 48 positive high risked patients, 41.67% are graduates, while 58.33% have undergraduate education. In terms of occupation, 14.58% are in business, 20.83% are farmers, 12.50% are housewives, 29.17% are in service jobs, 12.50% are students, and 10.42% are workers. Regarding marital status, 62.5% are married, 12.5% are separated, and 25% are unmarried.

[Table 6] details the prevalence of various comorbidities within the surveyed population. The most common conditions are body ache and fatigue, each affecting 20.83% of individuals. Abdominal pain, nausea, and diabetes each occur in 14.58% of the population. Other noted conditions include dark urine (10.42%), vomiting and hypertension (8.33% each), and loss of appetite and jaundice (6.25% each).

Figure 6: Study of Comorbidities in high-risk patients

Table 7: Risk Factor Distribution Among HBsAg-Positive Patients

Risk factors	Frequency (N)	Percentage (%)
Blood transfusion	6	12.50
surgery	1	2.08
dental procedure	1	2.08
tattoos done	2	4.17
multiple sexual contacts	4	8.33
organ transplant	0	0.00

Table 8: Prevalence study of HBV & HDV

Prevalence	HIV (%)	Thalassemia (%)
Prevalence of HBV	9	7
Prevalence of HDV	3.7	0

[Table 7] presents the distribution of various risk factors among HBsAg-positive patients. The most common risk factor identified is blood transfusion, reported by 12.50% of the patients. Multiple sexual contacts are noted by 8.33% of the patients. Tattoos are a risk factor for 4.17%, while both surgery and dental procedures are each reported by 2.08% of the patients.

[Table 8] shows the prevalence of HBV (Hepatitis B Virus) and HDV (Hepatitis D Virus) among patients with HIV and Thalassemia. Among HIV patients, the prevalence of HBV is 9%, while the prevalence of HDV is 3.7%. For patients with Thalassemia, the prevalence of HBV is 7%, and there are no cases of HDV reported.

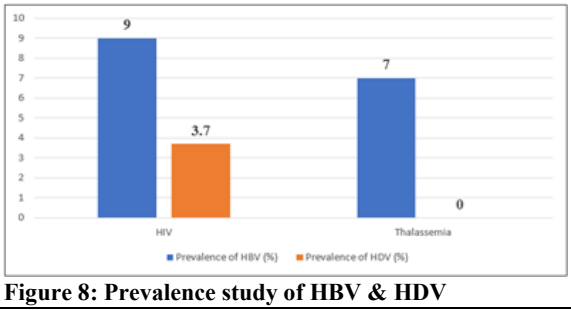

Figure 8: Prevalence study of HBV & HDV

DISCUSSION

The present prospective and observational study was conducted in a hospital-based population and among high-risk patients to evaluate the prevalence of HBV (Hepatitis B Virus) and HDV (Hepatitis D Virus) by assessing antibody titers. HBsAg-positive samples were isolated and reconfirmed using ELISA for Hepatitis B surface antigen. Subsequently, the positive ELISA (HBsAg) samples were tested for type-specific antibodies (IgM and IgG) to HDV using an IgM and IgG ELISA kit, following the manufacturer's instructions.

In this study, a total of 1200 patients were screened for HBsAg, comprising 600 from the general population and 600 high-risk patients. The high-risk group included 300 HIV patients and 300 Thalassemia patients. Among the high-risk population, 48 patients tested positive for HBsAg, with 27 (9%) being HIV patients and 21 (7%) being Thalassemia patients. Furthermore, 2 out of the 90 HBsAg-positive patients were HDV antibody-positive.

The study found a prevalence of HBV at 9% and HDV at 3.7% among HIV patients, and a 7% prevalence of HBV among Thalassemia patients. These results are comparable to those reported by Natasha Samsunder et al,[13] who found an HBsAg-positive seroprevalence of 4.0%, and Paulo Antonio Ferreira et al,[14] who reported a 7.5% HBsAg-positive rate, indicating moderate-to-high endemicity for HBV. In the present study, no significant relationship was found between age and gender among HBsAg-positive patients.

In the demographic data on education 41.67% are graduates and 58.33% are undergraduates. Regarding occupation, 14.58% are in business, 20.83% are farmers, 12.50% are housewives, 29.17% work in service jobs, 12.50% are students, and 10.42% are workers. The marital status, 62.5% are married, 12.5% are separated, and 25% are unmarried.

In the present study, the most common conditions were body ache and fatigue, affecting 10 (20.83%) cases. Abdominal pain, nausea, and diabetes each affected 7 (14.58%) patients, while vomiting and hypertension were reported by 4 (8.33%) patients. Loss of appetite and jaundice were each present in 3 (6.25%) cases. In contrast, a study by Sajini Souda et al,[15] found that 18.6% of patients had other comorbid conditions such as tuberculosis, diabetes, hypertension, HIV, or a history of alcohol abuse, which may have contributed to hepatic injury. These findings differ from those of the present study.

The most common risk factor identified is blood transfusion, reported by 12.50% of the patients. Multiple sexual contacts are noted by 8.33% of the patients. Tattoos are a risk factor for 4.17%, while both surgery and dental procedures are each reported by 2.08% of the patients. The comparable study by K T Sharma et al,[16] in his study reported history of blood transfusion was present in 5.6 % of cases.

In the present study, among HIV patients, the prevalence of HBV is 9%, while the prevalence of HDV is 3.7%. For patients with Thalassemia, the prevalence of HBV is 7%, with no cases of HDV reported. In a study conducted by K.T. Sharma et al., the seroprevalence of HDV infection was found to be 4.2%, which is higher compared to the present study. The prevalence of HBV and HDV is on a decreasing trend when compared with studies conducted in different parts of India, with HDV infection being rarer compared to the 1990s. Improved healthcare facilities, vaccination programs, and increased awareness may contribute to this improvement.

The current study showed a lower prevalence of both HBV and HDV. Various factors such as drug abuse, blood transfusions, dental procedures, organ transplants, tattoos, multiple sexual partners, HIV infection, occupation, and education were examined to understand the reasons behind the incidence of HBV infection.

CONCLUSION

HBV infection remains a significant public health concern, particularly in developing nations. However, there has been a gradual decrease in HBV prevalence due to increased awareness, higher vaccination rates, and improved healthcare facilities. Our study reports a seroprevalence of 7.5% for HBV, with higher rates observed among high-risk groups such as HIV-positive patients (9%) and individuals with Thalassemia (7%). HDV seroprevalence was found to be 2.22%, with one case identified among HIV-positive patients in the high-risk group.

We investigated various factors such as education, occupation, marital status, HIV status, multiple partners, blood transfusions, organ transplantation, dental surgery, and tattoos to understand their relationship with HBV infection incidence. Our findings underscore the importance of surveillance and highlight a significant opportunity for expanding programs aimed at controlling HBV for the betterment of public health.

Increasing awareness, promoting proper sanitation practices, leveraging novel technologies, and enhancing vaccination efforts are crucial strategies to further reduce the incidence of HBV and HDV infections. By implementing these measures, we can make substantial progress towards mitigating the burden of these infections and improving overall public health outcomes.

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