Prevalence of Hepatitis Delta Virus Infection among Hepatitis B Virus-Infected and Exposed Patients

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Abstract

Background: Hepatitis delta virus (HDV) infection is a cause of coinfection and superinfection among hepatitis B virus (HBV)-infected patients. The global prevalence of HDV may vary drastically depending on the geographical location. In India, serological techniques form the basis for the determination of HDV prevalence in majority of the studies with very limited literature based on molecular techniques. In addition, sparse data on HDV infection among HBV-exposed group, i.e., patients with total antibodies to core antigen (anti-hepatitis B core [HBc]) positive and negative hepatitis B surface antigen (HBsAg), are available. Objective: This study was aimed to determine the prevalence of HDV in both HBV-infected and HBV-exposed groups, utilizing both serological and molecular methods. Settings and Design: This was a retrospective cross-sectional study conducted from January till June 2018 where samples of 142 patients were retrieved and were categorized into two groups: Group A included patients with both HBsAg and anti-HBc positivity (n = 120/142 [85%]), i.e., confirmed HBV infection, and Group B included patients with anti-HBc positivity and HBsAg negativity (n = 22/142 [15%]), i.e., exposed to HBV. Materials and Methods: All the specimens were retrieved from −80°C and were tested for anti-HDV immunoglobulin (Ig) M (IgM), anti-HDV IgG, and HDV RNA. Results: HDV infection was observed in only one patient in Group A and none in Group B, making an overall prevalence of 0.78% (95% confidence interval = 0.02%–3.9%). The infected patient was reactive for both IgM and IgG with a viral load of 2log_{10} IUK/ml. Conclusion: The present study provides evidence that HDV infection is very low (0.78%) in this part of India. However further prospective studies with larger sample size are warranted.

Keywords: Hepatitis B virus, hepatitis delta virus, prevalence

Introduction

Hepatitis delta virus (HDV) was discovered by Mario Rizotto in 1977, which is a defective RNA virus belonging to the family Deltaviridae and genus Deltavirus. It utilizes the envelope of hepatitis B virus (HBV), i.e., hepatitis B surface antigen (HBsAg), for the transmission of infection. Both these viruses are transmitted parenterally; although in hyperendemic regions, HDV is spread by horizontal transmission. Infection with HDV can occur in two forms. Coinfection occurs along with acute HBV infection and mostly, the patients land up in acute hepatitis B (AHB), clearing both the viruses within 6 months. Superinfection with HDV occurs in chronic hepatitis B (CHB)-infected patients, and the clinical presentation mimics CHB with acute exacerbation (CHB-AE). Dual HBV–HDV infection results in a twofold increase in mortality and accelerates the progression to cirrhosis and hepatocellular carcinoma when compared to the monoinfection of HBV.
HBV-exposed patients, we inquisitively planned a study to find the true prevalence of HDV. This study would help us in updating the present knowledge on HDV prevalence.

**Objective**

The current study was undertaken to determine the prevalence of HDV using both serological and molecular techniques among HBV-infected and HBV-exposed groups.

**Materials and Methods**

This was a retrospective cross-sectional study conducted for a period of 6 months from January to June 2018 where the patients were selected from the Virology database with an inclusion criterion of HBV infection (who were anti-HBc reactive with or without HBsAg positivity but with negative anti-HBs) and exclusion criteria of patients with immunosuppression/HIV infection and negative for coinfection with other hepatitis A, B, C, and E viruses.
C, and E. All those with satisfactory inclusion criteria, patients whose archived blood samples were retrieved from −80°C, and patients whose sample volume of minimum 500 μl was available, wherever sufficient, were finally included in this study. A total of 142 samples were retrieved. Complete demographic and laboratory details of the patients (serological markers of HBV infection such as HBsAg, anti-HBc total, hepatitis Be antigen [HBcAg], immunoglobulin [Ig] M [IgM] antibodies for the HBV core antigen [anti-HBc-IgM], quantitative HBV DNA viral load, and liver function tests) were obtained from the hospital information system. The included population (n = 142) were further categorized into two groups: Group A included patients with both HBsAg and anti-HBc positivity (n = 120/142 [85%]), i.e., confirmed HBV infection, and Group B included those with only anti-HBc positivity (n = 22/142 [15%]), i.e., HBV exposed. Based on the persistence of HBsAg, Group A was further subdivided into AHB, CHB, and CHB-AE as per the defined criteria. AHB was defined as HBsAg and anti-HBc IgM positivity (S/Co ≥8) with disappearance of HBsAg within 6 months of detection or histopathologically proven. CHB was defined as the persistence of HBsAg for >6 months. CHB-AE was defined as HBsAg positivity for >6 months in addition to anti-HBc IgM positivity in the episode of exacerbation (S/Co <8). All the specimens were further tested for anti-HDV IgM, anti-HDV IgG, and HDV RNA. Anti-HDV IgM and IgG were performed using commercial enzyme immunoassay (Wantai Biopharm, Beijing, China) as per the manufacturer’s instructions. RNA extraction was done by QIA symphony DSP Virus/Pathogen Midi Kit (Qiagen, Hilden, Germany), and HDV RNA quantitation was done by reverse transcription-polymerase chain reaction (PCR) by Altona RealStar® HDV RT-PCR Kit (Altona Diagnostics, Hamburg, Germany) as per the manufacturer’s instructions. The presence of anti-HDV IgM was considered as an evidence of recent infection and that of anti-HDV IgG as past infection. Detection of HDV RNA was taken as the evidence of acute ongoing infection.

Statistical analysis
The analysis was carried out using SPSS version 23.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics were shown as mean with standard deviation or median with interquartile range as appropriate. P < 0.05 was considered statistically significant.

RESULTS
Among the 142 enrolled patients, 85% (n = 120) belonged to Group A and the remaining 15% (n = 22) belonged to Group B. Among Group A, AHB was documented in 7% (n = 10), CHB in 60% (n = 85), and CHB-AE in 18% (n = 25). The mean age was 42.57 (±15.5) years, with a male-to-female ratio of 4:1. Laboratory parameters of the studied population are depicted in Table 2.

Serological markers of hepatitis delta virus in Groups A and B
Anti-HDV IgM and IgG were found to be positive only in one patient belonging to Group A. In contrast, none of the patients in Group B showed any serological evidence of HDV infection.

Hepatitis delta virus RNA load in Groups A and B
HDV RNA remained undetected among all patients in Group B. The viral load of 2log10 IU/ml was documented in one patient of Group A with anti-HDV IgM and IgG positivity, making the overall prevalence of HDV infection 0.78% (95% confidence interval = 0.02%–3.9%) in the present study.

The patient who was infected with HDV was a known case of CHB and was diagnosed as CHB-AE with HDV superinfection. The patient had a clinical course of fulminant hepatitis landing up in acute liver failure and finally succumbed to the illness.

DISCUSSION
Almost 40 years has passed by after the discovery of HDV, yet it remains to be a major public health problem in regions where HBV is still endemic. HDV infection adversely affects the clinical course and outcome of HBV by worsening CHB and by increasing the rates of hepatocellular carcinoma. The prevalence of HDV differs widely depending on the target population and the geographical area evaluated. Although serology remains the mainstay to determine the prevalence, our study incorporates an additional molecular approach to ascertain the active infection. In the present study, only one patient from Group A was found to be positive for anti-HDV IgM and IgG with an RNA load of 2log10 IU/ml and none in Group B. Our results are in concordance with those of the study published by Jat et al. from India in 2015 where HDV RNA testing was also performed to study the prevalence.[24] In contrast to a study by Mhalla et al. where the prevalence of 4.6% was documented among isolated anti-HBc positive group, none of the patients in our study showed any evidence of HDV infection.[29] Hence, the overall prevalence of HDV was observed to be 0.78%.

As already known, HDV infection is still endemic in the Middle East, Central Africa, the Mediterranean, eastern part of Europe, Amazon Basin, and parts of Asia, with the prevalence still being high from 60.5% to 68.2% in the regions of Nigeria and Pakistan.[30–32] However, globally,
in recent years, a declining trend is observed drastically in other parts of the world.[4,33-36] To determine if it holds true for our country also, all the studies conducted on HDV prevalence beyond 1990 in India were included for a review. The English literature search was done in Medline (National Library of Medicine, Bethesda, Maryland, USA) using the following terms “Hepatitis Delta Virus,” “HDV prevalence,” “HDV infection,” “HDV co-infection,” “HDV super-infection,” “Delta virus hepatitis,” and “India.” Combinations such as “HDV prevalence in India,” “HDV infection in India,” and “HDV prevalence and India” were also used. Our review clearly revealed a declining trend in India with prevalence varying as high as 62.5% in the early 1990s to almost negligible in recent past years [Table 1].

The declining trend of HDV infection can be attributed to the decline in HBV infection owing to the improvement in the socioeconomic status, health-care development, awareness among the public about the parental modes of transmission of the viruses, enhanced screening for transfusion-transmitted infections, and, ultimately, the improved vaccination strategy incorporating the HBV vaccine in the National Immunization Schedule from 2002 and also making the birth dose mandatory from 2008.[37]

Limitations
The sample size was small with a shorter duration of analysis. Although ours is a retrospective study, complete patient details regarding their socioeconomic status, modes of acquisition of infection, or family history were not available in our study cohort. However, further prospective studies with larger sample size for a longer duration encompassing both serological and molecular techniques are warranted to understand the sequelae of HDV infection, severity of infection, and reasons for the declining trend in co/superinfection with HDV in the Indian population.

Conclusion
The present study provides evidence that prevalence of HDV infection is very low (0.78%) in this part of India. Yet more of prospective studies encompassing larger study population are warranted to understand the same.

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Conflicts of interest
There are no conflicts of interest.

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