

Original article

Prevalence of hepatitis D among the cases of hepatitis B infection in a tertiary care centre in Eastern India

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Abstract:

Context: The last few years data showed a low incidence rate of HBsAg positive cases at our tertiary care institution. This low incidence rate made us inquisitive to know the prevalence rate of HDV (Hepatitis D virus) infection among acute /chronic hepatitis B positive patient, as there is no previous study in this area of Bihar and adjoining areas of West Bengal in Eastern India .

Aims: To estimate the prevalence rate of HDV (Hepatitis D virus) infection among acute /chronic hepatitis B positive patient.

Methods and Material: The present study included consecutive patients clinically suspected to carry Hepatitis D virus along with jaundice caused by HBV and carriers of HBV with or without jaundice suspected for HDV super-infection in our tertiary care teaching hospital. All patients with positive HbsAg (newly diagnose) were screened for HDVAb by standard methods.

Results: The prevalence of Hepatitis D infection in patient suffering from Hepatitis B infection in our study was found to be 2%. . The age of the patients varied from 16 years to 74 years. Mean age of the patients were found to be 34.54 years with a standard deviation of 13.53 years. The maximum no. of patients (39%) belonged to the third decade followed by in the fourth decade (27%). Male and Female ratio in our study is 1:1.17.

Conclusions: The present study confirmed the trend in global decline in the prevalence of hepatitis D infection, which is true for India in all cases of HDV infection. This decreasing trend can be attributed to the result of global HBV vaccination, increasing awareness, improved prevention strategies and change in the socioeconomic conditions in a fast developing country like India.

Key-words: Hepatitis D, Prevalence, Eastern India, Co-infection, Hepatitis B

Introduction:

It was in 1977, an Italian doctor named Mario Rizzetto discovered a new nuclear antigen in the liver cells of patients infected with Hepatitis B Virus (HBV). This nuclear antigen was then thought to be a part of the hepatitis B antigen and was called the delta antigen. Subsequent experiments in chimpanzees showed that the hepatitis delta antigen (HDAg) was a structural part of a pathogen that required HBV infection to replicate. The entire genome was cloned and sequenced in 1986, and obtained its own genus: Delta virus (HDV). HDV is the only virus in the genus, Delta viridae. HDV is not classified into a viral family because it is a unique virus dependent on HBV. HDV is a co-infection of HBV. The envelope of HDV particles contains the Hepatitis B

surface antigen (HBsAg). The production and transmission of HDV is entirely dependent on HBV to provide HBsAg. Hepatitis D virus (HDV) is a sub-viral agent that requires a preexisting or concurrent infection with hepatitis B virus (HBV), which provides the coat protein for the HDV virion.

The last two years data showed a low incidence rate of HBsAg positive cases at our tertiary care institution. This low incidence rate made us inquisitive to know the prevalence rate of HDV (Hepatitis D virus) infection among acute /chronic hepatitis B positive patient, as there is no previous study in this area of Bihar and adjoining areas of West Bengal in Eastern India. It is apparent from various literature survey that there is a declining trend of prevalence of HDV super infection throughout

the world^[1] Over the last four decades the prevalence of HDV has declined in the western world and middle-east. It is also declining in the countries of the South East Asia including India. Different investigator groups have studied the prevalence of HDV super infection in the different parts of India like North (New Delhi, Chandigarh, Ludhiana), South (Chennai), West (Gujarat), Central (Indore) even in Andaman and Nicobar Islands. Apart from a study in Kolkata, two decades earlier, there is no representative study conducted in the Eastern India^[2] Hence the intention of this study is to evaluate the prevalence of HDV super-infection of HBV infected subjects in the sample group of this part of Eastern India **Subjects and Methods:**

Study design: The present study included consecutive patients clinically suspected to carry Hepatitis D virus along with jaundice caused by HBV and carriers of HBV with or without jaundice suspected for HDV super-infection in our tertiary care teaching hospital.

The basis of selection: We selected only those cases who were HBsAg positive even after three months. It has been observed that acute phase of super-infection is heralded by the appearance of IgM (Immunoglobulin M) which is replaced by IgG (Immunoglobulin G) within 2/4 weeks in continuation of acute phase IgG persists for some time. In chronic phase of super-infection IgG antibody persists along with IgM antibody. Hence IgG antibody can be identified in whole spectrum (acute and chronic phase) of super infection of HDV. Accordingly, we have selected IgG antibody against HDV as the preferred and sole marker of my study to assess the prevalence rate of HDV in HBV infected subjects. All patients with positive HbsAg (newly diagnosed) with or without high serum bilirubin irrespective of their age, were screened for HDVAb. Mostly patients presented with insidious onset of jaundice with high ALT (alanine transaminase) levels were screened for HbsAg (surface antigen of the hepatitis B virus) test showing positivity. All patients, who were chronic carriers of HbsAg were also tested for HDVAb.

The Study Sample: Blood samples were collected from the patients for HDVAb in a single vial of 5ml capacity without anti-coagulant. One hundred (100) HbsAg positive patients were screened for HDVAg. Eighty seven (87) patients were diagnosed with HbsAg positive having insidious rise of jaundice (Bilirubin & ALT) and thirteen (13) HbsAg positive patients detected earlier in our microbiology department, suffering more than two (2) months were subjected for HDVAb test.

Specimen Collection: All hospital service providers especially paramedical/phlebotomists were accordingly guided regarding this study. The proper collection of specimens with universal precautions were taken. Proper identification code and numbering with name of each and every patient's samples was repeatedly emphasized. Haemolysed blood samples were not accepted in this study.

Blood volume: 5ml blood sample was taken in a vacutainer without any anti-coagulant. The container was kept in slanting positions (for at least one hour at room temperature) to obtain a clear, unhaemolysed serum.

Transportation: Just after clot formation (within an hour) the sample was transported with proper history sheet and requisition slip having its own identification (registration) number and laboratory code number, name, date to the microbiology laboratory.

Serum separation: Immediately the serum was collected in a separate vial maintaining adequate precaution to avoid contamination and kept in at 2°C—8°C in a freezer.

Specimen Processing: All the frozen samples were allowed to thaw in a vertical position in the rack without shaking. Once again samples were centrifuged (1000 rpm for 15 min). It was kept for half an hour at room temp. Now the samples were separated in two tubes each one for HbsAg ELISA TEST and the other for HDVAb.

Test done for HBSAg: Immunochromatographic test was used as primary screening test for HbsAg. A confirmatory ELISA TEST was performed with HEPALISA (J Mitra and Co. PVT.LTD.). The

HEPALISA has a sensitivity of 0.1ng/ml which is more sensitive to immunochromatographic test. The HEPALISA is approved by WHO for the specificity to identify all the subtypes of HBsAg.

Procedure of HbsAg by ELISA:

Preparation of reagents: The incubator was pre warmed to 37°C. The foil pack was brought to room temperature (20°C-25°C) before opening, to prevent condensation on the microwell strips. The required number of wells needed for the assay were broken off and placed in the strip holder. The strip holder with the required number of strips were taken into account so that two negative and two positive control should be included in the run while opening the fresh kit. Unused wells were stored at 2°C-8°C with dessicant in an aluminum pouch with clamp and rod.

Preparation of working wash buffer: The buffer concentrate was checked for the presence of salt crystals. If crystals are present in the solution, resolubilization was done by warming at 37°C until all the crystal were dissolved. Twenty five ml of buffer for each strip were prepared before use. It was mixed well before use. 20ml of 25x wash buffer concentrate was mixed with 480ml of distilled or deionized water. The wash buffer remained stable for two month when stored at 2°C-8°C.

Preparation of working conjugate: The conjugate concentrate was diluted 1:50 in conjugate diluents. Working conjugate was not stored. A fresh dilution for each assay was prepared in a clean vessel. The quantity of working conjugate solution to be prepared was determined. Before use the solution was mixed thoroughly.

Preparation of working substrate solution: TMB substrate and TMB Diluent were mixed in 1:1 ratio to prepare the working substrate fresh.

Test procedure: The strip holder was fitted with the required number of HEPALISA strips. Then the assay control wells so arranged that well A-1 is the reagent blank. From the well A1 all controls were arranged in a horizontal or vertical configuration.

1. A-1 well was left as blank

2. 100µl negative controls were added in each well No B-1 & C-1 respectively.
3. 100µl positive controls were added in D-1 & E-1 wells.
4. 100µl of patient's sample were added in each well, starting from F-1
5. 50µl of working enzyme conjugate were added to each well except A1. The plates were rotated for 2-3sec for well mixing the sample & conjugate.
6. The plates were covered and incubated in an incubator at 37°C± 1°C for 60 minutes.
7. The wash buffer concentrate was diluted with distilled water to 1:25 dilution.
8. At the end of incubation period the plates were taken out from incubator and washed with working wash buffer.

Calculation of result: Computation of results were done in absorbance mode with cut-off control against negative control and blank. All the value above 0.120 were taken as positive value. All the value below 0.120 were taken as negative value.

Interpretation of Results: All the immunochromatographic positive results were cross checked with HEPALISA TEST reading. It showed value above the cut off control and so were confirmed to be positive.

Results:

The prevalence of Hepatitis D infection in patient suffering from Hepatitis B infection in our study was found to be 2%. One hundred cases of seropositive Hepatitis B were taken for study. The age of the patients varied from 16 years to 74 years. Mean age of the patients were found to be 34.54 years with a standard deviation of 13.53 years. The maximum no. of patients (39%) belonged to the third decade followed by in the fourth decade (27%). Male and Female ratio in our study is 1:1.17.

Discussion:

Results in our study showed prevalence of HDV detected by IgG antibody (ELISA method) is 2%. The following national studies conducted by previous renowned

workers have been taken into consideration for comparison of our work. The study done by Banker *et al*^[3] in 1992 reported the HDV prevalence to be 37.46% in HBsAg-positive patients in Mumbai. Also, there was a higher HDV prevalence of 63% in patients with fulminant hepatitis. In another study from Mumbai in 1995, Amarapurkar *et al*^[4] showed a prevalence of 16% in patients with acute viral hepatitis, 17% in asymptomatic HBsAg carriers and 19% in patients with chronic liver disease. Among the high-risk population, HDV prevalence was 20% in chronic renal failure patients, 29% in medical professionals and 38% in recipients of multiple transfusions. Jaiswal *et al*^[5] showed higher prevalence of HDV - 5.7% in patients with chronic liver disease, 1.9% in those with acute viral hepatitis, 15% in those with hepatic failure, and 2.3% in those with chronic renal failure in a study conducted in Indore, Central India from July 1992 to June 1998. Bhattacharyya *et al*^[2] had found the prevalence to be 3.3% in 1998 in a study in Kolkata. Only two cases were positive for HDV antigen among 60 HBsAg positive jaundice patients indicating a lower rate of prevalence of infection (3.3%). Chakraborty *et al*^[6] in 2005 found prevalence of hepatitis D in HBsAg-positive individuals from New Delhi to be 10.6 % in 2005 and concluded that delta infection not to be very common in Indian patients with HBV-related liver diseases, because (7.3%) had evidence of past infection (IgG positive, IgM negative) and 3.3 had recent infection (IgM anti-delta antibody positive). Shanmugam *et al*^[7] evaluated the seroprevalence of HDV among 153 individuals with HBV-related liver diseases in Chennai during 2008 and assessed any change in epidemiological pattern by comparing the results with seroprevalence figures reported previously. Of the 153 patients screened, nine (5.9%) were reactive to anti-delta antibodies, six (3.9%) presented an evidence of past infection (IgG anti-delta positive) and three (2.0%) showed anti-HDV IgM, suggestive of recent HDV infection. HDV infection are of two types (1) co infection, (2) superinfection. Co-infections are mostly acute. 95% cases are self-limiting.

Only IgM antibody is recognized in acute co-infection. In contrast, superinfection are mostly chronic. It has been seen that in both acute and chronic phase of superinfection, IgG antibody persist in the whole spectra of the disease. Hence we advocate IgG antibody, against HDV infection in HBV seropositive cases, should be investigated by ELISA method.

In this study the prevalence of HDV infection in HBV seropositive cases has been found to be 2%. From our study it is quite evident that in spite of so many factors like low socio economic status, migratory job habits, high exposure, drug abuses, the rate of HDV positivity among HBsAg positive patients has not increased. The rate of positivity is almost same or declining when compare to national level studies. However, considering the high incidence of HBsAg positivity we had to be careful and conduct extensive monitoring not only in our tertiary hospital but also in the adjoining private care clinics.

An important trend in worldwide HDV infection, is a global decline in the prevalence of hepatitis D infection, which is true for all cases of HDV infection. This decreasing trend is the result of global HBV vaccination, increasing awareness, improved prevention strategies and socioeconomic conditions. In Iran overall HBV, and therefore, HDV prevalence might have decreased^[8] Italy, which was considered a traditionally prevalent area for HDV and where the virus was first reported, has shown a steady decline in the prevalence of this infection from 23% in 1987 to 14% in 1992 and 8.3% in 1997.^[9,10] Taiwan is considered to be endemic for hepatitis B, but as a result of effective immunization, HBV prevalence has decreased markedly. Hepatitis D prevalence in Taiwan was very high in the 1990s and before, but the prevalence has decreased greatly since then and new cases of HDV infection are now encountered rarely^[11]. From June 1983 to May 1995, the prevalence decreased considerably by each 3-year period (23.7, 15.5, 13.1 and 4.2%, respectively). This change in the endemicity was ascribed to the effective preventive measures taken against sexually transmitted diseases

(STDs) and promiscuity and encouragement to use disposable^[11]

In India also, HDV infection does not seem to be very common and the trend is towards a lower side. It is suggested that the infection is switching towards low prevalence in this country. Since HDV is dependent on HBV for replication, control of HDV infection is achieved by targeting HBV infections. All measures aimed at preventing the transmission of HBV will prevent the transmission of hepatitis D. HBV vaccination is therefore recommended to avoid HBV-HDV co-infection. However, vaccination does not protect hepatitis B carriers from superinfection by HDV. India

introduced universal immunization against hepatitis B in 10 states in the year 2002, and in 2011, scaled up this operation countrywide. Recently a pentavalent vaccine, which also protects against HBV has been introduced in some states^[12]

Conclusion:

The present study confirmed the trend in global decline in the prevalence of hepatitis D infection, which is true for India in all cases of HDV infection. This decreasing trend can be attributed to the result of global HBV vaccination, increasing awareness, improved prevention strategies and changes in the socioeconomic conditions in a fast developing country like India.

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