International Journal of Advances in Medicine
Sharma KT et al. Int J Adv Med. 2022 Jun;9(6):703-708
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: https://dx.doi.org/10.18203/2349-3933.ijam20221355

Original Research Article

Hepatitis D virus seroprevalence in HBsAg positive patients attending a tertiary care hospital in Northeast India

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Received: 12 April 2022
Revised: 02 May 2022
Accepted: 09 May 2022

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ABSTRACT

Background: Hepatitis D virus (HDV) infection is present worldwide. Around 18 million people are estimated to be infected with HDV and can infect individuals with active HBV infection and cause severe liver disease. There is lack of data on the prevalence of HDV infection in the state and also in the region. The aim of the study was to determine the seroprevalence of HDV in HBsAg positive patients attending Regional Institute of Medical Sciences Hospital, Imphal, Manipur, India.
Methods: This study was carried out in a tertiary care hospital (Regional Institute of Medical Sciences, Imphal).The study was done for a period of 2 years from September 2016 to August 2018. A total of 119 HBsAg ELISA positive cases were included in the study.
Results: Out of 119 HBsAg positive cases, 5 cases were positive for hepatitis D antibodies, of which 3 were positive for anti-Hepatitis D virus IgM and 2 were positive for anti-Hepatitis D virus IgG. Seroprevalence of HDV infection was found to be 4.2%.
Conclusions: Seroprevalence of HDV infection was found to be 4.2% which is higher than the finding in some of the recent studies in the country.

Keywords: Hepatitis D virus, HBsAg, ELISA, IgM, IgG

INTRODUCTION

Hepatitis D virus (HDV) was first discovered in 1977 by Mario Rizzetto in Turin, Italy.[1] HDV is about 36 nm in size that requires Hepatitis B surface antigen (HBsAg) for their envelope and transmission.[2] HDV has circular, negative sense, single-stranded RNA genome, which is approximately 1700 nucleotides in length.[3] Eight phylogenetically distinct genotypes of HDV have been reported.[4]

HDV infection is present worldwide and estimated to have had infected around 18 million people. An important trend in HDV prevalence is its global decline. It has been decreased significantly in Europe since it was first reported in 1970s and 1980s. Now, the Asia-Pacific region seems to be where it is a major health concern but, there is lack of available data from this region.[5]

The transmission routes of HDV are similar to those of HBV and it has been shown that very little inoculums are sufficient for transmission.[6] These routes include intravenous transmission, sexual contact and nosocomial infections.[7]

HDV are transmitted only in existence of HBV as it requires hepatitis B virus surface antigen (HBsAg) for assembly of infectious viral particles. It can take two patterns, simultaneous HBV/HDV infection (co-infection) or HDV infection of an individual already chronically

infected with HBV (super-infection). The co-infection pattern ranges from mild to severe or fulminant hepatitis.[8] Co-infection is usually self-limited, with 20 % of cases progressing to cirrhosis. However, co-infection can also lead to more severe fulminant hepatitis compared to super-infection. Super-infection may present as exacerbation of disease in previously asymptomatic chronic hepatitis B infection (CHB) patients, worsening of disease in CHB patients, or as acute hepatitis. Super-infection is usually confirmed with positive HDV antibodies and negative IgM anti-HBc. 90% of super-infection cases acquires chronic hepatitis D and leads to cirrhosis in 5-10 years in 70 % of the patients. Cirrhosis incidence is three times higher with HDV infection than with HBV mono-infection.[9] Cirrhosis may continue for years and high percentage of patients will develop hepatocellular carcinoma (HCC).[10]

The associations of HDV with HBV coupled with its unique replication cycle have led to inadequate response to the treatment. The approach for eradication of HDV is the clearance of hepatitis B surface antigen (HBsAg), not just sustained HDV virological response (negative HDV RNA 6 months after stopping treatment).[11] If HBsAg remains positive, HDV remains infectious, even if the HBV or HDV viral titers are low. However, in some cases despite inhibition of HBV replication and, HBsAg clearance, the clearance of HDV has not been seen when oral antivirals against HBV were used alone. Lamivudine, ribavirin, famciclovir, adefovir, and entecavir have shown little or no effect when used alone to treat HDV, regardless of the duration of treatment.[12-16]

However, high dose alpha interferons (9 million units three times a week) for 12 months were associated with sustained loss of HDV replication and clinical improvement in up to 50% of the patient. The beneficial impact of treatment has been observed to persist for 15 years and was associated with reduction in grade of hepatic necrosis and inflammation and reversion of fibrosis. PEG interferon has also been shown to be effective in treatment.[17]

Despite the wide clinical implication of HDV infection, there is lack of data on the prevalence of HDV infection in the region. The aim of the study was to determine the seroprevalence of HDV in HBsAg positive patients attending a tertiary care hospital (Regional Institute of Medical Sciences Hospital, Imphal, Manipur, India) in North East India.

METHODS

This cross sectional study was conducted in a tertiary care hospital from September 2016 to August 2018 by taking blood samples from the HBsAg positive patients.

A total of 119 HBsAg positive patients were enrolled in the study. The aim of the study was to determine the seroprevalence of HDV IgM and IgG among the HBsAg positive patients and to analyse on the basis of various variables such as age, sex, presence of risk factors like intravenous drug use, multiple sex partners, history of transfusion of blood or blood products.

HBsAg positive patient who attended hospital (RIMS hospital) during the study period irrespective of gender, educational qualification and occupation were included and those who were not willing to participate were excluded from the study.

Study tools

Sandwich immunoassay for detection of HBsAg (Viruchek, Viola Diagnostic Systems, India), Enzyme Linked Immunosorbent Assay Test Kit (Erba Lisa SEN HBsAg) for detection of the surface antigen of Hepatitis B Virus, Enzyme Linked Immunosorbent Assay Test Kit for detection of anti-hepatitis D IgG (MyBioSource, Inc. San Diego, CA, USA), Enzyme Linked Immunosorbent Assay Test Kit for detection of anti-hepatitis D IgM (Wantai Biological Pharmacy Enterprise Co. Ltd.),Multiskan microplate reader (Lab Systems Diagnostics), ELISA microplate washer (Thermofisher Scientific).

Data collection

Data was collected using a structured questionnaire having information on variables such as age, sex and risk factors that may be associated with transmission of HDV, like history of drug abuse, blood transfusion, sexual contact etc. The participants were interviewed in an isolated room where confidentiality was maintained.

Specimen collection and laboratory tests

5 ml of peripheral venous blood were collected from all the study subjects under aseptic condition using sterile disposable syringe. The samples were transported to the laboratory as soon as possible. Sera were separated at room temperature and were stored at -20^{o}C in screw capped vials. Samples were subjected for detecting HBsAg using the principle of immunochromatography (two site sandwich immunoassay), following the manufacturer's guideline. HBsAg positive samples were separated and subjected to reconfirmation by ELISA for Hepatitis B surface antigen. Further those positive samples from ELISA (HBsAg) were subjected for the detection of type specific antibodies (IgM and IgG) to HDV using IgM and IgG ELISA kit. ELISA tests was performed and interpreted according to manufacturer's instructions.

Statistical analysis

Descriptive data was presented using Mean, Standard Deviation, etc. Chi square test was used to see any association between proportions. T test was used to see any association between two means. Statistical analysis was carried out using SPSS version 21 (IBM). The variables showing significant interactions were analyzed separately. The level of statistical significance was set at $p<0.05$.

Consent

Informed written consent was taken from all participants. Privacy and confidentiality were maintained in all cases.

Ethical issues

The study was approved by Institutional Ethics Board, Regional Institute of Medical Sciences, Imphal, Manipur, India.

RESULTS

A total of 119 HBsAg ELISA positive cases were included in the study. This study was aimed to determine the HDV antibodies (IgM and IgG) among the HBsAg positive patients and to analyse the seroprevalence on the basis of various variables such as age, sex, presence of risk factors like intravenous drug use, multiple sex partners, history of transfusion of blood and blood products.

Out of 119 patients, the number of male patients were 71 (60%) and female patients were 48 (40%). Out of 119 cases, IDUs constituted 22 (19%), history of blood or blood product transfusion was present in 18 (15%), 16 (13%) were spouse of high risk groups, 14 (12%) had history of multiple sexual partners.

Table 1 showing the description of hepatitis D antibody postive cases. Out of 119 HBsAg positive cases, 5 cases were positive for hepatitis D antibodies, of which 3 were positive for anti-hepatitis D virus IgM and 2 were positive for anti-hepatitis D virus IgG.

Hepatitis D antibodies were present in 3 (4.2%) out of 71 HBsAg positive male cases and 2 (4.2%) out of 48 HBsAg positive female cases. Out of 22 IDUs, 2 (9.1%) were anti-HDV antibodies positive, 1 (5.6%) out of 18 who had history of blood transfusion and 1 (6.2%) out of 16 who gave the history of being the spouse of high risk groups were anti-HDV antibodies positive (Table 2).

As shown in Table 3, maximum number of anti-HDV positive cases were from 31-40 years age group. Comparison with other studies has similar findings. Table 4 shows the prevalence of HDV according to different studies in India.

There was no significant association between presence of hepatitis D antibodies with the ages and the gender of the cases in this study as shown in Table 5 and 6 respectively. No significant association was found between presence of hepatitis D antibodies and variables such as IDUs, blood transfusion, spouse of high risk group, multiple sexual partners in this study (Table 7).

Table 1: Description of hepatitis D antibody positive cases.

Age in years	Gender	Anti-HDV IgM	Anti-HDV IgG	Associated risk factor
38	Male	Positive	Negative	IDU
42	Male	Positive	Negative	IDU
36	Male	Positive	Negative	Alcoholic, multiple partners
37	Female	Negative	Positive	Blood transfusion
55	Female	Negative	Positive	Spouse of high risk group

Table 2: Distribution of hepatitis D antibody according to gender and risk groups.

Characteristics		Cases	Positive (%)
Gender	Male	71	3(4.2)
	Female	48	2(4.2)
Risk groups	IDUs	22	2(9.1)
	Blood transfusion	18	1(5.6)
	Spouse of high risk group	16	1(6.2)

Table 3: Comparison of hepatitis D antibody positive cases in different age group in different studies.

Age in years	Murhekar et al[19]	Shah et al[18]	Present study
1-10	0	0	0
11-20	1	0	0
21-30	0	2	0
31-40	5	5	3
41-50	2	3	1
>50	0	2	1
Total	8	12	5

Table 4: Seroprevalence of hepatitis D in different studies.

Study	Year of publication	HBsAg +ve cases	Hepatitis D antibody positive cases (%)
Banker et al[20]	1992	331	124 (37.4)
Amarapurkar et al[21]	1992	254	44 (17.3)
Chakraborty et al[22]	2005	123	13 (10.6)
Saravanan et al[23]	2008	153	9 (5.9)
Shah et al[18]	2012	141	12 (8.5)
Chakraborty et al[24]	2015	100	2 (2)
Patel et al[25]	2016	150	0 (0)
Present study	-	119	5 (4.2)

Table 5: Association of age with presence of hepatitis D antibodies.

Hepatitis D antibodies	N (%)	Mean age±SD (in years)	P value
Positive	5 (4.2)	41.60±7.8	0.268
Negative	114 (95.8)	46.31±16.9	

Table 6: Association of gender with presence of hepatitis D antibodies

Gender	Hepatitis D antibodies N (%)		P value*
	Positive	**Negative**	
Male	3 (4.2)	68 (95.8)	1.000
Female	2 (4.2)	46 (95.8)	

Note: *-Fisher's exact test.

Table 7: Association of risk groups with presence of hepatitis D antibodies

Characteristics		Hepatitis D antibodies N (%)		P value*
		Positive	**Negative**	
IDUs	Yes	2 (9.1)	20 (90.9)	0.230
	No	3 (3.1)	94 (96.9)	
Blood transfusion	Yes	1 (5.6)	17 (94.4)	0.566
	No	4 (4)	97 (96)	
Spouse of high risk group	Yes	1 (6.2)	15 (93.8)	0.521
	No	4 (3.9)	99 (96.1)	
Multiple partners	Yes	1 (7.1)	13 (92.9)	0.471
	No	4 (3.8)	101 (96.2)	

Note: *-Fisher's exact test.

DISCUSSION

There has been mark decline in the prevalence of HDV. The following national studies by our previous renowned experts have been taken into consideration for the comparison. The study done by Banker et al in 1992 reported the HDV prevalence to be 37.46% in HBsAg positive patients in Mumbai.[20] Bhattacharya et al had found the prevalence of HDV to be 3.3% in 1998 in a study in Kolkata.[26] Chakraborty et al in 2005 found the prevalence of HDV to be 10.6% in New Delhi.[22] Saravanan et al evaluated the seroprevalence of HDV among 153 individuals with HBV related liver disease in Chennai during 2008.[23] Out of the 153 patients screened, nine (5.9%) were reactive to anti- delta antibodies.

In our study, the seroprevalence of HDV infection was found to be 4.2%. Different studies showed, deceasing trend in HDV infection in the world and in India also. Table 4 shows this declining trend, in studies done in different parts of India. HDV infection has been rear as compare to 1990s. Improving health care facilities, vaccination and awareness may be some of the reasons for this improvement. However the current study shows higher prevalence as compared to most recent studies in the country. Many factors such as drug abuses, multiple partners, HIV infection, geographical location, high incidence of HBV infection may play a role. It may be worth mentioning here, that there is no record on the prevalence of HDV in this part of the world. Prevalence of anti-HDV in female is same as the male in this study.

The seroprevalence of HDV was highest in age group of 31-40 years at 60% (n=3). Shah et al and Murhekar et al had similar findings.[18,19] Hepatitis D antibodies were detected in 2 (9.1%) IDUs and 3 (3.1%) non-IDUs. History

of blood transfusion was present in one case (5.6%), one (6.2%) give the history of being spouse of high risk group, and one (7.1%) had history of having multiple partners According to Gheorghe et al history of transfusion was present in 132 (31.4%), 8 (30.7%) was IDUs and 34 (28.3%) had history of having multiple partners.[27] However there is no association between HDV and variables such as age, sex and various risk factors.

There were certain limitations that should be considered. First, most of the participants attended the hospital for one symptom or another and many hepatic related. So, the resultant group may not fully represent the general population and may not reflect the actual picture. Second, the assays used in the study were from different manufacturer and data on their diagnostic performance were lacking.

CONCLUSION

The seroprevalence of HDV among HBsAg positive cases attending a tertiary care hospital in this far north eastern part of the country was found to be 4.2%.

ACKNOWLEDGEMENTS

We would like to thank all the participants in this study and also the laboratory technicians, department staffs and all the colleagues for their help and support.

Funding: This study was supported by the grant from DBT Nodal Cell, Tezpur University, Assam, Ministry of Science and Technology, GOI under a scheme titled "Research/Thesis Grant for MD/MS students from the North-Eastern region"(Sanction No. BT/Med/15/Vision-NER/2011 and memo no. TU/DBT-NC/MD/MS/P11-12/300).
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Sharma KT, Praveen S, Krossnunpuii, Saha S, Syiemiong B. Hepatitis D virus seroprevalence in HBsAg positive patients attending a tertiary care hospital in Northeast India. Int J Adv Med 2022;9:703-8.