

ORIGINAL PAPER

# Seroprevalence of Hepatitis D Virus in Patients with Hepatitis B Virus-Related Liver Diseases

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## ABSTRACT

**Purpose:** Hepatitis D Virus (HDV) infects patients that are already infected by hepatitis B virus (HBV). There is lack of data on the impact of Hepatitis D Virus (HDV) in patients with hepatitis B virus (HBV) in central Assam. This study was aimed at determining the seroprevalence of Hepatitis D Virus (HDV) in central Assam and does studies on HDV among Hepatitis B patients.

**Methods:** This study was carried out at a tertiary hospital. Out of 89 hepatitis B surface antigen (HBsAg) positive patients 44 were cases of acute viral hepatitis (AVH), 12 were fulminant hepatic failure (FHF), 19 were chronic hepatitis (CH), 12 were cirrhosis and 2 cases were hepatocellular carcinomas (HCC).

**Results:** Among 89 HBsAg positive patients 1 patient had showed positive for anti-HDV ELISA. Low prevalence rate was found in a middle aged business man. HBV and HDV infections together cause more severe liver damage.

**Conclusion:** Although many scholars have studied various aspects of Hepatitis delta virus infection including seroprevalence in India with different types of hepatitis related liver diseases, this study was to evaluate the seroprevalence of HDV infection patient with HBsAg carriers attending in Gauhati medical college and hospital “between” July 2006 to June 2007 A.D. The HDV infection is not uncommon.

**Keywords:** Hepatitis B virus, Hepatitis D Virus, HBsAg, anti HD antibody, Anti-HDV ELISA, liver cirrhosis, hepatocellular carcinoma

## INTRODUCTION

Hepatitis is a general term meaning inflammation of the liver. Hepatitis can be caused by a variety of hepatotropic viruses such as Hepatitis A, B, C, D, E, and G either alone or in concert. Infection with hepatotropic viruses results in acute viral hepatitis, chronic viral hepatitis or fulminant hepatic failure.<sup>1</sup> Hepatitis delta virus (HDV) is a member of group V, Genus Delta virus, and species Hepatitis delta virus.

The HDV genome consisted of a single stranded circular RNA structure and is able to fold on itself, with Watson and crick base pairing of approximately 70% of the nucleotides.<sup>2</sup> The surface protein of Hepatitis B virus (HBV) was discovered accidentally in 1965 during the search by an anthropology for polymorphic serum protein as genetic markers in the blood of an Australian aborigine and was called Australian antigen. Rizzetto had discovered two years later the association between the occurrence of the Australian antigen and serum hepatitis was detected.<sup>3</sup>

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Hepatitis delta virus and co-worker while they studied liver biopsy of patient of chronic liver diseases with positive hepatitis B surface antigen (HBsAg).

At first, Hepatitis delta virus infection was described in HBsAg carrier from southern Italy.<sup>4</sup> Hepatitis D or Delta hepatitis is caused by the hepatitis delta virus (HDV). HDV requires help of a hepadna virus like hepatitis B virus (HBV) for its own replication.<sup>2</sup> Eight phylogenetically distinct genotypes of HDV have been reported. Various genotypes are reported to be associated with different long term outcomes of infection. Genotype 1 is the most frequent and found in Europe, Middle East, North America and North Africa; Genotype 2 is seen in the Far East; Genotype 3 was reported in the Amazonian region of South America; Genotype 4 was isolated in Taiwan and Japan; and Genotype 5 to 8 have been identified in Africans.<sup>5,6</sup>

The single-stranded RNA genome of HDV is in several ways fundamentally different from other RNA viruses of animals.<sup>7</sup>

1. The RNA genome approximately 1700 nucleotides (nt), it is the smallest.
2. The genome has a circular conformation whereas the other viral genomes are linear.
3. The circle is able to fold on itself, with Watson and Crick base pairing of approximately 70 percent of the nucleotides, forming an unbranched rod-like structure.
4. On the genome is a domain of about 85 nt, which acts as a self-cleaving ribozyme.

To establish *in vivo*, HDV depends on helper functions provided by hepatitis B virus. The HDV virion is composed of the RNA genome and a hepatitis delta antigen (HDAg), both enveloped by HBSAg.<sup>7</sup> Several reports had indicated a declining trend in the occurrence of HDV infection in some geographical areas.<sup>8-11</sup> For example, while HDV was responsible for a high proportion of cases of acute and chronic liver disease in Southern Europe during the 1970s, its seroprevalence was reported to have declined substantially in 1997.<sup>10, 12</sup> Huo et al<sup>11, 12</sup> from Taiwan have reported a decrease in HDV infection in hepatitis B surface antigen (HBsAg) carriers from 23.7 in 1983 to 4.2 per cent in 1995.

The prevalence of hepatitis D shows a decreasing trend due to preventive measures like vaccination against hepatitis B and awareness campaigns with regard to risk

factors for the transmission of hepatitis B and D. Although preventive measures against hepatitis B including vaccination have decreased the prevalence of hepatitis D, there is no effective way of preventing HDV infection in HBV carriers in endemic areas. This can only be achieved by educating such individuals to prevent further exposures to risk factors.<sup>13</sup> In spite of the global trend of decline, significant and persistent transmission is present in some countries.

## MATERIAL AND METHODS

This study was to evaluate the seroprevalence of HDV infection patient with hepatitis B surface antigen (HBsAg) carriers attending in Gauhati Medical College and hospital “between” July 2006 to June 2007. A total of 89 hepatitis B surface antigen (HBsAg) positive patients with liver diseases attending Gauhati Medical College and hospital, Guwahati, Central Assam were included. These comprised 44 cases of acute viral hepatitis (AVH), 12 of fulminant hepatic failure (FHF), 19 of chronic hepatitis (CH), 12 of cirrhosis and 2 cases of hepatocellular carcinoma (HCC).

All patients were screened for HBV infection, which was established by positivity for surface antigen HBsAg. Under all aseptic care 5 ml of venous blood was collected in a sterile vial from the patient. Blood allow to clotting at room temperature. Serum is separated by centrifugation at 3000 rpm for 5 minutes and labelled the serum sample were stored at -70° c. Serum sample was tested by competitive enzyme linked immunoassay for Anti-HDV (IgG) to hepatitis using commercially available kit manufactured by Smartest Diagnostics ( M/S organics Ltd) , Israel with lot number 252046.

The serum samples were collected from 76 male and 13 female patients. Out of 89 HBV samples, 50 urban and 39 were from rural areas. The 89 HBV samples are broadly grouped into 5 groups. Out of 89 HBV samples 19 were students, 15 were engaged in services, 25 were businessman, 20 farmers, and 10 were housewives. HBsAg was detected by Hep-Alert rapid immunochromatographic card test (Ranbaxy Diagnostics, New Delhi, India) in human serum or plasma. The Competitive Enzyme linked immunoassay for Anti-HDV (IgG) to hepatitis Delta had > 98 % sensitivity and >98% specificity.

## OBSERVATION AND RESULTS

In this study a total of 89 cases of HBV related acute and chronic liver disease serum samples were collected. There were 76 males (85.39%) and 13 females (14.60%) included

in this 89 HBsAg serum positive cases. The male and female ratio is 5.84:1. This study group comprised of 44 patients with acute viral hepatitis (AVH), 12 patients with fulminant hepatitis, 19 patients with chronic active hepatitis (CH), 12 patients with cirrhosis (CRR) of liver and 2 patients with hepatocellular carcinoma (HCC). All the acute and chronic liver diseases were HBsAg positive.

ELISA tested the serological and clinically diagnosed 89 HBsAg positive serum samples for detection of hepatitis D virus antibody. Among the total of 89 HBV samples 50 (56.17%) were from urban and 39(43.82%) were from rural areas. The cases are broadly grouped into 5 groups. Among them 19 were students, 15 were engaged in services, 25 were businessman, 20 farmers, 10 were housewives.

**Table 1** Prevalence of HDV

Total number of case	HDV positive case	HDV negative case
89	01 (1.12%)	88 (98.87%)

Only 01(one) HDV seropositive case between 21-40 years age group was found with occupation of business. Among the total 89 samples only one HDV (1.12%) positive case and other 88 HDV (98.87%) negative cases were included in this study (**Table 1**). The HDV seropositive was found in 21 to 40 years age group. The age and HBV-HDV distribution are shown in **Table 2**.

**Table 2** Age and HBV & HDV positive distribution

Age (Years)	HBV case	HDV CASE
0-20	5	0
21-40	50	1
41-60	32	0
>60	2	0

## DISCUSSION

With the introduction of specific serological markers for Hepatitis A, B, or D and E infection, it is now possible to differentiate type A, type B, type C or type D infection from those with presumed non-A, non-B infection. In the present study 89 patients of various HBV-related liver diseases were studied for the presence of delta antibody.

The prevalence of delta infection in the present study was found to be 1.12% (1/89). Jain et al, 2013 had showed zero (0%) percentage HDV prevalence in North India among 43 HBV positive patient.<sup>14</sup> Li J, Wang J, Tian K, Wang Y, Zhang L, Huang H have conducted an epidemiological survey of hepatitis D viruses in IVDU

(intravenous drug users). The infection rate among IVDU was 2.22 % for HDV.<sup>15</sup> Amarapurkar DN et al, assayed the seroprevalence among 148 patients with HBV related acute viral hepatitis in Mumbai, India. They reported seropositive in 16% (23/148) patients.<sup>16</sup>

In present study one HDV seropositive case is found in FHF group. Celen M K and his co-workers reported high prevalence rate of HDV among fulminant hepatitis (27.5%) in Turkey.<sup>17</sup> Chakraborty P et al reported high prevalence in FHF group (20%) in Northern part of India.<sup>12</sup>

This low prevalence is identical with the results reported by other workers viz., 1.2% HDV infection in HBsAg Carriers, Ramia S, El- Zaatari M et al<sup>18</sup>, Lebanon; 4.2% HDV infection Huo et al<sup>11</sup>. However, some other workers from India have also reported a high prevalence of delta hepatitis 10.7% Singh V et al<sup>14</sup>; 10.6% Chakraborty P et al.<sup>12</sup> The difference in reference to the prevalence of delta infection in viral hepatitis as compared to the other workers may be related to the various factors (like I.V. drug user, prostitute, medical facility to the community, health education) in individual cases, which make them more susceptible.

The present study does not show seropositive case in acute viral hepatitis (AVH) group. Ramirez AM and his co-workers reported that HDV infections lower in acute viral hepatitis compared to chronic HDV infection in New Zealand.<sup>19</sup> They reported HDV prevalence of 3.8-4.8% in AVH and 28% in chronic hepatitis. Chakraborty P et al had also reported low prevalence (3.1%) among AVH in India.<sup>12</sup>

HDV infection was not found in hepatocellular carcinoma and cirrhosis patients in this study with a prevalence of 1.12%. But, Singh V et al reported 10.7% prevalence of HDV in Northern part of India. It is possible that the epidemiology of delta virus is undergoing a change over time with a trend towards decreasing prevalence.<sup>20</sup> In the present study, only one HDV seropositive was found in male population. Chen CJ et al reported that males had a higher prevalence than females in blood donors (2.7% versus 0), STD patients (8.2% versus 7.5%), and drug abusers (69.0% versus 57.1%) but the difference was not statistically significant.<sup>21</sup>

The factors responsible for such an epidemiological transition could be routine screening of blood and blood products for HBsAg, Extensive promotion of disposable needles and other medical instruments and sustained in epidemiology of delta agent in different parts of the world and even in different parts of India probably a host genetic susceptibility to this ubiquitous agent.

Some countries have witnessed a declining trend in the prevalence of HDV infection. According to L. Matthyssen et al prevalence of anti-HD antibody was 4.04% amongst 173 cases of HBsAg reactive patients.<sup>6,22</sup>

A decreasing trend of HDV infection from 15.1% in 1983 to 7.1% in 1992 had also been reported from Spain, Navascues CA et al.<sup>9</sup> From Taiwan, Huo et al had reported a decrease in HDV endemicity from 23.7% in 1983 to 4.2% in 1995.<sup>11</sup> The reduction in HDV in seroprevalence has been postulated to result from various sexually transmitted diseases, promotion of disposable needles and better control of HBV infection itself.

## CONCLUSION

The present study was undertaken to see the prevalence of hepatitis D virus in Indian patients with HBV-related acute and chronic liver diseases by serology.

In conclusion, HDV infection does not appear to be commonly in North-Eastern part of Indian patients. The results also suggest that the HDV epidemiology in this part of world may possibly be undergoing a transition with a trend towards declining prevalence.

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## REFERENCES

- Dienstag LG, Isselbacher KJ. Acute hepatitis. In: Wilson JD, Braunwald E, Hauser SL, Fauci AS, Longo DL, Jameson JL, Editors. Harrison's principles of Internal Medicine (Vol-2). 15<sup>th</sup> ed. New york: McGraw Hill Inc; 2001. p. 1721-36.
- Taylor JM. Delta Hepatitis. In: Mahy BWJ, Collier L Editors. Topley and Wilson's Microbiology and Microbial infection(vol-2). 10<sup>th</sup> ed. New york: Oxford University press; 2005. p. 1269-1275.
- Blumberg BS, Alter HJ and visnich S. A new antigen in leukemia sera. JAMA 1965;191:541-546.
- Blumberg BS and Gerstley BJ et al. A serum antigen (Australian antigen) in Down's syndrome, leukemia and hepatitis. Ann Intern Med. 1967;66:924-31.
- Shakil AO, Hadziyannis S, Hoofnagle JH, et al. Geographic distribution and genetic variability of hepatitis delta virus genotype. Virology 1997;234:160-167.
- Shah Latika J, Mulla Summaiya A. Prevalence Of Hepatitis D Virus (Hdv) In South Gujarat. J Microbiol Immunol infects 2008;41:227-230.
- Gerin JL In: Nishioka K, Suzuki S, Oda T eds. The Toxonomy of hepatitis delta Virus. Viral hepatitis and liver disease. Spingerverlag: Tokyo, Japan;1994. p. 63-65.
- Sagnelli E, Stroffolini T, Ascione A, Chiaramonte M, Craxi A, Giusti G, et al. Decrease in HDV endemicity in Italy. J Hepatol 1997;26: 20-24.
- Navascues CA, Rodriguez M, Sotorrio NG, Sala P, Linares A, Suarez A, et al. Epidemiology of hepatitis D virus infection: changes in the last 14 years. Am J Gastroenterol 1995;90:1981-4.
- Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S, et al. Chronic hepatitis D: a vanishing disease? An Italian multicenter study. Hepatology 2000;32:824-7.
- Huo TI, Wu JC, Lin RY, Sheng WY, Chang FY, Lee SD. Decreasing hepatitis D virus infection in Taiwan: an analysis of contributory factors. J Gastroenterol Hepatol 1997;12:747-51.
- Chakraborty P, Kailash U, Jain A, Goyal R, Gupta RK, Das BC, Kar P. Seroprevalence of hepatitis D virus in patients with hepatitis B virus-related liver diseases. Indian J Med Res 2005;122:254-257.
- Abbas Z, Jafri W, Raza S. Hepatitis D: Scenario in the Asia-Pacific region. World J Gastroenterol 2010;16(5):554–62.
- P Jain, S Prakash, S Gupta, KP Singh, S Shrivastava, DD Singh et al. Prevalence of hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus and hepatitis E virus as causes of acute viral hepatitis in North India: A hospital based study. Indian Journal of Medical Microbiology 2013;31(3):261-265.
- Li J, Wang J, Tian K, Wang Y, Zhang L, Huang H. Epidemiology of hepatitis B, C, D and G viruses and cytokine levels among intravenous drug users. J Huazhong Univ Sci Technolog Med Sci 2006;26:221-224.
- Amarapurkar DN, Vishwanath N, Kumar A, Shankaran S, Murti P, Kalro RH, et al. Prevalence of delta virus infection in high risk population and hepatitis B virus related liver diseases. Indian J Gastroenterol 1992;11:11-2.
- Celen MK, Ayaz C, Hosoglu S, Geyik MF, Ulug M. Anti-hepatitis delta virus seroprevalence and risk factors in patients with hepatitis B in Southeast Turkey. Saudi Med J 2006 May;27(5):617-20.
- Ramia S, El-Zaatari M, Sharara AI, Ramlawi F, Farhat B. Current prevalence of hepatitis delta virus (HDV) infection and the range of HDV genotypes in Lebanon. Epidemiol Infect 2007;135:959-962.
- Ramirez AM1, Lee SP, Woodfield DG. Hepatitis delta virus infection: a recently imported disease in New Zealand. N Z Med J 1987 Apr 22;100(822):235-7.
- Singh V, Goenka MK, Bhasin DK, Kochhar R, Singh K. A study of hepatitis delta virus infection in patients with acute and chronic liver disease from northern India. J Viral Hepat 1995;2:151-4.
- Chen CJ, Tseng SF, Lu CF, Lin HC, You SL, Chen CS, Hwang SJ, Hsieh SF, Hsu ST. Current seroepidemiology of hepatitis D virus infection among hepatitis B surface antigen carriers of general and high-risk populations in Taiwan. J Med Virol 1992;38:97-101.
- Matthyssen L et al. Organon Scientific Development Group, Netherlands, Viral Hepatitis and Liver Disease. 1988. p. 409-411.