Prevalence of Hepatitis D Among Patients with Hepatitis B Viral Infection Attending a Tertiary Care Centre of Nepal

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ABSTRACT

Introduction: Worldwide there is variation in prevalence of Hepatitis D viral infection. Superinfection and co infection with hepatitis B viral infection is known to occur in 15-20 million people.

Methods: This was a descriptive cross-sectional hospital based study carried out in NAMS, Bir hospital, Kathmandu, Nepal from period of January 2017 to June 2017. Consecutive patients of chronic hepatitis B viral infection of HBsAg positive with more than two-time upper normal limit of ALT were enrolled and tested for HDV IgG.

Results: Forty patients were enrolled during study period. Mean age was 30.9±12.2 years. Males were 28 (70%) and females 12 (30%). Most of the patients were asymptomatic for HBV infection 32 (80%). HBeAg negative chronic hepatitis was most commonly present in 31 (77.5%). Family history of Hepatitis B viral infection was seen in 7 (17.5%) and sexual promiscuity in 5 (12.5%) as the mode of acquisition of hepatitis B viral infection. HBcIgM was positive in three patients with mean HBV DNA of $4.97x10^5 \pm 4.5x10^5$ IU/ml in HBeAg positive group. HDV IgG was negative in all patients.

Conclusions: Coinfection and superinfection of hepatitis D virus were found to be uncommon at Bir hospital, Nepal.

Keywords: *HbsAg; HDV DNA; HDV IgG; Hepatitis B; Hepatitis D.*

INTRODUCTION

Hepatitis D virus (HDV) was first discovered in 1977. It is a defective virus which requires Hepatitis B virus (HBV) for replication and expression.[1] Global burden of HDV is 15- 20 million cases.[2]

Coinfection or superinfection of HDV with HBV is considered to be a severe form of viral hepatitis, leading to rapid development of cirrhosis.[3] Markers of HDV infection are antibody against HDV (IgM and IgG), detection of HDV antigen (HDV Ag), and HDV RNA quantification by polymerase chain reaction (PCR). Sensitivity and specificity of HDV IgG by enzyme linked imunosorbent assay (ELISA) are 98% respectively.[4]

We aimed to see the current prevalence of HDV in Hepatitis B infection in our setup.

METHODS

This is a cross sectional descriptive hospital based study carried out in National Academy of Medical sciences (NAMS), Bir hospital, Kathmandu. Informed written consent was taken from all patients. The study

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was approved by Institutional Review Board (IRB) of NAMS. Data was collected in structured proforma in period of March 2017 to August 2017. Any patient who presented to Bir hospital, Liver unit (OPD and indoor) with diagnosis of hepatitis B (HBsAg positive by ELISA) with two times upper normal limit of alanine aminotransferase (ALT) (Normal Upper limit of ALT 45 IU/ml) were included in study. Complete history was taken and clinical examination was done. Patients were specifically asked about history of blood transfusion, surgeries, hemodialysis, organ transplant, sexual promiscuity, intra-venous drug abuse (IVDA) and tattoo. All patients underwent two dimensional ultrasound of hepatobiliary system and selected patients underwent upper gastrointestinal endoscopy and their findings were recorded. Coexisting other diseases like Hepatitis C viral infection, Human immunodefienciency virus (HIV) infection, autoimmune hepatitis, wilson disease, hemochromatosis, viral hepatitis A and E, diabetes or alcohol abuse were excluded. Other tests like total serum billirubin, direct serum billirubin, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total protein, serum albumin, blood urea, serum creatinine and random blood glucose (RBG), prothrombin time (PT), International normalize ratio (INR) were sent. Patients were further evaluated for other markers of hepatitis B: hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (HBeAb), Antibody against core antigen of Hepatitis B virus (Anti HBcIgM) and HBV DNA quantification by polymerase chain reaction (PCR). Immunoglobulin G against hepatitis D virus (Anti HDV IgG) was sent as a marker of HDV infection. If the sample tested positive for HDV IgG, we planned to send for HDV RNA quantification by PCR. During study period serum was collected and stored at -80^{0}F before proceeding for HDV IgG by ELISA analysis (HDV Ab, Dia. Pro bioprobes srl).

Sample size was calculated as;

$$\text{Sample size (n)} = Z^2 \times \frac{P\,(100-P)}{e^2}$$

Assuming the prevalence of HDV (P) as 2% with Confidence Interval (CI) 95% (Z) and standard error (e) of 5%.

The calculated sample size was 31.

Data entry was done in Microsoft excel 2007 and analysis was done in SPSS version 23 and graph pad software. Variables were given in the form of ratio (%) and t-test was used for categorical variables.

RESULTS

A total of 46 patients were screened. Six patients were excluded among them two had drug induced liver injury, two were alcohol abuser, one had nonalcoholic steatohepatitis, and one had coexisting HIV infection. Prevalence of HDV among hepatitis B viral infection was zero. Most patients were males; M =28 (70%), F=12 (30%). Mean age of patients was 30.98±12.79 years with minimum age 15 years and maximum age 72 years (Table 1).

Table 1. Baseline characteristics of patients.

Variable	Value (Mean±SD)
Age (years)	30.98±12.28
Gender	M 28, F 12
SBP (mmHg)	108.72±12.77
DBP (mmHg)	70.62±8.8
Pulse (per/min)	90.4±12.60
Weight (Kg)	60.93±12.22
Abdominal girth (inch)	30.0±3.5

SBP = Systolic blood pressure, DBP = Diastolic blood pressure

Most patients were Chettri 9 (22.5%) followed by Madhesi 8 (20%) and Brahmin 7 (17.5%). Housewife were most common occupation 10(25%) followed by student 8 (20%). In this study, patients visited to Bir hospital from 26 different districts. Kathmandu district topped the list 5 (12.5%) followed by Sarlahi 4 (10%), Janakpur 3 (7.5%) and Dang 3 (7.5%). Thirty-one (77.5%) had HBeAg negative chronic hepatitis B followed by HBV related compensated cirrhosis of liver in 5 (12.5%) and acute flare of chronic Hepatitis B virus in 2 (5%). Symptoms at presentation were shown (Figure 1). At presentation, 12 (30%) patients had already on tenfovir or entacavir for chronic hepatitis B viral infection.

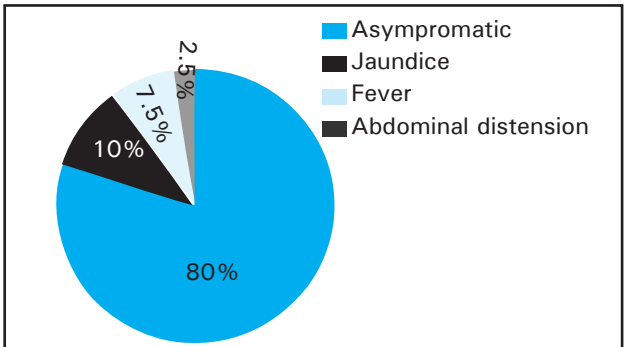

Figure 1. Common presenting symptoms.

Family history of Hepatitis B was present in 7 (17.5%). Sexual Promiscuity was present in 7 (17.5%). History of tattooing was present in 3 (7.5%) patients; however, 19 (47.5%) patients did not had any identifiable cause of hepatitis B viral infection. None of the patients had

past history of blood transfusion, organ transplantation, hemodialysis or intravenous drug abuser.

USG abdomen suggests 5 (12.5%) had cirrhosis of liver and 2 (5%) had cirrhosis of liver with features of portal hypertension and 6 (15%) patients had fatty liver. Other laboratory parameters were shown below (Table 2). Serological markers of Hepatitis B like HBeAg were positive in 11 (27.5) and HBeAb were positive in 29 (72.5%). Anti HBcIgM were positive in 3 (7.5%). Mean HBVDNA quantitification was $4.9x10^5 \pm 4.5x10^5$ IU/ml in HbeAg positive group.

Table 2. Lab Parameter of patients.

Variable	HBeAg (Mean ± SD) or n	HbeAb (Mean ± SD) or n	P value
Total serum Billirubin (mg%)	1.46 ± 1.24	0.8 ± 0.2	0.42
Direct Serum Billirubin (mg%)	0.39 ± 0.35	0.2 ± 0.12	0.51
ALT (IU/L)	211.5 ± 197.3	119.4 ± 20.4	0.46
AST (IU/L)	169.9 ± 167.8	86.93 ± 20.62	0.44
ALP (IU/L)	124.3 ± 23.9	112.27 ± 20.57	0.115
RBG (mg%)	103.27 ± 17.53	101.70 ± 22.08	0.833
PT (sec)	15.56 ± 3.24	13.89 ± 2.06	0.05
INR	1.24 ± 0.28	1.12 ± 0.13	0.70
HBcIgM	3	0	0
HBV DNA (IU/ml)	$4.9x10^5 \pm 4.5x10^5$	$1.1x10^4 \pm 2.4x10^3$	0.0001
Anti HDV IgG (ELISA)	0	0	0

DISCUSSION

Hepatitis D is defective virus and requires Hepatitis B for its replications. It is most prevalent in South America and Mediterranean belt. Superinfection and coinfection are the two different distinct manifestations occurring when Hepatitis B viral infection occur with hepatitis D viral infection. When associated with the co-infection, it can lead to acute hepatic failure and when associated with superinfection, to chronic liver disease.[5] HBsAg positive patients with HDV infection may develop hepatic failure and develop hepatocellular carcinoma as delta infection in HBV carriers is associated with more active and progressive disease, as suggested by clinical and histological evidence of high liver enzymes and a faster rate of developing cirrhosis.[6] At local level there was a single study which was carried out in 1991 by SM Shrestha and Tusuda F. Their results suggest no patients were found to be positive with hepatitis D.[7] However prevalence of HDV in India is variable. Various studies suggest that its prevalence ranged from zero to 37.4% in patients with hepatitis B viral infection. Highest reported prevalence was from Bombay 37.4% in year 1992.[8] In Ludhiana, its prevalence was 33% and most patients were children.[9] In New Delhi prevalence was 8.1% in 1996.[10] In a similar study conducted in 1998, prevalence of hepatitis D in Kolkata was 3.3 %.[11] Another study was conducted by Lal JS and colleagues in India in year 2014 involving large number of patients (n = 318) with Hepatitis B viral infection. These patients underwent three different modalities of tests (involving twice HDV IgG with two different manufacturers and HDV RNA by PCR). Surprisingly, no HDV infection was seen in this study.[12] They concluded that low rate of HDV infection was possibly due to recent decline in HDV transmission secondary to improved healthcare practices which includes universal precautions and of use of disposable syringes.[12] In a study from Bangladesh (n = 180), 21.8% of asymptomatic HBsAg carriers and 25.6% of symptomatic hepatitis B patients were anti-HDV positive.[13]

Similar to the above-mentioned study by Las JS and Shrestha SM, our study also showed a zero prevalence of HDV infection. However, our study had a lower sample size as compared to their study. We did not perform HDV RNA quantification by PCR as none of our participants were ELISA HDV IgG positive. In various studies the prevalence of HDV infection was high in IV abusers.[14] Similarly high prevalence was recorded in hemophiliacs.[15] This also may be one of the reasons for non-detection of HDV infection in our study as none of our patients were IVDA or hemophiliacs.

This study was aimed to find out prevalence of superinfection or coinfection of hepatitis D in patients

attending to one of the tertiary care centre of Nepal. Though it was small study, prevalence of HDV infection was zero. Our study has a limitation where patients might have acute hepatitis D viral infection however HDV IgM was not done.

CONCLUSIONS

The HDV infection in patients with HBV are not found in our setup. More epidemiological study is required whether to use HDV IgG in patients with hepatitis B viral infection in Nepal.

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Conflict of Interest: None.

REFERENCES

1. Jacobson IM, Dienstag JL, Werner BG, Brettler DB, Levine PH, Mushahwar IK. Epidemiology and clinical impact of hepatitis D virus (delta) infection. Hepatology 1985;5:188-91. [PubMed | Full Text]
2. Hadziyaannis SJ. Review: Hepatitis Delta. J Gastroenterol Hepatol. 1997;12:289-98. [PubMed | Full Text]
3. Caturelli E, Castellano L, Fusilli S, et al. Coarse nodular US pattern in hepatic cirrhosis: risk for hepatocellular carcinoma. Radiology. 2003;226:691-697. [PubMed | DOI]
4. 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(3):151-154. [PubMed | Full Text]
5. Lok AS, Lindsay I, Scheuer PJ, Thomas HC. Clinical and histological features of delta infection in chronic hepatitis B virus carriers. J Clin Pathol. 1985;38(5):530-533. [PubMed | PMC | Full Text]
6. Shrestha S, Tsuda F. Hepatitis delta virus infection in Nepal. Tropical gastroenterology. 1991;12(4):185-7. [PubMed]
7. Banker DD, Desai P, Brawner TA, Decker RH. Hepatitis delta virus infection in Bombay. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1992 Jul 1;86(4):424-5. [PubMed]
8. Ghuman HK, Kaur S. Delta-hepatitis. Indian journal of pediatrics. 1995 Nov 1;62(6):691-3. [PubMed]
9. Chakraborty P, Kailash U, Jain A, Goyal R. Seroprevalence of hepatitis D virus in patients with hepatitis B virus-related liver diseases. Indian J Med Res. 2005 Sep 1;122(3):254. [PubMed]
10. Bhattacharyya S, Dalal BS, Lahiri A. Hepatitis D infectivity profile among hepatitis B infected hospitalised patients in Calcutta. Indian J Public Health. 1998;42:108-112. [PubMed]
11. Jat SL, Gupta N, Kumar T, Mishra S, S A, Yadav V, Goel A, Aggarwal R. Prevalence of hepatitis D virus infection among hepatitis B virus-infected individuals in India. Indian J Gastroenterol. 2015 Mar;34(2):164-8. [PubMed | DOI]
12. Zaki H, Darmstadt GL, Baten A, Ahsan CR, Saha SK. Seroepidemiology of hepatitis B and delta virus infections in Bangladesh. J Trop Pediatr. 2003;49:371-374. [PubMed]
13. Weller IV, Karayiannis P, Lok AS, Montano L, Bamber M, Thomas H, Sherlock S. Significance of delta agent infection in chronic hepatitis B virus infection; a study in British carriers. Gut. 1983;24(11):1061-3. [PubMed | PMC | Full Text]
14. Ruggiero G, Cesaro G, Mazzella C, Gaeta GB, Miraglia E, Mastrullo L, Boffa ML, Spiteri D, Schiattarella V, de Biasi R. Liver disease in hemophiliacs: etiological and biochemical data on 159 cases from our geographical area. Hepatogastroenterology. 1985;32(1):57-60. [PubMed]