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Research Article

Differential Viral Load Indicates HDV Influences HBV Replication in HBV/HDV Co-Infected Patients

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Abstract

Viral hepatitis causes approximately 1.4 million deaths every year. Hepatitis B virus (HBV) is a partially double-stranded DNA virus and belongs to the family Hepadnaviridae. Hepatitis D virus (HDV) is a sub-satellite virus that infects patients already infected by the HBV because it depends on HBV surface antigen for its replication. HDV causes superinfection with different complexities as compared to the HBV infection alone. To compare the viral load of HBV and HDV to investigate the possible influence on each other's replication, a cross-sectional analytical study was conducted on 907 samples. HBV DNA was detected and quantified by the Abbott HBV Quantification kit, and HDV RNA was detected and amplified by *RoboGene*®. Screening of HDV RNA and HBV DNA by PCR and results were analyzed using SPSS. Out of 907 patients, 33% were HBV/HDV co-infected, 35% were HBV, 18% were infected with HDV & 14% were HBV/HDV negative. Out of these, 75.7% were males, and 24.3% were females. The patients were divided into three age groups: 5-20 years, 21-40 years & 41-80 years. Overall levels of HBV DNA PCR < 1000 IU/ml were found in 74% of HBV/HDV co-infected patients as compared to 30.8% of HBV mono-infected. This study indicates that the absence or the low viral load of HBV in HDV-positive patients might be due to a possible subdue of HBV by HDV in HBV/HDV co-infected patients. The age group of 20-40 years and the male gender is comparatively at high risk for these viral infections.

Keywords: Hepatitis B virus (HBV), hepatitis delta virus (HDV), suppression, HBV/HDV Coinfection

1. Introduction

Hepatitis is a major global health problem affecting millions of children and adults. The word 'Hepatitis' is derived from a Latin word that means inflammation of the liver(Riaz et al. 2011, Saudy et al. 2003). According to WHO estimates, 1.5 million new cases of Hepatitis B are reported annually, and 296 million individuals are living with Chronic Hepatitis B (CHB)(Organization 2022). Hepatitis B Virus (HBV) is the major cause of fatal liver diseases such as cirrhosis and hepatocellular carcinoma. One million people die yearly due to hepatocellular carcinoma, liver failure, and cirrhosis (Lee 1997).

An estimated 820 000 people died from HBV in 2019, primarily from cirrhosis and hepatocellular carcinoma (primary liver cancer)(Kumar, Das, and Jameel 2010, Abbas, Jaffery, and Anwar 2008, Organization 2022). Patients with HBV should be evaluated for the presence of coinfection with other viral infections, including human immunodeficiency virus (HIV), hepatitis D, and hepatitis C. These coinfections result in a poor

prognosis compared to HBV infection alone (Ryu, Bayer, and Taylor 1992).

HBV belongs to the family Hepadnaviridae. HBV is an enveloped, icosahedral particle with an average of 42 nm in diameter. The HBV genome consists of a partially double-stranded DNA of approximately 3.2 Kbp. The four overlapping open reading frames encode the envelope (pre-S/S), core (pre-core/core), polymerase, and X proteins(Riaz et al. 2011, Abbas, Jaffery, and Anwar 2008). Hepatitis Delta Virus (HDV) is a defective RNA virus, depending on HBV surface antigen for its expression and replication. In 1977, it was first described by Rizzetto et al.(Ryu, Bayer, and Taylor 1992, Williams et al. 2009)HDV is a small spherical particle with a diameter of 36 to 43 nm, and its genome size is 1.7 Kbp. HDV contains two forms of HDAg, short (HDAg-S) is a peptide of 24 kDa and 195 amino acids in length, and long (HDAg-L) is a peptide of 27 kDa and 214 amino acids in length (Khan et al. 2011, MD and Alavian 2005, Modahl and Lai 2000). According to an estimate, more than 15 to 20 million people have HDV infection worldwide (Lin et al. 2020). However, its prevalence is higher in Eastern Europe, Italy, and Western Asia and endemic in the Middle East (Sheldon et al. 2008).

HDV, with a universal distribution, has a rate of approximately 5% among HBV surface antigen (HbsAg) carriers worldwide(Jardi et al. 2001). The routes of HDV transmission are similar to those for HBV, including blood-borne, per mucosal, sexual, percutaneous, and perinatal transmission. However, patients having HDV/HBV coinfection may develop more severe acute diseases and higher risks of hepatocellular carcinoma (HCC), cirrhosis, and fulminant hepatitis than those having HBV infection alone(Roshandel et al. 2007). In addition, patients infected with HBV/HDV also have a higher rate of progression to serious complications and chronic disease(Sagnelli et al. 2000).

The presence of HDV is associated with the suppression of HBV replication and possibly infectivity. Therefore, it is reasonable to consider that HDV may facilitate the control of HBV and inhibits the replication of HBV (Mathurin et al. 2000, Jardi et al. 2001). The reason for this could be the host DNA-dependent RNA polymerase II inhibition by a large delta antigen of HDV involved in HBV replication.(Wu et al. 1991, Sheng et al. 2007, Riaz et al. 2011).

To evaluate the possible suppression of HBV in HBV/HDV coinfection, we have used molecular techniques such as real-time PCR to detect HDV RNA and HBV DNA in HBsAg-positive samples from patients of different geographical regions of Pakistan.

Table 1: Rate of HBV/HDV mono and coinfection.

Infection	No of Sample	Percentage
HBV/ HDV Positive	303	33%
HBV Positive	318	35 %
HDV Positive	164	18%
HBV/HDV NEGATIVE	122	14%
Total	907	

This table is representing the total number of mono and coinfection samples of HBV and HDV

2. Material & Methods

A total of 907 serum samples were collected with informed consent and stored in aliquots at -70°C.

2.1. HBV DNA Extraction and Real-Time PCR Amplification

HBV DNA was extracted from 500µl of serum using the Abbott m2000sp, an automated sample preparation system designed to purify nucleic acids from the samples. HBV DNA was quantified with the Abbott HBV Quantification kit using Abbott mrt200 amplification and detection system. Target region of this kit is the conserved region of HBV surface antigen, and the lower detection limit of kit is 15µl/ml.

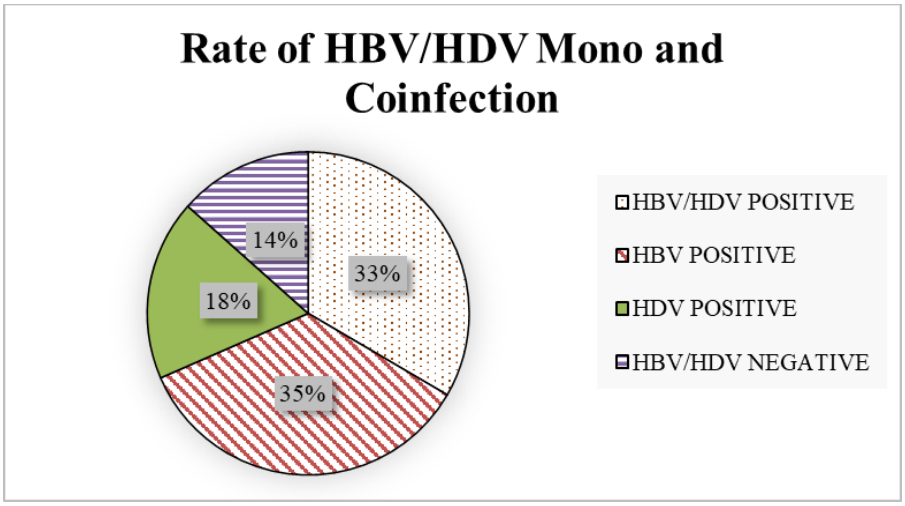

Figure 1: Rate of HBV/HDV mono and coinfection.

2.2. HDV RNA Extraction and Real-Time PCR Amplification

HDV RNA was extracted from 200µl of serum by spin column base *RoboGene*® Viral RNA isolation kit according to the manufacturer's procedure. HDV RNA was quantified with *RoboGene*® Hepatitis D Virus (HDV) RNA Quantification Kit by using Rotor-Gene 6000 Amplification and detection system. The lower detection limit of the kit is 500µl/ml.

2.3 Statistical Analysis

The descriptive statistics were reported using SPSS statistics 21. The prevalence of HDV/HBV was shown as percentages.

Table 2: Distribution of HBV and HDV infection in different age groups.

Infection	**Age 5-20 years n= 171 (18.9%)**		**Age 21-40 years n=585 (64.5%)**		**Age 41-80 years n=151 (16.6%)**		**Total n=907**	
	Male 127 (14%)	**Female 44 (4.7%)**	**Male 443 (48.8%)**	**Female 142 (15.6%)**	**Male 117 (12.9%)**	**Female 34 (3.7%)**	**Male 687 (75.7%)**	**Female 220 (24.3%)**
HBV/HDV Co Infection	66	12	159	27	37	02	262 (28.9%)	41 (4.5%)
HBV infection	25	14	145	65	49	20	219 (24.1%)	99 (10.9%)
HDV infection	21	9	89	24	15	06	125 (13.7%)	39 (4.2%)
HBV/HDV Negative	15	9	50	26	16	06	81 (8.9%)	41 (4.5%)

This table represent the distribution of mono and coinfection samples of HBV and HDV according to different age groups and gender.

3. Results

A total of 907 patients were tested for HBV and HDV. The number of HBV/HDV co-infected patients was 303(33%). HBV mono-infection was detected in 318(35%) patients. In comparison, HDV mono-infection was detected in 164(18%) patients. HBV/HDV negative patients were 122(14%). The rates of HDV and HBV positivity are shown in Table 1 and Fig 1.

Out of these 907 patients 687(75.7%) were males and 220(24.3%) were females. The patients were divided into three age groups. Patients 5-20 years of age were 171(18.9%), in this group there were 44(4.7%) females and 127(14%) were males. Patients 21-40 years of age were 585(64.5%), in this group there were 142(15.6%) females and 443(48.8%) were males. Patients 41-80 years of age were 151(16.6%) In this group there were 34(3.7%) females and 117(12.9%) were males (Table2 and Figure 2).

Overall levels of HBV DNA PCR < 1000 IU/ml were found in 346 (74 %) HBV/HDV co-infected patients as compared to 98 (30.8%) HBV mono-infection. This indicates that the presence of HBV/HDV coinfection suppresses HBV DNA levels. (Figure 3)

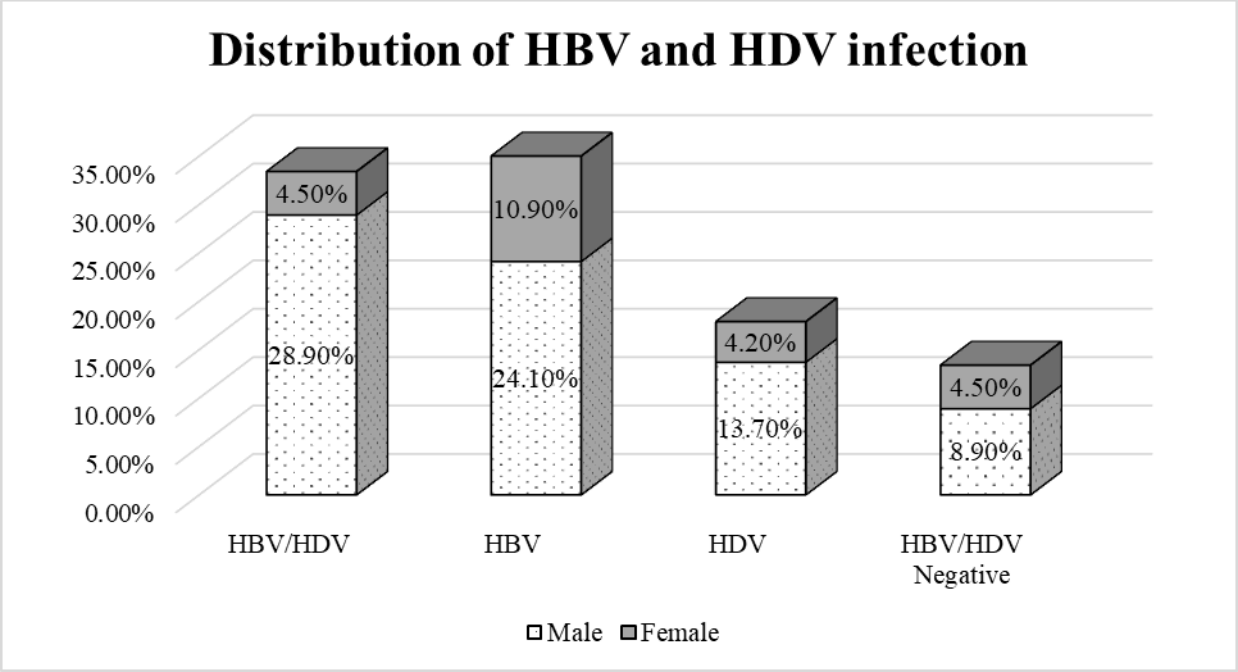

Figure 2: Distribution of HBV and HDV infection.

4. Discussion

HDV is a unique defective RNA virus. It needs helper HBV to provide surface antigen for its transmission and cause coinfection or superinfection in Chronic HBV carriers. Coinfection of HBV and HDV may have more severe acute disease and higher risks of fulminant hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)(Zaidi et al. 2010, Sagnelli et al. 2000, Le Gal et al. 2005). In the current study, we compared HBV viral load in HBV/HDV co-infected patients and HBV mono-infected patients using molecular methods to describe possible interference of HDV to suppress HBV replication.

Our present study showed several important findings 33 % of patients have HBV/HDV coinfection, which shows an extremely high prevalence. Several previous studies also show the same prevalence of HBV/HDV coinfection(Khan et al. 2011, Mumtaz et al. 2011). HBV and HDV coinfection has increased in Pakistan during the last decade, which has also been shown in our

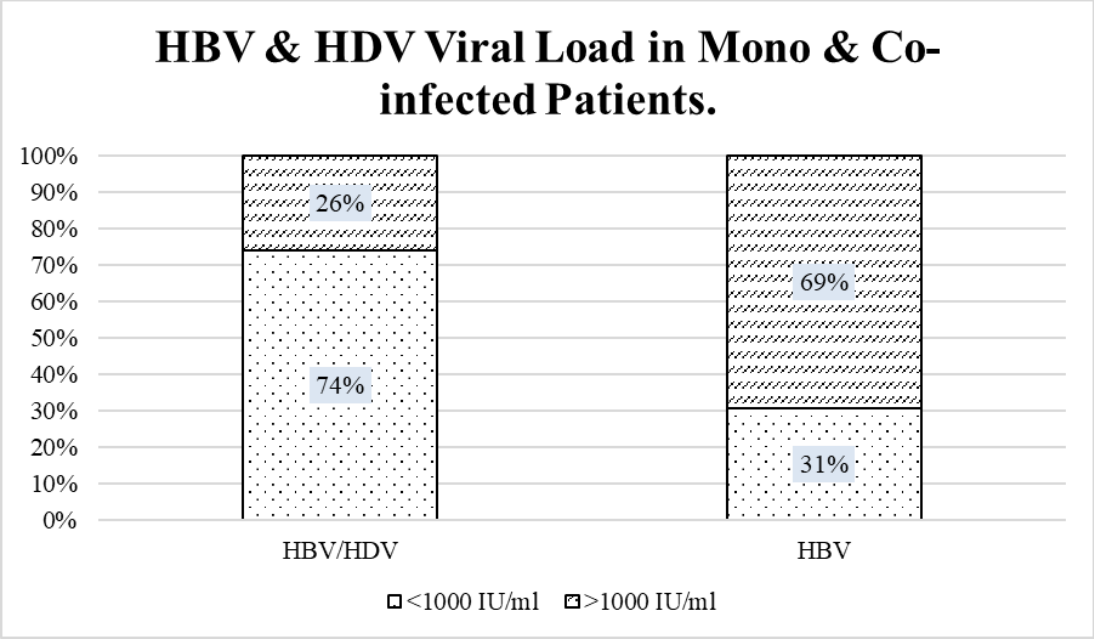

Figure 3: HBV & HDV Viral Load in Mono & Co-infected Patients.

studies, but on the other hand, the prevalence of coinfection of HBV and HDV is declining worldwide(Ibrahim Al Traif 2004, Chakraborty et al. 2005, Değertekin, Yalçin, and Yakut 2006). Our study also shows that coinfection or mono-infection of HBV and HDV was higher in males (75%) compared to females (24%), and the age group below 40 years is more affected than those above 40 years. These findings of the current study are similar to the finding of another study from Pakistan (Zaidi et al. 2010, Mumtaz et al. 2005).

Another important finding of the present study is that 18% of HDV-positive patients were found negative for HBV, which may be due to chronic carriers, superinfection, or an inhibitory effect of HDV, also described previously(Ibrahim Al Traif 2004, Chu and Liaw 1988). Our study also shows that HBV/HDV coinfection suppresses HBV. Overall levels of HBV DNA PCR< 1000 IU/ml were found in 38% HBV/HDV co-infected patients as compared to 31% HBV mono-infection which shows the suppression of the HBV virus and has been reported in many other studies as well(Riaz et al. 2011, Jardi et al. 2001, Mathurin et al. 2000, Mumtaz et al. 2011, Arribas et al. 2005). The presence of HDV is associated with suppressed HBV replication and possibly infectivity. Therefore, it is reasonable to speculate that HDV may facilitate the control of HBV. One possible explanation for this observation might be that both HDV p24 and HDV p27 proteins repress the HBV enhancers' Enh1 and Enh2 and inhibit HBV replication. In addition, HDV p27 transactivates the IFN-α inducible MX1 gene (also known as MXA), thereby inhibiting HBV replication (Williams et al. 2009, Mumtaz et al. 2011).

The absence or the low viral load of HBV in HDV-positive patients indicates a possible subdued of HBV by HDV in HBV/HDV co-infected patients. However, the age group of 20-40 years and the male gender is comparatively at high risk for these viral infections. Therefore, extra attention is required to prevent the spread of infection, particularly in this age group.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

The study was approved by the Institutional Review Board of Dow University of Health Sciences.

Consent Forms

Consent forms were signed by the participants and are available with the corresponding author.

Authors Contribution

SK conceptualized the study, collected samples and wrote the final manuscript, NJ, AL helped in analysis and writing the first draft, SK and SA did the experimental analysis and helped in initial manuscript writing.

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