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Seroprevalence of Hepatitis Delta virus (HDV) in Hyderabad, Sindh

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Abstract

Hepatitis is an infection with swelling and inflammation of the liver that may lead to cirrhosis. Viral hepatitis is an important public issue around the world, particularly in developing countries. The current study aimed to evaluate the seroprevalence rate of HDV in Hyderabad, Sindh. A total of 1054 HDV suspected patients were enrolled for the determination of HDV seroprevalence in Hyderabad. All samples were analyzed by competitive ELISA using DIA.PRO kit as per manufacturer's guidelines. Out of 1054, the prevalence rate of HDV was found to be 18.69% ($n=197$) whereas 81.31% ($n=857$) were negative. The gender wise distribution of positive samples demonstrated that 12.33% ($n=130$) were male patients and 6.36% ($n=67$) were female patients. In conclusion, the current study has demonstrated a high prevalence rate of HDV infections. Moreover, it was observed that the male patients were more susceptible to HDV infection as compared to female patients.

Keywords: Prevalence, Hepatitis; Hepatitis D virus; Delta Virus.

Introduction

HDV is a satellite virus of a spherical shape possessing a single-stranded circular RNA of negative polarity with 36 nm size (Pollicino *et al.*, 2011; Tseng *et al.*, 2010). Eight genotypes of HDV including HDV-1 (former genotype I), HDV-2 (former genotype IIa), HDV-3 (former genotype III), HDV-4 (former genotype IIb), and HDV-5 to HDV-8 have been reported across the worldwide based on phylogenetic analysis of the HDV (Mello *et al.*, 2023; Radjef *et al.*, 2004). Around 8 million people worldwide have been reported to be infected with HDV (Taylor, 2006). HDV replicates via double rolling circle mechanism. The delta hepatitis virus possesses an extraordinary ability to amplify its genome utilizing host RNA polymerases. Although the hepatitis delta virus is typically linked to a severe form of hepatitis, there is a very wide spectrum of clinical presentations, asymptomatic cases to fulminant hepatitis. Like its helper virus HBV, the HDV has been reported to spread by contact to contaminated blood or bodily fluids parenterally. Intrafamilial spread is naturally common in highly endemic regions (Tharwani & Hamid, 2023). HDV infection can occur in two ways: coinfection, which is the consequence of acute HDV and hepatitis B virus (HBV) infection, and superinfection, which is the result of HDV infection in individuals suffering from underlying chronic HBV (Shenoy & Fontana, 2024). Compared to coinfection with HBV and HDV, superinfection with HDV increases the probability of chronic HBV infection, which results in progressive illness and cirrhosis in about 80% of patients (Pan *et al.*, 2023). Because HBV/HDV co-infection frequently promotes the development of cirrhosis and hepatic fibrosis and raises the risk of hepatocellular carcinoma,

it is a clinically extremely detrimental condition (Wedemeyer, 2010). Given that HDV is satellite virus of HBV, all patients who test positive for HBsAg should also be screened for co-infection with HDV, at least once for confirmation of anti-HDV antibodies in the HBV infected patients. Since it appears that everyone infected with HDV generates anti-HDV antibodies, a negative result does not warrant testing for HDV RNA (Wedemeyer & Manns, 2010). On the other hand, positive anti-HDV antibody testing requires confirmation of ongoing HDV infection by detection of HDV RNA in serum. As the infection cures, anti-HDV antibodies could remain long after HDV RNA has disappeared (Kamal *et al.*, 2023). The current study aimed to assess the seroprevalence rate of HDV in the population of Hyderabad.

Materials and Methods

Sample collection, study area and duration of study:

The present prospective study was carried out for eight months from January 2015 to August 2015 at the Institute of Microbiology University of Sindh, Jamshoro, Pakistan. All individuals suspected of Hepatitis D were enrolled in this study. The blood samples were collected from patients attending various clinical diagnostic centre, laboratories and hospitals of Hyderabad, Sindh and processed at Institute of Microbiology, University of Sindh, Jamshoro.

Detection of Hepatitis D using Competitive ELISA:

HDV seroprevalence was assessed by Competitive Enzyme Immunosorbent assay (ELISA) in human plasma and serum using the HDV Ab Kit (DIA.PRO, Italy) as per manufacturers guidelines. The assay involves a two-step incubation competitive system. In 1st phase, the sample was added to the microplate to

allow the anti HDV antibodies to react with coated antigen. The microplate was washed twice followed by addition of an enzyme conjugated polyclonal antibody to HDV to bind to the free portion of the adsorbed antigen. The plate was washed twice, and a chromogenic substrate mixture was added to the wells of microplate. The microplate wells were added with 100µl of each calibrator (in duplicate), negative control (in triplicate), and positive control in single well and the test samples of HDV patients' serum in other wells. The microplate was then incubated for one hour at 37°C. The microplate was washed twice followed by addition of enzyme conjugate (100µl). The plate was again incubated for one hour at 37°C. The results were calculated based on a cut-off value using the formula:

Cut-Off = (NC + PC)/5, where NP stands for negative control, and PC stands for positive control. The data was interpreted as ratio between the cut-off value and the sample OD at 450nm and recorded for future analysis.

Results And Discussions

Trends in seroprevalence of HDV infection: For determination of HDV prevalence, a total of 1054 patients were enrolled in this study. The data was collected during an eight-month period from January 2015 to August 2015. The male patients comprised of (n=685) and (n=369) were female patients

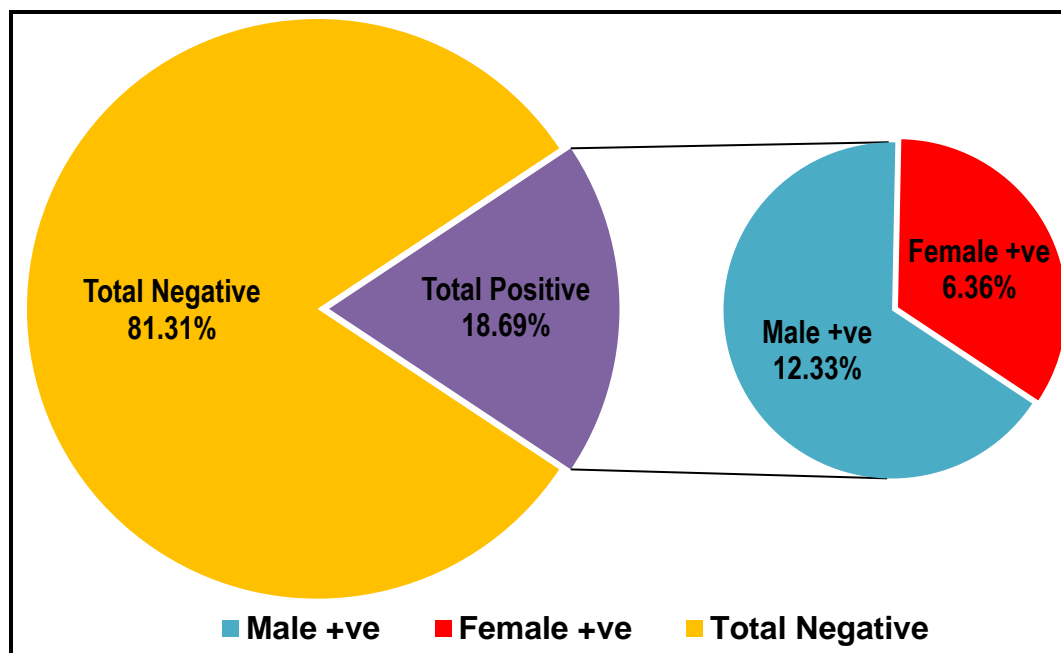


Figure 1. The pie chart demonstrating the overall seroprevalence of HDV in male and female patients enrolled in current study.

Previously published data has reported varying prevalences of HDV poses a major threat to global health and it has been reported to affect about 15 million individuals worldwide. Current study has demonstrated that overall prevalence of Delta virus was found to be 18.69% (n=197) which comprised 12.33% male population and 6.36% of female population. HDV was found more common in males as compared to females. A total of 81.31% of patients were negative for HDV (Fig. 01). The clinical studies published on HDV infection have indicated a variety of signs and symptoms including from rapidly progressive chronic liver disease to an asymptomatic carrier state and from benign acute hepatitis to fulminant hepatitis (Moatter et al., 2007). The finding of the current study revealed that the positivity rate of HDV (18.69%) is much lower as compared with the results of a previous study, who have showed 28% (Khan et al., 2011) HDV positivity in HBsAg positive patients, while in another study by Zaidi et al, had

demonstrated 88.80% HDV positivity (Zaidi et al., 2010) and Seetlani et al, showed an 58.60% prevalence of HDV infection (Seetlani et al., 2009). The decrease in HDV prevalence recorded in present study may possibly be due to introduction of HBV vaccination and awareness and improvement in treatment worldwide.

Gender wise prevalence of HDV: An interesting outcome of current study was indication that HDV infection rate was observed significantly higher in male patients (12.33%) as compared to the female (6.36%) patients. This finding was in agreement with the results of studies of Khan et al, (Khan et al., 2011) and Zaidi et al, (Zaidi et al., 2010), which showed that HDV infection is more common in males than in females. Tariq Moatter and his colleagues also found male dominance in their study (Moatter et al., 2007). High prevalence of HDV infection in males may possibly be due to risk behaviors, environmental exposure, and chronic HBV infection. Therefore, proper information about the risk factors of HDV

infection should be disseminated among health authorities, health care managers, practitioners, and the general public. Moreover, it is recommended to initiate

vaccination and awareness campaign programs to prevent HBV/HDV dual and HDV mono in the population of study area

Table 1. Seroprevalence trends of HDV (month-wise) among the patients enrolled in this study.

Month	Male (+ve)		Female (+ve)		Total HDV +ve		Total Sample
	(n=)	(%)	(n=)	(%)	(n=)	(%)	
January	13	13.68	7	7.37	20	21.05	95
February	13	8.55	12	7.89	25	16.45	152
March	10	7.69	11	8.46	21	16.15	130
April	15	10.71	9	6.43	24	17.14	140
May	18	13.74	6	4.58	24	18.32	131
June	17	13.60	9	7.20	26	20.80	125
July	27	14.06	8	4.17	35	18.23	192
August	17	19.10	5	5.62	22	24.72	89
Total	130	12.33	67	6.36	197	18.69	1054

Conclusion

The present study reports the seroprevalence of HDV in Hyderabad, Sindh. The data demonstrated that the rate of HDV was although higher in the region but comparatively lower than that of other studies conducted in recent past. Males were more affected with HDV as compared to females.

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