

RAPID COMMUNICATION

## Clinical presentation and genotype of hepatitis delta in Karachi

Tariq Moatter, Zaigham Abbas, Sabhita Shabir, Wasim Jafri

Tariq Moatter, Departments of Pathology, The Aga Khan University Hospital, Karachi 74800, Pakistan  
Zaigham Abbas, Sabhita Shabir, The Aga Khan University Hospital, Karachi, and Medicare Clinic, Karachi 74800, Pakistan  
Wasim Jafri, Departments of Medicine, The Aga Khan University Hospital, Karachi 74800, Pakistan

Correspondence to: Dr. Zaigham Abbas, Department of Medicine, The Aga Khan University Hospital, Stadium Road, Karachi 74800, Pakistan. zaigham@akunet.org  
Telephone: +92-21-4930051 Fax: +92-21-4934294  
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### Abstract

**AIM:** To assess the clinical presentation and genotypes of delta hepatitis in local population.

**METHODS:** In this prospective study, 39 consecutive patients who were positive for HBsAg and hepatitis D virus (HDV) antibody were included. The patients were divided in two groups on the basis of presence or absence of HDV RNA and a comparative study was done. Genotype of HDV was determined in PCR positive patients.

**RESULTS:** Overall there is male dominance, in which 34 patients out of 39 (87.2%) were male. Twenty (51%) patients were from the adjacent areas of three provinces; Sindh, Punjab and Balochistan indicating the higher prevalence of delta hepatitis in this mid region of Pakistan. Patients of all age groups were affected with delta hepatitis (median 31.5 years, range 12-75). HDV RNA was detectable in 23 patients (59%). All the HDV strains belonged to genotype I. HBV DNA was detectable only in 3 cases who were also HBeAg and HDV RNA positive. Patients with detectable HDV RNA were younger than patients with undetectable RNA; mean age  $29.7 \pm 12.8$  years vs  $36.8 \pm 15.2$ . There were no statistically significant differences in the clinical presentation and routine biochemical profile of patients with detectable or undetectable HDV RNA. Clinical cirrhosis was present in 19 (49%) patients; 12 with detectable RNA and 7 with undetectable HDV RNA ( $P = 0.748$ ). Decompensated disease was seen in eight patients; five and three respectively from each group. Four patients with undetectable RNA and two patients with detectable RNA had normal ALT and ultrasound abdomen.

**CONCLUSION:** HDV may infect at any age, usually

young adult males. Genotype I is prevalent. With time some of the patients become HDV RNA negative or asymptomatic carrier. Most of the patients have suppressed HBV DNA replication. Significant numbers of patients have cirrhosis.

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**Key words:** Hepatitis delta; Hepatitis B; Genotype; HBeAg; Pakistan

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### INTRODUCTION

Up to 5% of the world's population is infected with hepatitis B virus (HBV), and probably 5% of the HBV carriers have hepatitis D virus (HDV) superinfection<sup>[1]</sup>. It is estimated that 15 million people are infected with HDV worldwide<sup>[2]</sup>. Superinfection of HDV in persons with HBV infection leads to progressive disease and cirrhosis in approximately 80% of cases<sup>[3]</sup>. Cirrhosis develops at a younger age than in patients with chronic HBV infection alone<sup>[4]</sup>.

In Pakistan viral hepatitis remains a serious health problem. Recent data suggested that 16.6% of patients infected with hepatitis B virus also suffered from superimposed delta infection<sup>[5]</sup>. However, local data is based on serology of HDV and PCR studies were not done. It is not clear how many of local patients spontaneously recover from the infection and how these patients differ from patients with persistent infection. Moreover, the genotype of the virus in Pakistan is also unknown.

The nucleotide sequences of hepatitis D viruses (HDV) vary 5% to 14% among isolates of the same genotype and 23% to 34% among different genotypes<sup>[6,7]</sup>. Based on the analysis of HDV genomes from different areas of the world, three genotypes of HDV have been identified. Genotype I is the most prevalent worldwide and is associated with a broad spectrum of pathogenicity. It is predominant in the Europe, United States and the Middle East and has some geographically based subtypes<sup>[7,8]</sup>. In the Far East, the predominant genotype is genotype II.

Patients infected with this genotype have significantly lower ALT levels, more favorable outcomes and a trend to have lower serum HDV RNA levels as compared with those infected with genotype I<sup>[9]</sup>. However IIb variant may be associated with progressive disease<sup>[10]</sup>. Type III genotype is predominant in northern South America and has been associated with severe forms of hepatitis<sup>[11]</sup>.

We evaluated our patients in terms of genotype and prognosis and compared the HDV RNA positive patients with the negative group.

## MATERIALS AND METHODS

Thirty nine patients who were HBsAg and HDV antibody positive for more than six months were further tested for HDV RNA. These patients were divided into two groups; the first group included a total of 23 patients which were HDV RNA positive by PCR reflecting active ongoing delta infection while the second group consisted of a total of 16 patients with undetectable HDV RNA reflecting previous exposure to delta hepatitis or viral load less than the sensitivity of assay used for the HDV RNA. Markers for hepatitis B activity were done in all of these which included HBeAg, Anti-HBe antibody, and HBV DNA.

### Extraction and RT-PCR analysis of HDV RNA

HDV RNA was isolated from patients' serum samples by High Pure Viral RNA isolation kit according to the manufacturer's instructions (Roche Diagnostics, USA). RNA was eluted from spin columns provided with the kit in sterile nuclease free water and stored at -80°C until further analysis. Later, RNA samples were reverse transcribed into cDNA using 1st Strand cDNA Synthesis Kit for RT-PCR (Roche Diagnostics, USA). Briefly, cDNA mix consisted of reaction buffer containing 5 mmol/L MgCl<sub>2</sub>, RNA, random primers, 50 units RNase inhibitor and AMV reverse transcriptase. The reaction was carried out for 90 min at 42°C in a thermal cycler. The resulting cDNA was amplified with sequence specific primers for HDV according to the published method<sup>[12]</sup>. The amplified products were separated on a 1.5% agarose gel and a 400 bp product indicated the presence of HDV in the sample. To monitor the quality of the assay in each test run both negative and positive controls were included.

### HDV genotyping by RT-PCR and RFLP assay

Serum samples of patients which showed presence of HDV RNA were genotyped according to the published method of Ivaniushia *et al*<sup>[13]</sup> 2000. After PCR amplification with primers specific for HDV coding region, positive samples were digested overnight at 37°C with restriction enzyme *Sma*I. Genotype I, II and III were confirmed and classified according to the sizes of the fragments following *Sma*I digestion.

### Extraction and amplification of HBV DNA

HBV DNA was extracted using High Pure Viral Nucleic Acid isolation assay as indicated by the manufacturer (Roche Diagnostics). Sequences of primers, specific for core region of HBV genome, used for amplification were

previously reported<sup>[14]</sup>. Each PCR reaction consisted of reaction buffer, 0.2 mmol/L deoxyribonucleotides, 1.5 mmol/L MgCl<sub>2</sub>, 100 ng of each primer, and 2 U *Taq* polymerase in a final volume of 50 µL. All standard precautions were taken during the study to prevent cross contamination between PCR samples.

### Serological tests

HBe 2.0 assay (Abbott, USA), which is based on microparticle enzyme immunoassay technology, was employed for the detection of HBeAg levels in sera. For running the assay, the AxSYM system was used according to the manufacturer's instruction (Abbott, USA). Anti-HDV antibody test was performed by Murex competitive enzyme immunoassay as suggested by the manufacturer (Abbott, Chicago IL, USA).

### Clinical aspects

The clinical features of patients with detectable HDV RNA were compared with HDV RNA negative patients to determine the clinical impact of continuous presence of HDV RNA and outcome for HDV infection. Informed consent was taken and a standardized pro forma was completed regarding laboratory and serological data, complications of liver disease to include ascites, hematemesis and melena, portosystemic encephalopathy (PSE), hepatocellular carcinoma (HCC), and Childs-Pugh score. The study was approved by the Ethics Review Committee of the Medicare Clinic.

The patients were labeled suffering from clinical cirrhosis on the basis of clinical and ultrasound findings suggestive of cirrhosis (nodular surface, firm consistency, blunt liver edge, altered echotexture, dilated portal vein and splenomegaly) and evidence of hypersplenism (platelets < 15000/mm<sup>3</sup>).

### Statistical analysis

Results were analyzed using Special package for Social Sciences software (SPSS version 13.0, Chicago, IL, USA). Data was summarized as the means with standard deviations (for continuous variables), and as frequency and percentages (for categorical variables). Univariate analysis was done using Mann Whitney *U*-test, and Fischer exact test wherever appropriate. *P* < 0.05 was considered significant. All *P*-values were two sided.

## RESULTS

Overall there is male dominance, in which 34 out of 39 (87.2%) patients were male. These patients belonged to the different areas of country. Of those, 12 came from upper parts of Sindh Province, 15 were from the lower Sindh including Karachi, 8 from Balochistan Province and 4 from the lower parts of Punjab Province. Out of these, 20 (51%) patients were from the adjoining districts of upper Sindh, Punjab and Balochistan Provinces. Patients of all the age groups were affected with delta hepatitis, the disease was seen as early as the second decade of life to as late as the mid 70's (range 12-75, median 31.5, mode 22), but the majority of patients in both the groups were adults.

Table 1 Characteristics of hepatitis delta patients

	RNA detected (n = 23)	RNA not detected (n = 16)
Male:Female	20:3	14:4
Age (yr)	29.7 ± 12.8	36.8 ± 15.2
Clinical cirrhosis	12 (52%)	7 (44%)
Decompensated disease	5 (22%)	3 (19%)
Normal ultrasound and ALT	2 (9%)	4 (25%)
HBe antigen	3 (13%)	1 (6%)
HBV DNA	3 (13%)	0
Bilirubin (mg/dL)	1.8 ± 2.0	2.9 ± 5.6
ALT (U/L)	88 ± 45	125 ± 199
AST (U/L)	81 ± 52	150 ± 280
GGT U/L	99 ± 121	49 ± 31
ALP (U/L)	124 ± 56	100 ± 31
Albumin (g/dL)	3.4 ± 0.7	3.4 ± 0.9
Hemoglobin (g/dL)	12.3 ± 2.0	12.9 ± 1.8
Total leucocyte count (× 10 <sup>9</sup> /L)	6.1 ± 2.0	5.0 ± 1.8
Platelets (× 10 <sup>9</sup> /L)	143 ± 84	157 ± 85
INR	1.3 ± 0.2	1.2 ± 0.1

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transpeptidase; ALP: alkaline phosphatase; INR: international normalization ratio. Values are means ± SD, or n (%). There are no statistically significant differences among the two groups.

The clinical presentation of anti-HDV positive patients was jaundice in 11 (28%), ascites 3 (7.7%), encephalopathy 3 (7.7%), and hematemesis 2 (5.1%). Nine patients gave the history of hospitalization due to liver related indication. On examination the liver was palpable in 4 (10.3%) and splenomegaly in 17 (43.6%). Laboratory investigations included hemoglobin 12.5 ± 1.9 g/dL, total leucocyte count 5.7 ± 2.0 × 10<sup>9</sup>/L, platelets 151 ± 66 × 10<sup>9</sup>/L, INR 1.25 ± 0.16, bilirubin 2.2 ± 3.7 mg/dL, alanine aminotransferase (ALT) 103 ± 131 IU/L, aspartate aminotransferase (AST) 109 ± 179 IU/L, gamma glutamyl transpeptidase (GGT) 81 ± 101 IU/L, alkaline phosphatase 116 ± 49 IU/L.

HDV RNA was detectable in 23 patients (59%). All of these patients belonged to genotype I of the virus. Viral markers of hepatitis B replication, serum HBeAg and HBV DNA, were absent in a majority of the cases in both the groups. HBV DNA was detectable only in 3 cases. These patients had HBeAg positive disease and were also positive for HDV RNA. None of the HDV RNA negative patients were positive for HBV DNA though one such patient was positive for HBeAg.

Patients with detectable HDV RNA were younger than patients with undetectable RNA; age 29.7 ± 12.8 years vs 36.8 ± 15.2, though the difference was not statistically significant (95% CI-2.68-16.8). There was no statistically significant difference in the clinical presentation and routine biochemical profile of patients with detectable or undetectable HDV RNA (Table 1). Clinical cirrhosis was present in 19 (49%) patients; 12 (52%) with detectable RNA and 7 (43%) with undetectable HDV RNA (*P* = 0.748). Out of these, decompensated disease (history of hematemesis, ascites, and encephalopathy) was seen in 8 patients; 5 patients with detectable RNA and 3 without detectable HDV RNA. Four patients with undetectable RNA and 2 patients with detectable RNA had normal

ALT and ultrasound abdomen. These 6 patients were HBV DNA and HBeAg negative.

## DISCUSSION

Clinical studies of HDV disease worldwide indicate that there is a wide variation in the clinical presentation. The manifestations of HDV infection vary from benign acute hepatitis to fulminant hepatitis and from rapidly progressive chronic liver disease to an asymptomatic carrier state<sup>[15]</sup>. The clinical course was influenced by several factors, including the HDV genotype<sup>[9]</sup>. In our study all of the patients were infected with genotype I. This is new data from this region. Our previous study indicates, that in Pakistan, there is a predominant genotype of Hepatitis B is genotype D<sup>[16]</sup>. The association of genotype I of HDV with genotype D of HBV has been reported from other countries where HBV genotype D is predominant as is in Italy<sup>[17]</sup>, Turkey<sup>[18]</sup> and Egypt<sup>[19]</sup>. This genotype may cause a wide spectrum of pathogenicity. In the South East Asia, the predominant HDV genotype is II and HBV genotype B and C. Genotype II is associated with a less rapidly progressive course of chronic liver disease<sup>[9]</sup>.

Local data regarding the hepatitis D strain is based upon presence of anti-HDV antibody due to non-availability of PCR facility. The percentage of patients who spontaneously recover from hepatitis D is not known. A recent study involving the clinical presentation of hepatitis delta from Pakistan based on anti-HDV antibody, showed that HDV infection was present in 16.6% of hepatitis B infected patients in Pakistan, most commonly in younger males living in rural areas. Delta antibody positive patients had less severe clinical liver disease compared to delta negative, hepatitis B patients<sup>[5]</sup>. HDV RNA status of the patients was not known in that series. In our study again majority of HDV antibody positive patients, 34 patients out of 39 (87.2%), were male. Most of these patients were young adults and came from upper part of Sindh, lower Punjab and adjacent areas of Balochistan Province. These adjoining areas of three provinces form the hepatitis belt in Pakistan, the area with higher prevalence of hepatitis. In our study, clinical cirrhosis was present in about half of the patients so the present data adds further information on severity of liver disease associated with HDV genotype 1 in Pakistan.

It may not be difficult to predict whether HDV was acquired as a coinfection with HBV or as a superinfection in patients with existing chronic HBV infection. Natural history of HDV infection shows that coinfection evolves to chronicity only in a small number of patients and patients recover from both hepatitis B and D, while superinfection of HDV leads to progressive disease and cirrhosis in approximately 80% of cases<sup>[3]</sup>. Our patients were in the chronic phase. It can be assumed that most of our HDV RNA positive patients acquired the infection as a superinfection on hepatitis B.

In most cases of HDV infection, HBV replication is suppressed to very low levels by HDV<sup>[20,21]</sup>. In our series, all the cases were negative for HBV DNA except three who were positive for HBV DNA, HBe antigen and HDV RNA. It seems that HBV replication is not suppressed

by the presence of HDV RNA in a subset of subjects. A more ominous course toward liver decompensation has been documented in patients with active HBV and HDV replication<sup>[15]</sup> with each virus contributing to the liver damage, thereby resulting in more severe liver disease<sup>[22]</sup>. All the three of our patients who were both HBV DNA and HDV RNA positive, had clinical cirrhosis.

Our patients with detectable HDV RNA were younger than patients with undetectable RNA. Absence of HDV RNA in our 16 (41%) patients signifies that a proportion of patients lose the virus with increasing age or viral titres become too low to be detectable by the assay we use. Four patients with undetectable RNA and two patients with detectable RNA had normal ALT and ultrasound abdomen. These patients were HBV DNA negative. The former four represent spontaneous recovery while later two were probably the asymptomatic carriers. This state has been reported previously<sup>[15,23]</sup>.

In summary, HDV genotype I is prevalent in our patients. HDV may infect at any age, usually young adult males. With time, some of the patients become a HDV RNA negative or an asymptomatic carrier. Most of the patients have suppressed HBV DNA replication. A significant number of patients have cirrhosis.

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