

# HEPATITIS

## FREQUENCY OF HEPATITIS D CO & SUPER INFECTION IN HEPATITIS B PATIENTS

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**ABSTRACT... Objective:** To determine the frequency of co/super infection of hepatitis D in patients with hepatitis B related liver disorders. **Study Design:** Descriptive study. **Place and Duration of Study:** The hepatitis Centre Ghulam Mohammad Mahar Medical College Hospitals Sukkur & Khairpur, from January 2009 to December 2009. **Methodology:** All patient registered for HBV associated infections were selected. Blood was drawn from 200 patients who fulfilled the inclusion criteria. Those with an incomplete test profile were excluded. All clinical conditions were investigated through liver function tests, coagulation profile, abdominal ultrasonography, upper gastrointestinal endoscopy and liver biopsy. Liver cirrhosis and hepatocellular carcinoma (HCC) were diagnosed either on the basis of histology, or on a combination of radiological, endoscopic and laboratory data. Hepatitis B virus DNA was extracted from serum by real time PCR. Descriptive statistics were used for frequency and mean determination. **Results:** The 129 patients finally selected for statistical analysis included 108 (84%) males and 21 (16%) females. The ages ranged from 6-68 years (mean=31.5 ± 12.39 years). There were 70 (54.2%) patients of non-cirrhotic, chronic hepatitis (CLD), 38 (29.4%) carriers, 12 (9.3%) cirrhotic and 9 (6.9%) hepatocellular carcinoma (HCC) patients. Among the 129 patients, 45 (34.9%) were positive for double infection with HDV. These included 35 CLD cases, 7 cirrhotic and 3 carriers. **Conclusions:** The frequency of co/super infection of hepatitis D was found to be highest in HBV cirrhosis patients compared to patients having chronic liver disease (non-cirrhotics) and carriers.

**Key words:** Hepatitis D virus co/super infection, Hepatitis B virus, Cirrhosis, Chronic liver disease, Carrier, Hepatocellular carcinoma

### INTRODUCTION

In our region, hepatitis B infection (HBV) is hyper endemic, but the frequency of co/super infection with HDV is generally not known. The objective of the present study was to determine the frequency of the co/super infection of hepatitis D in patients with hepatitis B related liver disorders.

Presence of dual viral infections has been reported from various parts of the world. Since hepatitis B and D share the same mode of transmission, hepatitis B virus (HBV) combined infection with HDV is a fairly frequent occurrence among intravenous drug users and subjects with a high risk of parenteral infection, particularly in areas where all these viruses are endemic. It is generally considered a condition favoring the progression of liver cirrhosis and represents one of the most important risk factors for the development of hepatocellular carcinoma<sup>1,2</sup>. Hepatitis delta virus (HDV) is a RNA viral agent that obliges hepatitis B virus for packaging and transmission<sup>3</sup>. HBV provides the envelope (HBsAg)

proteins for the assembly, release, and spread of virions containing the HDV RNA genome. By itself, it is unable to replicate, initiate or infect others cells completely<sup>4</sup>.

### METHODOLOGY

Patients registered at the hepatitis centers of Ghulam Mohammad Mahar Medical College Hospitals Sukkur & Khairpur were included. The previously diagnosed HBV positive, 200 registered patients, irrespective of age or gender, reporting for a follow up at the outpatient department, were investigated for their current status and co/super infection with HDV during January 2009 and December 2009. Out of 200 patients, who were selected for study, 71 were excluded due to their incomplete test profile and follow up. Serum samples were collected from the study patients when they came for the follow up. Before taking the blood sample, a written consent and in case of under 18 years of age, a parental consent was obtained. Ten ml of blood was drawn from all patients and serum was separated. All sera were stored in aliquots of 200µl each at -70°C.

Clinical conditions for all patients were investigated through laboratory test results including liver function tests, coagulation profile, findings at abdominal ultrasonography, upper gastrointestinal endoscopy and liver biopsy. Liver cirrhosis and hepatocellular carcinoma (HCC) were diagnosed either on the basis of histology or on a combination of radiological, endoscopic and laboratory data of patients with HCC. The hepatitis B serology tests used were the enzyme immunoassay HBe/HBs antigen tests and hepatitis delta virus antibody tests. DNA extraction and amplification by PCR was done. All standard precautions were taken during the study to prevent cross contamination between PCR samples. Some of the samples were checked twice or thrice for confirmation. For statistical evaluation frequency and percentages were calculated from categorical variables like chronic liver disease (CLD), cirrhosis, HCC and carriers. Mean values with standard deviation were calculated for continuous variables. SPSS version 13 was applied for statistical analysis.

## RESULT

Among the 129 patients with complete test profile finally selected for statistical evaluation, 108 (84%) were males and 21 (16%) were females with male to female ratio of 5:1. The ages of the subjects ranged from 6 – 68 years (mean =31.5 ± 12.39 years). There were 70 (54.2%) chronic liver disease (non cirrhotic) patients, 38 (29.4%) were carriers, 12 (9.3%) were cirrhotics and 9(6.9%) were HCC patients. Among the 129 patients, 58.9% (n=76) were not co/super infected, though nine had developed HCC. Patients positive for double active infection were 45 (34.9%) for HBV/HDV. (Table-I).

Co/super infection with HDV was present in 45 (34.9%) patients. Viral markers of hepatitis B replication, HBV DNA, were present in all of the cases. Among the 70

patients with non-cirrhosis, 50% were co/super infected with HDV. Cirrhosis had developed in 12 (9.3%) patients, out of which 25% had been infected by HBV alone whereas, 58.3% had co/super infection with HDV. Among the 38 asymptomatic carriers, only 8% were co/super infected with HDV whereas the rest (92%) were not co/super infected. In HCC patients, 89% were not co/super infected and had HBV alone. Anti-HDV was positive in 50% (35/70) CLD patients (33males and 5females), 58% (7/12) cirrhotic patients and 8% (3/38) HBV carriers but none of the patients who had developed HCC were positive for anti-HDV.

## DISCUSSION

Despite widespread prevalence of hepatitis B in our population, the data regarding dual infection with HDV is scarce<sup>5</sup>. In this study, 34.9% of HBV patients who came for follow up had evidence of co/super infection with HDV. The assessment of the level of association of dual infection with different categories of HBV infections revealed that patients suffering from chronic liver disease and cirrhosis (50% and 58.2% respectively) were more likely to have dual infection with HDV compared to carriers and HCC patients. Generally, chronic HBV infection is considered as a situation suggested to be tested for HDV infection, especially if there is acute worsening of the liver condition. Drug abusers who share contaminated needles to inject themselves with illicit drugs are likely to become infected by HDV as well. Chronic HBV carriers who acquire HDV super infection usually develop chronic HDV infection. Cirrhosis and hepatocellular carcinoma (HCC) are two major long term complications of chronic HBV infection which developed in 21 patients out of 129. The effect of hepatitis delta virus (HDV) infection on the clinical course of cirrhosis is poorly defined, yet it has been reported that HDV infection increases the risk for mortality two-fold in patients with

**Table-I. Status of HBV, HDV Co/Super infection among chronic, cirrhotic, carrier and HCC patients.**

Infection	CLD (non cirrhosis) 70 (54.2%)	Carrier 38 (29.4%)	Cirrhosis 12 (9.3%)	HCC 9 (6.9%)	Total 129 (100%)
HBV (alone)	30 (42.9%)	35 (92%)	3 (25.0%)	8 (88.9%)	76 (58.9%)
HBV/HDV	35 (50.0%)	3 (8%)	7 (58.3%)	-	45 (34.9%)

cirrhosis type B<sup>6</sup>. The development of cirrhosis occurs more frequently in patients with episodes of decompensation and with repeated severe acute exacerbations. However, progression to cirrhosis can be relatively silent and can occur even in children.

Although cirrhosis develops during the process of HBeAg seroconversion, 68% of the complications of cirrhosis and of hepatocellular carcinoma occur after HBeAg seroconversion. These complications may still occur even after HBeAg seroclearance. Basically, the virus produces many of the same diseases as the hepatitis B virus; however, these problems such as cirrhosis are much more deadly and frequent. Out of the 12 patients (9.3%) found cirrhotic in this study, 7 were HDV positive, whereas, only 3 patients developed cirrhosis due to HBV alone. Similarly, regarding HCC, it has been reported that HDV co/super infection developed HCC at an earlier age than HBsAg carriers without HDV infection. Hepatitis D virus appears to represent a promotion factor for HCC in subjects with an oncogenic risk induced by HBV, increasing the risk for HCC three fold. In comparison to these reports, the 9 HCC patients in this study, ages 35-55 years, did not have dual infection.

It seems HBV alone was responsible for this complication in 8 patients. An association between HDV infection, with a higher rate of HCC, was also found in a Japanese study<sup>7</sup>; however, they could not conclusively confirm this association. Nevertheless, there seems to be a fair amount of evidence that HDV infection can lead to HCC. Asymptomatic carriers in this study were 38 (29.4%), out of which only 3 (8%) were co infected with HDV and 35 (92%) were not co infected. Super infection with HDV in HBV carriers in Western countries has been reported to aggravate the clinical course of chronic hepatitis B but the infection does not seem to affect the liver disease in Asian HBsAg carriers<sup>8,9</sup>. The less aggressive course of HDV infection in Asia than in Western countries may be explained by the distribution of different genotypes of HDV in specific geographic areas which were not checked in this study due to study limitations. In studies from other developing countries such as Taiwan and Vietnam where HBV infection is hyper endemic<sup>10,11</sup>, HDV infection has also been reported

to be generally infrequent in asymptomatic HBsAg carriers, whereas, it is extremely highly in intravenous drug users. They also reported it to be infrequent in CLD which is in contrast to this study. Different genotypes of the hepatitis viruses may influence the clinical outcome of the disease.

## CONCLUSIONS

The frequency of co-super infection of hepatitis D was found to be highest in HBV cirrhosis patients compared to patients having chronic liver disease (non-cirrhosis) and carriers. These findings prompt further investigation into the relationship between HDV genetic structures and their function and pathogenesis.

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