# COMPARATIVE ANALYSIS OF DISEASE ACTIVITY IN PATIENTS OF CHRONIC HEPATITIS B VIRUS, WITH AND WITHOUT SUPER-INFECTION WITH HEPATITIS D VIRUS; AN EXPERIENCE AT TERTIARY CARE CENTRE

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**Background:** The hepatitis D virus super-infection contributes significantly to the morbidity and mortality of hepatitis B virus infection. The objectives were to describe the incidence of Hepatitis D virus and comparative analysis of disease activity in patients of chronic hepatitis B virus, with and without super-infection of hepatitis D virus. **Methods:** This Cross-sectional comparative study was conducted at Department of Medicine and Gastroenterology Clinic Jinnah Postgraduate Medical Centre, Karachi, Pakistan from February 2007 to July 2007. HBsAg positive patients who attended our Gastroenterology clinic were selected for the study. After screening for Anti-HDV these patients were segregated in to Anti-HDV positive and negative groups**.** Data was analyzed on SPSS 12. **Results:** Eighty-four patients were selected. Seventy-three patients who fulfilled the inclusion criteria were enrolled in to the study. Anti-HDV was positive in 23 (31.5%) patients. Among these 23 anti-HDV positive, HDV-RNA was detected in 15 (75%) patients. The differences of age, gender, marital status and area of residence whether rural or urban were not significant between the two groups. HBV-DNA was significantly suppressed in majority of anti-HDV positive patients ($p$=0.019). Mean serum ALT levels were significantly higher in patients who had HDV infection ($p$=0.014). **Conclusion:** HDV infection was common in this series of patients with a frequency of 31.5%. All patients of chronic HBV should be screened for HDV whether they are asymptomatic HBV carriers or have chronic active hepatitis particularly when they have raised serum ALT.

**Key words:** Hepatitis B virus, Hepatitis D virus, chronic hepatitis, Pakistan.

## INTRODUCTION

Infection by hepatitis B virus (HBV) is worldwide public health problem. It is a significant cause of morbidity and mortality, especially in developing countries. It is estimated that 350 million people worldwide are chronic HBV carriers, representing approximately 7% of the total population.[1] Moreover it causes 1 million deaths annually.[2]

Hepatitis D or delta virus (HDV) is small, defective RNA virus that requires the presence of HBV infection for its replication and expression.[3] With worldwide distribution it is estimated that more than 15 million people are infected with HDV, which approximate 5% of all hepatitis B carriers.[4]

Although a decreasing or declining trend has been reported in the occurrence of hepatitis D infection from some geographical parts of the world like southern Europe and Mediterranean area by 1990, possibly due to the improvement in health delivery system including the vaccination strategies against the hepatitis B infection, disease prevalence is still high in most of the south Asian countries.[4,5] This is because of the socioeconomic and health related conditions that favor the occurrence of HBV and other related infections. A recently published large sample size study demonstrated that in Pakistan sero-prevalence of HDV infection is 16.6% and disease is more common in central part of the country.[5]

As HBV and HDV have the same transmission routes, dual infection may occur. HDV may therefore be acquired as a co-infection simultaneously with acute hepatitis B or as a superinfection in patients who are already infected with chronic HBV.[6] It is known that co-existent infection with HDV tends to accelerate the progress of chronic HBV infection to chronic hepatitis, cirrhosis and hepato-cellular carcinoma (HCC).[7]

HDV related chronic hepatitis (HBV) is difficult to treat. The response to Alpha-interferon (IFN-α), which still represents the only therapy for chronic hepatitis D, is limited, varies widely and occurs at lengthy duration of treatment even with higher doses of INF.[8]

Recently, pegylated IFN is being tried and could represent a reasonable therapeutic option in the long-term treatment required for chronic HDV.[8]

Though the presence of HDV infection in Pakistani patients with different types of liver diseases has been described in the past,[5,9] no study has been done to demonstrate the impact of HDV on the disease activity of chronic HBV infection only. Moreover before starting the treatment of chronic HBV infection, screening for anti-HDV is usually not done in this part of the world.

This study was conducted with the objectives to describe the incidence of HDV infection in patients with chronic HBV and to compare the disease activity in such patients on the basis of Anti-HDV positive and negative status.

## MATERIALS AND METHODS

This cross sectional comparative hospital based observational study was conducted in the Medical Unit III, Jinnah Postgraduate Medical centre (JPMC), Karachi, Pakistan over a period of 6 months between February 2007 and July 2007.

The hepatitis B surface antigen positive ($HBsAg^+$) patients who attended our clinic for their treatment, during the specified period, were selected for the study.

To assess the incidence of HDV infection, all $HBsAg^+$ patients were tested for the presence of delta agent antibody (Anti-HDV). These cases represent all the patients with chronic HBV infection seen in our clinic during the study period. The incidence of HDV was then described as the %age of anti-HDV positive patients among all the cases of chronic HBV during the study period.

All patients underwent a set of investigations such as, Full blood count (FBC), Liver function test, HBeAg, anti-HBc IgM, HBV DNA by polymerase chain reaction(PCR), anti-HDV; HDV RNA by PCR, anti-HCV, and ultrasound abdomen. The serological tests were performed using the commercially available ELISA kits.

HDV RNA is detected by in house real-time RT-PCR method while HBV qualitative and quantification are performed by real time PCR and Branch–DNA respectively.

Details of history, clinical assessment and serological as well as bio-chemical laboratory parameters were noted in all patients on a standardized questionnaire. Patients with acute HBV, triple infections (HBV, HDV, and HCV) and those with liver cirrhosis were excluded from the study.

Presence of $HBsAg^+$ was taken as exposure to HBV infection. Patients were considered to be suffering from acute HBV when positive for HBc-IgM while patients were documented as chronic HBV infection when positive for HBc-IgG and HBsAg. Positive HDV infection was diagnosed on the basis of Anti-HDV in the serum.

To asses the impact of HDV on clinical disease of $HBsAg^+$ patients, we divided the patients of HBV in to two groups, according to anti-HDV status, one with HDV positive and the other with HDV negative group.

A database on the basis of pre-filled proformas was developed on SPSS Version 12. The quantitative variables like age, serum albumin, serum ALT, and haemoglobin, were described by their means with standard deviations. The comparison of difference in means between the two groups was calculated by the Student's *t*-test**.** The categorical variables were represented as frequency and percentages and the differences in proportions were compared by chi-square test of proportions. Results were expressed as percentages, mean and standard deviation. Two- tailed tests were used thorough out and a *p*-Value of $<0.05$ was taken as significant for all Statistical analysis

## RESULTS

A total of eighty-four (84) patients with $HBsAg^+$ were analyzed over a period of 6 months. Four patients with triple infection (HBV, HCV, and HDV) and seven patients with acute hepatitis B (anti-$HBcIgM^+$) were excluded from the study. The remaining seventy three (73) patients who fulfilled the inclusion criteria were enrolled in to the study. There were 48 (65.8%) males and 25 (34.2%) females who ranged in age from 10–60 years. Most of the patients were young running in the second and third decades of their life.

Majority of the patients were married and belonged to the rural community. It was observed that the male patients were significantly younger as compared to female patients. In males, Mean±SD age was 23.88±10.96 years as compared with the Mean±SD age of 36.84±15.69 years in females ($p$=0.0001).

The Mean±SD value of serum alanine-aminotransferase (ALT) was 58.86±45.95 IU/L and that of albumin was 3.8±0.4 mg/dl.

$HBeAg^+$, which represents a marker of active replication of hepatitis B virus, was positive in 31 (42.5%) patients while in 42 (57.5%) patients it was negative. This showed that majority patients were $HBeAg^+$ negative. HBV-DNA was detected in 37 (50.7%) patients. This showed that HBV-DNA was detected in greater proportion of patients as compared to $HBeAg^+$.

**Table-1: Clinical characteristic of 73 study patients**

| Variable | Number | Percentage (%) |
|---|---|---|
| Male | 48 | 65.8 |
| Female | 25 | 34.2 |
| Married | 48 | 65.8 |
| Rural | 52 | 71.2 |
| Hepatomegaly | 14 | 19.18 |
| Hepatocellular Carcinoma | 2 | 2.74 |
| HBeAg (reactive) | 31 | 42.5 |
| Anti-HBe | 34 | 46.75 |
| Anti-HBcIgM | 7 | 9.58 |
| HBV-DNA (Detected) | 37 | 50.7 |
| Anti-HDV | 23 | 31.5 |
| HDV-RNA(Detected) | 15 | 20.5 |

Anti-Hbe was positive in 34 (46.75%) patients. Among anti-HDV positive patients, anti-HBe was detected in 15 (65.22%) patients as compared to 19 (38%) patients in anti-HDV negative patients ($p$=0.030 Table-2).

Hepatomegaly was present in 14 (19.18%) patients. All the remaining patients had normal liver size as was assed clinically as well as by ultrasound.

Hepatocellular carcinoma (HCC) was detected in 2 (2.74%) patients.

The frequency of Anti-HDV in patients of HBV was 23 (31.5%). Among the 23 patients who were anti-HDV positive, 15 (75%) had detectable HDV-RNA by polymerase chain reaction PCR). This showed that majority of anti-HDV positive patients had active liver disease with evidence of HDV replication.

Patients were divided in two groups on the basis of Anti-HDV status. Table-2 compared the parameters between patients who were Anti-HDV positive and those who were negative.

There were no significant differences regarding the sexes, or gender and marital status between the patients of two groups (Table-2). Similarly patients from rural areas are dominant in both groups and the difference was not significant ($p$=0.145).

HBeAg was positive in 31 (42.5%) patients. Among the 23 patients who were Anti-HDV positive, HBeAg was detected in 8 (34.78%) patients as compared to the HDV negative patients in whom it was detected in 23 (46%) patients. These differences in the frequency between the two groups were not significant statistically ($p$=0.37). HBV-DNA was detected in 37 (50.68%) patients. On further analysis it was observed that in those patients who were positive for Anti-HDV, Hepatitis B virus DNA was detected in only 7 (30.43%) patients as compared to 30 (60%) in Anti-HDV negative group. This showed that frequency of detection of HBV-DNA was significantly lower in Anti-HDV positive group (Pearson Chi-Square value; 5.509, $p$=0.019).

Serum ALT value was significantly higher in patients with Anti-HDV positive patients. In this group the Mean±SD value of ALT was 78.08±68.85 IU/L (Range 26–282 IU/L) as compared with Mean±SD value of 50.02±26.85IU/L (Range13–147 IU/L) in those who were Anti-HDV negative ($p$=0.014 Table-2). The differences in serum Albumin, Haemoglobin and ages of the patients between the two groups were not statistically significant.

**Table-2: Comparison between Anti-HDV positive and Anti-HDV negative groups**

| Variable | Anti-HDV Positive (n=23) No. (%) | Anti-HDV Negative (n=50) No. (%) | *p*-Value |
|---|---|---|---|
| Gender: Male | 17 (73.91) | 31 (62) | |
| Female | 6 (26.08) | 19 (38) | 0.319 |
| Married | 18 (78.26) | 30 (60) | 0.127 |
| Rural | 19 (82.60) | 33 (66) | 0.145 |
| Hepatomegaly | 6 (26.08) | 8 (10.95) | 0.309 |
| HBeAg (Reactive) | 8 (34.78) | 23 (46) | 0.368 |
| Anti-HBe | 15 (65.22) | 19 (38) | 0.030 |
| HBV-DNA(Detected) | 7 (30.43) | 30 (60) | 0.019 |
| Age (in years) | 30.52±13.24 | 27±14.49 | 0.368 |
| Serum ALT(IU/L) | 78.08±68.85 | 50.02±26.85 | 0.014 |
| Serum Albumin (mg/dl) | 3.10±0.65 | 2.81±0.61 | 0.195 |
| Haemoglobin | 12.02±1.37 | 12.50±0.95 | 0.088 |

## DISCUSSION

The global epidemiology of hepatitis D virus infection is changing. On one hand, the incidence of hepatitis D virus infection in the traditionally prevalent areas of Southern Europe such as Italy has declined from 23% to 8.3% over a period of 10 years from 1987 to 1997,[10–12] while on the other hand developing countries such as, Pakistan with a high prevalence of HBV infection and lack of preventive strategies including immunization against HBV, have become the prevalent areas for HDV infection.[5]

The results of our study showed that the seropositive rate of Anti- HDV was 31.5% which is slightly high as compared to some previous Pakistani studies.[9,13] Mumtaz *et al* reported that overall sero-prevalence of Anti-HDV in Pakistan is 16.6%. Moreover the authors also have stated that a large belt exist in the middle of the country where HDV seroprevalence ranges from 20% to 60%.[5]

Although not statistically significant a high incidence of HDV infection was found in male patients from rural areas. This finding was comparable with the past studies conducted locally[5,9] as well as in other parts of the world.[14]

The results of our study showed that HDV infection modifies to a certain extent the serological markers of chronic HBV. The characteristic features of patients with HDV superinfection were high positive rate, i.e., 69.45% of anti-HBe in serum and the low rate of HBV replication, as indicated by the detection of HBV-DNA in small proportion of about 30.43% of such patients. These results are consistent with the fact that HDV suppresses the synthesis/replication of HBV genome and serum HBV-DNA is often suppressed to very low or undetectable levels by the acute and chronic stage of HDV superinfection.[15,16] Lee *et al* reported that HBV-DNA was detected in 62% of their patients with out HDV superinfection while it was detected in only 10% of patients who had HDV superinfection.[17] Another study from Argentina reported that among the patients who were anti-HDV positive, 69% had anti-HBe.[18]

Numerous clinical studies have reported that patients with chronic HDV infection often show severe chronic hepatitis.[19,20] In this series of patients we observed that serum ALT was significantly higher in those patients of HBV who had HDV super-infection. The three possible explanations for this raised ALT are:

1. The Delta virus infection has increased or accelerated the severity of chronic HBV infection.[21]
2. The liver damage in such patients, who had raised ALT, was essentially due to HDV-replication.[5]
3. HBV and HDV were replicating simultaneously and each was contributing to liver damage, thereby resulting in more severe liver disease.[21]

But, in majority of HDV superinfection patients, anti-HBe was reactive, HBV-DNA was not detected, and in 75% of such patients HDV-RNA was detected reflecting on-going viral replication, we assumed that raised ALT in these patients was due to liver damage by HDV replication. This is in agreement with most of the previous studies.[20,21]

The relationship between persistent HBV infection and HCC has been well documented. The role of HDV infection in the development of HCC in chronic $HBsAg^+$ patients is not well defined.[22] In some studies a high frequency of dual HBV/HDV infection has been described in patients of HCC and it has been suggested that florid replication of both HBV and HDV and the resulting severe necro-inflammation may be an additional factor for promotion of HCC.[21,23] In our study only two patients had HCC. As this number was not large enough to reach the statistical significance, we could not compare this finding between the two groups and were unable to highlight the impact of HDV infection in the development of HCC.

## CONCLUSION & RECOMMENDATIONS

The high positivity rate of anti-HDV observed in the present study points out that HDV infection may be increasing in this part of the world with a threat of more severe HBV related liver diseases in the future. We suggest that prevention of HBV infection with hepatitis B vaccine is the best available tool to reduce the impact of this problem.

Furthermore, since the treatment strategies for chronic HBV associated with HDV are different as compared to chronic HBV alone and the long term prognosis of dual infection is poor, we recommend the screening for anti-HDV in all those $HBsAg^+$ patients who are going to receive the specific antiviral therapy so as to adjust the appropriate therapeutic regimen.

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