

# Triple Hepatitis: Frequency and Treatment Outcome of co/super-Infection of Hepatitis C and D Among Patients of Hepatitis B

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## ABSTRACT

**Objective:** To determine the frequency of hepatitis C and D in patients of chronic hepatitis B and the treatment response of hepatitis B in such patients.

**Study Design:** Case series.

**Place and Duration of Study:** Civil Hospital and Lyari General Hospital, Dow University of Health Sciences, from July 2003 to June 2005.

**Patients and Methods:** All patients of hepatitis B presenting during the study period were screened for triple infection by carrying out anti-HBc (IgG), anti-HCV and anti-HDV. Patients who were positive to all three were included in the study. Complete Blood Count (CBC); HBsAg; HBeAg; anti-HBc IgM; anti-HDV; anti-HCV; HBV DNA PCR; HCV RNA PCR; serum albumin; SGPT; serum bilirubin and ultrasound abdomen were acquired in all patients. All patients received pegylated interferon- $\alpha$  2a 180 mcg sc weekly x 48 weeks. Patients who were also positive for HCV RNA also received ribavirin 1000-1200 mg/d po x 24 weeks for genotype 3 and 48 weeks for genotype 1. Descriptive statistics were used for describing the data.

**Results:** Out of the 246 patients of HBV, 29 (11.8%) patients were also positive for anti-HBc IgG, anti-HDV and anti-HCV. After 48 weeks of therapy, the respective viral undetection by PCR was 4 (13.8%) in patients having only HBV DNA, 3 (10.3%) in patients with only HCV RNA and in patients who had both HBV DNA and HCV RNA positive, simultaneously HCV was cleared in 2 (6.9%) while HBV was not cleared in any case.

**Conclusion:** In patients coming with one hepatic infection, other infections should be sought as they share a common mode of spread and may affect the overall response to treatment.

**Key words:** Chronic hepatitis. Triple hepatitis. Pegylated interferon. Hepatitis B virus. Hepatitis C virus. Hepatitis D virus.

## INTRODUCTION

Chronic hepatitis due to different hepatic viruses is a common cause of liver related morbidity. Hepatitis B (HBV) and hepatitis C (HCV) are the main causes for chronic hepatitis.<sup>1-3</sup> It could lead to many complications. Cirrhosis, liver failure and hepatocellular carcinoma (HCC) develop in 15-40% of patients of HBV.<sup>4</sup> Over a million persons die annually due to HBV related complications.<sup>5,6</sup> Similarly, HCV also leads to many complications including HCC in 32% of infected patients.<sup>7</sup> It has been shown that super-infection of hepatitis A or E over HBV or HCV could lead to patient's deterioration and increase or precipitate encephalopathy.<sup>8</sup> Infection with multiple viruses leads to management problems with higher incidence of morbidity and mortality.<sup>9</sup>

Presence of dual and triple viral infections has been reported from various parts of the world. As hepatitis B, C and D share same modes of transmission, infection with more than one virus is possible.<sup>10</sup> There are reports from many parts of the world regarding multiple hepatitis virus infections.<sup>9,11</sup> In a recent report, increasing prevalence of HDV co-infection has been reported from South London.<sup>12</sup> Till date, there is no report of triple hepatic viral infection from Pakistan.

The objective of the current study was to determine and report the frequency of triple viral infections with hepatitis C and D in patients of chronic hepatitis B disease presenting at the study centres and the outcome of their treatment.

## PATIENTS AND METHODS

The study was conducted at Civil Hospital and Lyari General Hospital, Karachi, from July 2003 to June 2005. All patients of hepatitis B presenting during the study period were screened for triple infection by carrying out anti-HBc (total), anti-HCV and anti-HDV. Patients who were positive to all three were included in the study.

The investigations carried out in selected patients were Complete Blood Count (CBC); HBsAg; HBeAg; anti-HBc

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IgM; anti-HBc IgG; anti-HDV; anti-HCV; HBV DNA PCR; HCV RNA PCR; serum albumin; SGPT; serum bilirubin and ultrasound abdomen. The biochemistries were done by auto-analyzer; serological tests were carried out by ELISA. At the time of conduction of this study, facility of HDV RNA PCR was not commercially available in Pakistan, so this test could not be done. Patients were treated according to the PCR status of the infecting viruses.

Patients with both HCV RNA and HBV DNA positive, were treated with pegylated interferon- $\alpha$  2a 180 mcg sc weekly x 48 weeks and ribavirin 1000-1200 mg/d po x 24 weeks for HCV genotype 3 and 48 weeks for HCV genotype 1. Patients who were negative for both HBV DNA and HCV RNA were not given any anti-viral therapy. Patients who were anti-HBc (IgM) positive were included only if HBV DNA was still detected after 6 months of observation.

Data was entered and analyzed using SPSS. Frequencies and percentages of HBV, HCV and HDV were calculated. Continuous variables like age, SGPT and albumin level were analyzed by Student's 't' test. Standard error of difference, 95% confidence intervals and p-values were calculated. Box plot of SGPT at 4 weekly intervals was plotted. Patients who were still positive for either HBV or HCV PCR at the end of 48 weeks were re-coded in a new variable. The mean values of SGPT were compared for this new variable. Level of significance was set at  $\leq 0.05$ . SPSS version 16.0 was used for statistical analysis.

## RESULTS

During the study period, 246 patients of HBV presented for the treatment at our centre. Out of these, 29 (11.8%) patients, who were also positive to both anti-HDV and anti-HCV, were selected for the study. These included 23 (79.3%) males and 6 (20.7%) females. HBV DNA was detected by PCR in 16 (55.2%), HCV RNA by PCR was detectable in 7 (24.1%), among them one patient was of genotype 1 while 6 patients were of genotype 3, PCR for both HBV and HCV were detected in 4 (13.8%), all 4 patients were HCV genotype 3. The PCR for both viruses were undetectable in 6 (20.7%) of patients.

Twenty three (79.3%) patients were non-cirrhotic on presentation, while 6 (20.7%) patients were having cirrhosis at the time of presentation and all of them belonged to Child's Class B. Three (10.3%) patients were having anti-HBc IgM showing presence of acute hepatitis B infection at the time of presentation. They were inducted after 6 months as all three were still positive for HBV DNA after their self clearing period.

The mean SGPT at the time of presentation was  $199.7 \pm 70.7$  IU/dl while at 48 weeks its value was  $75.0 \pm 70.0$  IU/dl. The box plot of SGPT values is given in Figure 1. The difference between the mean SGPT

values at the end of 48 week in whom both HBV and HCV virus became undetectable ( $55.5 \pm 54.2$  iu/dl) and in whom any one of the virus was present ( $88.5 \pm 78.1$  iu/dl) was not significant (SE = 26.2; 95% CI = -86.3, 21.0;  $p=0.22$ ).

Among the 16 patients that were initially positive to HBV DNA, 4 (13.8%) patients became negative to HBV DNA and 1 (3.4%) was lost to follow-up while 11 (37.9%) were still positive at the end of 48 weeks.

Among the 7 patients who were initially positive to HCV RNA, 2 (6.9%) were lost to follow-up during the 48 weeks therapy. Two (6.9%) patients became HCV RNA negative while 3 (10.3%) patients were still harboring HCV RNA at the end of 48 weeks. Both the patients who cleared their virus were of genotype 3.

Only 1 (3.4%) out of 6 patients was lost to follow-up in patients who had both HBV DNA and HCV RNA detectable. HCV RNA became undetectable in 2 (6.9%) patients while HBV DNA remained detectable in all.

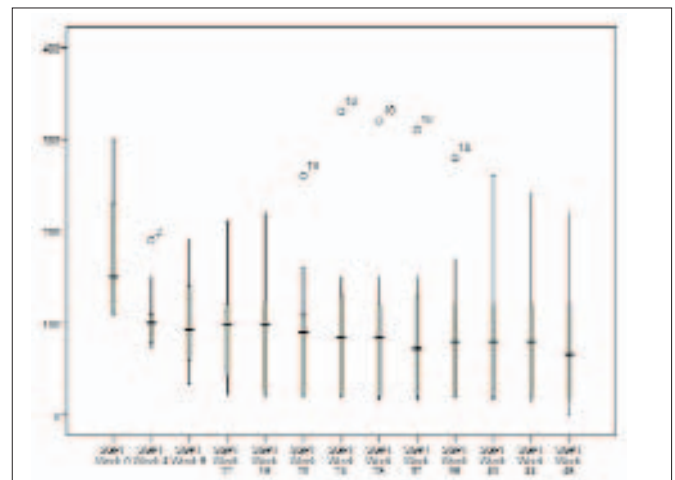


Figure 1: Box plot of SGPT levels over the period of 48 weeks of treatment.

## DISCUSSION

Much data is available on dual and triple hepatitis in HIV patients, though it is scanty on more than one hepatic viral infections.<sup>13</sup> Diagnosing multiple hepatitis viral infections, which share common routes of infections, is limited by low level of awareness among physicians and availability of simple diagnostic tests. Recently, good results were demonstrated by a single integrated protein microarray that could simultaneously determine in human sera two viral antigens (HBsAg, HBeAg) and seven viral antibodies (HBsAb, HBcAb, HBeAb, HCVAb, HDVAb, HEVAb, HGVAb) of human hepatitis viruses within 20 minutes.<sup>14</sup>

Despite improvements in the management, the case fatality rates (CFR) and standardized mortality ratios (SMRs) for all patients, carrying the diagnosis of liver

cirrhosis, have not improved much.<sup>15</sup> In a recent report CFR for cirrhosis were 16% within 30 days of admission, and nearly 34% at one year, with corresponding SMRs of 93 and 16.3, respectively. Moreover, because all-cause mortality among the general population progressively declined, the standardized mortality ratio in patients with liver cirrhosis rose significantly both at one month (from 77 to 112) and at one year (from 9.5 to 17.4).<sup>15</sup> Cirrhotic patients were also more likely to die of non-hepatic causes (SMR, 8.0), most notably: infections (SMR, 51.7), other digestive disorders (49.8), endocrine disorders (20), accidents (16.9), renal diseases (12.1), mental disorders (10.9), respiratory diseases (9.0), and suicide (9.0).<sup>15</sup> Presence of multiple viral infections could also increase mortality and morbidity in cirrhotic patients.<sup>16</sup>

The present study showed that 11.8% of patients of HBV presenting to our centre also had evidence of infection with HDV and HCV leading to triple viral infection. The treatment outcome of these patients was also poor ranging from 3.4-13.8% in various categories after 48 weeks of treatment with pegylated interferon. Poor response has been reported with high dropout rates in dual HBV and HCV infections.<sup>17</sup> Another study, conducted on smaller number of patients, did not show any improved response in patients receiving 3.0 MIU or 5.0 MIU dose of standard interferon.<sup>17</sup>

In this study, 20.7% of patients became negative for both HBV and HCV PCR but still had elevated SGPT in the absence of HDV RNA. It is strongly suspected that they were still harbouring the HDV virus. It again highlights the importance of this diagnostic test in the management of multiple virus infections.

A study from Taiwan, where hepatitis prevalence is high, has reported results of triple HBV HCV and HDV infections. Among the triple hepatitis patients, it reported the prevalence of HBV as 12.6%, HCV as 41.6% and that of HDV as 15.3%.<sup>18</sup> In this study, the HBV DNA was detected in 55.2% of patients followed by HCV RNA at 24.1%. This effect could be due to the fact that the authors primarily inducted the patients of hepatitis B and tested them for HDV and HCV while they primarily inducted the patients of chronic hepatitis and tested for all three viruses. Studies from Mongolia has reported the prevalence of triple infection at 30%.<sup>9,19,20</sup>

It has also been documented that in cases of dual and triple infections, one virus could have inhibitory effect on other. Like in HBV-HDV dual infections, the virological levels of HBV have been found lower than the HBV infection alone and similarly, the virological levels of HBV and HCV were lower than control in triple infections.<sup>21,22</sup> Others are of the opinion that dual/triple infections are more aggressive leading to early appearance of complications.<sup>23</sup> Virological responses are widely divergent and have dynamic profiles in triple

infections. This results in fluctuating viral levels during the therapy and follow-up period.<sup>24</sup> Some investigators have, therefore, suggested that yearly follow up of these patients, with viral loads, should be carried out.<sup>24</sup>

Treatment options are limited in triple infections as lamivudine alone or in combination with interferon have not shown much benefit in patients co-infected with HDV.<sup>25,26</sup> Very recently an indirect, beneficial but weak effect of HBV DNA suppression by new powerful nucleoside analogs on HDV RNA has been reported for the first time. Theoretically, a significant and sustained reduction in serum HDV RNA might only be seen when a reduction in HBV covalently closed circular DNA or HBV surface antigen is achieved. This could open new frontiers in the management of HDV if also supported by well designed large studies.<sup>27</sup> Till then, pegylated interferon in combination with ribavirin and prenylation inhibitor might offer the best hope in these cases.<sup>28,29</sup>

## CONCLUSION

In patients coming with one hepatic infection, other infections should be sought as they share common mode of spread. The response rates of multi-viral infections were lower in this series.

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