Original Article

Serological, Biochemical and Radiological Comparison of Triple Hepatitis (Hepatitis B, C & D Virus) and Dual Hepatitis (Hepatitis B & D Virus) at a Hepatology Clinic

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ABSTRACT

Objectives: To compare the serological, biochemical and radiological parameters of triple hepatitis (Hepatitis B, C & D) and dual hepatitis (Hepatitis B & D) infection.

Study Design: Cross Sectional Study.

Place and Duration of Study: This 3½ study conducted at Hepatology clinic and wards of Medicine Department, Chandka Medical College (C.M.C), Larkana from January 2008 to June 2011.

Materials and Methods: Blood sample of 1713 HBsAg positive patients were drawn, for detection of anti-HDV Antibodies, anti – HCV antibodies on ELISA, HBV DNA, HCV RNA and HDV RNA on PCR and liver function tests (LFT). Ultrasound of all patients was performed. Serological, biochemical and radiological parameters were compare in triple hepatitis and dual hepatitis patients by chi-square test. P value of less than 0.05 was taken as statistically significant.

Results: Of 1713 patients, anti-HCVAb was detected in 420 (24.5%) and anti-HDVAb in 1116 (65.1%). 268 (15.6%) had triple hepatitis and 848 (49.5%) had double hepatitis. HDV RNA was detected in 100% patients of TH positive as compared to 74.1% of DH ($p < 0.001$). TH patients tend to have normal liver span [OR: 11.28 (95% CI: 8.19 – 15.13). TH and DH patients, had features of advanced liver diseases, but no other statistically significant serological, biochemical or radiological difference was noted between both.

Conclusion: TH infection was documented in 15.6% and DH in 49.5% HBsAg positive patients. Infected patients had advanced liver disease. There was no statistically significant major difference noted in serological, biochemical or radiological parameters of TH and DH.

Key Words: Triple hepatitis, Dual hepatitis, HBsAg, HBV DNA, anti-HCV Ab, HCV RNA, anti-HDV Ab, HDV RNA.

INTRODUCTION

Hepatitis B virus (HBV) is a hepatotropic virus, belonging to hepadnaviridea family. It has infected approximately one third of world population and at least 350 million individuals worldwide are harboring this virus in active state. 80 – 85% individuals infected with it, clear it spontaneously, but the remaining go into chronic phase of infection. After a mean period of 15 – 25 years, 25 – 40% of these go on to develop cirrhosis and Hepatocellular carcinoma at the rate of 2 – 5% per year.[1,2,3] Pakistan comes in the category of intermediate prevalence zone for HBV infection where the prevalence of carrier of HBV is 2.11% to 10%.[4,5,6]

There are two important dilemma associated with HBV infection. First, there is no curative therapy available for it and secondly, HBV infected patients are the only individuals at risk of acquiring hepatitis Delta virus (HDV) infection. HDV is a defective virus which can only infect and replicate in the patients who are infected with HBV. This combined infection of HBV and HDV is called dual hepatitis (DH). When both viruses infect an individual simultaneously, the course of disease is not much different from isolated HBV infection. But, if super infection occurs, the rate of liver damage increases exponentially. This eventually leads to severe disease with higher rates of progression to cirrhosis and Hepatocellular Carcinoma.[7,8]

Three major problems are encountered with DH infection. First, rapid and aggressive progression of disease (hepatitis), second, no response to oral nucleoside / nucleotide drugs used for suppression of HBV proliferation, and third, marginal response (20%) to higher doses of interferon alpha. These problems make the management of dual hepatitis (HBV and HDV co infection or super infection) a challenging task.[9,l0,11,12,13]

This situation is even complicated if HCV infect an individual already harboring DH. This is called triple hepatitis (TH). The exact course and natural history of triple hepatitis is un-determined and treatment guidelines unclear regarding drugs, dosing and duration.[14,15,16,17] The magnitude of both entities i.e. DH and TH is reported to be very high in our country, ranging from 20 – 90% for DH and 3.4 – 11.8% for TH.[18,19,20,21,22] So, we designed this study to observe the serological, biochemical and radiological differences between dual and triple hepatitis infection. This study may help in understanding the characteristics of dual

and triple hepatitis.

MATERIALS AND METHODS

This was a hospital based cross-sectional and observational study for a period of three and half years from January 2008 till June 2011. The patients were enrolled from weekly Hepatology clinic and wards of Medicine Department, Chandka Medical College (C.M.C), Larkana. The study population / participants were the same that we studied for determination of frequencies of dual and triple hepatitis.[23]

Sampling Technique: Purposive sampling.

Inclusion Criteria: All new known HBsAg positive patients of either sex visiting Hepatology clinic of C.M.C Larkana for further evaluation and management were enrolled.

Exclusion Criteria:

- Patients younger than 15 years and older than 75 years.
- Patients previously diagnosed as seropositive for HCV antibodies (anti-HCV Ab) and / or HDV antibodies (anti-HDV Ab).
- Patients receiving oral or injectable therapy for viral hepatitis or previously non responders to such therapy.

Data Collection: Collection of data was started after approval of ethical review committee of Shaheed Mohtarma Benazir Bhutto Medical University, Larkana. Patients meeting our selection criteria were enrolled, study protocol was explained in detail and informed written consent was taken from each to draw blood sample and undergo ultrasonological evaluation. Blood samples were taken and sent to central laboratory C.M.C Larkana for detection of anti – HCV antibodies (anti-HCV Ab), Hepatitis D Virus antibodies (anti-HDV Ab) on ELISA, Hepatitis B Virus (HBV) DNA, Hepatitis C Virus (HCV) RNA and HDV (HDV) RNA by Polymerase Chain Reaction (PCR), and Liver Function Test as serum bilirubin, serum Alanine aminotransferase (ALT) and serum asparate aminotransferase (AST). LFTs were performed by Slectra – E Merck (Germany) auto-analyzing machine.

Ultrasound (US) examination of abdomen was done for liver parenchymal changes, liver size, portal vein (PV) size, spleen size and the presence of ascites. Ultrasound examination of abdomen was done by a senior radiologist with more than 10 years experience, at the Radiology Department C.M.C Teaching Hospital. Toshiba SSA-70 U/S machine was used to carry out U/S examination. A separate Performa was filled for each patient enrolled for the study to record the data of these investigations and demography. All investigations were performed at the laboratory of CMC teaching hospital.

Data Analysis: The collected data was transferred to and analyzed using SPSS version 19. Means of numeric response variables as age, serum bilirubin, serum ALT, serum AST, portal vein diameter and splenic size were calculated and compared in triple hepatitis and dual hepatitis. Categorical response variables as age (16-35years, 36-55years, 56-75years), gender (male, female), liver size (normal, decreased, increased), liver echo texture (normal, altered), portal vein diameter (normal, dilated) and ascites (present, absent) were compared in triple hepatitis and dual hepatitis patients by Chi-square test. Odd ratios (OR) and 95% Confidence Interval (CI) were calculated. Probability value (p-value) of less than 0.05 (<0.05) was considered to be statistically significant.

RESULTS

Table No 1: Demographic Profile of 1713 HBsAg Positive Patients

Characteristic	Number, n (%)
Age	
Mean ± SD	43.45 ± 14.83
Range	56 (72 - 16)
Age Categories	
16 – 35 Years	565 (33.0%)
36 – 55 Years	705 (41.2%)
56 – 75 Years	443 (25.8%)
Gender	
Male	1225 (71.5%)
Female	488 (28.5%)
Liver Function Test	
Serum Bilirubin (mg/dl)	1.82 ± 0.58 mg/dl
Serum ALT (IU/L)	67.96 ± 40.93 IU/L
Serum AST (IU/L)	172.16 ± 62.12 IU/L
Radiological Features	
Liver Size	
Normal (8 - 12 cms)	516 (30.1%)
Decreased (< 8cms)	814 (47.5%)
Increased (> 12 cms)	383 (22.4%)
Liver Echo Texture	
Normal	652 (38.1%)
Altered	1061 (61.9%)
Splenic Size (cms)	12.73 ± 1.81 cm
Portal Vein Diameter (cms)	1.63 ± 0.48 cm
Normal	1084 (63.3%)
Dilated	629 (36.7%)
Ascites	
Present	576 (33.6%)
Absent	1137 (66.4%)
Serological Test	
HBsAg Positive	1713 (100%)
Anti – HDV Ab Positive	1116 (65.1%)
Anti – HCV Ab Positive	420 (24.5%)
HBV DNA PCR Positive	308 (18%)
HCV RNA PCR Positive	148 (8.6%)
HDV RNA PCR Positive	896 (52.3%)
Dual / Triple Hepatitis	
Triple Hepatitis (HBV+HCV+HDV)	268 (15.6%)
Dual Hepatitis (HBV + HDV)	848 (49.5%)

A total of 1713 known HBsAg positive patients were enrolled in our study during the specified period with mean age of 43.45 ± 14.83 years. 1225 (71.5%) were male and 488 (28.5%) female. HBV DNA was positive in 308 (18%) patients. Anti – HCV Ab was documented in 420 (24.5%) and HCV RNA in 148 (8.6%). Anti – HDV Ab was documented in 1116 (65.1%) and HDV RNA in 896 (52.3%). Mean serum bilirubin, ALT and AST was 1.82 ± 0.58 mg/dl, 67.96 ± 40.93 IU/L and 172.16 ± 62.12 IU/L respectively. Liver span was decreased in 814 (47.5%), liver echo texture altered in 1061 (61.9%), portal vein dilated in 629 (36.7%), and ascites was present in 576 (33.6%) patients. 268 (15.6%) patients were HBsAg, anti-HCV Ab and anti-HDV Ab positive and were labeled as triple hepatitis. A total of 848 (49.5%) patients were HBsAg and anti-HDV Ab positive and were labeled as dual hepatitis. Table 1.

Table No 2: Comparison of Triple Hepatitis and Dual Hepatitis Patients

Characteristic	Triple Hepatitis (268)	Dual Hepatitis (848)	P values*	or (95%CI)
Age				
Mean ± SD	43.38 ± 14.33	43.85 ± 15.03	0.394	NA
Range	56 (72 - 16)	54 (71 - 17)		
Age Categories				
16 – 35 Years	86 (32.1%)	270 (31.8%)	0.939	1.01 (0.75 – 1.35)
36 – 55 Years	118 (44.0%)	342 (40.3%)	0.283	1.16 (0.88 – 1.53)
56 – 75 Years	64 (23.9%)	236 (27.8%)	0.204	0.81 (0.59 – 1.11)
Gender				
Male	220 (82.1%)	712 (84.0%)	0.471	0.87 (0.60 – 1.25)
Female	48 (17.9%)	136 (16.0%)		
Serological Test				
HBV DNA PCR Positive	60 (22.4%)	176 (20.8%)	0.568	1.10 (0.79 – 1.53)
HCV RNA PCR Positive	32 (11.9%)	00(0%)	0.234	NA
HDV RNA PCR Positive	268 (100.0%)	628 (74.1%)	0.001**	0.70 (0.67 - 0.73)

*Chi – square test (2-sided significance) **Statistically significant p values (<0.05)
+Significant and high odd ratios NA. Not Applicable

Table No.3: Comparison of liver function test of triple Hepatitis and dual Hepatitis Patients

Characteristics	Triple Hepatitis (268)	Dual Hepatitis (848)	P values*
Serum Bilirubin (mg/dl)	2.04 ± 0.44	2.02 ± 0.54	0.364
Serum ALT (IU/L)	83.84 ± 40.70	75.05 ± 47.35	0.137
Serum AST (IU/L)	212.05 ± 84.97	185.54 ± 55.6(	0.069

***Chi – square test (2-sided significance)**

Mean age of triple hepatitis positive patients was 43.38 ± 14.33 years as compared to 43.85 ± 15.03 years in dual hepatitis patients. HBV DNA was detected in 60 (22.4%) triple hepatitis ($p < 0.568$) as compared to 176 (20.8%) in dual hepatitis patients with odd risk of 1.10 (95% CI; 0.79 – 1.53). HCV RNA was detected in 32 (11.9%) triple hepatitis patients ($p < 0.234$). HDV RNA was detected in 268 (100%) triple hepatitis patients ($p < 0.001$) as compared to 628 (74.1%) patients in triple hepatitis negative patients with odd risk of 0.74 (95% CI; 0.71 – 0.78) as given in table 2.

Table No 4: Comparison of Radiological features of triple hepatitis and dual hepatitis patients

Characteristic	Triple Hepatitis (268)	Dual Hepatitis (848)	P values*	or (95%CI)
Liver Size				
Normal (8 - 12 cms)	164 (61.2%)	104 (12.3%)	0.001**	11.28 (8.19 – 15.53)+
Decreased (< 8cms)	104 (38.8%)	540 (63.7%)	0.001**	0.36 (0.27 – 0.48)
Increased (> 12 cms)	000 (0.00%)	204 (24.1%)	0.001**	1.41 (1.35 – 1.47)
Liver Echotexture				
Altered	148 (55.2%)	704 (83.0%)	0.001**	3.96 (2.93 – 5.35)+
Splenic Size (cms)	13.31 ± 1.55	13.46 ± 1.60	0.736	NA
Portal Vein (cms)	1.27 ± 0.20	1.25 ± 0.21	0.071	NA
Dilated	130 (48.5%)	415 (48.9%)	0.902	0.98 (0.74 – 1.29)
Ascites Present	110 (41.0%)	410 (48.3%)	0.067	0.74 (0.56 – 0.98)

*Chi – square test (2-sided significance) **Statistically significant p values (<0.05)
+Significant and high odd ratios NA. Not Applicable

There was not statistically significant difference in biochemical profile (serum bilirubin, serum ALT, serum AST) of triple hepatitis and dual hepatitis patients, as illustrated in table 3.

In patients having triple hepatitis, the liver span was normal in 164 (61.2%) as compared to 104 (12.3%) patients in dual hepatitis ($p < 0.001$) with odds of 11.28 (95% CI: 8.19 – 15.53). Liver echo texture was altered in 704 (83.0%) dual hepatitis patients as compared to 148 (55.2%) triple hepatitis with odds of 3.96 (95% CI: 2.93 – 5.35) as shown in table 4.

DISCUSSION

In our study, we observed that both triple and dual hepatitis patients had biochemical, serological and radiological evidence of advanced chronic liver disease. Triple hepatitis was documented in 15.6% HbsAg positive patients and dual hepatitis in 49.5%. The only serological difference between Triple Hepatitis and Dual Hepatitis was more frequent detection of HDV RNA. So, we assume that, HCV may have permissive effect on HDV, as it was detected in 100% patients of TH and 74% of DH ($p < 0.001$). There was no significant biochemical (LFT) difference noted between both Triple Hepatitis and Dual Hepatitis. Radiological comparisons were also statistically insignificant, except two important findings. TH patients tend to have normal liver size ($p < 0.001$) with odds of 11.28 (95% CI: 8.19 – 15.53) and DH patients were more likely to have altered echo texture of liver ($p < 0.001$) with odds of 3.96 (95% CI: 2.93 – 5.35).

Morsica G et al., (2009), studied the dual hepatitis and triple hepatitis infection in HIV positive individuals. He was of the view that HCV and HBV had suppressive effect on each other, while the presence of both viruses had permissive effect on HDV proliferation. So, HDV may be the predominant virus in triple hepatitis. Similar, observations were made by Jardi R et al.(2001). Liaw YF et al., (1998), while studying the virological and clinical course of triple hepatitis, found completely different results. He reported that triple hepatitis is associated with severe acute disease, but relatively benign, slowly progressive chronic infection. Clinical, biochemical and serological course of triple hepatitis infection was dominated by HCV infection.[24,25,26]

Mathurin P et al., in 2000 at France studied replication status and histological features of patients with triple and dual hepatic infection. Multiple infection was associated with a decrease of HCV replication. Cirrhosis was more frequently observed in patients with multiple infection. In patients with triple infection, serum HCV RNA and markers of HBV replication were absent in 80%, suggesting that HDV acts as a dominant virus. In patients with dual infection, HBV and HCV exerted an alternative, dominant replication.[27]

Whether HCV or HDV is the pre-dominant virus in triple hepatitis or what should be the ideal therapy for triple hepatitis, is still unknown and controversial issue. Our study and international literature bring focus to these issues.

CONCLUSION

- Triple and dual hepatitis is associated with advanced liver disease.
- Triple hepatitis and dual hepatitis seen in 15.6% and 49.5% respectively.
- HDV RNA was detected more frequently in triple hepatitis.
- Liver echo texture was altered more frequently in dual hepatitis.
- Triple hepatitis patients tend to have normal liver span.

Recommendations:

- ❖ Studies (multicentre) should be performed to answer the issues raised by our study, so that the collected data can be used to by international authorities, societies and associations to formulate recommendations for triple and dual hepatitis management.
- ❖ Community awareness regarding prevention of viral hepatitis should be intensified, so that we get rid of these silent killers (dual and triple hepatitis).
- ❖ Government should make immunization mandatory and compulsory, so that we can save our future from developing dual and triple hepatitis, because prevention is much better than cure.

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