CORRELATION OF VIROLOGICAL AND CLINICAL PROFILE OF PATIENTS WITH CHRONIC HEPATITIS B & C CO-INFECTION

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ABSTRACT

Background: Both HBV super-infection in patients with chronic hepatitis C and HCV super-infection in patients with chronic hepatitis B have been reported. In case of co-infection, hepatitis B and C interact with each other and affect immune responses resulting in active HCV with inactive HBV, active HBV with inactive HCV, both active and both inactive. The objective of our study was to find out and correlate the virological and clinical profile in our patients with chronic hepatitis B and C co-infection.

Material and Methods: This observational, descriptive and cross-sectional study was conducted at Medical 'A' Ward, PGMI, LRH Peshawar and Author's Private Hepatology Clinic, from July 2010 to June 2014. All patients with HBsAg and Anti-HCV Reactive by ELISA for more than six months were included in the study. Following investigations were carried out in these patients: Serum ALT, HBeAg, anti-HBe, HBV DNA PCR, HCV RNA PCR, and abdominal ultrasound. Data was entered and analyzed using SPSS version 16.0.

Results: A total of 130 patients were included in the analysis, out of which 81 (62.3%) were males. Mean age of patients was 40.52 ± 14.27 years. Majority of patients belonged to age-group of 21-30 years. Mean serum ALT of patients was 83.69 ± 60.48 U/L. Majority of patients belonged to ALT-Group of 41-80 U/L. Hepatitis C Virus was the dominant virus in 53% of patients. Chronic Hepatitis was the dominant clinical profile in 73% of patients.

Conclusion: Hepatitis C Virus is the dominant virus in our patients with BC co-infection. There is no statistically significant association between virological and clinical profile of these patients.

Key Words: Hepatitis B Virus, Hepatitis C Virus, BC Co-infection, Chronic Hepatitis, Cirrhosis, Hepatocellular Carcinoma

INTRODUCTION

Hepatitis B and hepatitis C viruses are the most common causes of chronic liver disease worldwide. Approximately 350 million people are infected with hepatitis B virus (HBV)¹ and another 170 million people with hepatitis C virus (HCV)². In Pakistan, overall national prevalence of hepatitis B is 2.5% and hepatitis C is 4.9%³. As hepatitis B and C share same modes of transmission, co-infection with these viruses is possible⁴. In Pakistan, overall national prevalence of hepatitis B and C co-infection is 0.1%³. Both HBV super-infection in patients with chronic hepatitis C⁵ and HCV super-infection in patients with chronic hepatitis B⁶

have been reported. In case of co-infection, hepatitis B and C interact with each other and affect immune responses^{7,8}, resulting in active HCV with inactive HBV, active HBV with inactive HCV, both active and both inactive.

If untreated, HBV as well as HCV can progress to cirrhosis and later on hepatocellular carcinoma (HCC). Chronic hepatitis B leads to cirrhosis and HCC in 5-40% of patients⁹, with an annual death rate of an over a million people worldwide¹⁰. Similarly, chronic hepatitis C leads to cirrhosis and HCC in 32% of patients, with an annual death rate of half a million people worldwide¹¹.

In case of chronic hepatitis B and C co-infection, morbidity and mortality are expected to be the highest in patients with of both active viruses as compared to in patients with both inactive viruses. What happens to the morbidity and mortality in case of one active and another inactive virus, largely needs to be determined, especially if there is any difference between patients with active HBV & inactive HCV and active HCV & inactive HBV. Therefore, the present study was conducted to find out the correlation of virological and clinical profile of patients with chronic hepatitis B and C co-infection in our setup.

MATERIAL AND METHODS

This observational, descriptive and cross-sectional study was conducted at principal author's private practice setup and Medical A Ward, PGMI, LRH Peshawar, over 4 years' time period (from July 2010 to June 2014). All patients with HBsAg and Anti-HCV Reactive by ELISA for more than 6 months were included in the study by convenience sampling. Patients, who were currently receiving or had received in past antiviral therapy, were excluded from the study. As per routine clinical practice protocol, following investigations were carried out in all of these patients: Serum ALT, HBeAg, anti-HBe, HBV DNA Qualitative PCR, HCV RNA Qualitative PCR, and abdominal ultrasound.

According to virological profile, patients were divided in four groups: 1) *Both Active*: those patients in whom both HBV and HCV PCRs were detected; 2) *HBV Active*: those patients in whom HBV PCR was detected and HCV PCR was not-detected; 3) *HCV Active*: those patients in whom HCV PCR was detected and HBV PCR was not-detected; and 4) *Both Inactive*: those patients in whom both HBV and HCV PCRs were not-detected.

Chronic hepatitis B patients were classified into: 1) *CHB-ITP (Chronic Hepatitis B Immune Tolerant Phase)*: those patients, who were having HBV-DNA PCR detected, HBeAg Reactive, Anti-HBe Non-Reactive, with normal

serum ALT; 2) *CHB-IRP (Chronic Hepatitis B Immune Reactive Phase)*: those patients, who were having HBV-DNA PCR detected, HBeAg Reactive, Anti-HBe Non-Reactive, with raised serum ALT; 3) *CHB-ICP (Chronic Hepatitis B Immune Control Phase)*: those patients, who were having HBV-DNA PCR not-detected, HBeAg Non-Reactive, Anti-HBe Reactive, with normal serum ALT; and 4) *CHB-IEP (Chronic Hepatitis B Immune Escape Phase)*: those patients, who were having HBV-DNA PCR detected, HBeAg Non-Reactive, Anti-HBe Reactive, with raised serum ALT.

According to clinical profile, patients were divided in three groups: 1) *Chronic Hepatitis*: those patients in whom ultrasound was showing normal liver with portal vein of normal size, spleen of normal size, and no evidence of ascites; 2) *Cirrhosis*: those patients in whom ultrasound was showing liver with coarse echogenic parenchyma to small nodular liver, increased portal vein diameter, enlarged spleen with or without ascites; and 3) *Hepatocellular Carcinoma (HCC)*: those patients in whom ultrasound showing space occupying lesion, confirmed as hepatocellular carcinoma by triphasic CT liver with raised serum alpha fetoproteins (AFP).

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Patients were enrolled after written informed consent was obtained. All patients consented to the study.

SPSS Version 16.0 was used to analyze the data. After entry, descriptive statistics was used to calculate Mean + SD (Standard Deviation) for numerical variables like age (in years) and ALT levels. Frequencies/Percentages were calculated for gender, virological and clinical profile of patients. Chi-square (in Crosstabs analysis) was used to identify significant association between virological and clinical profile of the patients.

RESULTS

A total of 130 patients with BC Co-infection were assessed during the study period. Among them 81(62.3%) were males and 49 (37.7%) were females. Mean age was 40.52 ± 14.272 years. Their age range was from 10 to 85 years. Majority of patients belonged to age-group of 21-40 years, followed by age-group of 41-60 years. Mean serum ALT was 83.69 ± 60.482 U/L. Their Serum ALT range was from 18 to 377 U/L. Majority of patients belonged to ALT-group of 41-80 U/L, followed by ALT-group of 81-120 U/L. Among the four virological profile groups, majority of patients belonged to "C Active" group, followed by "Both Active" group. Among the four HBV virological profile groups, majority of patients belonged to "CHB-ICP"-group, followed by "CHB-IRP"-group. Among three clinical profile groups, majority of patients belonged to "Chronic Hepatitis" group, followed by "Cirrhosis"-group (Table-1).

Table-1: Baseline Characteristics of patients

Parameters	Number of Patients	Percentage
Gender-wise distribution:		
- Males	81	62.3%
- Females	49	37.7%
- Total	130	100%
Age-wise distribution:		
- < 20 years	5	03.8%
- 21-40 years	63	48.5%
- 41-60 years	54	41.5%
- > 60 years	8	06.2%
ALT Levels:		
- < 40 U/L	23	17.7%
- 41-80 U/L	60	46.2%
- 81-120 U/L	28	21.5%
- 121-160 U/L	5	03.8%
- 161-200 U/L	7	05.4%
- > 200 U/L	7	05.4%
Virological Profile:		
- Both Active	28	21.5%
- HBV Active	15	11.5%
- HCV Active	69	53.1%
- Both Inactive	18	13.8%
HBV Profile:		
- CHB-ITP	1	00.8%
- CHB-IRP	31	23.8%
- CHB-ICP	87	66.9%
- CHB-IEP	11	08.5%
Clinical Profile:		
- Chronic Hepatitis	95	73.0%
- Cirrhosis	34	26.2%
- HCC	1	00.8%

Majority of patients with "Chronic Hepatitis" was females and majority of patients with "Cirrhosis" was males. Majority of patients with "Chronic Hepatitis" was in the age-group of 21-40 years whereas majority of patients with "Cirrhosis" was in the age-group of 41-60 years. Majority of patients with "Chronic Hepatitis" as well as "Cirrhosis" was having ALT in the range of 41-80 U/L (Table-2).

Table-2: Details of Clinical Profile of Patients

Parameters	Clinical Profile		
	Chronic Hepatitis	Cirrhosis	HCC
Gender-wise distribution:	52	28	1
- Males (n=81)	(62.2%) 43	(34.6%) 6 (12.2%)	(1.2%) 0
- Females (n=49)	(87.8%) 95	(26.2%) (0.8%)	1
- Total (n=130)	(73.0%)		
Age-wise distribution:	5 (100%)	0	0
- < 20 years (n=5)	55 (87.3%)	8 (12.7%)	0
- 21-40 years (n=63)	33 (61.1%)	21 (38.9%)	0
- 41-60 years (n=54)	2 (25%)	5 (62.5%)	1 (12.5%)
- > 60 years (n=8)			
ALT Levels:			
- < 40 U/L (n=23)	17 (73.9%)	6 (26.1%)	0
- 41-80 U/L (n=60)	46 (76.6%)	14 (23.4%)	0
- 81-120 U/L (n=28)	22 (78.6%)	6 (21.4%)	0
- 121-160 U/L (n=5)	2 (40%)	3 (60%)	1 (14.2%)
- 161-200 U/L (n=7)	2 (57.2%)	3 (42.8%)	0
- > 200 U/L (n=7)	4 (57.2%)		

Pearson Chi-Square test showed that there is no statistically significant association between virological and clinical profile of these patients, and similarly Phi and Cramer's V systemic measures showed that the strength of association

between virological and clinical profile is very weak (Table-3).

Table-3: Correlation of Virological and Clinical Profiles of patients

Virological Profile	Clinical Status of Patients		
	Chronic Hepatitis	Cirrhosis† !!*	HCC
Both Active† (n=28)	20 (71.4%)	8 (28.6%)	0
HBV Active!! (n=15)	13 (86.8%)	1 (6.6%)	1 (6.6%)
HCV Active* (n=69)	47 (68.1%)	22 (31.9)	0
Both Inactive (n=18)	15 (83.3%)	3 (16.7%)	0
Total	95	34	1

*Both-Active vs Rest = Pearson Chi-square p value = 0.552, Phi & Cramer's V p value = 0.552

!!HBV-Active vs Rest = Pearson Chi-square p value = 0.186, Phi & Cramer's V p value = 0.186

†HCV-Active vs Rest = Pearson Chi-square p value = 0.966, Phi & Cramer's V p value = 0.966

DISCUSSION

In our study, male to female ratio was 1.6:1 among 130 patients studied. Results of the current study revealed that 74% of chronic liver disease patients were in 3rd to 5th decade of life. Chronic hepatitis patients were a decade younger than cirrhotic patients, whereas HCC patient was a decade older at an average. Increasing age is a higher risk to have the disease, probably due to longer exposure to multiple risk factors^{12,13}. In our study, 62% of chronic liver disease cases were younger than 50-years of age, out of which 31% younger than 30 years of age; the percentage gradually decreased in the later decades. The reason could be an early childhood infection with the hepatitis viruses.

Patients with dual HBV-HCV infection may exhibit a wide spectrum of virologic profiles. Chronic hepatitis B patients who become super infected with HCV can undergo sero-conversion of HBeAg and HBsAg to respective antibodies¹⁴⁻¹⁶. Longitudinal studies have found 2.08% annual incidence of HBsAg seroconversion in BC co-infected patients as compared to 0.43% in patients with HBV monoinfection^{17,18}. Several authors have reported that HBV can reciprocally inhibit HCV replication as well¹⁹⁻²¹. Furthermore, mutual suppression of each other in BC Co-infected patients has also been reported²².

Although most dually infected patients appear to have both active HCV and inactive HBV replication, some patients indeed have high HBV DNA levels and undetectable HCV RNA. Because most available data are based on cross-sectional observations, the possibility cannot be ruled out that HBV and HCV can alternate their dominance during different periods of co-infection. A multicenter longitudinal follow-up study in Italy indeed revealed that patterns of virologic dominance in these cases vary and change over time⁷. This study enrolled 133 untreated HBV-HCV dually infected patients with or without hepatitis D virus (HDV). Out of 103 patients without HDV, 49 cases (47.5%) had active HCV/inactive HBV, 24 (23.3%) had both viruses active, 15 (14.6%) had active HBV/inactive HCV, and 15 (14.6%) had both viruses inactive. During 1-year prospective follow up, 32 subjects (31%) presented dynamic virologic profiles characterized by the fluctuation of HBV and/or HCV viremia levels at different time points. In summary, this study showed that the virologic patterns in HBV-HCV dual infection are widely divergent and have dynamic profiles. A careful longitudinal evaluation of the viremia levels of both viruses is essential for making a correct diagnosis. In our study, more than half of the patients (53%) were having active HCV/inactive HBV, 21% of patients were having both active viruses, 11% of patients were having active HBV/inactive HCV, and 14% of patients were having both inactive viruses, the pattern similar to the above-mentioned study⁷; anyhow, our study was cross-sectional and we did not evaluate these patients over time to look for fluctuating viremia.

Chronic hepatitis B and C co-infected patients can present in three possible clinical phases: *Chronic Hepatitis, Cirrhosis, and Hepatocellular carcinoma*. In the above-mentioned study⁷, out of 103 patients, 69 (67%) were having hepatitis and 34 (33%) were having cirrhosis. We found the same pattern in our study; 73% patients were having chronic hepatitis stage and 26% patients

were having cirrhosis. Majority of patients with hepatitis was females, in the age-group of 21-40 years, having active HCV with or without active HBV, and having ALT in the range of 41-80 U/L. These patients are at highest risk of progress onto cirrhosis and decompensated liver disease, and therefore, should be considered for treatment. In our study, Majority of patients with cirrhosis was males, in the age-group of 31-50 years, and having no specific trend regarding virological profile and ALT levels.

Many studies have shown that patients with hepatitis B and C co-infection have more severe liver disease, and are at an increased risk for progression to hepatocellular carcinoma (HCC), as compared to mono-infected patients^{4, 22-25}. We evaluated patients with co-infection without control group of mono-infected patients, but if we compare our findings, 26% patients having's cirrhosis, with other national studies^{26,27}, patients with hepatitis B and C co-infection do have more severe liver disease.

Our study did not reveal statistically significant association between virological and clinical profile of patients with chronic hepatitis B and C co-infected patients, as found in literature⁷. We found only one case of HCC, a male patient over 70 years of age. Because of the likely increased risk of developing HCC, co-infected patients should receive regular 6-month and possible more frequent screening with ultrasound of the liver and serum Alfa-Feto-Protein (AFP) levels.

CONCLUSION

Hepatitis B and hepatitis C co-infection is not uncommon, especially within areas of high prevalence of hepatitis B. We could not find any statistically significant association between virological and clinical profile of these patients, rather the strength of association between virological and clinical profile was very weak.

Dual infections present unique management challenges given the complex interaction of HBV and HCV, and the propensity for developing more severe liver disease. Treatment

decisions should be made based upon the determination of the "dominant" hepatitis virus. Caution must be exercised in treating co infected patients, as flares of the untreated virus may occur. No standard of care has been established for treatment of co infected patients, and larger randomized, controlled trials are needed to clarify the optimal treatment for such patients and the role of newer antiviral agents.

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