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Viral Infections Original Article

Pilot study of hepatitis b, c and human immunodeficiency viruses infections among patients with chronic liver diseases from north-east india attending a new tertiary care health set up at shillong

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ABSTRACT

Background: Chronic liver diseases (CLD) are major public health concerns in North-Eastern India. Association of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections in CLD patients result in atypical presentations with increased severity and duration of illness. Understanding of agent, host, clinical profiles and their co-relationship for better management and prevention of such diseases in the community are important challenges. **Aims:** To assess sero-prevalance of HBV, HCV, HIV and their co-infection/triple infection in CLD patients from North-East India. To determine risk factors predisposing to development of CLDs. To find out if any correlation exists between risk factors for CLDs and that of HBV/HCV/HIV infections. **Materials And Methods:** This study was conducted from December 2009 to June 2011 in North-Eastern Indira Gandhi Regional Institute of Health and Medical Sciences. Blood samples were collected from 57 clinically diagnosed CLD patients after obtaining Institutional ethical clearance. Detail clinical profile with relevant biochemical test results were recorded. Viral markers - hepatitis B surface antigen, hepatitis B e (HBe) antigen, anti-HBe and anti-HCV were assessed employing commercial ELISA kits. Specimens were subjected for detection and confirmation of HIV infection as per NACO Guidelines. **Results:** Male to female ratio was 1.85:1 with most cases in range of 31-50 years. HBV markers were detected in 35 (61.40%) and anti-HCV in 2 (3.5%) patients. Anti-HIV was reactive in 7 (12.28%) patients; 4 co-infected with HBV and 2 with HCV. **Conclusion:** HBV is still a major cause of CLD, followed by HCV in North-East India. Co-infection of HBV/HCV with HIV was low (7.14%) in comparison to rest of India. Confections of HIV with HBV/HCV in CLDs patients was observed to be major public health concern in terms of risk factors and transmission dynamics of these chronic diseases in North-East India.

Key words: *Chronic liver diseases, enzyme immunosorbent assays, hepatitis B virus, hepatitis C virus, human immunodeficiency virus*

INTRODUCTION

Chronic liver disease (CLD) is defined as hepatic injury lasting for at least 6 months and comprises of chronic hepatitis, which on progression leads to cirrhosis and ultimately to hepatocellular carcinoma (HCC).[1]

Approximately 2 billion people are infected with hepatitis B virus (HBV) including 360 million chronically infected cases.[2] HBV-related CLD claims around 1 million lives annually.[3] Europe has intermediate to high hepatitis B surface antigen (HBsAg) carrier rates.[4] In Asia, HBV is responsible for 75% of CLDs.[5] India has the second largest global pool (50 million cases) of chronic hepatitis B (CHB) infection.[3,4] Prevalence of HBV in CLD from India is reported to be 6-44%.[6-8]

Worldwide 170 million people are infected with hepatitis C virus (HCV). Approximately 20% of chronic HCV infection progress to cirrhosis with an increased risk for development of HCC.[9] Data from Europe indicate HCV prevalence as 0.1-6%.[4] Asian studies report anti-HCV reactivity in 8-64% of CLD patients.[10] Studies from India show prevalence of HCV ranging from 3% to 32% in CLD patients.[11,12] Co-infection of HBV and HCV in CLD is reported to be 3-79% while a single study reports no co-infection.[13,14] Studies from India show lower prevalence of HCV 3-31.5% in comparison to HBV 6-44% in CLD patients.[6-8]

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Globally, co-infection of hepatitis viruses with human immunodeficiency virus (HIV) is reported as 12-15%.[15] Indian studies report co-infection of HBV with HIV between 6-25% and HCV with HIV between 3-21%.[15-17]

Detection of viral markers are important in HBV and HCV infections for diagnosis, prognosis and monitoring of patients.[18] Molecular methods require proper laboratory settings, cumbersome procedures and expensive equipments, while enzyme-linked immunosorbent assay (ELISA) is commonly available, easy to perform, reliable, reproducible and cost effective tool in detection of viral markers especially for under-developed and developing countries.[19-22]

MATERIALS AND METHODS

Inclusion criteria were based on clinical, laboratory and radiological findings of CLDs. All cases of acute liver diseases and patients refusing to give consent were excluded from the study. Clinical presentations, past medical and surgical history, vaccination against HBV, family history, history of personal contacts with cases of hepatitis, findings of systemic examination, biochemical parameters, and risk factors were documented in detail. Ultrasonographic features of CLD included shrunken liver, hepatomegaly, fine nodular margins of liver, altered echo-texture, coarse parenchyma, chronic parenchymatous changes, splenomegaly, ascites and distended portal or splenic veins.

Five milliliters of venous blood sample was collected from each patient under aseptic precautions in a vacutainer after explaining the procedure and transported to central serology laboratory, Department of Microbiology, for further processing. In the case

of hemolyzed samples, recollection was done. The samples were kept at room temperature (24°C) for 30 min and then centrifuged at 4000 revs/min for 5 min. The serum obtained was aliquoted in eppendorf tubes and stored at −80°C until the tests were performed.

Commercially available third Generation ELISA kits were used for HBsAg (Standard Diagnostic Inc., Korea), hepatitis B e antigen (HBeAg) (Bio-Rad, France), anti-HBe (Bio-Rad, France) and anti-HCV (Standard Diagnostic Inc., Korea). Anti-HIV (Comb Aids: Span Diagnostics Ltd., India, AIDSCAN Trispot test: Bhat Bio-Tech India (P) Ltd., SD Bioline: Standard Diagnostic Inc., Korea) was tested as per National AIDS Control Organization guidelines.[23] All tests performed in triplicates and results recorded.

Statistical analysis

Chi-square test and relative risk calculated using IBM SPSS (Statistical Package for the Social Sciences), Version 22 (Manufacturer IBM Corporation, August 2013, USA).

RESULTS

In 1½-year period, 767 patients with liver diseases attended general medicine department, out of which 167 patients fulfilled inclusion criteria. Only 57 (7 outpatients and 50 inpatients) consented to be enrolled in this study, 37 (64.91%) males and 20 (35.09%) females. Male to female ratio was 1.85:1. Age ranged from 19 to 85 years. Most cases were in the range of 31-50 years.

Distribution of patients as per the geographical regions [Figures 1 and 2]. Majority presented with jaundice (93%) and fatigue (80%) [Figure 3].

Serum aspartate transaminase (AST) and alanine transaminase (ALT) levels were moderately elevated in all patients (195-890 IU/L). Liver enzyme levels were higher in patients with HBV infection than patients infected with HCV. Mean ALT level (630 IU/L) was higher in patients with HBV infection while, mean AST level (310 IU/L) was higher in patients with HCV infection [Table 1]. Ultrasonography reported cirrhosis in 30 (52.63%), varying degree of hepatic fibrosis in 24 (42.11%) and HCC in 3 (5.26%) patients.

Multiple risk factors predisposing to the development of CLDs were found in 18 (31.57%) patients. Chronic alcoholism and blood transfusion as risk factors in 10 (17.54%) and 5 (8.77%) patients, respectively. In 16 (28.07%) patients, no obvious risk factors that lead to CLD could be elicited [Figure 4].

The study showed detection of markers of HBV in 35 (61.40%), HIV in 7 (12.28%) and HCV in 2 (3.5%) among 57 CLD patients. Geographical distribution of patients showing HBV, HCV and HIV positivity are shown in Tables 2 and 3. HBV, HCV and HIV positivity as per the presentations and risk factors documented in the patients is shown in Table 4. No co-infection of HBV and HCV was detected in this study.

Three markers of HBV-HBsAg, HBeAg and anti-HBe were detected in 3 (5.26%) patients. Two markers - HBsAg and anti-HBe, HBsAg and HBeAg and HBeAg and anti-HBe were

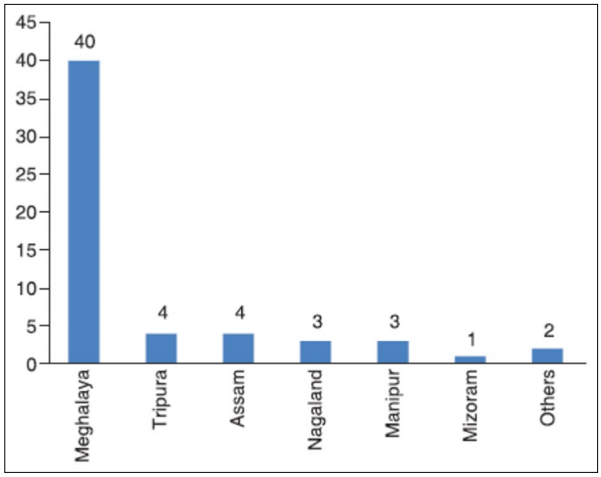

Figure 1: Distribution of patients as per the geographical regions

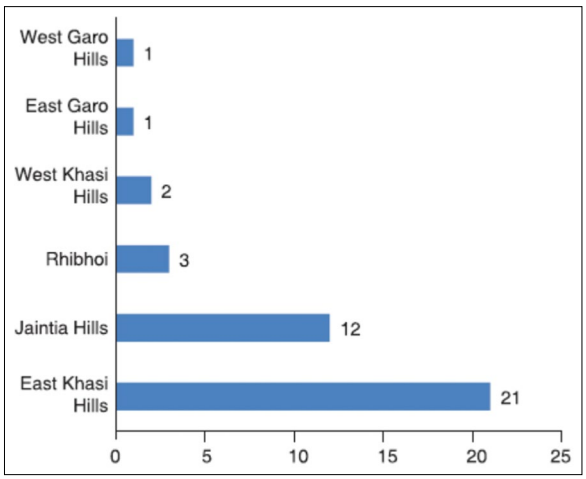

Figure 2: Distribution of patients within Meghalaya

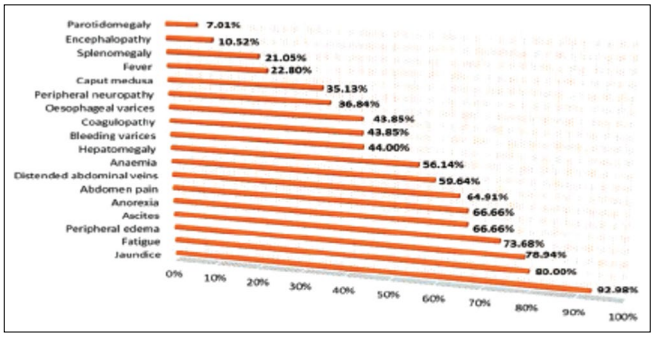

Figure 3: Clinical presentations of the patients

detected in 9 (15.80%), 4 (7.02%) and 2 (3.51%)patients, respectively. Lone anti-HBe, HBsAg and HBeAg were detected in other 11 (1929%), 5 (8.77%) and 1 (1.75%) patients, respectively. Among the HIV positive CLD patients, 4 were co-infected with HBV and 2 with HCV. 4 (7%) patients succumbed to the illness in the hospital.

DISCUSSION

Hepatitis B and C infections are among the most prevalent infectious diseases and are major causes of CLDs worldwide.[14,24] Both infections can cause a varied range of presentations including asymptomatic infections to those that progress to chronic hepatitis, cirrhosis and HCC.[24] These viruses are endemic in India and have an etiological role in acute hepatitis, of which 50-70% end up with CLD.[12]

In this study, 15 (26.32%) patients initially admitted for different ailments were incidentally diagnosed as cases of CLDs during the follow-up. Such findings of asymptomatic chronic hepatitis are reported in the literature.[25] Higher prevalence of CLD was seen in males, similar to other studies.[11]

Hepatitis B or C positivity accounted for 37 (64.91%) patients, which was significant ($P = 0.000001$) in comparison to negative group. There were 14 (37.84%) females and 23 (62.16%) males in sero-positive group; 6 (30%) females and 14 (70%) males in sero-negative group indicating higher prevalence of hepatitis virus infection in males as is the finding of other studies.[8,14] However, this gender difference was not statistically significant ($P = 0.554$). The mean age for males was 42 years while that of the female was 46 years similar to findings of Singh *et al.* and Arora *et al*.[11,14]

Fatigue (64%), ascites (58%), abdominal pain (50%) and jaundice (44%) were the most common reasons for hospital admission. Decompensated cirrhosis and its complications accounted for 22% of admissions similar to several studies.[12] Hepatic encephalopathy was seen in 6 (10.53%) patients similar to a study by Kumar *et al.* but higher prevalence (24%) have been reported from Aligarh.[8]

Mean aminotransferases levels were higher in patients with HBV infection in comparison to HCV infection ($P = 0.028$) similar to findings of other studies.[12,14] However, higher aminotransferase levels did not always correlate with the severity of infection in this study. In fact, most studies found no correlation between higher aminotransferases levels and severity of hepatitis infection.[12,14] This study detected higher mean AST levels than ALT levels in HCV infection in contrary to several other studies.[12,14]

Ultrasonography reported liver cirrhosis, portal hypertension and ascites in 22 (38.59%), hepatomegaly in 14 (63.64%) and splenomegaly in 12 (54.55%) patients. Early stage of liver cirrhosis with portal hypertension were observed in 6 (10.52%) and renal involvement in 2 (3.5%) patients. The patients showing renal involvement had severe liver diseases with widespread systemic manifestations and increased levels of blood urea and serum creatinine. HCC was diagnosed in 3 (5.26%) patients. Among the patients with cirrhosis, hepatitis markers were detected in 17 (77.27%) which was higher than a study from North India (40.6%).[7] All cases of HCC showed markers for HBV, a finding similar to a study from China.[26] Two cases with bilateral renal involvement were sero-positive for HBV markers corroborating with the report that HBV may cause chronic renal parenchymal changes and glomerulonephritis due to deposition of HBV antigen-antibody immune complex.[27]

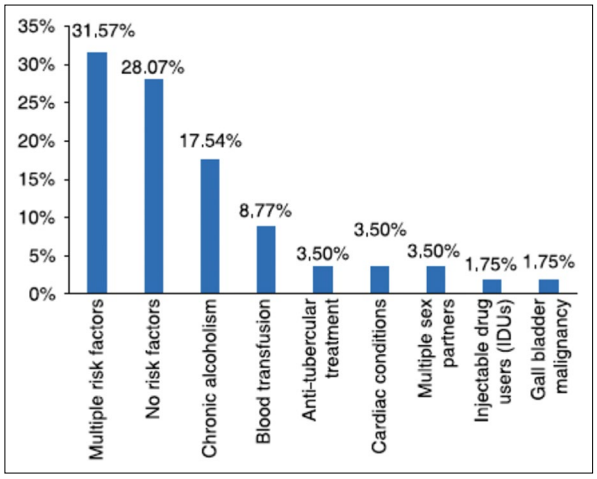

Figure 4: Risk factors in the patients

Patients exposed to multiple risk factors associated with the development of CLDs were also found to have high HBV (83.33%) and HCV (11.11%) sero-positivity rates. Exposure to multiple risk factors increased the risk of acquiring HBV/HCV/HIV infections by 1 and half times (relative risk 1.5516) in comparison to those with a single risk factor ($P = 0.0129$). Hence, CLDs and HBV/HCV/HIV infections share common risk factors.

Chronic alcoholism alone was a risk factor predisposing for development of CLDs in 10 (17.54%) patients and HBV sero-markers were detected in two of these patients. The remaining eight sero-negative patients were diagnosed as cases of chronic alcoholic hepatitis. Blood transfusion was the only known risk factor, which could have led to CLDs in 5 (8.77%) patients and all of them were positive for HBV markers. This finding outlines the possibility of high positivity rate among blood donors from

Table 1: Mean biochemical parameters in patients

Parameters	*HBV*	*HCV*	*Normal value*
Age (in years)			
Male	42	41	
Female	46	47	
Hb (g/dl)	8.1	9.2	12-18
Platelet (10^3 cells/mm^3)	85	90	150-400
Leucocytes (10^3 cells/mm^3)	2.9	3.1	4-11
Bilirubin (mg/dl)	11.5	7	0.2-1.0
Aspartate transaminase (IU/L)	610	310	0-40
Alanine transaminase (IU/L)	630	256	0-40
Alkaline phosphatase (U/L)	245	253	80-290
Albumin (g/dl)	2.1	2.9	3.5-5.0
Globulin (g/dl)	3.3	1.9	1.5-3.0
Prothrombin time (s)	33	27	12
Urea (mg/dl)	35	26	10-45
Creatinine (mg/dl)			
Male	2.7	1.7	0.9-1.5
Female	1.7	1.2	0.8-1.3

Hb=Hemoglobin, HBV=Hepatitis B virus, HCV=Hepatitis C virus

Table 2: Geographical locations of patients showing HBV, HCV and HIV positivity

North-East states	*HBV alone*	*HBV+HIV*	*HCV+HIV*	*HIV alone*
Mizoram	Nil	Nil	1	Nil
Manipur	2	Nil	Nil	Nil
Nagaland	2	Nil	1	Nil
Assam	3	Nil	Nil	Nil
Tripura	3	Nil	Nil	Nil
Meghalaya	21	4	Nil	1
Total	31	4	2	1

HBV=Hepatitis B virus, HCV=Hepatitis C virus, HIV=Human immunodeficiency virus

Table 3: Geographical locations of patients showing HBV, HCV and HIV positivity within Meghalaya

District	*HBV alone*	*HBV+HIV*	*HCV+HIV*	*HIV alone*
East Khasi Hills	13	2	Nil	Nil
Jaintia Hills	5	2	Nil	Nil
Rhibhoi	1	Nil	Nil	1
West Khasi Hills	1	Nil	Nil	Nil
East Garo Hills	1	Nil	Nil	Nil
Total	21	4	Nil	1

HBV=Hepatitis B virus, HCV=Hepatitis C virus, HIV=Human immunodeficiency virus

Table 4: HBV, HCV and HIV positivity as per the presentations and risk factors documented in the patients

CLD with different presentations and risk factors	*Total*	*HBV*	*Different combinations of HBV markers*	*HCV*	*HIV*
Chronic alcoholism	10	2	HBsAg (1), HBsAg+Anti-HBe (1)	Nil	Nil
Blood transfusion	5	5	HBsAg (2), HBsAg+HBeAg (1), Anti-HBe (2)	Nil	Nil
Injectable drug users+multiple sex partners	3	3	HBsAg+HBeAg (1), HBsAg+Anti-HBe (2)	Nil	Nil
Gall bladder cancer+HCC+b/l renal involvement	1	1	HBsAg+HBeAg+Anti-HBe (1)	Nil	Nil
HCC+anti tubercular drugs	1	1	HBeAg+Anti-HBe (1)	Nil	Nil
Anti-tubercular drugs	1	Nil		Nil	Nil
HCC+cardiomyopathy	1	1	HBsAg Anti-HBe (1)	Nil	Nil
Cardiomyopathy+b/l renal involvement	1	1	HBeAg (1)	Nil	Nil
No risk factor	16	6	HBsAg (2), HBsAg+Anti-HBe (2), Anti-HBe (2)	Nil	Nil
Multiple risk factors	18	15	HBsAg+HBeAg (2), Anti-HBe (7), HBeAg+Anti-HBe (1), HBsAg+Anti-HBe (3), HBsAg+HBeAg+Anti-HBe (2)	2	7
Total	57	35		2	7

HBV=Hepatitis B virus, HCV=Hepatitis C virus, HIV=Human immunodeficiency virus, CLD=Chronic liver diseases, HBsAg=Hepatitis B surface antigen, HBeAg=Hepatitis B e antigen, HBe=Hepatitis B e, HCC=Hepatocellular carcinoma

this region and emphasizes on strict vigilance of pretransfusion screening of blood and blood products.[28] All patients who had a history of injectable drug usages and multiple sexual partners were positive for HBV markers indicating a high prevalence of HBV in these high-risk groups. Hence, public awareness programs, health education on personal safety and safe sexual practice campaigns has to be organized by healthcare authorities to reach these groups.[6]

No risk factors for developing CLD could be elicited in 16 (28.07%) patients similar to a study from Chandigarh.[11] Of these, 6 (10.53%) patients were found to be positive for HBV markers similar to reports of other Indian studies.[8,14] The remaining 10 (17.54%) patients were diagnosed as cryptogenic hepatitis as no risk factors, or viral markers were found and such association has been documented in CLD patients.[11,13] Patients exposed to risk factors were 2 and half times (relative risk 2.4065) more likely to be infected with HBV/HCV/HIV in comparison to patients without risk factors and it was statistically significant ($P = 0.0072$).

Viral hepatitis cannot be diagnosed solely by clinical, biochemical and radiological parameters, therefore serological tests must be performed.[12] In the present study, HBV markers were detected in 35 (61.40%) cases, prevalence similar to other studies on CLDs.[11,12,14] HCV was detected in 2 (3.51%) patients, which is in contrast to earlier reports which showed prevalence ranging from 12% to 25.6% in CLD patients.[11] The lower prevalence of HCV could be due to small number of patients in this study. Considering the clinical, biochemical, radiological findings and hepatitis markers, these 37 (64.91%) patients were diagnosed as cases of chronic viral hepatitis.

Presence of all three HBV markers (HBsAg, HBeAg and anti-HBe) was detected in 3 (5.26%) patients. HBsAg alone was present in 5 (8.77%) patients, of which 4 (80%) showed severe liver diseases similar to a study from Northern India.[14] HBsAg alone may be present in patients with HBeAg-negative CHB infection and although their levels of HBV DNA tend to be lower than among patients with HBeAg-positive CHB, these patients can have progressive liver injury complicated by cirrhosis and HCC.[2]

HBsAg along with HBeAg was detected in 4 (7.02%) cases who showed severe liver disease with longer duration of hospital stay. Serum HBeAg concentration reflects virus replication, hepatitis activity and close correlation with virus load.[19] This was also true in our study as two patients who were sero-positive for both HBsAg and HBeAg showed high viral load for HBV DNA (real-time polymerase chain reaction using TaqMan probe) when tested elsewhere.

HBsAg and anti-HBe were detected in 9 (15.79%) patients, a finding similar to other studies.[14,25] Although HBsAg and HBeAg coexist frequently, HBeAg may be replaced by anti-HBe in some cases of exacerbation of CHB, which leads to persistence of HBV infection.[29]

HBeAg was detected as sole viral marker in 1 (1.75%) patient, and similar finding has been reported.[3] Such scenario can occur when escape mutants of HBV exist, or immune complexing of HBsAg are present, whereby only HBeAg can be detected.[3]

HBeAg and anti-HBe were detected in 2 (3.51%) patients as reported in previous studies where both markers can coexist. Anti-HBe sero-conversion does not necessarily indicate subsequent HBeAg clearance.[3,18,19] Patients with HBeAg and anti-HBe had severe liver disorder, which corroborates with findings of other studies that the mere presence of anti-HBe does not prevent the progression of disease to severe form.[25] A similar study from Japan had reported prevalence of HBeAg and anti-HBe in CLD as 1.85%.[18]

Anti-HBe alone was detected in 11 (19.30%) patients. In the presence of precore mutants, anti-HBe can be the only marker detectable.[2,19] The presence of only anti-HBe marker neither makes the patient noninfectious nor can it predict the severity of disease.[30]

There was no case of co-infection of HBV and HCV in this study, though, dual infection has been reported up to 12% in CLD.[8,11]

Exposure to multiple risk factors increases the risk of HBV, HCV and HIV co-infection and triple infection. Moreover, the modes of transmission of HBV, HCV and HIV are similar; hence, they are likely to be seen in the same patient.[9] The interactions are resulting from such co-infections often result in varied clinical presentations, increased severity and duration of CLD which ultimately affects the treatment culminating in an adverse outcome.[12,15,29]

Anti-HIV was reactive for 7 (12.28%) patients. Hepatitis viral markers were detected in 6 (11%) of these patients similar to reports from North India but lower when compared to West

India.[15,16] The relative risk of HBV/HCV positive patients acquiring HIV infection was 3.2432 and proportion of HBV/HCV infected individuals being infected by HIV was statistically significant (P = 0.000949). HCV co-infection with HIV was found in 2 (3.51%) patients with similar results reported by Gupta and Singh though; higher prevalence (21%) has also been reported.[16,17] Both of these patients had multiple risk factors, which could have contributed to higher rate of co-infection as similar reports have been documented in the literature.[4] Co-infection of HBV and HIV was seen in 4 (7.02%) patients similar to findings of Muralidhar *et al*.[31] However, both low and high prevalence 2.59-85% of co- infection has also been reported.[16,31]

Reduced survival rate with a high rate of hepatic decompensation was observed among HIV positive patients co-infected with HBV/HCV viruses similar to other studies.[8] Outcome of 4 patients was unfavorable as 2 males and 2 females succumbed to infection. One male patient was seropositive for HBsAg, HBeAg and HIV, which corroborated with findings of increased risk of mortality when both HBsAg and HBeAg existed with HIV.[32] The other male patient showed HBsAg, HBeAg and the anti-HBe positivity. Among the female patients, one was seropositive for anti-HBe alone while the other had both HBeAg and anti-HBe seropositivity. This reinforces the fact that anti-HBe is not a protective antibody and patients with anti-HBe can still have severe disease as also reported by other studies.[19,25]

CONCLUSION

Patients with CLDs have increased risk of infection with hepatitis viruses (statistically significant, P = 0.000001). HBV is still a major cause of CLD, followed by HCV in North-East India. Co-infection of HB/HC with HIV in CLD patients is detected to be low (7.14%) in comparison to other parts of India. CLD patients co-infected with hepatitis viruses and/or HIV have severe liver diseases and increased mortality rates in comparison to those without such associations.

Hepatitis B virus infected blood transfusion has been implicated in the development of CLDs in 8.77% of patients in this study. This study also reinforces the fact that anti-HBe is not a protective antibody.

Patients diagnosed with cryptogenic hepatitis (18%) may have been infected with HB and HC variants, hepatitis G virus and transfusion transmissible virus or may be having autoimmune hepatitis, metabolic disorders, etc. Our study highlights the importance of screening for these relatively rare causes of CLD.

ELISA is a reliable, reproducible and affordable method, which helps in timely detection of hepatitis virus infection. This leads to an early institution of therapy resulting in decreased morbidity and mortality in this group of patients.

Generalized weakness (64%), ascitis (58%), pain abdomen (50%) and jaundice (44%) were the major clinical features of the CLD patients included in the study. Cirrhosis of the liver with portal hypertension,[22] hepatomegaly[14] and splenomegaly[12] were seen in 39%, 64% and 55% patients, respectively. Majority of patients (77.27%) with cirrhosis showed association of HBV infection. All three CLD patients with HCC demonstrated the presence of HBV markers. Combination of factors like multiple sex partners, injectable drug usage and blood transfusion with chronic alcoholism were found to be major risk factors (83.33%) whereas chronic alcoholism alone was found in 3.5% patients in association with HBV. CLDs were of multi-factorial origin where majority were associated with a viral agent like HBV, followed by HCV.

Coinfections of HIV with HBV/HCV in CLDs patients were observed to be major public health concern in terms of risk factors and transmission dynamics of these chronic diseases in North-East India. Proper health awareness and education, early warning signal, altered health seeking behavior, strong vigilance system, political commitment and implementation of transfusion safety are present day needs to avoid impending devastations.

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