Original Article

Hepatitis B virus seroprevalence and its correlation with CD4 cells and liver enzymes among human immunodeficiency virus positive individuals at a tertiary care hospital in North-West India

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Abstract

Background: Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are global health concerns. Due to shared routes of transmission, co-infection is common. Their co-existence can cause severe liver complications and immunological impairment in infected individuals. **Aim:** To find the prevalence of HBV co-infection in HIV patients and to assess the pattern of liver enzymes and CD4 T-cell counts in HIV monoinfected and HIV/HBV co-infected patients. **Materials and Methods:** A total of 342 consecutive confirmed HIV positive treatment naïve patients were tested for hepatitis B surface antigen (HBsAg). Clinical staging was done according to Centers for Disease Control and Prevention classification guidelines. Liver function tests were performed by an autoanalyser. CD4 T-cells were estimated by FACS Calibur. **Results:** Hepatitis B virus co-infection was detected in 8.7% of HIV positive patients as compared to 1.42% in the HIV negative control group ($P < 0.05$). Majority of the HIV monoinfected and co-infected patients were below 38 years. HBsAg positivity was higher in males (9.4%) and the route of transmission was heterosexual. Categorical data revealed significantly higher proportion of alanine aminotransferase and aspartate aminotransferase (AST) in the co-infected patients compared to the monoinfected patients ($P < 0.05$). The HIV/HBV co-infected patients had significantly lower CD4 T-cell counts ($P = 0.03$) and significantly higher AST, alkaline phosphatase and serum bilirubin values ($P = 0.023$, $P = 0.029$, $P = 0.009$ respectively) than the monoinfected group. Males had 1.33 times higher risk than females for co-infection (odds ratio = 1.33; 95% confidence interval 0.57–3.10). **Conclusion:** The prevalence of co-infection was high. Raised levels of liver enzymes and lowered CD4 counts were seen in co-infected patients. These findings underscore the importance of HBV screening of all HIV positive individuals before initiating antiretroviral treatment.

Key words: Co-infection, CD4 T-cells, hepatitis B virus, human immunodeficiency virus, liver enzymes

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Introduction

Liver disease caused by chronic hepatitis B virus (HBV) is emerging as a significant cause of morbidity and mortality among human immunodeficiency virus (HIV)-infected individuals.[1] Since HIV and HBV have epidemiological similarities as regards route of transmission, patients of HIV have a high probability of getting co-infected with HBV. In the Middle East and Indian subcontinent an estimated 2–5% of the general population is chronically infected with HBV.[2] Approximately 10% of the HIV-infected population worldwide suffers from chronic hepatitis B.[3] Co-infection rates of HBV in HIV patients vary worldwide and largely depend upon the geographical location, risk groups, the type of exposure involved and the socioeconomic condition of that particular region.[4] In Europe and the United States of America, HIV/HBV co-infection is around 6–14%.[5,6] In India there are only few reports of the prevalence of HBV in HIV-infected patients. Though the mortality and morbidity rate from HIV/Acquired Immunodeficiency

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Syndrome (AIDS) have declined as a result of highly active antiretroviral therapy (HAART), liver disease due to chronic HBV infection has become a leading cause of death. In HIV/HBV co-infections, HIV infection causes increased rates of persistent HBV infection, cirrhosis, liver-related mortality and risk of hepatocellular carcinoma at lower CD4 T cell counts.[7]

There is paucity of data regarding correlation of liver enzymes and CD4 T-cells among HIV/HBV co-infected patients. Therefore, the present study was undertaken to find the prevalence of HBV co-infection in HIV patients in North-West India and to assess the pattern of liver enzymes and CD4 T-cell counts in both HIV-infected and HIV/HBV co-infected patients.

Materials and Methods

Study samples

The present study was carried out in the Department of Microbiology from January 2011 to March 2012. Patients suspected of HIV, attending clinics as well as direct walk-in patients, referred to the Integrated Counselling and Testing Centre under our department were screened for HIV after performing pretest counselling and informed consent. Our laboratory follows the World Health Organization (WHO) testing strategies and as a routine all confirmed HIV positive patients are referred to the antiretroviral therapy (ART) centre attached to the Medical College during post test counselling.

A total of 342 consecutive confirmed HIV positive (WHO strategy III) treatment naïve patients (age: 18–60 years) who were first time attendees at the ART centre were recruited for the study and anonymously tested for hepatitis B surface antigen (HBsAg). None of these patients gave a history of vaccination against HBV. None of the patients received antiviral therapy against HBV. Socio-demographic and risk factors were recorded on a structured proforma. Clinical staging of the disease was done according to 1993 Centers for Disease Control and Prevention classification (CDC) guidelines for adolescents and adults.[8] A total of 350, age and sex matched, HIV negative blood donors as controls were also included in the study.

Serology

All samples (patients and controls) were screened for HBsAg by enzyme linked immunosorbent assay test (Hepalisa J. Mitra and Co. Private Limited India). Samples positive for HBsAg by first test were retested for confirmation of results.

Biochemistry

Liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST) serum bilirubin, serum alkaline phosphatase (ALP) and serum albumin were carried out for all patients using a fully automatic autoanalyzer (Olympus AU 400 Clinical Chemical Analyzer, Japan) on the same day of blood collection. Normal range: Serum ALT: 10–40 U/L; serum AST: 20–40 U/L; serum bilirubin: 0.2–1.1 mg/dL; serum ALP: 25–120 IU/L; serum albumin: 3.5–5 g/dL. CD4 T-cell count estimation was done by FACSCalibur™ flowcytometer (Becton Dickinson, California, USA).

Statistical analysis

Continuous data was summarised as mean and standard deviation while categorical data was summarised as percentage. Odds ratio (OR) and Chi-square test was applied for analysis of categorical data whereas unpaired *t*-test was used for comparison of continuous data between the two groups, that is, HIV monoinfected and HBV/HIV co-infected. Pearson's correlation coefficient was found to assess correlation between two continuous variables. All statistical calculations were done by using MedCalc Statatical Software, version 14.12.0 (MedCalc Software bvba, MedCalc Ostend, Belgium). P <0.05 was taken as significant for interpretation.

Results

A total of 342 consecutive treatment naïve HIV positive patients were included in the study. Among the study subjects there were 232 males and 110 females (M: F ratio- 2.1:1). These patients had an age between 18 and 60 years (mean 33.5 ± 8.5 years). HBV co-infection was seen in 30 (8.77%) HIV positive patients. This rate was highly significant ($P < 0.05$) when compared to 1.42% in the control group.

Sociodemographic characteristics of study subjects

The subjects were divided into two groups: Those with HIV alone and those co-infected with HBV. The mean age of HIV-infected patients was 33 years (95% confidence interval (CI) ±0.92 years). Majority of the patients (74.3%) were < 38 years of age. In the HIV/HBV co-infected patients the mean age was 37 years (95% CI ±3.3 years). In this group 56.6% were below 38 years of age. There were 22 males and eight females. HBsAg positivity rate was higher in males (9.4%) as compared to females (7.2%) though not statistically significant ($P = 0.49$). Data on risk factors in the HIV monoinfected group revealed that 290/312 (92.9%) of the patients were heterosexual, 2/312 (0.64%) were recipients of blood products and in the rest it was unidentified while among the co-infected patients 30/30 (100%) were heterosexual [Table 1].

Effect of gender

The data revealed that males had 1.33 times higher risk than females for co-infection, however it was not found to

be statistically significant (OR = 1.33; 95% CI 0.57–3.10, $P = 0.50$).

Centers for Disease Control and Prevention classification staging and CD4 T-cell count

Patients were categorized according to CDC classification system into groups A, B and C. In the HIV-infected patients 65/312 (20.8%) were classified as group A, 141/312 (45.1%) as group B and 106/312 (33.9%) as group C. In the co-infected group 2/30 (6.6%) were classified as group A, 13/30 (43.3%) as group B and 15/30 (50%) as group C [Figure 1]. The mean CD4 T-cell count in the HIV-infected group was 310 cells/μL while in the HIV/HBV co-infected group it was 215 cells/μL. The CD4 T-cell profile between the HIV and HIV/HBV co-infected group was not significant ($P = 0.09$).

Table 1: Socio-demographic characteristics and CD4 levels of HIV monoinfected and HIV/hepatitis B virus co-infected patients

Parameter	HIV monoinfected No. (%) (n = 312)	HIV/HBV Co-infected No. (%) (n = 30)
Gender		
Male	210 (67.31)	22 (73.33)
Female	102 (32.69)	08 (26.67)
Age		
18-28	103 (33.01)	6 (20)
29-38	129 (41.34)	11 (36.67)
39-48	58 (18.59)	9 (30)
49-58	22 (7.05)	2 (6.67)
>58	0 (0)	2 (6.67)
Education status		
No formal education	206 (66.02)	10 (33.33)
Formal education	106 (33.97)	20 (66.67)
Occupation		
Unemployed	34 (10.8)	3 (13.6)
Industrial worker	32 (10.2)	2 (9.09)
Farmer	38 (12.1)	4 (18.1)
Driver	42 (13.4)	4 (18.1)
Service	31 (9.9)	1 (4.5)
Housewife	102 (0)	8 (26.6)
Others	33 (10.5)	0 (0)
Risk factors		
HRSC	290 (92.9)	30 (100)
BT	2 (0.64)	0 (0)
Unknown	20 (6.41)	0 (0)
CD4		
>500	65 (20.83)	2 (6.67)
201–500	141 (45.19)	13 (43.33)
<200	106 (33.97)	15 (50)

HRSC: High risk sexual contact; BT: Blood transfusion; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus

Profile of liver enzymes

The mean ALT, AST and ALP levels in HIV monoinfected patients were 37 U/L, 59 U/L and 236 IU/L respectively. The mean ALT, AST and ALP levels in HIV/HBV co-infected patients were 49 U/L, 78 U/L and 874 IU/L respectively. The baseline values are shown in Table 2. Co-infected patients had higher proportions of elevated values of AST (86.6% vs. 51.6%) and ALT (56.6% vs. 25.3%) than HIV alone [Figure 2]. The difference was statistically significant ($P < 0.05$).

Correlation of CD4 cells with liver enzymes

When Pearson correlation coefficient was calculated between CD4 T-cell count and liver enzymes, it was found that AST, ALT and ALP had significantly negative correlation in the HIV monoinfected group. In the co-infected group negative correlation was present similarly as in the HIV monoinfected group but was not statistically significant [Figure 3]. The HIV/HBV co-infected patients had significantly lower CD4 T-cell counts than the HIV monoinfected group and significantly higher AST, ALP and serum bilirubin values. The mean serum ALT was also higher in the HIV/HBV co-infected group but it failed to show statistical significance probably due to less number of co-infected patients [Table 2].

Discussion

This study investigated the seroprevalence of HBV in treatment naïve HIV positive patients and tried to correlate levels of liver enzymes and CD4 counts in HIV monoinfected and HIV/HBV co-infected patients. The prevalence of hepatitis B among the study group was 8.7% which is comparable to studies from different parts of India.[9,10] However, some studies have reported a lower rate of HIV/HBV co-infection.[11,12] In the present study the prevalence of co-infection was higher in males than in females (9.4% vs. 7.2%). The difference was not statistically significant ($P = 0.49$). This finding is comparable with studies from Africa and India.[13,14] This trend can be explained on the basis of higher rate of sexual promiscuity and other exposure risks in males. The major risk factor was heterosexual accounting for 92.9% of patients. This is in concurrence with other studies from India.[9,15] Majority of

Table 2: Correlation of CD4 and liver enzymes among HIV monoinfected and HIV/HBV co-infected individuals

Status (no.)	Mean±standard deviation				
	CD4	Total bilirubin	AST	ALT	Alk. PO4
HIV monoinfected (312)	310.35±231.17	0.67±0.44	59.4±43.91	37.58±31.69	236.21±267
HIV/HBV co-infected (30)	215.1±203.84	1.07±0.78	78.31±39.24	49.13±26.68	874.8±1524.6
Significance (2-tailed)	0.030	0.009	0.023	0.055	0.029

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Alk.PO4: Alkanine phosphatase; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus

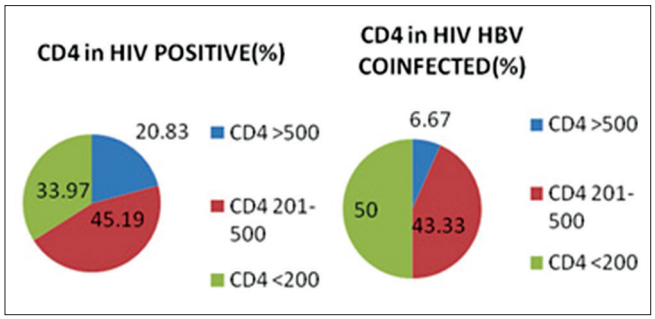

Figure 1: CD4 T-cell counts in human immunodeficiency virus (HIV) monoinfected and HIV/hepatitis B virus co-infected patients

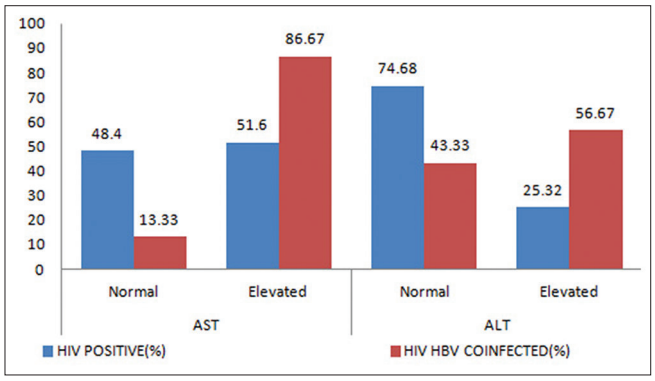

Figure 2: Categorical data of aspartate aminotransferase and alanine aminotransferase in human immunodeficiency virus (HIV) monoinfected and HIV/hepatitis B virus co-infected patients

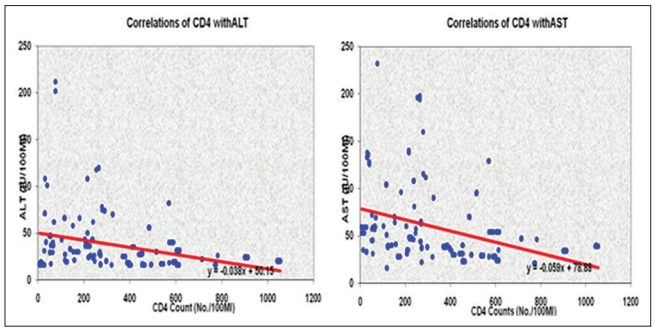

Figure 3: Correlation of CD4 T-cell counts with alanine aminotransferase and aspartate aminotransferase in human immunodeficiency virus patients

the HIV positive patients (74.3%) had an age between 18 and 38 years and a similar trend was seen in HIV/HBV co-infected patients (56.6%) indicating a common mode of transmission of these viruses which has been documented by other studies.[9,15]

In both the HIV monoinfected and HIV/HBV co-infected patients seropositivity was high in truck drivers and farmers. The poor educational status of these patients in the present study contributes to limited knowledge and awareness of HIV transmission and thus higher seropositivity rates.

The mean CD4 T-cell counts in the HIV monoinfected patients was 310 cells/μL while in the HIV/HBV co-infected group it was 215 cells/μL which is similar to a study from Gondar but lower mean CD4 T-cell values have been reported by a Nigerian study.[16,17] A study in India has reported a mean CD4 T-cell of 334 cells/μL in HIV monoinfected patients comparable with our study but a higher mean CD4 T-cell count of 294 cells/μL in co-infected patients.[9] In the present study 50% of HIV/HBV co-infected patients were in CDC stage C indicating that the incidence of HBV co-infection rises with HIV disease progression. Significant difference of co-infection existed between the symptomatic and asymptomatic groups of HIV-infected patients ($P < 0.05$). HIV disease reportedly leads to massive impairment of cell mediated responses and enhances the kinetics of hepatotropic viral replication.[9] This causes increased rates of chronicity, faster disease progression, prolonged HBV viremia and increased liver-related morbidity. Patients with AIDS apparently are less likely to clear HBV infection after exposure or more likely to reactivate a latent HBV infection or both.[18,19] Furthermore some HBV factors on HIV transcription favour enhanced HIV replication leading to faster CD4 T-cell decline in HIV/HBV co-infected individuals.[20]

Elevated transaminases are a marker of liver inflammation and have been shown to be common in HIV/AIDS patients. In the present study compared with people harbouring only HIV, more number of HIV/HBV co-infected patients had elevated liver enzymes. Categorical univariate analysis of transaminases in HIV co-infected and HIV monoinfected patients revealed elevated ALT in 56.7% versus 25.3% and AST in 86.6% versus 51.6% levels respectively. The difference was statistically significant ($P < 0.05$). Though ALP levels were elevated in both groups it was not statistically significant. A study among Nigerian patients showed abnormal liver enzymes in 87.6% patients out of whom only 2.3% were co-infected with Hepatitis B.[21] Another study from India showed elevated ALT levels in 43.3% of their co-infected patients.[15] In HIV positive patients the increase in hepatic enzymes could be secondary to multiple factors such as alcoholism, lipid lowering drugs, antibiotics, co-infection with hepatotropic viruses or opportunistic organisms as well as direct hepatic damage caused by HIV.[22] Even though more number of co-infected patients have elevated transaminases this elevation is mild and associated with higher risk of progression to cirrhosis.[23,24] This was apparent in the present study as there was only mild elevation of both ALT and AST in HIV/HBV co-infected patients and a mild elevation of only AST in the HIV monoinfected patients. This finding may be related to the impairment of immunity in advanced HIV, which despite higher rates of HBV replication, results in less inflammation and necrosis. Thus, in HIV-infected patients, HBV co-infection is an independent predictor for cirrhosis, hepatocellular carcinoma, and mortality.[25,26]

The present study has certain limitations. Firstly, this is a cross-sectional study unable to adequately establish a causal relationship between the time of exposure and subsequent infection. Secondly, the study was conducted with patients limited to a tertiary care referral hospital setting and not to a community setting. Thirdly repeated measurements of liver function tests were not done as it is known that liver-related morbidity can take a fluctuating course. This could have missed a few short lasting episodes of liver enzyme elevations.

Conclusion

We recommend that all HIV positive patients should be routinely screened for HBV markers before initiation of HAART as this practice would guide correct choice of drug combination. Also there should be a regular monitoring of liver enzymes and CD4 T-cell counts. This would help in reducing morbidity and mortality from antiretroviral drug associated hepatotoxicity among these patients.

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