Hepatitis B & C virus infection in HIV seropositive individuals & their association with risk factors: A hospital-based study

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Background & objectives: Hepatitis B and hepatitis C virus (HBV and HCV) cause acute and chronic hepatitis, and infections with HBV and HCV are common in HIV-infected patients. The present study was conducted to determine the co-infection of hepatitis B and C virus in stored serum samples of HIV-positive/negative individuals attending an Integrated Counselling and Testing Centre (ICTC) in north India and their association with certain risk factors.

Methods: This study included a total of 840 serum samples, of which 440 were from HIV seropositive individuals and 400 were from control individuals seeking voluntary check-up of HIV status at ICTC. Serum samples were used for the detection of HBV and HCV infection.

Results: HBV infection (11%) was found to be less in contrast to HCV (13%) amongst the HIV seropositive. In controls, HBV and HCV infection was two and three per cent, respectively. Co-infection of HBV and HCV was found in 15 of 109, and in controls, it was 2 of 15. Age group between 21 and 40 was significantly associated with HBV and HCV infection. Heterosexual contact was the leading mode of acquiring HBV and HCV infection.

Interpretation & conclusions: HBV and HCV co-infection was found to be significantly higher in HIV-positive individuals in comparison to normal population. Hepatitis virus infection leads to rapid progression of liver cirrhosis in HIV-infected patients. Routine check-up of HIV seropositive patients for hepatitis virus may be required to monitor clinical outcome.

Key words Co-infection - hepatitis B - hepatitis C - HIV

Hepatitis B virus (HBV) and hepatitis C virus (HCV) cause acute and chronic hepatitis. Around two billion population are infected with HBV, and approximately 170 million people suffer from HCV infection worldwide¹. Amongst the human immunodeficiency virus (HIV)-infected patients, about 2-4 million have been reported to have chronic HBV co-infection, while 4-5 million are co-infected with HCV². The severity of HBV and HCV infection depends on multiple factors including age, mode of transmission and immune status at the time of infection.

Globally, around 38.6 million of HIV infections are estimated to have occurred at the end of 2005³, while chronic HBV and chronic HCV were reported...
About 5 ml of whole blood was collected aseptically by venepuncture. The collected blood was allowed to clot; serum was separated by centrifugation at room temperature. Antibody to HIV infection was tested by three rapid diagnostic tests, each of different antigen or test principle. Testing algorithm adhered to National Guidelines on HIV testing specified by the National AIDS Control Organisation (NACO), Ministry of Health & Family Welfare, and Government of India. Detection of the HIV infection was done using approved test kits, namely, (i) Combaids (Arkray Healthcare Private Limited, Japan), (ii) Retroscreen (Tulip Diagnostics Private Limited, Goa), (iii) Instachk (Transasia Bio- Medicals Limited, Mumbai).

The HIV seropositive serum samples were labelled, coded and stored at −20°C. The coded samples were tested for HCV and HBV using ELISA kits.

Detection of HCV infection in HIV positive patients: Detection of the HCV infection was done using (Monolisa® HCV Ag-Ab ULTRA and Monolisa® Biorad, Gurugram, India), in serum samples of HIV-positive patients. Test was performed according to the manufacturer’s instructions. After the reaction was terminated, the optical density (OD) was measured at 450/620 nm using a ‘sunrise’ ELISA reader (Mumbai). The presence of antibodies to HCV and / or HCV capsid antigen was determined by comparing the absorbance for each sample with the cut-off value, which was calculated by dividing the mean of the OD readings for the three positive controls by four. Readings below the cut-off value were considered non-reactive; samples below the cut-off value by 10 per cent were retested. Samples above the cut-off values were considered initially reactive and retested in duplicate before a final interpretation was made. Results were compared with those of AxSYM HCV version 3.0 (Abbott GmbH, Wiesbaden, Germany), a microparticle enzyme immunoassay (MEIA) for the qualitative detection of antibodies to hepatitis C virus in human serum or plasma for the same samples.

Detection of HBV co-infection in HIV-positive patients: HbsAg was detected by ELISA using the Monolisa® Hbs Ag ULTRA kits, Bio-Rad as per manufacturer’s instructions. All samples were tested in duplicate.
Statistical analysis: All statistical analyses were performed using the software GraphPad InStat version 3.0 (GraphPad Software LaJolla, CA, USA). Chi-square test/Fisher’s exact test (SPSS Inc., Chicago, IL, USA) was used to compare the presence of HBV and HCV between HIV seropositive women/healthy controls.
The odds ratio and 95 per cent confidence intervals and multivariate analysis were done as a measure of the association between demographic details and different infections and their risk.

**Results**

The socio-demographic characteristics of the study population are given in Table I. Of the 440 HIV-positive individuals, 275 (63%) were males and 165 (37%) females. The mean age of the study group was 34±11.4 yr (range 2-70 yr); 340 (77%) were married, and partners of 182 (41%) were also seropositive. Most of the patients had the habit of smoking (51%) and alcohol (27%) intake. Data were also included for the 400 individuals attending the hospital for voluntary check-up of HIV status, who were found to be HIV negative and tested during the same period for HCV and HBV infection. There were 104 (26%) males and 296 (74%) females in this category.

**Presence of viral co-infections in HIV positive and negative individuals:** The co-infection with hepatitis viruses in HIV-positive patients was 14 per cent. The frequency of HBV infection in HIV-positive patients was 11 per cent, while it was two per cent in HIV-negative individuals (*P*<0.01) [95% CI 8.41 (3.5-19.8)]; HBV co-infection in HIV seropositives was 8-fold higher than in HIV-negative individuals (Table II). HCV co-infection amongst HIV seropositives was 13 per cent as compared to three per cent in HIV-negatives (*P*<0.001).

The infection with either HBV or HCV was seen in 21.4 per cent (94/440) in HIV-infected patients, it was 3.3 per cent in HIV-negatives (*P*<0.01), [95% CI 6.77 (3.7-12.2)]. Rate of co-infection was 6-fold higher in HIV patients compared to HIV-negative individuals.

**Association of age with presence of HBV and HCV infection in HIV seropositives:** The majority of the HIV-infected individuals were in the age group of 21-40 yr (34%). Mean age of HIV-positive patients was 34 yr. HBV and HCV co-infection with HIV was predominant in the age group 31-40 yr. The rate of infection was low in the age group of 1-10 yr (Fig. 1).

**Mode of transmission and rate of infection:** In the present study, heterosexual contact was the leading mode of acquiring HBV and HCV infection, at 42 and 44 per cent, respectively, followed by blood transfusion (4%) and homosexual contact (2-3%). Mother-to-child transmission accounted for approximately (1%), while the rate of transmission of infection was higher for HBV (4%) and HCV (8%) in patients inadvertently exposed to contaminated needle/syringe (NS) of infected person (Fig. 2).

**Discussion**

The present study highlighted the presence of HBV-HCV co-infection amongst the HIV-infected patients and comparing it with apparently healthy north Indian population. In our study, majority of HIV-infected individuals were in the age group of 21-40 yr (34%). Mean age of HIV-positive patients was 34 yr. HBV and HCV co-infection with HIV was predominant in the age group 31-40 yr. The rate of infection was low in the age group of 1-10 yr (Fig. 1).

**Table II. Presence of hepatitis B virus and hepatitis C virus in cases and controls**

| Infection       | HIV+ve (n=440) | HIV-ve (n=400) | *P*     | OR (95% CI) |
|-----------------|---------------|---------------|--------|------------|
| Hepatitis B+    | 50 (11)       | 6 (2)         | <0.001 | 8.419 (3.56-19.86) |
| Hepatitis B-    | 390 (89)      | 394 (98)      |        |            |
| Hepatitis C+    | 59 (13)       | 9 (3)         | <0.001 | 7.569 (3.56-16.05) |
| Hepatitis C-    | 381 (87)      | 391 (97)      |        |            |
| Hep B+ Hep C+   | 15 (14)       | 2 (13)        | 0.96   | 1.037 (0.212-5.064) |
| Hep B+/Hep C-   | 94 (86)       | 13 (87)       |        |            |

**Fig. 1.** Age-related distribution of hepatitis B and C virus in HIV-positive patients.
seropositive patient’s age was 21-40 yr and they were sexually active. This finding was in concordance to that reported previously\textsuperscript{13,14}. Some other demographic factors were also found to be significantly associated with HIV infection.

HBV (11\%) and HCV (13\%) co-infection in HIV seropositive was higher than in control population, which is in good agreement with previous studies from India\textsuperscript{15,16}. Mittal \textit{et al}\textsuperscript{17} reported low prevalence of HBV and HCV in Indian population. We observed a high prevalence of reproductive tract infections in HIV-seropositive women\textsuperscript{18}. The immunosuppressed patients fall into the high-risk group of acquiring HBV infection, while the transmission of HCV occurs more efficiently through percutaneous routes\textsuperscript{19}.

In HIV patients, the liver damage may be directly associated with HIV infection or it may be due to events such as prior hepatitis/intravenous drug abuse and alcoholism in already immunosuppressed patients\textsuperscript{7}. Probably, other factors such as malnutrition, sepsis or administration of possible hepatotoxic antiretroviral medication may also be responsible for liver damage\textsuperscript{20}. Highly active antiretroviral therapy recipients are more vulnerable to other infections and persistence of HBV and HCV infections. The presence of HIV infection makes the transmission of hepatitis viruses easier, through prenatal as well as sexual contact\textsuperscript{21}. Moreover, pregnant women are prone to infection, perhaps owing to low immunity and hormonal changes\textsuperscript{22}.

Our findings on HBV co-infection in HIV-infected patients were similar with the study reported by Mudawi \textit{et al}\textsuperscript{23} but slightly lower than those reported by Abera \textit{et al}\textsuperscript{24} from Sudanese and Ethiopian population, respectively.

The heterosexual contacts were found with higher prevalence of HBV and HCV in HIV-seropositive participants\textsuperscript{25}. In the present study, transmission of HCV and HBV from mother-to-child was found in one per cent HIV-positive women only. Besides, transmission of HCV infection was also higher amongst the patients exposed to contaminated needles and syringes as well as multiple use of single-use needles and syringes of infected person, which was in accordance with previous report from India\textsuperscript{26}. HCV co-infection was higher in HIV-positive male patients in comparison to female group, perhaps attributable to higher rate of sexual promiscuity\textsuperscript{15,27}.

In conclusion, our findings showed that the HIV seropositive individuals had a high risk of acquiring HBV and HCV co-infections predominantly through heterosexual contact. Therefore, it would be beneficial if the HIV seropositive individuals screened routinely for parallel HBV/HCV infection.

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