

# Proportion of syphilis and hepatitis B and C virus infections among the Integrated Counselling and Testing Centre attendees of a tertiary care hospital

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## Abstract

**Introduction:** Human Immunodeficiency Virus (HIV) affects the immune system of the body, causing a breakdown in its normal defenses and leaving it vulnerable to a host of life-threatening diseases. High-risk behaviors and routes of transmission for sexually transmitted infections such as syphilis, hepatitis B and hepatitis C are identical with HIV. This study was conducted to assess the proportion of syphilis and hepatitis B and C virus infections in HIV-positive and HIV-negative individuals, and their association with socioeconomic and other factors in Integrated Counselling and Testing Centre (ICTC) attendees, and to determine the association of absolute CD4+ T-lymphocyte count with these co-infections in HIV-positive individuals. **Materials and Methods:** The study was conducted in the Department of Microbiology of a tertiary care hospital. It included 100 HIV-positive individuals and 100 matched HIV-negative controls attending the ICTC. HIV-positive patients on antiretroviral therapy and patients with history of current/past treatment for chronic hepatitis infection were excluded from the study. Blood samples were tested for HIV, syphilis, and hepatitis B and C infections. **Results:** The prevalence of syphilis, hepatitis B, and hepatitis C was observed in 3.5%, 2%, and 10% of patients, respectively. The frequency of hepatitis B virus (HBV) infection in HIV-positive and HIV-negative individuals was 1% and 3%, respectively. Hepatitis C virus (HCV) infection among HIV-positive and HIV-negative patients was 16% and 4%, respectively. Syphilis was seen in 7% of the HIV-infected patients. The mean CD4+ count for the HIV-positive patients with either syphilis, HBV, or HCV infections was  $252 \pm 137.5$  cells/ $\mu$ l. Significant associations between HIV infection and education below high school, IV drug abuse, and multiple sexual partners were observed. **Conclusions:** The HIV-infected patients were observed to be at an increased risk of acquiring syphilis and HCV co-infections through the shared routes of transmission. Routine screening of these patients for concurrent syphilis and viral hepatitis may aid in prompt diagnosis and treatment with improved outcomes, which in turn may decrease the further spread of these infections.

**Key words:** Co-infection, hepatitis B, hepatitis C, HIV, syphilis

## Introduction

Human Immunodeficiency Virus (HIV) affects the immune system of the body, causing a breakdown in its normal defenses and leaving it vulnerable to a host of life-threatening diseases. High-risk behaviors and routes of transmission for sexually transmitted infections (STIs) such as syphilis, hepatitis B virus (HBV), and hepatitis C virus (HCV) infections are identical with HIV.

The rollout of highly active antiretroviral therapy (HAART) has had a major impact on HIV-associated mortality in low- and middle-income countries, leading to an increased

life expectancy in people with HIV, and the disease is now considered as a chronic manageable condition.<sup>[1]</sup> As people with HIV live longer on HAART, coexisting infections can contribute to the increasing morbidity and mortality among these patients. Ulcerative STIs provide a great opportunity for HIV transmission. Syphilis has also been seen to be associated with a decline in CD4+ T-lymphocyte count and an increased level of

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HIV-1 RNA in HIV-positive patients.<sup>[2-4]</sup> Moreover, these patients may experience a higher rate of syphilis treatment failure in comparison to the individuals without HIV infection. In case of co-infection with HBV or HCV, HIV modifies the natural history of the disease, leading to an increased percentage of carrier state, and also accelerates the HBV- and HCV-related liver disease.<sup>[1,5,6]</sup> About one-third of deaths due to liver diseases in HIV-infected patients are attributable to co-infection with either HBV or HCV.<sup>[7]</sup>

Around 38 million people are living with HIV worldwide.<sup>[8]</sup> According to the WHO global estimates for 2016, there were approximately 376 million new infections of curable sexually transmitted diseases, with syphilis accounting for 6.3 million of these cases.<sup>[9]</sup> Chronic hepatitis B and C infections were reported in 296 million and 58 million people worldwide, respectively.<sup>[10]</sup> About 2.6 million people living with HIV are co-infected with HBV and 2.9 million with HCV.<sup>[11]</sup> India, with a current population of 1.39 billion, is the second most populous nation in the world.<sup>[12]</sup> The country is carrying a burden of 2.1 million HIV cases, 40 million HBV carriers, and 6–12 million chronically infected HCV cases.<sup>[13,14]</sup> Given the global resurgence of syphilis and increasing prevalence of HIV-1 and syphilis co-infection,<sup>[15]</sup> and considering the high burden of HBV- and HCV-infected people in the country, seroepidemiological studies are critical to understand the pattern and distribution of these co-infections within the population.

The objectives of this study were to assess the proportion of syphilis and hepatitis B and C virus infections in HIV-positive and HIV-negative individuals, and their association with social, economic, demographic, and other factors in Integrated Counselling and Testing Centre (ICTC) attendees, and to determine the association of absolute CD4+ T-lymphocyte count with syphilis and hepatitis B and C co-infection in HIV-positive individuals.

## Materials and Methods

The study was conducted in the Department of Microbiology of a tertiary care hospital from October 2019 to March 2021. It was an observational and cross-sectional study, which included 100 HIV-positive individuals  $\geq 18$  years of age, and 100 age- and sex-matched HIV-negative controls attending the ICTC. HIV-positive patients, who had already been initiated on antiretroviral therapy, and patients with history of current or past treatment for chronic hepatitis B and/or chronic hepatitis C infection were excluded from the study. The study protocol was approved by the ethics committee of the institution.

Serological tests were done for HIV, syphilis, and hepatitis B and C infections for each individual, and absolute CD4+ T-lymphocyte count was performed for the HIV-positive individuals. Socioeconomic status was assessed using the revised Kuppuswamy's Socioeconomic Status Scale.<sup>[16]</sup>

### Collection of specimen

For serological assays, 4–5 ml of blood was collected aseptically by venepuncture. The collected blood was allowed to clot; serum was separated by centrifugation at room temperature. For the absolute CD4+ T-lymphocyte count, 3 ml of blood sample was collected in a K-EDTA vial.

### Laboratory diagnosis

Serodiagnosis of HIV infection was performed using upto three commercially available rapid assays, each of

different antigens or test principles. Detection of the HIV infection was done using approved test kits, namely, (i) COMBAIDS®-RS Dot Immunoassay, (ii) VoxPress HIV 1 and 2 Rapid Test, (iii) TREDRO HIV 1-2 Ab. The interpretation of result, i.e., HIV positive, HIV negative, and HIV indeterminate, was done as per the National AIDS Control Organisation (NACO) HIV testing strategies 2B or 3, as applicable.

For diagnosis of syphilis, serum samples were screened by the venereal disease research laboratory (VDRL) slide flocculation test. All sera, found to be reactive by VDRL, were confirmed by the *Treponema pallidum* particle hemagglutination (TPHA) test (Plasmatec TPHA Test Kit). Those patients, whose serum samples tested positive for both VDRL and TPHA, were considered to be having an active-syphilis infection.

Hepatitis B infection was diagnosed by the detection of HBs antigen (HBsAg) in the serum samples, by using a commercially available rapid kit (ABON Hepatitis B Surface Antigen Rapid Test Device). The presence of HBsAg indicated current HBV infection. As for hepatitis C infection, anti-HCV antibodies were detected by using a commercially available rapid kit (Standard Q HCV Ab Rapid Test Device) and their presence indicated current or resolved infection.

Absolute CD4+ T-lymphocyte count in HIV-positive patients was performed by flowcytometry, using a commercially available assay (CyFlow Counter and CD4% easy count kit).

### Statistical analysis

All statistical analyses were performed using the software SPSS version 25.0. Qualitative variables were analyzed using Chi-square or Fisher's exact test (SPSS Inc., Chicago, IL, USA).  $P < 0.05$  was considered statistically significant.

## Results

The sociodemographic characteristics and clinical profile of the study population are given in Tables 1 and 2. Of the 100 HIV-positive individuals, 76 (76%) were male and 24 (24%) were female. The mean age of the study group ( $n = 200$ ) was  $35 \pm 12.7$  years (range: 18–80 years), with 81 (40.5%) patients in the 26–35-year age group; 129 (64.5%) were married, 121 (60.5%) were employed, and 128 (64%) belonged to the middle class (upper-middle and lower-middle socioeconomic status). Out of 200 patients, 54 (27%) were illiterate, whereas among the HIV-positive patients, 34 (34%) of the patients had received no education, while 38 (38%) patients in this group were literate or had studied till secondary school. A statistically significant association was observed between HIV infection and education below high school ( $P = 0.001$ ).

Among the HIV-positive individuals, 30 (30%) patients had history of seropositive partners ( $P < 0.001$ ), while 21 (21%) of them had multiple sexual partners ( $P < 0.001$ ) and 20 (20%) patients were intravenous drug users ( $P < 0.001$ ).

The most common presenting manifestations among HIV-positive individuals were weight loss (61%), myalgia (31%), and lymphadenopathy (9%). Twenty-four (24%) of these patients also had history of tuberculosis and 10 (10%) had history of blood transfusion. Statistically significant associations were seen between HIV infection and myalgia ( $P < 0.001$ ), weight

**Table 1: Sociodemographic characteristics of study population**

| Characteristics               | HIV Positive (n=100) | HIV Negative (n=100) | P      |
|-------------------------------|----------------------|----------------------|--------|
| Age (in years)                |                      |                      |        |
| ≤40                           | 79                   | 69                   | 0.107  |
| >40                           | 21                   | 31                   |        |
| Gender                        |                      |                      |        |
| Female                        | 24                   | 24                   | 0.999  |
| Male                          | 76                   | 76                   |        |
| Educational Status            |                      |                      |        |
| High School & above           | 28                   | 51                   | 0.001  |
| Below High School             | 72                   | 49                   |        |
| Marital Status <sup>#</sup>   |                      |                      |        |
| Married                       | 58                   | 71                   | 0.237  |
| Unmarried                     | 32                   | 27                   |        |
| Employment Status             |                      |                      |        |
| Employed                      | 60                   | 61                   | 0.885  |
| Unemployed                    | 40                   | 39                   |        |
| Socioeconomic Status          |                      |                      |        |
| High                          | 66                   | 66                   | 0.118  |
| Low                           | 34                   | 34                   |        |
| Personal Profile              |                      |                      |        |
| Alcohol intake                | 29                   | 19                   | 0.098  |
| IV drug abuse                 | 20                   | 0                    | <0.001 |
| Multiple sexual partners      | 21                   | 0                    | <0.001 |
| Partner status (HIV positive) | 30                   | 0                    | <0.001 |
| Smoking                       | 23                   | 17                   | 0.289  |
| Tattooing                     | 3                    | 3                    | 0.999  |
| Tobacco chewing               | 24                   | 11                   | 0.016  |

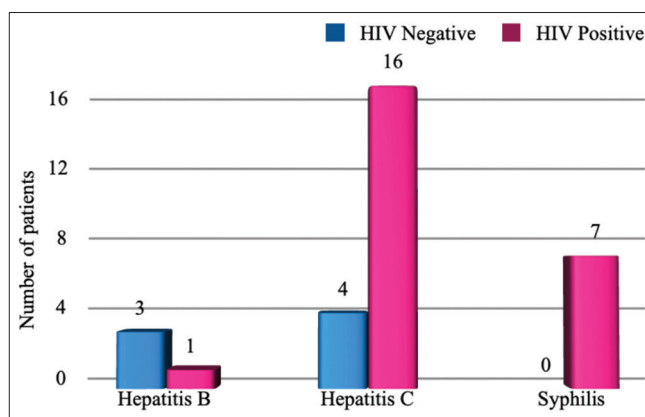
<sup>#</sup>n=188 (2 divorced and 10 widowed study-subjects were not included the analysis)

**Table 2: Clinical profile of study subjects**

| Clinical History  | HIV Positive (n=100) | HIV Negative (n=100) | P      |
|-------------------|----------------------|----------------------|--------|
| Blood transfusion | 10                   | 3                    | 0.005  |
| Genital ulcer     | 1                    | 0                    | 0.316  |
| Jaundice          | 4                    | 1                    | 0.171  |
| Lymphadenopathy   | 9                    | 0                    | 0.002  |
| Myalgia           | 31                   | 5                    | <0.001 |
| Oral thrush       | 2                    | 1                    | 0.561  |
| Seizure           | 4                    | 0                    | 0.043  |
| Sore throat       | 11                   | 1                    | 0.003  |
| Tuberculosis      | 24                   | 11                   | 0.016  |
| Weight loss       | 61                   | 11                   | <0.001 |

loss ( $P < 0.001$ ), lymphadenopathy ( $P = 0.002$ ), and history of blood transfusion and tuberculosis ( $P = 0.005$  and  $0.016$ , respectively).

Presence of co-infections in HIV-positive and HIV-negative individuals: The prevalence of syphilis, hepatitis B and C among both HIV-positive and -negative individuals was 3.5%, 2% and 10%, respectively [Figure 1]. The frequency of HBV infection in HIV-positive patients was 1%, while it was 3% in HIV-negative individuals ( $P = 0.312$ ). HCV co-infection among HIV-seropositive patients was 16% as compared to 4% in HIV-negative patients ( $P = 0.012$ ). Syphilis was only seen in the HIV-infected cohort ( $P = 0.007$ ). One of the HIV-negative patients was observed to be seropositive for both hepatitis B and C infections, and one HIV-positive patient was seropositive for syphilis and hepatitis B and C infections.

**Figure 1: Syphilis and hepatitis B and C virus infection among HIV-negative and HIV-positive individuals**

The infection with either syphilis, HBV, or HCV was seen in 22 percent (22/100) of HIV-infected patients, while it was 7 percent (7/100) in HIV-negative patients.

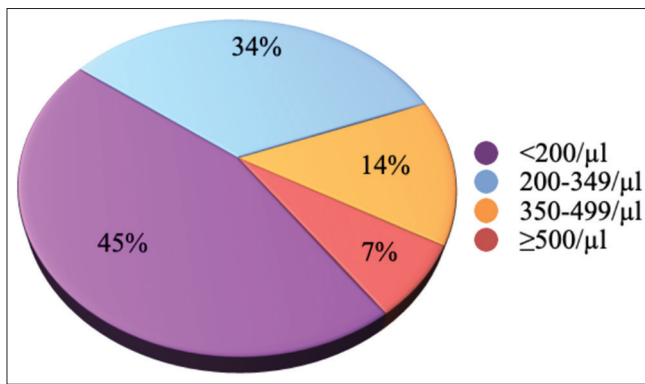
#### CD4+ T-lymphocyte count distribution among HIV-positive individuals

Most of the HIV-positive patients (45%) had CD4+ T-lymphocyte count  $<200$  cells/ $\mu$ l. The mean CD4+ count was  $238 \pm 148.4$  cells/ $\mu$ l [Figure 2].

#### Factors associated with the presence of syphilis and hepatitis B and C infections in study subjects

The sociodemographic characteristics of the patients infected with syphilis, hepatitis B, and/or hepatitis C infections are given in Table 3. Among the HIV-negative individuals, majority of the patients with hepatitis B and/or C were married males above 40 years of age, belonging to lower-middle and upper-lower socioeconomic classes. In case of HBV infection, these patients had attained education till high school or above, and were employed. The HCV infection was observed in patients educated below high school and mostly unemployed. Among the HIV-positive individuals, most of the patients co-infected with syphilis, hepatitis B, and/or hepatitis C were unmarried males belonging to 18–45 years of age group. Majority of these patients had education level below high school and belonged to lower-middle and upper-lower socioeconomic classes. A statistically significant association was observed between the presence of HCV infection and unmarried individuals ( $P = 0.035$ ).

Most of the patients with syphilis, HBV, and/or HCV co-infection in HIV-positive cohort gave history of intravenous drug abuse (54.5%), smoking (50%), alcohol intake (31.2%), and multiple sexual partners (31.2%). Sixty percent of IV drug users in our study were observed to have HCV infection. Statistically significant associations were observed between HCV infection and IV drug abuse ( $P < 0.001$ ) and smoking ( $P < 0.003$ ). In our study, most of the HIV-positive patients with syphilis, hepatitis B, and/or hepatitis C infection gave history of weight loss (59.1%), tuberculosis (40.9%), and myalgia (36.4%), while others had history of jaundice (13.6%), skin lesions (13.6%), lymphadenopathy (9.0%), and genital ulcer (4.5%). Statistically significant associations were observed between HBV infection and the presence of jaundice ( $P = 0.004$ ). In case of HCV infection, statistically significant associations were seen for jaundice ( $P < 0.001$ ), genital ulcer ( $P = 0.003$ ), tuberculosis ( $P = 0.03$ ), and myalgia ( $P = 0.037$ ).



**Figure 2:** CD4+ T-lymphocyte count distribution among HIV-positive individuals. HIV = Human immunodeficiency virus

The mean CD4+ count for the HIV-positive patients with either syphilis, HBV, or HCV infections was 252 ± 137.5 cells/μl, which was found to be statistically insignificant, when compared to HIV-positive individuals without these co-infections.

### Discussion

The present study highlighted the presence of syphilis, HBV, and HCV infections among the HIV-positive and HIV-negative individuals attending the ICTC of a tertiary care hospital in North India. The majority of the HIV-seropositive individuals in our study were in the 26–35-year age group, and were sexually active. Furthermore, 34% of these patients had received no education, while another 38% of patients in this group were literate or had studied till secondary school. A statistically significant association was observed between HIV infection and education below high school ( $P = 0.001$ ). The higher rate of illiteracy among the HIV cohort in our study could have contributed to the lack of awareness about sexually transmitted diseases and safe sex practices. These findings were in concordance with those reported previously.<sup>[17,18]</sup> Several clinical features, such as myalgia ( $P < 0.001$ ), weight loss ( $P < 0.001$ ), and lymphadenopathy ( $P = 0.002$ ), were also found to be significantly associated with HIV infection.

Syphilis (7%) and HCV (16%) co-infections in HIV seropositive were higher than in the control population, which is in good agreement with previous studies from India.<sup>[5,19]</sup> In a study conducted by Khan *et al.*, the prevalence of HBV, HCV, and syphilis co-infections in HIV-positive patients was reported as 4.4%, 1.4%, and 2.2%, respectively.<sup>[20]</sup> Sharma *et al.* conducted a similar study in a tertiary care hospital in Delhi and observed that 11% and 13% of HIV-seropositive patients were co-infected with HBV and HCV, respectively.<sup>[17]</sup> In another study conducted at a different tertiary care hospital in New Delhi by Sharma *et al.*, the prevalence of HBV infection was <10% in HIV-seropositive patients, while it was 1.56% for HCV infection.<sup>[21]</sup> The data reveal that observations have been highly variable for hepatitis B and C from different regions of the country. One of the major determinants of prevalence is geographical location, and different HIV-infected populations show marked variations in the prevalence of HBV and HCV co-infection depending upon the endemicity.<sup>[22,23]</sup> It is noticeable in our study that HCV seropositivity in HIV patients is higher than that of HBV, which may be a reflection of the population from which the patients were enrolled. In our study, intravenous drug users constituted 10% of the study population, which may have attributed to the higher prevalence of HCV infection.

**Table 3: Sociodemographic characteristics of individuals with syphilis, hepatitis B, and hepatitis C infections**

| Characteristics                   | Syphilis |     |       | Hepatitis B |     |       | Hepatitis C |     |       |
|-----------------------------------|----------|-----|-------|-------------|-----|-------|-------------|-----|-------|
|                                   | (+)      | (-) | P     | (+)         | (-) | P     | (+)         | (-) | P     |
| Age (in years) <sup>#</sup>       |          |     |       |             |     |       |             |     |       |
| ≤40                               | 6        | 142 | 0.472 | 2           | 146 | 0.269 | 15          | 133 | 0.914 |
| >40                               | 1        | 51  |       | 2           | 50  |       | 5           | 47  |       |
| Gender <sup>#</sup>               |          |     |       |             |     |       |             |     |       |
| Female                            | 0        | 48  | 0.13  | 0           | 48  | 0.421 | 3           | 45  | 0.321 |
| Male                              | 7        | 145 |       | 4           | 148 |       | 17          | 135 |       |
| Educational Status <sup>#</sup>   |          |     |       |             |     |       |             |     |       |
| High School & above               | 4        | 75  | 0.331 | 2           | 77  | 0.664 | 5           | 74  | 0.162 |
| Below High School                 | 3        | 118 |       | 2           | 119 |       | 15          | 106 |       |
| Marital Status <sup>##</sup>      |          |     |       |             |     |       |             |     |       |
| Married                           | 3        | 126 | 0.134 | 3           | 126 | 0.781 | 9           | 120 | 0.035 |
| Unmarried                         | 4        | 55  |       | 1           | 58  |       | 10          | 49  |       |
| Employment Status <sup>#</sup>    |          |     |       |             |     |       |             |     |       |
| Employed                          | 4        | 117 | 0.853 | 3           | 118 | 0.549 | 13          | 108 | 0.664 |
| Unemployed                        | 3        | 76  |       | 1           | 78  |       | 7           | 72  |       |
| Socioeconomic Status <sup>#</sup> |          |     |       |             |     |       |             |     |       |
| High                              | 4        | 128 | 0.927 | 2           | 130 | 0.499 | 9           | 123 | 0.276 |
| Low                               | 3        | 65  |       | 2           | 66  |       | 11          | 57  |       |

(+)Present; (-)Absent; <sup>#</sup>n=200; <sup>##</sup>n=188 (1 divorced HCV positive subject not included in the analysis)

Majority of the HIV-seropositive patients with syphilis, HBV, and/or HCV co-infection gave history of intravenous drugs abuse (54.5%), smoking (50%), alcohol intake (31.2%), and multiple sexual partners (31.2%). Sixty percent of IV drug users in our study were observed to have HCV infection. Statistically significant associations were observed between HCV infection and IV drug abuse ( $P < 0.001$ ). These findings are similar to those reported previously by Ionita *et al.*, wherein 16% of the IV drug users had HCV infection.<sup>[5]</sup>

The mean CD4+ count for the HIV-positive patients with either syphilis, HBV, or HCV infections was 252 ± 137.5 cells/μl. This is in contrast to the study by Chandra *et al.* which observed that the CD4+ count <200 cells/μl was seen in a significantly higher number of patients in HBV and HCV co-infection than in HIV alone.<sup>[24]</sup> This difference may be attributed to the “Test and Treat Strategy” implemented in India in 2017, in which newly diagnosed HIV-infected individuals are immediately started on anti-retroviral therapy, regardless of the CD4 count.

Our study was limited by the fact that it was performed at a single center and had a small sample size. Nonetheless, the findings of our study indicate that the HIV-infected patients in this region are at an increased risk of acquiring syphilis and HCV co-infections through the shared routes of transmission. Progression of HIV and enhanced immune suppression has an effect on natural history and pathogenesis of these co-infections. Therefore, it is recommended that HIV patients should be screened routinely for concurrent syphilis, HBV, and HCV infections. This would aid in prompt diagnosis and treatment with improved outcomes in these patients which in turn may decrease the further spread of these infections. Furthermore, in view of strong association between intravenous drug abuse and infection with HIV and HCV found in our study, specific interventions for harm reduction, such as needle/syringe programs and opioid substitution therapy, could be worth exploring. Finally, having multiple sex partners was

found to be significantly associated with HIV infection, thus highlighting the need for implementation of “behavior change communication” strategies.

### Conclusions:

The HIV-infected patients were observed to be at an increased risk of acquiring syphilis and HCV co-infections through the shared routes of transmission. Routine screening of these patients for concurrent syphilis and viral hepatitis may aid in prompt diagnosis and treatment with improved outcomes, which in turn may decrease the further spread of these infections.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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