

## Research Article

# A study on seroprevalence of hepatotropic viruses among HIV-positive individuals attending the integrated counselling and testing centre (ICTC) in Mayo hospital of Nagpur city, Maharashtra, India

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## ABSTRACT

**Background:** Infection with human immunodeficiency virus type-1 and its end stage, acquired immunodeficiency syndrome is the major public health challenge of modern time. A variety of exogenously acquired infectious agents appear to influence the pace of HIV replication, the destruction of CD4+ T cells, and HIV transmission to infants and sexual partners. More persistent elevations in plasma HIV levels have been seen in patients with chronic infections (such as those with tuberculosis and herpes and hepatitis viruses), and such co-infected patients have a more rapid loss of CD4+ T cells and an increased rate of progression to AIDS and death. Within India, variable co-infection rates have been reported from region to region. With the above background, the present study was undertaken to study the seroprevalence of hepatotropic viruses (Hepatitis B virus and Hepatitis C virus) in HIV-positive individuals attending the Integrated Counselling and Testing Centre (ICTC) in Mayo hospital of Nagpur city, Maharashtra, India.

**Methods:** The current prospective study was conducted in the department of microbiology, Indira Gandhi government medical college and Mayo general hospital, Nagpur (Maharashtra) from August 2005 to August 2007. All the patients attending ICTC were included in the study. 300 HIV negative matched controls were also included in the study. All the individuals who were positive for HIV infection were selected for this study and further tested for the presence of hepatitis B surface antigen (HBsAg) and anti-HCV antibodies. Data was compiled in MS excel. Then it was analyzed using online statistical calculator and chi square test were applied with value of P <0.05 was considered statistically significant for interpretation of finding.

**Results:** Maximum numbers of HIV positive patients (38.8%) were in age group of 31-40 years. Present study showed male preponderance in HIV positive patients. Males showed a slightly high seroprevalence of HBsAg (9.21%) compared to females (7.57%) among HIV positive patients. The difference in positivity of two viral agents studied in HIV positive patients was highly significant as compared to HIV-negative individuals (P <0.001).

**Conclusion:** On the basis of the findings that the co-infection of hepatotropic viruses (HBV and HCV) were significantly higher in HIV positive cases than controls, the study concludes that the chronic viral hepatitis is a serious concern in HIV-infected patients. Thus, there is an urgent need to ensure the screening of the same in HIV-infected patients.

**Keywords:** Co-infection, Hepatitis B virus, Hepatitis C virus, HIV, Maharashtra, Nagpur city

## INTRODUCTION

Infection with Human Immunodeficiency Virus type 1 (HIV-1) and its end stage, acquired immunodeficiency syndrome (AIDS) is the major public health challenge of modern times.<sup>1</sup>

HIV infection in humans is now a global pandemic. As per the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), AIDS has killed more than 25 million people since it was first recognized on December 1, 1981, making it one of the most destructive pandemics in recorded history.

A total of 39.5 million people were living with HIV/AIDS in year 2006. It is estimated that 90% of HIV infected persons live in developing countries. India, with a whopping 5.7 million cases of HIV infection, has distinction of harboring the second highest number of these patients in the world.<sup>2</sup>

A variety of exogenously acquired infectious agents appear to influence the pace of HIV replication, the destruction of CD4+ T cells, and HIV transmission to infants and sexual partners.<sup>3</sup> More persistent elevations in plasma HIV levels have been seen in patients with chronic infections (such as those with tuberculosis and herpes and hepatitis viruses), and such co-infected patients have a more rapid loss of CD4+ T cells and an increased rate of progression to AIDS and death.<sup>4</sup>

In the wake of recent advances in antiretroviral therapy for HIV-infection and the attendant reconstitution of the immune system, survival, free from life-limiting opportunistic infections has improved significantly. In place of these opportunists, infections with hepatitis B (HBV) and C virus (HCV) will become increasingly important problems in HIV-positive individuals.<sup>5</sup> Recent data from a European population showed that chronic viral liver disease represented the fifth most common cause of death for HIV-infected patients.<sup>6</sup>

Since the HIV shares the routes of transmission with that of the hepatotropic viruses, as a consequence, infections with HBV and HCV are expected in HIV infected patients. Co-infections of HBV and HCV with HIV have been associated with reduced survival, increased risk of progression to liver disease and increased risk of hepatotoxicity associated with anti-retroviral therapy.<sup>7,8</sup>

HBV co-infection with HIV modifies the natural history of HBV infection, increasing the percentage of patients likely to become HBV surface antigen (HBsAg) carriers and have a slower loss of serum HbeAg.<sup>9</sup> In HIV-HBV co-infection, there is an increase in persistence of HBV, increase in HBV viral load and increase in incidence of HBV reactivation and reinfection.<sup>10</sup>

For HCV, several studies have suggested that HCV infection is an independent predicting factor of mortality

in HIV infection<sup>11</sup> and increases the risk of progression to severe liver disease.<sup>12</sup> In addition, HCV infection has been shown to increase the risk of hepatotoxicity associated with highly active antiretroviral therapy (HAART).<sup>9</sup>

Among the HIV-infected patients worldwide, 2-4 million are estimated to have chronic HBV co-infection while 4-5 million are co-infected with HCV. In Europe and USA, HIV-HBV co-infection has been seen in 6%-14% of all patients while HIV-HCV co-infection has been variably reported ranging from 25% to almost 50% of these patients.<sup>13</sup>

Within India also, variable co-infection rates have been reported from region to region. HIV-HBV co-infection (HBsAg positives) is reported in 6% to 16% of all the patients. (14, 15) Similarly, HIV-HCV co-infection rates also vary from 2.4% to 30%.<sup>15</sup>

The literature regarding the prevalence of co-infection with HBV & HCV in India is sparse. Hence, the present study was undertaken to study the seroprevalence of hepatotropic viruses (Hepatitis B virus and hepatitis C virus) in HIV-positive individuals attending the Integrated Counselling and Testing Centre (ICTC) in Mayo general hospital of Nagpur city, Maharashtra, India.

## METHODS

The present prospective study was conducted in the department of microbiology, Indira Gandhi government medical college and Mayo general hospital, Nagpur (Maharashtra) India, from August 2005 to August 2007.

All the patients attending ICTC were included in the study and a detail history from all the patients were taken according to a predesigned proforma. All the patients were provided with HIV pretest counselling and the written consent was taken for testing. HIV testing was done as per NACO guidelines.<sup>16</sup>

All the individuals who were positive for HIV infection were selected for this study and further tested for the presence of hepatitis B surface antigen (HBsAg) and for IgG antibodies against hepatitis C virus (HCV). Three hundred healthy age and sex matched HIV-negative controls for HBsAg & HCV were also included in the study.

A total of 2250 & 300 blood samples were collected from study subjects and control respectively. All the serum samples were subjected to the detection of HIV-I and HIV-II antibodies as per NACO guidelines.<sup>16</sup> The serum samples found positive for HIV infection were further tested for the presence of HBsAg and anti HCV antibodies by using a third generation ELISA kit (QUALISA-HBsAg, by Qualpro diagnostic, Goa, India and Advanced HCV test kit by Intec Products, Inc,

Xiamen, China, respectively). Samples positive for HBsAg and/or anti HCV antibody by first test were retested for confirmation of results.

The serum samples of control cases were also tested for presence of HBsAg and anti HCV antibodies.

Data was compiled in MS excel and checked for its completeness and correctness. Then it was analyzed using online statistical calculator and chi square test were applied, with value of <0.05 was considered statistically significant for interpretation of finding.

## RESULTS

Out of 2250 patients attending ICTC, a total of 500 (22.22%) patients were positive for HIV antibodies. Age and sex matched HIV negative 300 persons (as a control) were also included in the study. Maximum numbers of HIV positive patients (38.8%) were in age group of 31-40 years, followed by age groups of 21-40 years which had 33.6% of HIV positive patients. Present study showed male preponderance in HIV positive patients. Out of total HIV positive patients, 63% were males and among HIV negative controls, 67.67% were males (Table 1).

**Table 1: Age & sex wise distribution of HIV positive patients and HIV negative controls.**

Age (years)	HIV positive (n=500)			HIV negative (n=300)		
	Male	Female	Total (%)	Male	Female	Total (%)
<15	28	17	45 (9.00)	16	9	15 (8.33)
15-20	10	6	16 (3.20)	7	3	10 (3.33)
21-30	115	53	168 (33.60)	79	27	106 (35.33)
31-40	102	92	194 (38.80)	64	49	113 (37.68)
>40	60	17	77 (15.40)	37	9	46 (15.33)
<b>Total</b>	<b>315</b>	<b>185</b>	<b>500 (100.0)</b>	<b>203</b>	<b>97</b>	<b>300 (100.0)</b>

**Table 2: Occupation-wise distribution & probable route of transmission of HIV in HIV positive cases.**

Occupation	HIV positive (n=500)		
	Male	Female	Total (%)
Agriculture /unskilled worker	129	40	169 (33.80)
Truck/auto/taxi driver & cleaner	53	0	53 (10.60)
Industrial/factory worker	25	0	25 (05.00)
Hotel staff	17	0	17 (03.40)
Service class	22	13	35 (07.00)
Business	11	0	11 (02.20)
Unemployed	31	8	39 (07.80)
Student	10	9	19 (03.80)
Housewife	0	107	107 (21.40)
Others	17	8	25 (05.00)
<b>Probable route of transmission of HIV</b>			
Heterosexual	275	160	435 (87.0)
MSM*	2	0	2 (0.40)
Vertical	25	15	40 (8.00)
Blood transfusion	5	5	10 (2.00)
IDU**	0	0	0 (0.00)
Unknown	8	5	13 (2.60)
<b>Total</b>	<b>315</b>	<b>185</b>	<b>500 (100)</b>

\*MSM - Men who have sex with men

\*\*IDU - Intravenous drug use

Among HIV positive cases, maximum number of cases, 169 (33.80%) were agricultural or unskilled workers (laborers) followed by housewives (21.40%), drivers/cleaners (10.60%) and service class people (7.0%). 87% acquired HIV through heterosexual contact and 0.4% acquired HIV through homosexual contact (MSM) among HIV positive patient. Vertical route of transmission was observed in 8% of HIV positive patients, whereas 2% of HIV positive patients acquired HIV via blood transfusion. In 2.6% patient's route of transmission of HIV could not be identified (Table 2).

**Table 3: Age-wise distribution of HBsAg & anti HCV positive cases among HIV positive patients and HIV negative controls.**

Age (years)	HIV positive (n=500)		
	Total	HBsAg positive (%)	Anti HCV positive (%)
<15	45	3 (6.67)	1 (2.22)
15-20	16	1 (6.25)	0 (0.00)
21-30	168	15 (8.93)	6 (3.57)
31-40	194	18 (9.28)	7 (3.61)
>40	77	6 (7.79)	2 (2.59)
<b>Total</b>	<b>500</b>	<b>43 (8.60)</b>	<b>16(3.20)</b>

Among HIV positive patients HBsAg positivity was highest in age group of 31-40 years (9.28%) followed by 8.93% of HBsAg positivity in age group of 21-30 years. Among HIV positive patients, anti-HCV antibodies were detected in maximum number of patients in age group of

31-40 years (3.61%) followed by patients in age group of 21-30 years (3.57%) (Table 3). Males showed a slightly high seroprevalence of HBsAg (9.21%) compared to females (7.57%) among HIV positive patients. Among

HIV positive patients, 2.86% males had anti-HCV antibodies, whereas 3.78% females had anti-HCV antibodies (Table 4).

**Table 4: Gender-wise distribution of HBsAg positive & anti HCV positive cases among HIV positive patients and HIV negative control.**

Gender	HIV positive (n=500)			HIV negative (n=300)		
	Total	HBsAg positive (%)	Anti-HCV	Total	HBsAg positive (%)	Anti-HCV
Male	315	29 (9.21)	9 (2.86)	203	2 (0.99)	0 (0)
Female	185	14 (7.57)	7 (3.78)	97	1 (1.03)	0 (0)
<b>Total</b>	<b>500</b>	<b>43 (8.60)</b>	<b>16 (3.20)</b>	<b>300</b>	<b>3 (1.00)</b>	<b>0 (0)</b>

**Table 5: Probable route of transmission in HBsAg & anti HCV positive cases among HIV-positive patients.**

Probable route of transmission	HIV positive (n=500)		
	Total	HBsAg positive (%)	Anti-HCV positive (%)
Heterosexual	435	39 (08.97)	15 (3.47)
MSM	2	0 (00.00)	0 (00.00)
Vertical	40	3 (07.50)	1 ( 2.50)
Blood transfusion	10	0 (00.00)	0 (00.00)
IDU	0	0 (00.00)	0 (00.00)
Unknown	13	1 (07.69)	0 (00.00)
<b>Total</b>	<b>500</b>	<b>43 (08.60)</b>	<b>16 ( 3.20)</b>

The HBsAg positivity was seen highest in HIV positive heterosexuals (8.97%). None of HIV positive MSM was HBsAg positive. One out of 13 (7.69%) HIV positive cases with unknown route of transmission were positive for HBsAg. Anti-HCV antibody positivity in HIV positive heterosexuals was 3.47% and in vertically acquired HIV positive patients it was 2.5%.

**Table 6: Seropositivity of different viral agents in HIV positive patients and HIV negative controls.**

Viral agent	HIV positive n=500 (%)	HIV negative n=300 (%)	Chi square test, df, P value
HBsAg	43 (8.60)	3 (1.00)	X <sup>2</sup> =19.984, df=1, P <0.0001
Anti-HCV antibodies	16 (3.20)	0 (0.00)	X <sup>2</sup> =9.796, df=1, P <0.001

Over all 8.6% patients among HIV positive were HBsAg positive whereas only 1% of HIV negative controls tested positive for HBsAg. Anti-HCV antibodies were present in 3.2% of HIV positive patients. None of the HIV

negative controls was positive for anti-HCV antibodies. The difference in positivity of the two viral agents studied in HIV positive patients was highly significant as compared to HIV-negative individuals. (p<0.001)

**DISCUSSION**

The last decade of millennium saw the emergence and re-emergence of many infectious diseases.<sup>17</sup> HIV infection, being the most dreaded amongst them, has become a global epidemic far more widespread than what was predicted few decades ago.<sup>14</sup> The geographic distribution, clinical course of the disease and the pattern of opportunistic infections in HIV infection, varies from country to country and also from patient to patient. In tropical countries like India, a handful of pathogens cause most of the opportunistic infections.<sup>18,19</sup>

The intimate relationship between the HIV-1 life-cycle and the activation state of cells supporting viral replication results in a dynamic interaction between co-infections and HIV-1 replication in dually infected people. More persistent elevations in plasma HIV levels have been seen in patients with chronic infections (such as those with tuberculosis and herpes and hepatitis viruses), and such co-infected patients have a more rapid loss of CD4+ T cells and an increased rate of progression to AIDS and death and also an increased risk of HIV-1 transmission to infants and sexual partners.<sup>4,20</sup>

Considering the scanty information regarding the co-infection of hepatitis viruses (HBV & HCV) with HIV from Nagpur region, which is reported to be one of the highly HIV prevalent regions in India by National AIDS Control Organization (NACO), with an average of 4-5 times higher HIV prevalence than other regions,<sup>16</sup> the present study was undertaken in the department of microbiology, Indira Gandhi government medical college and Mayo general hospital to know the seroprevalence of HBV & HCV, in HIV-positive individuals.

In present study 2250 patients attending the Integrated Counselling and Testing Center (ICTC) were screened for HIV antibodies. Out of which, 22.22% were found to be HIV-positive. Anvikar AR et al. also observed HIV seroprevalence of 19.90% in patients attending Voluntary Counselling and Testing Centre (VCTC) in Central India.<sup>21</sup>

In the present study 63% of HIV-positive individuals were males. Other studies have also reported male preponderance among HIV-positive individuals.<sup>14,18,22</sup>

The low number (37%) of females is not a true representation of the proportion of females as financial constraints and gender bias. The social stigma and neglect attached with the disease decreases the number of females attending the HIV clinics.

As expected, majority of the HIV-positive patients in the study belonged to the sexually active age group of 20-40 years, maximum being in age group of 31-40 years (38.80%), followed by age group of 21-30 years (33.60%). Similar are the findings in various studies which reported highest prevalence of HIV in age group of 31-40 years.<sup>18,23</sup> Moreover majority of the HIV-positive patients in this were indulged in agriculture/unskilled work (33.80%) or were housewives (21.40%). These findings concord with other studies.<sup>22</sup> Thus HIV seems to affect the economically productive, sexually active age group, having a tremendous impact on the livelihood of the affected family.

The predominant mode of transmission of HIV in present study was heterosexual contact (87.0%). This finding is in line with the findings of various studies, suggesting the heterosexual contact to be the most common mode of transmission of HIV.<sup>18-23</sup>

In this study, two (0.4%) HIV-positive patients were homosexuals and none of the HIV-positive patients was intravenous drug abuser. In present study risk factors or routes of transmission could not be identified in 2.6% of HIV-positive patients. The reason could be social taboos associated with the practice of risk factors.

About two third of patients with AIDS develop hepatomegaly and abnormalities in serum biochemical parameters of liver function.<sup>24</sup> Liver damage may be directly related to HIV infection or may result from conditions such as HBV and HCV co-infections, chronic alcoholism, hepatic tuberculosis, or intravenous drug abuse which are highly prevalent with HIV infection, or due to the effects of Anti-Retroviral Therapy (ART).<sup>8</sup>

Moreover, among HIV infected patients worldwide, an estimated 2-4 million have chronic HBV co-infection and 4-5 million have HCV co-infection.<sup>13</sup> Co-infection with HBV or HCV in HIV infected patients complicates the clinical course, management and may also adversely affect therapy for HIV infection.<sup>25</sup> The reported co-

infection rates of HBV and HCV in HIV patients have been variable worldwide depending on the geographic regions, risk groups and the type of exposure involved.<sup>23</sup>

As a result of highly active antiretroviral therapy (HAART) life expectancy of patients with HIV has increased and there has been a decline in opportunistic infections, consequently focus has been shifted to the management of concurrent illnesses such as chronic HBV and HCV infections which have the potential to increase long term morbidity and mortality.<sup>5</sup> As a result of the shared epidemiological factors, patients infected with HIV have a higher risk of both HBV and HCV infection as compared to those not infected with HIV.<sup>7</sup>

HBV Co-infection with HIV modifies the natural history of HBV infection, increasing the percentage of patients likely to become HBV surface antigen (HBsAg) carriers and have a slower clearance of serum HBeAg.<sup>9</sup>

The immune reconstitution following HAART for HIV plays an important role in the reactivation of HBV disease. This may be due to restored cellular immunity to hepatocytes expressing enhanced levels of viral antigen, but these flares have been followed by successful resolution of HBV.<sup>5</sup>

In India, HBV and HCV co-infection among HIV infected has been reported infrequently from different regions. However, our study indicated that HIV infected patients are at a high risk of viral co-infections as evident from the high prevalence of HBsAg (8.60%) and HCV antibodies (3.2%) in HIV-positive patients. ( $P < 0.001$ ) Nearly similar prevalence of HBsAg (chronic HBV infection) in HIV-positive individuals was reported by various studies.<sup>9,11,23</sup>

A low seroprevalence of HBsAg, 2.25% and 5.3% among HIV-positive patients were reported by Tripathi et al.<sup>26</sup> and Gupta S and Singh S<sup>8</sup> respectively, whereas HBsAg seroprevalence approaching as high as 30.4% among HIV-positive patients was reported by Tankhiwale SS et al.<sup>10</sup>

The finding that the prevalence of HIV-HBV co-infection is higher in men belonging the age group of 31-40 years, which is the normal sexually active age group, where the HIV positivity is reportedly higher as per Indian literature,<sup>8,18,19,23</sup> suggests that sexual route could be the common mode of transmission for both HBV and HIV, which was further confirmed by our findings showing that maximum (8.97%) HIV-HBV co-infection rate occurred in heterosexually acquired HIV positive patients than other modes of transmission. This finding is in accordance with that of previously conducted studies,<sup>10</sup> which also observed heterosexual contact to be predominant mode of transmission in HIV-HBV co-infected individuals.

HIV and HCV have similar routes of transmission, namely through blood and blood products, sharing of needles to inject drugs and sexual activity, enabling co-infection with these viruses a common event.<sup>5</sup> HCV may circumvent the host antiviral response by inhibiting the interferon induced Protein Kinase R (PKR) which inhibits HCV viral protein synthesis.<sup>27</sup> The altered cytokine production in HIV infection may ultimately lead to an aggravation of this already blunted endogenous interferon response against HCV in HIV-HCV co-infected individual.<sup>28</sup> This increased HCV viral load in co-infected patients results in worsened clinical outcomes, suggesting a cytopathic form of liver injury caused by direct toxic effects of HCV viral proteins on hepatocytes.<sup>5</sup>

The data from western studies revealed that the incidence of HCV co-infection has been variably ranging from 25% to almost 50% of HIV-positive patients.<sup>15</sup> There are studies reporting that HIV-positive patients were seven times more likely to have HCV infection than controls.<sup>29</sup>

In present study seroprevalence of HCV antibodies in HIV positive patients was found to be 3.2%, whereas none of the HIV negative controls was positive for HCV antibodies. ( $P < 0.001$ ) This finding was in concordance with the other studies.<sup>8,23,30</sup>

HCV prevalence in this study group was lower than other studies from western countries and other parts of India. This can be related to the type of risk groups in present study. Saha MK et al.<sup>31</sup> reported an HCV co-infection rate of 92% in intravenous drug users from Northeast India, whereas a study in Thailand showed that the prevalence of HCV co-infection was 88.2% in patients with IDU and 2.8% in patients without IDU ( $P < 0.001$ ).<sup>9</sup> The findings of these studies are in accordance with the fact that HCV is commonly transmitted through transfusion or intravenous drug abuse.<sup>32</sup>

The low HCV prevalence in present data could be attributed to the absence of IDUs and a very low number of transfusion associated HIV-positive patients in this study.

Majority of the investigators reported a higher prevalence of HIV-HCV co-infection in males as compared with females.<sup>9,25</sup> In present study HCV co-infection among HIV positive patients is reported higher in females. This finding is similar to that obtained by Gupta S and Singh S in their study. In India, the majorities of the women are in a monogamous relationship with their husbands and usually acquire HIV infection from their spouse. Therefore, as the risk for HCV acquisition in steady monogamous relationships is quite low, it becomes necessary to look into other factors like sharing of tooth brushes and other contaminated personal items with her husband who may be index for the HCV infection.<sup>8</sup>

As a blood borne infection, HCV potentially can be transmitted sexually and perinatally. HCV is transmitted via heterosexual contact far less efficiently than HIV.<sup>33</sup> Various studies have suggested that HCV could be well transmitted via heterosexual routes.<sup>34,35</sup> In current study also, majority of HIV-HCV infected patients probably have acquired the disease via heterosexual contact. The findings were further potentiated in the present study by having majority of HIV-HCV co infection in age group of 31- 40 years, which is the normal sexually active age group where the HIV positivity is reportedly higher as per previous studies.<sup>8,18,23</sup>

Thus it is clear that apart from other infections, HIV infected individuals have a high probability of getting co-infected with hepatotropic viruses (HBV and HCV). HIV disease progression and enhanced immunosuppression has a direct bearing on the natural history and pathogenesis of these viruses.<sup>7,8,36,37</sup> This study obtained significantly higher prevalence of HBsAg and anti HCV antibodies in HIV positive patients than in controls. It indicates that, careful monitoring of HIV infected patients for concurrent infection with hepatotropic viruses is therefore necessary in order to ensure that preventive measures should be targeted early and appropriately to decrease long term morbidity and mortality.

## CONCLUSION

On the basis of the findings that co-infection of hepatotropic viruses (HBV and HCV) were significantly higher in HIV positive cases than controls, the study concludes that the chronic viral hepatitis is a serious concern in HIV-infected patients. In view of the shared routes of transmission of these viruses, morbidity and mortality associated with hepatitis viruses is expected to become an even more critical problem in the immediate future. Thus the study suggests more careful and a vigilant screening of hepatotropic viruses in HIV-infected patients. The finding of the present study will be useful for public health authorities in priority settings and resource allocation under National AIDS control programme in Nagpur city, Maharashtra.

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