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# A Study on Herpes Simplex Virus Type-2 Specific Seroprevalence Among HIV Positive Patients of a Tertiary Care Hospital in Central India

KEYWORDS: HSV-2, HIV, Maharashtra, Seroprevalence


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**ABSTRACT** *India has the third highest number of estimated people living with HIV in the world. The intricacy of interaction between HIV and herpes viruses at the cellular level and the possibility of adverse outcome leading to heightened activity of either virus with disease progression is an alarming situation. Hence knowledge about the co-existence of HIV with other viruses is not only essential but is mandatory to monitor the disease progression and more importantly in the treatment of these infections, where the specific therapy is identified and available. 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 individuals who were positive for HIV infection were selected for this study and further tested for the presence of anti HSV-2 antibodies. Three hundred healthy age and sex matched HIV-negative controls for anti HSV-2 antibodies were also included in the study.*

*Present study showed male preponderance in HIV positive patients. Anti-HSV2 antibodies could be detected in 43.6% HIV positive patients and in 17.33% HIV-negative controls. The difference in positivity of both viral agents studied in HIV positive patients was highly significant as compared to HIV-negative individuals ($p < 0.001$).This study concludes that the seroprevalence of HSV-2 antibodies is significantly higher among HIV- positive patients as compared to HIV-negative controls, also that the increased seroprevalence of HSV-2 antibodies is observed in persons who acquire HIV-I infection through heterosexual route. These findings suggest that HSV-2 type specific serological testing in the Indian HIV-infected subpopulation could be an efficient strategy to diagnose clinically asymptomatic HSV-2 infections and therefore to reduce the risk of HSV-2 and HIV sexual transmission by convenient prophylactic counseling against unprotected intercourse.*

## INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is well established as the cause of acquired immunodeficiency syndrome (AIDS), and is continued to be the major public health challenge of modern times. (1, 2)

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. (3, 4)

India has the third highest number of estimated people living with HIV in the world. According to the HIV estimations 2012, the estimated number of people living with HIV/AIDS in India was 20.89 lakh, with an estimated adult (15-49 age group) HIV prevalence of 0.27% in 2011. (5)

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. (6) 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. (7)

A bi-directional interaction between HIV-1 and the "classic" sexually transmitted diseases (STDs) referred to as "epidemiological synergy" has been proposed as one explanation for the rapid spread of the HIV-1 epidemic. (8, 9) Among various STDs, prevalence pattern of herpes genitalis, caused by herpes simplex virus type-2 (HSV-2), is pivotal and NACO has already issued guidelines to test for this disease in AIDS as well as non–AIDS patients by serology. (10)

Many studies indicate that herpes simplex virus type-2 (HSV-2) seropositivity increases the risk of acquiring and transmitting HIV. It has been demonstrated that HIV-infected CD4 cells are recruited to HSV-2 infected lesions and that HSV-2 regulatory proteins may up regulate HIV replication, thus increasing the frequency and titre of mucosal HIV shedding. It also appears that individuals co-infected with HIV and HSV-2 have more frequent HSV-2 recurrences than individuals infected with HSV-2 alone, suggesting that reactivation is linked to immunosuppression. Thus HSV-2 infection should be targeted as a modifiable risk factor for HIV acquisition by testing, counseling and preventing acquisition through behavioural interventions, treatment and antiviral suppression. (11)

The intricacy of interaction between HIV and herpes viruses at the cellular level and the possibility of adverse outcome leading to heightened activity of either virus with disease progression is an alarming situation. Hence knowledge about the co-existence of HIV with other viruses is not only essential but is mandatory to monitor the disease progression and more importantly in the treatment of these infections, where the specific therapy is identified

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and available. The literature regarding the prevalence of co-infection of HIV with HSV-2 in India is sparse. Hence, the present study was undertaken to study the seroprevalence of HSV-2 in HIV-positive individuals attending the Integrated Counselling and Testing Centre (ICTC) in Mayo General Hospital of Nagpur City, Maharashtra, India.

**MATERIAL AND 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 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. (12)

All the individuals who were positive for HIV infection were selected for this study and further tested for the presence of anti HSV-2 antibodies. Three hundred healthy age and sex matched HIV-negative controls for anti HSV-2 antibodies 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. (12) The serum samples found positive for HIV infection were further tested for the presence of anti HSV-2 antibodies by using a third generation ELISA kit UBI MAGIWELTM Herpes 2 IgG kit, provided by UBI–United Biotech Inc, USA. Samples positive for anti HSV-2 antibodies by first test were retested for confirmation of results.

The serum samples of control cases were also tested for presence of anti HSV-2 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.

**OBSERVATIONS**

**Table No. - I**

**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) |
| Total | 315 | 185 | 500 (100.0) | 203 | 97 | 300(100.0) |

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-I)

**Table No. - II**

**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) |
| Probable route of transmission of HIV | | | |
| 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) |
| Total | 315 | 185 | 500 (100) |

* 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

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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-II)

**Table No. - III**
**Age-wise distribution of HSV-2 cases among HIV positive patients and HIV- negative controls**

| Age (years) | HIV positive (n=500) | | HIV negative (n=300) | |
|---|---|---|---|---|
| | Total | Anti-HSV -2 positive (%) | Total | Anti-HSV-2 positive (%) |
| < 15 | 45 | 9 (20.00) | 25 | 0 (00.00) |
| 15 – 20 | 16 | 5 (31.25) | 10 | 1 (10.00) |
| 21 – 30 | 168 | 65 (38.69) | 106 | 19 (17.92) |
| 31 - 40 | 194 | 89 (45.88) | 113 | 21 (18.58) |
| > 40 | 77 | 50 (64.94) | 46 | 11 (23.91) |
| Total | 500 | 218 (43.60) | 300 | 52 (17.33) |

Among HIV positive patients, anti-HSV-2 antibody positivity was highest in age group of >40 years (64.94%) followed by age group of 31-40 years (43.45%). (Table-III)

**Table No. - IV**
**Gender-wise distribution of anti-HSV-2 cases among HIV-positive patients and HIV-negative controls**

| Gender | HIV positive (n=500) | | HIV negative (n=300) | |
|---|---|---|---|---|
| | Total | Anti- HSV-2 positive (%) | Total | Anti- HSV-2 positive (%) |
| Male | 315 | 134 (42.54) | 203 | 33(16.26) |
| Female | 185 | 84 (45.41) | 97 | 10(19.59) |
| Total | 500 | 218 (43.60) | 300 | 52 (17.33) |

Among HIV positive patients, a total of 43.60% patients were positive for anti-HSV-2 antibodies. Overall, females (45.41%) had a higher prevalence of anti-HSV-2 antibodies than males (42.54%) among HIV positives. Among HIV negative controls, a total of 17.33% patients were positive for anti-HSV-2 antibodies. (Table-IV)

**Table No. - V**
**Probable route of transmission of HSV-2 among HIV positive patients**

| Route | HIV positive(n=500) | |
|---|---|---|
| | Total | Anti- HSV-2 positive (%) |
| Heterosexual | 435 | 204 (46.90) |
| MSM | 2 | 0 (00.00) |
| Vertical | 40 | 9 (22.50) |
| Blood transfusion | 10 | 2 (20.00) |
| IDU | 0 | 0 (00.00) |
| Unknown | 13 | 3 (23.07) |
| Total | 500 | 218 (43.6) |

Among HIV positive heterosexuals, anti-HSV-2 antibody positivity was 46.90%. In vertically acquired HIV positive cases, 22.50% were positive for anti- HSV-2 antibodies. In HIV positives via blood transfusion 20% were positive for anti–HSV-2 antibodies; whereas 23.07% of HIV positive with unknown route of transmission had anti-HSV-2 antibodies. (Table-V)

**Table No. VI**
**Seropositivity of HSV-2 in HIV positive patients and HIV negative controls**

| Viral agent | HIV positive n=500 (%) | HIV negative n=300 (%) | Chi square test, d.f., p value | Odds ratio (C.I. 95%) |
|---|---|---|---|---|
| Anti HSV-2 antibodies | 218 (43.6) | 52 (17.33) | $x^2$ = 57.856, d.f.=1, p<0.0001 | 3.69 |

Anti-HSV2 antibodies could be detected in 43.6% HIV positive patients and in 17.33% HIV-negative controls. The difference in positivity of both viral agents studied in HIV positive patients was highly significant as compared to HIV-negative individuals ($p < 0.001$). (Table-VI)

**DISCUSSION**

Persistent HSV-2 infections are one of the most common clinical presentations of HIV infection (6). Moreover, several studies have shown that HIV-induced immunologic impairment results in severe, persistent and recurrent genital herpes (13) and HIV-positive individuals are four times more likely than HIV-negative individuals to have reactivation of infection (especially perianal shedding in men and subclinical shedding in women), and are thus more likely to transmit HSV-2. (14, 15, 16) Most people worldwide with sexually acquired HIV have virologically active HSV- 2 infection. (6) Low CD4 counts and high HIV viral loads are associated with an increased frequency of HSV-2 reactivation. (17)

The reciprocal effects of HSV-2 infection on HIV epidemiology are also known. It is shown that prior HSV-2 infection is associated with an increased risk of acquisition of HIV. (18) It has been shown that there were significantly higher amounts of HIV-1 in plasma and in genital secretions in HSV-2 infected women who have sexually acquired HIV-1. (19).

Epidemiological studies suggest that sexually transmitted diseases (STDs) facilitate the sexual spread of HIV infection. The association appears to be especially strong for genital ulcer disease, with a 2-4 fold increased rate of HIV acquisition and transmission in presence of genial ulcers. (20) Genital herpes, which is caused by herpes simplex virus type-2 is the most commonly reported ulcerative sexually transmitted disease worldwide. (21) Genital ulcers by HSV -2 are associated with disruption of genital mucosa

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and are believed to shed and facilitate the transfer of HIV during intercourse. (19, 22)

Transactivator proteins encoded by all human herpes viruses can up- regulate the expressions of HIV by interacting with HIV long terminal repeat (LTR) sequences which are the main region of HIV genome responsible for controlling gene expression. These transactivator proteins, in addition of up-regulating the HSV-2 genome, can also up-regulate CD4 cell surface receptors for HIV, and cell surface Fc receptors which enables HIV to enter CD4 negative cell types. Thus ultimately herpes viruses lead to the expansion of the pool of HIV –infected cells in HIV HSV-2 co-infected individuals. (23)Thus high rate of HSV 2 infection may act as a co-factor in HIV acquisition and transmission. (11)

HSV-2 Seropositivity is a marker of genital HSV-2 infection and is associated with both symptomatic and asymptomatic shedding of HSV- 2 in the genital tract. (24) Some studies demonstrated higher proportions of subclinical and undiagnosed HSV- 2 infection in HIV- infected individuals and suggested that HSV-2 type specific serological testing in HIV infected subpopulation could be an efficient strategy to diagnose clinically asymptomatic HSV-2 infection. (25)

It has been reported that HSV-2 seroprevalence rates are higher in HIV- positive than in HIV-negative persons. (26, 27, 28) In the present study the HSV-2 Seropositivity of 43.6% was reported among HIV-positive patients, which was significantly higher when compared to HSV-2 Seropositivity of 17.33% among HIV-negative controls ($p< 0.0001$).

HSV-2 seropositivities of 37%, 40% and 42.9% among HIV-positive patients were reported by Bystricka M et al (1998), Bystricka M et al (2000) and Peters BP et al (2005) respectively. (10, 26, 29)

Present study observed HIV-HSV2 co-infection to be higher in females (45.41%) than in males (42.54%). In various other studies also HSV-2 Seropositivity among HIV-positives was associated with female gender. (27, 30) The association with female gender is believed to be both due to the biologic and behavioral factors, including exposure to infection of a large mucosal area in vaginal intercourse and sexual contact with older partners who are more likely to be HSV-2 Seropositivity. (30)

Some of the investigators have observed HIV-HSV2 co-infection to be higher in males. The reason might be that a bulk of patients in their study was constituted by homosexual males, and this group had the highest HSV-2 seroprevalence, ultimately giving the number of HSV-2 positive males to be higher than females. This reason of difference was further confirmed by the findings that the odds ratio (OR) for HSV- 2 Seropositivity, which was higher for males in univariate model, changed directions with adjustment for exposure category and became higher for females. (15, 21) Thus high HSV-2 seroprevalence among men in their studies was attributable to the high proportion of HSV-2 positive homosexual men among HIV- positive individuals, whereas in our study only two HIV-positive men were homosexuals and were both negative for HSV-2 antibodies.

Majority of investigators have found that HSV-2 seroprevalence in HIV positive increased with older age (>45 years), this simply reflects the cumulative number of sexual activity and duration of sexual activity. This means that there is a higher probability of acquiring HSV-2 infection with increasing duration of exposure to infectious agents. (15, 28, 30) Present study also revealed that HSV-2 Seropositivity among HIV-positive patients increased with increasing age and was highest (64.94%) in the age group of >40 years.

A marker of high risk sexual behaviors, (30) HSV-2 Seropositivity, increased the risk of HIV-1 acquisition and transmission through heterosexual intercourse by approximately two folds. (20, 31, 32) HSV-2 Seropositivity in present study was higher (46.90%) in heterosexually acquired HIV-positive patients than in HIV positive patients with other modes of transmission.

Heterosexual risk group was a strong predictor of HSV-2 seropositivity among HIV-positive in the study conducted by Ramaswamy M et al. (30) Heterosexual route as a predominant mode of transmission of HSV-2and HIV co- infection was also reported by various other studies. (22, 27)

Thus, these findings stress the need for a frequent and rigorous screening of anti-HSV-2 antibodies among HIV-positive patients, for a better understanding of the interactions between these viruses, which will be of a great help in early diagnosis and proper management of these co infected patients.

## CONCLUSION

Present study concludes that the seroprevalence of HSV-2 antibodies is significantly higher among HIV- positive patients as compared to HIV-negative controls, also that the increased seroprevalence of HSV-2 antibodies is observed in persons who acquire HIV-I infection through heterosexual route. These findings suggest that HSV-2 type specific serological testing in the Indian HIV-infected subpopulation could be an efficient strategy to diagnose clinically asymptomatic HSV-2 infections and therefore to reduce the risk of HSV-2 and HIV sexual transmission by convenient prophylactic counseling against unprotected intercourse. In light of the evidence for a bidirectional interaction between HIV-1 and HSV-2, and the potential role of HSV-2 infection in facilitating HIV transmission further highlights the need for including anti-HSV2 testing and therapy in the management of HIV-positive patients, especially for reducing the risk of transmission of HIV through herpetic lesions.

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