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Seroprevalence of Herpes Simplex Virus type-2 and risk factors among HIV infected and uninfected individuals in North India

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ABSTRACT

Background

Opportunistic infections (OIs) continue to be one of the most universal complications of HIV infected patients. In recent years, an interesting epidemiological association has emerged between herpes simplex virus type-2 (HSV-2) and HIV infection. HSV-2 seroprevalence rates are higher in HIV seropositive individuals than in those who are HIV seronegative.

Method

A prospective observational study was conducted at a tertiary care hospital in North India, to determine the seroprevalence of HSV-2 infection among HIV infected and uninfected individuals, and to assess their risk behaviour. The study group comprised 240 subjects:
Group A (Cases) – 120 HIV seropositive clients/patients.
Group B (Controls) – 120 age/sex matched HIV seronegative clients/patients.
HIV testing was carried out as per National AIDS Control Organisation (NACO) guidelines using E/R (ELISA/Rapid) tests. HSV-2 IgM and IgG antibody detection was carried out using HSV-2 IgM and IgG ELISA based kits.

Results

Only one subject in Group A tested HSV-2 IgM seropositive, whereas all Group B subjects were HSV-2 IgM seronegative. The number of HSV-2 IgG seropositive subjects in Group A was significantly higher ($p<0.02$) than the seropositive subjects in Group B, and the number of seronegative subjects was significantly higher ($p<0.01$) in Group B compared to Group A. On analyzing the sexual behaviour risk factors, it was found that subjects with a history of multiple sexual partners was significantly higher ($p<0.007$) in Group A than in Group B.

Conclusion

HSV-2 IgG seropositivity was significantly higher in group A as compared to Group B. The significantly higher number of HSV-2 IgG seropositive subjects in Group A had a history of unprotected sexual contacts, multiple sexual partners and were more likely to have an HIV positive spouse or parent.

Keywords: Co-infection, HIV, HSV-2, Seroprevalence

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INTRODUCTION

India has the second highest number of people living with Human Immunodeficiency Virus (HIV) in the world. Currently 21.17 million people in India are infected with HIV, with an estimated adult prevalence rate of 0.26%.[1] Opportunistic infections (OIs) and co–infections are the leading cause of deaths amongst the HIV infected individuals. Globally, commonly observed viral co-infections include Human Herpes Virus types 1 and 2 (commonly known as Herpes Simplex Virus types 1 and 2), Cytomegalovirus, Hepatitis B and C infections.[2] In the Indian subcontinent, HSV-1 and 2 and Cytomegalovirus (also

a herpes virus) are commonly responsible for opportunistic infections in HIV-infected populations.[3] These herpes viruses are usually acquired in childhood or young adulthood; they establish a state of asymptomatic latency but may reactivate to give clinical disease later in life, or following an HIV-induced decline in cell-mediated immunity.[4]

In recent years, an interesting epidemiological association has emerged between HSV-2 and HIV infection. HSV-2 seroprevalence rates are higher in HIV seropositive than in HIV seronegative individuals. Multiple mechanisms may explain these observations. Genital ulceration provides an entry site in HIV non-reactive persons, for example, in which the associated inflammation increases the number of activated cells that can be targeted by HIV. It has also been observed that symptomatic and asymptomatic HSV-2 reactivations may promote genital HIV shedding and thus increase HIV levels in blood.[5] These two viruses thus appear to be epidemiologically synergistic. Further HIV infections change the natural history of HSV-2 infection, and HSV-2 infection alters the course of HIV disease.[6]

METHOD AND MATERIALS

A prospective observational case-control study was conducted from November 2014 to March 2016 at a tertiary care centre in New Delhi, to determine the seroprevalence of HSV-2 infection among HIV infected and uninfected individuals, and to assess the risk behaviour in the two groups. A total of 240 subjects were divided equally into age and sex matched two groups:

1) Group A (Cases) – 120 HIV seropositive clients/patients
2) Group B (Controls) – 120 age and sex matched HIV seronegative clients/patients.

An HIV test was carried out as per NACO guidelines[7] using ELISA/Rapid (E/R) tests. Subjects with indeterminate results for the HIV test were excluded from the study. HSV-2 IgM and IgG antibody detection were performed in the two groups, using HSV-2 IgM and IgG ELISA based kit (Calbiotech, CA, USA). As per NACO guidelines,[7] all clients/patients underwent a pre-test counselling session and a written informed consent was obtained before drawing blood samples. The HIV test result was given to the clients/patients after the post-test counselling, as per NACO guidelines[7]. Another written informed consent form was obtained from the client/patient for undergoing the HSV-2 (IgM and IgG) antibody test. The demographic details and the probable risk factors for each subject were recorded in the pre-structured proforma. Regarding clinical history, the parameters recorded were: history of prior admission to hospital; past surgery; headache; nausea; vomiting; numbness; weakness; fever; diarrhoea; cough; sleep disturbance; neck rigidity; or dysuria (painful or difficult urination).

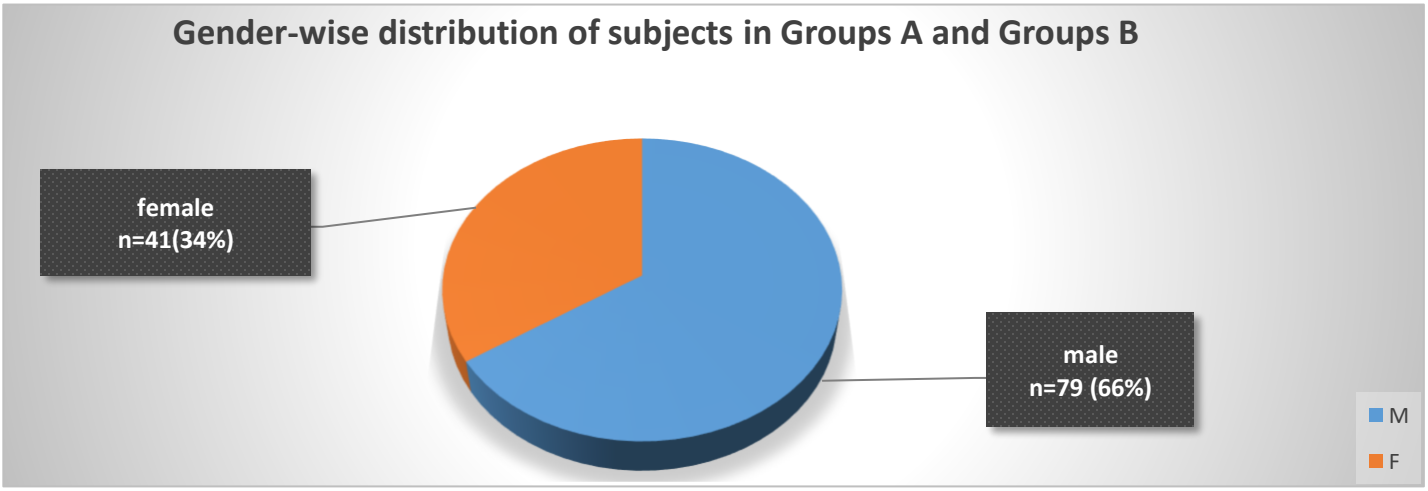

Fig 2 Comparison of groups A and B for HSV-2 IgG seropositive status and gender (n=number)

The results of the HSV-2 (IgM and IgG) antibody test were given to the client/patient. The clients/patients who tested seropositive for HSV-2 IgM and /or IgG antibodies were counselled and referred to a STI/ART (Sexually transmitted infection/Antiretroviral therapy) clinic for further management.

Clients/ patients who tested borderline positive for HSV-2 antibodies were contacted and requested to report back. A repeat HSV-2 antibody test was performed at a minimum interval of 2-4 weeks.

The data was analyzed by the latest version of SPSS version 16. Chi square tests and Fisher's exact tests were used for comparison of the proportions for determining statistical significance. A p value of <0.05 was taken to indicate a significant difference.

RESULTS

The gender distribution of Groups A and B is shown in Figure 1.

HSV-2 IgM Antibody

Among the HIV infected clients/patients (Group A) only one subject tested seropositive for HSV-2 IgM antibody, whereas none tested positive among the HIV uninfected clients/ patients (Group B). Two subjects tested borderline positive in Group B.

HSV-2 IgG Antibody

Group A

In Group A, a significantly higher (p<0.0001) number of subjects – 77 (64.2%) – were seropositive for HSV-2 IgG, whereas 38 (31.6%) tested seronegative.

The number of males was significantly higher in both seropositive (p<0.01) and seronegative groups (p<0.04). Nine subjects had borderline results initially and only four of these subjects came for a follow-up. A second sample was taken and all four of these subjects then tested seronegative. The other five subjects were lost to follow-up and thus were interpreted as borderline. (Table 1)

Group B

In Group B, 59 subjects (49.1%) were HSV-2 IgG seropositive, whereas 58 (48.4%) were seronegative. Four tested borderline initially on the first sample, only one of whom reported for follow-up; this subject tested seronegative for HSV-2 IgG on the second blood sample.

Thus only three clients (one female, two males) had borderline results for HSV-2 IgG. The sero status for HSV-2 IgG antibody in Group B subjects showed no significant difference between the seropositive and seronegative individuals (See Tab 1) .

Comparison of HIV infected and uninfected subjects

The number of seropositive subjects in Group A was significantly higher (p<0.02) as compared to seropositive subjects in Group B (64.2% and 49.1% respectively) (See figure 2). HSV-2 IgG seropositivity was higher in both males and females in Group A as compared to Group B subjects.

A significantly higher number (p<0.02) of male subjects tested seropositive for HSV-2 IgG in Group A as compared to Group B. (Again, see figure 2).

Table 1 Comparison of HSV-2 IgG Seroprevalence in Groups A and B

HSV-IgG serostatus	Group A		Group B	
	N	(%)	n	(%)
Positive	77	64.2	59	49.1
Negative	38	31.6	58	48.4
Borderline	5	4.2	3	2.5

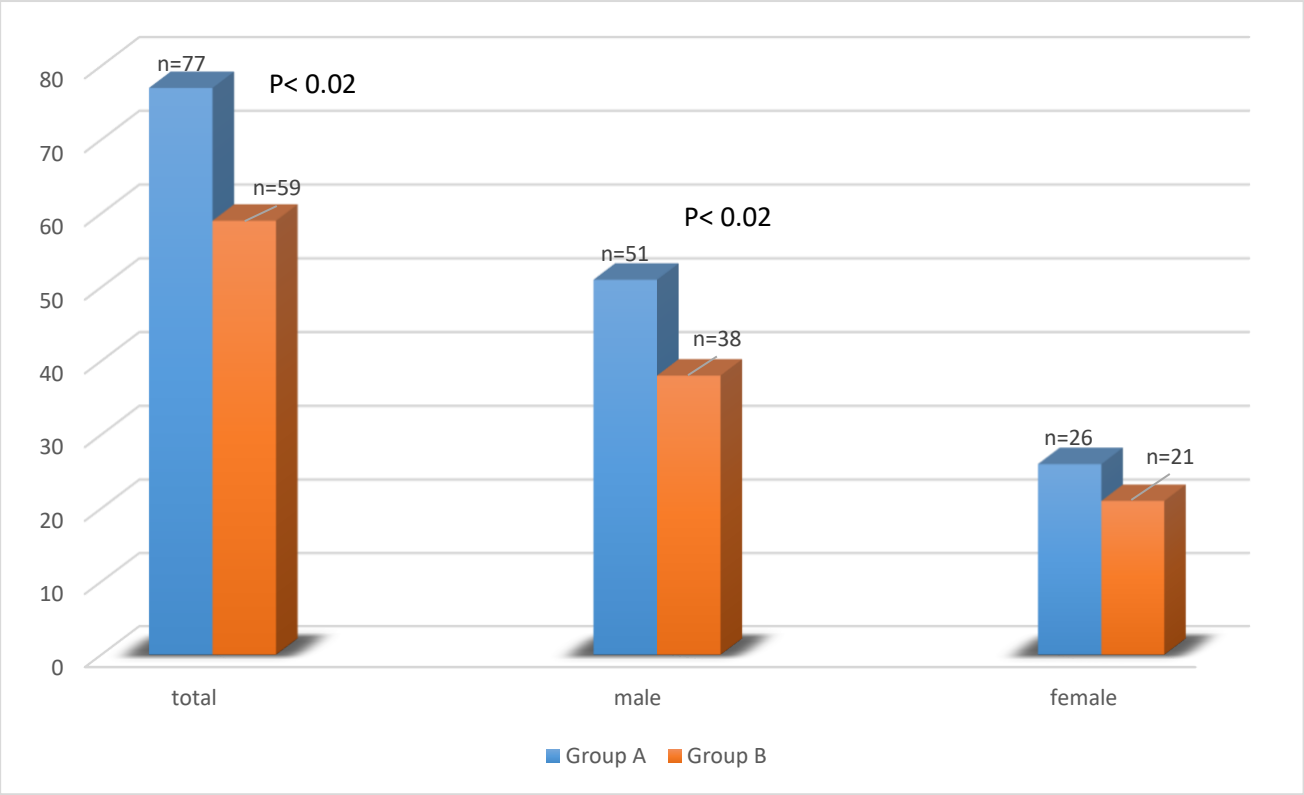

Fig 2 Comparison of groups A and B for HSV-2 IgG seropositive status and gender (n=number)

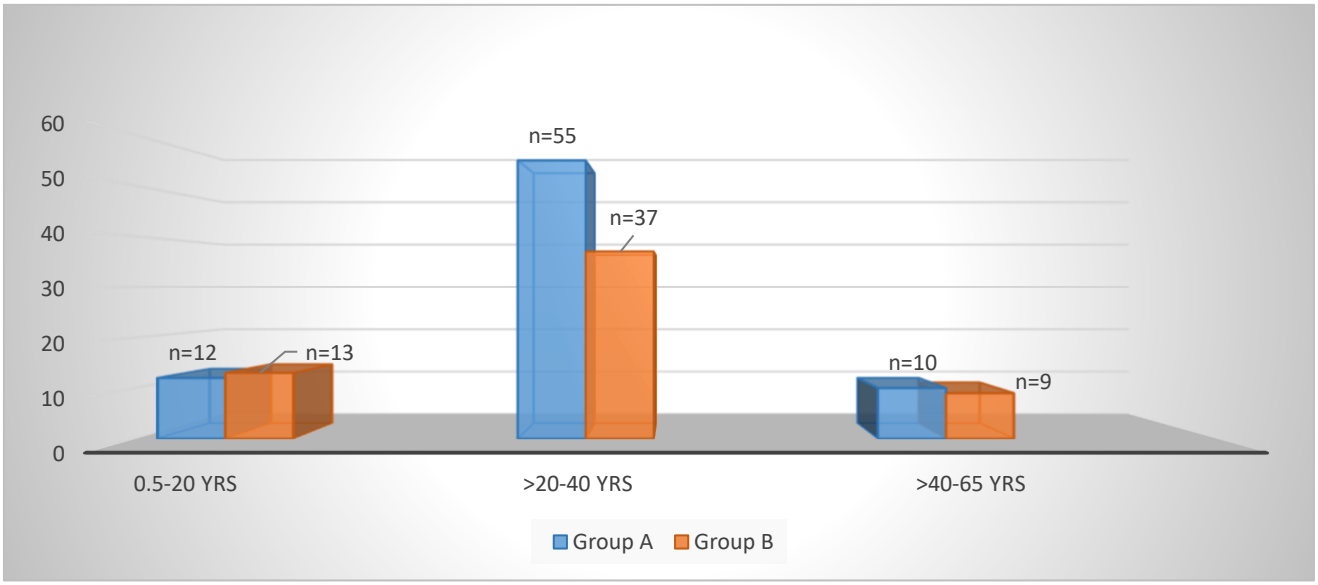

Fig 3 Comparison of groups A and B for HSV-2 IgG seropositive status and age (n=number)

The HSV-2 IgG seropositive status of Groups A and B subjects in relation to their age is shown in Figure 3. Seropositivity was observed to be higher in Group A subjects as compared to Group B in the 20–40 age group; however, the difference between the two groups was not statistically significant.

Risk behaviour

It was observed that a significantly higher number ($p<0.002$) of Group A seropositive subjects (25%) had an HIV positive spouse or parent as compared to Group B subjects (6.7%). In order to assess the possible modes of transmission, subjects were asked about their history of unprotected sexual contact, previous hospitalization, intravenous drug use and blood transfusion. A significantly higher number of Group A subjects gave a history of unprotected sexual contact ($p<0.01$) and previous hospitalization ($p<0.03$) as compared to Group B subjects (see Table 2), suggesting that these are significant risk factors.

Table 2 Correlation of HSV-2 IgG seropositive status with modes of transmission in Groups A and B

Modes of transmission	HSV-2 IgG seropositive			
	Group A		Group B	
	n	%	N	%
Previous Hospitalization	17	**14.1 (p<0.03)**	5	**4.1**
Unprotected sexual contact	62	**51.6 (p<0.01)**	41	**34.2**
Blood transfusion	2	1.7	3	2.5
Intravenous drug use	8	6.6	6	5

Five sexual risk factors were evaluated in the study: a history of multiple sexual partners; evidence of vaginal or penile discharge; genital ulcers; a past history of sexually transmitted disease (STD); and rash or itching. All five of these sexual risk factors were reported more frequently in Group A subjects as compared to Group B. However, only a history of multiple sexual partners emerged as a statistically significant parameter ($p<0.007$) (Table 3) but this may be influenced by the small sample size.

Table 3 Correlation of HSV-2 IgG seropositive status with sexual risk factors in Groups A and B

Sexual Risk Factors	Group A		Group B	
	n	%	N	%
Multiple sexual partner	55	45.8 ($p<0.007$)	28	**33.3**
Discharge	19	15.8	11	9.1
Genital Ulcer	5	4.2	3	2.5
Rash/Itching	14	11.6	6	5
STD	10	8.3	2	1.7

Clinical presentation:

Overall, the most commonly reported symptoms in the two groups were fever, diarrhoea and cough. Although all three symptoms were reported more frequently in Group A than in group B, fever was reported by a significantly higher number of Group A seropositive subjects as compared to Group B seropositive subjects ($p<0.007$) (Table 4, next page).

Table 4 Correlation of HSV-2 IgG seropositive status with clinical presentations in Groups A and B

Clinical Presentation	Group A		Group B	
	n	%	n	%
Fever	51	42.5 (p<0.007)	29	24.2
Cough	18	15	12	10
Diarrhoea	15	12.5	6	5

DISCUSSION

Amongst the HIV infected group, significantly higher (p<0.0001) numbers of subjects tested seropositive (64.2%) for HSV-2 IgG. Similar co-infection rates were reported by a studies previously undertaken in South India (60%) [8] and the USA(67.7%).[9] However, other authors have reported lower co-infection rates in India, between 43% and 50%.[10,11-14]

Globally, a similar study undertaken in Iran[4], reported a very low co-infection rate of 6.5%. This low co-infection rate compared to other countries could be because of local lifestyle factors in Iran such as social, cultural and religious limitations in sexual contact, and less prevalent experiences of multiple sexual partnerships in the country.[4] Mugo et al reported a higher prevalence rate, of 81%, in Kenya.[15]

In the HIV infected group, HSV-2 seropositivity was significantly higher among male subjects (64.6%) as compared to females. This was in contrast to most studies, where higher seropositivity has been reported among female subjects.[4,10,15,16] The high seropositivity in the present study could be because the majority of the study subjects were male (65.8%). Higher seropositivity was also reported in male subjects by Karad et al,[12] however this difference was not statistically significant. A higher number of subjects reported seropositive in Group A as compared to Group B (73.3% and 49.3% respectively); and in the >20–40 years age group. Rode et al[10] have also reported lower seropositivity among cases (33.3%) and controls (2.9%) in the same age-group. This low seroprevalence in both the groups as reported by Rode et al[10] could be due to overall low co-infection rates in the two groups. In the present study, HSV-2 IgG seroprevalence was high in the reproductive age group, which has also been reported by other studies.[9,13] This could be due to factors including multiple sexual partners and unprotected sexual contact among the reproductive age group. HSV-2 IgG seroprevalence rates in the present study were found to increase with age. While Stover et al[9] and Rode et al [10] reported a similar pattern, however, Janbakhash et al[4] have reported an opposite trend – increasing seroprevalence with decreasing age; however, this correlation was not statistically significant. In the present study a significantly higher number of HSV-2 IgG seronegative subjects had an HIV positive spouse/parent compared to the seropositive group (p<0.04). However, no other study has shown similar findings. Amongst the modes of transmission among HIV positive subjects, a history of unprotected sexual contact was the most frequently reported mode of transmission and was significantly higher compared to each of the other modes of transmission: a history of blood transfusion, intravenous drug use and previous hospitalization. Among subjects with a history of unprotected sexual contact, HSV-2 IgG seropositivity was significantly higher (p<0.0004). Stover et al[9] reported similar findings. Karad et al[12] in their study found that condom use showed significant protective effect.

Among HIV positive subjects, a history of multiple sexual partners emerged as the most frequently reported risk factor and was significantly higher when compared to each of the other sexual risk factors: a history of vaginal or penile discharge; genital ulcers; rash/itching; and past STD. No other studies have reported similar findings, however some[7,11] have reported contact with commercial sex workers as the strongest independent risk factor. On analyzing the sexual risk factors in detail, a history of multiple sexual partners emerged as the most frequently reported risk factor (p<0.04) compared to other combinations of risk factors, consistent with finding from Stover et al[9] and Janbakhash et al[4]. In the HSV-2 seropositive

group, 45.8% of subjects gave a history of multiple sexual partners. Reynolds et al[11] and Karad et al[12] reported higher rates of seropositivity in subjects with multiple sexual partners (80.6% and 63.6% respectively). Presence of genital ulcers was associated with seropositivity in the present study (4% females and 5.7% males) and a similar association was reported by Karad et al[12] (4.5% of HSV-2 IgG seropositive subjects had a history of genital ulcers), whereas Reynolds[11] reported significant correlation (51.7%) between HSV-2 IgG seropositive subjects and a history of genital ulcers. The present study found a history of genital ulcers to be higher in males than females with no significant association; this finding is not reported by other studies.

In the present study, HSV-2 IgM co-infection among HIV infected subjects was 0.8% whereas other studies undertaken in India[2,7,8,17] have reported higher co-infection rates, with HSV-2 IgM varying between 28.6% to 58.4%. This low prevalence rate could be due to absence of seroconversion or re-activation among the subjects. A study undertaken in Nigeria by Mawak et al[18] reported a high HSV-2 IgM prevalence rate of 55% in HIV infected individuals.

Infection with HSV-2 leading to genital ulceration could result in higher local HIV shedding, along with increased risk of transmission of HIV. Some studies have focused on the synergy of these two viruses at a cellular level, whereby HSV-2 infection may increase the risk of acquiring HIV by up-regulating HIV replication and recruitment of HIV target cells. The significant co-infection rate as reported by our study thus supports previous findings by other authors.

CONCLUSION

In conclusion, HSV-2 IgG seroprevalence was observed to be higher in Group A subjects as compared to Group B. Unprotected sexual contact, multiple sexual partners, vaginal and penile discharge, and rash/itching emerged as the most frequently associated risk factors with HSV-2 IgG seropositivity. The evidence from the above-mentioned study points towards a causal relationship between HIV and HSV-2 infection risk and has important public health implications. If the association between HSV-2 and HIV is indeed causal, then the high seroprevalence of HIV and HSV-2 suggests the need for suppressive HSV-2 treatment as a strategy to reduce HIV transmission in the population.

In India, more studies need to be carried out in this regard to establish a causal relationship for enhancing the pathogenicity.

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