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**Farzana**  
Assistant Professor, Dept of  
Dermatology, GIMSR,  
Visakhapatnam, Andhra  
Pradesh, India

**Sree Harsha Y**  
Assistant Professor, Dept of  
Dermatology, GIMSR,  
Visakhapatnam, Andhra  
Pradesh, India

**Ramprasad Y**  
Senior Resident, Dept of  
Dermatology, GIMSR,  
Visakhapatnam, Andhra  
Pradesh, India

## A study of seroprevalence of herpes simplex virus infection in STI clinic attendees

**Farzana, Sree Harsha Y and Ramprasad Y**

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### Abstract

**Background:** Herpes simplex virus infections are prevalent worldwide. They are caused by Herpes simplex virus Type-1 (HSV-1) and type-2 (HSV-2). HSV-1 causes orolabial disease. Genital herpes may be caused by either HSV-2 or HSV-1, large majority of cases being caused by HSV-2. HSV-1 is acquired during early childhood by most of the individuals. The acquisition of HSV-2 starts around puberty or thereafter.

The ability of the virus to successfully avoid clearance by the immune system by entering a non-replicating state known as latency leads to life long persistence of infection with unpredictable reactivation and transmission. Detecting antibodies plays an important role in identifying those infected. Type specific tests have been developed that are based on the protein Glycoprotein G from HSV-2 (gG2) or Glycoprotein G from HSV-1 (gG1). As very limited sequence homology exists between gG1 and gG2, assays based on detecting these type specific epitopes using ELISA can reliably differentiate between antibodies to HSV-1 and HSV-2.

### Objectives

1. To study the Seroprevalence of Herpes simplex virus infection in patients attending the STI clinic.
2. To study the risk factors and other socioepidemiological factors associated with HSV-2 seropositivity.
3. To study the seroprevalence of HSV infection in HIV positive individuals.
4. To study the proportion of subclinical seropositives for HSV infection.

**Material and methods:** The study was carried out from July 2017 to June 2019. All patients reporting to DVL OPD at GITAM institute of Medical sciences & Research, Visakhapatnam, Andhra Pradesh were examined and evaluated. 82 consecutive patients of both sexes with STI complaints, in the age group of 15-70 years were studied. Patients with genital herpes as well as patients with other STI complaints including both HIV positive and HIV negative patients were included in the study.

With prior permission from ethical committee and consent from patients, blood samples were collected and tested for HSV-1 IgG and IgM antibodies and HSV-2 IgG and IgM antibodies.

**Results:** Out of 82 patients 42 (51%) were females and 40 (49%) were males; 41 (50%) were HIV positive and the other 41(50%) were HIV negative.

Out of 82 patients, 39(47.6%) had genital herpes and 43 (52.5%) had other STDs which include genital candidiasis (18.3%), non specific urethritis (13.4%), Condyloma acuminata (14.6%) and genital molluscum contagiosum (6.1%).

Out of 82 patients, 2(2.4%) were seropositive for HSV-1 IgM and 68 (82.9%) were seropositive for HSV-1 IgG.

Out of 82 patients, 4(5%) were seropositive for HSV-2 IgM and 40(48.8%) are seropositive for HSV-2 IgG.

Out of 39 patients with genital herpes 21(53.8%) were seropositive for HSV-2 and out of 43 patients with other STDs without genital herpes 19 (44.2%) were seropositive for HSV-2, indicating that the proportion of subclinical seropositivity was 44.2% in our study

**Conclusion:** From our study we conclude that Herpes simplex virus infection is common in STI clinic attendees in our hospital. Prevalence of HSV-1 and HSV-2 serum antibodies provide an epidemiological measure of population burden of these infections. The high seroprevalence of HSV-2 in HIV patients potentiates the need for regular HSV-2 screening in HIV positive individuals and proper counselling and treatment of HSV-2 seropositives during outbreaks will efficiently lower the frequency of recurrences and decrease the transmission of both HSV and HIV infections.

**Corresponding Author:**  
**Farzana**  
Assistant Professor, Dept of  
Dermatology, GIMSR,  
Visakhapatnam, Andhra  
Pradesh, India

**Keywords:** HSV 1 & 2; HIV; seropositive; STI

### Introduction

Herpes simplex virus infections are prevalent worldwide. They are caused by Herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2). HSV-1 causes orolabial disease.

Genital herpes may be caused by either HSV-2 or HSV-1, large majority of cases being caused by HSV-2. HSV-1 is acquired during early childhood by most of the individuals. The acquisition of HSV-2 starts around puberty or thereafter [1].

The ability of the virus to successfully avoid clearance by the immune system by entering a non-replicating state known as latency leads to life long persistence of infection with unpredictable reactivation and transmission. Detecting antibodies plays an important role in identifying those infected. Type specific tests have been developed that are based on the protein Glycoprotein G from HSV-2 (gG2) or Glycoprotein G from HSV-1 (gG1). As very limited sequence homology exists between gG1 and gG2, assays based on detecting these type specific epitopes using ELISA can reliably differentiate between antibodies to HSV-1 and HSV-2 [2].

In the most recent data from NHANES, the prevalence of HSV-1 is 48.1% in the years 2015–2016 in the general population<sup>9</sup>. In STD clinics in US, about 60% have HSV-1 antibodies. Antibodies to HSV-2 are not routinely detected in sera until puberty and antibody prevalence rates correlate with indices of sexual activity. In the United States, the overall rate of HSV-2 seroprevalence from 2015-2016 is 12.1%<sup>9</sup>. Fewer population based studies have been conducted in Asian countries. HSV -2 antibody prevalence in pregnant women is 8% in India, 27% in Saudi Arabia [3]. In south Africa HSV-2 infection rate reaches 80% in women and 40% in men by age.

The existence of synergistic relationship between HSV-2 and transmission of HIV has been indicated by many observational and biological studies in which HSV-2 has been implicated as a cofactor in the acquisition and transmission of HIV. This synergistic relationship can be substantial in developing countries that have high prevalence of both viral infections [4].

### Aims and objectives

1. To study the Seroprevalence of Herpes simplex virus infection in patients attending the STI clinic.
2. To study the risk factors and other socioepidemiological factors associated with HSV-2 seropositivity.
3. To study the seroprevalence of HSV infection in HIV positive individuals.
4. To study the proportion of subclinical seropositives for HSV infection.

### Materials and methods

The study was carried out from July 2017 to June 2019. All patients reporting to Dermatology OPD at GITAM institute of Medical sciences & Research, Visakhapatnam, Andhra Pradesh were examined and evaluated. 82 consecutive patients of both sexes with complaints suggestive of STI, in the age group of 15-70 years were studied. Patients with genital herpes as well as patients with other STI complaints including both HIV positive and HIV negative patients were included in the study.

With prior permission from ethical committee and consent from patients blood samples were collected and tested for HSV-1 IgG and IgM antibodies and HSV-2 IgG and IgM antibodies.

### Collection of samples

The whole blood was collected for estimation of HSV-1 IgM and IgG antibodies and HSV-2 IgM and IgG antibodies. Using disposable syringes and with sterile precautions about 5cc blood was withdrawn by venepuncture. Blood was collected in dry sterile bottles without adding anticoagulant.

### Processing of samples

Blood was centrifuged and serum separated. The serum was then transferred into sterile vials and preserved at -20 degrees centigrade until testing. Estimations of HSV-1 and 2 IgM and IgG antibodies against glycoprotein G1 and G2 were done based on the principle of Enzyme Linked Immunosorbent Assay (ELISA) using four separate kits for each antibody type at a standard laboratory.

### Results

A total of 82 patients, 40 (49%) males and 42 (51%) females were included in the present study.

### Population characteristics

**Table 1:** Sex Distribution of Study Population

Sex	Total Population-82	
	No	%
Male	40	49%
Female	42	51%

**Table 2:** Age Distribution of Study Population

Age groups In Years	Total population 82					
	Male - 40		Female- 42		Total	
	No	%	No	%	No	%
11-20	2	5%	1	2.4%	3	3.6%
21-30	9	22.5%	17	40.5%	26	31.7%
31-40	19	47.5%	15	35.7%	34	41.4%
41-50	5	12.5%	8	19%	13	16%
51-60	4	10%	1	2.4%	5	6%
61-70	1	2.5%	0	0	1	1.2%
Total	40		42			82

Out of 82 patients, 56 (68.3%) patients were from rural areas and 26 (31.7%) were from urban areas. In our study 58 (70.7%) patients belonged to low socioeconomic status and 24 (29.3%) belonged to middle class.

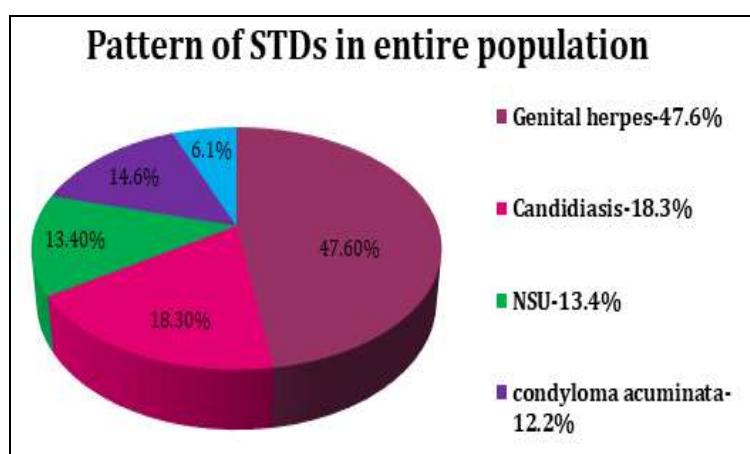
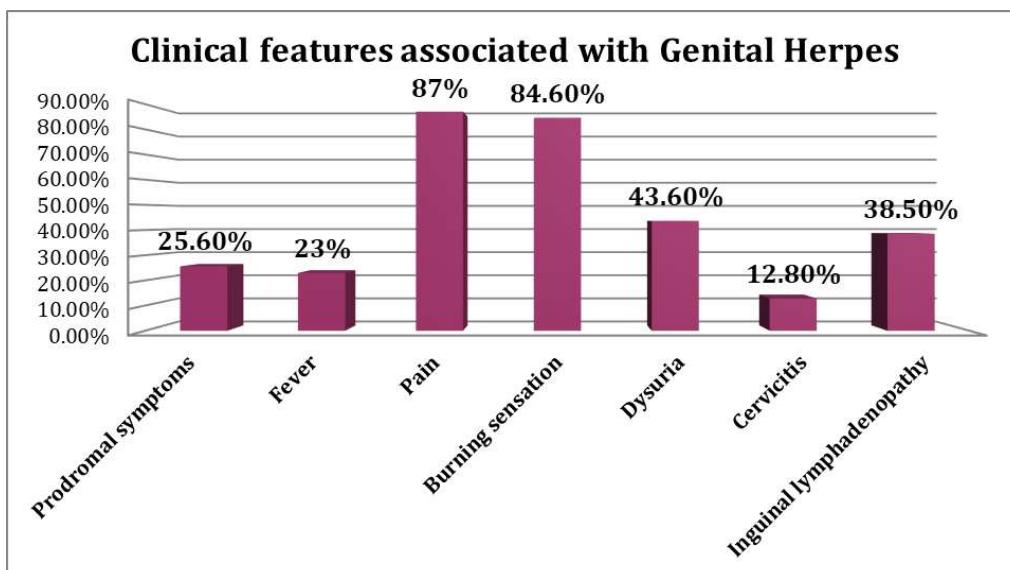
**Table 3:** Hiv Status of Study Population

HIV status	Total Population 82				Total	
	Male- 40		Female- 42			
	No	%	No	%	No	%
HIV Positive	16	40%	25	59.5%	41	50%
HIV Negative	24	60%	17	40.5%	41	50%
Total	40		42		82	

Of the 82 patients examined in the present study, 39 (47.6%) were clinically diagnosed of Genital herpes. There were 43 patients with other STDs which include 15 (18.3%) patients with genital candidiasis, 11 (13.4%) patients with non specific urethritis, 10 (12.2%) patients with condyloma acuminata, 4 (5%) with genital molluscum contagiosum and 3(3.7%) with erectile dysfunction.

**Table 4:** Pattern of Various STIs In the Study Population

Diagnosed STI	Total No of patients - 82					
	Male - 40		Female - 42		Total-82	
	No	%	No	%	No	%
Genital herpes	20	50%	19	45.2%	39	47.6%
Candidiasis	6	15%	9	21.4%	15	18.3%
Non specific urethritis	5	12.5%	6	14.3%	11	13.4%
Condyloma acuminata	6	15%	6	14.3%	12	14.6%
Genital MC	3	7.5%	2	4.8%	5	6.1%

**Fig 1:** Herpes genitalis – Superficial ulcers over inner aspect of prepuce**Fig 2:** Herpes genitalis in a HIV positive patient with extensive ulceration**Pie diagram 1:** Pattern of STDs in entire population**Graph 1:** Clinical features associated with Genital Herpes

**Table 11:** Serological Status of Hsv Antibodies of Entire Population

Type of Antibody	Total Population - 82			
	Positive		Negative	
	No	%	No	%
HSV-1 IgM	2	2.4%	80	97.6%
HSV-1 IgG	68	82.9%	14	17%
HSV-2 IgM	4	5%	78	95%
HSV-2 IgG	40	48.8%	42	51.2%

37(90.2%) out of 41 HIV positive patients and 31(75.6%) out of 41 of the HIV negative patients were seropositive for HSV-1 IgG. HSV-1 IgG seropositivity was more in HIV positive than HIV negative patients. 29 (70.7%) out of 41 HIV positive patients and 11(26.8%) out of 41 of the HIV negative patients were seropositive for HSV-2 IgG. HSV-2 seropositivity is high in HIV positive patients than in the HIV negative patients. The association of HSV-2 seropositivity with HIV infection is statistically significant with p value = 0. (Chisquare value = 15.814, degree of freedom = 1). Of the 43 patients with other STDs without genital herpes, HSV-2 IgG seropositivity was seen in 19(44.2%) individuals. Among them 5 (3.3%) out of 15 patients with genital candidiasis, 7 (63.6%) out of 11 patients with non-specific urethritis, 4 (33.3%) out of 12 patients with condyloma acuminata and 3(60%) out of 5 patients with genital molluscum contagiosum were positive for HSV-2 IgG antibodies.

## Discussion

Genital herpes is the commonest genital ulcerative disease [1]. Asymptomatic or subclinical viral shedding is an important aspect of clinical and epidemiological understanding of genital herpes as most episodes of sexual and vertical transmission appear to occur during such shedding [1]. Transmission of genital herpes usually occurs by sexual contact with a person who is shedding HSV subclinically. In addition, several studies have documented HSV-2 infection increases the risk of HIV infection among women by 3 fold and men by 2 fold [2].

In the present study, the serological profile of HSV infection in patients attending STI clinic and the association of seropositivity with HIV infection and other risk factors is noted. 82 consecutive STI clinic attendees of both sexes in the age group of 15-70 years were studied.

Of the 82 patients in the present study, there were 40(49%) male and 42 (51%) female patients. Most of the patients were in the age groups of 21-30yrs (31.7%) and 31-40yrs (41.4%). 41 patients (50%) were HIV positive and 41 (50%) patients were HIV negative. 58 (70.7%) patients were from low socioeconomic status and 56 (68.3%) were rural residents. Out of 82 patients, 32(80%) males and 28(66.7%) females were married and 37(45%) patients had multiple partners. All patients were heterosexual. Of the 82 patients, 39(47.6%) had genital herpes and there were 43 patients with other STDs which included 15(18.3%) patients with genital candidiasis, 11 (13.4%) patients with non specific urethritis, 12(14.6%) patients with condyloma acuminata, 5 (6.1%) patients with genital molluscum contagiosum.

Serological survey of STI clinic attendees in our hospital revealed that out of 82 patients, 2(2.4%) patients were positive for HSV-1 IgM antibodies while in a study by Subha Priya Venkateshwaran *et al.* [3] in Tamilnadu, 34.3% HSV-1 IgM positivity was seen. HSV-1 IgG antibodies

were positive in 68 (82.9%) patients in our study almost similar to 71.4% HSV-1 IgG positivity in a study by Subha Priya Venkateshwaran *et al.* [3] in Tamilnadu. In a study by K.N. Shivaswamy *et al.* 91.5% of STI clinic attendees were positive for HSV-1 IgG. Similar studies by P.N. Levett *et al.* and Rezaei *et al.* [4] showed varied results of 89% & 58.4% of HSV-1 IgG seropositivity. In STD clinics in United states, about 60% attendees have HSV-1 antibodies [4].

Prevalence of antibodies to HSV correlates inversely to socioeconomic status [5]. Serosurveys of western population in post world war II era found 80-100% of middle aged adults of low socioeconomic status had antibodies to HSV as compared with 30-50% of adults of high socioeconomic status. In our study 55(94.8%) out of 58 patients of low socioeconomic status had HSV-1 IgG antibodies as compared to 13 (54.2%) out of 24 patients of the high socioeconomic status showing statistical association of HSV-1 positivity with low socioeconomic status ( $p<0.001$ ). In our STD clinic attendees 44 (78.6%) out of 56 of rural population showed HSV-1 IgG antibodies as compared to 24(92.3%) out of 26 of urban population. HSV-1 IgG seropositivity is higher in uneducated (86%) than in educated (76%) individuals [6].

The frequency of HSV-2 antibody is higher among persons recruited from STD clinics [7]. Serological survey of STD clinic attendees in our hospital revealed that out of 82, 4(5%) patients were positive for HSV-2 IgM. In a study by Subha Priya Venkateshwaran *et al.* [4] in Tamilnadu 38.6% HSV-2 IgM positivity was observed.

Prevalence of antibodies to HSV correlates inversely to socioeconomic status [8]. In our study 31 (53.4%) patients of low socioeconomic status had HSV-2 IgG antibodies as compared to 9 (37.5%) in the middle class. In our STD clinic attendees 25 (44.6%) patients of rural population showed HSV-2 IgG antibodies as compared to 15(57.7%) of urban population. HSV-2 seropositivity is 50.9% among the educated patients and 44% in the uneducated.

HSV-2 seropositivity in relation to occupation was studied. In our study 3 out of 3(100%) of truck drivers, 3 among 5 (60%) businessmen, 10 among 19 (52.6%) housewives, 20 among 40 (50%) labourers, 1 of the 2 (50%) commercial sex workers and 1 among 6 (16.7%) students had HSV-2 antibodies.

In our study there is a wide disparity of seroprevalence of HSV-2 among the HIV positive (70.7%) and negative populations (26.8%) with a high seroprevalence of HSV-2 in HIV positives. As discussed above, our findings are consistent with several previous studies as well. This signifies that the burden of HSV-2 seropositivity is clearly associated with HIV positive individuals.

In the present study, of the 43 patients with other STDs without genital herpes, HSV-2 IgG seropositivity was seen in 19(44.2%) individuals. Among them 7 (63.6%) out of 11 patients with non-specific urethritis, 3(60%) out of 5 with genital molluscum contagiosum, 4(33.3%) out of 12 with condyloma acuminata and 5 (33.3%) out of 15 with genital candidiasis were positive for HSV-2 IgG antibodies.

## Conclusion

From our study we conclude that Herpes simplex virus infection is common in STI clinic attendees in our hospital. Prevalence of HSV-1 and HSV-2 serum antibodies provide an epidemiological measure of population burden of these infections. In developing countries where facilities for viral

culture, or Nucleic acid amplification tests are not available and more expensive, testing for HSV antibodies is a useful approach to detect those subclinically infected or to screen high risk groups like HIV positives.

The high seroprevalence of HSV-2 in HIV patients potentiates the need for regular HSV-2 screening in HIV positive individuals and proper counselling and treatment of HSV-2 seropositives during outbreaks will efficiently lower the frequency of recurrences and decrease the transmission of both HSV and HIV infections. The role of antiviral suppressive therapy in reducing viral shedding and transmission of infection in HSV-2 seropositive HIV individuals needs to be assessed. It is important that there is an accurate estimate of HSV-1 seroprevalence in adolescent and adult populations, in preparing for potential deployment of HSV vaccine as efficacy of vaccine has only been demonstrated in women with no previous antibodies to HSV-1 or HSV-2.

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