

Serological Profile of HSV-2 in STD Patients: Evaluation of Diagnostic Utility of HSV-2 IgM and IgG Detection

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ABSTRACT

Background and Objectives: The present study was undertaken to determine Herpes Simplex Virus-2 seroprevalence in sexually active adults aged 20-49 and to investigate the correlation with sociodemographic characteristics and to find its association with other sexually transmitted diseases especially HIV and also to assess the proportion of primary and reactivated HSV-2 cases.

Materials and Methods: This prospective study was carried out for a period of six months in a tertiary care hospital. Serum samples were taken from 91 patients attending the out Patient clinic of the Department of Venereology. The serological testing for HSV-2 was performed on all the specimens by using Euroimmun anti-HSV2 (gG2) IgM ELISA and IgG ELISA.

Results: Out of the 91 STD patients in the study group, 18 males (34.62%) and 14 females (36.84%) tested positive for HSV-2 antibodies. Seropositivity rate is 35.16%. More number of HSV-2 positive cases were seen among males, older age, rural residence, low socioeconomic status, single marital status,

irregular condom usage during the sexual intercourses with new partners and with higher number of sexual partners during lifetime. HSV-2 IgM alone was positive in three cases, HSV-2 IgG alone was positive in 26 cases and three had a positive HSV-2 IgM and IgG result. Addition of IgM testing increased rate of detecting seroconversion, 31.87%, when only IgG ELISA was used, to 35.16 % patients when IgM test was added. In the study group four cases tested positive for VDRL, and one of them was a known positive case. Among the 55 HIV positive cases in the study group, HSV 2 was positive in 17 cases and among the 36 HIV negative cases HSV 2 was positive in 15 cases. (30.91% and 47.22%). Though the number of HIV cases were high, HSV 2 positivity among them was statistically not significant.

Conclusion: The purpose of screening for HSV-2 is not only to identify seropositivity, but to help seropositive people identify symptoms and protect themselves from acquiring HIV and to protect their partners and seronegative people from acquiring HSV-2 and/or HIV.

Keywords: Herpes simplex virus-2, HIV, IgM ELISA, IgG ELISA, Seroprevalence

INTRODUCTION

Herpes simplex virus (HSV) infection is one of the most common viral sexually transmitted diseases worldwide, affecting more than one in five sexually active adults with an global estimate of 536 million infected persons and an annual incidence of 23.6 million cases among persons aged 15 to 49 y [1].

Herpes simplex virus type 2 (HSV-2) infections is considered to be almost exclusively sexually transmitted and may be symptomatic or asymptomatic. The majority of sexual HSV transmission occurs during asymptomatic periods because the patients are unaware of the asymptomatic virus shedding [2].

The potential role of HSV-2 infection in facilitating HIV transmission highlights the need for including anti-HSV-2 testing and therapy in the management of HIV positive patients, especially for reducing the risk of transmission of HIV through herpetic lesions [3].

The HSV infection may be identified directly by detection of the virus or one of its components or indirectly by assaying for specific serum antibodies of the viruses. After infection with HSV, specific antibodies are formed against the pathogen, which can be detected by various immunological methods. The most significant potential application of serology is to detect the silent carriers of HSV-2, especially in high-risk settings such as STD clinics [4].

The present study was done to evaluate the serological profile of HSV-2 among patients having sexually transmitted infections and to determine the utility of detecting HSV-2 IgM and IgG in such a high- risk group.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Venerology, Tirunelveli Medical College hospital, TamilNadu, India

from April 2013 to September 2013. The study protocol was approved by the ethical committee of the institution. The patients gave written informed consent to participate in the study.

Study population

Patients attending the out Patient clinic of the Department of Venerology of Tirunelveli medical college hospital were taken for study.

Inclusion criteria

1. All sexually active adults aged 20-49 attending the Outpatient clinic of the Department of Venereology.
2. Patients undergoing treatment for sexually transmitted diseases including HIV.

Exclusion Criteria

1. Unwillingness to participate in the study.

A predesigned structured Proforma having information on socio-demographic variables such as age, education, occupation, marital status, geographic location, annual income and risk factors that may be associated with sexually transmitted diseases like number of sex partners in previous one year, use of condom, alcohol consumption, commercial sex worker and type of sex partner were recorded. For all cases, the clinical history, signs & symptoms associated with genito-pelvic infections, including previous history of genital ulcer, pelvic pain, discharge, pain during sexual intercourse, genital warts, and swelling in the groin also recorded.

All the subjects were screened for other common sexually transmitted diseases by standard laboratory procedures / commercially available kits.

IgM Elisa Result	IgG Elisa Result	Number of Persons
Negative	Negative	59
Positive	Negative	3
Negative	Positive	26
Positive	Positive	3

[Table/Fig-1]: ELISA test results

Serology test	HIV positive(n=55)	HIV negative(n=36)
HSV 2 IgM positive	1	2
HSV-2 IgG positive	15	11
BOTH positive	1	2
Total	17	15

[Table/Fig-2]: Seroprevalence of HSV 2 in HIV patients

The history and treatment particulars of other sexually transmitted diseases were noted. The HIV status of the individual was recorded after obtaining consent.

Sample collection

From all the 91 patients, 7-8 ml venous blood samples were taken from the brachial veins under aseptic precautions and placed in vacutainers with no anti-coagulant, Serum samples were obtained by centrifuging the specimens for 8 min at 3000 rpm. Serums were forwarded to the Central Diagnostic Microbiology Laboratory immediately. Samples were then stored at -80 deg C till assay was carried out.

The serological testing for HSV-2 was performed on all the specimens by using Euroimmun anti-HSV2 (gG2) IgM ELISA and IgG ELISA.

Anti-HSV2 (gG2) IgM and IgG ELISA Test procedure

Semi quantitative in vitro assay for human antibodies of IgM and IgG class against the HSV-2 specific glycoprotein G2 in serum was performed according to the manufacturer's instructions.

STATISTICAL ANALYSIS

The collected data were edited for completeness, consistency and accuracy. They were analysed by parameters like mean and percentages. The differences of the above parameter were tested by chi square test.

RESULTS

A total of 91 patients attending the outpatient clinic of Department of Venereology, Tirunelveli Medical college Hospital who consented to participate in this study were analysed, of which 52 were males, 38 were females and one was a transgender. Maximum number of cases was seen in age group between 35 and 39 y. In the present study the lowest age was 21 y and highest age was 59 y. Most of them (63.77%) belonged to the lower socioeconomic status.

Among the 91 cases, 11 cases (12.09%) were single, 63 (69.23%) were married, 6(6.59%) were divorced and 11(12.09%) were widowers. Only one of the patients in the female was a commercial sex worker and she was married. The study group patients had attended the STD OPD for the following reasons. Among all the study subjects there were 8 (6.5%) of them who revealed that they had been diagnosed with any kind of STD at least once during the lifetime.

All patients were screened for syphilis by VDRL tests and four of them tested positive. All the 91 samples were tested for the presence of HSV2 specific IgM and IgG antibodies according to the manufacturer's instructions and the results are tabulated [Table/ Fig-1].

Thirty two samples tested positive for HSV-2 IgM or IgG or both and 59 samples were negative for both the tests. The seropositivity rate of HSV-2 is 35.16%. HSV-2 IgM was positive in six cases, amongst

which three of them had a positive HSV-2 IgG also. HSV-2 IgG was positive in 29 cases, amongst which three had a positive HSV-2 IgM result.

Among the 55 HIV positive cases, HSV 2 was positive in 17 cases and among the 36 HIV negative cases HSV 2 was positive in 15 cases [Table/Fig-2]. The HSV-2 seroprevalence and the patients sociodemographic characteristics are consolidated and tabulated in [Table/Fig-3].

DISCUSSION

HSV-2 is the virus most often responsible for genital herpes, a recurrent, painful, vesicular and ulcerative disease of the genitals in adults. Recent evidence demonstrates that all people infected with HSV-2 shed the virus asymptotically and the sexual contacts of individuals with symptomatic or asymptomatic HSV-2 are at-risk of becoming infected [5]. HSV-2 infection increases the risk of HIV acquisition by approximately threefold. Within co-infected patients, HSV-2 increases the plasma load of HIV, which may enhance HIV disease progression.

The clinical diagnosis of genital herpes is both insensitive and non-specific. Only approximately 20% of herpes infections have classic vesicular or ulcerative lesions. The clinical diagnosis can be confirmed by laboratory testing using culture, direct antigen tests, PCR, Cytology and type-specific serologies.

In this study, a total of 91 patients attending STD clinic were included, of which 52(57.14%) were males, 38(41.76%) were females and one was a transgender (1.1%). The median age was 37.9 y for males and 36.05 y for females. Most of them belonged to 35-39 age group. 18 males (34.62%) and 14 females (36.84%) tested positive for HSV-2 antibodies. Out of the 91 cases, 32 cases (35.16%) were seropositive. Difference in the rate of findings positive for HSV-2 antibodies between the two sexes and age groups was statistically not significant.

CDC reports that HSV-2 infection is more common among women than among men (20.9% versus 11.5% in 14 to 49 y olds). Infection is more easily transmitted from men to women than from women to men [6].

Suligo et al., shows that HSV-2 seroprevalence among persons attending an STD clinic in Italy is high (29.5%) and it increased with age, older age was independently associated with HSV-2 infection yet it did not differ by gender. It is believed that the age is closely connected with another risk factor, the duration of sexual activity, where, obviously, larger number of years of sexual activity represents greater risk [7].

Only 29.67% of the cases had a secondary or more level of education and out of 64, 26 cases (46.43%) who had lower level of education tested positive for HSV-2 antibodies and this is statistically significant($p=0.039$) [8]. Some studies had pointed out to the connection between lower education and HSV-2 seroprevalence though many did not find it significant [9-12].

In the study group 68 cases came from the rural areas (74.73%) and the remaining 25.27% resided within Corporation limits. Seventeen cases tested positive from the rural areas and 15 cases from the urban areas and was statistically significant. Most of the study group patients (63.77%) belonged to poor socioeconomic class, and 70.33% of the cases enrolled in the study were workers and daily labourers. The seropositivity among them was not statistically significant. In comparison to low income group, Biswas et al., observed significantly higher HSV-2 seroprevalence among middle or high income group [13]. Some studies have reported significant association between HSV-2 seropositivity and lower income [14].

Among the 91 cases, 11 cases (12.09%) were single, 63 (69.23%) were married, 6(6.59%) were divorced and 11(12.09%) were widowers. The seropositivity was higher (45.45%) among widowers and was almost equal among the single and married group but this difference was not statistically significant.

Sociodemographic Characteristics	Study population		HSV positive cases		HSV prevalence in the study population
	N=91	%	N=32	%	%
Sex					
Male	52	57.14%	18	56.25%	34.61%
Female	38	41.76%	14	43.75%	36.84%
Transgender	1	1.1%	-	-	-
Age Group					
20-24	6	6.59%	2	6.25%	33.33%
25-29	18	19.78%	7	21.88%	38.89%
30-34	12	13.19%	2	6.25%	16.67%
35-39	23	25.27%	7	21.88%	30.43%
40-44	14	15.39%	5	15.63%	35.71%
45-49	9	9.89%	2	6.25%	22.22%
>50	9	9.89%	7	21.88%	77.78%
Educational Level					
Secondary or More	27	29.67%	6	18.75%	22.22%
Less Than Secondary	64	70.33%	26	81.25%	46.43%
Marital Status					
Single	11	12.09%	4	12.5%	36.36%
Married	63	69.23%	23	71.88%	36.51%
Divorced	6	6.59%	-	-	-
Widowed	11	12.09%	5	3.13%	45.45%
Socioeconomic Status					
Lower	58	63.74%	19	59.38%	32.76%
Middle	28	30.77%	12	37.5%	42.86%
Higher	5	5.49%	1	3.13%	20%
Residence					
Rural	68	74.73%	17	53.13%	25%
Urban	23	25.27%	15	46.87%	65.23%
Occupation					
Govt./Non Govt Service	8	8.79%	1	3.13%	12.5%
Businesss	2	2.2%	1	3.13%	50%
Worker/ Labourer	64	70.33%	22	68.75%	34.38%
Student	2	2.2%	-	-	-
Unemployed	15	16.48%	8	25%	53.33%
Lifetime Number of Sexual Partners					
0	2	2.2%	-	-	-
1	48	52.75%	21	65.63%	43.75%
2-4	32	35.16%	7	21.88%	21.88%
5-9	6	6.59%	3	9.38%	50%
>10	3	3.3%	1	3.13%	33.33%

[Table/Fig-3]: HSV-2 seroprevalence distribution by subjects' sociodemographic characteristics

Yusuf et al., in his study observed a higher prevalence of HSV-2 infection among singles compared to married individuals and attributed it to the impact of other determinants, such as contact with commercial sex workers, early age of first sexual activity with HSV-2 seropositive persons and presence of other STIs. This report also indicated that there was a higher prevalence of infection among patients with more than one sex partner [6].

Higher HSV-2 seroprevalence was associated with marital status (with single subjects been under the greater risk), irregular condom usage during the sexual intercourses with new partners and with higher number (five or more) of sexual partners during lifetime in several surveys [15-18].

When an individual contracts herpes, the immune system responds by developing antibodies to fight the virus: IgG and IgM. IgM antibodies are usually detectable 9-10 d after exposure to the virus and lasts for 7-14 d, although they may remain detectable for even up to six weeks in certain individuals.

Out of the 91 samples, 32 tested positive for HSV-2 IgM or IgG or both and 59 samples were negative for both the tests. The seropositivity rate of HSV-2 is 35.16%. HSV-2 IgM alone was positive in three cases, HSV-2 IgG alone was positive in 26 cases and three had a positive HSV-2 IgM and IgG result. Addition of IgM testing increased rate of detecting seroconversion, 31.87%, when only IgG ELISA was used, to 35.16 % patients when IgM test was added. Literature states that tests for IgM in the early weeks following symptomatic genital HSV-2 infection markedly increased the detection of seroconversion though it appears to be ineffective at discriminating early from established infections for which it cannot be relied upon [19]. Three cases had a positive HSV-2 IgM and IgG result which indicates that genital herpes symptoms could be caused by either initial or recurrent infection.

Among the 55 HIV positive cases in the study group, HSV-2 was positive in 17 cases and among the 36 HIV negative cases HSV-2 was positive in 15 cases (30.91% and 47.22%). Though the number of HIV cases was high, HSV 2 positivity among them was statistically significant. Schneider et al., observed that among behavioural risk factors and other covariates in women and men, HIV infection exhibited the strongest association with HSV-2 [20]. The co- infected individuals appeared to be subject to high levels of asymptomatic HSV-2 genital infection, which could increase their genital infectivity for HIV-1 and therefore, the rates of HIV transmission to potentially exposed HIV-1-negative sexual partners [21]. Thus, it becomes essential to perform periodic, repeated and comprehensive screening of HIV patients for detection of HSV-2 antibodies [22].

Previous studies in India had shown much higher rates of HSV-2 sero conversion among women attending STD clinics and HIV voluntary counselling and testing centers. Kumarasamy et al., in Chennai, conducted a study among women at high risk for HIV and found the prevalence of HSV-2 infection to be 50% and a 1-year incidence to be 8% [23].

CONCLUSION

The purpose of screening for HSV-2 is not only to identify seropositivity, but to help seropositive people identify symptoms and protect themselves from acquiring HIV and to protect their partners and seronegative people from acquiring HSV-2 and/or HIV.

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