

# Seroprevalence of herpes simplex 2 Infection in patients attending STI clinic

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## Abstract

**Background:** HSV-2 is the primary cause of genital herpes. About 95% of genital infections due to HSV are predominantly caused by HSV-2. Serology is especially useful in recurrent, atypical lesions, culture negative cases and unrecognized infections. Laboratory confirmation of the diagnosis in patients who present with genital ulceration is mandatory. The present study was undertaken to determine the prevalence of HSV-2 antibodies among the patients attending STI clinic. **Material and Methods:** The study included clinically suspected 100 cases of genital herpes attending STI OPD at our institute. Serum samples were obtained from all the patients after written consent. A portion of serum sample was used for the detection of HSV-2 IgM antibody by HSV-2 IgM kits [DIA.PRO Diagnostic Bioprobe SRL, Italy]. **Results:** Out of 100 patients 36% were HSV-2 IgM ELISA positive and 64% were negative. Maximum (61%) ELISA positive cases were in the 21-30 years of age group. Male preponderance was apparently seen with 24 being males and 12 were females out of 36 HSV-2 seropositive cases. **Conclusion:** The seroprevalence of HSV-2 by IgM ELISA Test was 36%. The use of serological testing for diagnosis of genital ulcerative lesions is of course an indirect method, but it is useful in the situation where HSV is the most likely diagnosis of genital lesion and that the other causes of genital ulcerations are infrequent and have been ruled out.

**Key Words:** Genital herpes, Herpes Simplex Virus-2, IgM ELISA tests, prevalence.

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## INTRODUCTION

Genital herpes is a common sexually transmitted infection (STI) caused by herpes simplex virus (HSV). It is highly prevalent in human populations in many parts of the world. Data from various clinics across India suggests that rates of genital herpes among STD clinic patients range from 4% to 27.9% in different regions<sup>1</sup>. There are two types of HSV namely HSV-1 and HSV-2. Among these, HSV-2 is the primary cause of genital herpes.

About 95% of genital infections due to HSV are predominantly caused by HSV-2<sup>2</sup>. The infection is chronic, widespread, and infectious during both its symptomatic and asymptomatic periods<sup>3</sup>. It acts as a significant risk factor for acquisition and transmission of Human Immunodeficiency Virus (HIV) infection<sup>4</sup>. Genital herpes is usually a mild disease, however, the severity and frequency of clinical features may vary depending upon a number of viral and host factors. The infection in many individuals may go unnoticed and without any associated signs or symptoms. About 50% of patients who presents with first episode of clinical genital herpes have a true primary infection with either HSV-1 or HSV-2<sup>5</sup>. Whereas in rest of the cases, there is serological evidence of earlier infection usually with HSV-1 known as a non-primary first episode<sup>6</sup>. About a fourth of patients presenting with their first episode of clinical HSV-2 infection have serological evidence of previous HSV-2 infection, which suggests a recurrence in an individual with an asymptomatic first episode. Prior non genital

HSV-1 infection protects against acquisition of genital HSV-1 infection, but protection against genital HSV-2 is incomplete. The diagnosis of genital herpes is usually clinical. Laboratory tests for diagnosis of herpes virus infection include Tzanck smear, viral culture, serology and polymerase chain reaction (PCR). Serology is especially useful in recurrent lesions, healing lesions, atypical lesions, culture negative cases, unrecognized infections and in the evaluation of sexual partner, during pregnancy<sup>7</sup>. The most appropriate use of serologic testing is to differentiate primary from recurrent infection by comparing acute and convalescent antibody titers<sup>8</sup>. HSV antibodies form during the first several weeks after infection and remain indefinitely. Serological tests can be performed on blood or cerebrospinal fluid. Antibodies to HSV in blood can be detected by numerous techniques including complement fixation test, neutralizing antibody assays, enzyme-linked immunosorbent assay (ELISA) and western blot test. The presence of HSV-2 antibodies essentially confirms genital HSV infection as oral HSV-2 is uncommon without concomitant genital infection<sup>9</sup>. Occasionally patients may have HSV infections co-existing with other STDs. For this reason, laboratory confirmation of the diagnosis in patients who present with genital ulceration is mandatory. The present study was undertaken to determine the prevalence of HSV-2 antibodies among the patients attending STI clinic.

**MATERIAL AND METHODS**

The study included clinically suspected 100 cases of genital herpes attending STI OPD at our institute. All outdoor patients clinically suspected of herpes simplex virus 2 infection were included in the study. Serum samples were obtained from all the patients after written consent. A portion of serum sample was used for the detection of HSV-2 IgM antibody by HSV-2 IgM kits supplied by DIA.PRO Diagnostic Bioprobe SRL, Italy. It is an enzyme immunoassay for the detection of specific IgM antibodies against Herpes simplex virus type 2 antigens in human sera. The HSV-2 IgM test is based on the principle of an enzyme immunoassay (EIA). The assay is based on the principle of IgM Capture where IgM class antibodies in the sample are first captured by solid phase coated with anti hIgM antibody. After washing all other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a preparation of inactivated HSV-2, labeled with a HSV-2 specific antibody conjugate with peroxidase (HRP). After incubation, microwells were washed to remove unbound conjugate and then the chromogen/ substrate was added. In the presence of bound conjugate the colorless substrate is hydrolyzed to colored end product, whose optical

density may be detected and is proportional to the amount of IgM antibodies to HSV-2 present in the sample.

**RESULTS**

The maximum incidence of clinically suspected genital herpes cases were noted in the age group of 21-30 years age group (42%). Out of 100 cases of genital herpes patient 56 were male (56%) and 44 were females (44%) with male to female ratio of 1.2:1. Of the 56 male patients, 42(75%) were unmarried while 14(25%) were married with striking incidence of STD in unmarried male population. Of the 44 female patients, 37(84%) were married and 7(16%) were unmarried. Thus, in females there was preponderance of STDs in married ones. A total of 58 patients had recurrent episodes and 42 had first episode.

**Table 1: Age and Sex Distribution of Herpes Simplex Virus Type-2 Infection**

| Age and Sex distribution | No. of patients tested | No. of patients positive |
|--------------------------|------------------------|--------------------------|
| Age groups (years)       |                        |                          |
| 11-20                    | 17 (17%)               | 05 (13.8%)               |
| 21-30                    | 42 (42%)               | 22(61%)                  |
| 31-40                    | 19 (19%)               | 08(22.2%)                |
| 41-50                    | 11 (11%)               | 01(2.5%)                 |
| 51-60                    | 10 (10%)               | 00(0%)                   |
| > 60                     | 01 (1%)                | 00(0%)                   |
| Sex                      |                        |                          |
| Male                     | 56 (56%)               | 24(42.8%)                |
| Female                   | 44 (44%)               | 12(27.7%)                |

Out of 100 patients, 36% were ELISA positive and 64% were negative. Maximum ELISA positive cases were in the 21-30 years of age group (61%). Out of 56 male patients, 24 were found to be ELISA positive, while out of 44 female patients, 12 were found to be ELISA positive. However, this difference in ELISA positivity between male and female was not found to be statistically significant. ( $\chi^2=2.60, p=0.107, df=1$ ).

**DISCUSSION**

The sexually transmitted disease continues to prevail throughout the world. The genital ulcerative lesions are commonly seen everywhere irrespective of geographic location. Wide variations have been reported in the etiology of these lesions. Genital herpes caused by HSV-2 has emerged to become more frequent causative agent of genital ulcers. However, data in this context in our region is rather sparse. Therefore, the study was undertaken to evaluate the prevalence of HSV-2 infection in patients of genital ulcers. The maximum number of patients were in the age group of 20-30 years (40%) which corresponds to the age of maximum sexual activity in both males and females. The study done by Jaiswal and Bhushan reported

81.25% of patients in the same age group<sup>10</sup>. The study done by Mendiratta Vibhu and Harjai Bhawana<sup>11</sup> also reported genital herpes was common in 21-30 yrs of age group while study done by Shilpee *et al*<sup>12</sup> also shows most common age group was 25-30yrs. Thus, our findings are similar to these studies. In present study, 56 patients were male and 44 were female with male to female ratio of 1.2:1. In an earlier study by Verma *et al*<sup>13</sup> male to female ratio was found to be 9:1. The study done by Jain VK and Surabhi Dayal<sup>14</sup> showing a male: female ratio of 5.17:1. In an earlier study by Sarkar S, Shrimal A<sup>15</sup>, male to female ratio was 3:1. Slightly higher male to female ratio in our study may be due to most of our STD clinic attendee are male. Female patients with genital lesions may have attended gynecology services. Many female patients prefer to attend private clinics due to various reasons. In Indian set up, females generally do not seek medical attention for sexually transmitted diseases unless it is symptomatic or they are brought as contacts of their spouse. The presence of quiescent lesions or mildly symptomatic lesions in the concealed area for instance vagina or cervix may not bother the patient and she may not go to a doctor giving rise to poor female attendance. On the other hand, males seek medical advice for STDs more often and the lesions being present in the area visible to patients and on the genitals and even if mildly symptomatic make him seek medical advice, giving rise to high male/ female ratio. Out of 100 patients 36% were ELISA positive and 64% were negative. The seroprevalence of HSV-2 by IgM ELISA Test was 36%. In a study by Choudhry *et al*<sup>12</sup>, 30 patients out of 100 were ELISA positive (30%). The detection of serum antibodies to HSV-2 has been useful in epidemiological surveys evaluating the prevalence of the genital herpes infection. The prevalence of HSV-2 antibodies varies greatly amongst various groups. The factors responsible for this include age, sex, race, socioeconomic status, place of residence and the region. The prevalence of genital HSV-2 infection has been difficult to ascertain primarily because of the large percentage of subclinical cases and limitations of serologic assays for HSV-2 antibody detection. In India, the accurate national data on prevalence of genital herpes is lacking and very few studies have been undertaken in this respect. These large variations in the prevalence of HSV-2 infection may result from differences in sexual behavior, frequency of contact, abstinence during the period of viral replication or the use of barrier contraception. Maximum ELISA positive cases were in the 21-30 years of age group (61%). In the present study, like overall age prevalence of STDS, HSV-2 also displayed maximum prevalence (61.26%) in the age group of 21-30 years coinciding well with the degree of sexual activity. Mendiratta *et al*<sup>11</sup> also

reported genital herpes was common in 21-30 yrs of age group. In this study the male preponderance was apparently seen with 24 being males and 12 were females out of 36 HSV-2 seropositive cases, male is to female ratio being 2:1. This ratio is statistically not significant. However, this difference in ELISA positivity between male and female was not found to be statistically significant ( $x^2= 2.60, p=0.107, df=1$ ). The assays now available are fairly and can differentiate HSV-2 infections from that of HSV-1 eliminating the cross-reaction. The use of serological testing for diagnosis of genital ulcerative lesions is of course an indirect method. It is useful in the situation where HSV is the most likely diagnosis of genital lesion and that the other cause of genital ulcerations are infrequent and have been ruled out.

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