

Original Research Article

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Seroprevalence of Herpes Simplex Virus2 in Sexually Transmitted Diseases in a Tertiary Care Hospital in Visakhapatnam, India

Venkata Hemalatha Neeli, Perala Bala Murali Krishna*and Medidi Deborah Purushottam

Department of Microbiology, Andhra Medical College, Visakhapatnam, India

*Corresponding author

ABSTRACT

Keywords

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Herpes simplex virus type 2 (HSV2) infection is the primary cause of genital herpes and one of the most frequent sexually transmitted diseases world-wide. Subclinical shedding of HSV occurs in persons with a history of genital herpes and is instrumental in transmitting HSV infection. This study was carried out to find out the seroprevalence of HSV2 among sexually transmitted diseases patients, to examine the influence of certain factors associated with HSV2 infection and to evaluate the need for regular HSV2 seroprevalence surveys. Patients attending Sexually Transmitted Diseases (STD) out-patient department (OPD) with complaints of vesicles and ulcers over the genital region, either in their initial presentation or recurrence were examined for the presence of herpes virus infection by microscopy and serology. Majority of the STD patients with antibodies to HSV2 did not have history of genital herpes. This finding drives home the importance of screening in a community as the majority of persons infected with HSV2 infection is symptom-free and thus represent the major reservoir for HSV2 transmission. HSV2 seroprevalence is high among HIV seropositive individuals than among HIV seronegative individuals. HSV2 could thus be an important risk factor associated with HIV acquisition.

Introduction

'Venus' is the Goddess of love as per Greek mythology and that's the origin of the word 'Venereal Diseases'. The older terminology of 'venereal diseases' largely has been superseded in last 50 years by, 'sexually transmitted diseases' (STDs) and more recently by 'sexually transmitted infections' (STIs). Both ulcerative and non-ulcerative STIs have emerged as a major public health problem in all populations and socio-economic groups world-wide. Over 300 million new cases are reported annually and

75%-85% of them are in developing countries. The incidence and spread of STIs are greatly influenced by numerous factors such as availability of multiple sexual partners, the presence of asymptomatic infection, the frequent movement of people within populations and increasing affluence (Bhushan Kumar *et al.*, 2005).

The Herpes virus family contains several of the most important human viral pathogens. Clinically, the Herpes virus exhibits a

spectrum of diseases. The outstanding property of herpes virus is their ability to establish life-long persistent infections in their hosts and to undergo periodic reactivation. Their frequent reactivation in the immunosuppressed patients causes serious health complications (Jawetz *et al.*, 2013).

Herpes simplex virus type 2 (HSV2) infection is the primary cause of genital herpes and one of the most frequent sexually transmitted diseases world-wide (Anna Wald *et al.*, 1997, 2004; David *et al.*, 2004; Sgaier *et al.*, 2011). Subclinical shedding of HSV occurs in persons with a history of genital herpes and is instrumental in transmitting HSV infection to sexual partners and neonates (Anna Wald *et al.*, 1995).

HSV2 prevalence is increasing worldwide, and has become a prominent public health issue over recent years. More than 80 % of primary HSV2 infections are asymptomatic. Therefore the prevalence of the disease is best assessed by serological surveys. The prevalence of HSV2 antibodies is dependent on sexual activity, ranging from 0% in celibate adults to over 80 % in commercial sex workers (David *et al.*, 2004; Weatherall, 1996).

The main objectives of this study to find out the seroprevalence of HSV2 among sexually transmitted diseases patients. To examine the influence of certain factors associated with HSV2 infection. And also to evaluate the need for regular HSV2 seroprevalence surveys.

Materials and Methods

The present cross sectional study was conducted at King George Hospital (KGH), a tertiary care hospital in Visakhapatnam over a period of seventeen months, i.e., from

February 2014 to June 2015. The study group included 100 patients attending Sexually Transmitted Diseases (STD) out-patient department (OPD) with complaints of vesicles and ulcers over the genital region, either in their initial presentation or recurrence. Patients already on treatment with anti-viral agents were excluded from the study.

Their consent for participation in the study was obtained. Detailed history of each and every patient pertaining to the name, age, sex, address, education, occupation, income, socio-economic status, marital status, name and occupation of the spouse if married, multiple sexual partners (if any), whether belonging to any high risk group (such as CSWs, MSM, IV drug users, truck drivers), complaints and duration of symptoms, past history of genital herpes or a different STI, history of similar complaints of the partner was taken and examination findings were noted down. The diagnosis of herpes virus infection was made by microscopy and serology.

Microscopy

Tzanck smear examination

The vesicles were unroofed with a sterile needle.

Base of the lesions were scraped with sterile surgical blade.

The material thus obtained was smeared onto a clean glass slide.

The smears thus prepared were then fixed with gentle heat.

They were then stained with 1% aqueous solution of toluidine blue 'O' for 15 seconds.

The smears thus prepared were examined under oil immersion objective for multinucleated giant cells with faceted nuclei and homogeneously stained 'ground glass' chromatin, the "Tzanck cells" (Figure 1).

Serology

Collection of blood samples

Using sterile disposable syringes, under strict aseptic conditions, about 5ml of blood was withdrawn by venipuncture.

Blood thus collected was then transferred into sterile blood collection tubes (vacutainer tubes) with clot activator.

The blood sample was centrifuged; serum was separated and transferred into sterile vials.

Serum thus obtained was preserved at -70 °C.

All the samples were screened for HSV2 type specific IgM and IgG antibodies by ELISA [EUROIMMUN] and the results were noted.

Test Procedure of Anti-HSV-2 (gG2) ELISA (IgM and IgG)

Principle of the test

The test, based on the principle of ELISA detected the presence of human antibodies of IgM and IgG class against the HSV-2 specific glycoprotein G2 in the serum respectively. The test kits (two in number, one for IgM and the other for IgG) (Figure 2) contained microtiter strips each with 8 break-off reagent wells coated with purified glycoprotein G2. In the first reaction step, diluted patient samples were incubated in the wells. In the case of

positive samples, specific IgM and IgG antibodies bound to the antigens. To detect the bound antibodies, a second incubation was carried out using an enzyme-labelled anti-human IgM and IgG (enzyme conjugate), which was capable of catalyzing a colour reaction. The intensity of the colour formed was proportional to the concentration of IgM and IgG antibodies against HSV-2 glycoprotein gG2.

Preparation of the reagents was done as per the manufacturer's instructions.

All reagents were brought to room temperature before use.

The coated wells were opened after tearing off the protective wrapping and after the microplate has reached the room temperature.

The calibrator and controls were mixed thoroughly before use.

The serum samples for analysis were diluted 1:101 with sample buffer.

The wash buffer was diluted 1:10 with distilled water.

The enzyme conjugate was mixed thoroughly before use.

The bottle of chromogen/substrate solution was closed immediately after use as the contents are sensitive to light exposure.

Sample incubation (Figure 3)

100 µl each of the calibrator, positive and negative controls, and diluted patient samples sera were pipetted into the individual microplate wells and incubated for 30 minutes at room temperature. After incubation, the wells were emptied and

subsequently washed 3 times using 300 µl of working strength wash buffer per well for each wash. The wash buffer was left in each well for 30 to 60 seconds per washing cycle. The wells were then emptied. After washing, the microplate was thoroughly disposed of all liquid by tapping it on absorbent paper, with the openings facing downwards, to remove all residual wash buffers.

Conjugate incubation (Figure 4)

100 µl of enzyme conjugate (peroxide-labelled anti human IgM and IgG) was pipetted into each of the microplate wells and incubated for 30 minutes at room temperature. After incubation, the wells were emptied and washed as described earlier.

Substrate incubation: 100 µl of chromogen/substrate solution was pipetted into each of the microplate wells and incubated for 15 minutes at room temperature in the dark.

Stopping the reaction: 100 µl of stop solution was pipetted into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Photometric measurement: The color intensity was measured at a wavelength of 450 nm and a reference wavelength between 620 nm and 650nm within 30 minutes of adding the stop solution (Figure 5 and Figure 6 show ELISA reader and ELISA washer respectively). A homogenous distribution of the solution was ensured prior to measuring by slightly shaking the microplate.

Calculation of the results (Figure 7)

Results were evaluated semi-quantitatively by calculating a ratio of the extinction value

of the control or patient sample over the extinction value of calibrator and calibrator 2 for IgM and IgG respectively.

Ratio=

$$\frac{\text{Extinction value of the control or patient sample}}{\text{Extinction value of calibrator/ calibrator 2}}$$

Results were interpreted as follows:

Ratio < 0.8 : negative
Ratio ≥ 0.8 to <1.1 : borderline
Ratio ≥1.1 : positive

Both positive and negative controls served as internal controls for the reliability of the test procedure.

Results and Discussion

Among the 100 patients included in the study, 72% were men and 28% were women. Seroprevalance of HSV2 and its relation to the Tzanck smear positivity is shown in Tables 1 and 2 respectively.

Majority of the cases, 42% that were HSV2 seropositive belonged to 31-40 years of age-group (Diagram 1). In the study population, only 12 % individuals with antibodies to HSV2 gave history of symptoms suggestive of genital herpes (Diagram 2). Recurrence of the symptoms was reported by 35% of the seropositive individuals (Diagram 3).

Sixty-three percent of seropositives were married and 83% belonged to low socio-economic group (Diagram 4). 53% of them had multiple sexual partners (Diagram 5). 35% of seropositives were drivers by occupation (Diagram 6). Majority of them, 89% were not using any barrier methods (Diagram 7). Men who have Sex with Men (MSM) were 3% and the remaining were heterosexuals and all were sexually active. Association of HSV2 with HIV infection was also observed in 69% (Table 3).

In the present study, a higher seroprevalence of HSV2 was observed among the high risk groups compared to the general population.

Similar findings were observed by Steben and Sacks (1997); Nahmias *et al.*, (1990) and Santos *et al.*, (1996).

Table.1 HSV2 IgM and IgG seroprevalence (n=100)

HSV2 Seroprevalence	IgM	IgG
Positive	9	28
Borderline	1	8
Negative	90	64
Total	100	100

Table.2 Tzanck smear positivity (n=100) in relation to HSV2 seroprevalence

Tzanck smear	Study group	In HSV2 IgM antibody Positive	In HSV2 IgG antibody Positive
Positive	13	2	11
Negative	87	7	17
Total	100	9	28

Table.3 Association of HSV2 with HIV infection (n=100)

Association with HIV infection	Total	HSV2 IgM antibody			HSV2 IgG antibody		
		Positive	Borderline	Negative	Positive	Borderline	Negative
Reactive	13	0	0	13	2	7	4
Non-reactive	87	9	1	77	26	1	60

Fig.1 Tzanck smear showing multi-nucleated giant cells

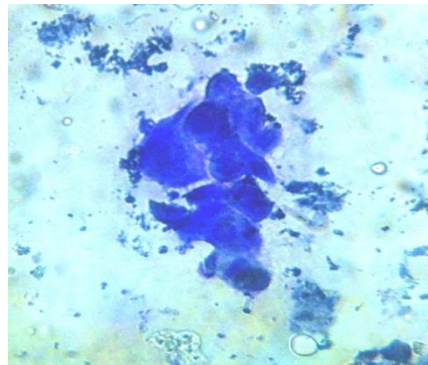


Fig.2 Contents of HSV2 IgM and IgG ELISA kits



Fig.3 HSV2 IgM and IgG ELISA - Diluted sera

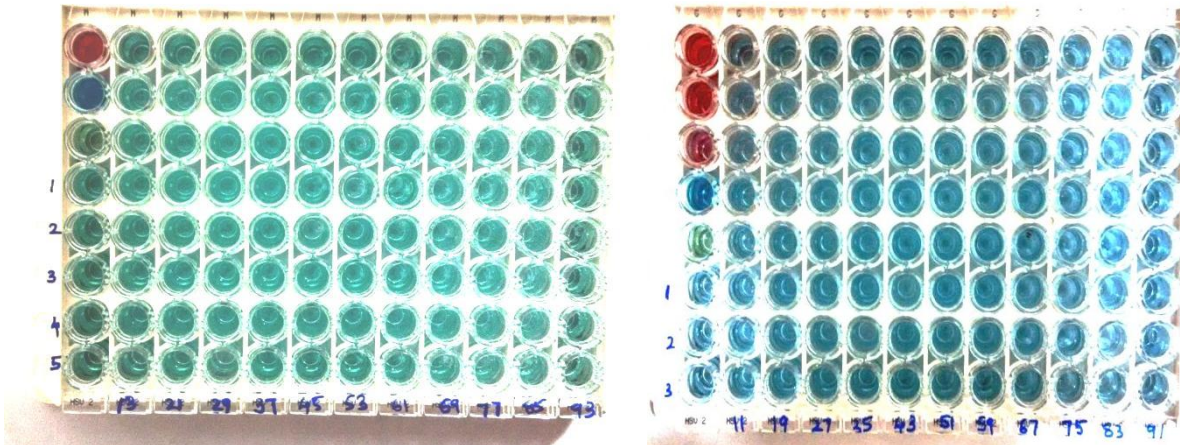


Fig.4 HSV2 IgM and IgG ELISA – Conjugate incubation

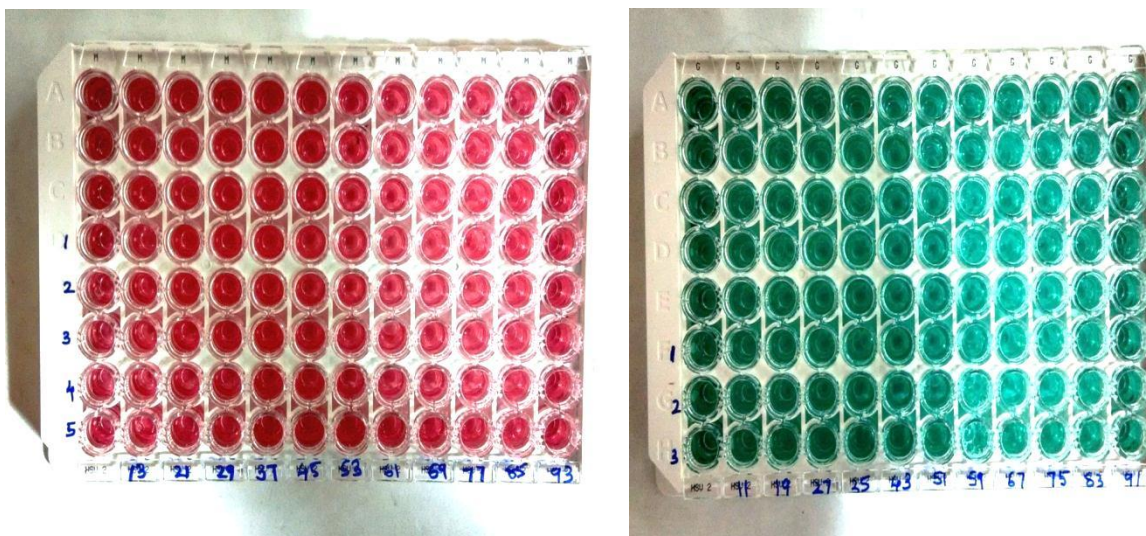


Fig.5 ELISA Reader



Fig.6 ELISA Washer

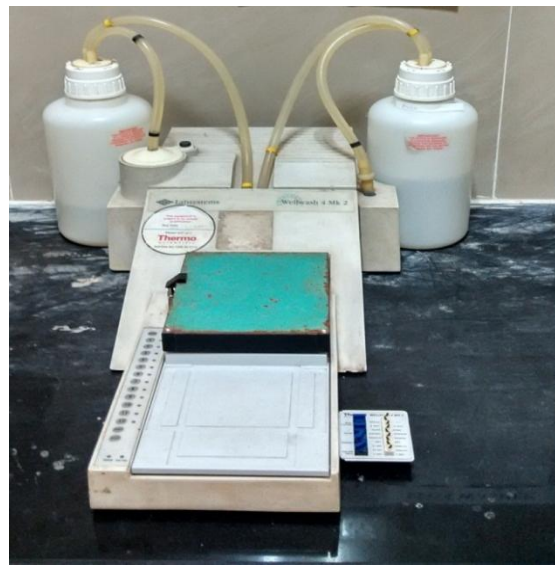
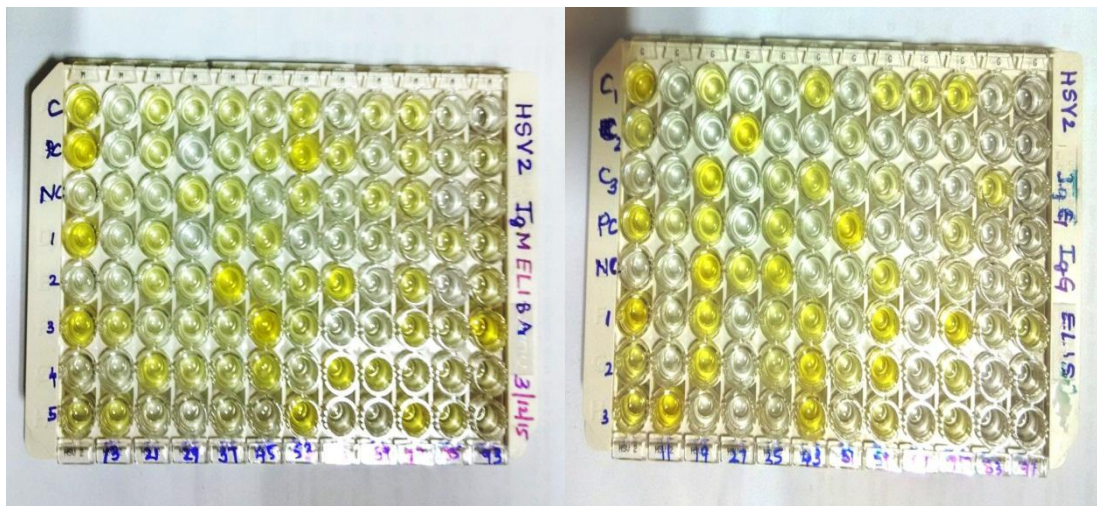


Fig.7 HSV2 IgM and IgG ELISA – Results



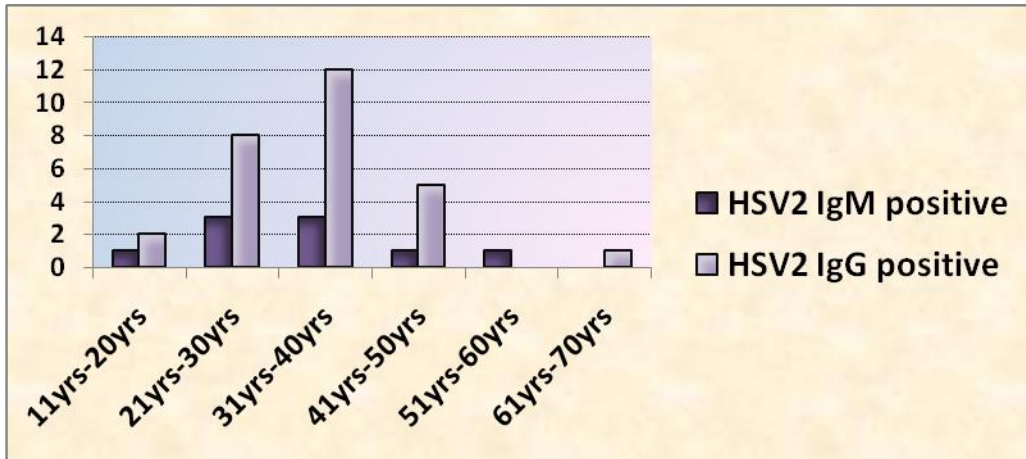


Diagram 1: Bar diagram showing HSV2 seroprevalence in relation to age

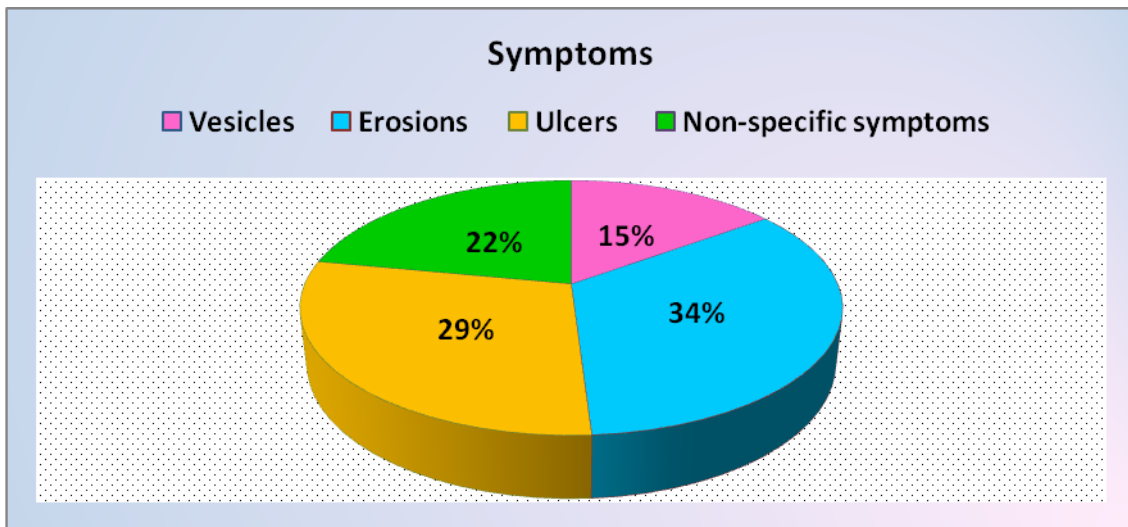


Diagram 2: Pie diagram showing symptom-wise distribution of cases

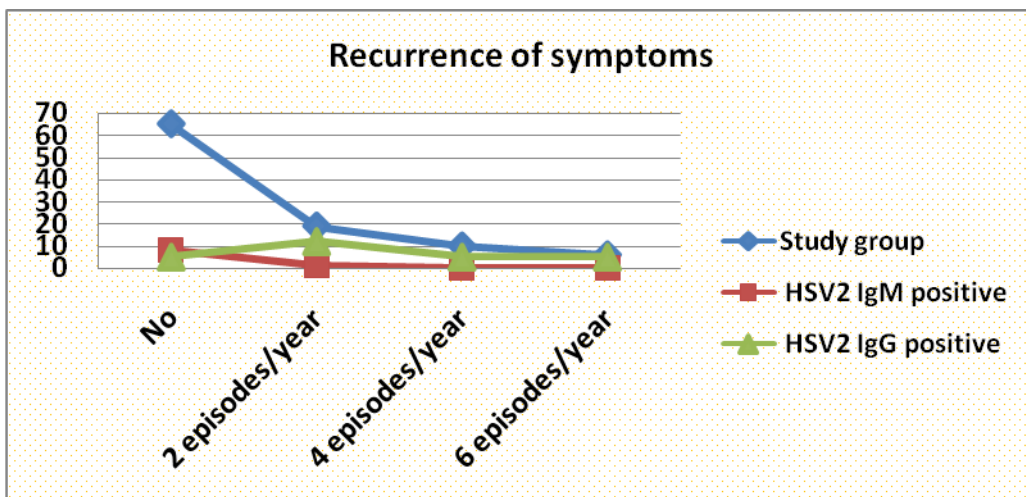


Diagram 3: Line diagram showing recurrence of symptoms

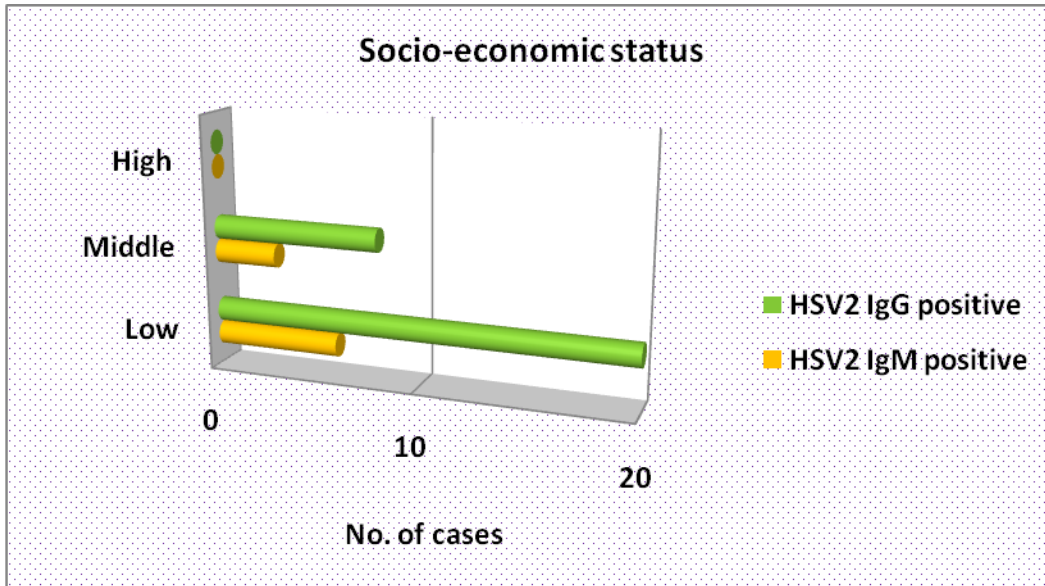


Diagram 4: Bar diagram showing HSV2 seroprevalence in relation to socio-economic status

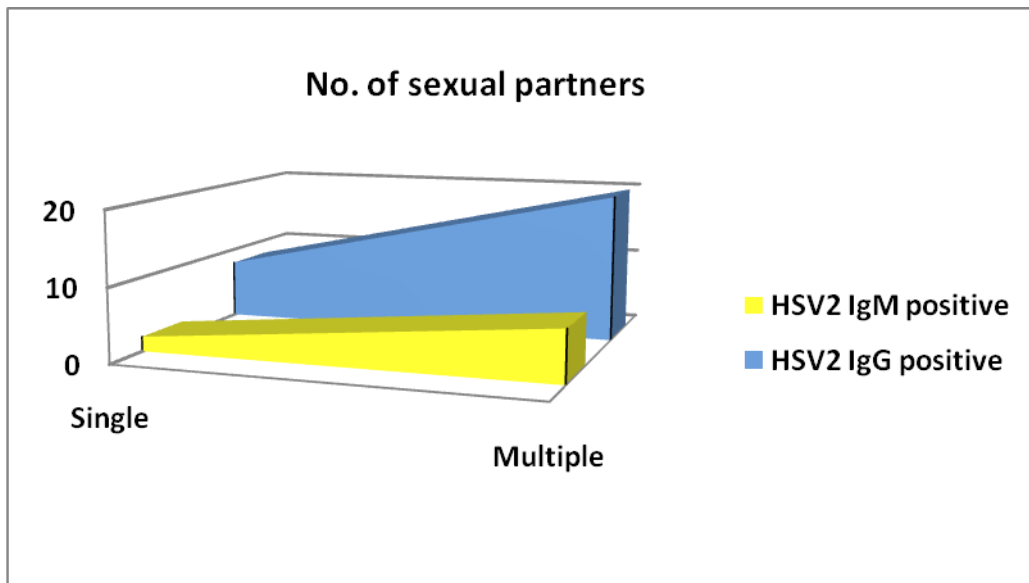


Diagram 5: Area diagram showing distribution of cases based on no. of sexual partners

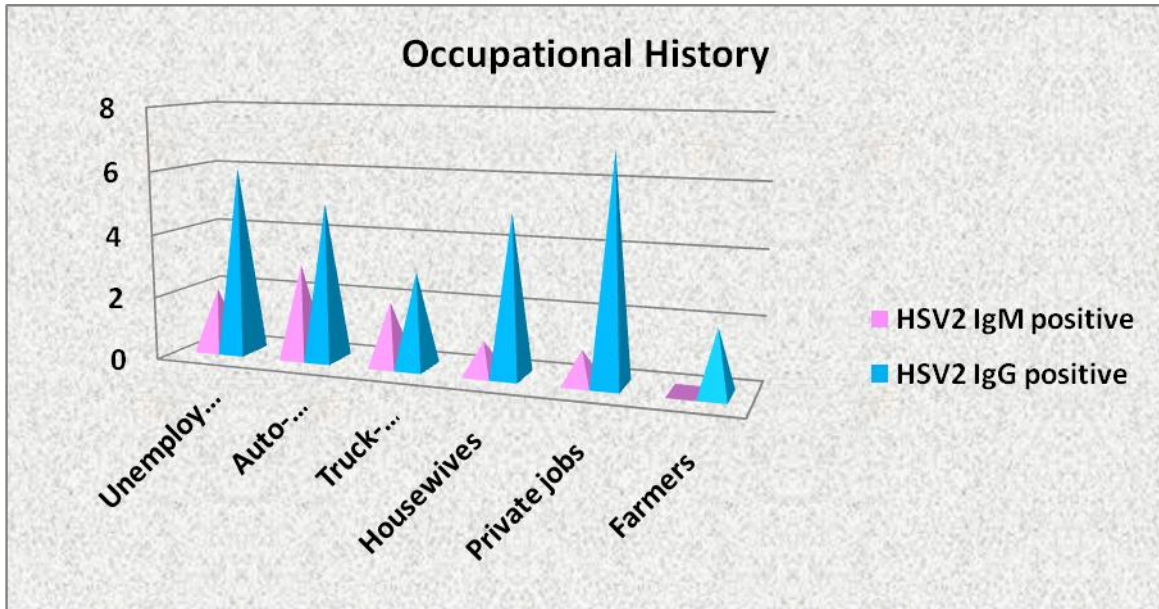


Diagram 6: Bar diagram showing distribution of cases based on occupation

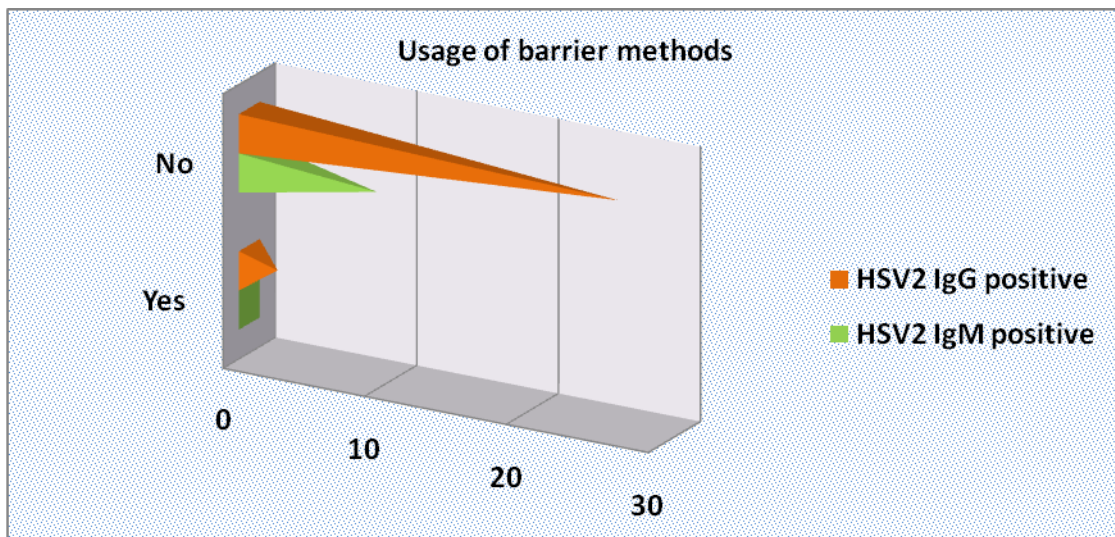


Diagram 7: Bar diagram showing HSV2 seroprevalence in relation to usage of barrier methods

In the study population, only 12 % individuals with antibodies to HSV2 gave history of symptoms suggestive of genital herpes. Similar findings were observed by Bassett *et al.*, (1994); Gottlieb *et al.*, (2002); Janier *et al.*, (1999); Varela *et al.*, (2001) and Wald *et al.*, (2000).

In the present study, the prevalence of antibodies to HSV2 was higher among STD

patients with multiple sexual partners (51%) than that of patients with a single partner (21%). A similar association was reported by Cowan *et al.*, (1994); Gottlieb *et al.*, (2002); Varela *et al.*, (2001); Fleming *et al.*, (1997) and Weiss *et al.*, (2001).

In the present study, the prevalence for antibody to HSV2 was higher in HIV seropositive individuals than that of HIV

seronegative individuals (69 % vs. 30%) with a ratio of 2.3:1. These findings were comparable with those of Steben and Sacks (1997); Bystricka *et al.*, (1998); Bystricka *et al.*, (2000); Gwanzura *et al.*, (1998); Mbopi – Keou *et al.*, (2000); Wutzler *et al.*, (2000); Lindan *et al.*, Russel *et al.*, (2001) and Wald and Link (2002).

In conclusion, majority of the STD patients with antibodies to HSV2 did not have history of genital herpes. This finding drives home the importance of screening in a community as the majority of persons infected with HSV2 infection are symptom-free. And thus represents the major reservoir for HSV2 transmission.

This study emphasizes the importance of studying the disease by serological methods. HSV2 seroprevalence is high among HIV seropositive individuals than among HIV seronegative individuals. Thus HSV2 could be an important risk factor in acquiring HIV infection.

High HSV2 seroprevalence in STD patients and its significant association with HIV infection demonstrates the need for regular HSV2 screening in STD patients. Asymptomatic individuals also can be serologically identified as carrying HSV2 and/or HIV infection.

Proper counseling and effective treatment for HSV/HIV will not only reduce the rate of transmission of HSV2 infection but also decrease the incidence of HIV infection. However, the findings made in the present study were to be confirmed in larger population-based studies so that more definitive conclusions can be made.

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