

Original Research Article

Seroprevalence pattern of Herpes Simplex Viruses (HSV-1 & 2) among STI vulnerable Women population

ABSTRACT

Background: Genital infections of Herpes Simplex virus are viewed as the indicators of sexual network in the society. Most of the infections caused by these viruses are asymptomatic and underdiagnosed.

Aim: To investigate the seroprevalences of HSV-1 and HSV-2 among the STI vulnerable women and to correlate them with clinical infections and co-positivities to other viral STIs.

Study design: A cross sectional retrospective study was carried out with female contacts of HIV / STI positive male partners, who were consulting the STD clinics of NGOs and OP section of STD department of Government hospital in Chennai, India between October 2006 and September 2008.

Methodology: The study included 138 STI vulnerable women along with age matched control group from whom socio-demographic and clinical data were collected. Serological screening was performed using ELISA for detection of IgM and IgG antibodies to HSV types 1 and 2 and co-positivities to HIV, HBV, HCV and CMV. Statistical analysis of results was carried out using Chi-square test.

Results: Overall seroprevalences of 79.71% and 74.32% were recorded for HSV-1 and HSV-2 respectively among the study group. Proportional increase in symptomatic cases were observed with individuals showing seropositivities to IgM, IgM+IgG (combined) and IgG antibodies to both types of HSV correlating to primary, reactivated and episodic infections. Observation of symptomatic cases among HSV-1 (48.0% vs. 4.05%) and HSV-2 (68.29% vs. 25.0%) IgG positive study and control groups corroborated the sexual transmission of these viruses. Significant percentages of co-positivities to other viral STIs were recorded with higher preponderance among HSV-2 seropositive individuals than those of HSV-1.

Conclusion: Comprehensive serological screening and correlation with the clinical data would be very helpful in demonstrating the impact of HSV infections. Wide screening of STI vulnerable women is necessary to estimate the actual burden of STIs and to adopt preventive intervention.

Key words: HSV, Seroprevalence, STI, HIV, Symptomatic, Seropositivity

1. INTRODUCTION

Herpes simplex virus (HSV) is one of the members of Herpesviridae family and an agent of widespread infections in humans, affecting 60-95% of the adult population worldwide [1]. There are two types of HSV, HSV-1 and HSV-2 which affect different parts of the body, and have different manners of transmission. Both of these viruses are transmitted by direct contact with infected secretions and their clinical manifestations and symptoms can overlap. These two viruses are highly identical with respect to genome and outer membrane morphology and share many immunogenic properties. Yet, they can be differentiated serologically based on the type-specific antibodies produced against their envelope glycoproteins viz., gG-1 (HSV-1) and gG-2 (HSV-2) [2].

HSV infections affect mainly humans and occur worldwide irrespective of seasonal variation [3]. A global estimate of 2012 stated that 3709 million (67%) and 417 million (11.3%) people were living with HSV-1 and HSV-2 infections respectively [4].

For infection initiation, HSV must come into direct contact with mucosal surfaces and epithelial cells of the skin through minor breaks. Subsequently, the virus enters into the host and spreads systemically. By means of retrograde transport, the virus establishes latent infection in sensory root ganglia, where it persists throughout life and cause periodical recurrent infections [5]. Reactivation of latent virus causes its 'shedding' (release) from the surface of the skin or mucosa [6]. Based on the immunological status of infected person, the virus can cause variety of infections. In most cases it causes asymptomatic (subclinical) or short-lived symptomatic infections [7]. Recurrent infections occur in individuals with pre-existing antibodies to the HSV type which is causing the prevailing infection [8]. Clinical symptoms characterized by ulcerative lesions occur at the site of infection in about 10–25% of primary infections [9]. In rare cases and in immunocompromised hosts, complications such as aseptic meningitis, blindness and encephalitis can occur [1].

Approximately 57 and 89% of individuals with a history of primary HSV-1 or HSV-2 infection, respectively, experience symptomatic HSV reactivation (recurrence) with symptoms lasting between 5 and 10 days. Individuals with genital HSV-2 infection experience about four recurrences per year whereas those with genital HSV-1 infection experience about one recurrence per year [10]. However, the virus shedding can facilitate its transmission from the infected individuals with or without symptoms [8].

Herpes simplex virus type 1 (HSV-1) is usually transmitted by nonsexual contacts and is acquired during childhood consequent with diminishing maternal passive immunity. The infection onsets very early in life along with characteristic orolabial ulcers [11]; and the seroconversion of infected individuals occurs late around the time they attain puberty [4]. Review of epidemiological studies indicate that global seroprevalence of HSV-1 is very high with an average of 40–60% in the normal population [12]. Substantial seroprevalence of HSV-1 has been reported in many countries such as Germany, Spain and Norway, where the number of affected individuals amounts to 50-85% [13]. However, the rate of incidences and seroprevalence of HSV-1, especially in Western countries, have declined in recent years owing to the improved standards of hygiene and living conditions [14]. Significant proportion of youth in these countries lack any nonsexual exposure to HSV-1 in their early life and enter sexual debut, thus facing the risk of acquiring genital infection [15].

Herpes simplex virus type 2 (HSV-2) is an important agent of sexually transmitted infection (STI) with the susceptible people being adolescents and adults [16]. In the affected individuals it causes lifelong infection and facilitates HIV transmission in high-risk group [17]. Nearly 80% of infections caused by HSV-2 are asymptomatic and among the range of diseases it causes, genital ulcer disease (GUD) is the most notable one. This disease is associated with recurrent, ulcerative and painful genital lesions and can lead to neonatal herpes if the virus is transmitted by infected pregnant women [18]. According to WHO, more than 400 million people aged 15–49 years around the world were seropositive for HSV-2 in 2012, with the annual incidences of 23 million cases. The global HSV-2 seroprevalence has been estimated to be between 10 and 40% [3] and ranges between various parts of the world viz., Asia (10 - 20%), Europe (5% - 25%), Latin America (20% - 40%), North America (15% - 25%), and sub-Saharan Africa (10% - 70%) [19, 20].

The risk factors leading to HSV-2 infection are comparable with other sexually transmitted infections (STIs): having multiple sexual partners, young age at sexual debut, intravenous drug abuse and previous exposure to STIs [21]. Individuals at higher risk for HSV-2 infection are among the group comprising female sex workers, HIV patients (60 - 95%) and those involving in sexual activities with partners having HIV / STIs. In the perspectives of epidemiologists, the HSV-2 seroprevalence constitute an implication in

public health assessment and could be considered as an index measure of risk behaviors paving way to STIs and HIV epidemics [19].

Indian women, especially from Tamil Nadu, are emotionally and sentimentally attached to their families. Owing to their sincerity towards spouse's welfare, they devote and subject themselves to sexual contact with their male partners, not attempting to know the health status of the latter. As a result, they become ill-fated victims of STIs. Many research studies have proposed that HSV infection, especially of genital origin, could be a significant risk factor for other STIs owing to its latent nature and the ability of disrupting the immune barriers of genitalia. Therefore, the present study was undertaken to screen STI vulnerable women (SVW) with a view to enlighten the pattern of seroprevalences of HSV types 1 and 2 and their association with other common STIs.

2. MATERIALS AND METHODS

2.1 Study population

This cross sectional retrospective study was conducted for a period of two years from October 2006 to September 2008 on selective group of sexually transmitted infections (STIs) vulnerable population. A total of 138 female contacts of HIV / STI positive male partners, who were consulting the STD clinics of NGOs and Out patients section of STD department of hospital for diagnosis or screening, constituted the study population. Volunteers of the age group ranging 20-55 were enrolled for the study from eight sources including Seven Non-Governmental Organizations (Manshree Deaddiction Center, Ashram Trust Chemical Addiction and Recovery Center, Wisdom Deaddiction Center, Sumana Goodwill Home, Freedom Foundation, Madras Christian Council of Social Service and Positive Network) and one Government General hospital located in Chennai city. Besides, age matched and healthy 250 female of general public, who were reportedly not indulged in any known STI risk behaviours, were inducted in the study as control group.

2.2 Collection of study information

For the purpose of collection of samples, the method of convenient sampling was followed. Sociological, demographical and clinical information pertaining to the study of interest were collected using a structured questionnaire. Sociological details such as age, education, marital status, family type, occupation and economic status were collected. Demographic information included type of sexual relationship, practice of condom use, injection drug abuse and other high risk behaviors. Clinical details such as symptoms observed, recurrence and episodic infections of HSV-1 & 2 were recorded through an interview with each volunteer of both study and control groups. Throughout the study anonymity was maintained with respect to the details of the volunteers.

2.3 Specimen collection and processing

From each volunteer enrolled in the study 2 ml of blood specimen was collected using sterile disposable syringe following aseptic procedure. This specimen was then decanted in sterile screw capped test tubes, labelled with unique code and then placed in thermocol ice box containing ice blocks. Specimens collected from different points were then transferred under cold chain to the Laboratory of Department of Microbiology, Presidency College (Aut.), Chennai, India. Blood specimens that have undergone lysis and of inadequate quantity were rejected. Each specimen was brought to the room temperature and then subjected to centrifugation at 2,500 rpm for 15 minutes using laboratory analytical centrifuge for the complete separation of serum. The serum samples were then transferred to a sterile vial of 1 ml capacity and preserved at 4°C (for immediate use) or at -20°C (for long term storage).

2.4 Serological screening of Specimens

Specimens were tested for seroprevalence of Herpes Simplex Virus types 1 & 2 (HSV-1 & 2) and their co-seropositivity to selected STI agents using certified diagnostic kits obtained from internationally reputed manufacturers. Serological screening for these agents was done with the Enzyme Linked Immunosorbent Assay (ELISA), the most accepted sensitive technique for serological diagnosis of diseases.

For the purpose of screening the serum specimens, different types of ELISA were used. Diagnostic kits were procured from two reputed manufacturers viz, Novatec Immunodiagnostica (Germany) and General Biologicals Corp. (Taiwan). The details of the agents tested, ELISA method followed, and particulars of

diagnostic kits are as given in the table 1. Care was taken to adhere to the criteria prescribed by the diagnostic kit manufacturers in order to obtain valid assay results.

Assay procedures for each ELISA were followed adopting the instructions given along with the diagnostic kits. Results of assays were observed and recorded using ELISA Microwell Plate Reader (Microlisa Plus, Micro Lab Instruments, India). The absorbance of all wells was measured at 450nm and the absorbance values for each control and patient sample was recorded in accordance with the distribution and identification plan. Results were validated and interpreted as positive or negative based on the mean absorbance values of the samples in comparison with positive and negative control samples.

Table 1. ELISA methods and Diagnostic kits used for Serological screening

Sl. No.	Agent Tested	ELISA Method	Brand Name & Product Code
1.	IgM Antibody to HSV type 1	Capture	Novatec HSV1M0500
2.	IgG Antibody to HSV type 1	Capture	Novatec HSV1G0500
3.	IgM Antibody to HSV type 2	Capture	Novatec HSV2M0540
4.	IgG Antibody to HSV type 2	Capture	Novatec HSV2G0540
5.	Antibodies to HIV type 1 & 2	Sandwich	General Biologicals HIV ASE 1+2;
6.	Surface Antigen of HBV	Sandwich	General Biologicals SURASE B-96
7.	Antibodies to HCV	Sandwich	General Biologicals SP-NANBASE B-96
8.	IgG Antibody to CMV	Capture	Novatec CMVG0110

2.5 Statistical analysis of results

The data of results from the serodiagnostic studies were statistically analyzed for their significance and reliability using Chi-square test for proportional differences and comparisons. A threshold of P value >0.01 and ≤ 0.05 was determined to be statistically significant and that of ≤ 0.01 as highly significant.

3. RESULTS

Serological screening of both study population – STI vulnerable women (SVW) (i.e., Female contacts of HIV / STI positive male partners) and control group was carried out for seropositivity to HSV-1, HSV-2 and other vital STI agents such as HIV, HBV, HCV and CMV. For a comprehensive and systematic understanding, the results of HSV screening are presented under three categories viz., single positivity to either IgM or IgG, dual positivity to both IgM and IgG and co-positivity to other vital STI agents. Besides, the data on seropositivity to HSV-1 and HSV-2 among symptomatic and asymptomatic individuals of study population and control group were also recorded.

3.1 Seropositivity to HSV-1 and HSV-2

Among the 138 volunteers of SVW screened 110 of them showed seropositivity to anti-HSV-1 IgM and IgG (79.7%) (Table 2; Fig.1). The percentage of seropositivity to HSV-1 for control group was 74.3%. The total positivity of study population to IgG and combined positivity to IgM+IgG were found to be statistically significant (Table 2). In contrast, only 67 individuals among the screened SVW were seropositive to anti-HSV-2 IgM and IgG (48.6%), while it was 10.1% for control group (Table 3; Fig.1). Statistical validation of seropositivity to HSV-2 indicated that these data were either significant or highly significant (Table 3).

Table 2. Seropositivity to HSV-1 among SVW and control groups

Study Group	No. of persons positive	Total positivity		Selective & combined positivity		
		IgM	IgG	IgM	IgM + IgG	IgG
SVW	110	35	100	10	25	75
Control	110	36	90	20	16	74
<i>P</i> value & Interpretation		0.839	0.037*	0.922	0.048*	0.672

*, Significant; #, Highly significant

Table 3. Seropositivity to HSV-2 among SVW and control groups

Study Group	No. of persons positive	Total positivity		Selective & Combined positivity		
		IgM	IgG	IgM	IgM + IgG	IgG
SVW	67	26	49	18	08	41
Control	15	07	09	06	01	08
<i>P</i> value & Interpretation		0.006#	0.002#	0.047*	0.041*	0.009#

*, Significant; #, Highly significant

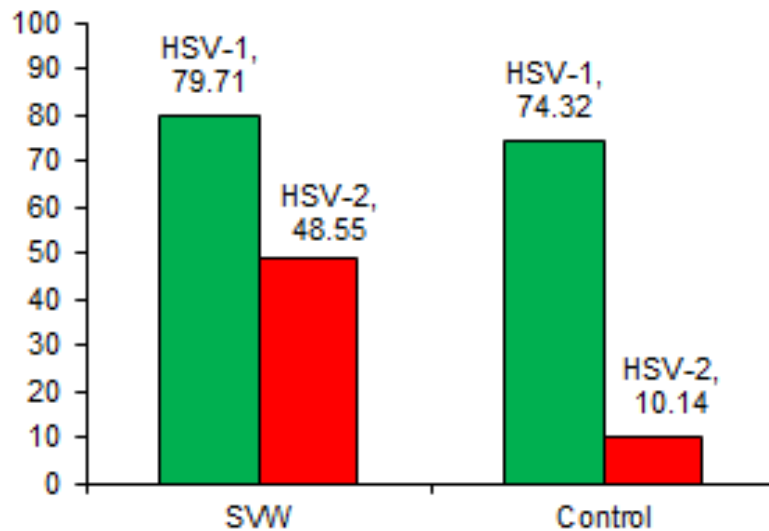


Fig 1. Seroprevalence (%) of HSV-1 and HSV-2 among SVW and Control group

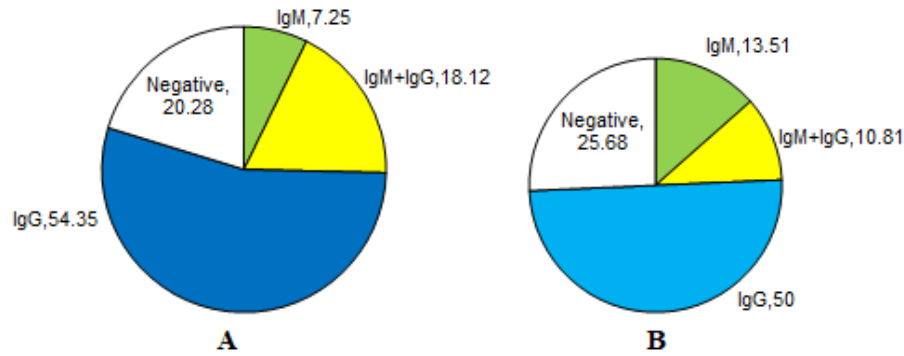


Fig 2. Selective and combined positivity (%) to HSV-1 among A) SVW and B) Control group

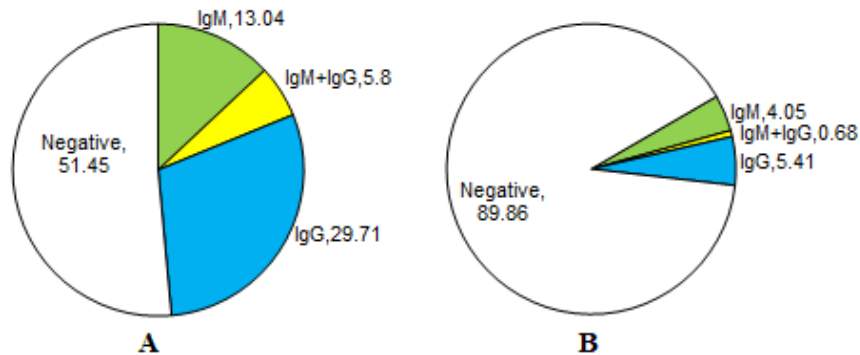


Fig 3. Selective and combined positivity (%) to HSV-2 among A) SVW and B) Control group

Further studies on the selective or combined positivity to IgM and IgG antibodies of both HSV-1 and HSV-2 seropositive study and control groups inferred interesting data. Percentages of positivity to IgM, IgM+IgG and IgG were in the ascending order for both HSV-1 seropositive SVW and control groups. However, the selective positivity to IgG was slightly higher among SVW (54%) than the control group (50%) (Fig. 2). In contrast, among the HSV-2 seropositive SVW more than half of them showed positivity to IgG followed by those positive to IgM and IgM+IgG. Although only ~10% of control group were seropositive to HSV-2, nearly 50% of them were positive to IgG followed by those to IgM and negligible percentage to IgM+IgG (Fig. 3).

3.2 Correlation of Seropositivity to clinical symptoms

Investigation of symptomatic cases among HSV-1 and HSV-2 seropositive SVW and control groups with selective and combined positivity to IgM and IgG gave rise to useful data. (Tables 4 & 5). Among the HSV-1 seropositive individuals, the percentage of symptomatic cases were higher among SVW who showed selective positivity to IgG (48%), followed by those positive to IgM+IgG and IgM alone (Fig. 4). Similar trend, but higher percentages of symptomatic cases were observed in the case of HSV-2 seropositive SVW with individuals positive to IgG and IgM+IgG encompassing >60% of symptomatic cases individually (Fig. 4). Detection of symptomatic cases among those selectively positive to anti-HSV-1 IgM and IgG was observed to be statistically significant (Table 4). However, data on the occurrences of symptoms among all the HSV-2 positive cases of SVW were highly significant (Table 5). As expected, symptomatic cases were negligible in the case of HSV-1 seropositive control group (Tables 4 & 5; Fig.4). Strikingly, significant portion (25%) of HSV-2 seropositive control group were symptomatic. Statistically, incidences of symptomatic cases among the individuals with single and combined positivity to IgM and

IgG in both HSV-1 and HSV-2 seropositive SVW and control groups were either significant or highly significant.

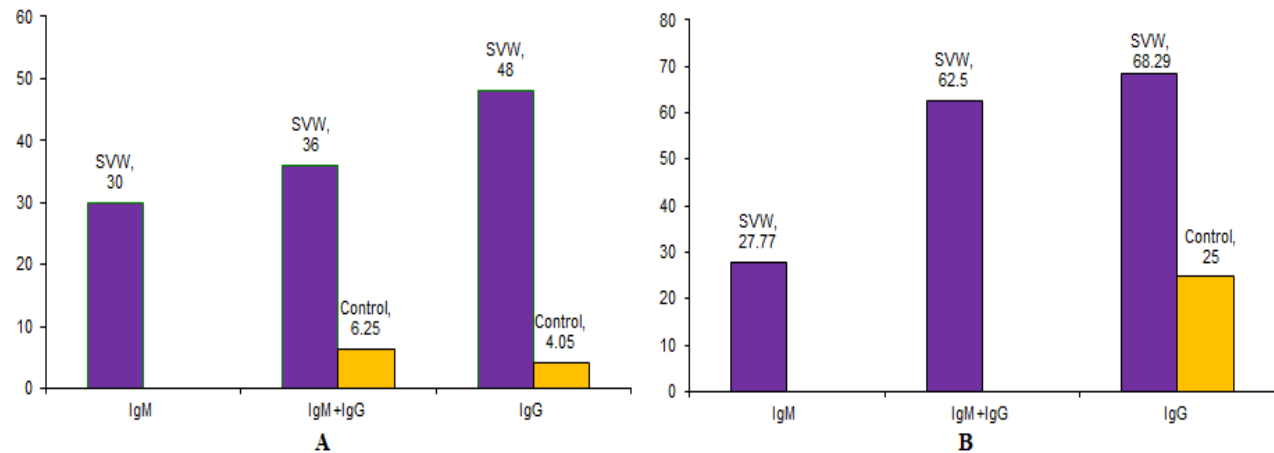


Fig 4. Incidence (%) of symptomatic cases among A) HSV-1 and B) HSV-2 seropositive SVW and control groups

Table 4. Correlation of HSV-1 seropositivity to clinical symptoms among SVW and control groups

Study Group	No. of persons positive	Persons with selective & combined positivity to					
		IgM		IgM + IgG		IgG	
		S	AS	S	AS	S	AS
SVW	110	03	07	09	16	36	39
Control	110	0	20	01	15	03	71
P value & Interpretation		0.001 [#]	--	0.023*	--	0.008 [#]	--

*, Significant; [#], Highly significant; S, Symptomatic; AS, Asymptomatic cases

Table 5. Correlation of HSV-2 seropositivity to clinical symptoms among SVW and control groups

Study Group	No. of persons positive	Persons with selective & combined positivity to					
		IgM		IgM + IgG		IgG	
		S	AS	S	AS	S	AS
SVW	67	05	13	05	03	28	13
Control	15	0	06	0	01	04	04
P value & Interpretation		0.008 [#]	--	0.008 [#]	--	0.009 [#]	--

*, Significant; [#], Highly significant; S, Symptomatic; AS, Asymptomatic

3.3 Co-positivity of HSV-1 and HSV-2 seropositive cases to other viral STIs

Studies on co-positivity of HSV-1 seropositive individuals to other STIs causing viruses revealed higher co-positivity to CMV in both SVW (77%) and control groups (51%) (Table 6; Fig.5). Followed by this, significant co-positivities (in descending order) were noted with HBV and HIV. However, only few cases of HSV-1 seropositive SVW, especially those with combined positivity and IgG selective positivity, were

found to be co-positive to HCV (Table 6; Fig.5). Overall validation of data indicated that co-positivities to HIV and HBV among HSV-1 seropositive cases were statistically highly significant (Table 6).

Table 6. Co-positivity (%) to other STI viruses among HSV-1 seropositive SVW and control groups

Anti HSV-1 antibodies detected	Study Group	Total no. positive	No. positive to other viral agents			
			HIV	HBV	HCV	CMV
IgM	SVW	10	2	1	0	7
	Control	20	0	0	0	9
IgM + IgG	SVW	25	2	4	2	19
	Control	16	0	1	0	8
IgG	SVW	75	16	20	3	59
	Control	74	0	5	1	39
<i>P</i> value & Interpretation			0.002 [#]	0.009 [#]	0.745	0.116

*, Significant; [#], Highly significant

Table 7. Co-positivity (%) to other STI viruses among HSV-2 seropositive SVW and control groups

Anti HSV-2 antibodies detected	Study Group	Total no. positive	No. positive to other viral agents			
			HIV	HBV	HCV	CMV
IgM	SVW	18	2	1	0	17
	Control	06	0	2	0	4
IgM + IgG	SVW	08	2	2	1	8
	Control	01	0	1	0	1
IgG	SVW	41	12	6	2	33
	Control	08	0	0	0	4
<i>P</i> value & Interpretation			0.006 [#]	0.381	0.036*	0.048*

*, Significant; [#], Highly significant

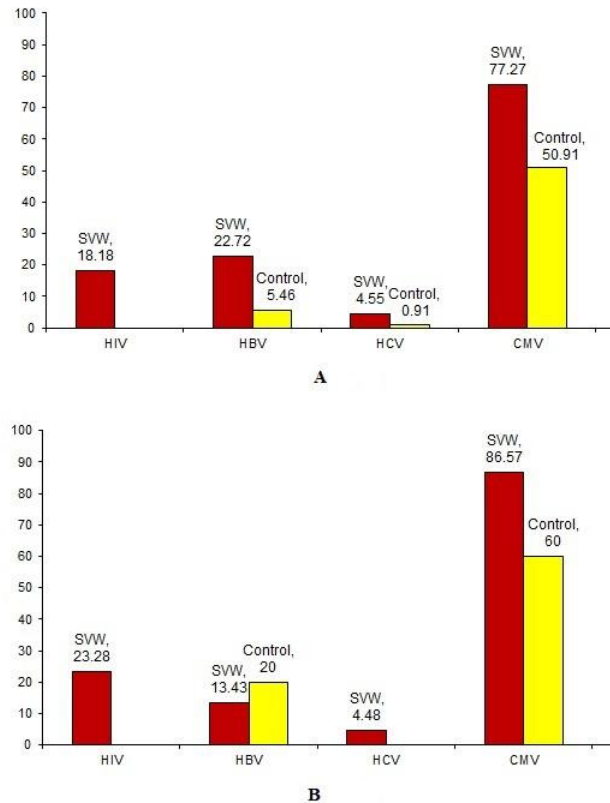


Fig 5. Co-positivity to other viral STIs among A) HSV-1 and B) HSV-2 seropositive SVW and control groups

As noted before, substantial co-positivity to CMV was observed with HSV-2 seropositive SVW (58 out of 67; 87%) and control groups (9 out of 15; 60%) (Table 7; Fig.5). Similar to the HSV-1 seropositive cases, highly significant co-positivity to HIV was noted with HSV-2 seropositive SVW (23%). None of the HSV-2 seropositive cases of control group were co-positive to HIV or HCV (Table 7; Fig.5).

4. DISCUSSION

Knowledge about HIV infection that eventually leads to AIDS syndrome is an eye opener for the study of pathogenic agents that endanger the immunity of human population. Some studies, based on mathematical modeling have demonstrated that seroprevalence of genital HSV is a statistical index of sexual network in a population [22]. Epidemiological assessment of this 'fore runner' HSV infection in the community could pave the way for combating life threatening STIs that afflict vulnerable populations.

The present study has attempted to decipher the prevalence of HSV types-1 and -2 infections among the study population (SVW) and control groups using a serology based test i.e., detection of antiviral antibodies by ELISA. Research studies suggest that these kind of type specific immunoassays facilitate the investigator to identify and distinguish the individuals with symptomatic infection and asymptomatic carrier status [1, 23]. Thus, these serological tests would be of immense use in deriving a comprehensive idea about HSV epidemiology.

The overall seroprevalence of HSV taking into account of both IgM and IgG positivities is one of the main outcomes of this study. The seropositivity of SVW to HSV-1 and HSV-2 was 79.71% and 74.32% respectively and the percentages of positivity in the control group were much lower. On comparison between SVW and the control group, the HSV-1 seroprevalence in the SVW is about 30.0% higher than the control group and the overall seropositivity to HSV-2 is much higher i.e., 64.0% (Fig. 1). An Iranian study demonstrated comparatively lower prevalence of HSV among general population with a total prevalence of 42.04% and individual prevalence of 25.7% (HSV-1) and 6.5% (HSV-2) [13]. Our report is in consonance with the data of Iranian study with respect to overall lower prevalence of HSV among

control group. A global review of 2015 estimated a HSV-1 seroprevalence of 60% of Indian general population also reiterates the same view [4]. The seroprevalence of HSV-2 of our control group (10.14%) is comparable to that of Mexican women (12.2%) [18].

Review of literature indicate that the seroprevalence of HSV-1 and HSV-2 among general population of both Western and Asian countries has been declining in recent decades. While decline of HSV-1 could be attributed to improved hygiene and socio-economic status [11], the changes in sexual behavior, increased condom use and emphasis of sex education in schools are endorsed for HSV-2 for the same [17]. However, higher general seroprevalence of 89.4% of HSV-1 have been documented in Middle East and North Africa [24]. Another recent study of India recorded seroprevalence rates of 89.0% and 0% respectively for HSV-1 and HSV-2 in the control group [25].

Only few studies have been conducted in India in this highly vulnerable group for HIV and STI. Both the studies were performed without control groups. The study conducted in Amritsar [26] had estimated the seropositivity to HSV-1 and HSV-2 in terms of combined positivity to HSV-1/-2 IgM. The seropositivity of SVW to HSV-1/-2 IgM in the Amritsar study was 16.67% while the seropositivity to IgM of HSV-1/HSV-2 among the SVW group in the present study is 43.49% (Fig. 3 & 4), thus indicating the rising level of HSV infection among SVW. It is interesting to note that the percentage of symptomatic cases among the HSV-1 IgM seropositive SVW of the present study is nearly similar to the observations made in Amritsar i.e., about 30.0%.

The percentage of HSV-1 IgG positivity of the SVW group of the present Chennai study (72.50%) (Fig. 2) is more similar to that of 72.0% of Birmingham [27] and 74.0% of U.S.A. [28]. The prevalence of HSV-1 is much higher in the reports of other countries i.e., in the range of 86.6% to 99.0%. Korr et al. [29] from their cross sectional investigation of non-institutionalized population reported a significantly higher HSV-1 seroprevalence among women than men (82.0% vs. 75.4%) and correlated that with increasing age and lower education level. Growing evidences point that, in recent years HSV-1 is surpassing HSV-2 in causing first episodes of genital herpes as most of the youth engage in their first sexual activity without any protective anti-HSV-1 antibodies [15, 30]. The likelihood attribute for higher genital HSV-1 infection in adolescents could be due to the lower seroprevalence of HSV-1 and changes in sexual behavior such as preferential oral sex.

In general, the infection by HSV-2 is considered as a marker of preceding sexual activity [17]. The percentage of positivity to HSV-2 IgG among the present study group is slightly of higher percentage i.e., 35.51 (Fig. 3), which is 3.0% lower than the observations made in 2007 by a Chennai based study [31]. Korr et al. [29] and Sanchez-Aleman et al. [18] from their respective investigations in Germany and Mexico documented that women of ages ranging 45-64 have more probable HSV-2 infection than men. Findings from our study is in agreement with their report and underscore that female sex and age are the important demographic characteristics associated with HSV-2 acquisition.

The distribution of prevalence of symptomatic cases of HSV-1 / HSV-2 seropositive individuals among three kinds of seropositive groups i.e., IgM, IgM+IgG and IgG is another important outcome of this study (Fig. 4). An intriguing feature in HSV infection is the slow onset of humoral immune response. The seroconversion and detection of type specific IgG antibodies may, in some cases, take 3 months since the onset of infection [32]. In such cases, detection of IgM antibodies, that are directed against HSV glycoproteins gA and gI, may be carried out. Serological assays for detection of IgM antibodies are considered as useful tools for diagnosis of early infection in IgG negative patients as well as recurrent infections [33]. It may be interpreted from our study that while detections of IgM and IgG antibodies are the indicatives of primary and past infections respectively, combined positivity (IgM+IgG) could represent the phase of reactivated episodic HSV infections.

Our study witnessed a gradual increase in symptomatic cases among the different seropositive study groups in the order from IgM through IgM+IgG to IgG. It may be interpreted that while the primary infections are associated with minimal infections, the past infections may have higher incidences of symptoms consequent to frequent and periodic episodes. Comparatively higher percentage of symptomatic cases have been observed with HSV-2 seropositive study group, especially with those who showed combined positivity and single positivity to IgG (Fig. 4). This may be attributed to higher exposure of SVW to STI / HIV infected male partners which could have contributed to enhanced reactivated and prolonged HSV-2 infections.

As expected, the symptomatic cases among the control group in both scenarios were remarkably lower in percentage or even nil. Individuals of both HSV-1 and HSV-2 seropositive control group who showed single positivity to IgM were asymptomatic. Our results corroborate the fact that the prevalence of HSV

are underestimated in most cases as many of these infections are asymptomatic not requiring health care intervention [17]. Interestingly, among HSV-1 seropositive group, symptomatic cases were recorded from those with combined positivity (6.25%) and single IgG positivity (4.05%) (Fig. 4). This could be due to the reason that these individuals may be uninfected during childhood and got infected during adolescent or adult age. Hence, these people with combined positivity and single IgG positivity experience symptomatic reactivated and recurrent HSV-1 infections. Paradoxically, statistically significant percentage (25%) of HSV-2 seropositive control group who showed single IgG positivity were symptomatic. These women, who had reportedly not involved in any risk activities, might have acquired infection through sex with their male partners without knowing infected or asymptomatic status of the same. From this striking observation, our study infers that there could be some prevalence of HSV-2 among the individuals of general population, who could be accidentally infected or may not be aware of the cause and source of STIs.

The study also has enabled to enumerate the percentages of co-positivity to certain viral STIs among HSV-1 and HSV-2 seropositive SVW (Fig. 5). In both the groups of HSV-1 and HSV-2 seropositive SVW, the co-positivities are distinctly of higher percentage than that of the control especially with reference to HIV, HCV and CMV. Most of the earlier studies have focused mainly on the co-prevalence of HIV with HSV-2. Comparable Tanzanian study by Msuya et al. [34] and a Chennai based study by Panda et al. (2007) have estimated a HIV co-positivity of 61.4% and 5.0% among HSV-2 seropositive women. Our study has recorded significant co-positivities of HIV among HSV-1 and HSV-2 seropositive SVW thus advocating synergistic relationship between these viruses in causing STIs (Tables 6 & 7; Fig. 5). However, the higher co-seropositivity of HIV among HSV-2 (23.28%) is in agreement with the reports of earlier studies that individuals with genital HSV-2 infection have three times more likelihood of acquiring HIV and the coinfection facilitates rapid progress to AIDS and more and longer recurrent GUDs [18, 35]. Significant co-positivity to HBV among HSV-1 seropositive SVW may be attributed to parenteral transmission of this virus through sexual contact. One case of HCV co-positivity in a HSV-1 seropositive control could have occurred through other routes of transmission such as surgical procedures, organ transplantation, etc., which was not investigated in our study. Singh et al. [36] from their study in Amritsar (India) have reported the seropositivities of HBV (32.14%) and HCV (17.5%) upon screening of female patients suspected to have blood stream viral infections which is higher than that of control group of our study (Tables 6 & 7; Fig. 5). However, significant co-prevalence of HCV among HSV-2 seropositive SVW confirms the sexual transmission of this virus. Substantial co-seropositivities to CMV among SVW (77-87%) and control group (50-60%) in both HSV-1 and HSV-2 seropositive cases could be attributed to higher global seroprevalence of this virus and underscores its ability of transmission by multiple routes. Concomitant with our finding Olsson et al. [37] have recorded 83% of seroprevalence of CMV among Swedish general population.

The women, on account of having wide mucosal lining constituting the external genitalia and consequent biological susceptibility, have higher probability of acquiring STIs than men. Reports indicate that most of the genital infections in men are asymptomatic and hence these underdiagnosed infected individuals contribute to transmission of STIs to women through sexual activities [29]. This is evident from some earlier reports which have documented that women, who had one life time sex partners, too had preponderance of HSV-2 antibodies [38]. Review of literature infers conflicting views on the use of condoms as a measure to prevent STIs. Studies indicate that condom offers only partial protection as it does not cover the whole genital area. Moreover, shedding of virus is possible in asymptomatic individuals without any visible genital lesions, which may infect the areas uncovered by condom [39]. Sanchez-Aleman et al. [18] have reported that the prevalence of HSV-2 has been higher (at least 1.8 fold) among the women than men in both the scenarios of consistent (7.9 vs. 5.2%) and inconsistent (16.6 vs. 10.3%) use of condoms. Thus, all of these reports reiterate that they are more vulnerable for genital infections than men.

Women play a vital role in contributing for building up a healthy society by virtue of their gifted reproductive ability. STIs, especially of HSV, not only cause morbidity of female genital system but can eventually compromise their reproductive health and endanger the neonates given birth by them. Although HSV vaccine development by the efforts of WHO and partnering research institutes are still underway [40], a full-fledged vaccine is not available until today to protect this vulnerable section. Therefore, our study insists that comprehensive global screening of women population and necessary intervention is the need of the hour for prevention and control of HSV and associated STIs in the society.

5. CONCLUSION

Type specific serological assays are useful tools for understanding the epidemiology of Herpes Simplex viruses in terms of associating their seroprevalences with symptomatic and asymptomatic infections. Substantial overall prevalences of HSV-1 (79.71%) and HSV-2 (74.32%) among the study group demonstrates their vulnerability to STIs. Low seroprevalences of both of these viruses among the control group is comparable to the studies conducted elsewhere. Relatively higher prevalence of HSV-1 among SVWs could be attributed to the changes in the epidemiology of this virus in recent decades and the sexual **behaviours**. The acquisition of HSV-2 infection is more influenced by demographic factors such as female sex and increasing age. Detection of IgM antibodies to HSV would be of immense help in the identification of asymptomatic cases. While combined seropositivity to IgM+IgG antibodies indicate periodic reactivations, the single positivity to IgG antibodies could be corroborated with prolonged and past HSV infections. Significant co-seroprevalence of HIV, HBV, HCV and CMV could be due to undermining of genital immune barrier by forerunner HSV infections. Symptomatic reactivated HSV-1 and HSV-2 infections among the control group portrays the biological susceptibility of women to acquire STIs even with a single infected male partner and existences of underdiagnosed HSV infected cases in the society.

CONSENT AND ETHICAL APPROVAL

Written consent informing the nature and purpose of study was obtained from each volunteer enrolled in the study. Proposal of this study was approved by the Ethical committee constituted by the Department of Microbiology, Presidency College (Autonomous), Chennai, India.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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