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Seroscreening of Men with Bisexual behavior for Genital infections with reference to HSV and other STI viruses

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A B S T R A C T

The men with bisexual behavior (MBS) contribute to a significant category of sexually transmitted infections (STIs) owing to their promiscuous sexual activities with other men and women. A total of 85 individuals of MBS group along with 153 control group volunteers were serologically screened for two types of HSV and other STI causing viruses by adopting ELISA techniques. The MBS group showed overall prevalence of 76% and 56% respectively for HSV-1 and HSV-2. Comparatively lower seroprevalence of HSV-1 among MBS than that of control group indicated the cross reaction and masking effects of symptomatic HSV-2 infections. Detection of selective positivity to IgM or IgG and combined positivity to both types of antibodies indicated primary and reactivated episodic infections of HSV respectively. Occurrences of symptomatic cases among MBS group showing selective and combined positivities to HSV-2 antibodies were statistically significant. Preponderance of symptomatic cases among HSV-1 seropositive MBS group (50-78%) than their HSV-2 counterparts (44-73%) correlated the preferential oral sex and the involvement of HSV-1 in genital infections. Co-prevalences of HIV (20 vs. 27%), HBV (22 vs. 25%), HCV (5 vs. 6%) and CMV (80 vs. 71%) in HSV-1 and HSV-2 seropositive MBS group attributed to high risk sexual behavior for STIs. Comparatively higher HIV prevalence in MBS group indicated the possible predisposing role of HSV-2 for infections by other STI viruses.

INTRODUCTION

Herpes simplex viruses (HSV) are neurotrophic DNA viruses known to cause devastating chronic infections in humans [1]. The two types of HSV viz., HSV-1 and HSV-2 are genetically and phenotypically related and follow different but overlapping patterns of pathogenesis and clinical manifestations. The infections by HSV viruses are acquired by contacting oral or genital secretions, lesions on the infected skin or mucosal surfaces [2]. By way of establishing latent and recurrent infections, the Herpes simplex viruses significantly affect the social and psychological wellbeing of infected individuals [3]. Notable susceptible groups who frequently acquire the HSV infections are the individuals with immunocompromised conditions, neonates and young children [4].

Among the types of HSV, the HSV-1 is commonly known for causing non-genital infections which in most cases is subclinical [5]. Clinical disease typically manifests in several anatomical regions such as mouth, skin, eye and central nervous system [6]. Vertical transmission of HSV-1 in pregnant women has been reported by many studies [7, 8].

Prevalence of HSV-1 is generally influenced by age, sex, geography, social and economic factors. A range of 60-90% of seroprevalence of HSV-1 among various populations of the world has been reported [9]. While the acquisition of HSV-1 occurs with the intimate contact from the family members in developing countries, it is delayed among the individuals of childhood to adolescent age in developed countries [10].

Owing to improved personal hygiene and socioeconomic status, the epidemiological scenario of HSV-1 has changed in recent decades. The substantial decline of its seroprevalence by about 30% among adolescents can be seen as a paradigm especially in developed countries [4]. As a consequence of sexual debut without protective antibodies, these younger generations are subjected to genital acquisition of HSV-1 [11].

HSV-2 is an important agent of sexually transmitted infections (STIs) and a leading cause of genital ulcer disease (GUD) worldwide. This virus causes chronic diseases which can eventually result in devastating physical and psychological morbidities. HSV-2 is transmitted both horizontally (through contact with genital secretions) and vertically (during pregnancy and at delivery) [12].

Genital herpes is highly contagious as the virus shedding can occur during asymptomatic primary and reactivated infections. Since most of the infections are subclinical and only one third of seropositive individuals are aware of their infectious status, the spread of the disease usually exceeds the documented epidemiological data [13]. HSV-2 infections clinically present painful ulcers in oral and anogenital regions, and notably, the disseminated neonatal disease and associated CNS complications are considered as serious manifestations with no cure [14].

Epidemiological risk factors associated with HSV-2 infections are female sex, old age, early sexual debut, multiple sexual partners, prior STIs, inconsistent condom use, low socioeconomic status and education level [15]. Antibodies to HSV-2 appear mainly after initial sexual activity and may increase with the advancement of age and number of sexual partners. Among the individuals who engage in high risk sexual activities, substantial seropevalence has been recorded with female sex workers (75%) and men who have sex with men (83%) [16]. Currently 19 million people around the world are infected with HSV-2 and among which 30% of cases are contributed by Sub-Saharan Africa [17].

The infection caused by HSV-2 gains its public health significance as it exhibits synergy with HIV infection. The reciprocal biological interaction between these two viruses cause complicated clinical conditions [18]. The disruption of mucosal barrier caused by the GUD of HSV-2 leads to local inflammation and recruitment of immune cells, thus favouring the entry and replication of HIV. On the other hand, HIV facilitates higher replication and genital shedding of HSV-2. Evidences suggest that coinfection with HIV renders fourfold increase of reactivation and faster decline of CD4 counts in HSV-2 infected individuals [6]. Considerable percentages of HIV coinfections have been recorded with low risk (25-50% in sub-Saharan Africa) and high risk populations (30-70% in Europe and 50-90% in Africa) [19].

Epidemiological studies have indicated that while the Herpes simplex viruses are implicated with devastating morbidities, the data on their prevalence are underreported owing to the nature of asymptomatic and clinically short-lived infections they cause. Besides, an efficacious vaccine to prevent HSV infection has not been made thus far [20]. In this scenario, serological methods of diagnosis would play a critical role in estimating the prevailing infections and their epidemiological prevalence in the community.

Men who have bisexual behavior (MBS), due to their engagement in multiple sexual activities with other men and women, could be a potential source of HSV infections and pose the risk of endangering vulnerable populations. Therefore, we carried out a serology based screening study to understand the epidemiological pattern of HSV-1 & -2 infections and coexisting STIs in this high risk population.

MATERIALS AND METHODS

Study population

This cross sectional retrospective study was conducted for a period of one year from December 2007 to December 2008 in Chennai city on men with high risk promiscuous bisexual behavior. A total of 85 men, who were reportedly engaged in sex with both heterosexual and homosexual partners, consulting the clinics of Non-Governmental Organizations (NGOs) and STD department of the hospital volunteered for this research study.

Individuals of this study population aged 20-65 were enrolled from four NGOs (Freedom Foundation, Sumana Goodwill Home, Positive Network and Madras Christian Council of Social Service) and one Government General hospital. The study also included 153 sex and age matched healthy individuals of general public as controls. The median age of study and control groups were 34.0 ± 6.46 and 35.0 ± 3.17 respectively.

Participant data and specimen collection

We adopted the method of convenient sampling for collection of study information from the participants viz., socio-demographical and clinical data. Using a structured data form of questionnaire, details such as age, education, marital and economic status, sexual relationship, number of partners, condom use and other high risk sexual activities, were collected. Clinical data on HSV-1 & 2 infections such as symptoms experienced, recurrence and frequency of infections were also obtained.

Through adhering to aseptic procedures, blood samples (5 mL) from each participant were collected and transported under cold chain to the Microbiology laboratory, Presidency College (Aut.), Chennai, India. Sera were extracted from each blood sample and labelled with unique code for identification. Serum samples were stored at -20^{o}C until further analysis.

Laboratory testing

Seropositivities of participants to HSV and other viral STI agents were tested using standardized commercial Enzyme Linked Immunosorbent Assay (ELISA) based diagnostic kits.

Serum samples were analyzed for detection of IgM antibodies to HSV-1 and HSV-2 (Novatec, HSV1M0500 and HSV2M0540), IgG antibodies to HSV-1 and HSV-2 (Novatec, HSV1G0500 and HSV2G0540), antibodies to Human immunodeficiency virus (HIV) types-1 & 2 (General Biologicals, HIV ASE 1+2), surface antigen of Hepatitis-B virus (HBV) (General Biologicals, SURASE B-96), antibodies to Hepatitis-C virus (HCV) (General Biologicals, SP-NANBASE B-96) and Cytomegalovirus (CMV) (Novatec, CMVG0110).

Serological assays were performed as per the instructions of manufacturers and the absorbance of values of reactions were read using ELISA Microwell Plate Reader (Microlisa Plus, Micro Lab Instruments, India) at 450nm. Positive, negative and equivocal results were interpreted with reference to the standard values of absorbance and validated by means of values of control sera.

Data Analysis

Data of the results were subjected to Chi-square test for univariate analysis and the statistical calculations were derived using SPSS software (v 20.0).

Data of continuous variables such as seropositivity, co-positivity and clinical symptoms were expressed as mean ± standard deviation. Tests were validated as significant (P>0.01 and ≤0.05) and highly significant (P≤0.01) based on proportional and comparative values.

Ethical approval

Protocol of this research study was approved by the Ethical committee constituted by the Department of Microbiology, Presidency College (Aut.), Chennai, India.

Informed consent was obtained from each volunteer of the study. Participant details, clinical data and laboratory test results were analyzed maintaining anonymity.

RESULTS AND DISCUSSION

The Men with Bisexual behaviour (MBS) are considered as individuals with abnormal behavior of having sex with men (homosexual) in addition to women (heterosexual). This kind of behavior is generally down looked in the society and not much attention is paid in the context of seroprevalence studies on sexually transmitted infections (STIs). Owing to the stigma and reluctance in participation in the survey based studies, our study faced a constraint of identifying sufficiently good number of individuals of this category and obtaining the data. As a result, the total number of individuals enrolled in this study group was only 85. However, with the available volunteers and along with the control group, our study could derive some significant findings and contribute to the existing literature.

The serological testing of study and control groups determined the seropositivity to HSV-1 and HSV-2 viruses in terms of detecting anti-viral antibodies of IgM and IgG categories. Since the tested individuals showed different degrees of positivities to both of these antibodies, the results were presented as selective positivity i.e., detection of either of these antibodies (IgM or IgG) and combined positivity i.e., detection of both antibodies together (IgM+ IgG).

Overall seroprevalence of HSV

The seroprevalences of the types HSV-1 and HSV-2, taking into account of the seropositivity to both IgM and IgG antibodies, among the study group (MBS) and the control group are shown in the fig. 1. There has been an interesting observation with respect to the seroprevalence of HSV-1, which was 9% higher in control group (82%) than that of MBS (76%). This lower value in the study group could be attributed to the cross reaction of anti-HSV-1 antibodies with the anti-HSV-2 antibodies and subsequent masking of the farmer due to the preponderance of HSV-2 in the study group. Thus, our study resonates with the findings of Korr et al. [21] linking reduced seroprevalence of HSV-1 to more symptomatic infections of HSV-2.

The sero-epidemiological study conducted by Drisu et al. [16] on the general population of Nigeria reported the HSV-1 prevalence of 96.4%, which is much higher than that of the control group in the present study. Comparatively higher prevalence of HSV-1 (76%) than the HSV-2 (46%) among the MBS group underscores the implication of promiscuous sexual activity by this group involving oral route where the existence of HSV-1 is high. This finding of our study agrees with the report of Biskup et al. [22], who has attributed the preponderance of HSV-1 among young men to the preferential oral sex over the vaginal sex during their intercourse.

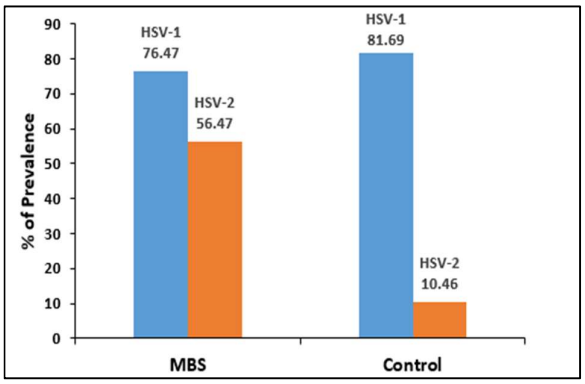

Figure 1: Seroprevalence of HSV among MBS and Control groups

As anticipated, the HSV-2 seroprevalence in MBS was 80% higher than that of the control group, which could be due to promiscuity and higher exposure of the study group to this STI virus. The seroprevalence of HSV-2 is considered as a biomarker of STIs in the population with the proxy of high risk sexual behavior [18]. Madebe et al. [14] argue that the individuals who engage with multiple sex partners have 3-folds higher risk of acquiring HSV-2 infections than the normal population. Thus, the data on HSV-2 seroprevalence among MBS group of our study is in agreement with the previous studies.

Seropositivity to anti-HSV antibodies

The noteworthy investigation of this study is the investigation of the selective (IgM / IgG) and combined (IgM+ IgG) seropositivity of the target and control groups. The category-wise seropositivities of MBS and control group individuals to HSV-1 and HSV-2 antibodies (in values) are presented in table 1. While the total positivity to HSV-2 antibodies were highly significant, similar pattern was observed with selective positivity to IgG and combined positivity among the control group. It may be noted that the selective positivity to IgM antibodies was insignificant indicating that there was no new infection with HSV-2 in the control group. The selective positivity to IgG antibodies may be attributed to the circulation of antibodies of the past infection which could have occurred by accidental contact with an infected partner. The fig. 2 and 3 depict the selective and combined seropositivities (%) of study and control groups to the antibodies of HSV-1 and HSV-2 respectively.

Table 1: Seropositivity to HSV antibodies among MBS and control groups

Agent screened	Study Group	No. of Persons screened	Total Positivity		Selective & Combined positivity		
			IgM	IgG	IgM	IgM + IgG	IgG
HSV-1	MBS	85	23	54	11	12	42
	Control	153	43	102	23	20	82
	P Value & Interpretation		0.863	0.626	0.753	0.821	0.982
HSV-2	MBS	85	18	39	09	09	30
	Control	153	07	09	07	0	09
	P Value & Interpretation		0.004#	0.000#	0.290	0.009#	0.006#

*-Significant; #-Highly significant

With reference to the individuals negative for the HSV-1 antibodies, percentage was 50% higher in MBS (26%) than the control group (18%) which might have occurred due to the cross reaction and the masking effect by the HSV-2 antibodies. Since the combined positivity is attributed to the index of reactivation of virus infection [17], its percentage in both the groups was proximal. Owing to the fact that the acquirement of HSV-1 during the early childhood and its common recurrent infections, the higher seropositivity to HSV-1 antibodies (>50%) is acceptable (Fig. 1).

The higher percentage of negative individuals (90%) and nil for combined seropositivity among the control group for HSV-2 antibodies clearly demonstrates that the risk behaviors play a pivotal role for the prevalence of STIs (Fig. 2). This view can be further substantiated with the occurrence of notable percentage of individuals with combined seropositivity (11%) among the study group who tend to have more reactivated episodic infections due to their bisexual behaviour.

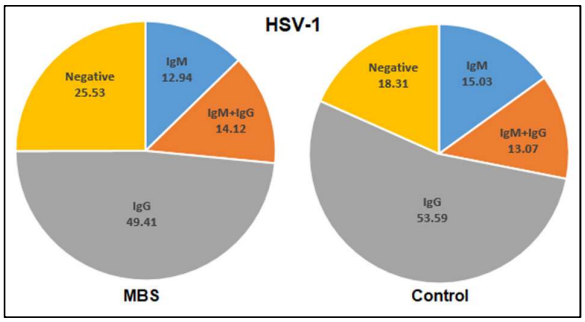

Figure 2: Selective and combined seropositivities (%) to anti-HSV-1 antibodies among MBS and Control groups

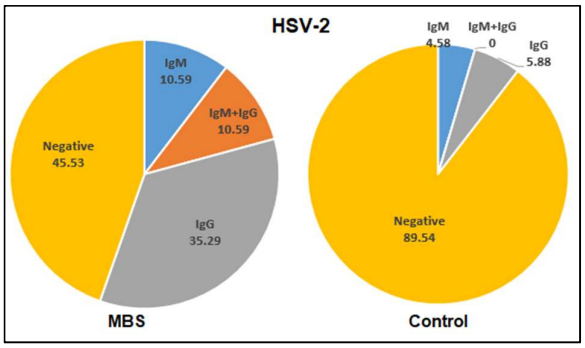

Figure 3: Selective and combined seropositivities (%) to anti-HSV-2 antibodies among MBS and Control groups

Survey of literature indicated that the research studies on the seroscreening of MBS group are scarce. A study conducted by Andreoletti et al. [23] in France investigated the seroprevalence of HSV-2 in this special high risk group of STIs.

However, this study reported the seroprevalence in terms of estimation of IgG antibodies in the target group. Besides, their study has another limitation of omitting the control group for the serological survey. In contrast, the present study made a comprehensive attempt of detecting selective and combined seropositivities to IgM and IgG antibodies to both types of HSV. While the seroprevalence of HSV-2, with reference to detection of IgG antibodies, was 36.7% in this France study, our study reports a higher prevalence of 45.8%. This value may be considered reliable as the present study determined the seroprevalence taking into account of both selective and combined positivities to anti-HSV-2 IgG antibodies.

Commensurate with the present study, a recent Nigerian study has investigated the seroprevalence of HSV-2 by way of detecting the IgM and IgG antibodies to the virus in the study group [17].

Correlation of symptomatic cases with HSV seropositivity

Another important part of this study was to investigate the proportion of symptomatic cases among the individuals seropositive to HSV-1 and HSV-2 antibodies. Tabulation of individuals with clinical symptoms and their selective and combined seropositivity to anti-HSV antibodies portrayed that the correlations are highly significant for HSV-1 seropositive cases (Table 2).

Concerning the HSV-2 seropositive individuals, while the occurrences of symptomatic cases were highly significant for the individuals with combined and selective positivity to IgG, it was significant for individuals with IgM selective positivity. It may be due to the reporting of small number of symptomatic cases among MBS (6) and control group (1), which is low for a statistical evaluation. Figure 4 explains the percentage of prevalence of symptomatic cases among the HSV seropositive target and control groups. Recently, Kadr et al. [24] have documented 3-folds higher association of HSV-1 (6%) with the symptomatic GUD than the HSV-2 (19%) among individuals engaging in high risk sexual behavior. Consonance with this report, the present study has recorded a comparatively higher portion of symptomatic cases among the HSV-1 seropositive MBS group (50-78%) than their HSV-2 counterparts (44-73%).

Table 2: Correlation of HSV seropositivity to clinical symptoms among MBS and control groups

Cases positive to	Study Group	No. of cases	Symptomatic selective & combined seropositive cases					
			IgM		IgM + IgG		IgG	
			S	AS	S	AS	S	AS
HSV-1	MBS	65	08	03	06	06	28	14
	Control	125	0	23	01	19	05	77
	P Value & Interpretation		0.005#	--	0.010#	--	0.007#	--
HSV-2	MBS	48	06	03	04	05	22	08
	Control	16	01	06	0	0	0	09
	P Value & Interpretation		0.044*	--	0.010#	--	0.008#	--

*-Significant; #-Highly significant; S-Symptomatic; AS-Asymptomatic

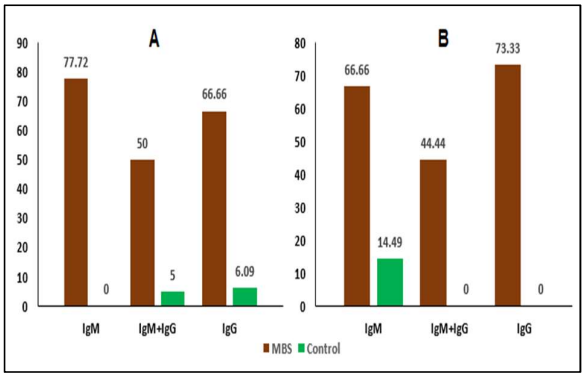

Figure 4: Prevalence (%) of symptomatic cases among A) HSV-1 & B) HSV-2 seropositive MBS and Control groups

While the control group reported low prevalence of symptomatic cases (5-6%) among HSV-1 based combined and selective IgG positive individuals, strikingly, 14% of HSV-2 IgM seropositive individuals of this group had reported having symptoms. Even though, these individuals of control group had not deliberately engaged in any risk behaviors of STI, development of symptomatic HSV-2 infection could be attributed to the transmission of virus from their sex partners

whose cause and source of infection is unknown. However, the selective positivity to anti-HSV-2 IgM antibodies by the symptomatic individuals of control group confirms that the infection is primary. Research studies report that during the primary or new infection, the antibodies of IgM type appear first [17]. The substantial reporting of symptomatic cases among the HSV-1 (50-78%) and HSV-2 (44-73%) seropositive individuals of MBS (Fig. 4) clearly explicates the impact of high risk sexual behavior on the seroprevalence of these viruses.

Co-prevalence of STI viruses among HSV seropositive cases

The results of seroscreening of HSV seropositive individuals of both MBS and control groups for the seroprevalence of other selected STI viruses revealed a significant relationship of infections of these viruses with that of HSV (Table 3). As can be seen in the fig. 5, both the HSV-1 and HSV-2 seropositive individuals of MBS group showed co-seropositivities to all the STI viruses chosen for the present study. The co-prevalence of these viruses in HSV seropositive MBS group could be due to their promiscuous behavior of engaging in high risk sex with multiple partners of both genders [25].

In general, the global seroprevalence of CMV has been reported to be in the range of 60-92% owing to its widespread nature, multiple routes of transmission and establishment of latency in lymphoreticular cells [26]. Interestingly, the prevalence of CMV among HSV-2 seropositive MBS group was 12% lesser than that of the HSV-1 counterparts as recorded in our study (Fig. 5). This comparatively lower prevalence may be due to higher frequency of reactivation of HSV-2 in this risk group than that of CMV. In consonance with the finding of our study, Naame et al. [27] have reported that the HSV-2 could suppress the seroprevalence of CMV in co-infected individuals owing to the faster replication of its DNA which has more G-C content than other viruses of Herpes family.

Research studies focusing on the epidemiology of HSV-2 virus have well documented that the infection by this virus is a forerunner of STIs caused by other viruses [28]. In particular, the recent research studies investigating the implications of HSV-2 infection on HIV infection have demonstrated the correlated pathogenesis of these two STI viruses [29, 30] in which the infection by HSV-2 predisposes to that of HIV. Nag et al. [31] have demonstrated that there is 34 times more likelihood of HSV-2 seropositivity in individuals with HIV infection. As the chronic infection by HSV-2 leads to GUD, the local immunity in the genitalia is compromised due to mucosal damage in this anatomical site. This paves the way for the entry of other STI viruses if promoted by high risk sexual activity [32]. The findings of our study on the co-seroprevalence of HIV, HBV, HCV and CMV among HSV-2 seropositive individuals corroborate with reports of contemporary research studies [33].

Table 3: Co-positivity of HSV seropositive MBS and control group to other STI viruses

Cases positive to	Antiviral antibodies detected	Study Group	Total no. positive	No. positive to other viral agents			
				HIV	HBV	HCV	CMV
HSV-1	IgM	MBS	11	2	3	1	10
		Control	23	0	2	2	11
	IgM + IgG	MBS	12	2	1	1	11
		Control	20	0	2	0	12
	IgG	MBS	42	9	10	1	31
		Control	82	0	3	1	43
	P Value & Interpretation			0.008#	0.034*	0.108	0.071
HSV-2	IgM	MBS	09	4	3	0	5
		Control	07	0	0	0	5
	IgM + IgG	MBS	09	3	5	1	6
		Control	0	0	0	0	0
	IgG	MBS	30	6	4	1	23
		Control	09	0	0	1	8
	P Value & Interpretation			0.093	0.004#	0.165	0.861

*-Significant; #-Highly significant

The co-positivities to HBV and HCV among HSV-1 seropositive control group of the present study could be due to other modes of blood borne transmissions such as organ transplantation, tattooing and catheterization

which were not recorded during patient history data collection. However, the co-prevalence of HCV (6%) among HSV-2 seropositive control group could be pointed to the sexual transmission from their female partners whose status of infection was unknown during the present study and needs confirmation with an extended study.

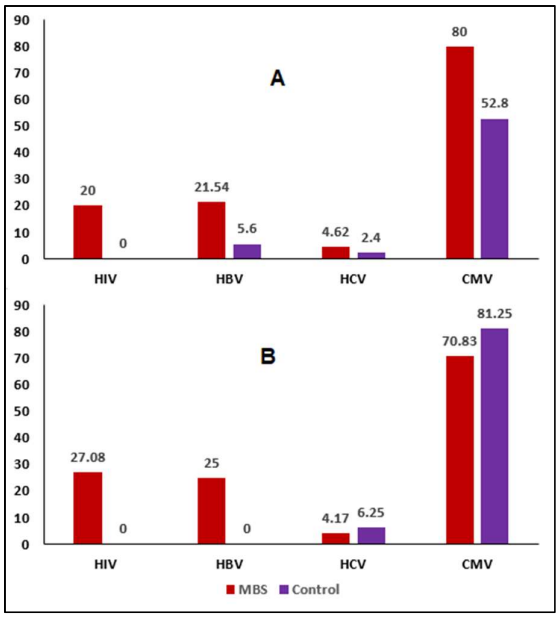

Figure 5: Co-seroprevalence (%) of STI viruses among HSV-1 (A) and HSV-2 (B) seropositive MBS and Control groups

Aligned with the investigations of the present study, Singh et al. [34] have demonstrated the co-seroprevalence of HBV, HCV and HSV-2 among the hospitalized patients in India.

CONCLUSION

The Men with Bisexual behavior tend to have significant seroprevalence of HSV-2 owing to their promiscuous behavior of sexual contact with both men and women. The HSV-1 seroprevalence in MBS group diminishes in relation to the higher symptomatic HSV-2 infections. Serological detection of selective and combined positivities to IgM and IgG antibodies facilitates a comprehensive seroprevalence study of HSV infections in the target and control groups. Comparative study with the control group demonstrates the implication of sexual risk behaviors in HSV-2 infections. Combined seropositivities to both types of antibodies could be correlated to the reactivation and episodic HSV infections. Higher symptomatic cases among HSV-1 seropositive MBS group may be a proxy of changing and preferential oral sexual activity over anogenital intercourse. Symptomatic cases of HSV-2 IgM seropositive individuals of control group could have acquired the infection through their sex partners, whose infectious status needs an extended investigation. The HSV-1 and HSV-2 seropositive MBS group show substantial co-seroprevalence of other STI viruses owing to their promiscuous high risk sexual activities. Genital ulceration, caused especially by HSV-2, diminishes the local immunity and predisposes to other viral STIs.

COMPETING INTERESTS

Authors have declared that no competing interests exist .

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