Original Article

DOI: 10.7860/JCDR/2023/60899.17474

Seroprevalence and Molecular Detection of Symptomatic and Asymptomatic Genital Herpes Simplex Virus 2 among HIV Positive Patients: A Cross-sectional Study

Microbiology Section

BETHA VVV TEJASWINI[1], MANO CHANDRIKA YARRA[2], SHAIK NASEEMA[3], I JAHNAVI[4]

ABSTRACT

Introduction: Genital Herpes Simplex Virus (HSV) infection is the most common infection in Acquired Immunodeficiency Syndrome (AIDS) patients, occurring in 60-90% according to World Health Organisation (WHO) reports. Early detection of HSV-2 infection, the introduction of chemotherapy and prophylaxis improve the lifespan of AIDS patients.

Aim: To detect the HSV-2 seroprevalence that is Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies in Human Immunodeficiency Virus (HIV) positive serum and molecular detection of HSV-2 Deoxyribonucleic Acid (DNA) in symptomatic HSV-2 patients.

Materials and Methods: The descriptive cross-sectional study was conducted in the Guntur Government Hospital with the help of the Antiretroviral Therapy (ART) Centre and Department of Dermatology, Venereology and Leprosy (DVL) in GGH, Guntur, Andhra Pradesh, India. A total of 100 blood samples from HIV positive individuals were collected and for those with symptoms on the external genitalia, a biopsy specimen from the ulcer and fluid from vesicles were taken. All the cases in the study group are on ART and without any antiviral treatment. A blood sample was collected for IgG (Calbiotech kit), IgM detection (Merilisa kit), and biopsy from genital ulcer for gene detection by real-time Polymerase Chain Reaction (PCR) (HELINI Biomolecules, Chennai). Statistical analysis was done by mean and percentages with p-value by calculating Chi-square test, Fisher's-Exact test to know significance of association between HSV-2 and HIV.

Results: Among the total 100 HIV positive patients, it was observed that IgG seroprevalence of HSV-2 in HIV positive individuals is 78%, while it is 19% for IgM. In the present study, 19 symptomatic cases showed DNA of HSV-2 in PCR, out of which, 17 were from genital ulcers and two from vesicle fluid.

Conclusion: As HSV-2 is a lifelong infection, serological testing provides the best method, to estimate its prevalence even in asymptomatic individuals.

Keywords: Acquired immunodeficiency syndrome, Human immunodeficiency virus, Immunoglobulin G, Immunoglobulin M, Real-time polymerase chain reaction

INTRODUCTION

Genital herpes simplex virus infection is a disease of major public health importance with markedly increasing prevalence throughout the world during the last four decades [1]. The morbidity of the illness, its high recurrence rates and its complications following primary genital herpetic infections such as aseptic meningitis, neuralgia paraesthesia, and dysaesthesia involving the lower extremities and perineum, have made the disease of great concern of patients and healthcare providers [1,2]. The ability of the virus to successfully avoid clearance by the immune system by entering a long non replicating phase known as latency leads to lifelong infection [3].

According to a World Health Organisation (WHO) report, an estimated 417 million people were living with HSV-2 infection and in India 10 million (2016). It often has a more severe, atypical presentation and more frequent recurrences. In advanced Human Immunodeficiency Virus (HIV) disease, Herpes Simplex Virus-2 (HSV-2) can lead to more serious complications such as meningoencephalitis, oesophagitis, hepatitis, pneumonitis, retinal necrosis or disseminated infection. As per WHO 2016, approximately 19 million people get newly infected with HSV-2 each year worldwide [4]. Among attendees in sexually transmitted diseases clinics in India, the seroprevalence of HSV-2 rate ranges from 7.9-18.9% in HIV seronegative individuals and it is 60-90% in People Living with HIV/AIDS (PLHA) [4,5]. It is known that acute or reactivated HSV-2 infection may stimulate HIV replication leading to the progression of HIV disease [6]. Both HIV and HSV-2 have shown to influence each other. It is associated with an increased risk of both HIV transmission (five-fold) and acquisition (2-3-fold) [5-9].

Diagnosis becomes difficult because of atypical presentation [10]. The infection is followed within a few days by the appearance of IgM antibodies, followed by IgG and IgA. IgG persists indefinitely [11]. As HSV-2 is a lifelong infection, serological testing provides the best method to estimate its prevalence. Enzyme Linked Immunosorbent Assay (ELISA) is much more sensitive and specific than the complement fixation test when type specific antigen preparation based on glycoprotein G is used [12]. HSV-2 type specific serological testing in HIV positive individuals are the best method to diagnose clinically asymptomatic HSV-2 infection and to reduce the risk of transmission [13]. PCR tests can be done on cells or fluids from a sore or on blood or other fluids such as spinal fluid. It has a high sensitivity of 99.99% and is used to differentiate between HSV-1 and HSV-2. The sample must be taken before acyclovir therapy is begun [11]. Early detection of HSV-2 infection, the introduction of chemotherapy and prophylaxis improve the quality of life and lifespan in AIDS patients. About the paucity of published reports in this Guntur region, since 2010 by Schneider JA et al., the present study was taken up to detect the HSV-2 seroprevalence that is IgG, and IgM antibodies to know the active infection of HSV-2 in HIV positive serum and detection of HSV-2 Deoxyribonucleic Acid (DNA) in symptomatic patients [5].

The present study was conducted to study the HSV-2 seroprevalence by IgG and IgM antibodies to know the active infection in HIV positive patients and the detection of HSV-2 DNA in symptomatic (genital ulcer) patients.

MATERIALS AND METHODS

This was a descriptive cross-sectional study done in Guntur Government Hospital (GGH) with the help of the ART Centre and DVL Department in GGH, Guntur, Andhra Pradesh, India. The duration of the study was from January to December 2017. An Institutional Ethical clearance certificate was obtained (GMC/IEC/073/2017). Informed consent was taken from every individual. Relevant information, such as age, sex, occupation, literacy, socio-economic status, demography, sexual behaviour and any treatment of antiviral drugs were noted.

Inclusion criteria: All the HIV positive individuals within the reproductive age group, who attended ART centre during the study period were included in the study.

Exclusion criteria: Individuals less than 18 years of age, who acquired HIV infection by vertical route, non cooperative and patients on antiviral treatment like acyclovir were excluded in the study.

Study Procedure

A total of 100 convenient blood samples from HIV positive individuals on ART and without any antiviral treatment were collected in given time period. Among those HIV positives, 19 presented with symptoms on the external genitalia, biopsy specimen from the ulcer and fluid from vesicles were taken.

For HSV-2 IgG and IgM detection by ELISA test, 3 mL of blood sample was collected and serum was separated. For molecular detection of HSV-2DNA by real-time PCR: 19 biopsy specimens from genital ulcers were placed into universal viral transport media (under the guidelines of US Public Health Service in the Department of Transportation and Interstate Quarantine Regulations) and sent to HELINI Biomolecules, Chennai for further processing. DNA was extracted and stored at -20°C for further processing.

ELISA test: Serological tests was done for all 100 serum samples. For HSV-2 IgG detection CALBIOTECH kit, an indirect ELISA method and for HSV-2 IgM Merilisa kit (Meril diagnostics), two step capture ELISA method was used. Calculation of IgG antibody index done by dividing Optical Density (OD) value of each sample by cut-off and for IgM, the mean of absorbance of all the three negative controls with value of +0.1 should be measured. Results were interpreted as for IgG <0.9 value considered as no detectable antibodies, 0.9-1.1 for borderline and >1.1 detectable antibodies and for IgM as when absorbance's of samples more than cut-off were considered reactive. The quality control used for IgG were OD of calibrator as >0.25 and antibody index of negative control <0.9 and for IgM mean of negative control as ≤0.1 and positive control as ≥0.7.

Real-time PCR for detection of HSV-2 DNA: The HSV-2 DNA extraction was done by PureFast® Viral DNA mini spin purification kit as per kit literature from 19 biopsies specimens. A 12.5 µL of elute was used in real-time PCR analysis.

Detection of HSV-2 DNA by using HELINI HSV-2 real-time PCR kit from HELINI Biomolecules, Chennai, India, used using real-time PCR model Agilent Aria Mx, USA. The detection mix is prepared by adding probe PCR master mix (10 µL), HSV-2 primer probe mix (2.5 µL), and purified DNA (12.5 µL), making a total reaction volume of (25 µL). Positive (12.5 µL) and negative (12.5 µL) (nuclease free water) controls were also added. The assay detection is based on TaqMan chemistry. As per the instruction manual sequences of primers for HSV-2 were 5′-CTACGACGCGTACCGGTCCGATG-3′ and 5′-GTGGTGCACGAACAGCGTGGTGA-3′. Primers are hybridised to HSV-2 DNA template. The target genes were FAM and HEX.

The result was plotted on a graph and the report has obtained from the machine after the completion of the procedure.

STATISTICAL ANALYSIS

Statistical analysis was done by mean and percentages and difference between proportions were analysed with p-value by calculating Chi-square test, Fisher's-Exact test to know significance of association between HSV-2 and HIV.

RESULTS

A total of 100 HIV positive cases were enrolled in the present study, for the detection of HSV-2 IgG, IgM, and HSV-2 DNA. Among these 100 cases, it was observed that the seroprevalence of HSV-2 IgG positive was 78%, 16% were both HSV-2 IgG and IgM positive, 3% were only IgM positive and 3% were both negative for IgG and IgM.

As shown in [Table/Fig-1], in 78% IgG seroprevalent cases, most of the HSV-2 IgG positive cases were seen in the age group 31-40 years 27 (34.6%) out of 78 and most of them belonged to lower, lower middle class labour, and followed by 41-50 years (32%). Among 19 seroprevalent HSV-2 IgM cases, most of the cases were seen in the age group 31-40 years (42.1%) in both sexes. Overall female preponderance was seen till the age group of 31-50 years, but male dominance was seen from 51-60 years. The lowest age noted was 21 years and highest age was 69 years and the mean age was 41.75. Among these 2% were unmarried and 5.1% were divorced females.

Age (years)	n	Male (%)			Female (%)		
		IgG positive	IgM positive	Sero negative	IgG positive	IgM positive	Sero negative
21-30	14	03 (8.82)	02 (20)	0	7 (15.9)	02 (22.22)	0
31-40	37	11 (32.35)	03 (30)	1	16 (36.36)	05 (55.55)	1
41-50	30	10 (29.4)	03 (30)	0	15 (34.09)	01 (11.11)	1
51-60	16	08 (23.53)	01 (10)	0	06 (13.63)	01 (11.11)	0
61-70	03	02 (5.88)	01 (10)	0	0	0	0
Total	100	34	10	1	44	09	2

[Table/Fig-1]: Age and sex-wise distribution of HSV-2 IgG and IgM positive.

All these patients were on antiretroviral treatment. The [Table/Fig-2] shows overall patients with a single partner were 61.8% and multiple partners were 38.1%. Males having >1 sexual partner were 36 (94.7%) positive for HSV-2 antibodies. Whereas seropositivity in females with single partners was 58 (93.5%). Literacy-wise, the highest prevalence (41.2%) was observed in illiterates, followed by the primary school group (35%) and secondary school group (15.4%) in both genders. Most of the labour and others were found from urban (59.3%) areas only. The p-value <0.001, so there was a significant relationship between increasing age and HSV-2 prevalence.

Demographic features	HIV positive cases n=100 (%)	HSV-2 seroprevalence n=97 (%)
Sex		
Males	45	44 (45.3)
Females	55	53 (54.6)
Occupation		
Labour	42	40 (41.2)
Housewife	24	24 (24.7)
Farmers	13	12 (12.3)
Others	11	11 (11.3)
Drivers	10	10 (10.3)

Literacy		
Illiterates	40	40 (41.2)
Primary school	35	34 (35)
Secondary school	16	15 (15.4)
College	09	08 (8.2)
Locality		
Urban	60	58 (59.3)
Rural	40	39 (40.2)
Sexual behaviour		
Single partner	62	60 (61.8)
Multiple partners	38	37 (38.1)
Marital status		
Married	93	90 (92.7)
Divorved	5	05 (5.1)
Unmarried	2	02 (2)
Socio-economic status		
Upper	02	02 (2)
Upper middle	04	03 (3)
Middle	36	34 (35)
Lower middle	33	33 (34)
Lower	25	25 (25.7)

[Table/Fig-2]: Demographic details of seropositive HSV-2 patients.
Others* include tailors, painters, shopkeepers, and factory supervisors

Among those HIV positives, 19 presented with symptoms of fever, myalgia, enlarged bilateral inguinal lymph nodes, discharge from multiple painful bilateral tiny vesicular ulcers and blisters on penis, vagina, cervical, rectal and perianal regions. In the present, among 97% of seroprevalent cases, 19 (19%) of cases were symptomatic with genital ulcers and vesicles showing HSV-2 DNA and IgM antibodies [Table/Fig-3].

HIV cases (%)	HSV-2 DNA positive	HSV-2 IgM positive	HSV-2 IgG positive	Seroprevalence (N=97)
Symptomatic (n=19)	16	Positive	Positive	16
	03	Positive	Negative	03
Asymptomatic (n=81)	NA	Negative	Positive	78
	NA	Negative	Negative	03

[Table/Fig-3]: Molecular detection and serological test results of HSV-2 DNA in symptomatic and asymptomatic herpes simplex virus 2 cases.

Among those 19 cases, only IgM is positive in 3 (15.7%) cases with vesicles, but both IgG and IgM are positive in the rest of the 16 (84.2%) cases with genital ulcers. In 19 symptomatic cases, males were 10 (52.63%) and females 9 (47.36%). There was a statistical significant association between HSV-2 in HIV patients with chi-square of 3.9857 and p-value of 0.045.

DISCUSSION

The HSV-2 is associated with an increased risk of both HIV transmission and acquisition, through numerous microscopic genital ulcers mostly containing CD4 cells, facilitating transmission of HIV [8,9]. HSV-2 is the most common genital infection and sero-epidemiological studies have documented worldwide distribution which varies across regions and populations [2].

The differentiating HSV-1 and HSV-2 ELISA is much more sensitive and specific than the complement fixation test when type specific antigen preparation based on glycoprotein G is used [12]. HSV-1 and HSV-2 can be differentiated either by using type specific primers or using common primers followed by analysis with restriction enzymes or hybridisation for each type [14].

In the present study, it was observed that the seroprevalence of HSV-2 IgG in HIV positive cases was 78%. There was a significant relation between HSV-2 and HIV as the p-value was <0.04. Similar studies showed a high prevalence of 83.3% by Shameem Banu AS et al., 61.5% by Nag S et al., from Tamil Nadu and West Bengal, India, 77.65% by Pennap GRI and Oti VB from Nigeria, 58% by Nakku-Joloba E et al., from Uganda 6.1% by Cohen JA et al., from the US, and 6.5% by Janbakhash A et al., from Iran [8-10,15-17].

While studying the gender variations in various studies in HSV-2 IgG positive cases there was a female predominance in the present study at 56.41%. Similar study from Andhra Pradesh showed 52.9% females in HIV positive individuals [18]. A study from Iran by Janbakhash A et al.,also showed the female predominance of 17.6% [8]. A study from Sudan by Ahmed NM et al., also showed the female predominance of 58% [19]. The estimated risk of susceptible females contracting HSV-2 from infected males is 80% following a single contact [2]. The severity of the primary infection and its association with complications are statistically higher in women than men. Systemic complications are common in both sexes approaching 70% of all cases. This gender difference for HSV-2 may be explained in part by the biological susceptibility of the female sexually transmitted disease, due to innate biological factors, such as possession of a large mucosal surface area of the female genital tract prone to infection and the receptor role of women in the act of sex with a consequent higher male to female transmission risk per exposure [17].

The present study was showing the highest prevalence of HSV-2 positive cases were among the age group 41-50 years, followed by 31-40 years, and 51-60 years. Similarly, a study from Nigeria, by Pennap GRI and Oti VB was showing the highest prevalence of HSV-2 positive cases among the age group 41-50 years (88.9%), followed by 31-40 years (by 79.6%), and 21-30 years (72%) [16], and a study from Tamil Nadu, India by Shameem Banu AS et al., 2011 was showing the prevalence of HSV-2 positive cases among the age group 21-30 years (50%) and 31-40 years (50%) [10]. The p-value <0.001, so there was a significant relationship between increasing age and HSV-2 prevalence. But for females, there were more cases from the 21-40 years age group than 51-60 years. There were no cases reported in females above 61 years. In most of the studies, females in reproductive age group have high seropositivity [8,10,17]. Seroprevalence of HSV-2 increases from 5.6% at 12-19 years of age to 24.3% by the age of 60 years among men [2]. Evidence of HSV-2 infection is found almost exclusively in adolescents and adults [11].

Seroprevalence rates vary greatly with geographical area and socio-economic groups. This is due to the high proportion of asymptomatic infections, and the influence of other factors; race (blacks (85.4%) more than whites) gender (women more than men), marital status (divorced more than single or married), and place of residence {cities (6.4%) more than sub-urban (4.3%)} [2,5,17,20-22].

In the present study, highest prevalence of HSV-2 positive cases were seen among labourers in both males and females 20 (45.4%). A similar study from Nigeria, by Mawak JD et al., was showing the highest prevalence of HSV-2 positive cases (60.5%) was seen among labourers [18,23]. A study from Nigeria, by Pennap GRI and Oti VB, shows highest prevalence of HSV-2 positive cases (80%) was seen among housewives [16]. Literacy-wise, it was observed that the highest prevalence (85%) was observed in illiterates, followed by the primary school group and secondary school group. Similarly, the study with the highest prevalence was seen as 100% illiterates, 93% in primary school, 75% in secondary school and 100% in college were seen Nag S et al., [15]. Among seropositive cases, single partners were seen in 38% of cases and 62% showed multiple partners which was similar to the study of Nag S et al., showing 53.8% of single partners and 46% of multiple partners [15]. This showing that polygamy or multiple sexual partners increase the acquisition of HSV-2 infection. Also, the number of sexual partners correlates directly

with the acquisition of HSV-2 infection. Thus, having multiple sexual partners, irrespective of sexual performance, correlates directly with the acquisition of HSV-2 infection [2] [Table/Fig-4] [15,24-26].

S. No.	Year	Author	Symptomatic		Asymptomatic	
			Males (%)	Females (%)	Males (%)	Females (%)
1	2015	Nag S et al., [15]	57.7	42.3	51.11	48.89
2	2014	Amudha VP et al., [24]	56.1	43.7	57.1	41.7
3	2018	Munawwar A et al., [25]	47.2	-	32.5	-
4.	2022	Alareeki A et al., [26]	46.8	-	27.8	63.2
5		Present study	52.63	47.36	35.8	64.19

[Table/Fig-4]: Comparison of seroprevalence (IgG) with symptomatic and asymptomatic HSV-2 cases [15,24-26].

In the present study, 52.63% of males with Genital Ulcerative Disease (GUD) were reported to the sexually transmitted disease clinics more than females (47.3%). Overall these study findings were similar to several other studies from India and meta-analysis conducted in Europe. Infection rates greatly exceed clinical disease rates and many perhaps most infections that are latently infected have no recollection of primary infection [11].

In the present study, seroprevalence of HSV-2 IgM was 19% and highest in the 31-40 years age group. Other studies, also showed the highest cases in the same age group and showed 56.6% IgM antibodies by Shameem Banu AS et al., in 2011 and 34.6% IgM antibodies by Nag S et al., 2015 showed [10,15]. IgM antibodies indicate that there was an active infection. Primary infections are mostly seen in younger age groups because they actively participate in sexual activities. Evidence of HSV-2 primary infection is found almost exclusively in adolescents and adults [11]. This indicates active infection, which may be primary, non primary, or reactivation of primary infection. Primary infection is followed within a few days by the appearance of IgM antibodies, closely followed by IgG and IgA. IgG persists indefinitely [27]. A study from Ahmedabad stated that, serum HSV-2 IgM can be used for periodically screening in Sexually Transmitted Diseases (STD) patient to know the trend, transmissibility and load of HSV [28].

The present study showed 100% positivity for HSV-2 PCR in 19 symptomatic cases. Similar studies from Tanzania by Nilsen A et al., and Ahmed HJ et al., were showing 83% and 63% PCR positivity respectively [29,30]. There was a wide variation of HSV-2 DNA detection from 10% in Shameen Banu AS et al., Tamil Nadu, India to 83% in Tanzania among GUD patients [10]. The sensitivity of DNA detection depends on the stage of lesions with higher sensitivity in vesicles than ulcers or higher sensitivity in first than recurrent episodes (whether the patient has first or recurrent episodes of disease) [29].

In the present study, more males than females in GUD cases were showing positive for HSV-2 PCR, but in other studies, it was female predominance. It may be because of the limited sample size and more asymptomatic cases in females due to sites of ulcers in inaccessible sites like the cervix, leading to less presentation to the STD clinics [9,11]. In females, the most active age from 21-30 years is most likely to excrete HSV-2, whereas those in the older age groups have a decreased frequency of viral excretion [2].

In the present study, IgG and IgM ELISA test was done on the PCR positive cases after one month of completion of treatment and the result was, there was the presence of IgG antibodies and the absence of IgM antibodies, which indicates that IgG antibodies persist for life after primary infection, and IgM antibodies produced after few days of infection and disappear by eight weeks [31].

Limitation(s)

The samples size was limited and there were more number of females in study group. No paired sera were taken and limited numbers of symptomatic cases were taken.

CONCLUSION(S)

The HSV-2 seroprevalence is high among the HIV patients and most of the cases are asymptomatic, so they cannot be identified. It indicates the requirement for regular screening of all HIV positive cases for HSV-2 antibodies. As there can be asymptomatic viral shedding in the genital fluids, which increases the transmission, prophylactic treatment of all HIV positive cases should be considered. Early treatment of HSV-2 infection by acyclovir or valacyclovir can decrease both HIV and HSV-2 viral shedding.

Authors contribution: BVVVT: Data collection and supervised the testing. YMC: Literature search and supervised the testing. NS: Literature search and manuscript preparation. IJ: Overall guidance.

REFERENCES

[1] Lawrence C, Anna W, Genital Herpes, King K Homes, Frederick P, Walter ES, Peter P, Judith. Sexually transmitted disease. 4th edition, edited by King K Homes, Frederick P, Walter ES, Peter P, published by McGraw-Hill companies, 2008, Pp. 399-438.

[2] Richard J Whitely, Bernard Roiziman. "Herpes simplex viruses" textbook of Clinical Virology. 2nd edition, edited by Douglas D Richman, Richward J Whitley, Frederick G. Harden, published by American Society for Microbiology, Washington; 2002, Pp. 375-401.

[3] Stanberry LR, Cunningham AL, Mindal A, Scott LL, Spruance SL, Aok FV, et al. Purpose for control of HSV-2 disease through immunization. Journal of Clinical Infectious Disease. 2000;30:549-66.

[4] World Health Organisation, Home/News/Fact sheet/Detail, Herpes simplex virus, 31 January 2017.

[5] Schneider JA, Lakshmi V, Dandona R, Kumar GA, Sudha T, Dandona L. Population-based seroprevalence of HSV-2 and syphilis in the Andhra Pradesh state of India. BMC Infect Dis. 2010;10:59.

[6] Jacob S, Gopal T, Kanagasabai S, Durairaj A, Sushi KM, Arumugam G. Herpes simplex virus 2 infection in HIV-seropositive individuals in Tamil Nadu, India. Int J Med Sci Public Health. 2015;4(3):404-07.

[7] Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ. 2008;86(10):805-12.

[8] Janbakhash A, Mansouri F, Vaziri S, Sayad B, Afsharian M, Abedanpor A. Seroepidemiology of herpes simplex virus type 2 (HSV-2) in HIV infected patients in Kermanshah-Iran. Caspian J Intern Med. 2012;3(4):546-49.

[9] Cohen JA, Sellers A, Sunil TS, Matthews PE, Okulicz JF. Herpes simplex virus seroprevalence and seroconversion among active duty US air force members with HIV infection. J Clin Virol. 2016;74:04-07.

[10] Shameen Banu AS, Lakshmi S, Kaveri K, Jayakumar S. Correlation of serology, tissue culture, and PCR in identification of herpes simplex type 2 infection among HIV patients. Journal of Clinical and Diagnostic Research in India. 2011;5(6):1190-94.

[11] Minson AC. Topley and Wilson's Microbiology and Microbial infections Virology. Vol. 1. edited by Brian WJ Mahy, Volker TerMeulen, 10th edition; Hodder Arnold; 2005:506-19.

[12] "Mackie and Maccartney textbook of practical medical microbiology edited by J. Gerald Collee, Andrew G, Fraser Churchill published by Livingstone 14th edition Pp. 650.

[13] Bernad Roziman, Herpes Viridae, editor Knipe DM, Howely PM, Textbook of Field Virology, 3rd edition published by Lippincott Williams and Wilkins, p-2221-2229.

[14] Roy M, Anderson FRS, Gilluan Urmin, Johnplay, "Fair Sexually Transmitted infections Richard medical microbiology 3rd editon, edited by Cedric Minus, Hazel, M. Doxkrell, published by Elesevier Mos by London, 2004, Pp. 550-553.

[15] Nag S, Sarkar S, Chattopadhyay D, Bhattacharya S, Biswas R, SenGupta M. Seroprevalence of herpes simplex virus infection in HIV coinfected individuals in eastern India with risk factor analysis. Adv Virol. 2015;2015:537939.

[16] Pennap GRI, Oti VB. Seroprevalence of HSV-2 among HIV patients accessing health care at Nigeria. Journal of Diagnostics. 2016;3:31-37.

[17] Nakku-Joloba E, Kambugu F, Wasubire J, Kimeze J, Salata R, Albert JM, et al. Sero-prevalence of herpes simplex type 2 virus (HSV-2) and HIV infection in Kampala, Uganda. Afr Health Sci. 2014;14(4):782-89.

[18] Anuradha K, Singh HM, Gopal K, Rama Rao GR, Ramani TV, Padmaja J. Herpes simplex virus 2 infection: A risk factor for HIV infection in heterosexuals. Indian J Dermatol Venereol Leprol. 2008;74:230-33.

[19] Ahmed NM, Ahmeed TB, Mohamed TM, Musa HH. Seroprevalence of herpes simplex virus 2 infection among HIV positive patients, Sudan. Journal of Human Virology and Retroviroloy. 2018;6(1):00187.

[20] Dargham SR, Nasrallah GK, Al-Absi ES, Mohammed LI, Al-Disi RS, Nofal MY. Herpes simplex virus type 2 seroprevalence among different national populations of middle east and north African men. Sexually Transmitted Diseases. 2018;45(7):482-87.

[21] Huai P, Li F, Li Z, Sun L, Fu X, Pan Q, et al. Seroprevalence and associated factors of HSV-2 infection among general population in Shandong Province, China. BMC Infect Dis. 2019;19:382.

[22] Otieno FO, Ndivo R, Oswago S, Pals S, Chen R, Thomas T, et al. Correlates of prevalent sexually transmitted infections among participants screened for an HIV incidence cohort study in Kisumu, Kenya. Int J STD AIDS. 2015;26:225-37.
[23] Mawak JD, Dashe, Atseye AB, Agabi, Zakeri H. Seroprevalence and co infection of herpes simplex type 2 and human immunodeficiency virus in Nigeria. Shiraz E Medical Journal. 2012;13:33-39.
[24] Amudha VP, Rashetha, Sucilathangam G, Cinthujah B, Revathy C. Serological profile of HSV-2 in STD patients: Evaluation of diagnostic utility of HSV-2 IgM and IgG detection. J Clin of Diagn Res. 2014;8(12):DC16-DC19.
[25] Munawwar A, Gupta S, Sharma SK, Singh S. Seroprevalence of HSV-1 and 2 in HIV-infected males with and without GUD: Study from a tertiary care setting of India. J Lab Physicians. 2018;10(3):326-31.
[26] Alareeki A, Osman AMM, Khandakji MN, Looker KJ, Harfouche M, Abu-Raddad LJ. Epidemiology of HSV-2 in Europe, systematic review, meta analyses, and meta regressions. J Lanepe. 2022;2022:100558.
[27] Lee F, Coleman R, Pereira L, Bailey PD, Tatsuno M, Nahmias AJ. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. J Clin Microbiol. 1985;22(4):641-44.
[28] Tada DG, Khandelwal N. Serum HSV-1 and 2 IgM in sexually transmitted diseases-more for screening less for diagnosis: An evaluation of clinical manifestation. J Glob Infect Dis. 2012;4(3):S01-04.
[29] Nilsen A, Kasubi MJ, Mohn SC, Mwakagile D, Langeland N, Haarr L. Herpes simplex virus infection and genital ulcer disease among patients with sexually transmitted infections in Tanzania. Acta Derm Venereal. 2007;87(4):355-59.
[30] Ahmed HJ, Mbwana J, Gunnarsson E, Ahlman K, Guerino C, Svensson LA et al. Etiology of genital ulcer disease and association with HIV in two Tanzanian cities, sexually transmitted disease. Sex transm Dis. 2003;30(2):114-19.
[31] Kurtz JB. Specific IgG and IgM antibodies responses in HSV infections, virology labouratories, Radcliffe infirmity. J Med Microbiol.1974;7(3):333-41.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Kamieni Institute of Medical Sciences, Narketpally, Telangana, India.
2. Senior Resident, Department of Microbiology, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India.
3. Assistant Professor, Department of Microbiology, NRI Medical College, Guntur, Andhra Pradesh, India.
4. Head, Department of Microbiology, Guntur Medical College, Guntur, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shaik Naseema,
Assistant Professor, Department of Microbiology, NRI Medical College,
Guntur, Andhra Pradesh, India.
E-mail: micronaseema@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 19, 2022
- Manual Googling: Jan 04, 2023
- iThenticate Software: Jan 16, 2023 (5%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 18, 2022**
Date of Peer Review: **Nov 26, 2022**
Date of Acceptance: **Jan 19, 2023**
Date of Publishing: **Feb 01, 2023**