



## Prevalence of Herpes Simplex Virus-2 Among Attendees in The HIV Clinic of University of Ilorin Teaching, Hospital, Ilorin, Nigeria

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### ABSTRACT

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Herpes simplex virus type 2(HSV-2) causes anogenital sores in humans. The aim of this study was to determine seroprevalence of HSV 2 among attendees of the HIV clinic of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. Blood samples were taken from 200 consenting adults and were tested for HSV-2 antibodies using ELISA. Socio-demographic data, socio-economic factors, behavioral characteristics and the health status of the subjects were obtained using the administered questionnaire. Results revealed that a seroprevalence of 49.5% (99/200) was obtained for subjects positive for IgM antibodies to the virus. Of the positive subjects, 67 (33.5%) were females while 32 (16.0%) were males (P=0.102). Subjects who were in the age range of 31-40 had the highest seropositivity while those in the age range of 61-70 had the lowest (p=0.002). High seropositivity was noticed with subjects residing in urban areas, 87 (43.5%) compared to 12 (6.0%) for rural dwellers (p=0.047). Trends of increasing seropositivity were observed in this order: divorced, widowed, separated, single and married (p=0.000). The group of subjects with tertiary education had the highest value HSV-2 prevalence (p=0.001) while subjects by occupational status reveals that the self-employed/business people had the highest prevalence of 57(28.5%) at p=0.045. Among the self-employed, traders 49 (24.5%) ranked highest at p=0.001. Respondents that reported yes to the use of condom and that without an answer was associated with the acquisition of the virus (p=0.000). Similarly, there was significance in relation to subjects that used condoms all the time and those that gave no answer to the use of condom to acquiring the virus(p=0.003). This study concludes that most of the infected subjects were asymptomatic to HSV-2. Therefore, strict adherence to safe-sex practice and hygiene is advocated.

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## **1. Introduction**

The virus Herpes simplex (HSV) commonly results in ulcerative disease of the mucosa in both the immunocompetent and the immunocompromised patients (Strick et al 2006). Herpes simplex virus type 2 (HSV2) is transmitted sexually and causes anogenital ulcers. One of hallmarks of HSV 2 infection is the periodic asymptomatic shedding of the virus (Corey et al 2004). Herpes simplex virus infection can last for a life time and virus can become latent permanently in the root ganglia of the nerve. The presence of HSV 2 in the system of a subject can induce the production of antibodies. Also, there could be cell mediated immune response that can modulate the severity of a recurrence. Such response might not be sufficient to eradicate HSV infection. Though origin of the herpes virus in humans is not very clear, their presence had been reported early in ancient Greek. Then they were called sores that creep or crawl (Spear 2004). The worldwide seroprevalence of HSV 1 is 67% (Looker et al 2015a) while that of HSV 2 is 11.3% (Looker et al 2015b).

HSV 2 associated diseases are prevalent and have been estimated to have affected 61-81.8% of human adults in Nigeria (Van Wagoner et al 2012, Ojinma et al 2012). Several factors like age, race, geographic location and other socio-economic factors affect the acquisition of the virus. Less industrialized nations have been documented to have higher seropositivity (Fatahzadeh and Schwartz 2007).

It has been discovered that among people living with HIV, HSV 2 is found to be common with prevalence that surpasses that of the general population (Whitley 2001). Encephalitis, acute retinal necrosis and aseptic meningitis are serious complications that may result from infection by HSV 2 (Matthew and Sapra 2020)

There is paucity of data on the infection of HSV 2 in people living with HIV in Ilorin, Kwara state, Nigeria where this study was carried out. Therefore, this research has the aim to create consciousness of the risk factors of acquiring the virus. Also, the study hopes to provide important data for HSV 2 and its co-infection with HIV in the study area in terms of prevention.

## **2. Methods**

This is a prospective study involving human participants. Approval for the research was granted by the ethical committee of the University of Ilorin Teaching Hospital with approval code ERC PAN/2013/07/1231. Subjects recruited included only HIV positive adults who signed the consent form and were attendees of the HIV Clinic of the University of Ilorin Teaching Hospital (UITH).

**Study Area:** This research was done at UITH, Ilorin, Kwara state Nigeria. Kwara is one of the states in North Central Nigeria and is located on latitude 8.50N and longitude 4.550E (Goggle Map 2011). The UITH is a 520 bedded tertiary hospital that serves as a referral center for healthcare facilities in Kwara state and neighboring states like Kogi, Niger, Oyo and Osun states.

**Sample collection:** Two hundred (200) blood samples were collected from consenting adult subjects. Each sample contained 5mls of blood drawn from each consenting subject using a 21 gauge needle to plain and pre labeled blood sample bottles. Then blood samples were spun to separate the serum which are then stored at 40C

**Serology:** Enzyme linked Immunosorbent Assay (ELISA) technique was employed as a diagnostic tool for the identification of IgM antibodies in the serum. All the stages of the test were carried out as instructed by the manufacturers (Dia Pro Diagnostics Bio probes). The diagnostic sensitivity of the kit has been tested in a clinical trial and value obtained >98%. The diagnostic specificity of the kit has determined in a performance evaluation study with a value >98%. Samples were diluted 1:101 by dispensing first 10µl of sample and then 1ml of specimen diluents and mix gently. From diluted serum, 100µl was inoculated into the wells and for control seronegatives samples obtained from subjects previously tested negative were used. Incubation of the inoculated micro plates was carried out at 370C for 60minutes. Then washing was done 5 times using 350µl of the washing solution provided. Then 100µl of enzyme conjugate was added to all the wells except that of blank. Micro plates were incubated at same temperature as a repeat. After the addition of chromogen/substrate mixture of 100 µl, micro plates were incubated for the third time at 18-24

OC for 20 minutes. Colour change observed was read with a spectrophotometer at 450nm using the micro well plate reader after addition of 100 µl of Sulphuric acid to all the wells.

Data collection: A semi structured questionnaire was used to collect necessary data. Data included socio-demographic characteristics, marital status, risk factors among others.

Data Analysis: The Statistical Package for the Social Science (SPSS) version 2.0 at significance level of  $p < 0.05$  and 95% confidence interval was used to analyze data obtained. Data included results obtained from serology and that from questionnaire administered.

### 3. Results

Assay results for HSV 2 revealed that out of the 200 subjects tested, 99(49.5%) of the respondent were positive for HSV 2. High seropositivity was recorded among the respondents that fell in the age bracket of 31-40 years the lowest was among age bracket of 21-30 years. ( $p=0.003$ ). Considering gender, HSV 2 positive subjects were females 67(33.5%) and males 32 (16.0%) ( $p=0.102$ ).

Most of the subjects 165 (82.5%) resides in the urban area while 35(17.5%) resides in the rural. Of the 99 (49.5%) HSV 2 positive subjects 87(43.5%) and 12 (6.0%) resides in urban the rural area respectively ( $p=0.047$ ).

A total of 30 (15.0%) respondent reported to have other disease as at the time of recruitment and 9 (4.5%) were positive for HSV 2 while 21 (10.5%) were not. On the other hand, out of the 163 (81.5%) respondent that reported had no other diseases as far as they know, 85 (42.5%) tested positive for HSV 2 while 78 (39.0%) were negative ( $p=0.041$ )

Table 1: HSV 2 Status in relation to demographic data

Age (Years)	Positive (%)	Negative (%)	P value
No answer	2(1)	0(0)	0.003
21-30	4(2)	19(9.5)	
31-40	40(20)	27(13.5)	
41-50	36(18)	28(14)	
51-60	11(5.5)	19(9.5)	
61-70	6(3)	8(4)	
Gender			
Male	32(16)	44(22)	0.102
Female	67(33)	57(28.5)	
Residence			
Urban	87(43.5)	78(39.0)	0.047
Rural	12(6.0)	23(11.5)	
Marital status			
Single	12(6.0%)	4(2%)	0.000
Married	72(36%)	61(30.5%)	
Divorced	2(1%)	0(0%)	
Widowed	4(2%)	26(13%)	
Separated	9(4.5%)	9(4.5%)	

Table 2: HSV 2 Status in relation to level of education and occupation

Level of Education	Positive (%)	Negative (%)	p value
None	15(7.5%)	24(12%)	0.156
Primary	19(9.5%)	23(11.5%)	
Secondary	32(16%)	20(10%)	
Tertiary	33(16.5%)	34(17%)	
Occupation			
Unemployed	34(17%)	20(10%)	0.045
Civil Servant	6(3%)	6(3%)	
Self- employed	57(28.5%)	75(37.5%)	
Military personnel	2(1%)	0(0%)	

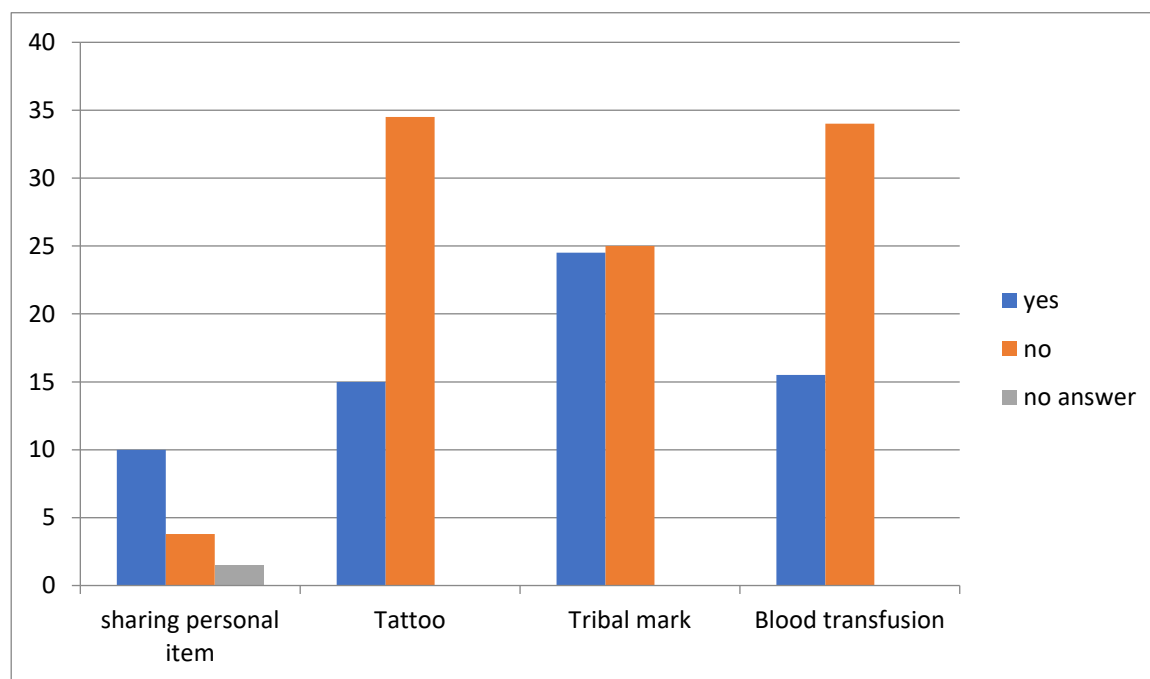


Figure 1: HSV 2 status of respondent by sharing of personal item, tattoo, tribal mark and blood transfusion (95% CI)

#### 4. Discussion

According to the results obtained, the prevalence of HSV2 in this location was 49.5% of the sample population. The prevalence in this research can be reported as co-infection of HSV 2 and HIV. This is because all subjects tested were already confirmed HIV positive. In line with this research is another study (Shivaswamy et al. 2005) on the prevalence of HSV1 and 2 in STD patients where a result of 82.9% was obtained. This also goes to confirms that an individual with a form of STD (e.g. HIV) is at higher risk of HSV infection and vice versa. Venkateshwaran et al. 2011 also reported high prevalence of HSV2 in HIV positive respondent. Though another study in Nigeria reported a lower prevalence of 33.3% (Anaedobe 2019).

The least age of respondent recruited for this study was from 21 to 30 but highest positivity to HSV 2 was recorded amidst the 31 to 40 age bracket (20%). Though, this was not significant at  $P > 0.05$ . The high seropositivity seen in the age group of 30-40 years may be due to early acquisition of the virus. Other factors that could be related with this age group are sexual activity with possibility of less protection and contact with broken skin or lesions. A study carried out by Geraldine et al (2018) reported that acquisition of HSV 2 increased with increase in age. Their work reported 13.3% for age group of 30-39 years. Considering gender, results from this research showed that gender was an important factor in the acquisition of HSV 2. Females had a higher positivity (33%) as compared to males (16%) and this was statistically significant. Report by Sharanya et al (2014) had similar findings in which they had females with higher incidence of HSV 2 as compared to males. Residence as to whether a subject is living in rural or urban location did not play a significant role in acquiring HSV 2 ( $p=0.05$ ). Similar findings were reported in Burkina Faso (Kirakoya et al 2011). So whatever prevention programs are organized, residents in both urban and rural places should benefit. The trend in the prevalence considering marital status noticed of respondent was single (6.0%), married (36%), divorced (1%), widowed (2%) (Table 1) and this correspond with the work of Shivaswamy et al. 2005 and Malkin et al. 2002. HSV2 positivity was found to be high amidst the groups with secondary and tertiary educational levels (16 and 16.5%) at  $P > 0.05$ . Study by Hind et al (2010) reported that education level can affect the acquisition of HSV. Majority of participants reported that they were self-employed. The indicated trades included but not limited to trading, driving, farming etc. Prevalence of 57 (28.5%) was recorded for the self-employed (Table 2) for HSV2 while the lowest was for military personnel though this was not found to be statistically significant. Previous studies show that occupations that expose people to higher sexual risk behavior and dentist are at higher risk of acquiring the virus (Lewis 2004).

Furthermore, another study by Pereira et al. 2012 suggests that genital lesions by HSV 2 are on the rise. The presence of tattoo did not affect acquisition of HSV 2. The acquisition of the virus was affected by the presence of tribal marks (Fig 1). This could be due to the low awareness and hygiene level of the traditional mode of inscription that is common within the locality. The blood transfusion status was not significant to the acquisition of HSV 2 status while sharing of personal items was significant (Figure 1). According to World Health Organization report (2020), sharing of personal items like blade, drinking glasses and utensils is important in HSV 2 acquisition

## **Conclusion**

Specific information regarding which of the infections (HIV or HSV 2) was primary in the subjects prior to this research can't be deduced since both infections compromise the immune system in one way or another, thereby creating an opportunity for secondary infection. However, this research reveals that prevalence of HSV 2 infection in HIV positive subjects is significant, so adherence to safe sexual practices among others should be maintained at all time since most carriers or infected individual are asymptomatic

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## **References**

- Anaedobe C.G., Ajani T.A. 2019. Co-infection of Herpes simplex virus Type 2 and HIV Infection among Pregnant Women in Ibadan, Nigeria. *Journal of Global Infectious Diseases*. 11(1): 19-24.
- Beydoun H.A., Dail J., Ugwu B., Boueiz A., Beydoun M.A. 2010. Socio-demographic and behavioural correlates of Herpes Simplex virus type 1 and 2 infections and co-infections among adults in the USA. *International Journal of Infectious Disease*. 14S3:154-160.

- Corey L., Walt A., Patel R. 2004. Once daily valacyclovir to reduce the risk of transmission of genital herpes. *New England Journal of Medicine*. 350 (1): 11-20.
- Fatahzadeh M., Schwartz R.A. 2007. Human herpes simplex virus infections; Epidemiology, pathogenesis, symptomatology, diagnosis and management. Newark, American Academy of Dermatology. 57(5); 737-63
- Kirakoya-Samadoulougou F., Nagot N., Defer M. C., Yaro S., Fao P., Langan Y., Meda N., Robert A. 2011. Epidemiology of herpes simplex virus type 2 infection in rural and urban Burkina Faso. *Sexually Transmitted Disease*. 38(2) 117-123.
- Lewis M.A.O. *International Dental Journal*. 2004. Herpes simplex virus; An Occupational Hazard in Dentistry 54(2); 103-11.
- Looker K.J., Margaret A.S., Turner K.M., Vickerman P., Gottlieb S.L., Newman L.M. 2015a. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. *PloS one*. 10(1):e114989.
- Looker K.J., Margaret A.S., May M.T., Turner K.M., Vickerman P., Gottlieb S.L., Newman L.M. 2015b. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. *PloS one*. 10(10):e0140765.
- Malkin J.E., Marand P., Malvy D., Ly T.D., Chanzy B., De Labareyre C., El Hasanai A., Hercberg S. 2002. Seroprevalence of HSV 1 and HSV 2 infection in the General Population, Sexually Transmitted Infections. 78;201-203
- Matthew Jr J., Sapra A. 2020. Herpes Simplex Type 2. In StatPearls[Internet]. StatPearls Publishing.
- McQuillan G.M., Kruszon- Moran D., Flagg E.W., Paulose-Ram R. 2018. Prevalence of Herpes Simplex virus Type 1 and 2 in Persons aged 14-49; United States, 2015-2016. US Department of Health and Human Services, National Center for Health Statistics.
- Ojinma U.R., Nnonuka E.N., Ozoh G.A.O., Onyekonwu C.L., Aguwa E.N. (2012). Herpes simplex virus type 2 infection among females in Enugu, Enugu state. *Nigerian Journal of Medicine*. 21(4) 394-403.
- Pereira V.S., Moizeis R.N., Fernandes T.A., Araujo J.M., Meissner R.V., Fernandes J.V. 2012. Herpes simplex virus type 1 is the main cause of genital herpes in women in Natal, Brazil. *European Journal of Obstetrics and Gynecology and Reproductive Biology*. 161; 190-3.
- Rajagopal S., Margaret A., Mugo N., Wald A. 2014. Incidence of Herpes Simplex Type 2 infections in Africa: A systematic review. *Open Forum Infectious Disease*. 1(2) doi.org/10.1093/ofid/ofu043.
- Shivaswamy K.N., Thappa D.M., Jaisankar T.J., Sujatha S. 2005. High seroprevalence of HSV-1 and HSV-2 in STD clinic attendees and non-high-risk controls. A case study control study at a referral hospital in south India. *Indian Journal of Dematology, Venereology and Leprology*. 71:26-30.
- Spear P.G. 2004. Herpes simplex virus; receptors and ligands for cell entry. *Cell Microbiology*. 6:40.
- Strick L.B., Wald A., Celum C. 2006. Management of herpes simplex virus 2 infection in HIV type 1-infected persons. *Clinical Infectious Diseases*. 43:347-356.
- Van Wagoner N. J., Brown E., Whitley R., Hook E.W. (2012) Predictors of Undiagnosed Herpes Simplex Virus Type 2 Seropositivity Among Persons Attending an HIV Care Clinic. *Sexual Transmitted Disease*. 39(11):857-9.
- Venkateshwaran S.P., Murungesan K., Sivaraj R. 2011. Seroprevalence of IgG and IgM antibodies in individuals with herpes simplex virus 1 and 2 infection in HIV positive and negative Individuals of South Indian population. *Journal of Applied Pharmaceutical Science*. 1(10):154-158.
- Whitley R. J. 2001. Herpes Simplex Viruses, Fields Virology, DM Knipe and PM Howley. Philadelphia, PA, Lippin-cott-Williams &Wikins; Ed 2;pp 2461-2509