Ann Ibd. Pg. Med 2020. Vol.18, No.1 37-43

SEROPREVALENCE AND RISK FACTORS OF HERPES SIMPLEX VIRUS TYPE 2 AMONGST PATIENTS' ATTENDING RETROVIRAL CLINIC IN FEDERAL MEDICAL CENTER ABEOKUTA, SOUTH-WEST NIGERIA

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ABSTRACT

Herpes simplex virus type 2 (HSV-2) is the primary cause of genital herpes worldwide and also has a role in facilitating human immunodeficiency virus (HIV) transmission. The aim of the study was to determine seroprevalence of HSV-2 among the subjects and associated risk factors.

In this cross-sectional study, 207 HIV Positive subjects attending a retroviral Clinic in Federal Medical Center, Abeokuta, Ogun state were interviewed and had blood samples taken. The patients were interviewed using a structured questionnaire and their serum samples were tested for HSV-2 specific immunoglobulin G (IgG) antibodies using enzyme-linked immunosorbent assay (ELISA) for consented participation. Prevalence of HSV-2 antibodies was ascertained and related to demographics and behavioral variables.

A total of 207 participants were recruited for this study comprising of Sixty-three (30.4%) male and 144 (69.6%) female HIV positive subjects. Seroprevalence of HSV-2 was 70.0%. Only 58(28%) had current history of genital ulcer. Logistic regression revealed a history of multiple sexual partners (P value=0.003), oral sex (P value=0.02) were found to be associated with HSV-2 acquisition in HIV patients. All other risk factors such as history of transactional sex, age at sexual debut and condom use were not significant. The results highlight the potential public health impact of HSV-2 particularly in a developing country like Nigeria where HSV-2 testing is not included in our testing and treatment protocol for HIV. This result should lead to commencement of HSV type-specific serological testing in the HIV infected population and also high risk groups should be targeted for behavioral modification messages.

Keywords: Seroprevalence, Risks, Factors, Herpes simplex, HIV.

INTRODUCTION

Genital herpes is an infection commonly caused by human herpes simplex virus type 2 (HSV-2). In Nigeria and in most regions of the world it has been described as a silent pandemic with different countries being at different stages of the epidemic[1].

Herpes Simplex Virus (HSV) is an infection that occurs globally and estimates of its prevalence differ widely depending largely on the patient population studied. HSV has largely been described as a very successful pathogen largely due to its high prevalence, successful sexual transmissibility rate, association with immune compromised patients and ability to cause recurrent disease. Herpes genitalis is the most frequent sexually transmitted disease (STD) amongst HIV positive patients, which when symptomatic, is characterized by periodic recurrences of painful genital ulcers.[2] Worldwide incidence of HSV ranges from roughly 65% to 90% and most persons seropositive for HSV type 2 (HSV-2) have an intermittent reactivation of the virus on mucosal surfaces[3].

Disruption of the epithelial surface and inflammation of HSV genital ulcers appear to increase the risk of HIV transmission. Acute or reactivated HSV infection may stimulate HIV replication, leading to the progression of HIV disease. On the reverse side, HIV-induced immunosuppression results in alterations in the natural history of HSV leading to more severe HSV outbreaks and more frequent viral shedding in persons co-infected with HIV and HSV compared with those \without HIV infection. The treatment of HSV can be more challenging in HIV-infected patients. Higher doses of antiviral drugs may be required, and

persons infected with HIV have an increased incidence of acyclovir resistant HSV-2.

In the United States, one in four sexually active adults had HSV-2 infection with a 31% increase in HSV-2 prevalence between 1978 and 1990. About 40-60% attendees in STD clinics have already acquired genital herpes and 20-35% of pregnant women are HSV-2 seropositive. Also Studies have shown that 22% of adults have antibodies to HSV-2 and that about 1.6million new cases of HSV-2 infection occur yearly in the United States.[4,5]

Comparably, high HSV-2 rates have been observed in sub Saharan Africa where HIV prevalence is the highest. In recent years, a parallel and interesting epidemiological association has emerged between HSV-2 and HIV infection. HSV-2 seroprevalence rates are higher in HIV positive than in HIV negative individuals and are especially high among HIV positive persons in sub-Saharan Africa. Multiple mechanisms may explain these observations; genital ulceration provides a site for HIV entry on HIV negative persons and the associated inflammation increases the number of activated cells that can be targeted by HIV.[6] Although symptomatic and asymptomatic HSV-2 reactivations may promote HIV shedding in the genital tract and increase HIV levels in blood[7].

Because asymptomatic HSV-2 infections in HIV-positive persons may be associated with increased transmission of HIV and may accelerate the course of HIV disease, screening should generally be offered to patients with documented HIV and without a history of genital herpes. HSV specific education and counseling should be provided for individuals with either HSV-2- negative or HSV-2-positive results, because HSV-2-negative, HIV-infected patients have a significantly increased risk of HSV-2 acquisition[1].

The HSV-2 pandemic is further reinforced by the HIV pandemic and *vice versa*; and it is becoming clear that the efforts at controlling the spread of HIV may remain ineffective if control of HSV-2 infection, along with other sexually transmitted infections (STI) is not integrated.

The diagnosis of HSV presents a challenge for public health programmes and for clinicians in developing countries.[1] The present study aims to determine the prevalence of HSV-2 antibodies and identify probable risk factors among HIV patients.

METHODOLOGY

This is a descriptive cross-sectional study design, carried out in the retroviral Clinic of the Federal Medical Center Abeokuta, Ogun state, Nigeria. The Federal Medical Center is a 250 bedded tertiary health institution and has a retroviral clinic serving about 800 patients. FMC Abeokuta is a tertiary Healthcare center which provides healthcare services to the people of Ogun state and neighboring Oyo, Ekiti, Osun and Ondo States and also serves as a referral hospital to secondary and tertiary hospitals across the country. The study population consists of the reproductive age group attending the retroviral clinic Community Medicine and Primary Care department of the Federal Medical Center Abeokuta.

Sample Size

The sample size was determined using the Fishers Formula

$$N = \frac{z^2pq}{d^2}$$

In this study, the proportion in the target population estimated to have a particular characteristic is 84%[1]

Where N=the desired sample size (where target population is greater than 10000)

Z = the standard deviation, usually set at 1.96

P = the proportion of patients estimated to be infected by *HSV-2*.

Q = 1.0 - p, i.e. 1.0-0.84 = 0.16

d = degree of accuracy desired usually set at 0.05

Therefore the minimum sample size will be

$$N = \frac{(1.96)^2(0.16)(0.84)}{(0.05)^2} = 207$$

The minimum sample size required for the study is 207

Selection of Subjects

Inclusion criteria

All patients in the reproductive age group attending the ART clinic.

Exclusion criteria

Non consenting patients or patients' relatives.

Ethical clearance

Ethical approval was obtained from the Institutional Review Board of FMC Abeokuta.

Methods of Data Collection

Verbal and written informed consent were sought and obtained from each of the subjects; thereafter relevant medical history, socio-demograhic data and other information obtained from the patients were entered into a semi-structured close-ended questionnaire.

Laboratory methods

The assay was carried out in the medical microbiology laboratory of FMC Abeokuta.

Specimen collection and transport: blood samples were collected in 5 ml plain vacutainer tubes and allowed to clot and sera separated by centrifugation at room temperature. Samples were stored in cryovials at -20C. The HSV-2 IgG assay procedure utilizes the enzyme-linked immunosorbent assay (ELISA) kit (IBL INTERNATIONAL HSV 2 IgG ELISA GMBH HAMBURG GERMANY). This is a glycoprotein G-based type-specific ELISA technique and test result is qualitative. All specimens and kit reagents were brought to room temperature and gently mixed. Procedures were performed in accordance with manufacturer's instructions. Quality control and test validation was included into the test protocols.

Principle of the test

This is a glycoprotein G-based enzyme-linked immunosorbent assay (ELISA) technique. Diluted serum samples of patients were added to wells precoated with HSV-2 antigens. Antibodies in the patient's sera are bound to the antigens to form immune complexes. Enzyme-conjugated antihuman globulin detects any such complexes. A chromogenic substrate was added to produce a coloured reaction which intensity depends on the amount of antibody in the complex. The absorbance of the microwell contents are read by a reader. The calibrators and controls are utilized in internal quality control and in qualitiative interpretation of the assay.

Materials and Reagents

Materials and reagents include 96 HSV-2 antigen coated Microwell strips; a 1 x 100ml vial of sample diluents; 6 x 2.0ml vials of calibrators (CAL1, CAL2, CAL3, CAL 4, CAL 5 and CAL 6); 1 vial of control serum; a 50ml bottle of washing buffer concentrate; a 16ml vial of polyclonal anti-IgG-Horseradish peroxidase conjugate; a 16ml vial of Tetramethylbezidine/ Hydrogen peroxide (chromogenic substrate); and a 15ml vial of sulphuric acid stop solution. Other materials include plate sealing foils, micropipettes, disposable pipette tips, distilled or de-ionized water, andabsorbent paper.

Procedures for HSV-2 igG assay: details of the assay procedure and precautions will be as stated in the manufacturer's instructions.

All specimens and kit reagents was brought to room temperature and gently mixed. The wash buffer was prepared by diluting the wash concentrate 1 in 10. The wells are pre-coated with type-specific HSV glycoprotein G antigens. Dilutions of the test serum, controls and calibrator are added into the appropriate wells. The assay was blanked using the sample diluents. The well contents are thoroughly mixed and incubated for 30 minutes at room temperature. HSV antibodies, if present in the sera, bind to the HSV antigens on the surface of the well. All liquid is then removed from all wells and washing was done three times with wash buffer. Enzyme-antibody conjugate was then added to each well and incubated for 30 minutes at room temperature. This was followed by another three-time wash step. Then a chromogenic substrate was added to each well and another incubation for 30 minutes at room temperature was done. The 0.2M sulfuric acid stop solution was then added. The intensity of the colour (OD) that develops was measured using an Elisa reader with a 450nm measurement filter. The absorbance is proportional to the concentration of the HSV antibodies. With each set of tests are 3 sets of calibrators (CAL1a and CAL1b; CAL2a and CAL2b; and CAL6) of concentrations 0 U/ml, 5 U/ml and 100 U/ml.

Quality Control and Interpretation of Results (qualitative)

The assay is validated if the absorbance(OD) of blank is less than 0.100; the mean absorbance of negative control less than 0.200, the cut off absorbance is between 0.150 and 1.30 and negative control absorbance > than cut off.

Interpretation of Results

The sample is positive if the absorbance is greater than 10% over the cut off. And negative when the absorbance is lower 10% below the cut off.

Data Analysis

Statistical analysis: all data was analyzed using the Statistical Package for the Social sciences (SPSS) version 15.0. Data was presented using frequency tables, charts, as appropriate and cross tabulation to study relationships and association between variables. Statistical significance was set at 5%. A logistic regression was conducted to identify factors independently associated with *HSV-2* infection.

RESULTS

A total of 207 participants took part in the study and we had 63 males and 144 females. The age range of the participants was from 14 to 68years, majority of the patients were in the 26-30 year age group. Majority of the respondents were from Ogun state 157 (75.8%) and 50(24.2%) from other states. The tribes of the participants ranged from Hausa 5(7.2%), Igbo 27(13%), Yoruba 161 (77.8%). Majority of the participants were Christians 124(59.9%). Majority of the participants were traders 70(33.8%), commercial sex workers constituted about 6.8% of the patients, civil servants 14.5% and students 18.8% (Table 1).

Table 1: Sociodemographic characteristics of participants.

	Frequency	Percentage
Age		
10-15	5	2.4
16-20	26	12.5
21-25	21	10.1
26-30	35	16.9
31-35	24	11.6
36-40	28	13.5
41-45	28	13.5
46-50	29	14.0
>50	16	7.7
Sex		
Male	63	30.4
Female	144	69.6
Location		
Ogun	157	75.8
Outside Ogun	50	24.2
Tribe		
Hausa	15	7.3
Igbo	27	13.0
Yoruba	161	77.8
Others	4	1.9
Religion		
Christianity	124	59.9
Islam	71	34.3
Traditionalist	12	5.8
Occupation		
Student	39	18.8
Commercial Sex Worker	14	6.8
Trader	70	33.8
Artisan	15	7.2
Teacher	25	12.1
Civil Servant	30	14.5
Others	14	6.8
Retroviral status of partners		
Positive	61	29.5
Negative	146	70.5

Less than a third of participants had genital ulcers, vaginal discharge, dyspaerunia, inguinal discomfort, recurrent ulcers occurred in 42(20.3%) of the patients (Table 2).

A total of 77(37.2%) participants had a history of multiple sexual partners, 75(36.2%) had a history of Alcohol intake, 47(21.3%) had a history of smoking. Of the respondents 46(22.2%) practiced anal sex, 67 (32.4%) oral sex and 60(29%) had a history of transactional sex. Over half of the participants had a history of condom use (Table 3).

Over two thirds of the participants 172 (83.1%) were currently on antiretroviral. A total of 145(70%) of the patients were HSV-2 seropositive and almost over 85% of the participants had CD4 count above 300 (Table 4).

Table 2: Common Clinical signs and symptoms among the participants

Papular Eruptions		
Yes	79	38.2
No	128	61.8
Genital ulcers		
Yes	58	28
No	149	72
Dysuria		
Yes	59	28.5
No	148	71.5
Vaginal/cervical discharge		
Yes	87	42
No	120	58
Dyspareunia		
Yes	58	28
No	149	72
Inguinal discomfort		
Yes	57	27.5
No	150	72.5
Recurrent ulcers		
Yes	42	20.3
No	165	79.7

Table 3: Risk factors for HSV-2 among the participants

Age at Sexual Debut		
<10	13	6.3
11-15	31	15.0
16-20	71	34.3
>20	92	44.4
Multiple sexual partners		
Yes	77	37.2
No	130	62.8
Condom use		
Yes	109	52.7
No	98	47.3
Alcohol intake		
Yes	75	36.2
No	154	63.8
Drug use		
Yes	53	25.6
No	154	74.4
Smoking		
Yes	42	20.3
No	165	79.7
Any form of cancer		
Yes	52	25.1
No	155	74.9
Anal sex		
Yes	45	21.7
No	161	77.8
Oral sex		
Yes	67	32.4
No	140	67.6
History of transactional sex		
Yes	59	28.5
No	148	71.5

Table 4: Seropositivity and CD4 count of the participants.

CD4 Count		%
<100	2	1
101-300	27	13.0
301-500	85	41.1
501-700	73	35.3
701-900	20	9.7
Seropositivity of HSV-2		
Positive	145	70
Negative	62	30
Currently on ARV		
Yes	172	83.1
No	35	16.9

Table 5: Age and sex in relation to HSV-2 seropositivity

	Positive	Negative	Total	P-Value
Age				
10-15	2(40%)	3(60%)	5(100%)	
16-20	13(61.9%)	8(38.1%)	21(100%)	
21-25	15(71.4%)	7(28.6%)	22(100%)	
26-30	25(73.5%)	9(26.5%)	34(100%)	
31-35	21(87.5%)	3(12.5%)	24((100%)	0.428
36-40	17(60.7%)	11(39.3%)	28(100%)	
41-45	20(71.4%)	8(28.6%)	28(100%)	
46-50	19(65.5%)	10(34.5%)	29(100%)	
>50	12(75.0%)	4(25.0%)	16(100%)	
Sex				
Male	41(65.1%)	22(34.9%)	63(100%)	0.302
Female	104(72.2%)	40(27.8%)	144(100%)	

Table 6: Clinical presentation and HSV-2

	Yes	No	Total	P-Value
Age at Sexual Debut				
<10	10(76.9%)	3(24.1%)	13 (100%)	
11-15	18(58.1%)	15(41.9%)	31(100%)	
16-20	51(71.8%)	20(28.2%)	71(100%)	0.708
>20	65(64.3%)	27(29.3%)	92(100%)	
Papular eruptions				
Yes	57(72.2%)	22(27.8%)	79(100%)	0.579
No	87(68.5%)	41(31.2%)	128(100%)	
Genital ulcer				
Yes	41(71.9%)	16(28.1%)	57(100%	0.695
No	104(69.1%)	46(30.1%)	150((100%)	
Dysuria				
Yes	40(67.8%)	20(32.2%)	60(100%)	
No	104(70.7%)	43(29.3%)	147(100%)	
Dyspaerunia				
Yes	41(71.9%)	16(28.1%)	57(100%)	0.585
No	104(68.7%)	46(31.3%)	150(100%)	
Recurrent ulcers				
Yes	28(68.3%)	13(31.7%)	41(100%)	
No	116(70.3%)	49(29.7%)	165(100%)	0.802
Multiple sexual partners				
Positive	58(76.3%)	18(23.7%)	76(100%)	
Negative	87(66.2%)	44(33.8%)	131 (100%)	0.125
Condom use				
Positive	76(69.7%)	33(30.3%)	109(100%)	0.953
Negative	69(70.1%)	29(29.9%)	98(100%)	
Any drug abuse				
Positive	34(64.2%)	19(35.8%)	53(100%)	
Negative	111(71.9%)	43(28.1%)	154(100%)	0.289
Anal sex				
Positive	32(71.1%)	13(28.9%)	45(100%)	0.785
Negative	112(69.4%)	50(30.6%)	162(100%)	
Oral sex				
Positive	42(63.6%)	24(36.4%)	66(100%)	
Negative	102(72.9)	39(27.1%)	141(100%)	0.178
History of transactional sex				
Positive	43(72.9%)	16(27.1%)	59(100%)	
Negative	102(68.5%)	46(31.5%)	148(100%)	0.665
Currently on antiretrovirals				
Yes	120(70.2%)	52(29.8%)	172(100%)	
No	24(68.6%)	11(31.4%)	35(100%)	

In relating age and sex, it observed the following that the most affected group is the 31-35 21(87.5%), while the least affected group was the 10-15 age group, there was no significant association between age and HSV-2 seropositivity (p value=0.428).

Forty one (65.1%) of the males were HSV-2 seropositive compared with One hundred and four (72.2%) observed among the females. Although more females were seropositive to HSV-2, There is no significant difference in the prevalence of HSV-2 infection between both sexes (x^2 =0.545, p-value= 0.302) (Table 5). Age at sexual debut was not a significant factor to HSV-2 Acquisition.

Fifty eight 58(28.2%) of the respondents who were seropositive had a history of multiple sexual partners. History of multiple sexual partners was not a significant factor to HSV-2 acquisition. (X^2=2.354, p value=0.125) (Table 6).

Thirty four (16.5%) of the respondent had history of drug use while 110(53.4%) did not. History of drug use was not a significant factor to HSV-2 acquisition. (X^2 =1.122, P value 0.289). Thirty two (15.5%) of the respondents had anal sex against 111(53.9%) who do not have anal sex. Anal sex was not a significant factor in HSV acquisition (X^2=0.483 p value=0.785) (Table 6).

Forty two (29.2%) of the respondents had a history of oral sex while 102 did not have a history oral sex. Oral sex did not contribute significantly to HSV acquisition. (X^2=1.813, p value=0.178). Forty three (29.9%) of respondents had a history of transactional sex while 101(70.1%) had no history of transactional sex (X^2=0.817, p value=0.665) (Table 6).

One hundred and twenty (83.3%) of the respondents were on antiretrovirals when compared to 24 (16.4%) who were not ARV there was no significance in HSV acquisition. (X^2 =0.036, p value=0.850) (Table 6).

DISCUSSION

Serological testing showed that the prevalence of HSV-2 was 70.0%.This result is consistent with HSV-2 prevalence reported in a multi-centre study in two sub-Saharan Africa cities: 90.0% in Cotonou Benin Republic; 84.1% in Yaoundé[1]. Majority of the participants affected belonged to 31-35yr age group showing affectation was in the young adult population and economically productive group this is in keeping with studies where the most affected group was the 30-32 age group.[8]

Globally, seroprevalence of HSV-2 is higher in the female sex compared to the males in this study the prevalence in females was 72.2% compared to 65.1% in males, this contrasts with studies from a study[9] where the prevalence was higher in the males.

In this study traders and commercial sex workers had a high seropositivity rate this is in keeping with reports from Jos.[10] The two groups have opposite motivations while the traders have the money to induce sexual activity, the CSW trades in sex for gratification thus exposing both groups to HSV-2 infection.

Age at sexual debut did not significantly affect HSV-2 infection, seropositivity was highest in the > 20 and lowest in the <10 group, this is in keeping with studies by Obasi *et al* but was in contrast to a study carried out in India[9] which showed that younger age at sexual debut increases risk of HSV-2 acquisition as these group are impressionable, immature and susceptible to the guile of older men.

Seropositivity was highest in the group with history of multiple sexual partners when compared to those who had no history of multiple sexual partners, this is in keeping with studies[9] which showed that >3 lifetime sexual partners may predispose to HSV-2 infection the reason for this is that the more the number of sexual contacts the higher the chances of contracting the infection. Amongst those who used condoms, seropositvity was higher in the group that did not use condoms compared to 69% who did use condoms this is in keeping with studies[9] which shows that despite improper condom some measure of protection is afforded by condom usage.

Seropositivity was higher in people who practiced anal sex than those who did not. Anal sex is a risk for both HIV and HSV 2 infection. 72.9% of those with history of transactional sex were seropositive compared to 68.5% who were seropositive with no history of transactional sex. There is a higher rate of HSV-2 seropositivity in patients who had a history of transactional sex this is supported by studies[11] where there was an increase in HSV-2 in patients who had transactional sex.

Over 41(70%) of all participants who presented with genital ulcers tested positive for HSV-2 compared to 103(69.1%) who had no genital ulcer but tested positive to HSV-2. Seropositivity was highest in the population who had no history of genital ulcer this proves that absence of an ulcer does not indicate absence of HSV-2 this is in keeping with studies that the abscence of genital ulcers does not preclude HSV-2 seropositivity as the prescence of HIV enhances the shedding of HSV-2 even in asymptomatic individuals.[9]

On logistic regression of the variables to rule out confounding risk factors only a history of multiple sexual partners and history of oral sex were risk factors for HSV-2 acquisition in HIV patients. This is in keeping with various studies which show that having multiple sexual partners lead to HSV-2 acquisition.

CONCLUSION

Sexually transmitted infections in particular HSV-2 infections are common in HIV-infected patients attending care in ART clinics in Nigeria. The implication of the findings is that patients should be regularly screened and subsequently treated for Herpes where found positive as several epidemiological and laboratory studies have suggested a biological plausibility of HSV-2 infection facilitating the acquisition and transmission of HIV-1. Recurrent episodes of HSV-2 may increase the susceptibility to HIV-1 infection in HSV-infected individuals by activating HIV-1 target cells in the genital tract. In resource-limited countries where both infections are prevalent efforts at symptomatic and diagnostic screening and treatment of HSV-2 could be part of the preventive measures for HIV-1. Furthermore, this study identified specific high risk behaviors – early onset of sexual debut, multiple sexual partners and oral sex – that could be used in crafting messages for HIV-1 prevention programs.

The strength of the study was that prevalence of HSV 2 in HIV patient was high even in asymptomatic patients, which brings about the need to have routine surveillance and treatment of HSV 2 in retroviral patients.

LIMITATION

There was no limitation.

RECOMMENDATIONS.

The Ministry of Health should ensure the integration of screening and treatment of HSV-2 in the current ARV protocols in the country and also initiate a mass campaign to discourage having multiple sexual partners and been involved in risky sexual behaviours.

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