

Seroprevalence of HSV-2 among Women of Reproductive Age in Zaria, Kaduna State

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Abstract

Introduction: HSV -2 infections is widespread and mainly sexually transmitted. Infection with Herpes simplex virus type 2 (HSV-2) is life long and there is no cure. It is estimated that up to 20 million people are newly infected with HSV-2 each year. Most people, who have the infection, however are unaware that they are infected.

Methodology: This work is therefore aimed at determining HSV-2 IgG status of the women used in the study serologically using Enzyme Linked Immunosorbent Assay (ELISA) and the risk factors associated with acquiring the virus.

Results: A total of 450 women were selected for the study and 370 of these women tested positive for IgG with a prevalence of 82.2%. Out of the three hospitals enrolled, subjects attending Gambo Sawabo General Hospital (GSGH) had the highest IgG prevalence of 98%, while the lowest prevalence was obtained in the subjects attending St. Luke's Anglican Hospital Wusasa –Zaria (SLAH) with a prevalence of 61.3%. The variation in prevalence was statistically associated with HSV-2 infection ($p=0.000$). IgG antibodies increased with age, with the highest prevalence recorded amongst subjects 45 years and above. Higher prevalence of IgG was detected amongst women who had single partners (85%), while the lower prevalence was recorded amongst women who had multiple partners (68.8%). A significant association was observed to exist between pregnancy and HSV-2 infection ($p=0.000$). In relation to level of awareness of the infection, 101 (22.4%) of the women have heard about the infection while 349 (77.6%) were not aware. Symptoms such as fever, blisters/ulcers on genital area, painful urination were significantly associated with the infection while vaginal discharge was not.

Conclusion: The findings in this study confirmed the presence of HSV-2 infection in Zaria metropolis, Kaduna state with seroprevalence comparable to rates reported in various towns and cities in Nigeria and other countries of the world. There is therefore the need for more public awareness/enlightenment to educate the general public especially women about the virus, the infection and how it can be prevented and controlled.

Keywords: HSV-2; Seroprevalence; ELISA

Introduction

Herpes Simplex Virus (HSV) is a neurotropic virus with a large linear, double-stranded DNA genome, protected by a capsid with icosahedral symmetry. It is surrounded by an envelope which consists of a lipid embedded glycoproteins bilayer and a proteinaceous region between the capsid and envelope called tegument [1]. The HSV belongs to the family of *Herpesviridae*, subfamily *Alphaherpesvirinae*, and genus Simplex virus [2,3]. It is a virus that has a very complex life cycle and stands out as one of the most common pathogens in the etiology of sexually transmitted diseases worldwide [4,5]. HSV-2 causes genital herpes presenting with genital lesions that are often very painful and can lead to substantial psychological morbidity [6]. The major public health importance of HSV-2 relates to its potential role in facilitating HIV transmission [7]. Most women become infected with HSV-2 in their reproductive and sexually active life [8]. The potential consequences of genital herpes go beyond the immediate physical and psychological impact to the possibility of transmission to a sexual

partner. Those women who are infected with HSV type 2 may be at greater risk for transmission and acquisition of HIV [9]. Furthermore, pregnant women infected with genital HSV (particularly those with a primary infection), can transmit the virus to the foetus, which can lead to serious neonatal complications, such as neurologic problems, brain damage and even death [10,11].

The estimated number of people aged 15-49 years living with HSV-2 worldwide in 2009 was put at 538 million and approximately 16% of the world's population fall within this age range [12]. The CDC estimates the annual new cases of herpes infection to be 776,000 in the U.S, with genital herpes being the most common [13]. The level of HSV-2 infection continues to rise globally varying between 65% and 90% [14], especially in women who are in their sexual and reproductive age; the virus has been found exhibiting co-infection with human papilloma virus in cervicitis and cervical cancer cases. HSV-2 exhibit an unusual high prevalence and incidence among young African women, and this may not be unconnected with engagement in risky sexual behaviours, including exchanging sex for money or goods occasioned by the poor economic conditions in the sub region [15-17].

The highest prevalence of genital herpes was found to be in sub-Saharan Africa, with a prevalence of 70%, among women and 55% among men [16,17]. The number of cases of genital herpes has gone up by over 30% in the last decade [18]. The aim of this study was to determine the seroprevalence of Herpes Simplex Virus-2, and the associated risk factors among women of reproductive age in Zaria, Kaduna state, Nigeria.

Materials and Methods

This research work was carried out in Zaria, which is an ancient city, home to the Zaria Emirate Council and the headquarters of the Zaria Local Government Area in Kaduna State, in Northern Nigeria. Its neighborhood is comprised of Sabon-Gari, Danmagaji - Wusasa, Zaria-city, Saye, Samaru, Bassawa, Kofan-Gaya, Shika, Giwa. Zaria is a very large heterogenous city with a population of about 1,490,000 of people with different ethnic mix in which Muslim Hausa and Fulani predominate with the others who come from different parts of the world [19]. It is second in size only to the state capital, Kaduna. The study was conducted in three hospitals located in Zaria metropolis, which include St. Luke's Anglican Hospital Wusasa, Gambo Sawaba Specialist Hospital, Zaria City and Major Ibrahim Bello Memorial Hospital at Sabon-Gari.

Study population

The study population includes women of reproductive age between 15-49 years old, attending the selected hospitals, irrespective of age, educational status and place of residence.

Study design

The study was a cross sectional where each of the selected hospitals was used as a Cluster Sample and equal number of samples was collected from all the women attending the selected hospitals, who gave their consent. A structured questionnaire was used to obtain information on socio-demographic variables such as age, education, occupation, type of family, geographic location, annual income, gestational weeks, and risk factors that may be associated with HSV-2 infection, such as number of sex partners in previous one year. The participants were interviewed confidentially and examined by qualified medical personnel.

Inclusion/exclusion criteria

This includes all consenting women of all categories within the age range of 15-49 years, who are attendees of the selected hospitals. Women below the age of 15 years, those above 49 years of age and those within the stipulated age range, who did not give their consent, were not included in the study.

Sample size determination

The sample size was determined, using the equation by Naing et al. [20] and a reported 44.3% prevalence of HSV-2 infections among pregnant women, attending antenatal clinics in Benin, Edo State Nigeria [12] at 95% confidence interval.

$$n = z^2 pq/d^2$$

Where, n=no of samples.

$$P = \text{prevalence rate of previous study} = 44.3\%$$

$$z = \text{standard normal distribution at 95\% confidence limit} = 1.96$$

$$d = \text{absolute desired precision of 5\%} = 0.05$$

$$q = 1 - p = 1 - 0.443 = 0.557$$

$$n = (1.96)^2 \times 0.443 \times 0.557 / (0.05)^2 = 379.17$$

The calculated sample size was 379.17, therefore about 450 blood samples were collected from the subjects attending selected hospitals.

Sample collection

The blood samples were collected in 5 ml plain vacutainer tubes through Venipuncture by qualified medical personnel using a 2 ml syringe. A wet swab with methylated spirit was used to disinfect the skin surface, prior to collection. The blood was allowed to clot, sera was separated into plain sample bottles using a micropipette. The sera were stored at -20°C until analyzed. Sera were analyzed using the Enzyme Linked Immunosorbent Assay-ELISA (Diagnostic Automation/Cortez Diagnostics USA) in accordance with the manufacturer's instruction.

Sample analysis using ELISA

All the test samples and the reagent kit components were brought out and allowed to stand at room temperature at 25-28°C for 30 minutes to equilibrate before the commencement of the assay. Prior to analysis of the samples, a one in forty dilutions was prepared by adding 5 µl of the test samples, negative control, positive control, and calibrator to 200 µl of sample diluents, these were mixed well and 100 µl of diluted sera, calibrator, and controls were dispensed into the appropriate wells. For the reagent blank, 100 µl of sample diluents was dispensed into 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well. The plates were incubated for 30 minutes at room temperature (28°C). The liquid was removed from all the wells and washing was repeated three times with washing buffer after which 100 µl of enzyme conjugate was dispensed in to each well, followed by incubation for about 30 minutes at room temperature. After the incubation period, the enzyme conjugate was removed from all the wells and washing was repeated three times with washing buffer. A total of 100 µl of Tetra Methyl Benzidine (TMB) chromogenic substrate was then dispensed in to each well and the plates were then incubated for 30 minutes at room temperature. At the end of Incubation, 100 µl of (2N) HCl was added to stop the reaction and the plates were read at 450 nm with a microwell reader and interpreted as either negative or positive.

Data analysis

Data generated from questionnaire and results obtained from laboratory analysis were entered into Microsoft excel and reduced to percentages by descriptive Statistical Package for Social Science (SPSS) version (21.0). Chi square was used to determine association between HSV-2 infection and the various parameters studied at 95% Confidence Interval and p value < 0.05 was considered significant.

Results

A total of 450 women were selected for the study and 370 of these women tested positive for IgG giving a prevalence of 82.2%.

The seroprevalence of HSV-2 Infection in relation to selected hospitals is shown in Table 1.

Hospitals	No Examined	No Positive (%)	No Negative (%)	P-Value
SLAH	150	92 (61.3)	58 (38.7)	0.000*
MIBMH	150	131 (87.3)	19 (12.7)	
GSGH	150	147 (98.0)	3 (2.0)	
Total	450	370 (82.2)	80 (17.8)	

Note: SLAH: St. Luke's Anglican Hospital, Wusasa - Zaria; MIBMH: Major Ibrahim Bello Memorial Hospital, Sabon-Gari; GSGH: Gambo Sawaba General Hospital, Zaria City. *p<0.05: Significant association exist; IgG: Immunoglobulin G.

Table 1: The seroprevalence of HSV-2 Infection in relation to selected hospitals.

Out of the three hospitals selected, subjects attending Gambo Sawabo General Hospital (GSGH) had the highest IgG prevalence of 98% (147/150), while the lowest prevalence was obtained in the subjects attending St. Luke's Anglican Hospital Wusasa -Zaria (SLAH) with a prevalence of 61.3% (92/150). The variation in prevalence was statistically associated with HSV-2 infection (IgG: $\chi = 73.003$, $p=0.000$). The seroprevalence of HSV-2 according to age group is shown in Table 2. IgG antibodies in this study increased with age, with the highest prevalence recorded amongst subjects 45 years and above. The presence of IgG ($\chi=21.947$, $df=2$, $p=0.001$) was significantly associated with age in this study.

Age group (Years)	No Examined	No Positive (%)	No Negative (%)	P-Value
16-20	115	83 (72.2)	32 (27.8)	0.001*
21-25	110	84 (76.4)	26 (23.6)	
26-30	73	64 (87.7)	9 (12.3)	
31-35	59	52 (88.1)	7 (11.9)	
36-40	47	43 (91.5)	4 (8.5)	
41-45	40	38 (95.0)	2 (5.0)	
Above 45	6	6 (100)	0 (0.0)	
Total	450	370 (82.2)	80 (17.8)	

Note: No: Number; (%): Percentage; *p<0.05: Significant association exist; df: degree of freedom (6).

Table 2: Seroprevalence of HSV-2 IgG among women with respect to age.

The seroprevalence of HSV-2 Infection in relation to risk factors is shown in Table 3. The distribution of HSV-2 antibodies was analyzed according to the number of sexual partners of the women and the result showed a marginal statistically significant association ($p=0.000$). The higher prevalence of IgG was detected amongst women who had single partners 85% (304/354), while the lower prevalence was recorded amongst women who had multiple partners 68.8% (66/96).

Analysis of the results according to protection used showed that those who didn't use protection during sex had the highest prevalence of 73.1% (38/52) antibodies to HSV-2 while the women who reported to have used protection had a prevalence of 68.8% (66/96). No significant association was observed to exist between the use of

protection and HSV-2 infection. Analysis of results according to pregnancy showed that those who were not pregnant had the highest prevalence to HSV-2 IgG 89.8% (272/303), while the pregnant women had the lowest IgG prevalence 66.7% (98/147). A significant association was observed to exist between pregnancy and HSV-2 infection ($p=0.000$).

In relation to level of awareness of the infection, 101 (22.4%) of the overall total were reported to have heard about the infection while 349 (77.6%) out of the 450 women were not aware of the infection. A total of 92 out of the 101 who have heard about the infection, were found to be seropositive for HSV-2 IgG with a prevalence of 91.1%, while 278 out of 349 who have never heard about the infection, tested positive with a prevalence of 79.7% ($p=0.008$) (Table 4).

Risk Factor	No Examined	No (%) Positive	Odds Ratio (OR)	Ratio	P-Value
No of Sexual Partners					
Single	354	304 (85.9)	2.764		0.000*
Multiple	96	66 (68.8)			
Use of Protective device during sex					
Yes	52	38 (73.1)	0.54		0.067
No	398	332 (83.4)			
Pregnancy					
Yes	147	98 (66.7)	0.228		0
No	303	272 (89.8)			
Total	450	370 (82.2)			

Note: Number of Sexual Partners: CI(IgG)=1.64-4.67; Use of Protection: CI(IgG)=0.28-1.05; Pregnancy: CI(IgG)=0.14-0.38

Table 3: Seroprevalence of HSV-2 IgG among women of reproductive age in relation to some risk factors.

Awareness	No Examined	No Positive (%)	Odds Ratio (OR)	P-Value
General Knowledge of Genital Herpes/HSV-2				
Yes	101	92 (91.1)	2.611	0.008
No	349	278 (79.7)		
Knowledge of Personal HSV-2 Status				
Yes	67	65 (97.0)	8.311	0.001
No	383	305 (79.6)		
Total	450	370 (82.2)		

Note: General Knowledge of Genital Herpes/HSV-2: CI(IgG)=1.26-5.43; Knowledge of Personal HSV-2 Status: CI(IgG)=1.99-34.69

Table 4: Seroprevalence of HSV-2IgG among women of reproductive age in relation to level of Awareness.

The seroprevalence of HSV-2 Infection in relation to some symptoms observed is shown on Table 5. Analysis of results in relation to fever as a symptom, about 87% (154/177) of women who reported

that they were not having fever as a symptom tested positive for HSV-2 IgG, while 79.1% (216/273) who responded to have fever, tested positive ($p=0.033$). In relation to vaginal discharge, 129 (28.7%) out of the 450 women indicated that they had vaginal discharge, where 101 out of the 129 tested positive for HSV-2 IgG with a prevalence of 78.3%. In relation to Blisters/ ulcers on genital area; 114 (25.3%) were reported to have Blisters and/or ulcers especially on their genital area while 336 (74.7%) did not have such symptom. A total of 88.6% (101/114) of women who had blisters/ulcers tested positive for HSV-2 IgG.

Symptom	No Examined	No Positive (%)	Odds (OR)	Ratio	P-Value
Fever					
Yes	273	216 (79.1)	0.57		0.03
No	177	154 (87.0)			
Vaginal Discharge					
Yes	129	101 (78.3)	0.7		0.17
No	321	269 (83.8)			
Itching/Burning sensation on Genital Area					
Yes	155	128 (82.6)	0.02		0.89
No	295	242 (82.0)			
Blister, Ulcers on Genital Area					
Yes	114	101 (88.6)	1.94		0.04
No	336	269 (80.1)			
Pain During Urination					
Yes	86	81 (94.2)	4.2		0
No	364	289 (79.4)			
Pain on thigh, Back, Knees and Buttocks					
Yes	261	211 (80.8)	0.8		0.37
No	189	159 (84.1)			
Total	450	370 (82.2)			
Note: Fever: CI(IgG)=0.33-0.96; Vaginal Discharge: CI(IgG)=0.42-1.17; Itching/Burning Sensation: CI(IgG)=1.24-3.92; Blister/sore on Genital Area: CI(IgG)=1.02-3.66; Pain During Urination: CI(IgG)=1.65-10.74; Thigh/Back Pain: CI(IgG)=0.48-1.31					

Table 5: Seroprevalence of HSV-2 among women of reproductive age in relation to some Symptoms.

As regards painful urination, 86 (19.1%) complained to be having this symptom while 364(80.9%) did not complain, 94.2% (81/86) of the women who had this complain tested positive for HSV-2 IgG, while 79.4% (289/364) who did not complaint, tested positive ($p=0.001$).

Discussion

In this study, the prevalence of HSV-2 IgG amongst women of reproductive age in Zaria was found to be 82.2%. The prevalence of HSV-2 IgG is quite high in the study area suggesting that factors that

allow its transmission remained inherent in the lifestyle of the populace. This prevalence was higher than the 53.3% reported in a similar work among pregnant women carried in the same area in 2014 [21]. The seroprevalence obtained is 82.2% which is comparable to that obtained in parts of the country like Jos where a prevalence of 87% was reported among patients attending Sexually Transmitted Infection Clinic [22]. The trend in prevalence has varied only slightly remaining between 80% and 95% in reports from other African countries [23]. This high prevalence showed that factors that prevent its transmission and effective control measures are not in place in these developing countries. Studies from Sweden, Switzerland and China presented a significant low seroprevalence value of 20.7% [24] and 10.8% [25] respectively among antenatal women. A significant association was observed between increasing age with seroprevalence of HSV-2 reaching 100% in those above 45 years ($p=0.001$). It must be reiterated that as the women advanced in age, the rate of exposure to risk factors increases, leading to the high prevalence observed. Aging is known to be associated with gradual decrease in immunity and could contribute to the high prevalence observed which is in agreement with the study carried out in Jos [22] who reported a higher prevalence among older age group 51–60 years and also with Aminu et al. [21] who reported a higher prevalence of HSV-2 infection among pregnant women of age group 43-47 years. Christenson et al. [26] also suggested that increasing age is a possible risk factor for HSV-2 infection and that this infection is lifelong with a high rate of transmission. The high prevalence obtained in this study could be due to sexual activities engaged in by the women, pregnancy, hormonal disturbances and other stressors which could predispose these women to the virus/ infection. The location of the hospitals was significantly associated with the exposure to the infection with GSSH having the highest prevalence. This hospital is located in the heart of the Zaria City and factors such as over-crowding and maintenance of multiple sexual partners are common. The prevalence was also high among the pregnant women though those that were not pregnant had a higher prevalence. In a sub-Saharan Africa based report 40% prevalence was documented among pregnant women while 30% was reported in Latin America, 17-22% North-America and 4-24% in Europe were HSV-2 infected [2].

However, a significant association was found to exist between the women attending the selected Hospitals and HSV-2 infection ($p=0.000$). This is to say that the virus is highly endemic in the communities where the Hospitals are situated and the fact that most of the subjects living in these communities do not have any knowledge and awareness about this virus, how it is transmitted. The use of protection was also found to be statistically associated with the infection, which is in agreement with Biswas et al. [27] who reported a significant association with never condom users compared to regular or occasional users in a study carried out in the Northern states of India. In contrast, Dada et al. [28] and Agabi et al. [22] also reported that the use of protection during sexual activity had no statistical significance to HSV-2 infection.

A significant association was observed between HSV-2 infection with knowledge of the virus and status of infection ($p<0.05$). This observation is similar to that made by Narouza et al. [29] who reported a lack of knowledge about the virus among general practitioners working in a conventry Ray and McMillan [30] also reported that public knowledge of genital herpes was incomplete and that misinformation about the virus was also observed among first time attendees at the Department of Genito-Urinary Medicine in Scotland. Matsiko [31] also reported a low awareness of the virus in Kayunaga

district, Uganda. Aaron et al. [32] also reported a lack of knowledge and poor attitude of the participants tested towards the virus as HSV-2 infection is most of the time asymptomatic.

Symptom such as fever was found to be significantly associated with HSV-2 infection this could arise as a result of immune response during the course of infection. This observation has been confirmed by Aminu et al. [21] who reported a seroprevalence of 54.3% amongst fever patients. Blisters and ulcers on genital area were found to be statistically associated with HSV-2 infection ($p < 0.05$). This finding agrees with earlier reports by Aaron et al. [32], who reported a 40.3% prevalence of HSV-2 infection and also observed a significant association between genital ulcerations with the virus infection, Biswas et al. [27] also reported a high HSV-2 seroprevalence among subjects with genital ulcers.

Painful urination was observed to be significantly associated with HSV-2 infection ($p < 0.05$). This finding disagrees with earlier report by Aaron et al. [32] who observed that 21% of his study population in India had painful urination and were seropositive in the course of the study, but there was no significant association with the infection.

Conclusion

This study confirmed the presence of HSV-2 infection in Zaria metropolis, Kaduna state with seroprevalence comparable to rates reported in various towns and cities in Nigeria and other countries of the world.

The study showed a significant association of HSV-2 infection with the selected hospitals where the study was carried out showing that the virus is highly endemic in these communities.

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