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Seroprevalence of Herpes simplex Virus Type 2 among Pregnant Women in Sokoto, Sokoto State, Nigeria

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Abstract

Herpes simplex virus type 2 (HSV-2) infections are one of the most common sexually transmitted infections among pregnant women worldwide. This study aimed to assess the seroprevalence of herpes simplex virus type-2 among pregnant women attending antenatal care at Maryam Abacha Women and Children's Hospital, Sokoto, Nigeria. A total of 200 blood samples were screened for HSV-2 among pregnant women. Socioeconomic factors were obtained from a questionnaire, and IgG/IgM antibodies were determined using an ELISA test kit. The Results have shown a prevalence of 6.59% among pregnant women. There was an association between tribe, religion, sexually transmitted Infection symptoms (STIs), history of genital ulcer, and the prevalence of herpes simplex virus type-2 IgM/IgG antibody. Based on age, all four groups were reactive, with the highest prevalence occurring in the age groups 21-30 years (9.1%), ≤ 20 years (6.1%), ≥ 40 years (5.6%), and 31-40 years (2.3%). Based on residence, urban settlers have a higher percentage (7.6%) than rural settlers (4.8%). Those with tertiary education (8.7%) have the highest percentage, followed by those with secondary education (8.2%). These findings make it necessary for all expectant mothers to have a serological test for HSV antibodies to detect early and treat congenital Infections as soon as possible.

KEY WORDS: herpes simplex virus type 2, elisa, pregnant woman and seroprevalance.

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is an envelope, double-stranded DNA virus. It is a universal virus belonging to the family Herpesviridae (Chayavichitsilp et al., 2009). It is the major cause of genital ulcers worldwide, causing anal and genital infections, and may cause infections in other areas of the body (Chayavichitsilp et al., 2009). Herpes Simplex Virus infection is contracted through direct contact with an active lesion or body fluid of an It is mostly transmitted infected person. through sexual intercourse. (Oluboyo etal., 2020). Clinical manifestations of HSV include: skin and mucous lining infections, genital herpes, herpetic whitlow, herpes encephalitis, cognitive deficits of bipolar disorder, and Alzheimer's disease (Dickerson et al., 2004).

Bujko *et al.*, (2004) reported that Herpes simplex virus-2 is one of the most common and relevant venereal diseases occurring in women. Cusini and Ghislanzoni (2001) reported that the prevalence of HSV infection increases with age,

with the highest occurrence among women at 40 years. It has been reported that age, educational status, parity, stage of pregnancy and history of blood transfusion were not significantly associated with the prevalence of HSV infection (Oti et al., 2017).

This infection appears to be related to the number of sexual partners, and with respect to gender, infection is said to be more frequent in women than in men (Smith and Robinson, 2002). Ethnicity, poverty, cocaine abuse, earlier onset of sexual activity, sexual behaviour, and bacterial vaginosis are conditions capable of increasing a woman's possibility of infection before pregnancy (Cherpes et al., 2003). Genital herpes is not routinely diagnosed in the laboratory in Sokoto; hence, delivery of babies by caesarean section is desirable. There is no data to guide HSV-2 prevention efforts like that available for other common Transmitted Infections (STIs). Testing for seroprevalence of antibodies to HSV-2 is one way to estimate the seroprevalence of genital herpes in a population. Records are, however, scanty on the seroprevalence of herpes simplex virus type 2 among pregnant women in Sokoto State. This triggers the research work to investigate the prevalence of herpes simplex virus type 2 among pregnant women attending Maryam Abacha Women and Children Hospital, Sokoto, Sokoto State. To determine the seroprevalence (IgG/IgM) of herpes simplex virus type 2 among pregnant women and to determine the associated risk factors with herpes simplex virus type 2 infections

METHODOLOGY

Study Area

The study was conducted at Maryam Abacha Women and Children Hospital, Sokoto, among pregnant women attending the antenatal clinic in the hospital. Patients come from different parts of the state to the hospital for medical attention. Maryam Abacha Women and Children Hospital is located at longitude 5.2460 °E and latitude 13.0522 ^oN. It is situated on Sultan Bello Road in Sokoto, near the Sokoto old market. The women and children's health-care clinic was built in 1997 and commissioned by Her Excellency, Maryam Sani Abacha, to address health issues that women and children face, such as vesicovaginal fistula (VVF). Since its the hospital has successfully inception, performed surgery in such cases (Lema et al., 2019).

Study Design

The study was a prospective cross-sectional hospital-based study where blood samples were collected from pregnant women attending the antenatal clinic of Maryam Abacha Women and Children Hospital, Sokoto.

Eligibility Criteria

Pregnant women attending the antenatal clinic of Maryam Abacha Women and Children Hospital, Sokoto, were eligible and recruited for the study.

Inclusion Criteria

Pregnant women attending Maryam Abacha Women and Children Hospital, Sokoto, who give consent to participate in the study.

Exclusion Criteria

Non-pregnant women, Pregnant women who do not give consent to the study, and Pregnant

women who were not attendees of the Maryam Abacha Women and Children Hospital, Sokoto.

Ethical Approval and Informed Consent

Ethical approval for the study was obtained from the Ministry of Health, Sokoto, with a reference number SMH/158/V iv. The study participants were informed about the purpose of the study.

Study Population and Sample Size Determination

The study includes 200 pregnant women attending an antenatal clinic in Maryam Abacha Women and Children Hospital, Sokoto State. Sample size for the study was determined using the method described by Fischer *et al.* (2020) in the equation.

$$N = [Z^2 P (1-P)]/D^2$$

Where N = Minimum sample size

Z = confidence interval at 95% (standard value of 1.96)

P = Prevalence rate

D = desire degree of accuracy at 95% confidence level 0.05

To calculate N using the 13% prevalence obtained by WHO (2022)

Therefore

 $N=[1.96^2 \times 0.13(1-0.13)]/0.05^2$

n=[3.8416x 0.13(1- 0.13)]/0.0025

n= 173.79

n=174.

The sample size calculated for the entire study population was 174. However, to make a sample size that will give a fair representation of the study population, 200 samples were collected among the pregnant women.

Application of Questionnaire

A well-structured questionnaire was used to collect the demographic data of the participants, risk factors, and birth history.

Sample Collection and Processing

Five (5) ml of venous blood were collected from each of the consenting pregnant women using

sterile needles, syringes, and clean sample bottles. Serum was obtained by centrifuging each blood sample at 1000 rpm (revolutions per minute) for 10 minutes. The sera were stored in the Chemical Pathology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto, at -20°C until ready for assay (Cheesbrough, 2006).

Detection of Herpes Simplex Virus Type-2 (HSV-2)

The serum samples were determined using an (Beijing North Institute ELISA kit οf Biotechnology Co. Ltd) following the manufacturer's instructions. The kits were intended for the determination of IgG/IgM classspecific antibodies against HSV-2 in human serum or plasma. The kit uses the capture ELISA principle to detect HSV-2 IgM. Purified antihuman IgM monoclonal antibody was precoated on the microplate. The HSV-2 IgM in the sample combines with the anti-human IgM monoclonal antibody first, then combines with the enzymelabeled antigen to form an antigen-antibody complex, and shows a blue color in the microplate. The kit was used for the specific detection of HSV-2 IgM antibody in the samples.

Detection of Herpes Simplex Virus Type-2 (HSV-2) using ELISA IgM

The manufacturer's procedure was strictly followed. The reagents provided were allowed to attain room temperature for 15 minutes before use. The wash buffer was diluted with distilled water at a ratio of 1:40 before use. The microtiter plate was set up with one well as a blank, two wells as negative controls, and two wells as positive controls. 100µl of sample diluents was dispensed into the respective wells except the blank well, negative control well, and positive control well. 100µl of the negative and positive controls were dispensed into their 10µl of samples were wells, respectively. dispensed into their wells and the content was mix by shaking the plate gently for 30 seconds.

The microplate was covered with a sealing paper and incubated in a microplate incubator at 37 °C for 20 minutes. After incubation, the plate cover was removed and discarded. Wash buffer was added to each well for 20 seconds, and this process was repeated 5 times using the washed buffer. After the final washing cycle, the plate was turned over onto blotting paper and tapped to remove any excess. 50µl of conjugate was added to each well except the blank; the microplate was covered with a sealing paper and

also incubated in a microplate incubator at 37 °C for 20 minutes. After incubation, the microplate was washed five times with the diluted wash buffer. 50µl of substrate solution A and 50µl of substrate B were added to each well, except the blank well, and then vortexed; the plate was covered and incubated at 37 °C for 10 minutes. 50µl of stop solution was added to each well except the blank well, and it was followed by mixing. The absorbance was read in an ELISA reader machine at a wavelength of 450 nm (Oluboyo et al., 2020).

The kit uses the indirect ELISA principle to detect HSV-2 IgG. Purified HSV-2 antigen was pre-coated on the microplate, the HSV-2 antibody in the sample combines with HSV-2 antigen first, then combines with enzymelabeled antigen to form antigen-antibodyantibody complex, and shows blue color in the microplate. The kit was used for the specific detection of HSV-2 IgG antibody in the samples.

Detection of Herpes Simplex Virus Type-2 (HSV-2) using ELISA IgG

The manufacturer's procedure was strictly followed. The reagents provided were allowed to attain room temperature for 15 minutes before use. The wash buffer was diluted with distilled water at a ratio of 1:40 before use. The microtiter plate was set up with one well as a blank, two wells as negative controls, and two wells as positive controls. 100µl of sample diluents was dispensed into the respective wells except the blank well, negative control well, and positive control well. 100µl of the negative and positive controls were dispensed into their 10µl of samples were wells, respectively. dispensed into their wells and the content was mix by shaking the plate gently for 30 seconds.

The microplate was covered with a sealing paper and incubated in a microplate incubator at 37 °C for 20 minutes. After incubation, the plate cover was removed and discarded. Wash buffer was added to each well for 20 seconds, and this process was repeated 5 times using the washed buffer. After the final washing cycle, the plate was turned over onto blotting paper and tapped to remove any excess. 50µl of conjugate was added to each well except the blank; the microplate was covered with a sealing paper and also incubated in a microplate incubator at 37 °C for 20 minutes. After incubation, the microplate was washed five times with the diluted wash buffer. 50µl of substrate solution A and 50µl of substrate B were added to each well, except for the blank well and the mix; the

plate was covered and incubated at 37 $^{\circ}$ C for 10 minutes. 50µl of stop solution was added to each well, except the blank well, and the mixture was gently shaken. The absorbance was read in an ELISA reader machine at a wavelength of 450 nm (Oluboyo *et al.*, 2020).

Interpretation of the result of HSV-2 IgG/IgM

If the mean negative control O.D. \leq 0.1 and the mean positive control O.D. \geq 0.8, the test is valid.

Cut-off O.D = the mean O.D value of the negative control \times 2.1

Positive results: Sample O.D.≥ cut-off O.D

Negative results: Sample O.D. < cut-off O.D

RESULTS

Seroprevalence of HSV Type 2 IgG/IgM antibody among pregnant women attending Maryam Abacha Women and Children's Hospital was studied in this research. Out of two hundred (200) blood samples collected, only 182 serums were able to be screened for herpes virus antibodies (IgM and IgG). 170 (93.41%) of the serum samples were negative for herpes simplex

virus IgM and IgG. The overall positive herpes simplex virus IgM and IgG serum samples were 12 (6.59%). Socioeconomic characteristics associated with HSV Type 2 among pregnant women were presented on the Table. 2. A total of 182 samples were examined, and the prevalence of HSV-2 IgG/IgM antibodies with age was 12 (6.59%). All four age groups were reactive, with the highest prevalence occurring in ages 21-30 years (9.1%), followed by ≤ 20 (6.1%), ≥ 40 (5.6%), and 31-40 (2.3%). However, there was no association ($X^2=0.019$, p=0.892) between age and HSV-2 prevalence. Based on religion, higher HSV-2 seroprevalence occurred in subjects practicing Christianity (21.4%) than those practicing Islam (5.4%). However, there is a significant association ($X^2=5.420$, p=0.020) between religion and HSV-2 prevalence. So also, Hausa have the highest proportion of HSV Type 2 IgG/IgM antibody (59.34%), while Igbo have the lowest proportion (1.10%). There is an association among the Igbo pregnant women, with a p-value < 0.05. So, pregnant women living in urban areas have the highest prevalence (7.6%), while those living in rural areas have the lowest prevalence (4.8%).

Table 2: Seroprevalence of HSV Type 2 IgG/IgM among pregnant women based on some sociodemographic factors

Variables	Total n (%)	Positive n (%)	Negative n (%)	x²-value	p-value
Age group (years)					
<20	33 (18.13)	2 (6.1)	31 (93.9)	0.019	0.892
21-30	88 (48.35)	8 (9.1)	80 (90.9)	1.726	0.189
31-40	43 (23.63)	1 (2.3)	42 (97.7)	1.665	0.197
>40	18 (9.89)	1 (5.6)	17 (94.4)	-	-
Religion				5.420	0.020
Islam	168 (92.31)	9 (5.4)	159 (94.6)		
Christian	14 (7.69)	3 (21.4)	11 (78.6)		
Tribe				6.187	0.013
Hausa	108 (59.34)	5 (4.6)	103 (95.4)		
Yoruba	12 (6.59)	1 (8.3)	11 (91.7)		
Fulani	39 (21.43)	4 (10.3)	35 (89.7)		
Igbo	2 (1.10)	1 (50.0)	1 (50.0)		
Others	21 (11.54)	1 (4.8)	20 (95.2)		
Residence				0.525	0.469
Rural	63 (34.62)	3 (4.8)	60 (95.2)		
Urban	119 (65.38)	9 (7.6)	110 (92.4)		

The seropositivity of HSV-2 IgG/IgM antibody based on occupation and educational level is

presented in Table 3. Out of 182 samples examined, the others (9.8%) had a higher

prevalence than other occupational groups. Statistically, there was no association (X^2 =1.186, p=0.276) between occupation and HSV-2 prevalence. Also, the educational level of the pregnant women attending antenatal care was not associated with HSV-2 IgG/IgM

prevalence (X^2 =0.189, p=0.664). A higher prevalence occurred with tertiary education (8.7%) than in other categories of educational level, and the lowest prevalence occurred among illiterates (2.2%).

Table 3: Prevalence of HSV-2 IgG/IgM antibodies among pregnant women based on Occupational and Educational level

Variables	No. of Samples (%)	Positive n (%)	Negative n (%)	x²-value	P-value
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Occupation					
Housewife	95 (52.20)	5 (5.3)	90 (94.7)	0.571	0.450
Civil Servant	24 (13.19)	1 (4.2)	23 (95.8)	0.264	0.607
Farming	12 (6.59)	1 (8.3)	11 (91.7)	0.063	0.802
Others	51 (28.02)	5 (9.8)	46 (90.2)	1.186	0.276
Educational Level					
Illiterate	45 (24.72)	1 (2.2)	44 (97.8)	1.855	0.173
Primary	41 (22.53)	3 (7.3)	38 (92.7)	0.045	0.832
Secondary	73 (40.11)	6 (8.2)	67 (91.8)	0.523	0.470
Tertiary	23 (12.64)	2 (8.7)	21 (91.3)	0.189	0.664

Risk Factors associated with HSV Type 2 IgG/IgM antibodies among pregnant women are presented in <u>Table 4</u>. Prevalence of HSV Type 2 IgG/IgM antibodies among women based on their gestational period, a higher HSV-2 IgG/IgM prevalence occurred in pregnant women in their third trimester (8.1%) than second and first trimesters. However, no association (X²=0.330, p=0.565) existed between the gestation period and HSV-2 prevalence.

Prevalence of HSV-2 IgG/IgM antibody according to parity is presented in <u>Table 5</u>. Higher HSV-2 IgG/IgM prevalence occurred in those with 3 parity pregnant women (9.4%) than those with 2, 1, and >4 parity had a prevalence rate of 7.4%, 6.3%, and 4.5%, respectively. No Significant association (X²=0.488, p=0.485) between parity and HSV-2 prevalence.

Table4: Prevalence of HSV-2lgG/lg Mantibodies according to gestational period

Trimester	Number of samples N	Percentage		X²value	P-value
	= 182 (%)	Positive (%)	Negative (%)		
First	36(19.78)	2(5.6)	34(94.4)	0.078	0.779
Second	84(46.15)	5(6.0)	79(94.0)	0.104	0.747
Third	62(34.07)	5(8.1)	57(91.9)	0.330	0.565
Total	182 (100.00)	12 (6.59)	170 (93.41)		

Table 5: Prevalence of HSV-2IgG/Ig Mantibody according to parity

Parity	Number of samples	Percentage		X ² -value	P-value
	N= 182 (%)	Positive (%)	Negative (%)	7	
1	44(24.17)	2(4.5)	42(95.5)	0.395	0.530
2	27(14.84)	2(7.4)	25(92.6)	0.034	0.853
3	32(17.58)	3(9.4)	29(90.6)	0.488	0.485
>4	79(43.41)	5(6.3)	74(93.7)	0.016	0.900
Total	182(100.00)	12(6.59)	170(93.41)		

DISCUSSION

The prevalence of genital herpes simplex virus (HSV) infection in pregnant women is a significant concern due to the potential risks it

poses to both the fetus and the newborn. HSV-1 and HSV-2 infections can lead to neonatal herpes, which can be severe or even fatal if not properly managed. Therefore, assessing HSV infection in pregnant women is essential for

appropriate clinical management and epidemiological tracking.

This study revealed a relatively low prevalence of 6.59% of HSV-2 among pregnant women in Sokoto, Nigeria (6.59%) is notably lower compared to similar studies in other Nigerian States, where seroprevalence rates have been significantly higher. For instance, a study in a tertiary health facility in Keffi, Central Nigeria, reported a seroprevalence of 35.5% (Oti et al., 2017), while another study in Benin, Nigeria, recorded a prevalence of 46.3% (Kalu et al... 2015). Similarly, a study conducted in Ethiopia reported an HSV-2 seroprevalence of 7.8% among antenatal attendees, which aligns closely with this finding. However, differences in testing methods (eg, ELISA & PCR) and sample size may account for minor variations. These variations highlight the diverse epidemiological patterns of HSV-2 across different regions. Furthermore, HSV-2 seroprevalence in other parts of Africa and the Americas is reported to be much higher, with figures ranging from 60 to 90% in various rural and urban populations (Mihret et al., 2002; Fleming et al., 1997).

The higher seroprevalence observed in these regions could be attributed to factors such as more frequent promiscuous sexual behavior, a larger number of sexual partners, and higher rates of other sexually transmitted infections (STIs), which increase susceptibility to HSV-2 infection. The comparatively lower prevalence in Sokoto could be attributed to cultural practices, levels of sexual education, and healthcare accessibility in the region.

In contrast, studies in some Asian countries show a lower prevalence of HSV-2 infection, with figures ranging from 10 to 30% (Weiss, 2004). In India, a study conducted at a general gynecology clinic found a seroprevalence of 23.3% (Maitra and Gupta, 2007), while a study in Delhi reported 7% and 8.6% in two urban communities (Chawla *et al.*, 2008).

Comparatively, a study from Jigawa, Nigeria, found a prevalence of 18.3% (Amoo et al., 2020), and similar figures have been observed in other Nigerian states, such as Ibadan (33.3%) and Keffi (35.5%) (Anaedobe and Ajani, 2019; Oti et al., 2017). These differences can be attributed to the variations in the local prevalence of the virus, exposure rates, and socio-cultural behaviors related to sexual health.

The 6.59% seroprevalence observed in this study is comparable to that reported in other regions

of Nigeria and internationally. For example, a prevalence of 6.1% and 6.7% was found in pregnant women in Kano, Northern Nigeria, and India, respectively (Muhammad et al., 2021; Aaron et al., 2013). However, this study's prevalence is higher than that reported in studies from other regions, such as 2.8% in Porthacourt Nigeria (Okonko et al., 2023), 4.2% in Turkey (Mehmet et al., 2016), and 4.76% in Baghdad (Hussan, 2013). These discrepancies may be influenced by factors such as the rate of exposure to the virus, the local epidemiology of HSV-2, and the immune status of the population.

The highest prevalence was observed among pregnant women aged 21-- 30 years (9.1%), while the lowest was among those aged 31-40 years (2.3%). This finding is consistent with studies by Apurba et al. (2013) and Okonko and Cookey (2015), which also reported higher seropositivity in women aged 26 - 30 years. These also corroborate with other studies showing that HSV2 prevalence tends to peak in younger, sexually active women. For example, research in Tanzania reported similar agerelated trends, attributing higher prevalence in this age group to increased sexual activity and biological vulnerability to STIs. contrary to some previous reports (Cusini and Ghislanzoni, 2001), this study did not find a statistically significant correlation between age and HSV-2 infection. This suggests that infection can occur at any age if there is exposure to the virus, which is supported by the findings of Oti et al. (2017), who reported the highest prevalence of HVS in pregnant women aged less than 20 years and the least in older age groups, 31-40 years.

Regarding sociodemographic factors, our study found no statistically significant correlation between HSV-2 seroprevalence and factors such as place of residence (rural vs. urban), educational level, and occupation. There is a significant association between religion and HSV2 prevalence, with Christians showing a higher prevalence of 21.4% than Muslims, 5.4%. This trend might reflect differences in sexual health education, cultural practices, socioeconomic factors. Tribal analysis revealed significant prevalence among Igbo women (50%), which could be linked to smaller sample sizes, leading to skewed data. This warrants further investigation with a larger sample size (Chawla et al., 2008). These findings align with those of Rathore et al. (2010), who also reported no significant associations. In contrast, other studies have found significant associations

between sociodemographic factors and HSV-2 infection (Chawla et al., 2008).

CONCLUSION

Results from this study have shown a prevalence of 6.59% of HSV among pregnant women. There was an association between tribe, religion, sexually transmitted Infection symptoms (STIs). history of genital ulcer, and the prevalence of herpes simplex virus type-2 IgM/IgG antibody. The highest prevalence was among women aged 21-30 years (9.1%). Urban settlers had a higher percentage (7.6%), and those with tertiary education had the highest percentage (8.7%). Pregnant women in the third trimester (8.1%) have a higher percentage. Those with parity 3 have the highest percentage, followed by those with parity 2 (7.4%). These findings make it necessary for all expectant mothers to have a serological test for HSV antibodies to detect early and treat congenital Infections as soon as possible.

RECOMMENDATION

Based on the findings of this study, the following recommendations are suggested:

- It is crucial to implement routine screening for HSV-2 in antenatal care settings. Early detection can help identify at-risk individuals and enable timely interventions to reduce the risk of neonatal transmission.
- Awareness campaigns should be launched to educate the general population, especially pregnant women, about the risks of HSV-2. Providing information on preventive measures, such as safe sexual practices, can help reduce the spread of the virus.
- Special attention should be given to women with known risk factors for HSV-2, such as a history of genital ulcers, multiple sexual partners, early age of first intercourse, and previous STIs. Tailored interventions, including counseling and appropriate antiviral therapy, could significantly reduce the transmission risk and improve pregnancy outcomes.

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