Serological Evidence of Herpes Simplex Virus Type 1 & 2 IgM Antibody among Pregnant Women in Port Harcourt, Rivers State, Nigeria

ABSTRACT

A hospital-based cross-sectional survey was adopted to randomly analyze 90 pregnant women attending the antenatal clinic at the University of Port Harcourt Teaching Hospital (UPTH) in Port Harcourt, Nigeria. Enzyme-linked immunosorbent Assay (ELISA) was used to assess HSV-1 & -2 IgM antibodies in the samples obtained. Chi-square analysis was used to determine the association of the infection with socio-demographic factors. Of the 90 subjects, 2(2.2%) were seropositive for HSV-1& -2 IgM antibody while 44(48.9%) were observed to be seronegative for HSV-1 & -2 IgM antibody. No statistical association existed between the prevalence of HSV-1 & -2 IgM antibodies and the socio-demographic factors studied ($p>0.05$). Higher prevalence of HSV-1 & -2 IgM antibody was found in age group 20-29 years (4.7%), married (2.2%), pregnant women with tertiary education (2.6%), traders (6.5%), Christians (2.5%), monogamous family type (2.5%), pregnant women in their second trimester (4.5%), nulliparous (3.0%), history of abortion (3.3%), history of STDs (16.7%) and HIV seronegativity (2.3%). This study has further confirmed the presence of Herpes Simplex Virus primary or recurrent infection among pregnant women as reflected by the prevalence obtained for HSV-1 & -2 IgM antibodies. The study also showed that the prevalence of HSV-1 & -2 IgM was significantly associated with marital status. This observation necessitates the need for serological evaluation in all pregnant women for HSV antibodies for early diagnosis and treatment to prevent congenital infections in the fetus.

Keywords: HSV, Prevalence, IgM, ELISA, UPTH

1. INTRODUCTION

The Herpesviridae family is a group of enveloped, double-stranded DNA viruses known to cause persistent infections in humans. Among the subfamily of Alphaherpesvirinae, there are five genera and 45 species, with human alphaherpesvirus 1 and 2 being ubiquitous members of the Simplexvirus genus, commonly referred to as herpes simplex type 1 and 2 (HSV-1 and HSV-2) (Hammad, &Konje, 2021). HSV-1 is primarily associated with orofacial infections, while HSV-2 is linked to genital infections. However, HSV-1 can also cause genital ulcers. These viruses are recognized as significant contributors to sexually transmitted infections (STIs), with a high prevalence worldwide, affecting 60-95% of the adult population. The infections are often subclinical or asymptomatic and depend on the host's immune system and the frequency of viral entries (Hosseini et al., 2023).

HSV-1 is typically transmitted through non-sexual contact, such as mouth-to-mouth contact, leading to oral ulcers, while HSV-2 is transmitted through sexual contact, resulting in genital ulcers. Nonetheless, HSV-1 can also be a causative agent of genital ulcers. According to the World Health Organization (WHO), in 2017, 67% of people under the age of 50 were globally infected with HSV-1, and 13% of individuals aged 15-49 were infected with HSV-2. The WHO

report estimated that approximately 313 million women were infected with HSV-2 in 2017. HSV infections pose significant risks during pregnancy, as both types can be transmitted from mother to neonate, potentially leading to mortality and severe morbidity (Khardr et al., 2018; Allen et al., 2020).

During pregnancy, the placenta acts as a balancing mechanism, providing tolerance to the fetus while protecting against pathogens. Vertical transmission of viruses from mother to fetus is generally restricted due to physical and immunological barriers at the maternal-fetal interface. However, certain viruses can exploit unknown strategies to weaken the placental barrier, leading to severe maternal and fetal health issues, particularly through vertical transmission (Yu et al., 2021). Although the risk of vertical transmission during pregnancy is rare (less than 1% of cases), active lesions or asymptomatic shedding of the virus significantly increases the risk of intrapartum vertical transmission (Aaoye et al., 2021).

HSV acquisition during pregnancy can result in various complications, including spontaneous abortion, prematurity, preterm birth, stillbirth, severe neurologic injuries, and congenital and neonatal herpes (Shi et al., 2018). While the global incidence of HSV-1 is increasing, improved sanitation in some societies has reversed this trend. However, adolescents'encounter with HSV before puberty can increase the risk of post-puberty contagion. Furthermore, approximately 80% of mothers with infected newborns are unaware of their HSV infection history, emphasizing the need for investigating and

HSV infection triggers the production of lifelong antibodies, and immunological laboratory tests can detect previous asymptomatic HSV-1 or HSV-2 infections, as well as identify current infections in symptomatic patients. Given the high genetic similarities between HSV-1 and HSV-2, there is antigen resemblance between the two serotypes. This cross-reactivity means that antibodies produced due to infection with one serotype can extensively cross-react with the other. Determining the specific IgM antibody against HSV-2 and HSV-1 in pregnant women provides a reliable method to estimate the population-based seroprevalence of IgM antibodies among pregnant women. The prevalence of HSV antibodies varies across countries and populations (Alkharsah et al., 2022). This research work aims to investigate the prevalence of herpes simplex virus 1 and 2 IgM antibodies among pregnant women in Port Harcourt, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Port Harcourt, Nigeria. Port Harcourt City is highly congested as it is the only major city in the state. The Greater Port Harcourt urban area spans eight local government areas that include PortHarcourt local government, Okrika, Obio/Akpor, Ikwerre, Oyigbo, Ogu/Bolo, Tai and Eleme local governments respectively. Port Harcourt features a tropical monsoon climate with lengthy and heavy rainy seasons and very short dry seasons (Mbakwem-Anieboet al.,2012). Only the months of December and January truly qualify as dry season months in the city. The harmattan, which climatically influences many cities in West Africa, is less pronounced in Port Harcourt. Port Harcourt's heaviest precipitation occurs during September with an average of 370 mm of rain. December on average is the driest month of the year; with an average rainfall of 20 mm. Temperatures throughout the year in the city are

relatively constant, showing little variation throughout the year. Average temperatures are typically between 25°C-28°C in the city.

2.2. Study Design

A hospital-based cross-sectional survey design was adopted for the present study which seeks to survey the prevalence of HSV 1 and 2 antibodies among pregnant women in Port Harcourt, Nigeria.

2.3 Study population

The target population constituted all the pregnant women attending the antenatal clinic for a routine antenatal check-upatthe University of Port Harcourt Teaching Hospital (UPTH) from January 2019 to November 2019.

2.4 Sample and Sampling Technique

From the study population, a total sample size of 90pregnant women wasrandomly selected and enrolled into the study. The demographic details relevant to the study were obtained from the clinic records as shown in Table 1. The information obtained was stratified as follows; the ages of the pregnant women were grouped into four groups- 20-29 years, 30-39 years and 40-49 years; the marital status was classified as being married or single; the educational status was grouped into primary, secondaryand tertiary education while occupational status was grouped as students, unemployed/housewife, civil servants, trading, artisans and business executives. The gestation periods were classified based on the trimesters the women were in at the time of the study; first, second or third trimesters. Parity was classified as 0, 1-2, 3-4, and 5 and above. The type of family was classified as monogamous or polygamous. The history of abortion was classified as yes or no. The history of STDs was classified as yes or no. Finally, religion was classified as none, Christianity and Islam.

2.5 Sample collection, preparation and storage

A specimen of 5ml venous blood was aseptically drawn from the enrolled subjects into sterile EDTA tubes. The blood was allowed to separate and the plasma aspirated into sterile Eppendorf tubes. Samples were identified with codes to avoid misinterpretation of results. Haemolysed and visibly hyperlipemic samples were discarded as they could generate false results. Samples containing residues of fibrin or heavy particles were also discarded as they could give rise to false results. Sera and plasma were stored at +2°- 8^0C for up to five days after collection. For longer storage periods, samples were stored frozen at -20^OC.

2.6 Serological Analysis

Serum samples were analyzed for HSV-1 & -2 IgM using the ELISA kit manufactured by DIA.PRO Diagnostic Bioprobes (Milano) – Italy. ELISA tests were performed according to the manufacturer's instructions.The HSV-1 & -2 IgM ELISA assay was based on the principle of IgM capture where IgM class antibodies in the samples are first captured by the solid phase coated with anti-hIgM antibody.Results were interpreted according to the manufacturer ‘s guide. The test results were calculated using the mean OD450nm value of the negative control (NC) and a mathematical calculation, to define the following cut-off formulation:Cut-Off = NC + 0.250. The values found were used to interpret the results as the ratio of the sample OD450nm and the cut-

off value (or S/Co) according to the following:**S/Co** <1.0 as Negative, 1.0 – 1.2 as Equivocal and >1.2 as Positive.

2.7 Method of Data Analysis

The data were recorded and analyzed using a Microsoft Excel spreadsheet (Microsoft Corporation). The seroprevalence was calculated as the number of serologically positive samples divided by the total number of samples tested. The Chi-square test was used to determine associations between seropositivity and socio-demographic factors. The level of statistical significance was set at $P \leq 0.05$.

3. RESULTS

3.1 Analysis of the Total Study Population

The total number of pregnant women included in this study was 90. The socio-demographic data for these samples were stratified and shown in Table 1.The age ranges from 20-49 years. The age groups 30 – 39 years constituted the largest populations making up 66.7%, followed by age groups 20-29 years (26.7%) while age groups 40-49 years were the least (6.7%). Married pregnant women predominated the study constituting 98.9% of the sample compared to 1.1% of the population that were single. Based on educational background, 1.1%, 13.3%and 85.6% of the pregnant women were found to have acquired no formal education, secondary education and tertiary education respectively. A lower percentage of 4.4% were artisans, 10.0% were students, 13.3% were unemployedanda larger percentage of 34.4% of them were traders. A higher percentage of 90.0% were Christians and 10.0% had no religion. A higher percentage of 83.3% were married to a monogamous family. Forty-four (48.9%) of them were in their second trimester, 42.2% were in their third trimester and 8.9% were in their first trimester. In terms of parity, a higher percentage of 42.2% fell within 1-2 parity. A higher percentage of 66.7% had no history of abortion and 33.3% had a history of abortion. A higher percentage of 93.3% had no history of STDs and 6.7% had a history of STDs. A lower percentage of 3.3% were HIV seropositive and 96.7% were HIV seronegative (Table 1).

3.2 Prevalence of HSV 1&2 IgM antibody

Of the 90 specimens tested, only 2 (2.2%) were seropositive for HSV 1&2 IgM antibodies while most of the specimens (97.8%) tested seronegative(Table 1).

3.3 Prevalence of HSV 1&2 IgM antibody with age

Table 1 shows the rate of seropositivity of HSV 1&2 IgM antibody according to age. With age, only two groups out of the three age groups were reactive. One (4.7%) in the age group 21-30years and 1 (1.7%) in the age group 31-40 years were found to be seropositive. The other age group$\geq$41 years showed no presence of HSV 1&2 IgM antibody. There was no significant relationship between the age groups and the prevalence of IgM antibodies against HSV 1&2 (χ^2= 4.359, df = 2, p>0.05).

3.3 Prevalence of HSV 1&2 IgM antibody with marital status

As shown in Table 1, a higher seroprevalence of HSV 1&2 IgM antibodyof 2.2% was observed for the pregnant women who were married. Those that were singles showed no presence of

HSV 1&2 IgM antibody. Statistically, marital status was significantly associated with the prevalence of IgM antibody against HSV 1&2 (χ^2= 221.5, df = 1, p<0.05).

Table 1: Prevalence of HSV-1 & -2 IgM and the socio-demographic characteristics of the study participants

Socio-Demographic Characteristics	Groups	No. Tested (%)	No. Positive for HSV IgM
Age	21-30	24(26.7)	1(4.7)
	31-40	60(66.7)	1(1.7)
	41 & above	6(6.7)	0(0.0)
Marital Status	Married	89(98.9)	2(2.2)
	Single	1(1.1)	0(0.0)
Educational Status	Primary	1(1.1)	0(0.0)
	Secondary	12(13.3)	0(0.0)
	Tertiary	77(85.6)	2(2.6)
Occupational Status	Student	9(10.0)	0(0.0)
	Unemployed	12(13.3)	0(0.0)
	Civil servants	29(32.2)	0(0.0)
	Trading	31(34.4)	2(6.5)
	Artisans	4(4.4)	0(0.0)
	Business Executive	5(5.6)	0(0.0)
Religion	Christianity	81(90.0)	2(2.5)
	Others	9(10.0)	0(0.0)
Family Type	Monogamous	75(83.3)	2(2.7)
	Polygamous	15(16.7)	0(0.0)
Gestation Period	1st Trimester	8(8.9)	0(0.0)
	2nd Trimester	44(48.9)	2(4.5)
	3rd Trimester	38(42.2)	0(0.0)
Parity	0	34(37.8)	1(3.0)
	1-2	38(42.2)	1(2.6)
	3-4	18(20.0)	0(0.0)
History of Abortion	Yes	30(33.3)	1(3.3)
	No	60(66.7)	1(1.7)
History of STDs	Yes	6(6.7)	1(16.7)
	No	84(93.3)	1(1.2)
Total		**90(100.0)**	**2(2.2)**

3.4 Prevalence of HSV-1& -2 IgM antibody with educational background

While none of the pregnant women withno formal education, primary and secondary education tested positive, only 2(2.6%) with tertiary education were found to be seropositive as shown in Table 1. The level of education of the pregnant women attending antenatal care had no significant relationship with the prevalence of IgM antibodies against HSV 1&2 (χ^2= 1.653, df = 2, p>0.05).

3.5 Prevalence of HSV-1& -2 IgM antibody with occupational status

Table 1 shows the prevalence rates of HSV 1&2 IgM antibodies according to occupational status which was found to be 6.5% (n=2) for traders. Statistically, there was no significant relationship between occupation and prevalence of IgM antibody against HSV 1&2 (χ^2= 3.264, df = 3, p>0.05).

3.6 Prevalence of HSV 1&2 IgM antibody withreligion

Two (2.5%) of the pregnant women who were seropositive for HSV 1&2 IgM antibodies were Christians as highlighted in Table 1. However, no significant association was found between religion and the prevalence of IgM antibodies against HSV 1&2 (χ^2= 5.297, df = 2, p>0.05).

3.7 Prevalence of HSV 1&2 IgM antibody with family type

Two (2.7%) of the pregnant women who were seropositive for HSV 1&2 IgM antibodies were married to a monogamous family as highlighted in Table 1. However, no significant association was found between the family type and the prevalence of IgM antibodies against HSV 1&2 (χ^2= 5.297, df = 2, p>0.05).

3.8 Prevalence of HSV 1&2 IgM antibody with gestation period

Two (4.5%) of the pregnant women who were seropositive for HSV 1&2 IgM antibodies were in their two trimesters as highlighted in Table 1. However, no significant association was found between the gestation period and the prevalence of IgM antibodies against HSV 1&2 (χ^2= 5.297, df = 2, p>0.05).

3.9 Prevalence of HSV 1&2 IgM antibody with parity

While none of the pregnant women with 3-4 parity tested positive for HSV 1&2 IgM antibody, only 1(3.0%) with nulliparity and 1 (2.6%) with 1-2 parity were found to be seropositive as shown in Table 1. The parity of the pregnant women attending antenatal care had no significant relationship with the prevalence of IgM antibody against HSV 1&2 (χ^2= 1.653, df = 2, p>0.05).

3.10 Prevalence of HSV 1&2 IgM antibody with History of Abortion

A higher prevalence of HSV 1&2 IgM antibody was found in pregnant women with a history of abortion (3.3%) compared to those with no such history (1.7%) as shown in Table 1. The history of abortion in pregnant women attending antenatal care had no significant relationship with the prevalence of IgM antibody against HSV 1&2 (χ^2= 1.653, df = 2, p>0.05).

3.11 Prevalence of HSV 1&2 IgM antibody with History of Abortion

A higher prevalence of HSV 1&2 IgM antibody was found in pregnant women with a history of STDs (16.7%) compared to those with no such history (1.2%) as shown in Table 1. The history of STDs in pregnant women attending antenatal care had no significant relationship with the prevalence of IgM antibodies against HSV 1&2 (χ^2= 1.653, df = 2, p>0.05).

3.12 Prevalence of HSV 1&2 IgM antibody with History of Abortion

A higher prevalence of HSV 1&2 IgM antibody was found in HIV seronegative pregnant women (2.3%) compared to HIV seropositive pregnant women (0.0%) as shown in Table 1. The HIV status of pregnant women attending antenatal care had no significant relationship with the prevalence of IgM antibodies against HSV 1&2 (χ^2= 1.653, df = 2, p>0.05).

4. DISCUSSION

The overall prevalence rate of HSV 1 and 2 IgM among pregnant women was found to be 2.2%, suggesting a relatively low acute HSV infection in the studied population. This result aligns with previous studies reporting similarly low rates. Okonko et al. (2015) reported a prevalence rate of 2.8%, and Oluboyo et al. (2020) reported a rate of 2.2% for HSV-2. However, this result contrasts with the higher prevalence rates reported by Hosseini et al. (2022) at 5.4% for HSV-1 and 92.7% for HSV-2. Alaa et al. (2019) also reported higher rates of 4.4% for HSV-1 and 7.7% for HSV-2, which do not support the present study's findings.

Marital status was found to be statistically significantly associated ($P < 0.05$) with HSV seroprevalence, indicating that married women may be at a higher risk of HSV infection. This association might be attributed to married women being infected by their husbands, leading to acute HSV infections. In contrast, the study did not find significant associations ($P > 0.05$) between HSV seroprevalence and age, education, occupation, and gestation period.

The study observed that pregnant women between the ages of 20-39 years had a higher presence of HSV IgM antibodies, suggesting that this age group is more prone to acute infections. Additionally, the rate of HSV IgM antibodies was higher in married pregnant women compared to single pregnant women. However, this discrepancy may be attributed to the difference in sample sizes, with only 1.1% of the sample being single and 98.9% being married.

HSV seroprevalence rates were higher in pregnant women with tertiary education (2.6%). This finding is consistent with Okonko et al. (2015), who reported a rate of 2.0% for tertiary education. However, Hosseini et al. (2022) reported a higher prevalence rate of 58.8% for secondary education. Pregnant women engaged in trading also exhibited a higher rate of HSV IgM antibodies (6.5%), which aligns with Ugadu et al. (2022) findings of an 8.2% prevalence rate among traders.

Pregnant women in their second trimester showed a higher prevalence rate of HSV 1 and 2 IgM antibodies (4.5%). This suggests that women in their second trimester are at an increased risk of primary or recurrent HSV infections, raising concerns about neonatal transmission. This finding contradicts Amar et al.'s (2015) report of a higher rate of 3.3% in the first trimester, as well as Hosseini et al. (2022) rate of 88.2% in the third trimester and Okonko et al. (2015) rate of 6.8% in the third trimester.

5. CONCLUSION

This study has further confirmed the presence ofherpes simplex virus type 1 & 2 primary or recurrent infection among pregnant womenas reflected by the prevalence obtained for HSV IgM antibody. The study also showed that the prevalence of HSV IgM was significantly associated with marital status.This observation necessitates the need for serological evaluation in all pregnant women for HSV antibodies for early diagnosis and treatment to prevent congenital infections in the fetus.

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