

Serological Evidence of Herpes Simplex Virus Type 2 IgM Antibody among Expectant Mothers Attending a Tertiary Healthcare Facility in Port Harcourt, Nigeria

ABSTRACT

Background: Herpes simplex virus (HSV) is the common viral infection that causes herpes and for which there is now no known cure. The two subtypes, HSV-1 and HSV-2, are known to induce a wide range of symptoms, from acute to long-term. HSV infections typically do not cause any symptoms, yet they can be fatal to expectant mothers and their unborn children. The purpose of this study is to look into the serological evidence of herpes simplex virus 2 IgM antibodies in expectant mothers in a tertiary healthcare facility in Port Harcourt, Rivers State, Nigeria.

Study Design: Ninety expectant mothers who visited the antenatal clinic at a tertiary health facility in Port Harcourt, Nigeria, were randomly analyzed as part of a hospital-based cross-sectional study.

Place and Duration of Study: The University of Port Harcourt Teaching Hospital (UPTH), in Rivers State, Nigeria from January 2019 to November 2019.

Methods: The HSV-2 IgM antibodies in the collected samples were evaluated using the Enzyme-Linked Immunosorbent Assay (ELISA). The method was followed as directed by the manufacturer. The relationship between the infection and sociodemographic variables was evaluated using chi-square analysis.

Results: Of the 90 expectant mothers, two (2.2%) had HSV-2 IgM antibody seropositive, and forty-four (48.1%) had HSV-2 IgM antibody seronegative. There was no significant correlation ($p > 0.05$) found between the sociodemographic characteristics examined and the prevalence of HSV-2 IgM antibodies. Age groups 20–29 years old (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%), and history of STDs (16.7%) were found to have higher prevalence of HSV-2 IgM antibody.

Conclusions: The 2.2% prevalence of HSV-2 IgM antibodies obtained in this study further confirms the occurrence of primary or recurrent HSV-2 infection in pregnant women. According to the study, there was a substantial correlation between a history of sexually transmitted diseases (STDs) and the prevalence of HSV-2 IgM. This finding makes it necessary for all expectant mothers to have a serological assessment for HSV antibodies to detect early and treat congenital infections in the fetus as soon as possible. To manage the viral seropositivity observed in expectant mothers in Port Harcourt, Nigeria, screening for herpes simplex viruses is advised.

Keywords: HSV-2, Prevalence, IgM, ELISA, UPTH

1. INTRODUCTION

The herpes simplex virus (HSV) is the common cause of herpes, a viral infection for which there is currently no known cure (Xu et al., 2019; Nath et al., 2021). HSV-1 and HSV-2 are the two subtypes that are known to induce a wide range of symptoms, from acute to long-term (Nath et al., 2021). Although herpes simplex virus infections are typically asymptomatic, they can be fatal for expectant mothers and their unborn children (Gomaa et al., 2022). It is established that they can lead to chronic infections in people (Hammad & Konje, 2021; Hosseini et al., 2023).

HSV-2 is more commonly connected to genital infections, whereas HSV-1 is mainly linked to orofacial infections. Except in cases where the immune system is weakened, most cases of genital herpes caused by the Herpes simplex virus type 2 are asymptomatic (Oluboyo et al., 2020). Nonetheless, according to Oluboyo et al. (2020), infections may result in hydrops, stillbirth, low infant birth weight, premature delivery, and fetal growth retardation. according to Oluboyo et al. (2020), infections may result in hydrops, stillbirth, low infant birth weight, premature delivery, and fetal growth retardation. With a high incidence of 60–95% in the adult population worldwide, these viruses are known to play a substantial role in the development of sexually transmitted diseases (STIs) (Hosseini et al., 2023). According to Hosseini et al. (2023), the frequency of viral incursions and the host's immune system determine how subclinical or asymptomatic the infections are.

Expectant mothers are the most susceptible individuals to HSV infections since up to 80% of HSV infections are asymptomatic (Whitley & Baines, 2018) and a significant portion of HSV infections have no or moderate symptoms without being detected (Gomaa et al., 2022). Clinical signs including vaginal lesions and herpetic ulcers occur in a small percentage of patients (Looker et al., 2017; Gomaa et al., 2022).

Sexual contact can spread HSV-2, which can cause genital sores. The World Health Organization (WHO) estimates that 13% of people between the ages of 15 and 49 had HSV-2 infection in 2017. According to the WHO report, 313 million women worldwide were thought to have contracted HSV-2 in 2017 (Sert et al., 2020; Wang et al., 2020; Hosseini et al., 2023). Given that both forms of HSV can be passed from mother to child and result in significant morbidity and mortality, HSV infections provide serious dangers to expectant mothers (Khardr et al., 2019; Allen et al., 2020).

Neonatal are the population most severely impacted, as they develop HSV infection from the virus they were exposed to at birth (Ugadu et al., 2022). The placenta serves as a balancing mechanism for the fetus during pregnancy, protecting it from viruses and giving it tolerance. Because of the physical and immunological barriers at the maternal-fetal interface, viruses cannot normally spread vertically from mother to fetus.

Neonates, who get HSV infection after being exposed to the virus at birth, are the population most severely impacted (Ugadu et al., 2022). Preterm delivery, spontaneous abortion, congenital

and neonatal herpes, severe brain damage, and preterm birth are only a few of the issues that can arise from HSV acquisition during pregnancy (Shi et al., 2018; Mahant et al., 2019; Tookey et al., 2020; Hosseini et al., 2023). The placenta functions as a balancing system throughout pregnancy, giving the fetus tolerance while guarding against infections. Physical and immunological barriers at the maternal-fetal interface typically limit the vertical transfer of viruses from mother to fetus. However other viruses can employ unidentified tactics to breach the placental barrier, which can seriously harm both the mother and the fetus, especially when vertical transmission occurs (Yu et al., 2021). Even while there is a very small (less than 1% of cases) risk of vertical transmission during pregnancy, the risk of intrapartum vertical transmission is greatly increased by active lesions or asymptomatic virus shedding (Aaoye et al., 2021).

Although HSV-1 cases are rising globally, certain societies have reversed this trend due to improved cleanliness practices (Hosseini et al., 2023). On the other hand, post-puberty contagion may be more likely in adolescents who have had HSV exposure before puberty (Kahdr et al., 2019; Hosseini et al., 2023). Additionally, according to Hosseini et al. (2023), 80% of mothers whose newborns have been infected do not know their history of HSV infection. This highlights the importance of tracing and monitoring women of childbearing age, particularly before and during pregnancy, as a critical step in preventing the complications of HSV infection, such as neonatal herpes (Woestenberg et al., 2016; Hosseini et al., 2023).

HSV is extremely contagious and can spread through any kind of physical interaction. Furthermore, asymptomatic infections may also result in viral shedding (Nath et al., 2021). Therefore, to stop the infection from spreading, HSV must be detected accurately and early (Nath et al., 2021). Herpes can be identified by looking for antibodies in the blood or by looking for signs of the virus in lesions (Nath et al., 2021). There are several detection methods depending on point-of-care (POC) and laboratory technologies (Nath et al., 2021). Nucleic acid amplification, microscopy, and various biochemical assays are examples of laboratory procedures (Kozel & Burnham-Marusich, 2017; Ray et al., 2017; Li et al., 2019; Muller & Zheng, 2019; Nikolic et al., 2019; Slinger et al., 2019; Nath et al., 2021). On the other hand, microfluidics-based assays that allow for on-the-spot testing are part of POC procedures (Nath et al., 2021).

Lifelong antibodies are produced by HSV infection, and immunological laboratory testing can identify active infections in symptomatic patients as well as past asymptomatic HSV-1 or HSV-2 infections (Nath et al., 2021). Antigen resemblance exists between the two serotypes of HSV-1 and HSV-2 due to their considerable genetic similarity (Alkharash et al., 2022). Consequently, antibodies generated as a result of one serotype infection have a significant cross-reaction with the other serotype (Alkharash et al., 2022). Antibodies generated as a result of infection with one serotype can significantly cross-react with the other due to this cross-reactivity (Alkharash et al., 2022).

An accurate way to determine the population-based seroprevalence of IgM antibodies among expectant mothers is to identify the particular IgM antibody against HSV-2 in these individuals. Different populations and nations have different prevalence rates of HSV antibodies (Alkharash et al., 2022). The purpose of this research study is to examine if expectant mothers in a tertiary

healthcare facility in Port Harcourt, Rivers State, Nigeria, have serological evidence of herpes simplex virus 2 IgM antibodies.

2. MATERIALS AND METHODS

2.1. Study Area

The research was carried out in Nigeria's Port Harcourt. Because it is the only large metropolis in the state, Port Harcourt metropolis is extremely crowded. The Port Harcourt local government, Okrika, Obio/Akpor, Ikwerre, Oyigbo, Ogu/Bolo, Tai, and Eleme local governments are among the eight local government units that make up the Greater Port Harcourt urban region. According to Mbakwem-Aniebo et al. (2012), Port Harcourt has a tropical monsoon climate with long, intense rainy seasons and brief dry ones. In the city, the only months that qualify as the dry season are December and January. In Port Harcourt, the harmattan, which affects the climate of many West African cities, is less noticeable. September is when Port Harcourt experiences the most precipitation, with an average of 370 mm. With 20 mm of rainfall on average, December is the driest month of the year. There is minimal variance in the city's annual temperature, which remains generally consistent. In the city, the average temperature is normally between 25°C and 28°C.

2.2. Study Design

The current study, which aims to assess the serological evidence of HSV-2 antibodies among expectant mothers in Port Harcourt, Nigeria, used a hospital-based cross-sectional survey approach.

2.3 Study population

The target group consisted of all expectant mothers who visited the University of Port Harcourt Teaching Hospital (UPTH) prenatal clinic for a standard prenatal check-up between January and November 2019. Ninety pregnant women were chosen at random and recruited into the study. Table 1 displays the demographic information pertinent to the study that was taken from the clinic records.

2.4 Serological Analysis

The HSV-2 IgM was measured in serum samples using an ELISA kit made in Italy by DIA.PRO Diagnostic Bioprobes (Milano). The ELISA tests were carried out in compliance with the manufacturer's guidelines. The HSV-2 IgM ELISA test was developed using the IgM capture principle, which states that samples containing IgM class antibodies are first captured by a solid phase that has been coated with an anti-IgM antibody. In line with the manufacturer's instructions, the results were interpreted. The following cut-off formulation was created using the test findings, the mean OD450nm value of the negative control (NC), and a mathematical calculation: $\text{Cut-Off} = \text{NC} + 0.250$. The ratio of the sample OD450nm to the cut-off value, or S/Co, was calculated using the values found, and the findings were interpreted as follows: S/Co < 1.0 is considered Negative, 1.0 – 1.2 is considered Equivocal, and > 1.2 is considered Positive.

2.5 Method of Data Analysis

A Microsoft Excel spreadsheet was used to record and evaluate the data (Microsoft Corporation). The number of samples that tested positive for serology divided by the total number of samples examined yielded the seroprevalence. To determine relationships between seropositivity and sociodemographic characteristics, the Chi-square test was employed. $P \leq 0.05$ was designated as the statistical significance threshold.

3. RESULTS

Of the 90 expectant mothers that were analyzed, only 2 (2.2%) had HSV-2 IgM antibodies, while the majority of the specimens (97.8%) had seronegative results (Table 1). Of the three age groups, only two showed signs of reactivity with age. One (1.7%) and one (4.7%) of the age groups 21–30 and 31–40 years, respectively, were found to be seropositive. The second age group, which was above 41, did not have any HSV-2 IgM antibodies. The frequency of IgM antibodies against HSV-2 did not significantly correlate with age groups ($p = 0.7264$).

Married pregnant women had a 2.2% higher seroprevalence of HSV-2 IgM antibodies, as indicated in Table 1. There were no HSV-2 IgM antibodies present in the singles. According to statistics, there was no significant correlation ($p = 0.8795$) between married status and the prevalence of IgM antibodies against HSV-2. Only 2 (2.6%) of the pregnant women with university education were found to be seropositive, as indicated in Table 1, whereas none of the women with no formal education, primary education, or secondary education tested positive. The incidence of IgM antibodies against HSV-2 did not significantly correlate with the educational attainment of the expectant mothers receiving prenatal care ($p = 0.8414$).

According to occupational position, the prevalence rates of HSV-2 IgM antibodies were 6.5% ($n=2$) for dealers. According to statistics, there was no discernible correlation between IgM antibody prevalence against HSV-2 and occupation ($p = 0.5649$). Christians made up two (2.5%) of the pregnant women who tested positive for HSV-2 IgM antibodies. Religion and the incidence of IgM antibodies against HSV-2, however, did not significantly correlate ($p = 0.6336$).

Of the pregnant women seropositive for HSV-2 IgM antibodies, two (2.7%) were married into a monogamous household. Nonetheless, a statistically insignificant correlation was discovered between the kind of family and the occurrence of IgM antibodies against HSV-2 ($p = 0.5224$). Of the pregnant women who tested positive for HSV-2 IgM antibodies, two (4.5%) were in the second trimester of their pregnancy. The gestation time and the incidence of IgM antibodies against HSV-2, however, did not significantly correlate ($p = 0.3433$). Only one (2.6%) and one (4.0%) of the pregnant women with 1-2 parity and nulliparity were found to be seropositive, however none of the women with 3-4 parity tested positive for HSV-2 IgM antibodies. There was no discernible correlation ($p = 0.7713$) between the parity of expectant mothers receiving prenatal care and the incidence of IgM antibodies against HSV-2.

Table 1 also indicates that pregnant women with a history of abortion had a higher prevalence of HSV-2 IgM antibody (3.3%) than pregnant women without a history of abortion (1.7%). The prevalence of IgM antibodies against HSV-2 did not significantly correlate with the history of abortion in pregnant patients receiving prenatal care ($p = 0.6131$). Pregnant women with a

history of sexually transmitted infections had a higher incidence of HSV-2 IgM antibodies (16.7%) than those without a history (1.2%). The prevalence of IgM antibodies against HSV-2 was significantly correlated with the history of STDs in pregnant patients receiving prenatal treatment ($p = 0.013$).

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

Socio-Demographic Characteristics	Groups	No. Tested (%)	No. Positive for HSV-2 IgM (%)	P value
Age	21-30	24(26.7)	1(4.7)	0.7264
	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)	0.8795
	Single	1(1.1)	0(0.0)	
Educational Status	Primary	1(1.1)	0(0.0)	0.8414
	Secondary	12(13.3)	0(0.0)	
	Tertiary	77(85.6)	2(2.6)	
Occupational Status	Student	9(10.0)	0(0.0)	0.5649
	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)	0.6336
	Others	9(10.0)	0(0.0)	
Family Type	Monogamous	75(83.3)	2(2.7)	0.5224
	Polygamous	15(16.7)	0(0.0)	
Gestation Period	1 st Trimester	8(8.9)	0(0.0)	0.3433
	2 nd Trimester	44(48.9)	2(4.5)	
	3 rd Trimester	38(42.2)	0(0.0)	
Parity	0	34(37.8)	1(3.0)	0.7713
	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)	0.6131
	No	60(66.7)	1(1.7)	
History of STDs	Yes	6(6.7)	1(16.7)	0.013
	No	84(93.3)	1(1.2)	
Total		90(100.0)	2(2.2)	

4. DISCUSSION

Pregnant women had an overall prevalence rate of 2.2% for HSV-2 IgM, indicating a low incidence of acute HSV infection in the population under study. This outcome is consistent with earlier research that found comparable low rates. For HSV-2, prevalence rates of 2.8% were reported by Okonko et al. (2015) and 2.2% by Oluboyo et al. (2020). This result, however, is in contrast to the higher prevalence rates for HSV-2—92.7%—reported by Hosseini et al. (2023). The results of the current study are not supported by reports from Alaa et al. (2019) and Gomaa et al. (2022) reporting higher rates of 7.7% and 6.4%, respectively, for recurrent HSV-2 infection among pregnant women in Ismailia City, Egypt.

Research findings varied widely. For example, three distinct research in Iraq (6.2%, 2.2%, and 0.6%, respectively; Mahmood & Najem, 2020; Hasan et al., 2013; Abdulkhaliq et al., 2017), 1.2% in Brazil (Lima et al., 2021), and 2.1% in India (Amar et al., 2015) reported similar results. However, other research, including those conducted in Saudi Arabia (Al-Hakami et al., 2020), Turkey (Özdemir et al., 2011), Sudan (El-Amin et al., 2013), and India (Rajendiran et al., 2021) revealed that none of the individuals under investigation had a recent HSV-2 infection with a negative IgM titre.

According to the study, pregnant women in the 20–39 age range had greater levels of HSV-2 IgM antibodies, which may indicate that they are more vulnerable to acute infections. In the 24-to-33-year-old age range, Hosseini et al. (2023) found a comparable but greater occurrence. Research performed in South Africa has shown similar results (Abbai et al., 2018; Sharma & Ganga, 2022). Similar to other studies, the current study demonstrated a link between HSV infectivity and young ages, which could be explained by greater sex drive in this age range (Hosseini et al., 2023). Furthermore, this study's dominating age range was 31–40 years, as opposed to 20–29 years in earlier research (Mezher et al., 2018; Hosseini et al., 2023).

Compared to unmarried pregnant women, married pregnant women had a greater incidence of HSV IgM antibodies. With only 1.1% of the sample being single and 98.9% being married, the difference in sample sizes could be the cause of this mismatch.

Tertiary-educated pregnant women had higher rates of HSV seroprevalence (2.6%). This result is in line with the findings of Okonko et al. (2015), who reported a 2.0% rate for higher education. Hosseini et al. (2023) did note that pregnant women with a diploma or less had a higher prevalence rate. A larger percentage of HSV-2 IgM antibodies (6.5%) was also present in pregnant women who traded, which is consistent with findings from Ugadu et al. (2022) that indicate an 8.2% prevalence rate among traders.

HSV-2 IgM antibody frequency was greater in second-trimester pregnant women (4.5%). This raises concerns about neonatal transmission since it indicates that women in their second trimester are more likely to get a primary or recurrent HSV infection. This result is in opposition to the greater rate reported by Amar et al. (2015) in the first trimester, the rate of 88.2% in the third trimester reported by Hosseini et al. (2023), and the rate of 6.8% in the third trimester reported by Okonko et al. (2015).

Furthermore, HSV-2 infection is a major cause of genital HSV, and one of the consequences of this infection is abortion (Saheduzzaman et al., 2021; Hosseini et al., 2023). Interestingly, there was no significant correlation found between the prevalence of IgM antibodies against HSV-2

and the history of abortion among pregnant women receiving antenatal care. Pregnant women with a history of abortion had a higher incidence of HSV-2 IgM antibodies than those without a history. This finding runs counter to that of Hosseini et al. (2023), who noted a greater incidence in expectant mothers who had never had an abortion.

Pregnant women who have a history of sexually transmitted infections may be more susceptible to HSV infection since the only factor that was found to be statistically substantially associated with HSV serological evidence was STD history. This correlation may be explained by acute HSV infections in pregnant women brought on by spouses or partners. On the other hand, the research did not discover any noteworthy correlations between the HSV-2 serological evidence and factors such as age, parity, gestation time, education, occupation, family type, marriage status, or history of abortion.

5. CONCLUSION

The prevalence of HSV-2 IgM antibody in pregnant women has been further validated by this study to indicate the existence of primary or recurrent herpes simplex virus infection. The study also revealed a strong correlation between STD history and HSV IgM prevalence. This finding makes it necessary for all expectant mothers to undergo serological evaluation for HSV-2 IgM antibodies to detect early and treat congenital infections in the fetus as soon as possible.

REFERENCES

1. Aaoye, M. O., Olokoba, A. B., Oluwole, A. A. & Olowookere, S. A. (2021): Prevalence and correlates of herpes simplex virus type 1&2 among pregnant women attending a tertiary hospital in Nigeria. *Journal of clinical and experimental microbiology* 22(1) 63-70.
2. Abbai NS, Govender S, Nyirenda M. Herpes simplex virus-2 infections in pregnant women from Durban, South Africa: prevalence, risk factors and co-infection with HIV-1. *South African J Infect Dis.* 2018;33(5):1-7.
3. Abdulkhaliq, R.J., Mohammed, S.T., and Abbas, A.A.H., 2017. Seroprevalence of rubella, cytomegalovirus, herpes, and *Toxoplasma gondii* in recurrent aborted women in Baghdad. *Pak. J. Biotechnol.*, 14(3): 359-364.
4. Alaa, T., Abdel-Rahim, M. E., Azza, B., Isam, M. E., Khalid, A. (2019). Molecular detection of Herpes virus type 1&2 among pregnant women in Khartoum state, Sudan *International Journal of Scientific Research in Science, Engineering and Technology* 4(10) 2394-4099.
5. Al-Hakami, A.M., Paul, E., Al-Abed, F., Alzoani, A.A., Shati, A.A., Assiri, M.I. and Chandramoorthy, H.C. 2020. Prevalence of toxoplasmosis, rubella, cytomegalovirus, and herpes (TORCH) infections among women attending the antenatal care clinic, maternity hospital in Abha, Southwestern Saudi Arabia. *Saudi Med. J.*, 41(7): 757. <https://doi.org/10.15537/smj.2020.7.25121>
6. Alkharash, K. R., Wannan, N. H., Reem Al Dossary, R. A., Obeid, O. E., Hunasemarada, B. C., Qahtani, N. A., El-Badry, A. M. (2022). Prevalence of Herpes simplex virus 1&2 antibodies among individuals screened in a tertiary hospital in Eastern province of Saudi Arabia *Journal of Medicine and Life* 15(10) 1272- 1277.
7. Allen, U. D., Robinson, J. L., Bitim, A. & McDonald, J. (2020). Prevention and management of neonatal herpes simplex virus infection. *Canadian Paediatrics Society Infectious Disease and Immunization Committee*, 9, 310-334.

8. Amar, O.A.O., Bajaj, H.K., Gupta, N., Singla, A., and Masih, H., 2015. Prevalence of herpes simplex virus in pregnant women from Gangetic plain region of Allahabad, India. *Adv. Microbiol.*, 5(6): 404. <https://doi.org/10.4236/aim.2015.56041>
9. El-Amin, E.O., Elamin, O.E., Ahmed, R.A.M., Abdulla, A.K., Elamin, S.E., and Elhaj, H.I. 2013. Sero-prevalence of herpes virus infection in Sudanese pregnant women. *Trop. Med. Health Surg.*, 1: 1-4.
10. Hammad, W. A. B. & Konje. C. (2021). Herpes simplex virus infection in pregnancy – An update. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, 259, 38-45.
11. Hosseini, S. D., Yasaghi, M., Mobasheri, E., Razavi Nikoo, H., & Tabarraei, A. (2023). Molecular and Serological Epidemiology of Herpes Simplex Virus Type 1 and 2 in Pregnant Women of Gorgan City, North East of Iran. *Journal of reproduction & infertility*, 24(1), 35–42. <https://doi.org/10.18502/jri.v24i1.11907>
12. Khardr L, Harfouche M, Omori R, Schwarzer G, Chemaitelly H, Abu-Raddad LJ. The epidemiology of herpes simplex virus type 1 in Asia: systematic review, meta-analyses, and meta-regressions. *Clin Infect Dis.* 2019;68(5):757–72.
13. Kozel, T.R.; Burnham-Marusich, A.R. Point-of-Care Testing for Infectious Diseases: Past, Present, and Future. *J. Clin. Microbiol.* 2017, 55, 2313–2320
14. Li, C.; Li, Y.; Yang, Y.; Wang, J.; Zhu, C.; Tang, S.; Pang, C.; Tang, W.; Cai, Q.; Li, Z.; et al. The Detection and Characterization of Herpes Simplex Virus Type 1 in Confirmed Measles Cases. *Sci. Rep.* 2019, 9, 12785
15. Lima, L.R., Dos-Santos, P.J.D.S., De Almeida, N.A., De Meneses, M.D., Aguiar, S.F., Fernandes, C.A., and de Paula, V.S. 2021. Seroprevalence of human alphaherpesvirus 1 and 2 among pregnant women infected or uninfected with Zika virus from Rio de Janeiro, Brazil. *J. Med. Virol.*, 93(6): 3383-3388. <https://doi.org/10.1002/jmv.26665>
16. Looker, K.J., Magaret, A.S., May, M.T., Turner, K.M.E., Vickerman, P., Newman, L.M., and Gottlieb, S.L. 2017. First estimates of the global and regional incidence of neonatal herpes infection. *Lancet Glob. Health*, 5(3):e300-e309. [https://doi.org/10.1016/S2214-109X\(16\)30362-X](https://doi.org/10.1016/S2214-109X(16)30362-X)
17. Mahant S, Hall M, Schondelmeyer AC, Berry JG, Kimberlin DW, Shah SS. Neonatal herpes simplex virus infection among Medicaid-enrolled children: 2009–2015. *Pediatrics.* 2019;143(4):e20183233.
18. Mahmood, M.N., and Najem, M.H., 2020. Detection of infection by Toxoplasma and Herpes simplex virus 2 among women exposed to abortion in Ninawah province/ Iraq. *Tikrit J. Pure Sci.*, 25(4): 31-35.
19. Mezher MN, Mejbil FA, Hussein HK. Detection of Herpes Simplex-2 Virus in Women with Spontaneous Abortion in Al-Najaf City/Iraq. *J Pharm Sci Res.* 2018;10(1):110–13.
20. Muller, W.J.; Zheng, X. Laboratory Diagnosis of Neonatal Herpes Simplex Virus Infections. *J. Clin. Microbiol.* 2019, 57, e01460-18
21. Nath, P.; Kabir, M.A.; Doust, S.K.; Ray, A. Diagnosis of Herpes Simplex Virus: Laboratory and Point-of-Care Techniques. *Infect. Dis. Rep.* 2021, 13, 518–539. <https://doi.org/10.3390/idr13020049>
22. Nikolic, D.; Kohn, D.; Yen-Lieberman, B.; Procop, G.W. Detection of Herpes Simplex Virus and Varicella-Zoster Virus by Traditional and Multiplex Molecular Methods. *Am. J. Clin. Pathol.* 2019, 151, 122–126

23. Okonko, I. O., Cooney, T. I. (2015). Seropositivity and determinants of immunoglobulin-G (IgG) antibodies against Herpes simplex virus (HSV) types -1 and -2 in pregnant women in Port Harcourt, Nigeria. *Journal of African Health Science* 15(3) 737-747.
24. Okonko, I.O., Cooney, T. I., Okerentugba, P. O. & Frank-Peterside, N. (2015). Serum HSV-1 and -2 IgM in Pregnant Women in Port Harcourt, Nigeria, *Journal of Immunoassay and Immunochemistry*, 36(4): 343-358
25. Oluboyo, B. O., Fayuko, O.P., Akele, R.Y., Akinseye, F. J. & Oluboyo, A. O (2020). Prevalence of herpes simplex virus type 2 antibodies among pregnant women attending federal teaching hospital, Ido-Ekiti, Nigeria. *World Journal of Advance Research and Review* 6(1) 200-206.
26. Ozdemir, M., Kalem, F., Feyzioglu, B., and Baysal, B., 2011. Investigation of viral pathogens during pregnancy in a city region in Turkey. *Anatol. J. Clin. Investig.*, 5: 78-81.
27. Rajendiran, P., Saravanan, N., Ramamurthy, M., Sankar, S., David, N., Nair, A., and Sridharan, G. 2021. Seroprevalence of TORCH- S infections among pregnant woman: A study from Vellore District (South India). *Med. Glob. Surv.*, 12(2): 170-170. https://doi.org/10.4103/jnsbm.JNSBM_103_20
28. Ray, A.; Daloglu, M.U.; Ho, J.; Torres, A.; Mcleod, E.; Ozcan, A. Computational sensing of herpes simplex virus using a cost-effective on-chip microscope. *Sci. Rep.* 2017, 7, 4856.
29. Saheduzzaman M, Sharmin Z, Al Hossain A, Hoque MM, Das MK, Rahman MA. Relation of IgM antibody with Herpes simplex virus type-2 among women with spontaneous abortion and normal delivery. *J Brahmanbaria Med College.* 2021;3(2):14–17.
30. Sert UY, Ozgu-Erdinc AS, Saygan S, Engin-Ustun Y. Herpes simplex infection during pregnancy, results of a tertiary referral centre in Turkey. *Z Geburtshilfe Neonatol.* 2020;224(1):22–5.
31. Sharma P & Ganga RT. A study to assess the prevalence of Herpes simplex type 2 (HSV-2) infections in pregnant women in a tertiary care hospital. *Int J Health Sci.* 2022;6(S1):11938–45.
32. Shi, T. L., Huang, L.J., Zhong, Y.Y., Yang, J. J., Fu, T., Lei, X. F. & Chen, Q. (2018). The risk of herpes simplex virus and human cytomegalovirus infection during pregnancy upon adverse pregnancy outcome. Meta-analysis. *Journal of Clinical Virology.* 104, 48-55.
33. Slinger, R.; Amrud, K.; Sant, N.; Ramotar, K.; Desjardins, M. A comparison of the Quidel Solana HSV 1 + 2/VZV Assay, the Focus Diagnostics Simplex HSV 1 & 2 Direct Assay and the Luminex Aries HSV 1&2 Assay for detection of herpes simplex virus 1 and 2 from swab specimens. *J. Clin. Virol.* 2019, 113, 35–38
34. Tookey PA, Mahdavi S, Peckham CS. Surveillance of neonatal herpes in the British Isles 2004–2006. *F1000Research*, 2020;9(163):163.
35. Ugadu, I. O., Nwuzo, A. C., Ugbo, E. N., Njoku, O. M., Njoku, C. J. & Ayomoh, E. E. (2022). Seroprevalence of Herpes Simplex Virus Type-1 and 2 among Pregnant Women Attending Antenatal Care at Mile Four Hospital, Abakaliki, Ebonyi State, Nigeria. *Nigerian Journal of Microbiology*, 36(1): 6190 – 6197.
36. Wang C, Zhou YH, Yang HX, Poon LC. Intrauterine vertical transmission of SARS-CoV-2: what we know so far. *Ultrasound Obstet Gynecol.* 2020;55(6):724–5.
37. Whitley, R., and Baines, J., 2018. Clinical management of herpes simplex virus infections: Past, present, and future. *F1000Res.*, 7. <https://doi.org/10.12688/f1000research.16157.1>

38. Woestenberg PJ, Tjhi JH, de Melker HE, van der Klis FR, van Bergen JE, van der Sande MA, et al. Herpes simplex virus type 1 and type 2 in the Netherlands: seroprevalence, risk factors and changes during a 12-year period. *BMC Infect Dis.* 2016;16:364.
39. Xu, X.; Zhang, Y.; Li, Q. Characteristics of herpes simplex virus infection and pathogenesis suggest a strategy for vaccine development. *Rev. Med. Virol.* 2019, 29, e2054
40. Yu, W., Hu, X. & Cao, B. (2021). Viral infections during pregnancy: The big challenge threatening maternal and fetal health. *Journal of Maternal-Fetal Medicine* 4(1) 72-86.

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