Seroprevalence of Herpes Simplex Virus Type-1 and 2 among Pregnant Women Attending Antenatal Care at Mile Four Hospital, Abakaliki, Ebonyi State, Nigeria

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Abstract: Herpes simplex virus type (HSV-1 and 2) infections are one of the major sexually transmitted infections among pregnant women worldwide. The aim of this study was to assess seroprevalence of herpes simplex virus type-1 and 2 among pregnant women attending antenatal care at mile four Hospital Abakaliki Nigeria. Blood samples were aseptically collected from 88 pregnant women who gave informed consent and completed a self-administered questionnaire. Blood samples were screened for HSV-1 and 2 specific IgG antibodies using an Enzyme Linked Immunosorbent Assay (ELISA) test kit. The haematological indices were determined using Mythic 22 machine method. The Chi-square test was used to determine associations between seropositivity and socio-demographic variables. The overall prevalence of HVS types was 55(62.50%), thus 36(40.91%) were positive for HSV-1, 19(21.59%) for HSV-2 and 17(19.32%) for HSV 1 and 2 co-infected. The prevalence of HSV type 1 and 2 were observed to be higher among pregnant women within the age of 24-29, (18.18%);(11.36%), within their third trimester, (23.86%);(12.50%), and zero parity, (15.91%);(10.23%) respectively. There were no significant changes in the haematological parameters tested in all age groups except for the pack cell volume which was lower than the normal range as a result of the pregnancy. Statistical analysis showed that prevalence of HSV-1 and 2 were significantly associated with age, occupation, trimester, gravidity and parity ($P \leq 0.05$). This study observed the potential public health impact of HSV-1 and 2 and co-infection among pregnant women in Abakaliki Nigeria and especially considering the possible risk of congenital transmission, thus there is need for frequent educating the pregnant women about the danger of HSV.

Key words: Seroprevalence, Herpes simplex virus type-1 and 2, Pregnant women, Abakaliki.

INTRODUCTION

Herpes simplex virus 1 and 2 (HSV-1 and-2), otherwise called human herpes virus 1 and 2 (HHV-1 and HHV-2), are two viruses from the herpes virus family, *Herpesviridae* that infect people (Alzahrani *et al*., 2013). Herpes simplex infection types 1 and 2 are noteworthy human pathogens causing clinically undefined facial and genital sores (Okonko *et al.,* 2015). HSV-1 is the typical reason for orolabial infection (gingivostomatitis or herpes labialis), while HSV-2 is the significant reason for genital infection. They can spread when an infected individual is producing and shedding the virus by inoculation, kissing and sex. However, either virus can infect either location (Alzahrani *et al*., 2013).

The frequency of herpes simplex infection (HSV) has been expanding relentlessly in ongoing decades, and worries about prenatal HSV infection are developing among women of reproductive age as a result of the danger of transmission of the infection to their children during pregnancy, with devastating outcomes to the fetus (Nizami, 2004). Information from a few forthcoming preliminaries has demonstrated that the significant hazard to the fetus results from primary genital HSV infection. More than 95 % of infected infants are conceived by women who are ignorant that they have genital herpes (Looker *et al.,* 2008).

The most seriously influenced populace is neonates, who acquire HSV infection after exposure to the virus during birth.

The most destroying direct effect of maternal genital herpes is the neonatal infection (Kalu, 2014). Genital herpes in pregnancy is related with unmistakable dangers of meningoencephalitis and scattered herpes in the neonate. Be that as it may, these contaminations bring about lasting neurological harm for some newborn children regardless of suitable antiviral treatment (Looker *et al.*, 2008). Past examinations have recommended that genital HSV infection acquired during pregnancy is related with preterm labour intrauterine growth retardation, and spontaneous abortion (Nizami**,** 2004). Information on the prevalence of HSV 1 and 2 among pregnant women in Abakaliki is lacking and needs to be studied to get the recent data. Thus, this research is aimed at assessing the seroprevalence of herpes simplex virus type-1 and 2 among pregnant women attending antenatal care at mile four hospital, Abakaliki Nigeria.

MATERIALS AND METHODS

Study Area and Population

The area of study for this research was Mile Four Hospital, Abakaliki Ebonyi State, Nigeria. Abakaliki, Ebonyi State is situated 6^{o} 20^{o} N longitude, 8^{o} 6^{o} E latitude of the equator. The study population was 88 selected pregnant women accessing antenatal care at Mile Four Hospital, Abakaliki who gave informed consent. The socio-demographic information of the participants was obtained by a structured questionnaire prior to sample collection.

Ethical Approval

Ethical Approval for this study was obtained from the Ethical Review Committee on Human Research, Mile Four Hospital, Abakaliki Ebonyi State Nigeria with (Reference Number: RE/M4H/28/19).

Sample Collection

After obtaining a written informed consent from each participant, 5 ml of blood samples were collected by vein puncture into a sterile ethylene diamine tetraacetic acid (EDTA) tube, allowed to clot for 30 minutes and centrifuged at 3000 rpm for 5 minutes. The samples were securely and clearly labelled with codes corresponding to a subject name to prevent mismatch and misinterpretation of result. All the sera were transferred into a new labelled cryovials and stored at -20°C until assay for the ELISA analysis. Samples were transported to the Department of Human Virology, Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development Idu, Abuja Nigeria in cold chain (ice packs) for the determination of HSV-1 and 2 IgG antibodies.

Enzyme Linked Immunosorbent Assay (ELISA) Technique

Plasma from each samples were assayed for Herpes simplex virus-1 and 2 using a commercially available ELISA kit according to the manufacturer's specifications (Kalu, 2014). Two wells were left as negative control, two wells as positive control and one well empty as blank control. Corresponding samples were numbered on the microtitre plate. Fifty (50 µl) microlitre of Negative control and positive control were added into the negative and positive control wells respectively**.** Forty (40 µl) microlitre of sample dilution buffer were added in the sample well and 10 µl of sample to the sample well. Wells were mixed with gentle shaking. The wells were sealed with closure plate membrane. It was incubated for 30 minutes at 37 ^{o}C. The concentrated washing buffer was diluted with distilled water. Closure plate membrane was carefully peeled off, aspirated and refilled with wash solution. The wash solution was discarded after washing for 30 seconds. The washing procedure was repeated for 5 times after which 50 µl HRP-conjugate reagent was added to each well except the blank control well. It was incubated for 30 minutes at 37 ^{o}C and wash solution was refilled and discarded after resting. Washing procedure was repeated for 5 times after which 50 µl chromogen A and 50 µl Chromogen B were added to each well and mixed well with gentle shaking.

Incubated at 37 ^{o}C for 15 minutes (light was avoided during colouring). Fifty (50) µl of stop solution were added to each well to terminate the reaction. (The colour in the well changed from blue to yellow). Absorbance optical density at 450 nm was read using a micro titre plate reader. The value of the blank control well was set as zero. Assay was carried out in 15 minutes after adding stop solution. The test results were calculated by means of the mean optical density 450 nm value of the negative control (NC) and a mathematical calculation, in order to define the following cut-off formulation: Cut-Off = Average value of negative control + 0.15 (Kalu, 2014; Okonko *et al.*, 2015).

Haematological Analysis

The following haematological parameters were ascertained using Mythic 22 OT (Murex diagnostic Technologies, Philippine): White blood cell count, differential count which include neutrophils, lymphocytes, monocytes, eosinophils, basophils, pack cell volume (PCV) and platelets count (Osazuwa *et al*., 2016). Exactly 2 ml of blood was collected into EDTA container. The sample was mixed very well using sample roller mixer while the rollermixer was missing the sample. The mythic 22 OT machine was switched on and calibrated. The sample container was opened and 30 µl of sample was dispensed through the flow cell**.** After two minutes the results were displayed on the screen and results were printed out

Statistical Analysis

The data were recorded in and analyzed using Microsoft Excel spreadsheet (Microsoft Corporation). Chi-square test was used to determine associations between seropositivity and socio-demographic variables. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

Eighty eight (88) pregnant women were screened for HSV-1 and 2 IgG antibodies, out of which 36(40.91%) were positive for HSV-1, 19(21.59%) were positive for HSV-2 and 17(19.32%) were HSV 1 and 2 co-infected, while the overall prevalence of HSV types was 55(62.50%) (Figure 1). The age group between 24- 29 years had the most positive results 16(18.18 %) for HSV-1, 10(11.36 %) for HSV-2, 8(9.09 %) for HSV-1 and 2 co-infected. However, 36-41 years had the least positive result 3(3.41 %) for HSV-1, 1(1.14 %) for HSV-2 and HSV-1&2 co-infected respectively. There was significance difference between the age groups and HSV status of the subjects. Based on occupation, traders had the highest prevalence 15(17.65 %) for HSV-1, 7(8.24 %) for HSV-2, followed by the artisans 9(10.59 %) for HSV-1, 5(5.88 %) for HSV-2 while the farmers and the House wife had the least prevalence of 2.35 % each (Table 1).

In this study, women in the first trimester recorded zero percent prevalence for HSV-1, HSV-2 and HSV-1 and 2 co infection. Women in the second trimester recorded 10.22 % prevalence for HSV-1 and 7.95 % prevalence for HSV-2 and 6.82 % for HSV-1 and 2 co infection. Women in the third trimester recorded 23.86 % prevalence for HSV-1 and 12.50 % prevalence for both HSV-2 and HSV-1 and 2 co infection. Those pregnant women within the first month of their gravidity recorded the highest prevalence 10(11.36 %) for HSV-1, 7(7.95 %) for HSV-2 and HSV-1&2 co-infected respectively, while those whose gravidity was three months recorded the zero prevalence of for HSV-2 and HSV-1&2 co-infected. Subjects with the zero number of births had the highest prevalence 14(15.91 %) for HSV-1, 9(10.23 %) for HSV-2 and HSV-1&2 co-infected respectively. Those with two number of birth had zero prevalence for HSV-2 and HSV-1&2 co-infected respectively (Table 2). Pregnant women between the age of 18- 23 years old, 24-29 years, 30-35 years and 36-41 years who were negative to HSV-1 and 2 were compared with those who were positive for HSV-1, HSV-2 and HSV-1 and 2 co infection with relation to their level of white blood cell, lymphocyte, monocyte, neutrophile, esinophile, basophiles, pack cell volume and platelets.

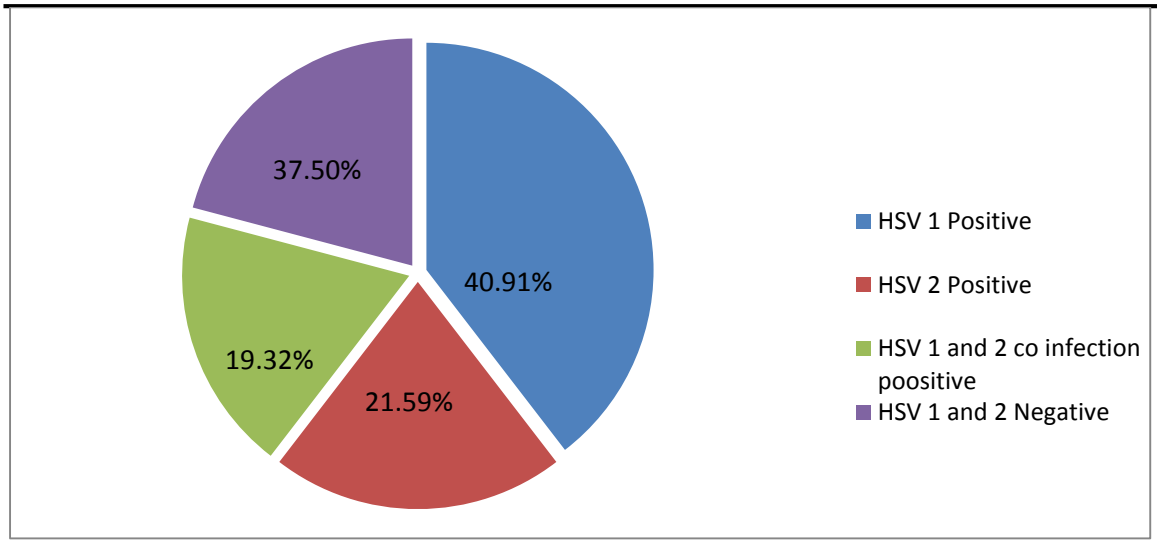

Figure 1: Prevalence rate of Herpes Simplex Virus among pregnant women attending antenatal clinic in Mile Four Hospital, Abakaliki

Table 1: Prevalence of HSV-1, HSV-2 and HSV-1&2 co-infected with relation to demographic distribution of the pregnant women

Demography	No. screened	HSV-1 +ve(%)	HSV-2 +ve(%)	HSV-1&2 +ve(%)
Age Distribution				
18 -23	14	6 (6.82)	2 (2.27)	2 (2.27)
24 -29	40	16 (18.18)	10 (11.36)	8 (9.09)
30 -35	24	11 (12.50)	6 (6.82)	6 (6.82)
36-41	10	3 (3.41)	1 (1.14)	1 (1.14)
Total	**88**	**36 (40.91)**	**19 (21.59)**	**17 (19.32)**
Occupation Distribution				
Student	8	3 (3.53)	0 (0.00)	0 (0.00)
C/servant	23	5 (5.88)	4 (4.71)	3 (3.53)
Trader	35	15 (17.65)	7 (8.24)	7 (8.24)
Farmer	2	2 (2.35)	2 (2.35)	2 (2.35)
H/wife	5	2 (2.35)	1 (1.18)	1 (1.18)
Artisans	15	9 (10.59)	5 (5.88)	4 (4.71)
Total	**88**	**36 (40.91)**	**19 (21.59)**	**17 (19.32)**

Table 2: Frequency of HSV-1, HSV-2 and HSV-1&2 co-infected with relation to trimesters and gravidity among pregnant women

Frequency	No. screened	HSV-1 +ve(%)	HSV-2 +ve(%)	HSV-1&2 +ve(%)
Trimester				
1	5	0 (0.00)	0 (0.00)	0 (0.00)
2	30	15 (17.05)	8 (9.09)	6 (6.82)
3	53	21 (23.86)	11 (12.50)	11 (12.50)
Total	**88**	**36 (40.91)**	**19 (21.59)**	**17 (19.32)**
Gravidity				
1	32	10 (11.36)	7 (7.95)	7 (7.95)
2	17	7 (7.95)	3 (3.41)	3 (3.41)
3	16	5 (5.68)	0 (0.00)	0 (0.00)
4	9	4 (4.55)	4 (4.55)	3 (3.41)
5	6	5 (5.68)	2 (2.27)	2 (2.27)
6	4	3 (3.41)	1 (1.14)	1 (1.14)
7	4	2 (2.27)	2 (2.27)	1 (1.14)
Total	**88**	**36 (40.91)**	**19 (21.59)**	**17 (19.32)**
Parity(Number of births)				
0	32	14 (15.91)	9 (10.23)	9 (10.23)
1	19	8 (9.09)	4 (4.55)	3 (3.41)
2	13	2 (2.27)	0 (0.00)	0 (0.00)
3	10	5 (5.68)	3 (3.41)	2 (2.27)
4	7	3 (3.41)	1 (1.14)	1 (1.14)
5	7	4 (4.55)	2 (2.27)	2 (2.27)
Total	**88**	**36 (40.91)**	**19 (21.59)**	**17 (19.32)**

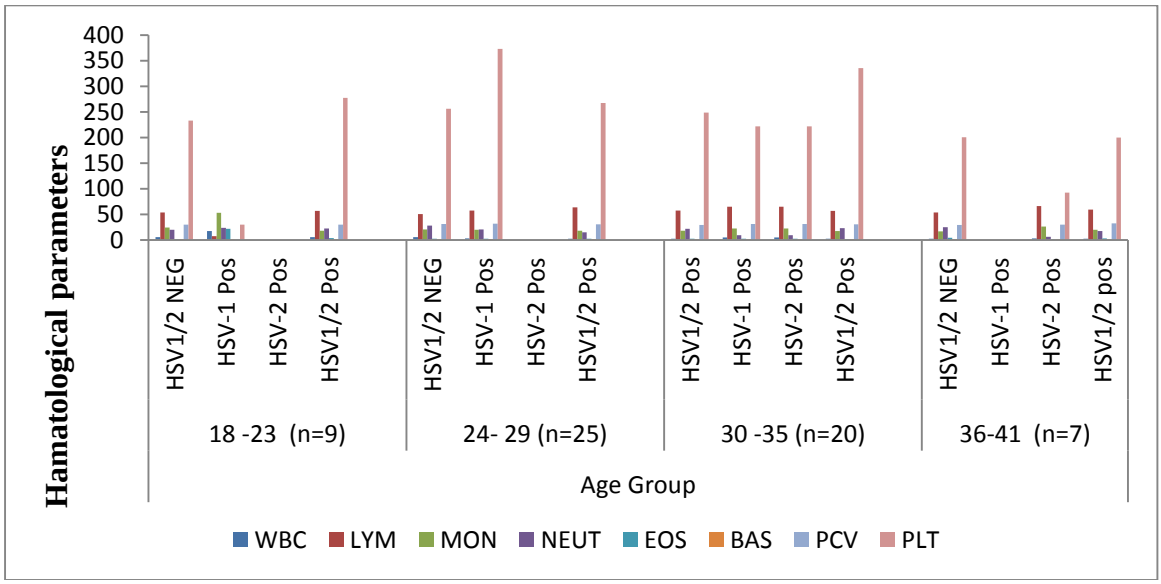

Figure 2: Impact of HSV on haematological parameters with relation to the immunological parameters of pregnant women

Keys: WBC= White Blood Cell, LYM= Lymphocyte, MON= Monocyte, NEU= Neutrophils, EOS= Eosinophils, BAS= Basophil, PCV= Pack Cell Volume, PLT= Platelet

DISCUSSION

In this study, the overall seroprevalence of herpes simplex virus type was observed to be 55(62.50%) and the HSV -1 infection was found to be the highly prevalence with frequency of 40.91 %. This agrees with 48 % and 34 % percentage prevalence recorded by Beydoun *et al.* (2010) and Nizami *et al.* (2004) respectively and disagrees with 96.6 % prevalence recorded by Kalu *et al.* (2014) and 96.4 % reported by Uteno *et al.* (2018). The differences could be because of the study group. The prevalence of 21.59 % for HSV-2 recorded in this study is relatively high especially when compared to similar study 4.2 % reported in Turkey by Mehmet *et al.* (2016) and Hashido *et al.* (1998) in their study on pregnant women in Japan reported the seroprevalence of HSV-2 as 7.0 %. Our research finding is also in line with seroprevalence findings of 20.7 % by Yahya *et al.* (2008) and 26 % by Diawara *et al.* (2008) found among pregnant women in Tanzania and Senegal, all of sub-Saharan Africa region. Prevalence of 42.4 % was found among female sex workers as reported by Chukwu *et al.* (2019) and it akins with the observation of the present study which reported 40.91%. Agabi *et al.* (2010) reported 87 % positivity for HSV 2 which higher than the report of this study. The differences between the two studies could be explained by the differences in the sexual behaviours among subjects screened.HSV-1 and 2 co infection of 18.68% as reported was in consonance with the work of Beydoun *et al.* (2010) which recorded 12 % prevalence for HSV1 and 2 co infection. It is believed that the high prevalence of HSV infection observed in this study may be explained in part by the low level of awareness of the prevalence and risk factors of this viral infection and lack of sensitization of the general population to avail themselves for the HSV screening tests.

Findings from this study showed that age may be a determinant factor for one to have HSV. Highest prevalence of HVS was recorded among pregnant women within the age of 24-29 years (18.18%) and least in older age group 36-41 years (3.41 %). This agrees with the work of Oti *et al.* (2017) who reported the highest prevalence of HVS in pregnant women with aged less than 20 years to be 28.1 % and least in older age groups 31- 40 years (19.3 %). It disagrees with Amar *et al.* (2015) which reported highest prevalence of 40.1 % in older age groups and least 20.2 % prevalence in younger age groups. Younger participants were more likely to acquire HSV-1 infections compared with older participants and less likely to develop recognized disease. Differences in HSV type may reflect differences in sexual practices by age, with younger participants more likely to engage in oral than vaginal sex (Nizami, 2004).

This study also observed that pregnant women within their third trimester had higher prevalence of HSV 23.86 % and least among subjects in their first trimester with zero percent prevalence. This report is in consonance with the outcome of Amar *et al. (*2015) in India who reported 26.0% among subjects in their third trimester and 1 % among subjects in their first trimester but disagreed with the work of Idress and Elhag, 2015 in Sudan who recorded highest prevalence among subjects in their second trimester 25.0% and least among subjects in their third trimester (2.0%) prevalence. With regards to occupation, traders had the highest prevalence rate (17.65%) followed by artisans (10.59 %). This finding is in disagreement with the work of Ibrahim *et al.* (2015) who reported that farmers had the highest prevalence (22.70%), followed by labourers who recorded 20.50 %. Oti *et al.* (2017) reported that artisans had the highest seroprevalence (90.0%), followed by housewives (80.6%), students (67.8%), civil servants (61.9%) and farmers (58.8%).Gravidity in the study had a significant impact on the individuals being infected with HSV infection.

Women whose gravidity was one month recorded the most prevalence of 11.36 % and those whose gravidity was greater than or equal to two had 2.27 % prevalence. This is in agreement with Osazuwa *et al.* (2016) who

reported gravidity one month as 10.1 % and those whose gravidity was greater than or equal to twoas 2.90 % prevalence and in disagreement with Okonko *et al.* (2015) who reported more prevalence among subjects whose gravidity was greater than or equal to two as 24 % and whose gravidity was one month as 10 %.

The HSV infection was highest among women with zero parity 15.91% prevalence and least with women with more parity 2.27% prevalence. This disagrees with the findings of Oti *et al.*, 2017 who reported that infection was highest with prevalence of 38.5 % among those pregnant women whose parity was three to four and least among women with parity more than four with 20.0% prevalence. The danger of mother to child spread of HSV is utmost, if a seronegative female acquire primary or secondary genital herpes close to the delivery, proceeding to antibody development.

White blood cell count, lymphocytes count, monocytes count, basophils count, platelet count, eosinophils count in all age groups, all fell within the normal range except for the pack cell volume which was lower than the normal range and this could be as a result of the pregnancy. This is in agreement with the work done in Bangladesh by Mohammad *et al.* (2018) who reported that WBC, lymphocytes and RBS levels were found with moderately increased values between both the sexes.

In conclusion, seroprevalence of HSV-1 infection was observed to be 40.91%, HSV-2 was 21.59%, HSV- 1 and 2 co infection was 19.32 %. There was significance difference between the age groups and HSV status of the subjects at P≤ 0.05. There were obvious changes in the haematological indices of the subjects who were seropositive to herpes simplex virus. Occupation, trimester, gravidity and parity play a significant role in transmission of HSV-1 and 2 among pregnant women. There is need for frequent education for the antenatal women about the health impact of HSV types, since this can be transmitted from mother to child and also capable of causing stillbirth.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

Agabi, Y. A., Banwat, E. B., Mawak, J. D., Lar, P. M., Dashe, N., Dashen, M, M., Adoga, M. P., Agabi, F. Y. and Zakari, H. (2010). Seroprevalence of Herpes Simplex Virus Type-2 among Patients Attending the Sexually Transmitted Infections Clinic in Jos, Nigeria. *Journal of Infectious Disease*, **4**: 572-576.

Alzahrani, A. J., Obeid1, O. E., Almulhim, A. A., Obgyne, A. B., Jordanian, B.O., Awari, A. T., Feryal, A. A. and Hatim, K. A. (2013). Prevalence of Herpes Simplex Virus Antibodies. *International Journal of Medicine and Biomedical Research*, **6**: 362-431.

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. *Journal of Advances in Microbiology*, **5:** 404- 408.

Beydoun, H. A., Dail, J., Ugwu, B., Boueiz, A. and Beydoun, M. A. (2010). Socio-demographic and Behavioural Correlates of Herpes Simplex Virus type-1 and 2 infections in the USA. *International Journal of Infectious Disease*, **14:** 154-160.

Chukwu, C. O. O., Reuben, R. C., Opara, R. C., Chukwu, I. D. and Akintola. R. I**.** (2019). Seroprevalence of Herpes Simplex Virus Type 2 among Female Sex Workers in Federal Capital Territory, Nigeria. *The Tropical Journal of Health Sciences*, **26:** 2-5.

Diawara, S., Kane, C. T., Legoff, J., Gaye A. G. and Mboup, S. (2008). Low Seroprevalence of Herpes Simplex Virus type 2 among Pregnant Women in Senegal. *International Journal of Sexually Transmitted Disease and AIDS*.**3**: 159-160.

Hashido, M., Lee, F. K. and Nahmias, M. (1998). An Epidemiologic Study of

Herpes Simplex Virus type-1 and 2 Infection in Japan based on Type Specific Serological Assays. *Journal of Epidemiology Infection*, **120:** 179-86.

Ibrahim, A., Adamu, I. A. and Abubakar, H. (2015). Seroepidemiology of Herpes Simplex Virus Type-2 (HSV-2) among Incarcerated Population of Potiskum Medium Security Prison Postiskum, Yobe State: A Study of Prevalence and Associated Risk Factors. *International journal of current Microbiology and Applied Science*, **4**(3): 632-637.

Idress, H. E. A. and Elhag, W. I. (2015).Seroepidemiology of Herpes Simplex Virus Type-2 among Pregnant Women attending Ibrahim Malik Teaching Hospital, Khartoun State, Sudan. *American Journal of Research Communication*, **3**(11): 149-160.

Kalu, E. I. (2014). Seroprevalence of Herpes Simplex Virus Infections among Pregnant Women Attending Antenatal Clinic in Benin, Nigeria. *International Journal of Tropical Disease and Health*, **4**(1): 70-81.

Looker, K. J., Garnett, G. P. and Schmid, G. P. (2008).An Estimate of the Global Prevalence and Incidence of Herpes Simplex Virus Type-2 Infection. *Bull World Health Organisation*, **86:** 737-816.

Mehmet, O., Tasbent, F. E., Terzi, H. A., Cetinkol, Y., Uysal, E. B., Asik, G., Parlak, M., Gulhan, B. and Ciftci, I. (2016). Seroprevalence of Major Viral Pathogens during Pregnancy: A Multicenter Study in Turkey. *Journal of Advances in Clinical and Medical Microbiology*, **1:** 1-7.

Mohammad, A., Akram, H. M., Najmul, Hasan, Omar, F., Farha, M . J., Mohammod, J. I. and Mohammad S. K. (2018). The Effect of Herpes Simplex Virus Infection on Different Blood Parameters: a Transverse Study**.** *Journal of Dental and Medical Sciences*, **17**: 2279-0861.

Nizami, D. (2004). Serological Evaluation of HSV-1 and HSV-2 Infection in Pregnancy. *Turkey Journal of Medical Science*, **34**: 97-101.

Okonko, I. O. and Cookey, 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.

Okonko, I. O., Cookey, T. I., Okerentugba, P. O. and Frank-Peterside, N. (2015). Serum HSV-1 and 2 IgM in Pregnant Women in Port Harcourt, Nigeria. *Journal of Immunoassay and Immunochemistry*, **36:** 343–358.

Osazuwa, F., Eghafona, N. O. and Okoji, R. (2016).Cellular Immune Status and Inflammatory Markers in Herpes Simplex Virus Type-2 Sero-Positve Pregnant Women. *American Journal of Biomedical Sciences*, **2**: 66-185.

Oti, V. B., Usman, B. A., Pennap, G. R. and Eno-Ibanga, C. K. (2017). Seroprevalence of Herpes Simplex Virus Type-2 (HSV-2) among Pregnant Women Accessing Antenatal Care in a Tertiary Healthcare Facility in Central Nigeria. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 1(**4**): 1-6.

Uteno, I. D., Francis, E. O., Babatunde, I. G., Gregory, A. U., Alaba, M. E., Dimkpa, U., Ifeanyichukwu, K. O., Oyarazi, A. M., Nasiru, O. A. and Nwaribunwanne, E. U. (2018). Seroprevalence, Type – Specific of Herpes Simplex Virus and Associated Risk Factors among Women of Child Bearing Age in Kogi State, Nigeria. *Journal of Infectious Diseases*, **14:** 60–65.

Yahya, M., Evien-Olsen, K., Matee, B., Fylkenes, M. I. and Haans, L. (2008). HIV-1, HSV-2 and Syphilis among Pregnant Women in a Rural Area of Tanzania: Prevalence and Risk Factors. *Journal of Infectious Disease*, **8:** 75-80.