



Research Article

Sero-Prevalence of Herpes Simplex Virus Type 2 and Human Immunodeficiency Virus Co-Infection Among Pregnant in Jalingo, Taraba State, Nigeria

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Abstract

Herpes simplex virus type 2 (HSV-2) and Human Immunodeficiency Virus (HIV) co-infection presents a significant public health challenge, particularly among pregnant women, due to their synergistic interaction and potential for adverse maternal and neonatal outcomes. This study determined the seroprevalence of HSV-2 and HIV co-infection among pregnant women in Jalingo, Taraba State, Nigeria, and identified associated socio-demographic and obstetric factors. A cross-sectional study was conducted among 227 pregnant women randomly recruited from Federal Medical Centre and the Specialist Hospital, Jalingo. Blood samples were analyzed for HSV-2 IgG antibodies using ELISA and HIV antibodies using rapid diagnostic tests. Molecular confirmation of HSV-2 was achieved through PCR targeting the UL41 gene, with amplicons resolved by agarose gel electrophoresis. Socio-demographic data were collected using structured questionnaires. HSV-2 seroprevalence was 7.0% (16/227), while HIV prevalence was 16.3% (37/227). Co-infection was observed in 2.2% (5/227) of participants. Among HSV-2-positive women, 31.3% were also HIV-positive, while 13.5% of HIV-positive women tested positive for HSV-2. The highest co-infection rate occurred in the 25–34 years age group (5.62%). Women from polygamous families demonstrated significantly higher co-infection rates (19.23%) compared to monogamous families (0%) ($\chi^2 = 26.42$, $p < 0.001$). Unemployment was associated with elevated HIV prevalence (21.31%). PCR confirmed HSV-2 DNA through amplification of the 192-base pair UL41 gene fragment in all five randomly selected seropositive samples. This study documents moderate HSV-2 and substantial HIV prevalence among pregnant women in Jalingo, with socio-demographic factors significantly influencing infection risk. The findings advocate for integrating routine HSV-2 screening into antenatal care programs alongside universal HIV testing. Targeted interventions addressing vulnerabilities of high-risk groups, particularly young women, those from polygamous families, and the unemployed, are essential to mitigate mother-to-child transmission and improve maternal and neonatal health outcomes in northeastern Nigeria.

Keywords: *Herpes simplex virus type 2, Human Immunodeficiency Virus, co-infection, seroprevalence, pregnant women, Nigeria.*

Introduction

Belonging to the *Herpesviridae* family, herpes simplex virus type 2 (HSV-2) is an enveloped pathogen carrying a linear double-stranded DNA genome. As the primary etiological agent behind genital ulcer disease worldwide, it predominantly establishes infections in the anogenital tract, though it retains the capacity to affect other anatomical sites (Tayyar and Ho, 2023). Recognized as one of the most widespread sexually transmitted infections globally, HSV-2 manifests symptomatically as recurrent, painful genital sores; however, a striking 80% to 90% of seropositive individuals remain unaware of their infection due to clinically silent viral reactivation and shedding (Looker *et al.*, 2020). This high rate of

asymptomatic carriage poses a significant public health challenge, as unchecked transmission within communities can drive prevalence rates to persistently elevated levels unless effective interventions are implemented (Stone *et al.*, 2023).

A complex, bidirectional interplay exists between HSV-2 and HIV. Notably, the prevalence of these two infections is directly correlated, with HSV-2 co-infection accelerating HIV replication and augmenting genital shedding in those already infected, thereby amplifying their infectiousness (Tu *et al.*, 2022). Furthermore, the presence of HSV-2 elevates both plasma and genital HIV viral loads (Domercant *et al.*, 2017), while the accompanying mucosal barrier disruption and local T-cell activation create a favorable environment for HIV acquisition and onward transmission (de Jong, 2010). Given its ulcerogenic nature and widespread prevalence, HSV-2 is frequently cited as a significant biological co-factor in the HIV epidemic (Shahnazaryan, 2020).

Geographically, the burden of HSV-2/HIV co-infection varies considerably, with rates ranging from 30% to 70% in Europe and reaching as high as 50% to 90% in various African cohorts (Kalu, 2014). Numerous epidemiological investigations have confirmed a robust association between the two viruses; a landmark meta-analysis by Freeman *et al.* (2006) estimated a 3.1-fold increase in the relative risk of HIV-1 acquisition among HSV-2-seropositive individuals (Kenyon *et al.*, 2013). Studies across both high-risk and general populations consistently show substantial incidence, such as the 41% co-infection prevalence documented among South African women (Abbai *et al.*, 2015). Key risk determinants linked to HSV-2 acquisition include advancing age, early menarche, lower educational status, and a greater number of sexual partners (Kirakoya *et al.*, 2011; Glynn *et al.*, 2014; Kapiga *et al.*, 2013; Abbai, 2015). It is crucial to point out, however, that many of these identified risk factors have been derived predominantly from high-risk female participants enrolled in prior HIV prevention research.

The transmission of HSV-2 typically occurs through direct mucocutaneous contact with infected secretions, oral fluids, or herpetic lesions (Corey *et al.*, 2008). Importantly, the infection is most often passed on by partners who are entirely asymptomatic and lack visible lesions, underscoring the insidious nature of the virus. Comparative studies reveal that genital HSV shedding occurs on approximately 10.2% of days in asymptomatic carriers, compared with 20.1% of days in those with symptomatic infections (Mertz, 2008). In the context of pregnancy, genital herpes takes on heightened clinical significance due to the grave risks it poses to both the developing fetus and the neonate, as well as its well-documented synergy with HIV acquisition and transmission (Sripriya, 2013; Jacob *et al.*, 2014).

The most severe neonatal outcomes are observed when primary maternal infection occurs late in gestation, as there is insufficient time for the development of protective maternal antibodies for passive transfer to the fetus (Qutib *et al.*, 2010). Indeed, the risk of maternal urinary tract infection peaks in the final trimester (Mehmet *et al.*, 2016). The consequences of neonatal infection are devastating: disseminated disease carries a mortality rate approaching 90%, while neonatal meningoencephalitis proves fatal in roughly 50% of cases, with most survivors enduring permanent neurological sequelae despite antiviral intervention (Looker *et al.*, 2015). Alarming, even in resource-rich nations, neonatal herpes mortality exceeds 50% (Corley, 2009). Furthermore, concurrent HIV and HSV infections negatively influence pregnancy outcomes, significantly raising the incidence of spontaneous abortion, preterm delivery, intrauterine growth restriction, and low birth weight (Trinh *et al.*, 2023).

Adding another layer of concern, HSV-2/HIV co-infection during pregnancy may increase the risk of mother-to-child HIV transmission by as much as 25% (Van Wagoner *et al.*, 2023). Although several antivirals, namely acyclovir (the pioneering drug, now available generically), valacyclovir, famciclovir, and penciclovir, are effective against HSV (LaFemina, 2009), evidence regarding their efficacy in reducing HIV acquisition remains mixed. Nonetheless, high-dose antiviral regimens have been suggested to lower HIV viral loads in co-infected patients (Mugwanya *et al.*, 2011). Early detection and prompt therapeutic management of HSV-2 during pregnancy are paramount for mitigating the risk of vertical transmission and preventing disseminated maternal disease. Additionally, for women presenting with primary HSV-2-related genital ulceration at the onset of labor, performing a cesarean section remains a critical measure to minimize neonatal exposure and transmission risk (Corley, 2009).

METHODOLOGY

Study Area and Design

This cross-sectional study was conducted in Jalingo, the capital city of Taraba State, located in north-eastern Nigeria. The state lies between latitudes 7.9869°N and longitudes 10.9807°E, encompassing a total land area of approximately 54,473 km². According to 2018 population estimates, the city had approximately 418,000 inhabitants. Participant recruitment was carried out at two major tertiary healthcare facilities within the metropolis: the Federal Medical Centre (FMC), Jalingo, and the Specialist Hospital, Jalingo.

Study Population, Enrollment Criteria, and Ethical Considerations

The study population comprised 227 pregnant women attending antenatal clinics at the aforementioned hospitals, who were selected through a random sampling approach. Enrollment was contingent upon voluntary participation and the

provision of written informed consent, which was obtained from all subjects before data and specimen collection, following a protocol approved by the institutional ethics review board. Inclusion was restricted to pregnant women who consented to partake, whereas those who declined were systematically excluded from the study. Ethical clearance for the research protocol was formally granted by the Taraba State Ministry of Health (approval reference: TRSHREC/2024/APP/001).

Sample Size Determination

The minimum required sample size was calculated using the Kish and Lisle formula for cross-sectional prevalence studies: $n = Z^2 p(1-p) / d^2$ where Z represents the standard normal deviate for a 95% confidence interval (1.96), p denotes the anticipated prevalence, and d is the allowable margin of error set at 0.05. Based on an 18.3% HSV-2 prevalence previously documented in a comparable Nigerian cohort from Jigawa State (Amoo *et al.*, 2020), the sample size was derived as follows: $n = (1.96)^2 \times (0.18) \times (0.82) / (0.05)^2 = 227$.

Blood Specimen Collection and Processing

Approximately 5 mL of venous blood was aseptically drawn from each participant via sterile needles and syringes, and immediately dispensed into labeled EDTA-containing Vacutainer tubes. Plasma was separated from whole blood by centrifugation at 1,500 rpm for 10 minutes. The resulting plasma aliquots were carefully transferred into sterile, pre-labeled cryovials, stored at 4°C in a portable cool box with ice packs, and subsequently transported to the Virology Laboratory of FMC, Jalingo, for serological and molecular analyses.

HIV Serological Screening

Initial HIV screening was conducted using the Determine™ rapid test kit (Alere, USA). Samples yielding non-reactive results were classified as HIV-negative. Conversely, reactive samples underwent confirmatory testing using the Uni-Gold™ HIV rapid test. For the confirmatory assay, two drops of whole blood were applied to the sample port of the Uni-Gold device, followed by the addition of two drops of the manufacturer-provided running buffer.

HSV-2 IgG Antibody Detection via Enzyme-Linked Immunosorbent Assay (ELISA)

Detection of HSV-2-specific IgG antibodies was performed using a commercially available glycoprotein G (gG)-based ELISA kit. Briefly, 100 µL of each serum sample was dispensed into the designated pre-coated microwells, alongside 10 µL of positive and negative controls. The plate was incubated at 37°C for 30 minutes. Following incubation, the plate cover was discarded, and the wells were washed five times with diluted wash buffer (30–60 seconds per wash cycle). Subsequently, 10 µL of enzyme conjugate was added to each well and incubated for another 30 minutes at 37°C to facilitate specific binding. An additional washing step removed unbound conjugate. A 100 µL volume of substrate solution was then introduced to each well, generating a chromogenic reaction catalyzed by the bound enzyme complex. The resultant color intensity, proportional to the antibody concentration, was measured spectrophotometrically as absorbance at an optical density of 450 nm using an EIA plate reader, strictly adhering to the manufacturer's recommended protocol.

Socio-demographic Data Collection

A structured, pre-tested questionnaire was administered to all participants to obtain relevant socio-demographic and obstetric information, including maternal age, family structure, marital status, educational attainment, employment status, gestational age, and parity.

Molecular Identification of the HSV-2 UL41 Gene

Viral DNA Extraction

From the pool of HSV-2 IgG-seropositive specimens, five samples were randomly selected for molecular confirmation. Viral DNA was extracted using the ZR Viral DNA Kit™ (Zymo Research, USA) according to the manufacturer's specifications. In summary, 400 µL of ZR Viral DNA Buffer was combined with 100 µL of serum in a 1.5 mL microcentrifuge tube, vortexed briefly, and allowed to stand at room temperature for 10 minutes. The resulting mixture was transferred onto a Zymo-Spin™ IC Column within a collection tube and centrifuged for 1 minute. The flow-through was discarded, and the column was washed twice with 300 µL of DNA Wash Buffer, with a 1-minute centrifugation step following each wash. Finally, the column was placed into a clean microcentrifuge tube, 10 µL of DNA Elution Buffer was applied directly to the column matrix, and after a 1-minute incubation at room temperature, the purified DNA was eluted by centrifugation for 1 minute.

DNA Quantification and Quality Assessment

The concentration and purity of the extracted DNA were determined using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific), measuring absorbance at 260 nm and calculating the 260/280 nm absorbance ratio to ensure sample quality.

Polymerase Chain Reaction (PCR) Amplification

Amplification of the HSV-2 UL41 gene, yielding a 192-base pair (bp) product, was carried out using previously described primers (Hayatudeen and Aminu, 2021): forward primer 5'-CATTCCGGCAGATTGTAG-3' and reverse primer 5'-CACCAATCCGCACGTAGAT-3'. The PCR reaction was assembled in a total volume of 25 μ L, comprising 12.5 μ L of a 5X master mix (containing 0.5 μ L FIREPol® DNA polymerase [Solis BioDyne], 7.5 μ L MgCl₂ to achieve a final concentration of 1.5 mM, and 0.4 mM each deoxyribonucleoside triphosphate [dNTPs]), 0.5 μ L of each primer, 1.25 μ L dimethyl sulfoxide (DMSO; Sigma), 8.25 μ L nuclease-free water, and 2 μ L of the extracted DNA template. Amplification was conducted in a VWR XT96 Gradient Thermal Cycler (Germany) using the following cycling profile: an initial denaturation at 95°C for 5 minutes, followed by 36 cycles of denaturation at 95°C for 15 seconds, annealing at 52°C for 15 seconds, and extension at 72°C for 30 seconds, with a final extension step at 72°C for 5 minutes.

Detection of Amplified Products

The amplified PCR products were resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide. Bands were visualized under ultraviolet (UV) light using a gel documentation system. A 100 bp DNA ladder was included as a molecular weight marker, and positive amplification was confirmed by the appearance of a distinct band at the expected 192 bp position.

Statistical Analysis

All collected data were entered and analyzed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages. Associations between HSV-2 seropositivity and socio-demographic risk factors were assessed using Pearson's chi-square (χ^2) test at a 95% confidence level, with statistical significance defined as a p-value < 0.05 (Abaka *et al.*, 2024).

RESULTS

The seroprevalence of HSV-2 Immunoglobulin G (IgG) and HIV among pregnant women is presented in Fig 1. Results showed that out of the total samples analyzed, 16 (7.0%) were found positive for HSV-2 virus antibodies, while 211 were negative; 37(16.3%) were HIV positive, and the remaining 190(83.7%) were found to be negative. Therefore, positive samples appeared yellow and negative appeared colorless after the ELISA test (Plate 1).

Sero-prevalence of HSV-2 and HIV co-infection among 227 pregnant women is presented in Table 1. Results showed that 5 (2.2%) of the total population of 227 pregnant women were co-infected with both viruses, a significant finding given the reciprocal biological interaction between HSV-2 and HIV.

Socio-demographic distribution associated with HSV-2 and HIV co-infection among pregnant women is presented in Table 2. Results showed that among the 227 pregnant women, the highest prevalence of HIV is in the 25-34 age group (19.10%), and this group also had the highest HSV-2 (10.11%) and HSV-2/HIV co-infection rates (5.62%) ($\chi^2 = 5.35$, $p = 0.25$). Women from polygamous families have a much higher rate of HSV-2/HIV co-infection (19.23%) compared to those from monogamous families (0%) ($\chi^2 = 26.42$, $p = 1.84$). Single women showed a higher prevalence of HSV-2 (30.00%) and HSV-2/HIV co-infection (10.00%) compared to married women ($\chi^2 = 2.69$, $p = 0.26$); women with lower educational attainment (Primary and Secondary) showed a slightly higher prevalence of HSV-2 and HSV-2/HIV co-infection; however, the differences were not significant ($\chi^2 = 2.91$, $p = 0.82$).

Results also showed that unemployed women had the highest rates of HIV (21.31%) and HSV-2/HIV co-infection (2.46%), but there was no clear pattern in HSV-2 distribution ($\chi^2 = 5.37$, $p = 0.44$). Furthermore, the highest rates of HIV (22%) and HSV-2/HIV co-infection (4%) were observed in women in their second trimester, but no co-infection was observed in the first trimester ($\chi^2 = 0.44$, $p = 0.80$), primiparous women showed slightly higher rates of HSV-2 (12.07%) and HSV-2/HIV co-infection (3.45%) compared to those who are Grand multiparous ($\chi^2 = 4.26$, $p = 0.37$).

Result of the MolThe resultecular confirmation of HSV-2 positive samples using agarose gel electrophoresis of the reaction products from PCR showed five lanes; Lane 1 for a DNA ladder of 100 base pairs. Lanes 2-6 are DNA samples with a recorded molecular weight of 192 base pairs, respectively (Fig 8).

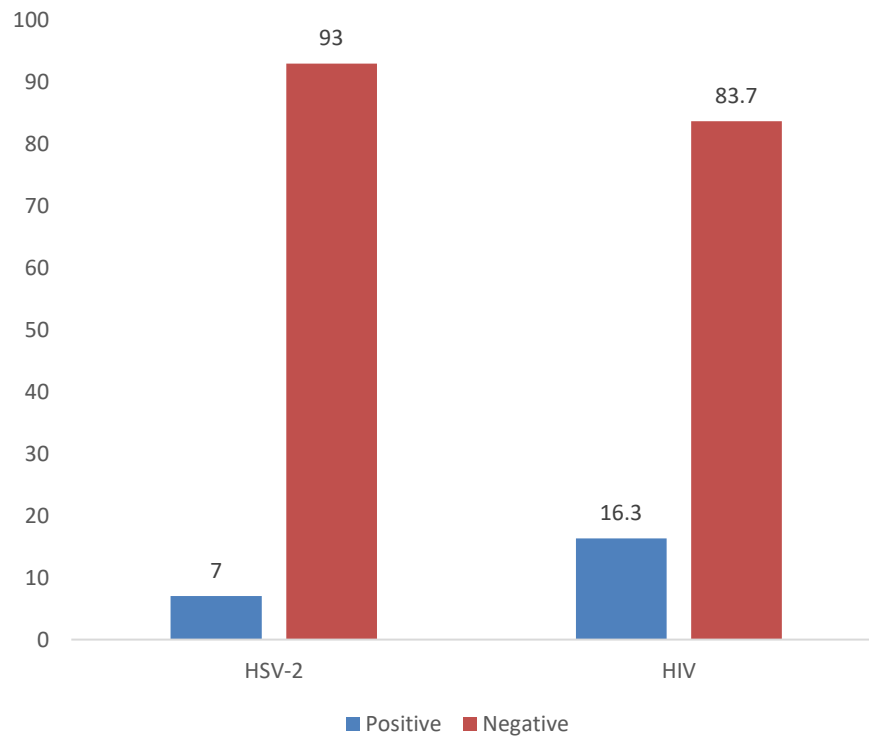
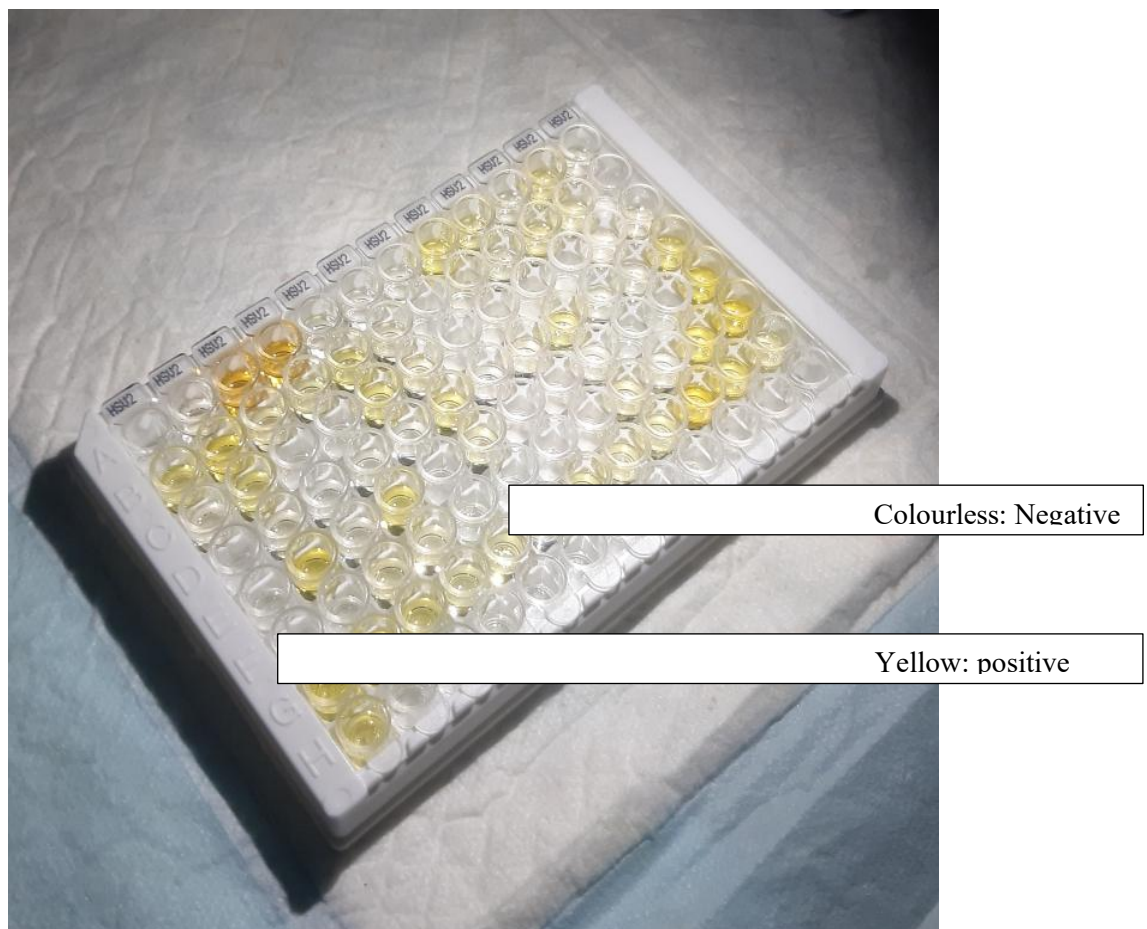


Fig 1: Seroprevalence of HSV-2 and HIV among pregnant women



**Plate 1. HSV-2 Elisa test result
(Key: Yellow: Positive, Colorless: Negative)**

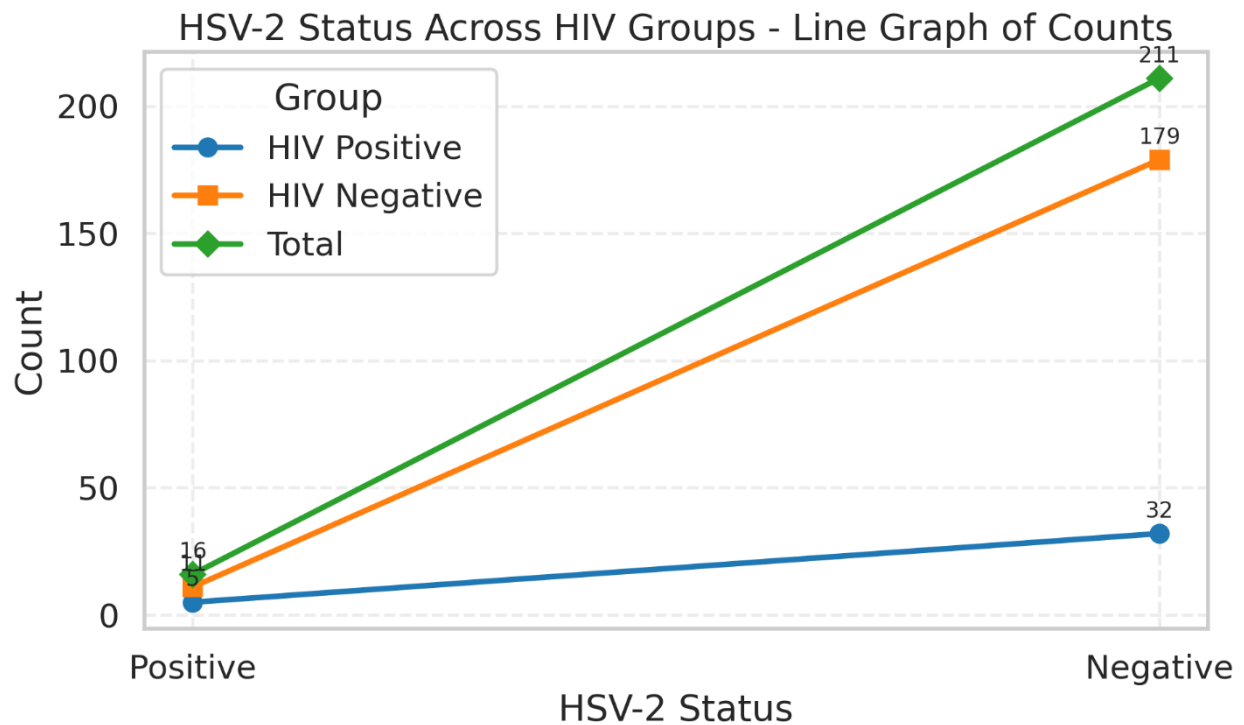


Figure 2: Association between HSV-2 and HIV Seropositivity among the Study Participants (N = 227)

Percentages for the HIV-positive and HIV-negative columns were calculated using their respective column totals (37 and 190), whereas percentages in the Total column were calculated using the overall study population (N = 227).

$\chi^2=1.76$, p- value 0.18, Odds Ratio: 2.54, Risk Ratio: 2.06, Relative Risk Ratio

Table 1: Socio-demographic characteristics associated with HSV-2 and HIV co-infection among pregnant women (n = 227)

Variable	Total n (%)	HSV-2 Positive n (%)	HIV Positive n (%)	HSV-2/HIV Co-infection n (%)	χ^2	P-value
Age group (years)					5.35	0.25
15–24	69 (30.4)	4 (5.80)	10 (14.49)	0 (0.00)		
25–34	89 (39.2)	9 (10.11)	17 (19.10)	5 (5.62)		
≥35	69 (30.4)	3 (4.35)	10 (14.49)	0 (0.00)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		
Family type					26.42	1.84×10^{-6}
Monogamous	201 (88.5)	14 (6.97)	34 (16.92)	0 (0.00)		
Polygamous	26 (11.5)	2 (7.69)	3 (11.54)	5 (19.23)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		
Marital status					2.69	0.26
Married	217 (95.6)	13 (5.99)	35 (16.13)	4 (1.84)		
Single	10 (4.4)	3 (30.00)	2 (20.00)	1 (10.00)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		

Table 2: Educational and employment characteristics associated with HSV-2 and HIV co-infection among pregnant women (n = 227)

Variable	Total n (%)	HSV-2 Positive n (%)	HIV Positive n (%)	HSV-2/HIV Co-infection n (%)	χ^2	P-value
Highest educational qualification					2.91	0.82
Primary	38 (16.7)	2 (5.26)	2 (5.26)	1 (2.63)		
Secondary	80 (35.2)	7 (8.75)	14 (17.50)	1 (1.25)		
Tertiary	90 (39.6)	6 (6.67)	16 (17.78)	2 (2.22)		
Informal education	19 (8.4)	1 (5.26)	5 (26.32)	1 (5.26)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		
Employment status					5.37	0.44
Employed	24 (10.6)	1 (4.17)	3 (12.50)	1 (4.17)		
Self-employed	81 (35.7)	8 (9.88)	8 (9.88)	1 (1.23)		
Unemployed	122 (53.7)	7 (5.74)	26 (21.31)	3 (2.46)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		

Table 3. Obstetric characteristics associated with HSV-2 and HIV co-infection among pregnant women (n = 227)

Variable	Total n (%)	HSV-2 Positive n (%)	HIV Positive n (%)	HSV-2/HIV Co-infection n (%)	χ^2	P-value
Gestational age					0.44	0.80
First trimester	17 (7.5)	0 (0.00)	0 (0.00)	0 (0.00)		
Second trimester	50 (22.0)	6 (12.00)	11 (22.00)	2 (4.00)		
Third trimester	160 (70.5)	10 (6.25)	26 (16.25)	3 (1.88)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		
Parity					4.26	0.37
Primiparous	58 (25.6)	7 (12.07)	10 (17.24)	2 (3.45)		
Multiparous	93 (41.0)	3 (3.23)	17 (18.28)	1 (1.08)		
Grand multiparous	76 (33.5)	6 (7.89)	10 (13.16)	2 (2.63)		
Total	227 (100)	16 (7.05)	37 (16.30)	5 (2.20)		

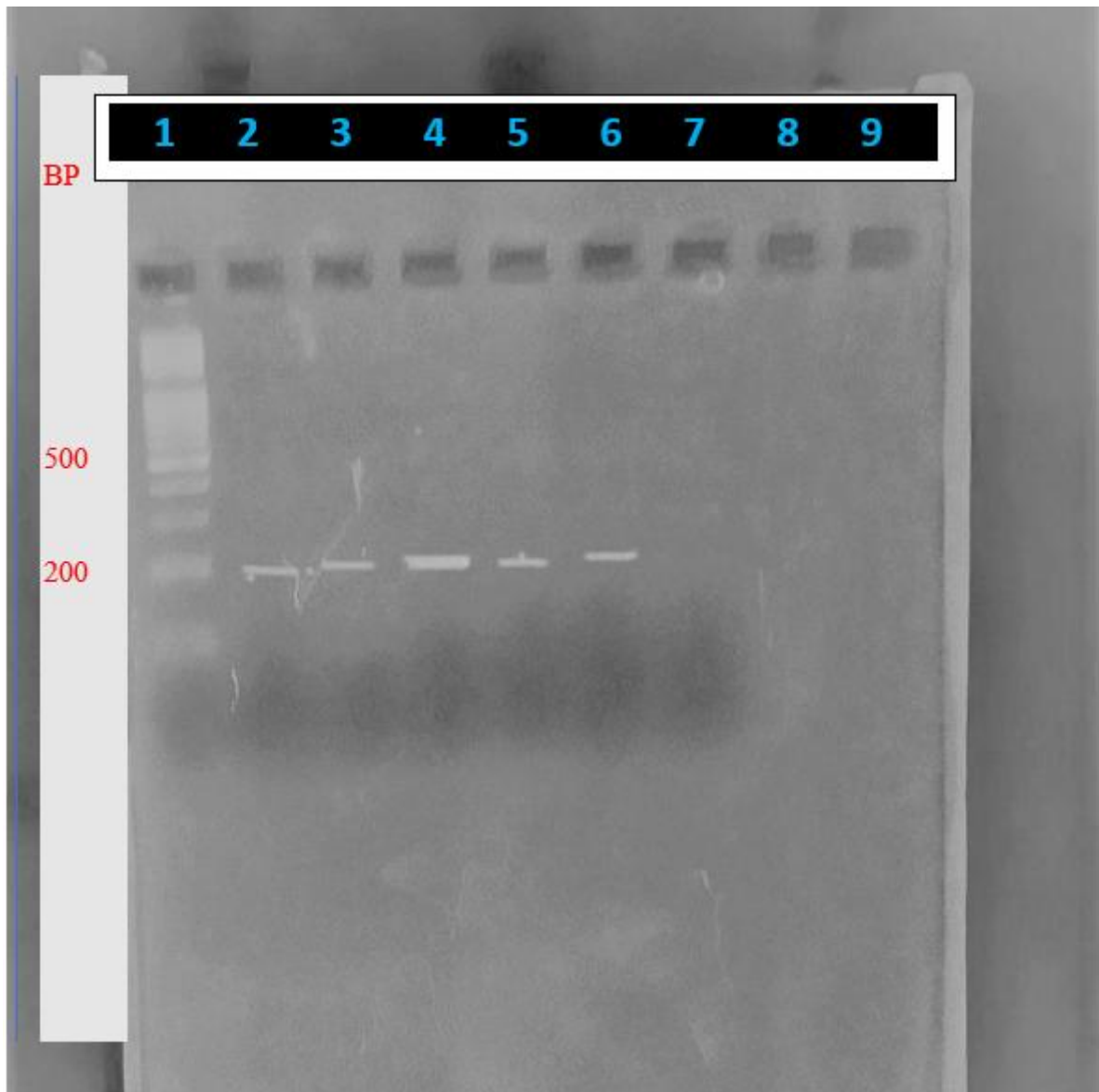


Fig 3: Gel electrophoresis picture stained with ethidium bromide showing positive amplification of a 192 base pairs product of the HSV-2 Gene under the U.V transilluminator.

Lane 1: DNA ladder (100 bp), Lane 2-6: DNA samples

DISCUSSION

Genital herpes constitutes a significant global public health challenge, imposing not only severe physical pain on affected individuals but also profound psychological and social distress (Ikokuwu *et al.*, 2023). The implications are particularly critical during pregnancy; maternal HSV-2 infection can lead to neonatal herpes, a preventable yet serious contributor to infant morbidity and mortality (Hammad & Konje, 2021).

In the current study conducted in Jalingo, the seroprevalence of HSV-2 IgG antibodies among pregnant women was determined to be 7.0%. This figure is congruent with rates documented in other global populations, such as 7.5% in India and Kashmir (Weiss, 2004; Rathore *et al.*, 2010), 7.6–8.4% in Italy (Straface *et al.*, 2012), and 6.5–6.8% in Saudi Arabia (Obeid, 2007). Conversely, it exceeds rates observed in Kisumu, Kenya (3.95%; Nyiro *et al.*, 2011) and Turkey (4.4–5%; Ozdemi *et al.*, 2011), but remains lower than the prevalence reported in Bangladesh (9.91%; Nabi *et al.*, 2012), China (10.8%; Chen *et al.*, 2007), the United States (21%; Weiss, 2004), and Brazil (21%; Lima *et al.*, 2017). Within the African continent, higher estimates have been recorded in Ethiopia (32%; Anjulo *et al.*, 2016), Uganda (53%; Nakubulwa *et al.*, 2016), and South Africa (59%; Perti *et al.*, 2014). In Nigeria, our findings are lower than those reported in Ibadan (33.3%; Anaedobe *et al.*, 2019), Keffi (35.5%; Otti *et al.*, 2017), Benin (44.3%; Kalu, 2013), and Enugu (77.8%; Ojinmah *et al.*, 2012), though they align closely with the 18.3% rate observed in Jigawa (Amoo *et al.*, 2020). Notably,

the overall prevalence in this study falls below the global HSV-2 seroprevalence estimate of 15% for women (Looker *et al.*, 2015).

The epidemiology of HSV-2 IgG varies considerably across nations and demographic groups, shaped by factors such as sexual behavior (McQuillan *et al.*, 2006), gravidity (Looker *et al.*, 2017), duration of sexual activity (Weiss, 2004), geographic residence (Looker *et al.*, 2015), educational attainment (Chao *et al.*, 2004), occupation (Morrow *et al.*, 2003), and socioeconomic status (Smith *et al.*, 2010). Although the 7.0% prevalence observed here is modest relative to some international data, it nonetheless underscores a substantial public health concern, particularly given the adverse neonatal outcomes linked to HSV-2 infection during pregnancy (Kimberlin, 2004).

Regarding HIV, the seroprevalence in our study population was 16.3%. This rate is markedly higher than the 2.4% reported among pregnant women in Lokoja (Kolawole *et al.*, 2016) and the national North-Central (6.84%) and South-West (6.27%) averages in Nigeria, yet it is slightly below the 17.04% prevalence documented in the South-East zone (Ozim *et al.*, 2023) and the 19.63% rate in Ibadan (Anaedobe *et al.*, 2019). The elevated HIV prevalence observed here raises serious concerns regarding mother-to-child transmission (MTCT), a persistent public health challenge in sub-Saharan Africa. This is consistent with other regional reports, such as a 30.8% prevalence among pregnant women in South Africa (Dinh *et al.*, 2015) and 11.2% in Tanzania (Msuya *et al.*, 2006).

The co-infection rate of HSV-2 and HIV among the 227 pregnant women was 2.2%, which aligns with findings from Lokoja (2.4%; Kolawole *et al.*, 2016) and Jos (2.8%; Mawak *et al.*, 2012), but is lower than rates observed in Jigawa (5.3%; Amoo *et al.*, 2020), Senegal (7%; Diawara *et al.*, 2008), Ibadan (38.9%; Anaedobe & Ajani, 2019), Benin (65.6%; Kalu, 2013), Brazil (73%; Caldeira *et al.*, 2013), and the Central African Republic (91%). This relatively low co-infection prevalence may be attributable to differences in study populations, as earlier investigations often involved commercial sex workers or STI clinic attendees.

Importantly, the finding of a 2.2% co-infection rate aligns with recent literature emphasizing that HSV-2 potentiates HIV acquisition through genital ulcerations that facilitate viral entry (James *et al.*, 2023; Patel *et al.*, 2022). In our cohort, 31.3% of HSV-2-positive women were also HIV-positive, while 13.5% of HIV-positive women tested positive for HSV-2. These figures corroborate the assertion that HSV-2 contributes to both HIV persistence and progression through viral reactivation (Kumar *et al.*, 2022).

Socio-demographic analysis revealed that women aged 25–34 years exhibited the highest rates of HSV-2/HIV co-infection, consistent with peak reproductive and sexual activity years that increase STI exposure (Adebayo *et al.*, 2021; Musa *et al.*, 2020). This age-related trend mirrors global patterns where HSV-2 prevalence peaks in this demographic (Weiss, 2004; Looker *et al.*, 2017), while lower prevalence in those aged ≥ 35 may reflect reduced sexual activity or enhanced preventive behaviors (James *et al.*, 2022; Patel *et al.*, 2021).

Marital status and family structure were also influential: women from polygamous households had a substantially higher co-infection rate (19.23%) compared to those from monogamous settings (0%), likely due to increased sexual network complexity and unprotected intercourse (Olawale & Ayoola, 2022). Single women also demonstrated elevated HSV-2 (30%) and co-infection (10%) rates, potentially attributable to multiple or transient partnerships (Mburu *et al.*, 2021). Educational attainment showed a modest inverse relationship with infection risk, with lower education levels associated with higher STI prevalence, possibly due to limited sexual health literacy and socioeconomic constraints (Njoku *et al.*, 2020). Unemployment correlated with the highest HIV (21.31%) and co-infection (2.46%) rates, reflecting socioeconomic vulnerabilities and increased engagement in high-risk behaviors such as transactional sex (Adebayo *et al.*, 2021).

Gestational age and parity also influenced infection patterns. The second trimester was associated with the highest HIV (22%) and co-infection (4%) rates, potentially due to pregnancy-induced immunological modulation (Ogbuagu *et al.*, 2022). Primiparous women showed slightly higher HSV-2 (12.07%) and co-infection (3.45%) rates compared to multiparous women, possibly reflecting limited reproductive health experience (Mburu *et al.*, 2021).

Molecular confirmation of HSV-2 was achieved via PCR and agarose gel electrophoresis, which demonstrated specific amplification of the 192-base pair fragment corresponding to the HSV-2 UL41 gene. The use of a 100-base pair DNA ladder in Lane 1 enabled accurate size determination, and the consistent band intensity across lanes 2–6 confirmed optimal PCR conditions, including appropriate annealing temperatures and cycle numbers. This reproducibility underscores the assay's high sensitivity and specificity for detecting HSV-2 DNA in clinical samples, affirming its utility for both diagnostic and research applications (Wang *et al.*, 2016; Garcia *et al.*, 2017; Johnson & Whitley, 2018).

Conclusion

This comprehensive sero-epidemiological investigation has provided critical insights into the burden of HSV-2 and HIV co-infection among pregnant women in Jalingo, Taraba State, Nigeria. The study documented an HSV-2 seroprevalence of 7.0% and an HIV prevalence of 16.3%, with a co-infection rate of 2.2% among the 227 pregnant women enrolled. These findings occupy an intermediate position within the spectrum of global and regional estimates, being lower than many African reports yet exceeding rates documented in several Asian and Middle Eastern populations. The molecular confirmation of HSV-2 DNA through PCR amplification of the UL41 gene, yielding the expected 192-base pair product, validates the serological findings and underscores the reliability of the diagnostic approach employed. The identification of a 31.3% HSV-2 positivity rate among HIV-infected women and 13.5% HIV positivity among HSV-2-seropositive women substantiates the well-established biological synergy between these two viruses, wherein HSV-2-induced genital ulceration facilitates HIV acquisition and transmission, while HIV-induced immunosuppression may promote HSV-2 reactivation.

The socio-demographic and obstetric determinants identified in this study illuminate the populations most vulnerable to these infections and their co-occurrence. Women in the 25–34 years age bracket, representing the peak reproductive and sexually active years, demonstrated the highest infection rates, while those from polygamous households exhibited a disproportionately elevated co-infection prevalence of 19.23%, likely reflecting complex sexual networks and increased exposure risk. The elevated HIV prevalence of 21.31% among unemployed women underscores the nexus between socioeconomic vulnerability and infection risk, potentially mediated through engagement in transactional sex or limited access to preventive healthcare services. Notably, the absence of co-infection during the first trimester, coupled with peak rates in the second trimester, suggests pregnancy-related immunological modulation that warrants further investigation. The higher infection rates observed among primiparous women compared to multiparous women may reflect younger age, limit reproductive health experience, or evolving sexual behaviors. These demographic patterns provide essential evidence for designing targeted interventions that address the specific vulnerabilities of these high-risk subgroups.

The findings of this study carry substantial public health and clinical implications for maternal and neonatal health in Jalingo and similar settings across sub-Saharan Africa. The documented co-infection prevalence, while relatively modest, represents a significant concern given that HSV-2 has been demonstrated to increase mother-to-child HIV transmission risk by up to 25% through enhanced viral shedding and mucosal disruption. The study strongly advocates for the integration of routine HSV-2 screening into existing antenatal care programs, alongside universal HIV testing, to facilitate early detection and prompt intervention. Such integrated screening approaches would enable timely administration of antiviral prophylaxis to HSV-2-positive women, reduce the risk of neonatal herpes, and potentially lower HIV transmission rates. Furthermore, the successful molecular characterization of HSV-2 in this population demonstrates the feasibility of PCR-based diagnostics in resource-limited settings, paving the way for enhanced surveillance and monitoring of viral epidemiology. Ultimately, this research provides a foundational evidence base for public health policymakers to strengthen antenatal screening protocols, implement targeted health education campaigns addressing the sexual and reproductive health needs of vulnerable groups, and thereby mitigate the dual burden of HSV-2 and HIV on maternal and neonatal health outcomes in northeastern Nigeria.

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Competing Interests

The authors declare that there are competing interests.

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