INCIDENCE OF VERTICAL-TRANSMISSION OF HIV AND ITS CORRELATION WITH MATERNAL GESTATIONAL AGE AT ANTENATAL BOOKING IN PORT-HARCOURT, NIGERIA

Abstract

Background: Pregnancy associated with human immunodeficiency virus (HIV)-infection poses risk to the fetus due to vertical-transmission. This can be prevented through administration of antiretroviral drugs. Our objective was to investigate the incidence of vertical-transmission of HIV and its correlation with maternal gestational age at antenatal booking with immediate commencement of antiretroviral therapy in Port-Harcourt, Nigeria.

Methods: All antenatal attendees and their HIV-exposed newborns were screened for HIV-infection between April, 2016 and May, 2017 using qualitative rapid ELISA kits and HIV-DNA PCR technique. The HIV-positive antenatal attendees were placed on daily single-dose triple antiretroviral regimen (efavirenz, lamivudine, tenofovir, 600/300/300mg tablet) with multivitamins from the first day of booking and continued afterward. The HIV-exposed babies were placed on 5ml daily single-dose triple antiretroviral chemoprophylaxis on the first day of delivery and continued until blood collection at 6 weeks for HIV diagnosis. Statistical Package for Social Science (SPSS) software (version 17.0) was used for data analysis.

Results: We found 4.34% overall prevalence of HIV-1 infection among the antenatal attendees and 7.57% incidence of mother-to-child transmission. There was significant difference between gestational age at booking with commencement of ART and the number of HIV-positive babies ($\chi 2$=7.113, *df*=2,

P<0.05). There was no vertical transmission among the attendees who booked at first trimester, 35.7% at second trimester and 64.3% at third trimester. There was no statistically significant gender difference (P>0.05) between the number of infected males, 42.9% and females, 57.1%.

Conclusion: High incidence of vertical-transmission of HIV was obtained from those HIV-positive mothers who registered late for antenatal care. Therefore, strong advocacy for early entry into antenatal care is solicited.

Key words: Incidence, HIV-infection, antenatal attendees, vertical-transmission, Port-Harcourt, Nigeria

Introduction

The impact of human immunodeficiency virus (HIV) infection is being felt by all nations worldwide. Globally, there are 36.9 million people living with HIV as at the end of 2017 (1), of which, as much as 68% are living in Sub-Saharan Africa (SSA). Nigeria is ranked second, after South-Africa among all the countries with HIV burden worldwide and currently has an estimated average of 3.2 million people living with HIV, of which, 58% are women and 270,000 are children and 220,000 are new infections (2,3). HIV burden distribution by states in Nigeria indicates that there are about 400,000 persons living with HIV in Rivers State with a prevalence of 15.2% (the highest prevalence in all the states), of which, 6% are pregnant women (2-5). Pregnancy associated with HIV infection stands the risk of having repeated abortions, premature birth, intrauterine growth retardation, still birth, congenital abnormalities and vertical transmission.

The World Health Organisation (6) defined vertical transmission of HIV also known as perinatal transmission or mother-to-child transmission (MTCT) of HIV as the transmission of HIV from an HIV infected woman to her child during pregnancy, labor, delivery or breast feeding. It constitutes the third most common route of HIV transmission globally. More than 90% of the world's 2.3 million children living with HIV get infected through vertical transmission. Nigeria accounts for about 30% of the global burden of MTCT; with a MTCT rate of about 32% and 75,000 new infant HIV infections per annum; making Nigeria one of the 22 focus countries of the Global Plan to Eliminate MTCT (6). The high burden of MTCT of HIV in Nigeria is due to high rates of heterosexual transmission, high prevalence of HIV in women of reproductive age, high total fertility rate, low PMTCT coverage and prolonged breastfeeding culture (4)**.** The number of pregnant women living with HIV in Rivers State or elsewhere stands the risk of transmitting the HIV virus to their off-springs if not prevented. Early diagnosis of HIV infection among HIV-exposed newborns would provide a critical opportunity for early treatment to improve quality of life and child survival. According to **WHO** (6) HIV-exposed or infected babies without intervention are much more prone to rapid rate of disease progression as well as have up to 50% chance of dying before their second birthday. There is, therefore, the need for early diagnosis and timely institution of appropriate measures of intervention.

To the best of our knowledge, there have been no previous or recent attempts to determine the incidence of vertical transmission of HIV and its correlation with maternal gestational age at antenatal booking with immediate commencement of antiretroviral therapy in Port-Harcourt, Rivers State, Nigeria. The objective of this study was therefore to determine the incidence of vertical transmission of HIV

infection and its correlation with maternal gestational age at antenatal booking with immediate commencement of antiretroviral therapy in Port-Harcourt, Nigeria.

MATERIALS AND METHODS

Study design/locations

This was a prospective cross-sectional study conducted among apparently healthy pregnant women who were attending antenatal clinics for the first time during this current pregnancy; of which, those who tested HIV-positive and the babies born to these HIV-positive mothers (HIV-exposed babies) formed the main study population. All the antenatal attendees had counseling on HIV testing and antiretroviral therapy (ART) for the infected and their HIV-exposed babies. The study was carried out both at University of Port-Harcourt Teaching Hospital (UPTH) and Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt. University of Port-Harcourt Teaching Hospital is a tertiary healthcare facility located along the East-West Road with 600 bed space capacity. While BMSH is a government owned 375 bed spaces general hospital located in the old Government Reserved Area (GRA). The two hospitals were chosen because they are not only the biggest but also offer prevention of mother-to-child transmission (PMTCT) of HIV services with large turnout of antenatal attendees.

Ethical approval and informed consent

Ethical approval was obtained from the facilities' Research Ethics committee (REC) while informed consent was obtained from each HIV-positive pregnant woman and the mothers on behalf of their babies.

HIV Screening

(a) HIV diagnosis in pregnancy

All the antenatal attendees were screened for HIV infection using commercial qualitative rapid immunoassay test kits, Determine HIV-1/2 (Abbott Laboratories, Illinois, USA), Unigold HIV test kit (Trinity Biotech Manufacturing, Bray, Ireland) and Stat-Pak HIV test kit (Chembio Diagnostic Systems USA) and performed according to the Nigeria national algorithm for HIV screening, which involves the use of three test kits- two for serial testing and one to resolve discordant results (as tie breaker). The manufacturer's standard operating procedures (SOPs) were strictly followed. Serial testing involves the use of one test kit at a time. Samples reactive to the first test kit are subjected to further test with a different test kit and those with discordant results were retested with a third kit as a tie-breaker. Quality control was monitored by the inclusion of kit controls with every batch. HIV-seropositivity was defined as a reactive result on two of the test kits. Subjects not reactive were considered HIV-negative.

(b) HIV diagnosis of HIV-exposed babies

HIV diagnosis of all HIV-exposed babies (babies born to HIV-positive pregnant women) was carried out using Roche Amplicom version 1.5 HIV-1 DNA Polymerase Chain Reaction (PCR) technique (Roche Diagnostics, Bassil, Switzerland) and dried blood spots (DBS) prepared from the babies' blood according to manufacturer's instructions. The protocol used was in accordance with the Nigeria National Early Infant Diagnosis algorithm which stipulates that HIV-DNA PCR test should be carried out on all HIV-exposed infants at six weeks of age.

DBS preparation

Blood was collected on EDTA tubes and then spotted onto a Whatman 903 filter paper to fill at least two preprinted circles (approximately 50µl of blood per spot). After drying for at least 4 hours in an air conditioned room or overnight at room temperature, DBS were individually inserted in a gas-impermeable bag (Bitran bag, VWR, USA) with a desiccant, and then stored at room temperature until analysis.

DNA extraction

Genomic DNA was isolated from DBS by extraction with the polyvalent cationic resin, chelex 100 ((Biorad, Marnes-la-Coquette, France). Each spot was punched for 3 pieces with a ¼ inch hole puncher into a 2ml screw-cap tube. After washing for 1 hour with a Triton X-100 Buffer (1X PBS, 0.5% Triton X-100), 230µl of 10% chelex solution was added and incubated at 100°C for 30 minutes. After centrifugation, 200µl of DNA-containing chelex supernatant were then transferred into a new tube that was used directly for PCR amplification.

DNA PCR testing methodology

Testing is performed on 5 mm disks of the DBS which are punched into sterile 2ml cryovials and washed in specimen wash solution for 30 minutes twice to remove hemoglobin. Working extraction solution, a detergent solution containing proteinase K and HIV-1 internal control (IC), was then used to extract and lyse the DNA containing leukocytes from the disks. Fifty microlitres (50µl) of the extracted DNA solution was added to an equal volume of working mastermix and then amplified for 35 cycles with a final hold stage at 72^{OC} for 15 minutes at which the amplified products were denatured. Denatured amplicon was hybridized in separate HIV-1 and HIV-1 IC target specific probe coated microwell plates, washed in buffer, conjugated to Avidin-Horseradish Peroxidase, washed again and a

substrate added to give a colored complex. Stop solution is added to the colored complex after 10 minutes incubation and detection is completed by colorimetric reading at 450nm. Any value, 0.2 A450 are considered negative, 0.2 A450 and, 0.8 A450 are considered indeterminate and 0.8 A450 are positive. Duplicate repeat testing is performed on indeterminate specimen and results interpreted using 0.2 A450 as the cutoff point.

Antiretroviral therapy Regimen

One hundred and eighty-five (185) of the antenatal attendees who tested positive for HIV were immediately placed on daily single-dose Triple Regimen Antiretroviral chemotherapy with multivitamins on the same day of antenatal booking and followed up until delivery and then along with their HIV-exposed newborns who were placed on 5ml daily single-dose Triple Regimen Antiretroviral chemoprophylaxis until 6 weeks postnatal life when blood sample was collected for HIV DNA-PCR analysis. The triple regimen consisted of Efaverenz, Lamivudine and Tenofovir, (600/300/300mg tablet). This is the recommended first line regimen for HIV-positive pregnant or breastfeeding women and their infants in Nigeria **(FMOH, 2016).** All the HIV-exposed babies were exclusively breastfed until blood samples collection.

Haematological tests

Some basic routine haematological tests (packed cell volume (PCV), haemoglobin electrophoresis, and ABO and Rhesus blood groups) were carried out on the 185 HIV-positive pregnant women by standard operating procedures.

Data analysis

Data analysis

The data was analysed using Statistical Package for Social Science (SPSS) software (version 17.0, SPSS, Chicago, USA). Statistical comparison between groups was made using Pearson's Chi square test or, where appropriate Fisher's exact test and odds ratios (OR) were calculated with 95% confidence intervals (CI) to measure the strength of association between variables and HIV. Student's t-test was used to test for difference among two continuous variables. The level of significance was set at $P < 0.05$. Percentages mean and standard deviations were used for the descriptive data.

RESULTS

Of the 4,262 consecutive antenatal attendees screened for HIV-1 and 2 between April, 2016 and May, 2017 a prevalence of 4.34% (185/4262) was obtained. At the individual health facilities, UPTH gave a prevalence of 4.53% (98/2,162) and BMSH, 4.14% (87/2,100) (Table 1). The 185 HIV-positive antenatal attendees were between 14 and 39 years of age (mean 27.4 ±4.3 years) and were positive for HIV-1 alone. The 28 to 34 years age group had the highest prevalence (59.5%) and the least prevalence (1.6%) occurred among 14 to 20 years age group (Table 2). HIV prevalence increased with age from 1.6% among 14-20 years age group to 25.4% for those aged 21-27 years and then to 59.5% among the 28-34 years age group and thereafter decreased with age. However, HIV infection occurred in all age groups.

Table 1 General Characteristics of the Total Antenatal Attendees Screened For HIV-1 and 2 at UPTH and BMSH

Study Site	Total number of antenatal attendees	Age Range and Mean (years)	Number of HIV Positive Women	Number of HIV Negative Women
UPTH	2,162	16-42 (27.4±4.3)	98 (4.53%)	2,064 (95.47%)
BMSH	2,100	14-43 (26.6±3.1)	87 (4.14%)	2,013 (95.86%)
TOTAL	4,262	14-43 (27.3±3.9)	185 (4.34%)	4,077 (95.66%)

The mean gestational age at booking of the HIV-positive antenatal attendees (Table 3) was 26.3±1.7 weeks. There were significant differences in the number of attendees in the 3 trimesters (X^2=6.212, df=2, P<0.05) with 57.3% in their second trimester (13-24 weeks). The least in attendance were those in their first trimester of pregnancy (12.4%). All the HIV-DNA PCR positive babies were from HIV-positive pregnant women who booked late for antenatal care (in their second and third trimesters of pregnancy), of which, a significant difference (*P*<0.05) in the number of infected babies was found between second and third trimesters. This suggests that an early entry into antenatal care with early commencement of ART (if indicated) is critical for suppression of vertical transmission.

Of the 185 HIV-positive expectant mothers, 7 (3.8%) were anaemic (PCV < 30%) (Table 4). None was severely anaemic (PCV < 21%). Four (4.6%) of the anaemic HIV-positive expectant mothers were at BMSH (n=87) while the remaining 3 (3.1%) were at UPTH (n=98). There was no significant difference in the number of anaemic HIV-positive expectant mothers at both facilities (P>0.05). The 7 anaemic HIV-positive women were specially placed on daily single-dose antiretroviral Triple Regimen consisted of Efavirenz, Lamivudine and Tenofovir (i.e. Zidovudine

was substituted with Efavirenz). This was in line with the guidelines for HIV-positive anaemic pregnant women.

Table 2 Frequency Distribution of HIV-Positive Expectant Mothers According to Age Groups

Age Groups	Frequency (%)		
	BMSH (n=87)	UPTH (n=98)	COMBINED (n=185)
14-20	1 (1.1)	2 (2.0)	3 (1.6)
21-27	31 (35.6)	16 (16.3)	47 (25.4)
28-34	48 (55.2)	62 (63.3)	110 (59.5)
35-41	7 (8.1)	18 (18.4)	25 (13.5)
TOTAL	87 (100)	98 (100)	185 (100)

The combined ABO blood group phenotypes distribution in the 2 health facilities were as follows: O =162.2 %(n=115), A= 25.4% (n=47), B =19.7% (n=18) and AB= 2.7% (n= 5). The Rhesus blood group gave the following distribution pattern: O positive 60.0% (n=111), O negative 2.2% (n=4); A positive 23.2% (n=43), A negative 2.2% (n=4); B positive 9.2% (n=17), B negative 0.5% (n=1) and AB positive 1.6% (n=3), AB negative 1.1% (n=2). These gave a total of 174 (94.0%) Rhesus "D" positives and 11 (6.0%) Rhesus "D" negatives (Table 5). Chi-Square ($\chi 2$) analysis showed no significant difference in the distribution of HIV status and the different ABO and Rhesus blood types [$\chi 2=5.387$, df=7, P=0.364]. The differences merely reflected the population frequencies of those blood groups. The cross tabulation

of the Rhesus positive and negative individuals showed no association with HIV status.

The haemoglobin (Hb) electrophoretic patterns of the HIV-positive antenatal attendees revealed 79.0% Haemoglobin A (HbAA), 19.5% Haemoglobin AS, 1.1% Haemoglobin AC and 0.5% Haemoglobin S (HbSS) (Table 6). However, there was no correlation between HIV infection and maternal haemoglobin type (P>0.05). As shown in Table 7, a total of 14 babies representing 7.57% (14/185) were HIV-1 DNA PCR positive. This gave an overall mother-to-child transmission rate of 7.57%. Five (5) of the 14 babies representing 5.75% (n=87) were at BMSH while the remaining 9 (9.18%) were at UPTH (n=98). Student t-test did not show significant difference at both facilities (P>0.05). One hundred and seventy-one (92.43%) were HIV negative (82 at BMSH and 89 at UPTH). Except for two babies that were delivered by caesarean section (CS), every other baby was delivered vaginally.

Table 3 Frequency Distribution of HIV-Positive Antenatal Attendees According to Gestational Age at Booking

Gestational Age (in weeks)	Frequency (%)		
	BMSH	UPTH	COMBINED

≤ 12 (First Trimester)	12 (13.8)	11 (11.2)	23 (12.4)
13-24 (Second Trimester)	49 (56.3)	57 (58.2)	106 (57.3)
25-36 (Third Trimester)	26 (29.9)	30 (30.6)	56 (30.3)
TOTAL	87 (100)	98 (100)	185 (100)

Table 4 Frequency Distribution of HIV-Positive Expectant Mothers According to Anaemic (PCV < 30%) and Non-Anaemic (PCV ≥ 30%) Groups

Anaemic Status	**Frequency (%)**		
	BMSH (n=87)	**UPTH** (n=98)	**COMBINED** (n=185)
Anaemic (PCV<30%)	4 (4.6)	3 (3.1)	7 (3.8)
Non-Anaemic (PCV≥30%)	83 (95.4)	95 (96.9)	178 (96.2)

Table 5 Frequency Distribution of HIV-Positive Expectant Mothers According to ABO and Rhesus Blood Group Phenotypes at both UPTH and BMSH

Blood Group		Frequency (%) BMSH (n=87)	UPTH (n=98)	COMBINED (n=185)
A	Rh 'D' Positive	18 (20.7)	25 (25.5)	43 (23.2)
A	Rh 'D' Negative	3 (3.4)	1 (1.0)	4 (2.2)
B	Rh 'D' Positive	7 (8.0)	10 (10.2)	17 (9.2)
B	Rh 'D' Negative	1 (1.2)	0 (NIL)	1 (0.5)
AB	Rh 'D' Positive	1 (1.2)	2 (2.0)	3 (1.6)
AB	Rh 'D' Negative	1 (1.2)	1 (1.0)	2 (1.1)
O	Rh 'D' Positive	53 (60.9)	58 (59.2)	111 (60)
O	Rh 'D' Negative	3 (3.4)	1 (1.0)	4 (2.2)
TOTAL		87 (100)	98(100)	185(100)

[$\chi 2=5.387$, df=7, P=0.364(P>0.05)].

Table 6 Frequency Distribution of HIV-Positive Mothers According to haemoglobin (Hb) Types (Hb Electrophoretic Pattern)

Hb Types	Frequency (%)		
	BMSH (n=87)	UPTH (n=98)	COMBINED (n=185)
AA	67(77.0)	79(80.6)	146 (78.9)
AS	19 (21.8)	17 (17.4)	36 (19.5)
AC	1 (1.2)	1 (1.0)	2 (1.1)
SS	NIL	1 (1.0)	1 (0.5)
TOTAL	87 (100)	98 (100)	185 (100)

($\chi 2$=6.748, *df*=3, *P*=0.431).

Table 7 Incidence of HIV-1 DNA PCR-positivity Among HIV-exposed Babies at both UPTH and BMSH

Study Site	Total Number of HIV Positive Mothers	Number of HIV-Positive Babies	Number of HIV-Negative Babies
BMSH	87	5 (5.75%)	82 (94.25%)
UPTH	98	9 (9.18%)	89 (90.82%)
TOTAL	185	14 (7.57%)	171 (92.43%)

(P>0.05)

The incidence of HIV-1 DNA PCR-positive babies in relation to maternal gestational age at booking and commencement of antiretroviral therapy (ART) revealed that 16.1% (9/56) were from those who booked and commenced ART at

their third trimester of pregnancy (Table 8). This was followed by those who booked and commenced ART in their second trimester of pregnancy, 4.7% (5/106). While those who booked early and commenced ART in their first trimester of pregnancy had no HIV-1 DNA PCR-positive babies (zero percent, 0%). All the HIV-1 DNA PCR positive babies were tested at 6 weeks of postnatal life. There was a statistically significant difference between the gestational age at booking and HIV-1 infection in the babies ($\chi 2$=7.113, *df*=2, *P*<0.05).

Of the 14 HIV-1 DNA PCR positive babies 42.9% (6/14) were males, and the remaining 8 representing 57.1% were females, giving a male to female ratio of 1.0:1.3 (Table 9). Although not statistically significant (P>0.05), slightly more females than males were infected. In relation to blood groups (Table 10), almost all the HIV-1 DNA PCR-positive babies 92.9% (13/14) belong to blood group O Rhesus (D) positive. While the remaining one baby (7.1%) belongs to blood group A Rhesus (D) positive. There was no association between the babies' blood groups and HIV positivity (Student T-test =1.547; P=0.468).

In terms of haemoglobin (Hb) types (Hb electrophoretic patterns), 12 (85.7%) of the 14 HIV-1 DNA PCR positive babies had haemoglobin A (HbAA) and the other 2 babies (14.3%) had haemoglobin AS electrophoretic pattern (AS genotype). None of the HIV-positive babies had abnormal haemoglobin type (Table 11). There is no association between haemoglobin type and HIV-positivity (Student T-test=1.647; P=0.558).

Table 8 Incidence of HIV-Positive Babies (PCR-DNA Positive Babies) According to Mothers Gestational Age at Booking

Frequency (%)

Gestational Age (in weeks)	BMSH	UPTH	COMBINED
≤ 12 (First Trimester)	NIL n=12	NIL n=11	NIL n=23
13-24 (Second Trimester)	1 (2.0) n=49	4(7.0) n=57	5(4.7) n=106
25-36 (Third Trimester)	4(15.4) n=26	5(16.7) n=30	9(16.1) n=56
TOTAL	5(5.8) n=87	9(9.2) n=98	14(7.7) n=185

($\chi 2$=7.113, *df*=2, *P*<0.05).

Table 9 Frequency Distribution of HIV-Positive Babies According to Sex

Sex	Frequency (%)		
	BMSH (n=5)	UPTH (n=9)	COMBINED (n=14)
Male	3 (60)	3 (33.3)	6 (42.9)
Female	2 (40)	6 (66.7)	8 (57.1)
TOTAL	5 (100)	9 (100)	14 (100)

(P>0.05)

Table 10 Frequency Distribution of PCR-DNA HIV-Positive Babies According to ABO and Rhesus Blood Group Phenotypes

Blood Group		Frequency (%) BMSH (n=5)	UPTH (n=9)	COMBINED (n=14)
A	Rh 'D' Positive	1 (20)	NIL	1 (7.1)
A	Rh 'D' Negative	NIL	NIL	NIL
B	Rh 'D' Positive	NIL	NIL	NIL
B	Rh 'D' Negative	NIL	NIL	NIL
AB	Rh 'D' Positive	NIL	NIL	NIL
AB	Rh 'D' Negative	NIL	NIL	NIL
O	Rh 'D' Positive	4 (80)	9 (100)	13 (92.9)
O	Rh 'D' Negative	NIL	NIL	NIL
TOTAL		5 (100)	9 (100)	14 (100)

Student T-test=1.547; P=0.468

Table 11 Frequency Distribution of HIV-Positive Babies According to haemoglobin (Hb) types (Hb Electrophoretic Pattern)

Hb Types	Frequency (%) BMSH (n=5)	UPTH (n=9)	COMBINED (n=14)
AA	4 (80)	8 (88.9)	12 (85.7)
AS	1 (20)	1 (11.1)	2 (14.3)
AC	NIL	NIL	NIL
SS	NIL	NIL	NIL
TOTAL	5 (100)	9 (100)	14 (100)

(Student T-test=1.647; P=0.558)

Discussion

The overall 4.34% prevalence of HIV-1 infection found among the antenatal attendees in this study suggests that HIV infection is still a major public health problem in our environment, especially among women of reproductive age. Probable reasons for this prevalence may be unfaithfulness of either of the spouse to one another, low perception to risk, nonchalant attitude towards preventive measures, naivety of being infected and poverty in the community. Our current prevalence rate is not comparable to the 15.2% prevalence credited to Rivers state by the National Agency for the Control of AIDS; Nigeria (2) and World Health Organization (5)**.** The difference might be that our study made no attempts to cover the entire Rivers State. This notwithstanding, the study facilities are not only the biggest in the state but also with the largest turnout of antenatal attendees that cut across every social strata. Our current prevalence rate is also lower than some earlier reports within and outside Nigeria (7,-9). A comparable rate of 4.1% had been reported in Yenagoa (10), 3.0% in Port Harcourt (111) and **5.6**% in Tanzania (12). In contrast, several other studies have reported lower rates, 0.8% (13), 2.4% (14) and 0.3% (15)**.** These lower rates may suggest a steady decline in HIV-1 infection among pregnant women in general. While the different results may indicate differences in socio-cultural practices, level of poverty in the community among others. This corroborates the importance of establishing our own population based values and mount sensitization campaign on the need for attitudinal change towards reducing the spread of HIV/AIDS in the general populace.

This study had the highest HIV prevalence (59.5%) among 28-34 years age group. The reasons may be because this age group constitutes about 70% of the total

number of pregnant women worldwide and among the most sexually active. This finding is in agreement with many earlier studies in Nigeria (7,16) and elsewhere in Africa (12). The least prevalence rate (1.6%) occurred among 14-20 years age group. This is in line with a previous Tanzanian study (17) and a report from Ethiopia (18). However, our prevalence rate in this age group was much lower than what was reported by Kumurya and Sule (18) and Oladeinde *et al.,* (19). Low prevalence rate in this age group is in tandem with normal distribution pattern usually reported in generalized epidemic. No maternal age group was completely free from HIV infection.

Most of the antenatal attendees (87.6%) booked late for antenatal care (≥13 weeks gestational age) in this study (Table 3). Some previous studies (20,21) had also reported that greater number of pregnant women enrolled late for antenatal care. Clinical guidelines recommend that pregnant women should enter antenatal care within the first 10 to 12 weeks of pregnancy, ideally within 10 weeks. Our study found only 12.4% enrollment within the first trimester suggesting that early booking within the first trimester appears to be much less common in the general population. This finding is comparable to the 14% reported by Okunlola *et al.,* (22) and the 14.9% by Enabudoso and Obhielo (20) but lower than the 67.1% observed in Italy (23). Sustained public enlightenment campaign about the benefits of early antenatal booking, which include early diagnosis and management of pregnancy-associated disorders, and implementation of preventive measures against adverse pregnancy outcome should be established. Although this study did not investigate the possible reasons for late antenatal booking, it may include ignorance about the right time to start, limited information on antenatal care, financial constraints,

and no permission from spouse as well a busy work schedule (24). It could also be due to incessant strike actions by healthcare workers, especially in Nigeria (21).

The proportion of anaemic HIV-positive antiretroviral naïve expectant mothers in this study (3.8%) was lower than those reported by Ohihoin *et al.,* (25). The prevalence rate is inconsequential as it is within the generally accepted population reference range of anaemia among normal pregnant or non-pregnant women.

Our current study did not find statistically significant differences [$\chi 2=5.387$, df=7, P=0.364)] between the HIV-positive antenatal attendees and their various ABO and Rhesus blood types (Table 5). However, the predominance of blood group O may suggest the presence of certain intrinsic factors other than mere reflection of its natural distribution in the populace. In contrast to the findings of non-association in this study, some earlier works have reported that blood group "O" had maximum association for HIV (26, 27). In addition, Ravi and collaborators (28) had also observed that blood group O Rh-D positive patients were more susceptible to HIV infection. Abdulazeez *et al.,* (29) on the other hand, had reported that blood group AB individuals were more prone to HIV-1 than HIV-2 while HIV-1 prevalence was significantly higher among rhesus D positive subjects than their rhesus D negative counterparts. Another contradictory result was from Onsten *et al.,* (30) who reported a higher frequency of HIV infection in blood group B compared to non-B donors. On the other hand, Mohammadali and Pourfathollah (31) reported a significant association between HIV infection and blood group A. However, the mechanism(s) underlying those seeming associations have not been unraveled. According to Swedish scientists (32), the risk of being infected by HIV may be determined by the presence of the

carbohydrate based blood group moiety P^k. Their result shows that individuals with high P^k levels exhibited a greater natural resistance to HIV infection but the mechanism underlying it and which blood group has this polysaccharide and how much quantity is available in each of the ABO blood groups is not yet clear. More researches are therefore encouraged, especially at the molecular level to unravel the densities of CD4, chemokine co-receptors, cytokines and other antigenic determinants such as P^k as higher or lower densities of these receptors on the cells membrane may influence their susceptibility or resistance.

Test for association between haemoglobin type and HIV infectivity suggests a null relationship ($\chi 2$=6.748, *df*=3, *P*=0.431). This is similar to the findings of (27) but is in contrast to the findings of Buseri and Okonkwo (33), who reported association between haemoglobin SC and HIV-1 infection.

The 7.57% overall incidence of mother-to-child transmission in this study is similar to several other findings within and outside of Nigeria (8, 34) where mother-baby pairs received some form of antiretroviral therapy or prophylaxis for the prevention of mother-to-child transmission. While, our prevalence rate is higher than what was reported by Anoje *et al.,* (35), (36) and (37); it is lower than the findings of (33, 38, 39). In spite of this, our current rate is a far cry from the desired zero to less than 1% advocated for good PMTCT outcome worldwide. In Africa, geographical differences in the prevalence rate of MTCT of HIV have been linked to circulating viral genotypes in different areas with subtype B found predominantly in countries reporting lower MTCT rates and non-B subtypes and recombinant forms (CRFs) having a higher efficiency of MTCT.

Although the prevalence rates of 5.75% and 9.18% at BMSH and UPTH respectively were not statistically significant (P>0.05) it corroborates the fact that

mother-to-child transmission of HIV remains one of the most efficient and prominent ways of paediatric HIV transmission in Port Harcourt, Nigeria. These findings also give credence to the need to offer routine screening to all HIV-exposed newborns as recommended in the WHO guidelines (40).

All the HIV-DNA PCR positive babies were from HIV-positive expectant mothers who booked late for antenatal care in their second and third trimesters of pregnancy, of which, a significant difference ($P<0.05$) in the number of infected babies were found. This suggests that a timely entry into antenatal care with early commencement of ART (if indicated) is critical for suppression of vertical transmission.

This study observed slightly more infected female babies than males, though not statistically significant ($P>0.05$), which is in congruence with the findings of (35). Possible explanations to this sex ratio bias include Y-chromosome bearing sperms being faster but less resilient to unfavorable conditions in the mother's reproductive tract than X-chromosome bearing sperms which are slower but survive longer (41); spontaneous abortion may be biased towards males (42) and might in general be more common than abortions of female fetuses (43).

However, the ultimate explanations in sex ratio changes include the Trivers-Willard hypothesis, which suggests that if a female is in a poor condition, or of a low social status, it is beneficial to her to invest into the offspring sex that is less vulnerable (44). The reproductive success of male offspring, in a society where access to breeding partners is limited (through dominance hierarchies and male-male competition) tends to be more variable and resource sensitive. Some males are thus highly successful breeders while others are not.

Although females can benefit by investing into the offspring with higher reproductive variance, they will not be able to do so if they lack the resources. Therefore, vertebrate females subjected to physiological and psychological stress like HIV infection, or in worse body conditions gain a selective advantage by producing female offspring, since male offsprings are thought to be more costly to produce and raise and are less likely to attain a high social status and lifetime reproductive success if born to a stressed, subordinate female (45). Hence , physiologically stressed females (as in HIV infection), would be better off producing female offspring under conditions of stress, as daughters are more likely to survive than sons (46) and the reproductive success of daughters is usually not dependent on social status. If the HIV-positive babies are to survive to adulthood, the finding in this study of 57.1% females supports the finding that HIV infection is progressively feminized mostly in Africa (3).

In this study, 92.9% of the HIV DNA-PCR positive babies belong to blood group O Rhesus (D) positive (92.9%). This seeming association of blood group “O” with HIV infection is in line with some earlier works that reported maximum association between blood group “O” and HIV infection (26,27), whereas Buseri and Okonkwo (33) reported blood group A to be most predominant among HIV-positive infants. There is no clear evidence for the predominance of Blood group O over other blood groups. It is probable that the parents of the babies are homozygous for blood group O but the blood groups of the spouse (the biological fathers of the babies) were not tested. The 7.1% prevalence of blood group A among the HIV DNA-PCR positive babies in this present study is not in accordance with the 42.1% reported among HIV-positive infants in Sokoto, North Western Nigeria (33). This study did not find any blood group B and AB either Rhesus (D) positive or negative among the

babies and the reason for the absence is not clear. In this study there was no association between haemoglobin type and HIV-positivity (P>0.05). This is in contrast to Buseri and Okonkwo (33) who reported abnormal haemoglobin SC to be associated with HIV-positivity.

Despite full adherence to the ART by all HIV-positive pregnant women probably because of treatment simplicity some infants became infected probably because (a) the HIV transmission occurred before treatment, (b) inefficient suppression of maternal viral replication by ART, or (c) unique characteristics of the infecting maternal HIV strain, such as decreased susceptibility to zidovudine. One limitation of this study was our failure to carry out susceptibility testing of the isolates from these mother-infant pairs. This not withstanding high-level resistance to ART was unlikely, considering the relatively short duration of the maternal treatment, and the women were not exposure to ART before now. Another limitation was the failure to determine the maternal viral load to ascertain whether infection in these babies was due to poor or absent viral suppression. Future studies should address these limitations.

Our current incidence rate of vertical transmission calls for more concerted efforts to improve all limiting factors to reduce the rate to at least less than one per cent (<1%). Some PMTCT programs have achieved less than 1% of pregnancies experiencing vertical transmission. It is difficult to ascertain the level of success of this study due to absence of control groups and there were no previously established baselines in the two healthcare facilities to compare intervention outcomes. This study has provided for the very first time a platform to compare

or monitor the efficiency of the ART regimens and the general prevention of mother-to-child transmission of HIV services in the facilities.

Conclusion: High incidence of vertical-transmission of HIV was obtained especially among HIV-exposed babies whose mothers registered late for antenatal care. Therefore, strong advocacy for early entry into antenatal care is solicited.

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