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Early infant diagnosis of HIV-I infection using dried blood spots among children born to seropositive mothers in Federal Medical Centre (FMC) Lokoja

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Abstract

Early diagnosis of Human immunodeficiency virus (HIV) in infants provides a critical opportunity to strengthen follow-up of HIV- exposed children using dried blood spots and assure early access to antiretroviral treatment for infected children. This study aimed to determine the prevalence of HVI-1 infection in infants born to HIV-seropositive mothers. Early infant diagnosis of HIV sub-type I was carried out using on dried blood spots of 286 babies born to HIV-I seropositive mothers attending the Federal Medical Centre, Lokoja - Kogi State, Nigeria, between the months of July to December, 2013. Data obtained was analyzed using Gene Amp PCR System 9700. The overall rate of HIV-I vertical transmission from infected mothers to their babies was 14.5%. High transmission rates 63.5%was seen in babies whose mothers could not get any form of interventions with the least transmission rates seen in babies whose mothers either took HAART or were one form of ARV or the other (0 – 1.0%). Babies who took nevirapine as prophylaxis after delivery had lower rate (1%) of transmission. From the 30 women that mix-fed their babies, 6.7% transmission rate was recorded.Lack of antiretroviral drugs by HIV-I positive pregnant women was found to be associated with high rate of HIV-I transmission ($p<0.05$). Early intervention of mother to child transmission of HIV-1 infection using Highly Active Antiretroviral Therapy, exclusive breastfeeding practice as well as constant visit to Tertiary Hospitals for counseling and management of HIV infection reduced the rate of infection among the infants born to seropositive mothers.

Keywords:HIV; Highly Active Antiretroviral Therapy; Mother–to-Child Transmission; Exclusive Breastfeeding; Tertiary Hospital Visitation

1. Introduction

Mother-to-child transmission of HIV (MTCT) remains the most prevalent source of pediatric HIV infection. In 2010 alone, an estimated 390,000 children were infected with HIV, 90% of whom live in sub-Saharan Africa [1]. Pediatric HIV threatens to reverse gains made in controlling child mortality in African countries with high HIV seroprevalence. HIV infection accounts for more than 20% of child deaths in southern Africa compared with approximately 3% globally [2]. Established modes of HIV transmission globally include the following; (1) sexual contact (vaginal, anal, or orogenital); (2) percutaneous (from contaminated needles or other sharp instruments) or mucous membrane exposure to contaminated blood or other body fluids; and (3) mother-to-child transmission during pregnancy, around the time of labor and delivery, and postnatally through breastfeeding. In the absence of documented sexual transmission or parenteral or mucous membrane contact with blood or blood-containing body fluids, transmission of HIV rarely has

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been demonstrated to occur in families or households or as a result of routine care in hospitals or clinics. Transmission of HIV has not been documented in schools or child care settings[3]. In recent years the number of women accessing programs that aim to prevent mother-to-child transmission of HIV has steadily increased [4]. Most prevention of mother-to-child transmission (PMTCT) programs have concentrated monitoring and evaluation efforts on measuring process indicators such as acceptance rate of HIV testing and counseling or proportion of HIV-positive women provided with antiretroviral drugs. Many approaches – with their advantages and limitations – have been suggested to measure outcomes of PMTCT [5]. One of the indicators proposed to measure PMTCT outcomes is the number of "infant infections averted" that can also be interpreted as the rate of MTCT. Early infant diagnosis and interventions of antiretroviral therapy are the most important tools to reduce AIDS cases in children[6].

Humans are the only known reservoir of HIV, although related viruses, perhaps genetic ancestors, have been identified in chimpanzees and monkeys. Because retroviruses integrate into the target cell genome as proviruses and the viral genome is copied during DNA replication, the virus persists in infected people for life, [3].

UNAIDS/WHO (2011 reported an estimated 39.5 million (34.1 - 47.1 million) people living with HIV globally, with an estimated 4.3 million (3.6 - 6.6 million) new HIV infections and 2.9 million (2.5 - 3.5 million) deaths due to AIDS. The report further had it that out of this number of people living with HIV, 37.2 million are adults, out of which 17.7 million are women, while children under 15 years of age constitute 2.3 million.

Sub-Saharan Africa with just over 10% of the world's population is heavily affected by the HIV virus with over 60% of all the people living with HIV (24.7 million) while 2.8 million new cases were estimated to have taken place within the same period, (UNAIDS Epidemic update; December, 2011). Only India and South Africa have more people infected with HIV than does Nigeria where an estimated 2.9 million (1.7-4.2 million) people were living with the vims in 2005, also an estimated 4.4% infection rate among pregnant women was reported within the same year, (UNAIDS Epidemic update, December, 2011). Although HIV prevalence rates are much lower in Nigeria than in other African countries such as South Africa (32%), the size of Nigeria's population meant that by the end of 2005, there were an estimated 2,900,000 people living with HIV/AIDS. This is the largest number in the world after India and South Africa, (UNAIDS Epidemic update, December, 2011). The epidemic shows considerable variation, with state-wide prevalence ranging from as high as 10% in Benue (in the North Central zone) and 8% in AkwaImbom (South South zone) to under 2% in Ekiti, Oyo (both in the South West zone), and in Jigawa (North West zone). In some states, HIV prevalence among pregnant women is higher in rural than in urban areas, while in others the reverse is being found, (UNAIDS Epidemic update, December, 2011).

In the North central states, Jos has an estimated 8.5% among Antenatal women, [7].

This study aimed to determine the prevalence of HVI-1 infection in infants born to HIV-seropositive mothers in Federal Medical Centre (FMC) Lokoja, Nigeria.

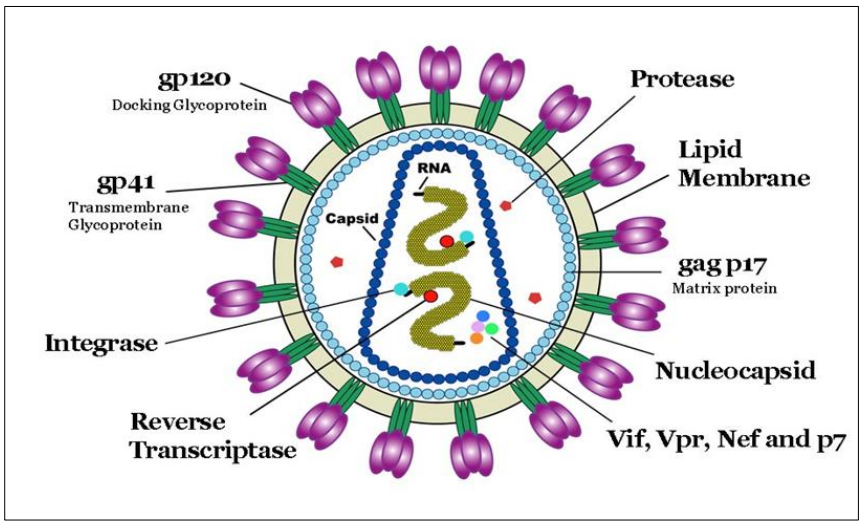

Figure 1 Structure of Human Immunodeficiency Virus (HIV) [8].

2. Material and methods

2.1. Study period and location

The study was carried out between the month of July to December, 2013. Samples were collected from Federal Medical Centre (FMC) Lokoja, Nigeria and while the analysis was done in Our Lady of Apostles (OLA) Hospital, Jos, Plateau State, Nigeria.

2.2. Sample size

The minimum sample size was obtained from Bennett's formula to calculate confidence limit as follows:

$$N = \frac{Z^2 pq}{d^2}$$

Where: N = Minimum sample size

z = Score at 95% Confidence limit

P = Local Prevalence rate (HIV is 4.0% = 0.51)

q = Complimentary prevalence (1-p)

d = level of precision to be adopted (0.05)

Substituting in the values into the above equation

$$N = \frac{(1.96)^2 \times 0.04\ (1 - 0.04)}{(0.05)^2}$$

The Minimum sample size = 59.0, however, a total of 268 samples were used for the study.

2.3. Inclusion criteria

All babies born to confirm HIV-1 seropositive mothers below the age of 18 months whose parents consented were recruited for the study.

2.4. Exclusion criteria

Babies born to confirmed HIV-1 seronegative mothers did not form part of this study.

2.5. Specimen collection

BDS samples were collected from infants born to HIV seropositive mothers. The samples were directly spotted onto DBS card from heel, big toe or finger prick depending on the age of the infants.

The DBS samples were air dried at room temperature. This can be stored at -20oC freezing point in an air tight bad contain silicate and descant.

Early infant diagnosis (EID) is recommended at age 6 weeks postnatal then at age 6 months and 12 months, 18 months to all HIV exposed infants.

2.6. Sample preparation, transportation and analysis

Infant dried blood spot (DBS) samples were collected using Lasec® collection kits with 5 spots and subsequently couriered to the OLA Hospital, Jos, Plateau State, Nigeria where the samples were analyzed with the centralized HIV deoxyribonucleic acid polymerase-chain reaction (DNA PCR) testing, using the Roche® brand of PCR machine.

3. Results

Table 1 Prevalence of HIV-I in Babies Born to HIV-I Positive Mothers in Lokoja according to Gender

Sex	No. Tested	No Positive	Percentage Positive
Male	132	5	3.8
Female	154	8	5.2
Total	286	13	4.5

$X^2 = 4.25$ $P>0.05$

In this study, various risk factors were considered including distribution of babies according to gender, place and mode of delivery and mode of feeding. Antiretroviral drugs intervention was also considered to be administered to mothers and babies or babies alone.

In this research, the number of children where 286 in comprising of males and females. Out of 286 children tested, 132 babies were males with transmission rate of (3.8%) and 154 babies were females with transmission rate of (5.8%) in table 1. There was no statistical significance (P>0.05) in the distribution of HIV-1 among babies of different genders. This study is similar to the finding in the study conducted at Lagos in Nigeria, where there was no significance difference between the male and female according to gender [9].

Table 2 Antiretroviral drug combinations administered to HIV-I positive mothers and infants HIV-I status

Drug Combination	No. Tested	No Positive	Percentage Positive
None	144	12	8.3
AZT, 3TC, NVP	58	0	0
TDF, 3TC, EFV	25	0	0
TDF, 3TC, NVP	18	0	0
A3T, 3TC, EFV	12	0	0
A3T, 3TC, LPV/r	6	0	0
NVP alone	23	1	4.3
Total	286	13	4.5

$X^2 = 71.0$ $p<0.05$

Table 2 shows antiretroviral drugs combination administered to the HIV-I seropositive mothers either before pregnancy or during labour and delivery. A transmission rate of 12 (8.3%) was observed out of 144 women who did not take any form of antiretroviral. Those that were on nevirapine (NVP) single dose were 23 and the transmission was(4.3%).

Fifty-eight women were on a form of highly active antiretroviral drugs (HAART) AZT (Zidovudine), ZTC (Lamivudine) and NVP (Nevirapine). Transmission was not observed. Twenty-five women were on three drugs therapy, TDF (Tenofovir), ZTC (lamivudine), EFV (Efaverenz). Transmission was not observed, eighteen women were on another three drugs therapy, TDF (Tenofovir), ZTC (Lamivudine), NVP (Nevirapine) no transmission was observed, twelve women were on another combination of therapy, AZT (Zidovudine), ZTC (Lamivudine), EFV (Efaverenz), no transmission was observed. Another six women were on three drugs combinations. AZT (Zidovudine), ZTC (Lamivudine), PI/r (Ritonavir), no transmission was observed. The prevalence of HIV-1 infection among mothers who were not on any form of antiretroviral therapy regime was higher. This study is similar to the finding in the studies conducted at Lagos in Nigeria and Zambia (Anoje*et al.,* 2011[9] and Mnyani*et al.,* 2009)[10] respectively. Therefore, there was statistical significance (*$p<0.05$*) in the combination of drugs administered.

Table 3 Nevirapine single dose Administration to Babies born to HIV-I positive mothers and their HIV-I status

Nevirapine administered	No. Tested	No Positive	Percentage Positive
None	182	12	6.6
Yes	104	1	1.0
Total	286	13	4.5

Table 3 shows Nevirapine single dose administered to babies born to HIV-I positive mothers and their HIV-I status. A total of 182 babies were not privileged to have the nevirapine single dose antiretroviral drug. Out of this (6.6) were positive for HIV-I.on the other hand 104 babies were administered the NVP single dose as prophylactic antiretroviral after birth out of which (1.0%) of them had HIV-I infection. None taking of NVP single dose by babies born to HIV-I positive mothers the transmission rate was higher. There was statistical significance (P<0.05) among babies who were not in any form of antiretroviral drugs than who were on drugs. This study is similar to the finding in the studies conducted at Lagos in Nigeria and Zambia [9] and [(10].

Table 4 Place of delivery of HIV-I positive mothers and HIV-I status of their infants

Place of Delivery	No. Tested	No Positive	Percentage Positive
Home	46	5	10.9
PHC	32	4	12.5
Private Hospital	66	2	3.0
Secondary Hospital	51	1	2.0
Tertiary Hospital	73	1	1.4
Total	286	13	4.5

$X^2 = 8.4$ $p>0.05$

Table 4 shows the various places of delivery and the infant HIV-I status. Forty-six of the women most of whom did not take any antiretroviral medication delivered at home out of which (10.9) transmission rate was seen. Thirty-two of the women had their babies in the PHC centre, 4 (12.5) transmission rate was recorded. Sixty-six women accessed health care for their delivery at the private health centre out of which (3.0%) acquired the virus from their mothers. Fifty-one of the women delivered in the secondary health care and (2.0%) transmission rate was observed. Seventy-three women delivered in the tertiary health care centre and (1.4%) transmission rate was observed. The percentage transmission rate was high in deliveries at home (10.9%) while deliveries at home (10.9%) while deliveries at the tertiary health services centres recorded the least percentage transmission rate (1.4%). In this research, there was no statistical significance (*p>0.05*) among the places of delivery.

Table 5 Mode of delivery of HIV-I positive mothers and their infants' HIV-I status

Mode of Delivery	No. Tested	No Positive	Percentage Positive
Elective C/S	78	0	0
Emergency C/S	55	4	7.3
Spontaneous vaginal delivery	135	9	6.7
Total	286	13	4.5

$X^2 = 11.8$ *p<0.05*

Table 5 reflects the various modes of delivery and the infant HIV-I status. Seventy-eight women delivered through scheduled caesarean section and out of this(1.3%) were positive for HIV-I, fifty-five women delivered through emergency caesarean section, (5.5%) of them transmitted HIV-I virus to their new born babies. Majority of the women who delivered through spontaneous vaginal delivery were one hundred and thirty-five with a transmission rate of

(6.7%). Women who delivered through scheduled caesarean section had less transmission rate compared to other deliveries. Higher transmission rates were associated with spontaneous vaginal deliveries (6.3%) and emergency caesarean section (7.3%) as a result of lack of antiretroviral drug intervention. In this study, there was statistical significance (*p<0.05*) among modes of delivery.

Table 6 Mode of feeding of Babies Born HIV-I positive mothers and HIV-I status of the infants

Mode of feeding	No. Tested	No Positive	Percentage Positive
Breast milk substitute	45	8	17.8
Exclusive breast milk	193	3	1.6
Mix feeding	30	2	6.7
Total	286	13	4.5

Table 6 presents the various modes of feeding exhibited by the cohort of women in this study. Most women opted breast milk substitute (BMS) and least transmission rate was recorded in this group 8 (17.8%) when compared to transmission in other modes of feeding. One hundred and ninety-three mothers were found to have practiced exclusive breastfeeding to the babies. Transmission rate was (1.6%). There was no statistical significance (P>0.05) among the modes of feeding in this study. But according to [9] and [10] there was significance difference in mode of feeding.Exclusive breast feeding was found to have contributed immensely to the rate of HIV-I vertical transmission. Thirty women mixed fed for their new born babies, transmission rate was (6.7%).

4. Discussion

This study shows an overall HIV-I transmission rate of 4.5% among the cohort of women attending the Federal Medical Centre (FMC) to access antiretroviral drugs either for themselves or their babies. Although it was reported that the rate of HIV-I vertical transmission from infected mothers to their new born can be as high as 30 – 45%[9] and[10], this refers to situations where intervention is not instituted at all, [11] The transmission rate from those who did not take any antiretroviral drug in this study is (8.3%).

From the result obtained, there was no significant difference in the transmission between the exposed male and the female babies.

The use of antiretroviral drugs and scheduled caesarean section were the most significant factors that had an impact on transmission rate, (P<0.05). Thus, when HAART is administered and the baby is delivered by scheduled caesarean section the chances of HIV-I transmission from mother-to-child is very low. Least transmission rates were observed in those women who were on highly active antiretroviral (HAART) drugs (nevirapine, zidovudine, lamivudine). 0% shows that pregnant women HIV-I positive mothers who are on HAART stand the chance of not transmitting the virus to their infants. The results above fall in line with that of [9] who reported an estimated transmission rate of 30 – 45 amongwomen in Cross-River and Akwa Ibom who had no drug intervention while others who were on HAART and other antiretroviral regiments thus recording a transmission rate of 0 – 3.4%.Torpey K. *et al.,* 2011 [12] and Anoje C.*et al.,* 2012 [9] reported a transmission rate of 0% in women who received antiretroviral drug during pregnancy and delivered by scheduled caesarean section,thus drug intervention and caesarean section delivery are very vital in lowering transmission rates as was observed in this study.

Nevirapine single dose prophylactic treatment administered to the newly born infant also shows a positive impact in reducing the transmission rate. 1 0f 104 (1.0%) as against that seen in those who did not take any, 12 of 182 (6.6%). Transmission rate in the NVP group is similar to the finding reported by[10] in ZAmbia, then Nigerian National PMTCT pilot, 11.0%[9] and Chama *et al.,* (2012) [13] who reported a transmission rate of 65% in Cross-River. NVP single dose prophylactic administration is just being used in this environment and resistance testing has not been done hence this could have been the reason why the transmission rate was high. These HIV-I positive mothers gave birth to their babies in various places and these seems to be variations in transmission rates according to these different places. Forty-six of the women most of whom did not take any antiretroviral medication delivered at home out of which 10.9% transmission rate was seen. Thirty-two of the women had their babies issue the primary health care centres (12.5%) transmission rate was recorded. Sixty-six women accessed health care for their delivery at the private health centre out of which 3.0% acquired the virus from their mothers. Fifty-one 51 of the women delivered in the secondary health care centre

and 2.0% transmission rate was observed. Tertiary health care centre had the highest record of deliveries, seventy-three and transmission rate was 1.4%. The percentage transmission rate was higher in deliveries at home (10.9%), while deliveries at the tertiary health service centre recorded the least percentage transmission rate (1.4%).This could be as a result of certain procedural interventions during deliveries such as avoidance of invasive techniques rendered by well trained personnel who are mostly found in the tertiary than the other health delivery services in this setting.

From this study, mode of delivery has also been found to have an impact on vertical transmission rate of HIV-I virus from mother-to-child. Elective C/S recorded 0% transmission rate, emergency C/S (7.3%) and spontaneous vaginal delivery (6.7%). It is on record that 15 – 25% of exposed babies will acquire the infection during the process of labour and delivery if no interventions are taken (Anderson, 1997). [14] Most babies who become infected during this period do so by sucking, imbibing or aspirating material blood or cervical secretions that contained HIV, [15] Factors such as duration of membrane rapture, acute chorioamnionite, and invasive delivery techniques that breach the baby's and or mother's skin thus increasing the infant's contact with the mother's blood have been associated with higher risks MTCT during labour and delivery, (WHO, 1990).(16) Deliveries through scheduled elective caesarean section before the onset of labour and rapture of membranes, indicated a very high significant effect in reducing transmission rate reducing transmission rate to (0%). This agrees with the report of [17] , [9] who separately reported a lower transmission rate in elective C/S when compared to other delivery methods and recommended elective C/S as the most important for risk reduction in women with detectable viremia before the expected delivery date. Important points to note include: accurate assessment of gestational age, necessary infrastructure and staffing both for the procedure itself and for subsequent material and neonatal care. Transmission rate was high (7.3) in women who delivered through emergency caesarean section. For emergency C/C have to be carried out on each of these women, either labour should have started which implies that the new born would have been probably exposed to the mother's blood and fluid which may translate into higher risk of infection or a problem could have occurred that led the gynecologist to opt for emergency C/S.Out of 135 deliveries through spontaneous vaginal delivery, 9 (6.7%) of the babies were HIV-I positive. This confirms the result of earlier study reported a transmission rate during labour and delivery 10 – 25%[18] and [6] Vaginal delivery have been recommended in situations where there has been prenatal care throughout the period of pregnancy, the viral load is undetectable i.e. less than 1000 copies/ml at 36 weeks of pregnancy, [9], [9] and [6]. Various modes of feeding were emphasized by different women and transmission rate also varied according to these various modes of feeding is an indication that there could have a substantial transmission during delivery and postpartum period. Risk of MTCH through breast milk is said to be depended on a number of factors,[19] pattern of breastfeeding whether exclusive or mix breast health (mastitis cracked nipple inflammation etc.) breastfeeding during whether prolonged short, maternal viral load, maternal immune status and maternal nutritional status.

Out of 45 women that opted for breast milk substitute, 17.8% were positive. Furthermore, of the 193 women that practiced exclusive breastfeeding, 1.6% of the babies tested HIV positive (Table 6). This was similar to the study report byMselati P.*et al.,* 1995[20] and Isaac *FA et al.,* 2011,[15] that also reported that transmission through breast milk accounts for 10-25% infection.

Our study further revealed that xix feeding had transmission rate of 2 (6.7%). Mix feeding possess greater danger of mother-to-child transmission of HIV, this occurs because mix feeding often lead to diarrhea diseases which enhance transmission from infected breast milk through the already inflamed villi of the intestinal wall as a result of the diarrhea. There was no statistical significance (P>0.05) according to this study.

5. Conclusion

Early intervention of mother to child transmission of HIV-1 infection using Highly Active Antiretroviral Therapy (HAART), exclusive breastfeeding practice as well as constant visit to Tertiary Hospitals for counseling and management of HIV infection reduced the rate of infection among the infants born to seropositive mothers.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors hereby declare no conflict of interest.

Statement of ethical approval

Approval/clearance for this research dated 17th October, 2013 was obtained from the Ethical Committee of the Federal Medical Centre Lokoja, Kogi State, Nigeria.

Statement of informed consent

Informed Consent forms were signed by the mothers of the infants used in this study.

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