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Early Infant Diagnosis of HIV Infection in Southeastern Nigeria: Prevalence of HIV Infection Among HIV-Exposed Babies

La Première Diagnose de Bébé de VIH Infection dans le Nigeria Au sud-est : la Prédominance de VIH Infection Parmi les Bébés VIH exposés

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ABSTRACT

BACKGROUND: **Vertical transmission of HIV-1 is responsible for a high level of infant mortality necessitating early infant diagnosis. Serologic tests are not useful because of persistence of maternal antibodies in infants. Amplification of the integrated viral genome by PCR is the preferred method of diagnosis of HIV infection in these children.**

OBJECTIVE: **To determine the prevalence of HIV among a cohort of HIV-exposed babies.**

METHODS: **HIV-exposed infants were recruited for DNA PCR (early infant diagnosis). Babies were enrolled from six weeks of age. Relevant data were collected with the aid of a proforma. Mothers were given pre-test counselling. Heel or finger prick samples of blood on Whatman filter paper were used for DNA PCR testing.**

RESULTS: **Data on the initial 304 babies enrolled for DNA PCR were analyzed. Seven (3.6%) of 192 mother-baby pairs who had received requisite prophylactic anti-retrovirals (PARV) were PCR-positive. In 23 (8.7%) PCR-positive babies, their mothers received PARV but the babies had no post-exposure prophylaxis (PEP), while two (12.5%) of 16 babies who had received PARV without their mothers turned out PCR-positive. Thirty-nine (53.4%) of 73 mother-baby pairs who had no PARV were infected. Exclusive breastfeeding (EBF) rate was 35.5%. In these babies five (18.5%) were infected, while 288 (75%) of babies were exclusive formula fed (EFF), out of which 11 (4.8%) were infected. Forty-seven (15.5%) of the babies were mixed-fed, and 32 (68.0%) of them were infected.**

CONCLUSION: **Prophylactic ARV in mothers and babies gave a marked reduction in Mother-to-Child-Transmission (MTCT) rate. Feeding BMS conferred a superior protection against (MTCT) than EBF. WAJM 2009; 29(1): 3–7.**

Keywords: **Early infant diagnosis of HIV, DNA PCR, dried blood spot.**

RÉSUMÉ

CONTEXTE: *la transmission verticale de VIH 1 est responsable d'un haut niveau de mortalité de bébé nécessitant la première diagnose de bébé. Les épreuves de Serologic ne sont pas utiles à cause de la persistance d'anticorps maternels dans les bébés. L'amplification du génome viral intégré par PCR est la méthode favorisée pour la diagnose de VIH infection chez ces enfants.*

OBJECTIF: *déterminer la prédominance de VIH parmi une cohorte de bébés VIH exposés.*

MÉTHODES: *les bébés VIH exposés ont été recrutés pour l'ADN PCR (la première diagnose de bébé). Les bébés ont été inscrits de six semaines d'âge. Les données pertinentes ont été recueillies à l'aide d'un proforma. On a donné le fait de conseiller pré-d'essai aux mères. Le talon ou les échantillons de piqûre de doigt de sang sur Whatman pénètrent le papier ont été utilisés pour l'ADN la mise à l'essai de PCR.*

RÉSULTATS: *les Données sur les 304 bébés initiaux se sont inscrites pour l'ADN PCR ont été analysés. Sept (3.6 %) de 192 paires de bébé-mère qui avaient reçu anti-retrovirals prophylactique requis (PARV) étaient PCR-positifs. Dans 23 bébés PCR-positifs (de 8.7 %), leurs mères ont reçu PARV mais les bébés n'avaient aucune prophylaxie de post-exposition (l'ALLANT), pendant que deux (12.5 %) de 16 bébés qui avaient reçu PARV sans leurs mères a retourné PCR-positif. Trente-neuf (53.4 %) de 73 paires de bébé-mère qui n'avaient aucun PARV ont été infectés. Le taux (EBF) allaitant exclusif était 35.5 %. Dans ces bébés cinq (18.5 %) ont été infectés, pendant que 288 (75 %) de bébés étaient (EFF) nourri de formule exclusive, dont 11 (4.8 %) ont été infectés. Quarante-sept (15.5 %) des bébés ont été mélangé-mangés et 32 (68.0 %) d'entre eux ont été infectés.*

Conclusion : ARV prophylactique dans les mères et les bébés a donné une réduction marquée de la 'mère à la transmission d'enfant' (MTCT) le taux. L'Alimentation de BMS a conféré une protection supérieure contre (MTCT) qu'EBF. ***WAJM 2009; 29 (1) : 3–7.***

Mots clé : *la première diagnose de bébé de VIH, l'ADN PCR, a séché la tache de sang.*

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Abbreviations: BMS, Breast milk substitutes; **DBS,** Dried blood spots; **DNA,** Deoxyribonucleic acid; **EBF,** Exclusive breastfeeding; **EFF,** Exclusive formula feeding; **HIV,** Human immunodeficiency virus; **MF,** Mixed feeding; **PARV,** Antiretroviral [Prophylaxis]; **PCR,** Polymerase chain reaction; **PEP,** Post-exposure prophylaxis; **PMTCT,** Prevention of mother-to-child transmission; **RNA,** Ribonucleic acid.

INTRODUCTION

Vertical transmission is the main source of human immunodeficiency virus (HIV) infection in children with an estimated 2000 vertically-acquired HIV infections occurring daily globally, mostly in sub Saharan Africa, Eastern Europe and Central Asia.[1] Effective interventions for Prevention of mother to child transmission (PMTCT) of HIV infection exist and where freely available, MTCT rates of 1–2% are achievable.[2] Morbidity and mortality rates for HIV type 1 (HIV-1) vertically exposed infants remain unacceptably high on the African continent.[3, 4]

In order that timely care be given to preserve the immune system of the affected infants, access to an early and accurate diagnosis of HIV infection, must be made available to all infants born to HIV seropositive mothers (HIV-exposed infants). Early diagnosis of HIV-1 infection in these infants has proved difficult with conventional serologic (antibody) tests. This is due to the fact that such infants often harbor transplacentally transferred maternal antibodies for up to 15 months of age.[5]

Virologic tests such as virus cultures, and RNA or DNA PCRs aid early diagnosis. However, virus cultures are time-consuming, require a biosecurity laboratory, and have a poor sensitivity. [6] The diagnostic gold standard laboratory test for HIV-1 infection is the HIV-1 qualitative DNA PCR. [7]

Screening technology using dried whole blood spots , used for over 50 years to screen for metabolic disorders in neonates, has been successfully extended to PCR-based detection of human immunodeficiency virus.[7] Since 1991, Dried Blood Sports (DBS) have been used for the detection of HIV-1 genome by PCR with good sensitivity and specificity. [8] Dried blood spots on filter paper facilitate the collection, transport, and storage of blood samples for laboratory use, reducing the need for venous blood sampling and refrigeration of blood samples.

This paper is the first of a series describing infant outcomes using the DBS technique for early infant diagnosis of HIV. This study was carried out to determine the prevalence of HIV among HIV-exposed babies delivered in and referred to Nnamdi Azikiwe University Teaching Hospital, Nnewi, for care.

SUBJECTS, MATERIALS, AND METHODS

The Nnamdi Azikiwe University Teaching Hospital, Nnewi (N.A.U.T.H.) is supported by the U.S. Presidential Emergency Program for AIDS Relief (PEPFAR) through the auspices of the Institute of Human Virology, Nigeria, and is one of the Federal Government primary ARV Centres.

DNA PCR testing in N.A.U.T.H. commenced on 20th March 2007. This paper analyzed the HIV infection status of the initial 304 HIV-exposed babies recruited for diagnosis by the DBS method. Two consecutive concordant results obtained at designated time intervals, (depending on age at presentation), were required to determine infection status of any child. No discordant results were encountered.

Sources of recruitment of the babies included:

- N.A.U.T.H. labour ward and Special Care Baby Unit. PMTCT services are provided in N.A.U.T.H. and its out-station comprehensive health centres in Umunya, Ukpo, Neni, and Oba, all in Anambra state, Southeast Nigeria. Babies are referred to the hub at age six weeks.
- PMTCT sites in government primary and secondary health care facilities in Anambra State and beyond (other than those above).
- PMTCT sites in mission and private hospitals in Anambra State of South-Eastern Nigeria.
- Exposed babies of HIV-positive mothers whose status was discovered post delivery (Referrals from N.A.U.T.H. free counseling and testing centre).

Inclusion criterion used was that babies must be aged at least six weeks at the first PCR test. All children more than 17 months of age were excluded and sent for serologic tests. All mothers were pre-test counseled and a proforma completed to collect data on date of birth, age and sex of baby, source of referral, HIV status of parents, ARV prophylaxis given to mother, mode of delivery of baby, post-exposure prophylaxis given to baby, infant feeding details since birth and suggestive symptoms of HIV infection in the baby.

Oral informed consent was obtained from the mothers after counseling before blood samples were taken from the babies. Ethical approval was duly obtained from the hospital's Ethnics Committee.

All mothers who received prenatal care in NAUTH were placed on highly active antiretroviral therapy (HAART). Women diagnosed in labour received single dose (200mg) nevirapine. In the peripheral centres linking up to NAUTH, mothers received zidovudine from gestational age of ≥28 weeks till delivery, and single dose (200mg) nevirapine in labour. All exposed babies who received post-exposure prophylaxis had single dose nevirapine (2mg/kg body weight) and six weeks of zidovudine (4mg/kg twice a day). A baby was said to be mixed-fed if he/she was concurrently fed breast milk and other feeds especially infant formula.

The categorization and management of the babies are outlined in Figure 1.

The thumb or forefinger or heel of the child was wiped with alcohol swab and allowed to dry completely. A lancet was placed on the selected finger or heel and the trigger depressed to make a skin puncture. The first drop of blood was wiped away as it contained tissue fluids. A gentle pressure was applied to collect the next few drops of blood to fill five circles on Whatman filter paper (903) for each child. The dried blood spot (DBS) cards were placed horizontally on a drying rack for about three hours.

Viral DNA was assayed by the method of the Roche Amplicor HIV-1 DNA Amplication test version 1.5. [9]

Data analysis was done with the software SPSS version 11.5 and the Chi-square statistic was used with 0.5 level of significance.

RESULTS

Table 1 outlines the DNA-PCR results according to the age and sex distribution of the children. Fifty (16.4%)

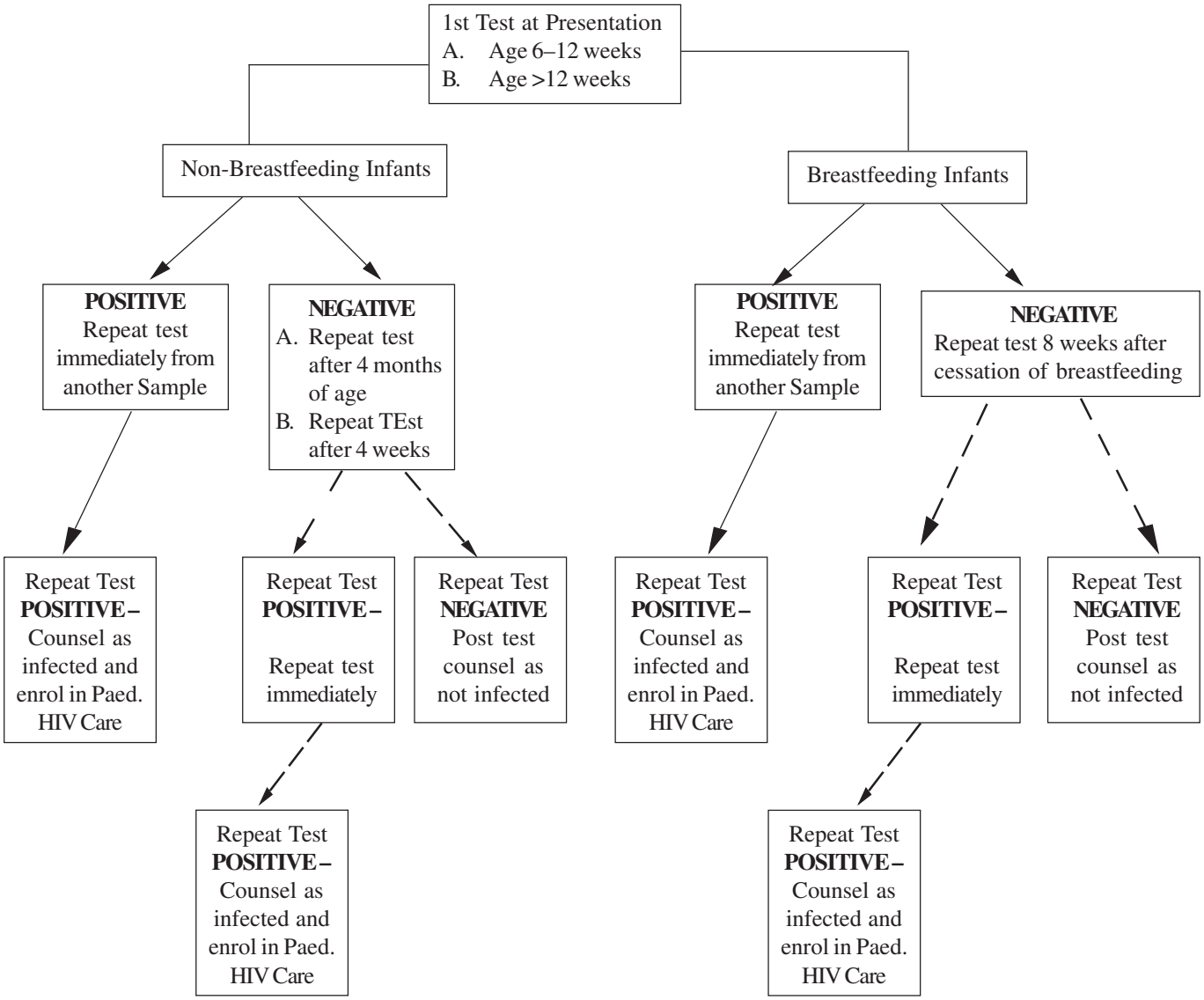

Figure: Flow Chart for Infant and Young Child HIV Diagnosis with DNA PCR

out of the 304 children were PCR-positive.

Four categories of babies were recognized:

i. One hundred and ninety-two mother-baby pairs received ARV prophylaxis. Seven babies in this category turned out PCR-positive (3.6%). These were the true PMTCT failures. However, one baby in this group had received PEP late (beyond 72hours of life), while another converted from PCR-negative (in the first test) to positive (in the second) after cessation of breastfeeding.

ii. Twenty-three mothers received ARV prophylaxis, while their babies had no PEP. Two of these babies turned out PCR-positive (8.7%).

iii. Sixteen babies received PEP, while their mothers had no ARV prophylaxis. Two of these babies turned out PCR-positive (12.5%). In this category one baby had received PEP beyond 72hours of age.

iv. Seventy-three mother-baby pairs were not opportuned to get ARV prophylaxis. In this group 39 babies were PCR-positive (53.4%).

Forty-seven babies (15.5%) were mixed-fed (i.e. given breast milk and infant formula ± cereals). Thirty-two (68.0%) of them turned out PCR-positive. Twenty-five percent (76 out of 304) of babies were breastfed. Of this number 39(51.3%) became PCR-positive. Two hundred and twenty-eight babies (75.0%) were never breastfed, out of which 11 (4.8%) were PCR-positive.

Only 35.5% (27 out of 76) of babies were exclusively breastfed. Five (18.5%) of exclusively breastfed babies were found to be PCR-positive.

Among the PCR-negative babies born to mothers who received ARV prophylaxis none was mixed-fed. (See Table 2). Among the PCR-negative children, those mixed-fed were drawn from the babies who did not receive any ARV prophylaxis.

Table 1: HIV-1 DNA PCR positivity according to Sex and Age Groups of Babies

Final PCR Result			Age at Diagnosis (Mothers) in Months			
			≥ 6	6.1– 12	> 12	Total
Negative	Sex	F	97	19	12	128
		M	94	21	11	126
	Sub-total		191(62.8%)	40(13.2%)	23(7.6%)	**254(83.6%)**
Positive	Sex	F	10	11	3	24
	M	9	8	9	26	
	Sub-total		19(6.3%)	19(6.3%)	12(3.9%)	**50(16.4%)**
	Total		**210**	**59**	**35**	**304**

Table 2: Mixed Feeding and HIV Infection Status of Babies

PCR Result	Mixed Feeding in the babies		Total	P value
Negative ARV prophylaxis to mother				
No	34(70.8%)	14(29.2%)	48	0.000
Yes	206(100.0%)	0(0.0%)	206	
Total	**240(94.5%)**	**14(5.5%)**	**254**	**0.209**
Positive ARV prophylaxis to mother				
No	14(32.6%)	29(67.4%)	43	
Yes	4(57.1%)	3(42.9%)	7	
Total	**18(36.0%)**	**32(64.0%)**	**50**	
Negative PEP to baby				
No	39(73.6%)	14(26.4%)	53	
Yes	201(100.0%)	0(0.0%)	201	
Total	**240(94.5%)**	**14(5.5%)**	**254**	
Positive PEP to baby				
No	13(31.0%)	29(69.0%)	42	0.214
Yes	4(66.7%)	2(33.3%)	6	
Yes but beyond 72hrs		1(50.0%)	1(50.0%)	2
Total	**18(36.0%)**	**32(64.0%)**	**50**	

DISCUSSION

It remains unclear why some infants become infected while others do not, despite significant exposure to HIV-1 *in utero,* during delivery and while breastfeeding. The rates of mother-to-child transmission of HIV vary by geographical locations ranging from 7% in Europe [10] to 40% in Africa and 48% in India [11] prior to maternal antiretroviral (ARV) prophylaxis. In this study a little over half of the HIV-exposed babies got infected among mother-baby pairs not opportuned to receive ARV prophylaxis. This compares with an overall MTCT rate of 45% observed by a study in Ibadan in Southwestern Nigeria.[12]

Prevention relies on reducing maternal HIV-1 levels via the use of ARV drugs in pregnancy and delivery and ARV prophylaxis to the infant. ARV drugs reduce viral replication and can reduce mother-to-child transmission of HIV either by lowering plasma viral load in pregnant women or through post-exposure prophylaxis in their newborns.[13]

The Roche Amplicor assay has been found to have a sensitivity of 100% and a specificity of 99.6%.[14] Sensitivity of DNA PCR increases with age, being low at birth and rising to 100% when assayed between the ages of two – six months,[15] hence the choice of age four months for the second PCR test.

Prevention of mother-to-child transmission of HIV in the United States and Europe has been a tremendous success, such that transmission rates of less than 2% have been achieved.[16] A study in Côte d'Ivoire [17] evaluating a two-tiered approach to use of ARVs in pregnant mothers (either HAART or short-course ARV PMTCT regimens) gave an overall rate of peripartum HIV transmission as 2.2%. In Kano, North central Nigeria, a study [18] carried out to evaluate the interventions offered to HIV-positive women at the Aminu Kano Teaching Hospital, gave a prevalence rate of 2.54% and 96.0% of the babies received breast milk substitutes.

The MTCT rate of 3.6% among mother-baby pairs who had received ARV prophylaxis in this study is higher than what was obtained in Kano probably because 75.0% of our babies (as against their 96.0%) received breast milk substitutes. The overall transmission rate for women on HAART in Maiduguri in Northeastern Nigeria was 9.1% while those who received single-dose nevirapine in labour were 33.0%. [19] This differed appreciably from findings in Lagos, southwest Nigeria [20] where the rate of transmission of HIV was 11.0% among women who had some intervention and 30.0% for those without any intervention. In the latter two cases, details of infant feeding were not given. It must be noted that infant feeding has a pivotal role in PMTCT.

Breastfeeding is a major health-promoting factor in infants and children in developing countries, but the risk of MTCT of HIV by this route is challenging traditional and health policies in low resource countries. The rate of mixed feeding is high and so the risk of MTCT is increased. [21] This lends credence to our finding of an overall MTCT rate of 51.3% among breastfed babies, and 68.0% among mixed fed babies.

Exclusive breastfeeding carried a significantly lower risk of HIV transmission than mixed feeding and a similar risk to no breastfeeding in Durban, South Africa. [22] This was the case among exclusively breastfed babies in this study, with a transmission rate of 18.5%. However, when compared to exclusively

breastfed babies, those who were never breastfed had a MTCT rate of just 4.8% suggesting that use of breast milk substitutes may be preferable.

Another study in Durban, South Africa showed a stepwise increase in the transmission rate of HIV with duration of exclusive breastfeeding of one to three months.[23] Breastfed infants who concurrently received solids and or infant formula were significantly more likely to acquire infection than were exclusively breastfed children. [24] Sixty-eight percent of our babies in this category became infected.

Conclusion

The DBS DNA PCR assay for HIV-1 is invaluable in early diagnosis of HIV in babies born to HIV-positive mothers. Antiretroviral prophylaxis in mothers and babies gave a 15-fold reduction in MTCT rate. Feeding breast milk substitutes conferred a superior protection against MTCT than exclusive breastfeeding. Exclusive formula feeding is advocated, in view of the very low exclusive breastfeeding rate observed with concomitant high mixed feeding rate and attendant unacceptably high MTCT rate.

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