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EARLY INFANT DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION USING DRIED BLOOD SPOTS AT A TERTIARY CARE HOSPITAL IN RIVERS STATE, NIGERIA

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ABSTRACT

Children born to HIV-positive mothers are likely to acquire maternal antibody which has made early infant detection of HIV challenging, using conventional screening methods. This study was aimed to provide early diagnosis of HIV infection in infants born to infected mothers using molecular method in comparison to the screening method. This study covered 134 infants born to HIV-sero-positive mothers attending antenatal clinics at the Braithwaite Memorial Specialist Hospital (BMSH) in Port Harcourt, Nigeria, between October 2016 and September 2017. Following ethical approval and informed consent, the infants Sero-status was determined using the National serial screening algorithm to identify anti-HIV-1 and anti-HIV-2 antibodies in the blood. Concordance positive test was regarded as positive; while discordant results were resolved using the third test kit as tie breaker. Early infant detection (EID) of viral DNA using dried blood spots (DBS) samples was performed using Roche COBAS ® Ampliprep/COBAS TaqMan HIV-1 Quali Test instrumentation. Of the 134 infants, aged between 6 weeks and 18 months tested, 52 were males and 82 were females. 76 (56.7%) tested sero-positive with the rapid diagnostic test (RDT) while 16 (11.9%) tested positive with the polymerase chain reaction (PCR) method ($P<0.05$) using the dried blood spots (DBS) samples. The overall result showed that the infants within the age bracket of 1 to 4 months had the highest positive cases with the RDT- 68 (58.1%) and PCR results 14 (12.0%). This was closely followed by 9 to 12 months which recorded 57.1% for RDT but no target detected with the PCR method. On gender-related occurrence of HIV among the infants tested, Eight male infants were positive in the PCR out of the fifty-two infants tested, while eight (9.8%) out of the eighty-two (82) female subjects also showed positive results with PCR method. With the RDT method, 47(57.3%) and 29 (55.8%) tested positive among the females and the males respectively. The result showed a great disparity in prevalence between the two methods of detection. Within the early weeks of life, maternal antibodies prevailed in the infants' blood which declined at later period of life, leading to false positive results and this is of great concern. Early identification of HIV in infants, based on Viral DNA detection should be encouraged in our clime. This will improve management and outcomes, reduce side effects of unnecessary administration of Anti Retroviral Therapy (ART), as well as conserve scarce resources.

KEYWORDS: Early Infant HIV-Detection, DBS, Maternal-antibodies, Viral DNA.

1. INTRODUCTION

The Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS) is by far the world's most important public health concern, particularly in sub-Saharan Africa.[1] HIV/AIDS is known to occur in nearly every part of the world, and the WHO,[2] estimates that about 40 million of the world's populations are affected, with about 23 million already living with the virus in sub-Saharan Africa.[3] Of these, approximately 40% are women, and an estimated 1 million children aged <15 years are expected to be infected with HIV; it has also been shown that approximately 90% live in developing countries.[4]

Of the ten countries worldwide with the greatest number of infected children, the top nine are all in sub-Saharan Africa.[5] Studies have shown that infants are at high risk of acquiring infection from their mothers during pregnancy.[6] Although, several modes of transmission of HIV is known to exist- such as sexual contact, blood transfusion, sharing of contaminated instruments, breast feeding, the most significant however, is the transmission of the virus from an infected mother to her infant.[7] Perinatal transmission of the virus still remains an important mode of transmission and accounts for virtually all new HIV infections in children in many parts of the world.[8] It has been estimated that about 69,000 infants contract HIV infections from their mothers

annually in Nigeria during pregnancy, delivery, and breastfeeding periods.[1] The child in the womb (uterus) depends on maternal antibody as a means of body defense. This low molecular weight antibody-IgG, which is abundant in the plasma of the mother, has the ability to cross the placenta to the foetus and confer immunity. Therefore, all children born to HIV-positive mothers have HIV antibodies in their blood at birth. However WHO[4], estimates that only about one third of children born to HIV-infected mothers are likely to be actually infected. Proper and early identification of HIV status in children born to HIV-infected mothers is crucial for the health and growth of such children as it will afford the chances of reducing the risk of further transmission through breast feeding as well as permit early initiation of antiretroviral therapy in infected infants. Detection of HIV in most infected infants by age 1 month and in virtually all infected infants by age 6 months is possible using HIV DNA PCR technology and this has proved very sensitive for the diagnosis of HIV infection during the neonatal period.[4] Until recently, early infant detection of HIV in our facility had depended on diagnosis based on HIV antibody testing which has been shown not to be a reliable indicator of HIV infection in infants because of maternal antibodies which tend to persist in the circulation beyond 18 months of age. Consequently, this study was aimed at the early infant detection using molecular methods which detects the viral DNA rather than antibodies in the blood.

2. MATERIALS AND METHODS

Following ethical approval and informed consent, One hundred and thirty-four (134) infants aged between 6 weeks to 18 months, born to HIV- infected mothers and attending Paediatric clinics at Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria, from October 2016 to September 2017, were selectively tested in this study.

Two methods were used in this study: Firstly, the national serial testing algorithm for non-cold chain dependant diagnostic kits- Determine test kit (Abbot, USA) and Unigold test kit (Trinity Biotech, USA) to identify antibodies to HIV using whole blood samples. Concordant positive tests were regarded as confirmed positive while discordant results from the two kits were resolved using a third test kit- Statpak (ChemBio Diagnostic Inc. USA) as tie breaker.

Secondly, Dried Blood Spots (DBS) samples were collected from the same infants from their heels, toes, and finger pricks based on their age and body weight. A drop of the blood or 70 μl of whole blood, using a pipette, was spotted unto a whatman-903 filter paper or card. The circle on the filter paper was filled completely by the drop of the blood and at least four circles were made per card. The DBS from all the infants born to HIV- infected mothers, whether sero-positive or negative with the screening method were again processed by DNA PCR Technology using Roche COBAS ® Ampliprep/COBAS TaqMan HIV-1 Quali Test instrumentation.[9] Test was performed following the manufacturer's instructions.

2.1 Statistical Analysis

Statistical analysis was performed using the computer software Statistical Package of Social Science (SPSS) for windows version 20 (SPSS Inc., Chicago IL, USA). Descriptive analysis was used to describe the proportion of HIV infected infants by age groups and gender.

3. RESULTS

Of the 134 samples tested, 76 (56.72%) tested sero-positive with the rapid diagnostic method while 16 (11.9%) tested positive with the PCR method using Dried Blood Spots (Table 1). The result showed that the highest sero-positive cases occurred in the age bracket of 1 to 4 months (58.1%) closely followed by 9 to 12 months (57.1%). Similarly, the DBS PCR reaction test results for HIV showed occurrence rate of 14 (12.0%) among infants within the age brackets (1 to 4) months, while 2 (22.2%) within the age bracket of 5 – 8 months were found to be positive. Table 2 shows gender-related occurrence of HIV among the infants tested. Eight male infants were positive in the PCR out of the fifty-two infants tested, while eight (9.8%) out of the eighty-two female subjects showed positive results with PCR method. With the RDT method, 47(57.3%) and 29 (55.8%) tested positive among the females and the males respectively.

Table 1: Occurrence of HIV based on Rapid Diagnostic Test (RDT) and PCR methods.

AGE GROUP (MONTHS)	NO. Tested	RDT Method No. Positive (%)	PCR Method No Positive (%)	P-value
1-4	117	68 (58.1)	14 (12.0%)	
5-8	9	4 (44.4%)	2 (22.2%)	
9-12	7	4 (57.1%)	0 (0%)	
13-16	1	0 (0%)	0 (0%)	
17-20	0	0	**0**	
TOTAL	**134**	**76 (56.72%)**	**16 (11.9%)**	**<0.05**

Table 2: Gender-related Occurrence of HIV based on Rapid Diagnostic Test (RDT) and PCR methods.

Gender	Age (Month)	NO. TESTED	RDT Method No. Positive (%)	PCR Method No. Positive (%)	P-value
Male	1-4	44	27 (61.4%)	5 (11.4%)	
	5-8	5	2 (40%)	1 (20%)	
	9-12	3	0 (0.0%)	2 (66.7%)	
	13-16	0	0	0	
	17- 20	0	0	0	
Sub Total		**52**	**29 (55.8%)**	**8 (15.4%)**	
Female	1-4	73	43 (58.9%)	5 (6.8%)	
	5-8	2	2 (100%)	2 (100%)	
	9-12	6	1 (16.7%)	1 (16.7 %)	
	13-16	1	1 (100%)	0 (0%)	
	17- 20	0	0	0	
Sub Total		**82**	**47(57.3%)**	**8 (9.8%)**	
TOTAL		**134**	**76 (56.72%)**	**16 (11.9%)**	**<0.05**

4. DISCUSSION

In this present study, a total of one hundred and thirty-four (134) HIV-exposed infants, aged between 6 weeks and 18 months were recruited and tested. Of this number, fifty-two (52) were males and eighty-two (82) were females. There was a significant ($p<0.05$) discordance of occurrence based on the detection methods. Whereas 76 (56.72%) of the 134 subjects tested positive with the rapid diagnostic methods, only 16 (11.9%) tested positive using the nucleic acid detection method. This disparity is huge and therefore raises serious concerns in terms of overuse of antiretroviral therapy and stewardship, diagnostics and general resource allocation. The PCR methods, which have become a gold standard, detects the viral nucleic acid rather than antibodies and therefore is more specific compared to the RDT.

Studies of this nature have been undertaken in other parts of the world. In Equatorial Guinea, a study carried out by Alvarez, *et al.*,[10] compared four commercial virological assays (CVAs) for EID using dried blood specimens (DBS) to detect the presence of HIV nucleic acids (proviral DNA, Viral RNA). In their study, DBS from 68 infants born to HIV-1-infected women were tested. Two HIV-1-infected infants (2.9%) were detected by the four CVAs while 49 (72%) resulted negative, although their result is at variance with the present study where only one CVA was used for EID.

Another study in Luanda, Angola by Martin, *et al.*,[11] showed that out of 139 HIV-1-exposed infants that were tested using a new DNA PCR assay and dried blood spots, three out of the 139 samples were HIV-1-DNA positive. Their detection rates are lower compared to the reported percentage in this present study, where 16 infants tested positive out of 134 DBS sample tested using DNA PCR. The high percentage of HIV-1-infected infants in this study may probably be attributed to non-adherence and follow-up of HIV-infected mothers during pregnancy and after delivery. Some other investigators have also reported higher detection rates. In Ethiopia, a study carried out by Wondafrash and Hiko,[12] showed that out of 138 HIV-exposed infants and children tested using DNA/PCR, 98 (71%) of them were diagnosed for HIV infection. Furthermore, a study by Chohan, *et al.*,[13] using commercially available Roche Amplicor ® HIV-1/DNA Test version 1.5 assays showed that out of the 187 filter paper dried blood spots (FP-DBS) collected from infants defined as HIV-1 positive, 178 FP-DBS tested positive by the in-house single round HIV *pol* PCR FP-DBS assay, while 8 infants FP-DBS samples were confirmed as HIV-1 negative.

The higher sero-prevalence rate obtained in the age bracket 1-4 months (Table 1) can be attributed to higher amount of maternal antibodies in this group compared to older infants. The results of the RDT and PCR of the infants based on gender are presented as in Table 2. Out of the 134 infants tested, 52 were males while 82 were females, yielding 15.4% and 9.8% positive results respectively by PCR and 55.8%) and 57.3% for males and females respectively by the rapid antibody screening. Gender did not influence the test outcome of either method used in this study. This also corroborates the study by Wondafrash and Hiko,[12] with HIV detection rates of 49.0% and 51.0% among males and females respectively in their study population.

5. CONCLUSION

The result showed a great disparity in prevalence between the two methods of detection. Within the early weeks of life, maternal antibodies prevailed in the infants' blood which declined at later period of life, leading to false positive results and this is of great concern. Early identification of HIV in infants, based on Viral DNA detection should be encouraged in our clime. This will improve management and outcomes, reduce side effects of unnecessary administration of Anti Retroviral Therapy (ART), as well as conserve scarce resources.

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