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Development and Implementation Challenges of a Quality Assured HIV Infant Diagnosis Program in Nigeria Using Dried Blood Spots and DNA Polymerase Chain Reaction

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Abstract

Nigeria has one of the highest HIV burdens as well as mother-to-infant transmission rates in the world. A pilot program using polymerase chain reaction (PCR)-based testing of dried blood spot (DBS) specimens was implemented to enable early identification of HIV-infected infants and timely referral and linkage to care. From February 2007 to October 2008, whole blood was collected by finger prick to prepare DBS from infants < 18 months presenting in six public mother-and-child health facilities in Lagos, Nigeria. The DBS were tested using the Roche Amplicor HIV-1 DNA Test, v1.5. To monitor laboratory testing quality, all of the PCR-positive and 10% of the PCR-negative DBS were retested by the same method at another reference laboratory. Three hundred and sixty-five randomly selected infants were screened using HIV rapid tests (RT) according to the national algorithm and RT-negative and PCR-positive specimens were also tested using Genscreen enzyme-linked immunosorbent assay (EIA) (Bio-Rad, France). The turnaround time (TAT) from sample collection, testing, and dispatching of results from each health facility was monitored. A total of 1,273 infants with a median age of 12.6 weeks (1 day to 71.6 weeks) participated in the program and 280 (22.0%) were PCR positive. HIV transmission levels varied greatly in the different health facilities ranging from 7.1% to 38.4%. Infants aged 48 to 72 weeks had the highest level of PCR positivity (41.1%). All PCR-positive specimens were confirmed by retesting. The mean turnaround time from DBS collection to returning of the laboratory result to the health facilities was 25 days. Three infants were found to be HIV antibody

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negative by rapid tests but were positive by both PCR and the fourth generation EIA. The DBS-based PCR program accurately identified all of the HIV-infected infants. However, many programmatic challenges related to the laboratory and TAT were identified.

Introduction

Nigeria has a high HIV prevalence (4.1%) and 3.3 million infected individuals; in 2010 it was estimated to account for 30% of the global burden of mother-to-child transmission of HIV.[1] A continuum of programs including prevention of mother-to-child transmission (PMTCT), early infant diagnosis (EID), and timely referral of infected infants to care is critical to reduce infant morbidity and mortality.[2] Nigeria has intensified its efforts to reduce new HIV infections in children younger than 15 years.[3] In 2004, only 431 out of 1,688 pregnant women from 11 PMTCT pilot sites accessed primarily single-dose nevirapine antiretroviral therapy (ART).[4] By the end of 2013, 53,626 HIV-infected pregnant women across the country received effective triple ART (personal communication).

Identification of HIV-infected infants by serology before 18 months of age is difficult. This is because of the presence of maternal antibodies[5,6] that are acquired transplacentally and can persist for as long as 18 months. Definitive diagnosis requires testing viral nucleic acid or antigen, which is technically complex.[7–10] Fourth generation antigen-detecting HIV rapid assays are available but their performance is poor.[11] Polymerase chain reaction (PCR) to detect viral nucleic acid is commercially available and has been adopted in resource-poor settings and is recommended by the World Health Organization (WHO) to diagnose HIV-infected infants.

In 2006, the Nigerian Institute of Medical Research (NIMR) was using a quantitative plasma RNA viral load assay to detect HIV-infected infants on a small scale.[12] To establish a larger-scale EID service, the Nigerian Federal Ministry of Health (FMOH) with the assistance of the U.S. Centers for Disease Control and Prevention (CDC) and U.S. Agency for International Development (USAID), with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), conducted a demonstration project in 2007 in Lagos using dried blood spots (DBS) and a DNA PCR assay.[13] Here we report the prevalence of HIV transmission in participating hospitals and the challenges encountered.

Materials and Methods

Study population

Infants enrolled in this study were aged less than 18 months and were either (1) known HIV-exposed infants referred from the PMTCT program or other settings in the facility or (2) sick infants whose HIV status was not necessarily known but who presented with signs and/or symptoms suggestive of HIV.[14] Some major signs and symptoms include growth failure, failure to thrive, wasting, failure to attain typical milestones, and recurrent bacterial, fungal, or viral infections.

Ethical approval and informed consent

All testing followed approval from the National Health Research Ethics Committee, Nigeria and the U.S. CDC Institutional Review Board in Atlanta, Georgia. Parents/guardians of infected children provided written informed consent for study participation. Consent allowed for storage and future analysis of stored specimens.

Study participant description

Infants (n = 1,273) younger than 18 months of age attending PMTCT and pediatric facilities in six mother-and-child health (MCH) facilities in Lagos between February 2007 and October 2008 were recruited. The hospitals were Isolo General Hospital (IGH), Lagos State University Teaching Hospital (LASUTH), Lagos Island Maternity Hospital (LIMH), Massey Street Children Hospital (MSCH), Nigerian Institute of Medical Research (NIMR), and Surulere General Hospital (SGH). Nurses and attending physicians were trained on the collection, storage, and transportation of DBS specimens. Information on gender, age, infant co-trimoxazole use, infant feeding choice, and PMTCT service utilization was entered in a standardized PCR test request form.

Specimen collection

Whole blood was collected from the fingers, heels, or toes of HIV-exposed infants and spotted onto five circles on a Whatman 903 card (Whatman, Cape Town, South Africa), dried overnight at ambient temperature, sealed in humidity-free bags with desiccants, and sent to the NIMR's EID laboratory. High volume sites that were specialist hospitals for pediatric (MSCH) and pregnant women (LIMH) used professional courier services for delivery of DBS to the NIMR laboratory. General health facilities, IGH and LASUTH, with moderate specimen volume used their own hospital vehicles. SGH with the smallest specimen volume used designated health providers to hand deliver samples (Table 1). Samples collected in the NIMR clinic were hand-delivered to the laboratory on the day of collection because they were both located on the same campus. The DBS specimen collection dates, receipt dates in the laboratory, and result report dates were recorded in an Excel spreadsheet in NIMR. Laboratory results were returned to the clinics using the same sample delivery mechanisms.

Serologic testing

To evaluate the utility of using serologic rapid tests (RT) to rule out HIV infection in infants who lost maternal antibodies and had not generated autologous antibodies, 365 HIV-exposed infants were selected by purposeful randomization and screened using RT. The purpose of this screening exercise was to determine at what age rapid antibody testing could be used to replace DNA PCR in infant diagnosis. Use of RT to determine HIV infection was performed on whole blood according to the recommendations of the World Health Organization and U.S. CDC.[15] Samples were tested by Determine (Abbott, Tokyo, Japan) and UniGold (Trinity Biotech, Jamestown, NY) or Stat-Pak (Chembio Diagnostic Systems, Medford, NY) in parallel. Samples with discordant results were further tested by Bundi RT (BUNDI International Diagnostics Ltd., Aba, Nigeria) as the tiebreaker. DBS found to be RT negative and PCR positive were tested using Genscreen (Bio-Rad, France), a fourth

generation EIA. Elution of antibodies and retrospective testing from archived DBS cards were carried out as previously described.[13] The optical density (OD) reading of the antibodies was conducted using an ELX800 universal microplate reader. This EIA was used later only to relate the discordance between the RT-negative and PCR-positive samples.

Qualitative PCR testing of DBS

A 6-mm DBS disk was dislodged from the Whatman 903 paper with a clean mechanical paper punch. Each disc was tested using the Roche Amplicor HIV-1 DNA Test, v1.5 (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Briefly, DNA was isolated from the disk using reagents provided in the Roche Amplicor DNA kit in a final solution of 100 μl. Then 50 μl of extracted DNA was added to the PCR master mix and subjected to amplification and detection. To ensure testing accuracy, all samples with positive results were retested using a second 6-mm punch from the same DBS card prior to issuing results to the hospital. HIV-negative DBS were not retested prior to issuing of the results. In addition to the internal kit controls, NIMR used positive and negative DBS controls provided by the International Laboratory Branch, Division of Global HIV/AIDS, U.S. CDC.[16] Finally, all PCR-positive and 10% of randomly selected negative DBS were sent for retesting to the Institute of Human Virology of Nigeria (IHVN) laboratory in Jos, Nigeria. Discordant results between NIMR and IHVN were further retested at the International Laboratory Branch, Division of Global HIV/AIDS, U.S. CDC. The staff of both NIMR and IHVN laboratories received EID PCR laboratory training in 2007 by U.S. CDC staff and received passing grades in the DBS proficiency testing program in 2008, which was supplied by the U.S. CDC.

Results

Demographics and HIV-1 vertical transmission

In the 20 months between February 2007 and October 2008, 1,273 infants younger than 18 months were recruited in the six MCH facilities (Table 1). NIMR recruited the largest number of infants at 446 (35%) followed by 322 (25.3%) at MSCH and 183 (14.4%) at LIMH. IGH and SGH had the lowest recruitment numbers of 115 and 43, respectively. Of the 1,138 (89.3%) infants with gender recorded, the male-to-female ratio was 1.25:1 (632:506).

This pilot protocol recommended DBS sample collection at 6 weeks of age. The median age of all infants tested was 12.6 weeks (ranging from birth to 71.6 weeks). LIMH had the youngest median age of infants (6.9 weeks), whereas MSCH had the oldest (26.3 weeks). Overall, 101 (7.9%) samples were collected from infants 6 weeks or younger (Table 2). In total, 22% (280) of the infants were PCR positive. Infection rates varied significantly (p = 0.0000) by hospital, with the highest in LASUTH (38.4% or 63/164) followed by MSCH (36.8% or 118/322). LIMH had the lowest rate of 7.1% (13/183) (Table 1).

The 280 HIV-infected infants were significantly older (median = 24.2 weeks; range = birth to 71.6 weeks) than the 993 uninfected infants (median = 9.4; range = birth to 71.1 weeks) ($p < 0.0001$). Seventy-nine percent of the HIV-infected infants were older than 12 weeks.

Older infants (48 to 72 months) had the highest infection rate (41.1%). Three of the 12 specimens (25%) collected from infants at birth were identified as HIV infected.

Quality assurance of PCR testing

The molecular biology section of the NIMR laboratory had previous experience conducting quantitative PCR tests using plasma specimens to monitor antiretroviral treatment of adults with HIV. They also had 18 months experience conducting qualitative PCR using whole blood for a few sites. This made it easy to quickly adopt the use of DBS for the Roche qualitative PCR assay; retesting of positive specimens was also conducted in an experienced laboratory at IHVN. Therefore, the concordance between the two laboratories was 98.4% (374/380). The six discordant samples were sent to the U.S. CDC for subsequent retesting and were all determined to be positive.

Service performance characteristics

The efficiency of the EID system was measured by the time spent at each processing stage. MCH facilities collected and forwarded DBS cards to the laboratory, the laboratory conducted the diagnostic tests, and then the results were returned to the MCH facilities. The mean number of days from collection of DBS from all the health facilities to delivery at the laboratory was 5.3 days (range = 0–10 days). A mean of 8 days was required for the NIMR laboratory staff to complete PCR testing and 12.2 days to return results to the MCH facility of origin. Therefore, the mean turnaround time (TAT) from sample collection to results return to all the health facilities was 25 days (range = 13–39 days) (Table 3). As expected, the NIMR clinic had the shortest TAT because the pediatric clinic and NIMR laboratory were both located on the same campus.

Use of antibody disappearance to rule out transmission

In our study, 20 (aged 6 to 72 weeks) of the 365 randomly selected infants screened by HIV rapid tests were found to be antibody negative. Also, all but three of the antibody-negative infants were found to be PCR negative (Table 4). The ages of these three antibody-negative but PCR-positive infants were 8, 23, and 24 weeks. These three samples were also tested positive using the fourth generation EIA. The OD readings for these samples were 0.335, 0.424, and 0.829 while the cut-off value of the test run was 0.301.

Discussion

Early diagnosis of HIV infection in infants is essential to provide timely treatment and reduce mortality.[17–20] This pilot study demonstrated high detection accuracy and encouraging concordance of PCR results between the NIMR and IHVN laboratories. As in other resource-limited countries, the Nigerian national guidelines recommend that EID testing begin at 6 weeks of age, when infants first return to healthcare facilities for immunization after birth.[14] In this project, the prevalence of HIV transmission was 22% among infants in the six participating MCH facilities at a median age of 12.6 weeks. This prevalence is similar to that previously reported in Nigeria in 2006[12] and illustrates the urgent need for a more effective PMTCT program to reduce vertical transmission.[21,22]

It was noted that the transmission rates in infants from LASUTH and MSCH were significantly higher than those from other facilities. This is not surprising since they are major referral hospitals; thus, sick children tended to be referred to them for better care. Lower rates of HIV infection were found in younger infants, who had been tested closer to the recommended age of first PCR at 6 weeks (Table 2). The higher rate of HIV infection observed in older infants could also be partially due to the increased duration of breastfeeding and choices such as mixed feeding, which could increase transmission.[23] These findings may also reflect a selection bias by the facility doctors/nurses as the diagnostic program was newly implemented; thus there were many infected but older infants whose HIV infection status needed to be confirmed. As the program develops it will be important to ensure that HIV-exposed infants are routinely screened for EID earlier per guidelines and infected infants found early so that appropriate HIV care and treatment can be initiated.

Due to the transplacental maternal HIV-1 antibodies in infants younger than 18 months, serological diagnosis is challenging. However, if a negative HIV-1 antibody result is obtained in infants 6 months or older, a presumptive diagnosis of no HIV-1 transmission can be made.[24] Nevertheless, in a subset (365) of infants in this study, we identified three infants aged 8 to 24 weeks with undetectable HIV-1 antibodies by RT who were in fact infected, as determined by positivity on repeat PCR and fourth generation EIA. The fourth generation EIAs test for both HIV antibodies and p24 antigen. In Nigeria, RT is performed in exposed infants older than 9 months and our findings suggest that negative RT results should be treated with greater caution than is the current practice. The frequency of such occurrences in Nigeria has not yet been determined. An HIV-exposed infant with a negative RT but with signs and symptoms suggestive of HIV should be retested with PCR. Some other studies have also reported similar findings of false-negative results using rapid test for infant diagnosis.[25]

In our study, the median age of first testing (12.6 weeks) was double the age recommended by Nigerian national guidelines. Programmatic efforts to identify HIV-positive women early and enroll them in PMTCT must be intensified to ensure that infants receive prophylaxis and early PCR tests.[19,24] Additionally, records in the centralized laboratory demonstrated significant prelaboratory and post-laboratory inadequacies and delays. Two facilities took over a week to deliver DBS to the EID laboratory. It is important to increase the delivery frequency and heighten MCH staff's awareness of timely forwarding of DBS cards. It is also critically important to deliver PCR results immediately to MCH clinics.

This study did not report on patient and caregiver receipt of test results, but it is reasonable to expect that any delays in returning a test result to clinics will contribute to increased loss to follow-up, decreased retention in care, and poorer linkages to care for infected infants with increased morbidity and mortality.[26–28] Some countries use electronic reporting systems; Nigeria is currently using the short message service (SMS) technology to facilitate rapid notification of results and bring PCR-positive infants into care. It would also be beneficial to enhance the database in the centralized EID laboratory to facilitate monitoring each segment of the events to identify and correct deficiencies in the MCH facilities and laboratory.[29]

The recent development of point-of-care (POC) nucleic acid devices[30] and the increased detection rate of infected infant *in utero* by testing "at birth" samples[31] are encouraging. POC devices can use toe or finger-prick blood in MCH facilities to diagnose infection in exposed infants. In view of the TAT recorded in this study, Nigeria may need to consider the use of nucleic acid-based POC in its testing algorithm.

As Nigeria continues to scale up its EID program, as additional devices become available, and as more PCR laboratories are established, attention should be given to issues of test result quality assurance. In most cases the testing system is first developed and QA issues are dealt with later as an afterthought. Having accurate data from the start of the program means that MCH staff and parents of infants would trust the test results much more. In this project, donors and National AIDS Control program managers saw the value in the system in which they invested.

Conclusions

We demonstrated that using a DBS collection network with six healthcare facilities and quality assured central DNA PCR testing could produce accurate diagnostic results for HIV-exposed infants. However, we also highlighted challenges, including late entry of infants into EID services and overly long time intervals between sample shipment and reporting of results to clinics. These limitations should be addressed to ensure timely linkage of HIV-infected infants to life-saving HIV care and treatment in Nigeria. The outcome of this pilot study has informed the roll-out of EID of the HIV program in Nigeria, using a network of PCR laboratories and DBS collection sites. Further studies on the reliability of rapid test kits to rule out HIV infection in older children are recommended.

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Table 1

Gender, Age, and Polymerase Chain Reaction Results for 1,273 Infants Recruited from Six Mother-and-Child Health Facilities in Lagos, Nigeria, 2007–2008

		Gender[a]			
MCH facility	***Infants*** **N**	***Male*** **N (%)**	***Female*** **N (%)**	***Age in weeks median (range)***	***Positive PCR***[b] **N (%)**
SGH	43	22 (52.4)	20 (47.6)	8.8 (3.4–60.6)	12 (27.9)
IGH	115	63 (57.8)	46 (42.2)	8.9 (2.0–71.1)	17 (14.8)
LASUTH	164	74 (54.8)	61 (45.2)	16.2 (0–71.3)	63 (38.4)
LIMH	183	89 (50.6)	87 (49.4)	6.9 (0–58.0)	13 (7.1)
MSCH	322	168 (56.2)	131 (43.8)	26.3 (0–71.6)	118 (36.8)
NIMR	446	216 (57.3)	161 (42.7)	11.3 (0–71.1)	57 (12.8)
Total	1,273	632 (55.5)	506 (44.5)	12.6 (0–71.6)	280 (22.0)

[a] 135 infants had gender information missing: IGH (6), LASUTH (29), LIMH (7), MSCH (23), NIMR (69), and SGH (1).

[b] Proportion of infected infants per facility is significantly different ($p = 0.000$).

MCH, mother-and-child health; PCR, polymerase chain reaction; IGH, Isolo General Hospital; LASUTH, Lagos State University Teaching Hospital; LIMH, Lagos Island Maternity Hospital; MSCH, Massey Street Children Hospital; NIMR, Nigerian Institute of Medical Research; SGH, Surulere General Hospital.

Table 2

Ages and DNA Polymerase Chain Reaction Positivity of 1,273 Infants Recruited from Six Mother-and Child Health Facilities in Lagos, Nigeria, 2007–2008

Age (weeks)	*Infants* N (%)	*PCR positive* N (%)	*Percent positive*
At birth	12 (0.9)	3 (1.1)	25.0
> 0–6	89 (7.0)	11 (3.9)	12.4
> 6–12	523 (41.1)	45 (16.1)	8.6
> 12–24	248 (19.5)	81 (28.9)	32.7
> 24–36	153 (12.0)	47 (16.8)	30.7
> 36–48	114 (9.0)	38 (13.6)	33.3
> 48–72	134 (10.5)	55 (19.6)	41.1
Total	1,273 (100.0)	280 (100.0)	22.0

TABLE 3

Median Days between Each Step of the Early Infant Diagnosis Process for 1,273 Infants Recruited from Six Mother-and Child Health Facilities in Lagos, Nigeria, 2007–2008

		Median time (days)			
Facility	***Method of DBS transportation***	***Delivery to laboratory***	***PCR***	***Return to facility***	***TAT***
IGH	Hospital vehicle	8	5	26	39
LASUTH	Hospital vehicle	4	8	13	25
LIMH	Courier	4	7	9	20
MSCH	Courier	6	8	9	23
NIMR	Hand delivery	0[@]	8	5	13
SGH	Physician hand delivery	10	9	11	30
Mean		5.3	8	12.2	25

Laboratory records on the service time were incomplete; these results are from those with complete records.

[@]Clinic and laboratory colocated on same campus.

DBS, dried blood spot; PCR, polymerase chain reaction; TAT, total turnaround time; IGH, Isolo General Hospital; LASUTH, Lagos State University Teaching Hospital; LIMH, Lagos Island Maternity Hospital; MSCH, Massey Street Children Hospital; NIMR, Nigerian Institute of Medical Research; SGH, Surulere General Hospital.

TABLE 4

Polymerase Chain Reaction Results and Age Distribution of 20 Rapid Test-Negative Infants from Six Mother-and-Child Health Facilities in Lagos, Nigeria, 2007–2008

	Negative rapid test[a]		
Age (weeks)	***Positive PCR***	***Negative PCR***	***Total***
> 6 to 12	1[b]	2	3
> 12 to 24	1[c]	0	1
> 24 to 36	1[d]	2	3
> 36 to 48	0	6	6
> 48 to 72	0	7	7
Total	3	17	20

[a] Tested for HIV antibodies by two rapid tests.

[b] One infant aged 8 weeks.

[c] One infant aged 23 weeks.

[d] One infant aged 24 weeks.