Oral Fluid Human Immunodeficiency Virus Tests

Improved Access to Diagnosis for Infants in Poorly Resourced Prevention of Mother to Child Transmission Programs

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Background: Perinatal exposure of infants in low resource settings generates the bulk of pediatric human immunodeficiency virus (HIV) disease globally. The HIV status of these infants is established by testing serum for anti-HIV antibodies at 12 months of age in Prevention of Mother to Child Transmission (PMTCT) programs because polymerase chain reaction testing is unavailable. The diagnostic accuracy of 2 oral fluid (OF) HIV tests has not been previously evaluated in children.

Methods: A serum and 2 OF HIV tests were performed at 12 months of age in a cohort of 321 vertically exposed children in a prospective, longitudinal study at a secondary level hospital in Johannesburg, South Africa during a 14-month period preceding October 2003. The 3 HIV tests were performed independently of each other by personnel blinded to the child's true HIV infection status, the reference standard used for comparison.

Results: HIV testing was performed at a median age of 12.1 months. The true HIV infection status of 310 of 321 (97%) children was determined. In comparison with serum testing results, OF HIV tests reduced the percentage of children requiring repeat HIV tests from 45% to 8–12%. The abilities of OF and serum to predict an HIV-uninfected status were comparable with negative predictive values >99%. Interpretation of HIV tests in conjunction with simple clinical assessment further improved the predictive value of the test.

Conclusions: OF HIV tests perform well in children and have the potential to increase accessibility and acceptability of HIV diagnosis for infants in the context of PMTCT programs in low resource settings.

Key Words: human immunodeficiency virus, oral fluid human immunodeficiency virus test, sensitivity and specificity, diagnosis, low resource setting, child

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Human immunodeficiency virus (HIV)-1 antibody assays in subjects 13 years of age and older are accurate and yield comparable results whether performed on oral fluid (OF) or serum.[1–7] The primary reactivity to HIV antigens in OF, detected by enzyme-linked immunosorbent assays (ELISA), is caused by specific IgG.[1,2,5] The concentration of IgG in OF is substantially lower than in serum, raising a concern of false negative HIV ELISA results when low titers of HIV antibodies are present, such as in early seroconversion.[1,2,5–8] The converse applies in Prevention of Mother to Child Transmission (PMTCT) programs in developing countries where HIV ELISA testing of infants is recommended at 12 months of age because detecting small titers of waning maternal HIV antibodies yields high numbers of false positive HIV ELISA results. All infants with positive tests therefore undergo repeat testing at 15–18 months to allow for seroreversion.[9] Repeat testing of infants, which further delays diagnosis and increases testing costs, could be minimized by OF HIV tests that fail to detect small titers of antibodies.[10] Additional potential advantages of OF HIV tests in low resource settings include increased access to testing because minimal skill is required for collection in comparison with venesection, sample stability up to 21 days where transportation from remote areas is necessary and sample safety in comparison with handling blood.[1,2,5,7,11,12] Because OF collection is less invasive than blood sampling, HIV testing is less traumatic for and more acceptable to infants and their mothers.

We report the first description of OF HIV testing in children and the first demonstration of a unique indication for OF HIV testing of vertically exposed infants in poorly resourced PMTCT settings.

METHODS

HIV-exposed children attending the PMTCT clinic at Coronation Women and Children's Hospital (CWCH) in Johannesburg, South Africa who were 11 months of age or older between September 2002 and October 2003 were eligible for inclusion. The OF tests were piloted on a convenience sample of older children from the pediatric HIV clinic

at CWCH. The study was approved by the Ethics Committee of the University of the Witwatersrand, and informed consent was obtained from the mother. The reference standard against which the performance of each HIV test was measured was the infant's true HIV infection status at 12 months of age determined according to Centers for Disease Control guidelines in conjunction with a clinical assessment.[9] A true HIV-negative status was demonstrated by seroreversion or at least 2 negative HIV DNA polymerase chain reaction (PCR) tests. Two positive serum HIV ELISA tests beyond 18 months of age or 2 positive HIV DNA PCR tests denoted true HIV infection.

HIV DNA PCR (Roche Amplicor HIV-1 DNA version 1.5; Roche Diagnostic Systems, Inc., Branchburg, NJ) testing, which is not routinely available in PMTCT clinics in the developing world, was performed as part of an infant diagnostic study during the same period.[10] Each child had a serum HIV ELISA (AXSYM System; Abbott) and 2 OF tests performed at the same visit. OF testing was performed according to the manufacturer's instructions using the OraSure collection device and Oraquick Rapid HIV-1/2 antibody test (Orasure Technologies, Inc.; Bethlehem, PA). It was difficult to maintain the recommended position of the OraSure collection device between the lower gum and cheek, and the device frequently was above the tongue during the 2-minute collection period. Laboratory testing was performed with the OF Vironostika Microelisa system (Organon Teknika Corp., Durham, NC) licensed for use with OraSure.[1,7] OraQuick is a qualitative, rapid test that detects HIV antibodies in oral fluid collected by swabbing the porous flat pad across the outer gum line and yields a result within 20 minutes.[3,4,11] All OraQuick tests were performed by the same clinic nurse who was blinded to the HIV status of the children. Serum and OF testing was performed in different laboratories to maintain anonymity. A pilot sample of 3 HIV-uninfected and 16 HIV-infected children 18.6–88 months of age (median, 40 months) were excluded from further analysis because they were older than children typically presenting for testing at a PMTCT follow-up clinic. Both OF tests demonstrated 100% sensitivity and specificity in these 19 children.

RESULTS

The HIV infection status of 310 children, 36 HIV infected and 274 HIV uninfected, was established. Serum and OF HIV ELISA (OraSure) test results were available for all children, and OraQuick results were available for 254 children, of whom 29 were HIV-infected. Eleven children who underwent OF testing were excluded from the analysis because their true HIV infection status was incompletely determined. None of the 11 children had OF test results that appeared discordant with other indicators of HIV infection status for that child, namely, the clinical assessment or HIV ELISA or PCR test result. Breast-feeding had been discontinued in all children by age 3 months.[10] The predominant viral subtype in this cohort was subtype C.[13] The HIV prevalence in the children 11–18 months of age was 6%, which is consistent with the HIV transmission rate of 9% documented at this PMTCT service when the demise of the remaining 3% of infants, before age 12 months, is factored in.[10] The performance of the 3 HIV tests in the remaining 291 children is shown in Table 1. High titers of maternal HIV antibodies, roughly estimated from the absorbance values of the serum HIV ELISA tests, did not predict false positive OF test results.[14] One-half of the false positive OF results occurred in children (n = 11) with nonreactive serum HIV ELISA results. When the serum HIV ELISA test was reac-

TABLE 1. Results Achieved by 3 HIV Tests in Determining True HIV Infection Status of 291 Perinatally Exposed Children 11–18 Months of Age

	HIV-Infected (Median Age, 12.2 mo; Range, 11–14)	HIV-Uninfected (Median Age, 12.1 mo; Range, 11.2–18)	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Serum HIV ELISA-positive	20	112	132* (45)†				
Serum HIV ELISA-negative	0‡	159	159				
Total	20	271	291	100	59	15	100
OraSure							
OF HIV ELISA-positive	19	18	37* (12)				
OF HIV ELISA-negative	1‡	253	254				
Total	20	271	291	95	93	51	99.6
OraQuick							
Rapid OF test-positive	13	6	19* (8)				
Rapid OF test-negative	2‡	214	216				
Total	15	220	235	87	97	68	99.1

*Number of children requiring repeat HIV testing.
†Numbers in parentheses, percent of total.
‡Number of false negative results.
PPV indicates positive predictive value; NPV, negative predictive value.

tive, low absorbance values were recorded (<3). Six OraQuick tests and 18 OF HIV ELISA (OraSure) tests yielded false positive results, but only 2 patients tested falsely positive on both OF tests with discordant OF tests in the remaining 20 patients (Table 1).

The 3 false negative results recorded for OF testing occurred in 3 patients (Table 1). One patient tested positive on a subsequent OraQuick test done 2 months later, and the other 2 infants were unavailable for further testing. All 20 HIV-infected children, including the 3 children with false negative OF test results, had overt clinical features compatible with HIV infection at the time of HIV testing.

DISCUSSION

HIV testing is a "key weapon" in overcoming the acquired immunodeficiency syndrome epidemic.[4,6,7] By the end of 2003, Sub-Saharan Africa was home to 90% of the 2.1 million children estimated to be HIV-infected worldwide.[15] Lack of access to HIV PCR testing represents a major barrier to HIV diagnosis for infants in poorly resourced settings. HIV-exposed infants wait 12–18 months before their HIV infection status can be established by reliance on detection of HIV antibodies in blood. High loss to follow-up rates at 12 months of age, exceeding 70% of infants known to be HIV-exposed, mean that the majority of exposed infants are diagnosed only if they present to a health care facility when they become ill.[10,16] In the CWCH PMTCT infant diagnostic study, 35% of HIV-infected children died of HIV-related illness before 12 months of age.[10] Without access to an HIV diagnosis before 12 months of age, one-third of HIV-infected children have no access to comprehensive HIV care including antiretroviral therapy now available in South Africa.

The false positive rate of both OF HIV tests is markedly reduced in comparison to that of the serum HIV ELISA. When HIV testing is performed on serum, 45% of all children tested must undergo repeat testing according to PMTCT guidelines (Table 1). The majority of children undergoing repeat testing (84%) are HIV-uninfected. Hence almost one-half of all HIV-exposed infants who do present for HIV testing at 12 months of age must return for retesting, compounding loss to follow-up rates.[16] OF HIV tests substantially reduce the need for repeat testing to 8–12% of all children, and more than one-half of children requiring repeat testing (52–69%) are HIV-infected. Thus the number of children on whom a definitive HIV diagnosis can be made from their initial test at 12 months of age is increased ~4-fold by testing OF instead of serum, and the number of repeat tests on HIV-uninfected children is minimized. The finding of positive OF tests in the absence of detectable HIV antibody in serum is unexplained. Although this does not detract from the utility of OF HIV tests in the PMTCT setting at 12 months of age given that all positive tests are repeated, further investigation of OF HIV tests in children older than 18 months is warranted. For instance, false positive OF HIV test results may have contributed to the higher than expected HIV prevalence noted in South African children who underwent OF HIV testing using the OraSure device.[17]

Despite lacking diagnostic accuracy, clinical examination is an important tool in assessing the HIV infection status of children in low resource settings even in the absence of HIV tests and the presence of other diseases, such as tuberculosis, that mimic HIV.[18–20] Where HIV tests are available, clinical assessments serve as a safety net to alert primary health care personnel to HIV infection in children with postnatal transmission or in instances where HIV test results are incorrect. A single negative serum HIV ELISA test at 12 months denotes an HIV-uninfected child. The negative predictive value of both OF HIV tests is <100% which risks HIV-infected children going undetected; however, concurrent clinical examination should raise suspicion of a false negative test and prompt additional testing. Because the negative predictive value of a test is influenced by the prevalence of the disease, the negative predictive value of OF tests is reduced when HIV transmission rates are higher than the 9% described here.[7] Both sets of OF HIV test results, and in particular the number of false negatives noted for OraQuick, require validation in different settings and in larger numbers of HIV-exposed and -infected children before diagnostic algorithms using OF testing can be applied.

When the OraSure collection device was used previously in children as young as 3.5 years in surveillance of other viral diseases, it was generally well-tolerated, and adequate OF was collected.[21] The same was true for the 12-month-old children in this study despite the placement difficulties. The rapid OF HIV test was easier to use in young children than the OraSure device and negates the need for a follow-up visit to receive the test result.[3,4,6,12] HIV viral subtypes vary across geographically distinct PMTCT settings and OF HIV tests have been validated for multiple viral subtypes.[6,11]

An HIV test with a sensitivity of 87–95% is not ideal, but neither are the current diagnostic strategies available to children in poorly resourced areas. Alternative HIV tests should be assessed in perspective and according to their merits in PMTCT programs at a country level so that HIV diagnosis of infants can advance beyond the current recommendations.[18,20] The higher cost of consumables required for OF testing (2.6 times more than serum) is offset by reducing the requirements for personnel skilled in venesection of young babies and for repeat tests, including repeat clinic visits.[7] Better resourced PMTCT programs may opt for earlier infant diagnosis using a single HIV DNA PCR test or the less costly but similarly accurate ultrasensitive p24Ag assay at 6 weeks of age in conjunction with clinical assessments.[13] Such a policy would identify infants with rapidly progressive disease for antiretroviral therapy before 12 months of age. An

early infant diagnosis would obviate the need for HIV-exposed but uninfected, non-breast-fed infants to attend PMTCT follow-up clinics to 12 months of age for HIV testing. At CWCH, where breast-feeding rates are very low, the overall cost of achieving an HIV diagnosis at 6 weeks of age was less than at 12 months despite using the more expensive HIV DNA PCR test (unpublished data).

Some PMTCT programs recommend a serum HIV ELISA test at 9 months of age in an attempt to determine a negative HIV infection status earlier in life.[16] This approach would be enhanced by using OF instead of serum, but investigation in 6- to 12-month-old exposed infants is necessary.

If further experiences confirm our observations, OF HIV tests in vertically exposed children might provide the best option for early diagnosis of HIV status when viral detection assays are unavailable and represent a step toward increasing the accessibility of HIV diagnosis and treatment to all children.[18]

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