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Short report

False-negative HIV tests using oral fluid tests in children taking antiretroviral therapy from Harare, Zimbabwe

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Abstract

Introduction: Rapid diagnostic tests (RDT) for HIV infection have high sensitivity and specificity, but in the setting of longstanding antiretroviral therapy (ART), can give false results that can lead to misinterpretation, confusion and inadequate management. The objective of this study was to evaluate the proportion of falsely negative results of a RDT performed on oral fluid in HIV-infected children on longstanding ART.

Methods: One hundred and twenty-nine children with known HIV infection and receiving ART were recruited from the HIV Clinic at the Harare Central Hospital, Zimbabwe. HIV testing was performed on oral fluid and on finger-stick blood.

Results and Discussion: Children included in the study had a median age of 12 years (IQR 10–14) and 67 (51.9%) were female. Median age at HIV diagnosis was 5 years (IQR 3–6) and the median time on ART was 6.3 years (IQR 4.3–8.1). The oral fluid test was negative in 11 (8.5%) patients and indeterminate in 2 (1.6%). Finger-stick blood test was negative in 1 patient. Patients with a negative oral fluid test had a higher CD4 cell count (967 vs. 723 cells/mm3, \( p = 0.016 \)) and a longer time on ART (8.5 vs. 6 years, \( p = 0.016 \)).

Conclusions: This study found that a substantial proportion of false-negative HIV test results in children on longstanding ART when using an oral fluid test. This could lead to misinterpretation of HIV test results and in the false perception of cure or delayed diagnosis.

Keywords: HIV; misdiagnosis; rapid diagnostic test; oral fluid test; children

Introduction

Rapid diagnostic tests (RDT) for HIV infection using whole-blood specimens have been used globally since 2005 [1]. These RDTs have high sensitivity, are easy to perform, require little or no infrastructure, and have a relatively low cost and a rapid turn-around time making them optimal for low-resource, high HIV burden settings. However, as with any test, the performance of the test will depend on its inherent sensitivity and specificity and the prevalence of the condition being tested for. The problem of false-positive test results especially in the context of low HIV prevalence is well recognized. Serial testing with a highly sensitive test followed by a confirmatory test with high specificity addresses this issue [2].

Although RDTs have been widely used both in health facilities and in community-based HIV testing and counseling approaches, a key barrier remains the reliance of a client making contact with a provider and receiving the test result from the provider, who may be known to the client. In recent years, there has been increasing interest in promoting self-testing as a strategy to address these barriers. Self-testing would enable individuals to undergo HIV testing confidentially and without concern about unwanted disclosure of their status to others. A recent meta-analysis of studies including adults at risk for HIV infection showed that HIV RDTs performed on blood had sensitivities and specificities exceeding 98–99% [3]. Oral fluid tests (OFTs) are RDTs that detect salivary HIV antibodies, and have been shown to have comparable performance to blood-based RDTs. As with blood-based RDTs, a positive OFT result can be confirmed by a subsequent blood-based test. In 2012, the first OFT received approval by the Food and Drug Administration as a home-use HIV kit for self-testing. The use of an OFT as a self-testing strategy has been demonstrated to be highly acceptable and accurate in Africa [4,5].

OFTs are particularly attractive for use in children because of their non-invasiveness. Studies have demonstrated a slow but persistent loss of HIV-specific antibodies in highly suppressed HIV-infected children and adolescents that may lead to false-negative results in blood-based RDTs [6]. HIV antibody titres in saliva are lower than antibody titres in blood,
which may make OFTs more prone to false-negative results [3]. This appears to be more frequently encountered in the setting of longstanding ART and in individuals receiving pre-exposure prophylaxis (PrEP) [7,8]. We recently observed several cases of false negative HIV tests using OFT among children and adolescents taking antiretroviral therapy. Although this has already been described to occur in adults, there are no studies focusing on the paediatric population [9]. To further investigate this, we systematically evaluated the performance of the OFT compared to the blood-based RDT among perinatally HIV-infected children aged 7–18 years established on ART.

**Methods**

The study was conducted in 2016 and was nested within an ongoing clinical cohort study among perinatally HIV-infected children on ART. Children with HIV who had been receiving ART for at least 18 months were recruited from the HIV Clinic at the Harare Central Hospital, Zimbabwe. HIV testing was performed using Ora-Quick ADVANCE HIV I/II™ OFT (OraSure Technologies, Bethlehem, USA) for oral fluid and concurrently using a finger-prick whole-blood sample (Alere Determine HIV 1/2, Alere Technologies, Jena, Germany). Testing was performed as per the instructions of the manufacturer by trained nurses. The nurse who performed the test was blinded to the result of the other test. CD4 count was assessed using the Alere PIMA CD4 analyser, and viral load was measured using GeneXpert HIV-1 Viral Load (Cepheid, Sunnyvale, CA). Demographic details, age at ART initiation and duration of ART use were collected.

Statistical analysis was performed using STATA version 14 (Stata-Corp, TX, USA). The Mann–Whitney U-test and Student’s t-test were used to evaluate for differences between groups for continuous variables. For categorical variables, the χ² test was used. Multivariable logistic regression was used to examine for factors associated with a false negative OT. The level of significance was set at α = 0.05.

Ethical approval for the parent study was obtained from the Medical Research Council of Zimbabwe, the Biomedical Research and Training Institute Institutional Review Board and the London School of Hygiene and Tropical Medicine Ethics Committee. Written informed consent from guardians and assent from participants were obtained. Specific verbal consent was also obtained to perform OFTs and finger-prick samples.

**Results and discussion**

In total 129 participants were enrolled, with median age 12 years (IQR 10–14), and 67 (51.9%) being female (Table 1). The study participants had been diagnosed with HIV infection at a median age of 5 years (IQR 3–6) and the median duration on ART was 6.3 years (IQR 4.3–8.1). At the time of the OFT, the median CD4 cell count was 747 cells/mm³ (IQR 474–989) and 30 (34.9%) had a viral load exceeding 1000 copies/ml. The OFT was negative in 11 (8.5%) patients and indeterminate in two (1.6%). Finger-prick blood tests were negative in one patient (0.8%) who also had a negative OFT. Patients with a negative OFT had a higher CD4 cell count (967 vs. 723 cells/mm³, p = 0.016), a longer time on ART (8.5 vs. 6 years, p = 0.018) and were more likely to be girls (76.9% vs. 49.1%, p = 0.057). Furthermore, children with a negative OFT had a median age at ART initiation of 4.5 years, while those with a positive test had a median age of 6.2 years although this was not statistically significant (p = 0.138). Only 5 (3.9%) children were started on ART within their first year of life. There was no association between age at ART initiation and a false-negative OFT result. While this was a pre-defined variable to be included in multivariable analysis, this was not done due to a strong collinearity with duration of ART. Notably, 64% of those with a positive OFT had a viral load <1000 copies/ml compared to 78% of those with a false-negative OFT result.

This study shows that a substantial proportion of children and adolescents receiving ART have a false-negative or indeterminate HIV test result using an OFT. Significantly more false-negative results occurred using an OFT compared to a whole-blood-based HIV RDT. While false-negative RDT results, can be due to technical issues such as inappropriate performance and self-interpretation of the test, this was not the case in this study where HIV testing was performed by...
Table 2. Factors associated with false-negative or indeterminate oral fluid-based HIV test

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4.66 (0.96; 22.47)</td>
<td>4.21 (0.81; 21.89)</td>
</tr>
<tr>
<td>Duration of ART (years)</td>
<td>1.30 (1.03; 1.64)</td>
<td>1.31 (1.01, 1.69)</td>
</tr>
<tr>
<td>CD4 count &gt;750 cells/µl</td>
<td>10.00 (1.24; 80.61)</td>
<td>9.50 (1.13; 79.62)</td>
</tr>
</tbody>
</table>

Additional text...

...other significant findings and implications...
writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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**References**