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An HIV1/2 point of care test on sputum for screening TB/HIV co-infection in central India - Will it work?

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ABSTRACT

Objective: To determine whether the OraQuick® HIV-1/2 Assay (OraSure Technologies, Inc.,Bethlehem, PA, USA) in sputum is a valid tool for HIV surveillance among TB patients. **Methods:** A cross sectional study was carried out on sputa of patients diagnosed with tuberculosis. Sputa were tested for antibodies to HIV using OraQuick® HIV-1/2 Assay (OraSure Technologies, Inc.,Bethlehem, PA, USA). The results were compared with results of serum ELISA. **Results:** Compared to serum ELISA, the OraQuick® HIV-1/2 Assay in sputum specimens reported 90% sensitivity (9/10) and 100% specificity (307/307), with a positive predictive value of 100% (95% *CI*: 66.37%-100.00%) and a negative predictive value of 99.68%(95% *CI*: 98.20%-99.99%). **Conclusions:** This testing method may provide a useful strategy for conducting HIV surveillance in possible co-infected TB patients at peripheral centres. Since there is no investment on infrastructure, it may be possible for paramedical health professionals to carry out the test, particularly in areas with low HIV endemicity.

1. Introduction

It is estimated that about 1 billion people in Asia and Pacific region are infected with tuberculosis, of whom presently about 2.5 million are also infected with HIV[1]. Hospital based HIV seroprevalence studies amongst tuberculosis patients from different regions of India have shown a great variation in prevalence[2]. HIV infection is considered as potent risk factor for fueling progression to active tuberculosis in people already infected with Mycobacterium tuberculosis. Though there has been global scale up of rapid HIV testing on blood with encouraging uptake, even in tuberculosis patients, it has to be kept in mind that TB diagnosis and treatment are often performed entirely on an out-patient basis and require no blood testing. In such a setting, and in view of the low endemicity of HIV in our area, we felt that we could utilize the ease of availability of sputum samples in tuberculosis patients to screen for the presence of TB/HIV coinfection in sputum samples without an immediate need for collection of blood samples. We hypothesized that such a screening test might help increase uptake of testing, since there is no blood collection involved. Currently, there is cross referral of TB suspects and patients to the National AIDS Control Programme, from the Revised National TB Control Programme, for a blood test to detect HIV infection. This may lead drop out of patients during the referral process, particularly given the stigma associated with HIV infection. This is a major cause for concern in the attempts to carry out routine HIV testing in this population. In such cohorts, simple, non-invasive, rapid, point of care tests for HIV on easily available clinical samples have the potential

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to overcome these issues, and to add to the strength of existing HIV screening programs.

The OraQuick® HIV-1/2 Assay (OraSure Technologies, Inc., Bethlehem, PA, USA) is a visually-read qualitative immunochromatographic test for the detection of antibodies to HIV-1 and -2 in gingival secretions and blood. Its availability for use on sputum would reduce health care worker exposure to blood and be useful for HIV surveillance among TB suspects. The purpose of this study was to determine whether the OraQuick HIV-1/2 Assay in sputum is a valid tool for HIV surveillance among TB patients.

2. Material and methods

A cross sectional study was conducted between May 2008 and October 2008 at our hospital and the local TB Hospital. The project was approved by the Institutional Review Board of our institute.

2.1. Inclusion and exclusion criteria

Adult participants with fever, chronic cough of more than 3 week duration with or without haemoptysis, loss of weight and history suggestive of high risk behaviour (*ie*., multiple sexual partners, intravenous drug usage, occupations like long distance truck driving), who were suspected to be TB/HIV co-infected, were included in the study. Such patients had sputum smear examination for acid fast bacilli and sputum culture for mycobacteria carried out as a part of their diagnostic work up. Patients with sputa smear positive for acid fast bacilli and/or culture positive for mycobacteria were also included in the study. Patients requiring life support, or with psychiatric illnesses (from whom a valid consent could not be obtained) were excluded. 317 participants fulfilled the inclusion criteria and were included in the study.

2.2. Collection of samples, and assessment of client preference

After informed consent, participants underwent pre test counseling followed by post test counseling, as per guidelines of the National AIDS Control Organization, India. Their sputum samples were collected. HIV antibody testing was conducted on the collected sputum specimens using OraQuick® HIV-1/2 Assay (OraSure Technologies, Inc., Bethlehem, PA, USA). Blood samples collected for testing of HIV antigen and antibody by ELISA. In patients who gave consent for collection of an extra 10 mL of blood, blood was collected for detection of HIV by PCR. The preference of participants for Oraquick testing was assessed by a face-to-face interview.

2.3. Lab procedures

HIV testing was performed according to the method used in an earlier study[3] using OraQuick® HIV-1/2 Assay. OraQuick® HIV-1/2 Assay is a visually read qualitative immunochromatographic test for the detection of antibodies to HIV-1 and -2. The porous flat pad of the QraQuick device was stirred in the sputum specimen for at least 60 s, placed in the developer vial and read after 20 to 60 min. If the control line was visible, the test was interpreted. An HIV-non reactive result was one that showed a single positive control line, and an HIV-reactive result was one that showed two lines: a line for the positive control and a line for the test sample.

The OraQuick® HIV-1/2 Assay results were confirmed by using two ELISAs on serum, Anti-HIV TETRA ELISA (Biotest, Landsteinerstr.5, D-63303 Dreieich, Germany), GENSCREEN® PLUS HIV Ag-Ab (BIO-RAD 3, Bd Raymond Poincare, 92430 MARNES LA COQUETTE, France).

In this study, we chose two ELISAs as reference standard, and reconfirmed with a third ELISA (4th Generation Microlisa HIV Ag & Ab, J.Mitra & Co. Pvt. Ltd., New Delhi, India). We provided risk reduction counseling and asked the patients to return after 3 months, should they suspect exposure.

2.4. PCR

PCR for HIV was performed on blood samples from the 102 patients who gave consent for collection of an extra 10 mL blood for the PCR test using COBAS AMPLICOR HIV-1 monitor test version 1.5 kit (Roche Diagnostic Systems, Branchhburg, NJ, USA). The tests were carried according to the manufacturer's guidelines.

2.5. Data analysis

Using the three serum ELISAs as a gold standard, Abramson, J. H. WINPEPI (PEPI-for-Windows) was used to determine sensitivity, specificity and confidence intervals.

3. Results

3.1. Description of the study population

The study sample included 317 participants. The median age was 38 years (range 14-77). Of 317 participants, 235(74.13%) were male. According to the categories of treatment as defined by the Revised National Tuberculosis

Control Program (RNTCP) of India (RNTCP at a glance), 174 participants (54.88%) belonged to category-Ⅰ, 138(43.53%) belonged to category-Ⅱ and five (1.57%) belonged to category-Ⅲ.

3.2. Diagnostic accuracy of OraQuick tests

Compared to serum ELISA for HIV, the OraQuick® HIV-1/2 Assay in sputum specimens reported 90% sensitivity (9/10) and 100% specificity (307/307), with a positive predictive value of 100% and a negative predictive value of 99.68% (Tables 1&2).

Table 1
Comparison of results of serum ELISA for HIV and OraQuick® HIV-1/2 assay in sputum.

Results of serum ELISA	Results of Oraquick assay on sputum		Total
	Positive	Negative	
Reactive	9	1	10
Nonreactive	0	307	307
Total	9	308	317

Table 2
Performance characteristics of OraQuick® HIV-1/2 assay in sputum.

Performance	Value in % (95% *CI*)
Sensitivity	90.00 (55.50-99.75)
Specificity	100.00 (98.81-100.00)
Positive predictive value	100.00 (66.37-100.00)
Negative predictive value	99.68 (98.20-99.99)

3.3. Results of PCR for HIV

Of the 102 samples tested for HIV by PCR, there was concordance between PCR, triple ELISAs on serum and OraQuick on sputum in 95 samples. Six samples were negative by triple ELISAs and by OraQuick on sputum and serum, but were reactive by PCR. One sample was non reactive by PCR and by OraQuick on sputum and serum but positive by triple ELISAs.

3.4. Client preference for HIV testing modalities

The OraQuick® HIV-1/2 Assay on sputum was preferred by 65% of the participants over ELISA. Of 317 participants, 111(35%) reported discomfort, at the time of blood sample collection for ELISA, and for PCR. Preference was defined as an unequivocal choice of one test modality over another.

3.5. Prevalence of HIV infection using reference standard

Of 317 participants tested for sputum by OraQuick® HIV-1/2 Assay, nine were reactive, and confirmed by dual ELISAs. Of the nine co-infected patients, five belonged to category-I and four to category-II. Prevalence of HIV infection was 3.15%, with 95% CI 1.52%-5.72%.

4. Discussion

Due to the increasing availability of interventions for HIV-infected persons in resource-poor settings, the ideal situation is to have all patients accept HIV testing in order to prevent HIV transmission and to access care programs. Detection of HIV in TB cases is the first step towards bringing patients that are co-infected into care and prevention. Currently, the RNTCP[4] accepts cross referral of patients between DOTs centres and Integrated Couselling and Testing Centres for detection of HIV infection.

In our study, in patients with tuberculosis, only 10 samples were HIV positive. This is probably because of the low HIV prevalence in our area. Significant parts of our country have low HIV prevalence, and this study would be relevant especially in such areas. In this study, Oraquick was found have 90% sensitivity and 100% specificity in sputum, with a positive predictive value of 100% and a negative predictive value of 99.68%.

Additionally, client preferences were definitely higher (65%) for OraQuick® HIV-1/2 Assay on sputum rather than a test involving collection of a blood sample (35%). This was, in a large part, due to the fact that it is a non invasive test. With a positive predictive value of 100% in an area of low endemicity for HIV, and higher client preferences, the test may have utility in screening for coinfection in sputum samples in TB cases and suspects. In the current scenario, it certainly cannot replace a blood test, but may help prevent loss to follow up of patients cross referred between the TB and AIDS control programmes. This, in itself will help boost detection rates of patients coinfected with TB and HIV.

TB/HIV co-infection was detected in 5 category-Ⅰ and 4 category-Ⅱ patients. Given that there were only 5 category-Ⅲ patients who were included in the study. It is difficult to ascertain the significance of the apparently higher incidence of TB/HIV co-infection in category-Ⅰ and category-Ⅱ patients.

In 6 patients, HIV infection was detected by PCR but not but by ELISA on serum or OraQuick on sputum. This could be explained by the fact that PCR can pick up viral nucleic acid which appears much earlier in the course of the disease than viral antibodies and antigen, unless infection is caused by transfusion of infected blood. In one patient, however antibodies were detected by ELISA, but not by OraQuick on serum or sputum, probably due to the higher sensitivity of the ELISA technique. One sample was non reactive by PCR and by OraQuick on sputum and serum but positive by triple ELISAs. This may be due to the detection of different

analytes by PCR and ELISA and/or due to a false positive ELISA result. A Western Blot test might help resolve this issue.

In a pilot study, on a limited number of samples, we reported a good diagnostic accuracy of the Oraquick test on sputum samples, and had stressed the need to test a larger cohort in Central India[5]. A study from South India[6], however, reported an inadequate performance on sputum among a low HIV prevalence population. A study from Botswana, reported a sensitivity of 97.1% and specificity of 98.3% on initial sputum specimens[3]. This study had been carried out on a much larger cohort (377 patients) in an area with a very high prevalence of HIV infection (84% of the cohort were HIV positive by serum ELISA).

Our study found an HIV prevalence of 2.83% in contrast to the 0.11% prevalence in our region[7]. Our hospital based estimate is obviously higher because it is a selected sample of suspects with TB/HIV co-infection.

Community based estimates are urgently needed to determine the performance of OraQuick for screening HIV infection in TB patients, particularly in view of the recent report of false positive results occurring in a performance study of OraQuick oral-fluid rapid HIV tests in Minnesota[8]. Another study had reduced specificity in stored sputum samples[9].

This testing method may be useful for conducting HIV surveillance in possible co-infected TB patients on sputum specimens collected as a part of the RNTCP. Since there is no investment on infrastructure, it may be possible for paramedical health professionals to carry it out at Microscopy Centres on sputum samples, particularly in areas with low HIV endemicity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Declarations

PD, NPP and SD designed the study protocol, PD, SD, KK and KKM carried out the clinical assessments, PM, NP, MV and ZUH carried out the oraquick tests, ELISAs and PCRs, and interpreted the results, PD, NPP, SD, PM, NP, KK and KKM analysed and interpreted the data, PD, NPP, SD, PM and MV drafted the manuscript. All authors read and approved the final manuscript. PD, SD and NPP are guarantors of the paper.

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