Detection of human immunodeficiency virus using oral mucosal transudate by rapid test

Bhuvan Jyoti, Parvathi Devi
Department of Dental Surgery, Ranchi Institute of Neuro-Psychiatry and Allied Sciences, Ranchi, Jharkhand, 1Department of Oral Medicine and Radiology, Teerthankar Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

Abstract
Context: On the cusp of the fourth decade of the acquired immunodeficiency syndrome epidemic, the world has turned the corner - it has halted and began to reverse the spread of human immunodeficiency virus (HIV). Oral fluid-based Rapid tests have potential advantages over blood-based tests hence the time is ripe for their use to scale up HIV screening and testing programmes. Aims and Objectives: To detect HIV using oral mucosal transudate (OMT) and to assess the sensitivity and specificity of the HIV Rapid test using OMT. Settings and Design: OraQuick Rapid HIV-1/2 Diagnostic test was evaluated in sera and oral fluids from 83 subjects. Materials and Methods: The study group comprised of 50 HIV seropositive subjects and the control group comprised of 33 seronegative subjects. Serum samples were collected using the standard phlebotomy technique and oral samples were collected using OraQuick Rapid HIV 1/2 Antibody test OMT collecting device. Statistical Analysis Used: The statistical analysis was done using statistical package for social sciences version 16.0, SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412. The Sensitivity, specificity, positive predictive value, negative predictive value were used. Results: All the subjects who tested either positive/reactive or negative/non-reactive with Western blot/enzyme-linked immunosorbent assay (ELISA) produced similar results with Rapid test using OMT in study, our study also revealed that the subjects whether on anti-retroviral therapy or not had 100% sensitivity and specificity with the Rapid test using OMT. Conclusions: The Rapid test using OMT is highly accurate as the diagnostic efficacy in our study was 100% for HIV antibody detection and produced similar results to that of conventional Western blot/ELISA tests.

Key words: Human immunodeficiency virus, oral mucosal transudate, rapid tests, saliva, serum

INTRODUCTION
Acquired immunodeficiency syndrome (AIDS) is a pandemic disease caused by human immunodeficiency virus (HIV). Globally, 34.0 million (31.4 million-35.9 million) people were living with HIV at the end of 2011 (from Global Report 2012 by UNAIDS).[1] India has the third largest number of people living with HIV/AIDS. As per the 2011 HIV estimates, there are an estimated 21 lakh people currently living with HIV/AIDS in India with an adult prevalence of 0.27%.[2] To detect undiagnosed HIV with accuracy, time and cost-effective manner, high quality Rapid point-of-care HIV tests are useful tools. Oral fluid-based Rapid tests have advantages over blood-based tests because of their convenience, ease of sample collection, non-invasiveness, cultural acceptance, high accuracy and rapidity.[3] The aim and objectives of this study was to detect HIV using oral mucosal transudate (OMT) and to assess the sensitivity and specificity of the HIV Rapid test using OMT.
MATERIALS AND METHODS

The present study was done in Kanpur to detect the HIV using OMT by Rapid test. Approval from the Ethical Committee was taken for the study. To calculate sample size, we used alpha = 0.05, medium effect size = 0.5 and set the power at 0.80 of Chi-square for comparing across HIV positive and negative participants. Power analysis resulted that at least a total of 80 sample size was required (Cohen 1988). Participants were selected on the basis of purposive sampling technique. Hence, the study sample comprised of total 83 subjects, of which the study group consisted 50 subjects, 27 females and 23 males, age ranging from 23 to 58 years and the control group comprised of 33 seronegative subjects, 13 females and 20 males, age ranging from 22 to 56 years. All the subjects were informed and explained about the study and the relevant data was collected and proforma was filled. The consent form was obtained from all the subjects. For the known HIV/AIDS patients (study group) information regarding the test results were collected from their medical records. Out of the 50 study subjects, 36 subjects had three Rapid tests - parallel testing algorithm - (Combaids Rs, Retroscreen and Bi-dot kit used) followed by enzyme-linked immunosorbent assay (ELISA) (from various diagnostic laboratories) and 14 subjects had Western blot (immunoblot analysis) test results (all of the subjects in the study group found it to be a disincentive getting another phlebotomy done). Serum samples for the control group were collected using standard phlebotomy technique and subjected to ELISA testing (Quick Assay by Biotek EL × 800 Absorbance Microplate Reader-12-well microplates, wavelength range used 450 nm and 630 nm). Oral samples of both study and control groups were collected using OraQuick Rapid HIV 1/2 Antibody test OMT collecting device that included test device and developer solution [Figure 1]. OMT sample were collected, by placing the flat pad of the test device above the teeth against the outer gum while gently swabbing the upper and lower gum line back and forth with the cloth end of the swab one time around applying moderate pressure. Both sides of the flat pad are used for this procedure [Figure 2]. The flat pad of the test device is then placed into a vial containing a premeasured amount of developer solution and allowed to develop [Figure 3]. The device is inserted all the way into the vial until the flat pad of the test device touches the bottom of the vial and then timer is set for 20 min. This Rapid test kit is a visually read, qualitative immunochromatographic test for the detection of antibodies to HIV-1 and HIV-2. The internal control line (C) should appear as a reddish-purple line in the window of the test device. Presence of the reddish-purple line adjacent to the test line (T) along with the control line (C) is suggestive of reactive specimen [Figure 4]. Fluid from the surface of the gums enters the device through the porous flat pad then flows onto a test strip. As it migrates across the strip, it hydrates
and mixes with a red-colored reagent (protein A bound to colloidal gold). Immunoglobulin G (IgG) antibodies in the specimen bind to the reagent. If in turn the bound IgG antibody recognizes synthetic HIV-1 or HIV-2 antigen immobilized on the strip enclosed in the housing, a colored line forms in the ‘T’ (test) area of the result window. If not, no line forms there, then considered as non-reactive specimen [Figure 5]. The results were recorded and the statistical analysis was done using statistical package for social sciences (SPSS) version 16.0 statistical Analysis Software. The Sensitivity, specificity, positive predictive value, negative predictive value were used and the values were represented in number (%) and mean ± standard deviation (SD).

RESULTS

Out of 50 subjects in the study group, 10 (20%) subjects were between 20 and 29 years age group, 25 (50%) subjects were in 30-39 years age group, 13 (26%) subjects were in 40-49 years age group and 2 (4%) subjects were in the 50-59 years age group. Out of 33 subjects in the control group, 10 (30.3%) subjects were between 20 and 29 years age group, 12 (36.4%) subjects 30-39 years age group, 8 (24.2%) subjects were in the 40-49 years age group and 3 (9.1%) subjects were in the 50-59 years age group. The mean age of the subjects was 35.72 ± 7.87 years for study group and 35.73 ± 8.20 years for control group [Table 1]. Total mean age with standard deviation observed was 35.72 ± 7.95 years. According to age and gender in study group, in the 20-29 years age group 3 (30%) males and seven (70%) females, 30-39 years age group 12 (48%) males and 13 (52%) females, 40-49 years age group 7 (53.9%) males and 6 (46.2%) females and in 50-59 years age group 1 (50%) male and 1 (50%) females were present. According to age and gender in control group, out of 33 subjects, 7 (70%) males and 3 (30%) females in the 20-29 years age group, 8 (66.7%) males and 17 (33.3%) females were between 30 and 39 years age group, 4 (50%) male and 4 (50%) females were between 40 and 49 years age group and 1 (33.3%) male and 2 (66.7%) females were between 50 and 59 years age group.

Table 1: Mean age of subjects in study group and control group

| Variables | Study group | Control group |
|-----------|-------------|---------------|
| Age (in years) | Mean±SD     | Mean±SD       |
| Age (in years) | 35.72±7.87  | 35.73±8.20    |
| SD=Standard deviation |

Table 2: Description about the socio-demographic characteristics of the subjects among study group and control group

| Variables                | Study group (n=50) | Control group (n=33) | Chi-Square value |
|--------------------------|--------------------|----------------------|------------------|
| Education                | Illiterate         | 09                   | 00               |
|                         | High school        | 13                   | 08               |
|                         | Intermediate       | 19                   | 06               |
|                         | Graduation         | 08                   | 17               |
|                         | Post-graduation    | 01                   | 02               |
| Marital status           | Married            | 31                   | 22               |
|                         | Single             | 14                   | 11               |
|                         | Separated/divorced | 05                   | 00               |
| Occupation               | Unemployed         | 14                   | 14               |
|                         | Employed           | 36                   | 19               |
| Residence                | Rural              | 17                   | 08               |
|                         | Semi urban         | 25                   | 14               |
|                         | Urban              | 08                   | 11               |
| Family type              | Nuclear            | 37                   | 24               |
|                         | Joint              | 13                   | 09               |
| Income                   | >5000              | 28                   | 17               |
|                         | 5000-2500          | 15                   | 10               |
|                         | <2500              | 07                   | 06               |

Figure 4: The reactive specimen Figure 5: The non-reactive specimen
oral manifestations of HIV in the study group are shown in Table 3. Diagnosis of oral lesions was done based on presumptive criteria of European Community-Clearinghouse, 1993. In the high-risk group, heterosexuals including sex workers were 28, subjects who had male-to-male sexual contact were 10, male-to-male sexual contact and injection drug use were nine and three were injection drug users. In the study group, all 50 subjects presented with oral manifestations of AIDS. It was found that the study subjects distributed amongst the high-risk group had either one or more than one oral manifestations of HIV. Out of 28 heterosexual 11 patients presented with more than one oral lesion. Oral candidiasis with angular cheilitis was most prevalent among heterosexual group and among male-to-male sexual contact (n = 10) seven subjects presented with more than one oral lesion. Male-to-male sexual contact and injection drug user (n = 9) two patients presented with more than one oral lesion. Among injection drug users (n = 3) two subjects presented with more than one oral lesion. Highly active anti-retroviral therapy (ART) has been shown to reduce the opportunistic infections, viral load and progress of some malignancies associated with advanced HIV infection. All 23 patients on ART were 100% reactive toward Rapid test using OMT [Table 4]. Hence, in the present study, the sensitivity, specificity, positive predictive value and negative predictive value was 100% [Table 5]. All the subjects who tested positive/reactive with Western blot/ELISA produced accurate results with Rapid test using OMT in study group and all the subjects who

![Figure 6: Distribution of subjects according to age and western blot/ enzyme-linked immunosorbent assay reactivity in study group](image1)

![Figure 7: Distribution of subjects according to age and western blot/ enzyme-linked immunosorbent assay reactivity in control group](image2)

| Table 3: High-risk group and their association with common oral manifestations of HIV in the study group |
|---------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Common oral manifestations detected in HIV subjects study group n=50 | Heterosexual including sex workers (n=28) | Male-to-male sexual contact (n=10) | Male-to-male sexual contact and injection drug user (n=9) | Injection drug user (n=3) |
| Candidiasis with angular cheilitis | 09 | 03 | 04 | 01 |
| Linear erythematous gingivitis | 02 | 00 | 01 | 00 |
| Xerostomia | 01 | 00 | 00 | 00 |
| Aphthous ulcer | 04 | 00 | 01 | 00 |
| Necrotizing ulcerative periodontitis | 01 | 00 | 01 | 00 |
| Candidiasis with linear erythematous gingivitis | 05 | 03 | 00 | 00 |
| Necrotizing ulcerative periodontitis with aphthous ulcer | 02 | 01 | 00 | 00 |
| Xerostomia with aphthous ulcer | 03 | 01 | 01 | 01 |
| Linear erythematous gingivitis with xerostomia | 01 | 02 | 01 | 01 |
| HIV=Human Immunodeficiency virus | | | | |
tested negative/non-reactive with ELISA produced accurate results with Rapid test using OMT. This study also revealed that irrespective of whether the subjects are on ART or not, sensitivity and specificity of the Rapid test using OMT was 100%.

DISCUSSION

There has been an attraction since long time to oral fluid as a specimen for detection of various analytes because of the inexpensive, safe and non-invasive methods for sample collection compared with blood testing. Today, the testing of oral fluid for antibodies, antigens and other analytes is a well-established and accepted form of clinical care, monitoring and research throughout the world including those for HIV diagnosis. Saliva is a complex mixture of parotid, submandibular and sublingual and minor salivary gland secretions mixed with mucin, bacteria, leukocytes, sloughed epithelial cells and gingival crevicular fluid. Oral fluid testing has number of diverse applications including detection of antibody to bacterial and viral infections. It is also been used in the detection of viral antigen, drugs of abuse, levels of steroid hormones and for the therapeutic monitoring of drugs.

OMT is the fluid derived from the passive transport of serum components through the oral mucosa into the mouth. It is also called as gingival crevicular fluid. The concentrations of IgG and other serum components in this fluid are significantly higher than in whole saliva. Consequently, collection of oral fluid from gums was identified as the most promising source of fluid for diagnostic testing. The testing device utilizes a clean untreated swab made of a soft absorbent material that targets those OMT-rich areas in the mouth when used as instructed. After a brief brushing along the gum lines, the oral fluid on the swab is immersed in the sample buffer and finally the result is recorded. There is no need for sharps, which have the potential for cross contamination among patients when used improperly and present a danger to health-care personnel. Because of the low concentration of antigens in saliva, HIV infection is much less of a danger from saliva than from blood. The presence of secretory leukocyte protease inhibitor (SLPI) may be another factor contributing to the safety of saliva as a diagnostic specimen. SLPI expresses antiviral activity against free HIV-1 in a model using monocyte and lymphocyte derived tumor cell lines. Because SLPI does not interfere with transcytosis of cell associated virus, the inhibitory effect is restricted to HIV-1 in OMT.

Studies of correlations between serum and OMT have shown that saliva is easily obtainable reliable diagnostic specimen for antibodies.

Pai conducted two studies with sample size of 15 and 146 subjects and Delaney et al. and Zelin et al. used 327 and 47 subjects respectively. O’Connell et al. considered 101 as study sample. The sample size in our study was most similar to Pai et al. and O’Connell et al.

Pai in one study considered age range of 18-38 years and 18-88 years with median age of the 34 years in the second study.
had considered subjects above 16 years of age. O’Connell et al.\cite{9} in their study considered the mean age of 38.9 ± 9.8 for the study group. In the present study, an age range of 20-59 years was considered for the study sample with the total mean age of 35.72 ± 7.95 years. The mean age being 35.72 ± 7.87 years for study group and 35.73 ± 8.20 years for control group and this is similar to Pai et al.\cite{11}

In the present study, all subjects in the age range 20-29 years, 30-39 years, 40-49 years, and 50-59 years in study group showed 100% reactivity toward Rapid test using OMT. All 23 patients in the present study with ART were 100% reactive toward Rapid test using OMT, thus indicating that ART has no impact on detecting the HIV infection by Rapid test using OMT. As there were no studies reported in relation to ART patients for detection of HIV, comparison was not possible.

Gender distribution in the present study was 46% males and 54% females in the study group and 60% males and 40% females in the control group. O’Connell et al.\cite{12} in their study showed 85% males and 15% female in the study group. Pai\cite{3} considered 100% females as the testing was done on women in labor in rural India. Pai et al.\cite{9} in their second study considered 74% males and 26% females.

In the present study, all the 23 male subjects and 27 female subjects in the study group were 100% reactive towards Rapid test using OMT with 100% true positive results and no false negative results, showing 100% sensitivity. All the 20 male subjects and 13 female subjects in the control group were 100% non-reactive with 100% true negative results and no false positive results showing 100% specificity. Pai\cite{3} reported 100% sensitivity and 100% specificity in one study. However, in another study Pai et al.\cite{9} reported sensitivity of 93.3% and specificity of 100%. Delaney et al.\cite{10} in their study found OraQuick sensitivity with oral fluid to be 99.1% and specificity to be 99.6%. Zelin et al.\cite{11} observed sensitivity to be 100% and specificity to be 99.87%. Whereas O’Connell et al.\cite{12} in their study reported sensitivity to be 96% and specificity to be 100%. In the present study, the sensitivity and specificity are 100%, which is comparable to Pai.\cite{3}

In the present study, there were 100% true positive results and no false positive results; hence, the positive predictive value was 100%. There were 100% true negative results and no false negative results; hence, the negative predictive value was 100%. Pai\cite{3} reported 100% positive predictive value and negative predictive value. Zelin et al.\cite{11} observed positive predictive value to be 97.78% and negative predictive value to be 100%. In our study, positive predictive value is in accordance with Pai\cite{3} and negative predictive value with Pai et al.\cite{9} and Zelin et al.\cite{11}.

Though, it was a small sample size we found that the Rapid test using OMT is highly accurate as the diagnostic efficacy in our study was 100% for HIV antibody detection as that of the conventional Western blot/ELISA test. One large study reported a 36% increase in testing with consequent increase in the detection of previously undiagnosed HIV infected individuals with the Rapid tests. In the context of global efforts to scale-up HIV testing, Rapid test using oral fluid is highly accurate and feasible. The simple and non-invasive antibody tests such as OraQuick using OMT can increase HIV testing and diagnosis. Furthermore, it offers a real alternative to the currently used point-of-care tests like finger prick and also has potential to increase HIV testing and diagnosis in culturally diverse population.

Oral point-of-care HIV tests can well be accepted by clients and have reported high diagnostic performance. Although the diagnostic accuracy of oral fluid based tests is high, their accuracy and clinical utility depends on the quality control procedures that should be strictly followed to get a quality and accurate results. Further like all Rapid tests, oral point-of-care HIV test results must be considered as preliminary and require confirmation with reference standard test such as ELISA, Western blot/immunoflourescent assay.

**CONCLUSION**

For nearly 20 years, ELISA with Western blot as the confirmatory test, constituted the gold standard for HIV testing. The availability of several fast, accurate, easy-to-use and cost-effective HIV antibody tests play an integral role in encouraging more widespread HIV screening. Rapid HIV anti-body tests are feasible in a variety of clinical and non-clinical venues, but quality assurance must be maintained to ensure that results are accurate, providing greater access to testing, prevention and care services for persons newly infected and living with HIV, which can reduce the number of new infections and lead to reductions in HIV-associated morbidity and mortality. In the present study: HIV was detected successfully using OMT by Rapid test. The OraQuick Advance Rapid test kit showed 100% sensitivity and specificity. As the sample size in this study
was small, future clinical trials and research is needed to firmly establish the efficacy and wide scale usages of OMT for detection of HIV. Rapid testing facilitates patients receiving their test results the same day, usually at the encounter where the test specimen was collected. OMT Rapid testing for HIV detection can be expanded to both urban and rural settings. OMT Rapid testing has client preferences for HIV testing and removes practical disincentives in priority population. OMT Rapid test is easy to perform and cost-effective (US$ 4 per kit in India) method of providing HIV testing services in community based settings. We now have a growing technological armamentarium to help us identify HIV, which is acting as adjunct for successful screening, accurate diagnosis and effective follow-up of HIV patients.

REFERENCES

1. Global report: UNAIDS report on the global AIDS epidemic 2012. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf. Last accessed: July 17, 2013.

2. HIV Estimations 2012 Report Released. Press Information Bureau, Government of India, Ministry of Health and Family Welfare, 2012. Available from: http://www.pib.nic.in/newsite/PrintRelease.aspx?relid=89785. Last accessed: July 17, 2013.

3. Pai NP. Rapid oral fluid-based point-of-care HIV testing: Applicability in developing countries. Indian J Med Res 2007;126:171-3.

4. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. Hillsdale, New Jersey: Lawrence Erlbaum Associate Inc.; 1988.

5. Classification and diagnostic criteria for oral lesions in HIV infection. EC-clearinghouse on oral problems related to HIV infection and WHO collaborating centre on oral manifestations of the immunodeficiency virus. J Oral Pathol Med 1993;22:289-91.

6. Granade TC, Phillips SK, Parekh B, Gomez P, Kitson-Piggott W, Oleander H, et al. Detection of antibodies to human immunodeficiency virus type 1 in oral fluids: A large-scale evaluation of immunoassay performance. Clin Diagn Lab Immunol 1998;5:171-5.

7. Mohan H. Textbook of Pathology. 5th ed. India: Jaypee; 2005.

8. Hofman LE. Human saliva as a diagnostic specimen. J Nutr 2001;131:1621S-5.

9. Pai NP, Joshi R, Moodie EE, Taksande B, Kalantri SP, Pai M, et al. Profile of adults seeking voluntary HIV testing and counseling in rural Central India: Results from a hospital-based study. AIDS Care 2008;21:294-300.

10. Delaney KP, Branson BM, Uniyal A, Kerndt PR, Keenan PA, Jafa K, et al. Performance of an oral fluid rapid HIV-1/2 test: Experience from four CDC studies. AIDS 2006;20:1655-60.

11. Zelin J, Garrett N, Saunders J, Warburton F, Anderson J, Moir K, et al. An evaluation of the performance of OraQuick ADVANCE Rapid HIV-1/2 Test in a high-risk population attending genitourinary medicine clinics in East London, UK. Int J STD AIDS 2008;19:665-7.

12. O'Connell RJ, Merritt TM, Malia JA, VanCott TC, Dolan MJ, Zahwa H, et al. Performance of the OraQuick rapid antibody test for diagnosis of human immunodeficiency virus type 1 infection in patients with various levels of exposure to highly active antiretroviral therapy. J Clin Microbiol 2003;41:2153-5.

Source of Support: Nil. Conflict of Interest: None declared.