QUALITATIVE IMMUNOASSAY FOR DETECTION OF ANTIBODIES TO HIV 1/2 IN ORAL MUCOSAL TRANSUDATE - A DIAGNOSTIC STUDY

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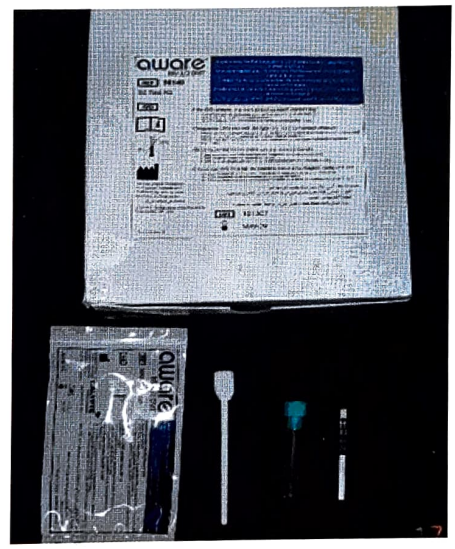
ABSTRACT

This study aims at qualitative analysis of antibodies to HIV in Oral mucosal fluid and discusses the accuracy and reliability of these tests in diagnosing HIV infection. 25 patients were selected under the category of clinically suspected cases of HIV infection. 25 cases Of Known HIV patients are used as control group and are tested for Oral Mucosal Transudate diagnostic test. The sensitivity of the OMT rapid screening assay was 93.93% and specificity was 100%. The positive predictive value was computed to be 100% with a negative predictive value of 87.4%. Receptor Operator characteristic curve which is plotted against sensitivity and specificity of OMT Test clearly shows the area under the curve is 0.970 indicating that OMT test is better useful as a screening test for oral diagnostic purpose.

Oral fluid can be used as an efficient Screening tool for diagnosis of HIV infection. Further longitudinal studies, increased sample size is required to test the efficacy of Oral Fluid / Saliva in diagnosing HIV infection and using OMT test as a screening test in practice.

INTRODUCTION

Acquired Immuno Deficiency syndrome is a fatal disease caused by a



Retro virus called Human Immuno Deficiency Virus which is a RNA virus and is characterized by profound Immunosuppression together with development of opportunistic infections, Secondary Neoplasm's and neurologic manifestations[1].

Centers of Disease control modified AIDS definition2 in 1992 as "All people infected with HIV who have a CD4 lymphocyte count of < 200 cells per micro liters irrespective of clinical manifestations / who have one of the following 3 clinical conditions, Pulmonary tuberculosis, Recurrent pneumonia and Invasive cervical cancer".

LABORATORY TESTS

Laboratory diagnosis for diagnosis of HIV includes specific tests for HIV as well as tests for immunodeficiency3-5.

A. Immunological tests

B. Specific Test for HIV infection

A. The Immunodeficiency of the individual can be established by the following parameters.

a. Total Leukocyte / Lymphocyte count

b. T cell subset assay: Absolute CD4 & Tcell Count, T4: T8 ratio.

c. Platelet Count

d. Raised IgG / IgA level

e. Lymph node biopsy

B. Specific Tests for HIV infection:

The specific test for detecting HIV infection depends on demonstration of HIV antigens and its components, antibodies, isolation of Virus. The antibody test which is current in practice is ELISA, Rapid serum tests, Rapid Salivary and Western Blot[2].

Oral Tests for HIV antibody

Various Oral Tests to detect HIV antibody either in Whole saliva or Oral Mucosal Transudate have become popular in recent years though it have been introduced long before. The possibility that saliva and oral fluids could be used for HIV screening and Diagnosis of HIV infections have been known since 1986. Oral Screening tests for HIV uses either IgA antibody of saliva or IgG antibody of Oral fluids. The advantages over serum screening test are ease of collection, safety, compliance and cost. Antibodies to Human Immuno deficiency virus type 1 have been found in various body fluids such as saliva, breast milk, cervical secretions and semen. The advantage of rapid HIV tests particularly with oral

fluid specimens, include increased availability of testing among populations at high risk for HIV infection and increased receipt of test results among those tested

MATERIALS AND METHODS

Armamentarium:

- Diagnostic Instruments
- Universal barriers: Disposable gloves, Masks, Protective goggles
- Timer
- Test KIT: Aware HIV 1/2 OMT Kit consisting of
  - Foil pouch containing Oral fluid test strip and desiccant.
  - Capped test tube containing oral fluid sample buffer
  - Oral fluid sample collection swab
- Biomedical Waste Disposal system

AWARE ORAL MUCOSAL TRANSUDATE TEST:

A qualitative, visually read, in vitro immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1(HIV- 1) and Type 2 (HIV-2) in human oral mucosal transudate specimens. Aware HIV 1/2 OMT test is introduced by Calytype Biomedical corporation, USA. It is intended for use as appoint of care aid in the clinical diagnosis of HIV infection. The test may be used as a component of a multi- test rapid algorithm in conjunction with other approved HIV antibody assays. Individuals infected with HIV produce antibodies against the HIV viral proteins. Testing for the presence of these antibodies in bodily fluids (blood, urine, and oral fluid) is an accurate aid in diagnosis of infection. The Aware HIV 1/2 OMT test is composed of a single - use test device, an oral fluid specimen, collection swab and a tube of oral fluid sample buffer. The test utilizes a proprietary lateral flow immunoassay procedure. The assay test strip is compromised of several materials that provide the matrix for the immunochromatography of the specimen. The assay strip contains recombinant regions of HIV -1 gp41 and HIV -2 gp 36 transmembrane proteins and a goat anti human IgG F (ab') 2 fragment antibody in- capture procedural control immobilized onto the nitrocellulose in the test zone and the control zone, respectively. The oral fluid swab is placed vertically into the test-tube of fluid buffer. The liquid in the swab is expressed out and the used swab is discarded. The assay strip is then placed vertically into the test tube containing the specimen / buffer mixture. As the diluted specimen migrates up the assay test strip, it rehydrates a reddish Protein A- colloidal gold reagent on the strip and IgG specimen becomes bound to the protein A / colloidal gold particles to form IgG / conjugate complex. The complex then migrates up the strip and first encounters the test zone of the assay strip containing the HIV antigens. If the specimen contains antibodies to HIV, IgG/conjugate complex binds to the antigen and becomes immolized at the antigen line in the test zone and a reddish colored line appears. In a validity performed test, this indicates a reactive result. The intensity of the line is not proportional to the amount of antibody present in the specimen. The Specimen/conjugate mixture continues to migrate up the assay strip until it encounters the Control Zone. The Control Zone contains anti human IgG F (ab') 2 fragments immobilized in a line on the assay test strip. The complex bound to the immobilized F (ab') 2 fragments and a reddish colored line appears. The appearance of the control line is evidence that the test functioned properly and the sample contained human IgG. A reddish purple control line will appear in the control Zone during the performance of all valid tests, whether or not the sample is reactive or negative for antibodies to HIV 1/2. The specimen continues to migrate past the control Zone into the final absorbent pad, which helps draw the specimen / conjugate mixture through the strip and clear any background color. The Test results are interpreted after 20 min-

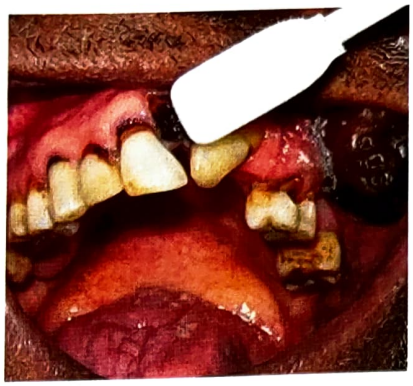
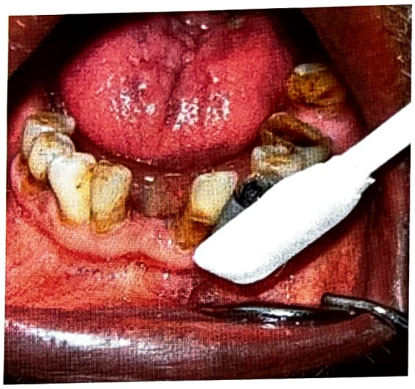


FIG 2



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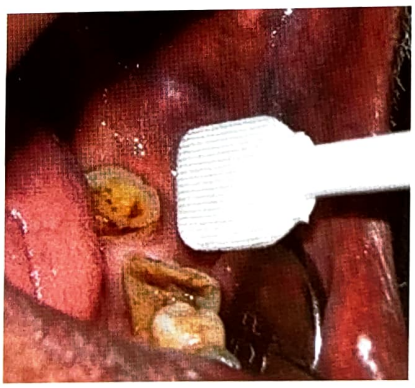

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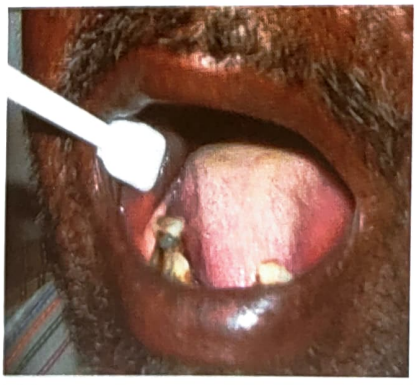


FIG 5

utes but not more than 45 minutes after introduction of the assay strip to the diluted specimen.

Procedure

Oral fluid sample collection swab is removed from the foil. Moderate pressure is applied while gently swabbing the upper gingiva back and forth with the cloth end of the swab. Beginning at one end of the mouth, swabbing is done gently and slowly until reaching the other corner of the mouth, covering the buccal mucosa. The other end of the swab is turned and is used for swabbing the lower gingiva. Immediately the swab is placed in the tube containing the sample buffer. **(Fig 2-5)**

The swab handle is grasped firmly and plunged up and down 6-8 times in the sample buffer rubbing both sides of the swab against the sides of the tube **(Fig 6)**.

The swab is carefully removed and discarded in a biomedical waste container. Now the foil pouch is opened and the assay test strip is placed in the tube containing diluted specimen with arrow in the assay strip pointing downwards. Timer is set. The results are read after 20 minutes. **(Fig 7)**

Interpretation of Results:

The Results are interpreted as follows by appreciating the test line and control line in the test strip. **(Fig 8)**

Reactive: The test is read as positive or reactive if Two lines appear in the assay strip (i.e.) in the test zone and control zone.

Non-reactive: The test is read as Negative or non reactive if only one line appear in the assay strip (i.e.) in the control zone only.

Invalid: The test is considered invalid if no control line is present. The invalid test should be repeated using a new test device and a second specimen.

Precautions

Tests should be performed at ambient temperature 15-30 degrees. Universal precautions have to be taken care of when handling body fluids and assay strips. Patient asked not to drink, eat or smoke before the tests. Biomedical waste disposal system should be maintained to dispose the test specimens.

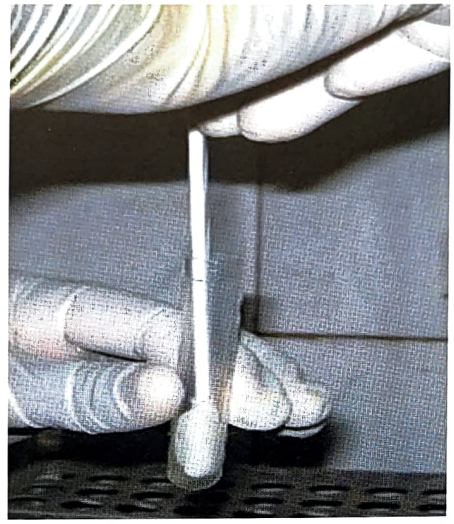


FIG 6

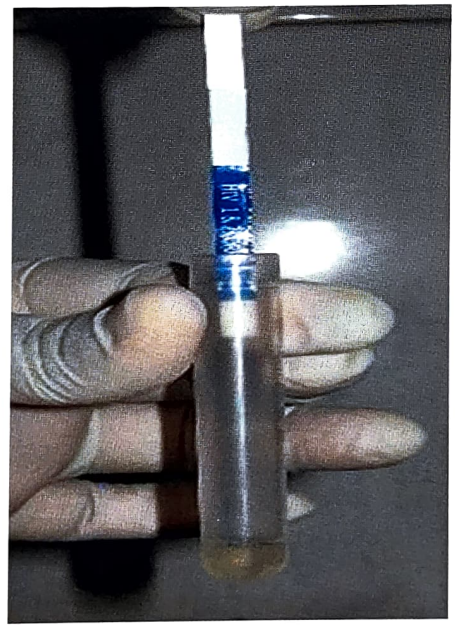


FIG 7

FIG 8

Results and Analysis:

This study is evaluated to test the sensitivity, specificity, positive predictive value and negative predictive value of Calypte's AWARETM Oral mucosal transudate rapid test for detecting antibodies to HIV 1/ 2 in oral fluid and is compared with standard serum rapid test approved by National AIDS control Organization. Sample sizes of 50 patients were selected and grouped in two groups namely Known Sero-positive (Group1) and suspected cases of HIV (Group 2). Group 1 includes 25 cases (50%) of HIV Known Sero-positive HIV infected individuals who came to the dept of Oral Medicine and Radiology for various dental treatments. Group 2 includes 25 cases (50%) of clinically suspected cases of HIV infection. All patients were under age group 20- 60yrs. Out of 50 patients the mean age group of Group 1 were 36 and Group 2 was 37. Out of which 35 were males 15 were females. With their informed consent and Pre counseling Oral Mucosal transudate test were carried out and the results were analyzed using SPSS software. From the statistical analysis the sensitivity of the OMT rapid screening assay was 93.93% and specificity was 100%. The positive predictive value was computed to be 100% with a negative predictive value of 87.4%. Receptor Operator characteristic curve (Graph 1) which is plotted against sensitivity and specificity of OMT Test clearly shows the area under the curve is 0.970 indicating that OMT test is better useful as a screening test.

Discussion:

The main aim of this study is to evaluate the efficacy of Oral Fluid in diagnosing and screening individuals with HIV infection and to compare the Sensitivity and Specificity of Oral Mucosal transudate test with standard serum screening test. Epidemiological studies need easier tools to evaluate

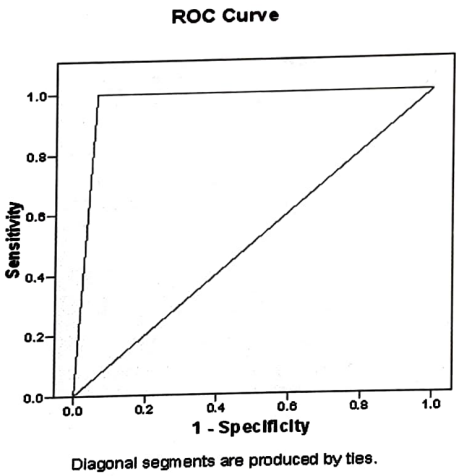
HIV prevalence particularly in high risk groups under difficult field conditions. Testing Saliva/ Oral mucosal antibody with accurate immunoassay could serve as an alternative to serum assays. Salivary assays could be efficient and non invasive epidemiological tool for HIV testing.

Whole saliva is a mixture of oral fluids and includes secretions from both minor and major salivary glands , in addition to several constituents of non-salivary origin such as gingival crevicular fluid (GCF), expectorated bronchial and nasal secretions , serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components and food debris. There are several ways by which the serum constituents that are not part of normal salivary constituents can reach saliva. A serum molecule reaching saliva by diffusion must cross five barriers; the capillary wall, interstitial space , basal cell membrane of the acinus cell/duct cell, cytoplasm of the duct cell, luminal cell membrane. Serum constituents are also found in whole saliva as a result of GCF outflow (Eliaz Kaufman, Ira B. Lamster 2002)6. Depending on the degree of inflammation in gingival, GCF is a serum transudate or an inflammatory exudate that contains serum constituents. This Study aims at detection of IgG anti HIV antibody in oral fluid and hence called as Oral Mucosal Transudate test. The antigens to HIV-1(gp 41) and HIV-2 (gp 36) were impregnated in the assay strip to test the presence of HIV antibody to either HIV type 1 virus or Type 2 virus. So this test qualitatively analyses the presence of antibodies to HIV infection of both type 1 and type 2. In this study sample size of 50 cases were selected and divided in two groups namely Known Sero-Positive patients and Suspected cases of HIV infection. Oral examination is an important part of any physical examination and nowhere is this more important than in the case of suspected HIV infection (Greenspan.D 1993)7. As by saying "Mouth is a mirror of systemic diseases" oral manifestations of HIV affected individuals are typical and in most cases be an indicator of poor immunosuppression and HIV infection. Suspected cases were selected accordingly by the presence of any one or more of the following conditions namely

OMT * Serum assay Crosstabulation

			Serum assay		Total
			Positive	Negative	
OMT	Positive	Count	31	0	31
		% within Serum assay	93.9%	.0%	62.0%
	Negative	Count	2	17	19
		% within Serum assay	6.1%	100.0%	38.0%
Total		Count	33	17	50
		% within Serum assay	100.0%	100.0%	100.0%

Sensitivity of OMT Test: (31/33) × 100 = 93.93 %
The percentage of the results that will be positive when HIV is present.
Specificity of OMT Test: 100%.
The Percentage of the results that will be negative when HIV is not present
Positive Predictive Value: 100%
Negative Predictive Value: 89.4%
ROC Curve: (Receiver Operative Characteristics Curve) Graph1



High Risk group individual
Homosexuals
Extramarital Sex
Intravenous drug users
Sex Workers
Children of HIV infected individuals
Occupationally Risk groups

General manifestations
Chronic Cough for more than 6 months
Recurrent Fever for more than 6 months
Sudden Weight loss of about 10kgs
Fatigue/ Tiredness
H/O tuberculosis
Others
Severe Dermatological Lesions
Malignancies
Respiratory and others serious infections

Oral manifestations:
Oral candidiasis
Psedomembranous
Atrophic/ erythemaotus
Angular Cheilitis
Non Healing Ulcers
Recurrent Apthous Ulcers.
Severe Periodontits/ Gingivitis with necrosis.
Others Malignancies

According to this study the sensitivity of Oral Mucosal Transudate test is 93.93 %, specificity is 100%, the positive predictive value is 100% and negative predictive value is 89.4%.

Receptor Operator characteristic curve of this study plotted against the sensitivity and specificity showed and area of 0.90 under the curve which clearly indicated that this test can be used as a Screening test to diagnose HIV infection. The Dental clinicians are more prone to HIV cross infections and it is necessary for the dental clinicians to cross check individuals affected with HIV infection to prevent HIV cross infection. Oral Mucosal transudate test , an easy, simple , non invasive method for screening HIV infection would be a valuable tool and a chair side diagnostic aid for Oral medicine Specialist to screen patients undergoing dental treatment and for those clinically suspected cases of HIV infection. Application of Oral Fluid as a diagnostic aid for HIV infection would bring a remarkable change in dentistry8 and also it would be a better screening tool for epidemiological studies and screening for HIV infection in high risk group individuals8. In this study the specificity of HIV infection is 100% indicating that this test is a valid screening test. However large sample size and further longitudinal studies is necessary to use this test as a screening test and validating this test as standard test for HIV infection.

SUMMARY AND CONCLUSION:

HIV rapid testing devices are easy to use, do not require refrigeration and are less expensive to run, portable with a longer shelf life. They are more adaptable for field use and screening of high risk and far to reach populations mostly located in resource poor settings8,9,10. Infact, they have reduced the time between testing and release of results to minutes from days or weeks when compared with other assays. HIV 1/ 2 OMT evaluation result falls within the WHO recommended range of tests to be used for HIV diagnosis. In this Study sensitivity and specificity of HIV 1/ 2 OMT test is evaluated in a sample size of 50 patients divided in two groups namely, known Sero-positive and suspected cases of HIV infection. From the results it's very clear that Oral mucosal transudate test can be used as a screening test for large scale high risk group individuals in a community, suspected patients in dental health care settings. Though a confirmatory testing is required using a standard serum test before disclosing positive result to a patient. Cross infections in a dental community or health care workers can be readily minimized when Oral mucosal transudate test is used in dental care patients. CDC continues to encourage the use of rapid HIV tests11-13, 8 because they increase the number of persons who are tested and who receive their tests results.. The main advantage of rapid HIV oral fluid tests includes increased availability and acceptability of testing among populations at high risk for HIV infection and is simple, easy, non-invasive, user friendly4 and prevents cross infection to health care workers. From this study we conclude that Oral fluid can be used as an efficient Screening tool for diagnosis of HIV infection8. Further longitudinal studies, increased sample size is required to test the efficacy of Oral Fluid / Saliva in diagnosing HIV infection and using OMT test as a screening test in practice.

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