Original Research Article

"Screening of Suspected HIV-AIDS Patients: A Comparative Study Evaluating HIV-ICT Device and ELISA"

Abstract

Objectives:

To evaluate the sensitivity and specificity of Immunochromatographic device in comparison with Enzyme Linked Immuno-Sorbrent Assay.

Material and Methods:

It was a descriptive comparative study certified by Ethical Review Board of Allama Iqbal Medical College Lahore. Study was conducted at the Department of Pathology, Allama Iqbal Medical College Lahore. A total of 106 study subjects were included by using convenient sampling method within the duration of 4 months. Samples were processed in ELISA section, Department of Pathology, Allama Iqbal Medical College. Data was entered and analysed by using SPSS 22.0. A p-value of ≤ 0.05 was considered as statistically significant.

Results:

Out of 106 patients 28 samples had been reported as positive with HIV–ELISA whereas, HIV ICT devices reported 21 cases as positive. On the other hand 78 samples stood negative with HIV-ELISA and 85 samples remained negative with HIV-ICT device. For HIV ICT device, the calculated sensitivity was 71.4% and the Specificity was 98.7%. The Positive Predictive Value (PPV) was 95.2% whereas the Negative Predictive Value (NPV) was 90.6%.

Conclusion:

The ICT device is a rapid, reliable and valid device with shortest turn-around time and can be used in emergency settings and in low resource settings. Although, the device showed high sensitivity and specificity, but it cannot be taken as an ultimate diagnostic tool for HIV screening. Final diagnosis should be based on anti HIV 1/2 ELISA, Western Blot and PCR findings (Gold standard diagnostic assay).

Key words:

Human Immunodeficiency Virus (HIV); Acquired Immunodeficiency Syndrome (AIDS); Injection Drug Users (IDU); Immunochromatographic Device (ICT); Enzyme Linked Immunosorbent Assay (ELISA).

Introduction

AIDS is a retroviral disease caused by Human Immunodeficiency Virus (HIV) characterized by depletion of CD4+ T-Lymphocytes, which later leads to immunosuppressant, opportunistic infections, secondary neoplasms and neurologic manifestations [1]. As the epidemiologic pattern of the disease unfolded, it became clear that an infectious agent transmissible by sexual (homosexual and heterosexual) contact and blood or blood products was the most likely etiologic cause of the epidemic [2].

In 1983, HIV was isolated from a patient with lymphadenopathy and by 1984 it was demonstrated clearly to be the causative agent of AIDS [3]. Although AIDS was first described in United States, it has now been reported in virtually every country in the world. Worldwide, more than 22 million people have died of AIDS since the epidemic was recognized in 1981. About 42 million people are living with the disease, and there are estimated 5 million infections each year. Worldwide 95% of HIV infections are in developing countries, with Africa alone

carrying more than 50% of the HIV burden. AIDS still represents the fifth most common cause of death in adults between the age of 25 and 44 [1].

Prevalence of HIV reached 31% amongst the Injection Drug Users (IDUs) in 2007 in Karachi, Pakistan making them the most vulnerable group. Males migrating from rural to urban areas for earning usually get involved in unsafe sexual practices being helped by the emergence of "red light areas" in the metropolitan cities. Professional blood donors and inadequate blood screening techniques worsen the scenario [4].

Rapid diagnostic tests (RDTs) are diagnostic assays designed for use at the point-of-care (POC) testing and can be adapted for use in low-resource settings. There are over 60 types of rapid HIV tests being used around the world [5]. A Rapid Diagnostic Test is low-cost, simple to operate and read, sensitive, specific, stable at high temperatures, and works in a short period of time [2]. Rapid HIV tests also referred to as rapid/simple (r/s) test devices these tests are based on one of four immunodiagnostic principles: particle agglutination, immunodot (dipstick), immunofiltration and immune chromatography [6]. Immunochromatographic device tests are better than other rapid assays by making HIV diagnostic test a one-step assay [7].

Iweala, (2004) reviewed different diagnostic tools for the detection of HIV and stated that the HIV diagnostic tests that detect host antibody specific to the virus include the enzyme immunoassay (EIA, also commonly referred to as the enzyme-linked immunosorbent assay), Western blot (or immunoblot), the immunofluorescence assay (IFA), rapid tests, salivary tests, urine tests and the detuned assay. Predictive value of the EIA and of HIV screening tests in general, or the likelihood that the assay will accurately determine a person’s true infection status, depends on the prevalence of HIV infection in the population. In general, the higher the

prevalence of HIV infection in the population, the higher the positive predictive value of the assay. [7]

Butto, (2010) studied different diagnostic tools for HIV and stated that Rapid tests can present some problems of sensitivity [8]. Kwenti, (2011) conducted a study to determine the validity of the results obtained by immunochromatographic rapid strip test to diagnose hepatitis C virus infection in HIV-positive patients and compared it with the results obtained by more sensitive and specific methods like ELISA and PCR. Evaluation of the rate of false positives with the rapid strip test using ELISA as the gold standard gave a rate of 6·3% [9].

Deguchi, (2012) conducted a study to evaluate the clinical performance of a new assay against immunochromatographic assay (ICA) for HIV Ab detection, ELISA for Ag/Ab combination assay and chemiluminescent enzyme immunoassay (CLEIA) for Ab detection and were evaluated with the immunochromatographic assay for Ag/Ab detection. The study found that HIV Ag/Ab ICA showed 100% clinical specificity and was better than 99.8% of the existing ICA. The CLEIA and ELISA showed 100% and 99.8% specificity, respectively [10].

Therefore, the present study has been designed to estimate the prevalence of HIV in patients presenting in 04 months of duration at Jinnah Hospital Lahore/Allama Iqbal Medical College and to detect the sensitivity and specificity of HIV ICT device in comparison with HIV-ELISA.

Material and Methods

It was a descriptive comparative study certified by Ethical Review Board of Allama Iqbal Medical College Lahore. Study was conducted at the department of pathology, Allama Iqbal Medical College Lahore. Blood samples from a total of 106 study subjects were collected by

using convenient sampling method within the duration of 04 months. Suspected cases of HIV infection presenting in Department of Pathology, without any discrimination of age or gender were included in this study. Three ml of blood sample from these patients was drawn according to the WHO protocol. Serum was separated for HIV screening by ELISA and ICT device. Samples were processed in tertiary care AIDS referral centre and ELISA section, Department of Pathology, Allama Iqbal Medical College Lahore. ICT device and ELISA kit used, both were standardized and commercial.

ICT device (Alere Global, USA) determines HIV-1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2. Sample was added to the sample pad. As the sample migrated through the conjugate pad, it constituted and got mixed with the selenium colloid-antigen conjugate. This mixture continued to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site.

In HIV detection ELISA kit (BioTech Services, Pakistan), a specific antigen was attached to solid phase by passive adsorption or with antigen specific antibody. Test serum containing specific antibody was added. Enzyme labeled antiglobulin specific for the test serum was added. Chromogenic enzyme substrate was then added. The color developed was proportional to the amount of antibody present in the test serum. Statistical analysis was done using SPSS version 22.0. Independent student's t-test had been applied for both study groups. A p value of ≤ 0.05 was considered as statistically significant.

Results

This observational study was undertaken for a period of four months at the Department of Pathology, Allama Iqbal Medical College, Lahore. Blood samples from a total of 106 patients, fulfilling the study criteria had been included in the study. The samples were collected from out patients department, Jinnah Hospital Lahore and patients presented in the Department of Pathology, Allama Iqbal Medical College and also from Laboratory staff members who were highly suspected for HIV infection specifically the staff dealing with the patients from Punjab AIDS Control Program (PACP) working in the Flowcytometry section.

Fortunately, we have found those highly suspected staff members negative with HIV. However, samples from Jinnah Hospital were mostly reported Positive and the reason sorted behind was that these samples were taken from prisoners and they were mostly intravenous drug users (IDUs) and were involved in extra marital contacts.

The mean age of study population was 34.63 ± 9.79 years, ranging between 19 to 60 years (Median 32.0 and Mode 42.0). Out of 106 samples 83 were males and 23 were females i.e. 78.30% and 21.70% respectively.

Out of 106 patients 28 samples had been reported as Positive with HIV–ELISA whereas, HIV ICT Devices reported 21 cases as Positive. On the other hand 78 samples stood Negative with HIV-ELISA and 85 samples remained Negative with HIV-ICT Device (Table 1).

All the above mentioned statistical data after the application of appropriate statistical tools has eventually aided us with the calculation of sensitivity and specificity of HIV-ICT Device against the HIV-ELISA assay. For HIV-ICT device, the calculated sensitivity is 71.4% and the Specificity is 98.7%. The Positive Predictive Value (PPV) is: 95.2% whereas, the Negative Predictive Value(NPV) is: 90.6% (Table 1)

In a ROC Curve the true positive rate (sensitivity) is plotted in function of the false positive rate (specificity) for different cut off points of a parameter. Each point on the ROC curve represents a sensitivity / specificity pair corresponding to a particular decision threshold (Figure 1).

Discussion

The main purpose of the present study was the screening of suspected HIV/AIDS patients and the evaluation of the performance of HIV-ICT device by comparing it with ELISA. After the application of appropriate statistical techniques, results showed the sensitivity and specificity of HIV-ICT device against the HIV-ELISA assay. The calculated sensitivity was 71.4% and the Specificity was 98.7%.

Cordes and Ryan (1995) compared Enzyme-linked Immunosorbent assay (ELISA) and Western blot assay which are commonly used laboratory tests for HIV infection. Results found that both detect antibodies to HIV but ELISA tests have greater than 98% sensitivity and specificity for HIV-ELISA results are based on detection of antigen-antibody complexes by using antibodies labeled with an enzyme that produces a color change in the presence of a specific substrate. Enzyme Linked Immunosorbent assay was also taken as gold standard in the present study [11].

Hua (2006) studied the sensitivity, specificity and the accuracy of the dot immunochromatography assay (DICA) for HBsAg, Anti-HCV and Anti-HIV methods. The plasma specimen of 502 patients were tested for HBs Ag,Anti-HCV and Anti-HIV by DICA and ELISA. The sensitivity and specificity of the two approaches were compared. The study found that the sensitivity and specificity of DICA are both slightly lower than those of ELISA. Results showed that as compared with ELISA, 2 false negative and 5 false positive were found in 502 specimens in HBsAg test by DICA. The sensitivity was 96.4%, while the specificity was 98.9%,

and the accuracy was 98.6%. Nine false positive were found in 502 specimens in Anti-HCV test by DICA, whose sensitivity was 100%, and the specificity was 98.2%, the accuracy was 98.2%.2 false positive and no false negative were found in 502 specimens in Anti-HIV test by DICA, the specificity was 99.6% and the accuracy was 99.6%. False positive and false negative were found in HBsAg test. The sensitivity of Anti-HCV and Anti-HIV tested by DICA accorded with ELISA But the specificity of Anti-HCV and Anti-HIV tested by DICA is slightly lower than those by ELISA. The study suggested that final report should be based on ELISA. The present study also proved that the sensitivity and specificity of the ICT device is less than ELISA [12].

Kwenti (2011) conducted a study to determine the validity of the results obtained by immunochromatographic rapid strip test to diagnose hepatitis C virus infection in HIV-positive patients and compared it with the results obtained by more sensitive and specific methods like ELISA and PCR. Among 350 HIV-positive patients, 25 (7·1%) patients were found to be positive with the rapid strip test of which 3 (12%) were positive with ELISA and all 3 (100%) positive with the ELISA were also positive with PCR. Evaluation of the rate of false positives with the rapid strip test using ELISA as the gold standard gave a rate of 6.3%. Meanwhile in the control group, after screening with the rapid strip test, 39 (11·1%) were positive of whom 6 (15·4%) were positive with the ELISA and 3 (50%) of the 6 positive with the ELISA were also positive with the PCR. Evaluation of the rate of false positives with the rapid strip test in the control group using ELISA as the gold standard gave the rate of 9·6% [9].

ICT devices are highly useful in emergency settings and point of care (POC) testing. False positive results with this immunochromatographic rapid strip test for the diagnosis of hepatitis C virus, HBs Ag and HIV infection are frequent. Therefore, it reinforces the need for a confirmatory test prior to treatment in hospital settings. It has already been documented that a

positive result for above mentioned conditions got with an immuno-chromatographic rapid strip test does not warrants that treatment should begin due to possibility of false positive or false negative results. Therefore the presence of the disease should be investigated further using a more sensitive and specific assay prior to treatment. Although PCR and western blotting (WB) assays are very expensive to be incorporated into hospital settings, an ELISA which is less expensive and more affordable can be implemented to give more valid results. Moreover, a negative result does not exclude the presence of the infection. If symptoms persist, then the infection should be investigated further with a PCR and WB assays. It is important that diagnosis should be done together with the patient medical history. The present study has also proved ELISA to be more specific and sensitive than ICT devices.

Conclusion

The ICT device is a rapid, reliable and valid device with shortest turnaround time and can be used in emergency settings and point of care (POC) testing. Moreover, it is highly useful in low resource settings. The device showed high sensitivity and specificity, but it cannot be taken as an ultimate diagnostic tool for HIV screening. Final diagnosis should be based on anti HIV 1/2 ELISA, Western Blot and PCR findings with the correlation of clinical picture of the suspect.

References

1. Kumar V, Abbas AK, Fausto RN. Robbins Basic Pathology. 8thed. (2007). New Dehli, Elesvier.

2. Sirisak N. Diagnostic value Immunochromatographic Test over clinical predictors for Tuberculosis in HIV patients. Dove Press Journal 2011; 3:237-244.

3. Fauci AS, Lane HC. Human immunodeficiency virus disease: AIDS and related disorders. In: Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL, eds. Harrison's

Principles of Internal Medicine, 17th ed. USA: The McGraw-Hill companies, Inc. 2008; 1137-203.

4. Rai MA, Warraich HJ, Ali SH, Nerurkar VR. HIV/AIDS in Pakistan: the battle begins. Retrovirology 2007; 4: 22.

5. Paul SM, Grimes-Dennis J, Burr CK, Diferdinando GT. Rapid diagnostic testing for HIV: clinical implications. New Jersey Medicine 2003; 100 (9):11–14.

6. Ekwueme DU, Pinkerton SD, Holtgrave D, Branson BM. Cost comparison of three HIV counseling and testing technologies. American Journal of Preventive Medicine 2003; 25:112-121.

7. Iweala IO. HIV diagnostic tests: an overview. Contraception 2004; 70: 141–147

8. Butto S, Suligoi B, Belasio FE, Raimondo M. Laboratory diagnostics for HIV infection. Annali dell'Istituto Superiore di Sanità 2010; 46 (1) : 24-33.

9. Kwenti ET, Njouom R, Njunda AL, Lucien H, Kamga F. Comparison of an Immunochromatographic Rapid Strip Test, ELISA and PCR in the Diagnosis of Hepatitis C in HIV Patients in Hospital Settings in Cameroon.Clinical Medicine and Diagnostics 2011; 1(1): 21-27.

10. Deguchi M, Yoshioka N, Kagita M, Kita M, Asari S, Honda T, Kobayashi E. Evaluation of human immunodeficiency virus-1/2 antigen/antibody immunochromatographic assay. Clinical Laboratory 2011; 58: 11-12.

11. Cordes RJ, Ryan ME. Pitfalls in HIV testing.Application and limitations of current tests. Postgraduate Medicine 1995; 98 (5):177-80.

12. Hu DJ, Dondero TJ, Rayfield MA, George JR, Schochetman G, Jaffe HW, Luo CC, Kalish ML, Weniger BG, Pau CP. The emerging genetic diversity of HIV. The importance of

global surveillance for diagnostics, research, and prevention. Journal of American Medical Association 1996; 275(3):210–216.

Bhanu Mehra, Sonali Bhattar, Preena Bhalla, and Deepti Rawat, "Rapid Tests versus ELISA for Screening of HIV Infection: Our Experience from a Voluntary Counselling and Testing Facility of a Tertiary Care Centre in North India," ISRN AIDS, vol. 2014, Article ID 296840, 5 pages, 2014. doi:10.1155/2014/296840

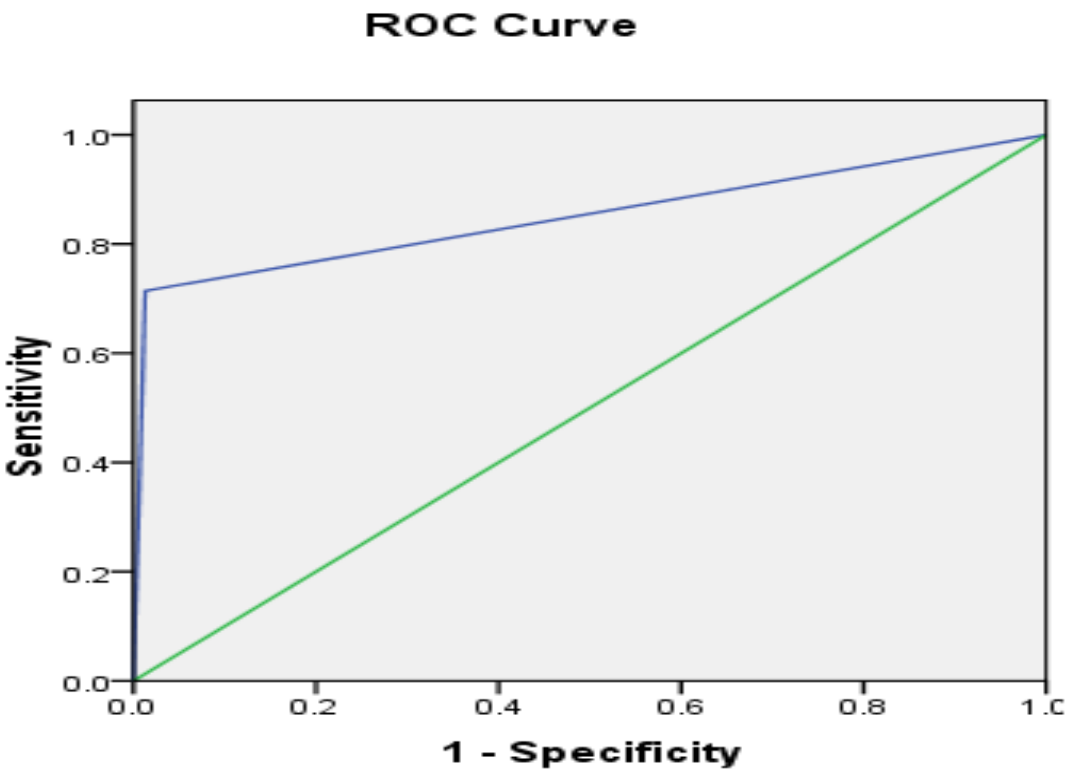
Table 1: Comparison of ICT Device with ELISA (n=106)

ELISA	
Positive Cases 28	(26.4%)
Negative Cases 78	(73.6%)
ICT	
Positive Cases 21	(19.8%)
Negative Cases 85	(80.2%)
Sensitivity	71.4%
Specificity	98.7%
PPV*	95.2%
NPV**	90.6%

*Positive Predictive Value: PPV

**Negative Predictive Value: NPV

Figure 1: Receiver Operating Characteristics (ROC) Curve (n=106)



Diagonal segments are produced by ties.

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