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The Ninth International Scientific Academic Conference
Under the Title "Contemporary trends in social, human, and natural sciences"
المؤتمر العلمي الاكاديمي الدولي التاسع
تحت عنوان "الاتجاهات المعاصرة في العلوم الاجتماعية، الانسانية، والطبيعية"
17 - 18 يوليو – تموز 2018 - اسطنبول – تركيا
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Detection of HIV by molecular and serological methods from Indian patients

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Abstract: Diagnosing HIV infection early allows a person to make more informed decisions . The "window period" refers to the maximum amount of time it may take for a person's body to create HIV antibodies after HIV infection. HIV antibodies must be present in order for the HIV antibody test to accurately detect HIV antibodies in someone's blood. If someone is "in the window period," there is a chance that even though they may have been infected with HIV, the test won't be able to detect the infection and will give a negative result .Window period may differ from individual to individual. Identifying early acute infection in HIV is very important to limit onward transmission of HIV-1, we explored the sensitivity of the Rapid tests available in the market with ARCHITECT assay.

This project describes studies on the evaluation of human immunodeficiency virus (HIV) enzyme-linked immunosorbent assays (ELISAs) and simple rapid HIV assays for use in HIV testing**.** We used Rapid TRIDOT test and compared it with CIA(ARCHITECT) on 20 HIV positive and 20 HIV negative controls

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and we observed that rapid tests tend to give false positive or false negative reactions when compared to CIA .

Keywords: HIV , Real time PCR , CIA(ARCHITECT) , Genosens HIV-1 RT-PCR , Rapid test.

1. Introduction: Globally, it is estimated that more than 33.3 million people are living with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) in 2009, over 50% were women , transmission of HIV (MTCT) from mother to child accounts for about 90% of HIV infection in new born and young kids. Over 370,000 infants acquire HIV infections globally each year with roughly more than 1000 children acquiring HIV each day [(1)].

HIV-infected infants and young children have high risk of mortality because of rapid advancement of disease [(2)].

It is projected that up to 30% of untreated HIV-infected children die before one year and more than 50% die before the age of 2 years [(3)], there is urgent need to identify and enroll them into care and treatment programs.

Early Infant Diagnosis (EID) of HIV finds early HIV status and management to comprehensive AIDS care and treatment of infected infants. EID of HIV is carried out by detection of infant blood RNA or DNA. In places where resources are limited, for EID venous blood has been used to carry out DNA polymerase chain reaction (PCR) test of HIV in children less than 1.5 years. Numerous studies have reported the transfer from venous blood to dried blood spot (DBS) specimen .

HIV-specific antibody detection is the most commonly used approach for the diagnosis of HIV infection. However, antibodies usually appear about 3-4 weeks after initial HIV infection .Several types of assays for HIV antibody detection have been developed and promoted for HIV screening and diagnosis [(4)]. Enzyme-linked immunosorbent assay (ELISA) is the most commonly used technique for screening purposes in developed countries, followed by confirmatory testing most commonly by using conventional Western blot (WB). There are many different commercially available ELISAs for detection of antibodies to HIV. In 1985, first-generation indirect ELISAs employed whole virus antigens obtained from cell cultures which were

bound to the solid phase on the bottom of the wells of microtitre plate . The first generation ELISAs were sensitive but less specific with capacity to detect early HIV antibodies slightly more than 40 days after

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infection [5] . The secondgeneration ELISAs used an indirect format, HIV recombinant antigens and 28 peptides bound in solid phase . The assays had increased specificity and good sensitivity that reduced the window period to detect antibodies as early as 33-35 days after infection [6]. In 1990s, due to diverse HIV variability, ELISAs were introduced which also included antigens from HIV-2 and new antigens from viruses of the HIV-1 groups M, N and O [7]. Third generation ELISAs which used antigen sandwich technique and included recombinant HIV-1 and HIV-2 proteins and/or peptides bound on a solid phase either in the bottom of microplate or a bead were introduced in 1994 . These ELISAs had higher sensitivity and specificity and reduced the window period to about 22 days after infection. Fourth-generation ELISAs that can detect both HIV p24 antigens and antibodies have been introduced recently. These assays offer advantages of early detection of acute HIV infection by reducing the window period to almost the levels of the detection of HIV RNA.[8]. Fourth generation ELISAs have been used in developed countries [9] and introduced in resource-limited settings in recent years.

The objective our objective for this study was to compare a low end ELISA test like RAPID test ,e.g TRIDOT with an advanced ELISA test e.g CIA and then compare both the results with a Nucleic acid test like Real time PCR .To address this ,we collected 20 HIV positive specimens and 20 HIV negative specimens and conducted the experiments.

2. Materials and methods

2.1. Samples and methods used for the study

1. Blood samples from HIV infected subjects and blood samples from healthy volunteers were collected from outpatients from a local hospital in Secunderabad.
2. Roche RNA extraction kit(for extraction of RNA from HIV infected plasma),
3. Genosens HIV-1 RT-PCR kit(for reverse transcription of HIV RNA and real time PCR for implication of HIV LTR gene)
4. Tridot Rapid kit(for screening HIV1 and HIV 2 antibodies)
5. ABI7500 FastDx real time PCR and Stratagenes`s MX3000P(for doing real time PCR)

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6. Abbott`s ARCHITECT machine for Ag-Ab combo assay(Fourth generation ELISA machine

2.2. Study Design

- 20 HIV positive serum /blood samples were collected from a local hospital in Secunderabad
- 20 Blood samples were collected from healthy controls who were not having HIV infection.
- Rapid tridot tests were done on all the 40 samples
- The fourth generation CIA test was done on all 40 samples using ARCHITECT.

The 20 HIV positive samples were further subjected to a confirmatory test by RT-PCR

3. Results:

The samples collected from both male and female individuals ranging between the age of 20 to 43 years from local hospital, Secundrabad. Out of the total 20 patient samples tested for HIV-1 and /or HIV-2 infection,18 proved to be seropositive for HIV-1 with no case of HIV detected using Tridot method, but using CIA and RT-PCR method all 20 proved to be HIV positive.

In AG-AB combo assay of ARCHITECT, the assay result is presented as ratios of specimen signals to the cut-off values (S/CO), where an S/CO ratio $\geq$ 1.00 is considered reactive. Though these assays have been shown to reduce the HIV window period by 4-5 days compared with 3rd generation HIV-Ab detection assays. Discrepant results have been published on their specificities in population groups with low HIV prevalence. Some chronic infections may cause false positive HIV results. The only findings that related to the false positivity of the HIV test result could be that the patient was suffering from chronic HBV infection. There are reports of false positive result after HBV vaccination or treatment with hepatitis B immune globulin; therefore it may be assumed that false positivity may be due to chronic HBV infection.

It is suggested that, laboratories in low prevalent countries like Nepal, Bangladesh, srilanka, etc. using 4th generation CMIA should interpret their result cautiously. In addition, this types of discrepant result reemphasis the need of confirming HIV positive test result by WB or LIA.

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Table 1. Comparison of Rapid test results with CIA-ELISA test

Patient ID	RDT(tridot)	CIA (ARCHITECT)
Case 1	+	36.33
Case 2	-	2.17
Case 3	+	56.30
Case 4	+	32.12
Case 5	+	9.85
Case 6	+	40.02
Case 7	+	10.11
Case 8	+	2.17
Case 9	+	4.65
Case 10	+	21.19
Case 11	+	13.15
Case 12	+	24.36
Case 13	+	35.15
Case 14	-	40.02
Case 15	+	10.5
Case 16	+	2.99
Case 17	+	2.68
Case 18	+	31.70
Case 19	+	26.55
Case 20	+	12.68
Control 1	-	0.05
Control 2	-	0.45
Control 3	-	0.17
Control 4	-	0.34
Control 5	-	0.67
Control 6	-	0.25
Control 7	-	0.56
Control 8	-	0.01
Control 9	-	0.07
Control 10	-	0.89
Control 11	-	0.78
Control 12	-	0.98
Control 13	-	0.34
Control 14	-	0.45
Control 15	-	0.36
Control 16	-	0.78
Control 17	-	0.26
Control 18	-	0.33
Control 19	-	0.45
Control 20	-	0.01

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Table 2. Comparison of Rapid test results with RT-PCR test

Patient ID	RDT(tridot)	RT-PCR (Genosens Kit) Copies of RNA
Case 1	+	3,410,685
Case 2	-	96,000
Case 3	+	8,635,135
Case 4	+	4,531,313
Case 5	+	1,011,021
Case 6	+	56,66,316
Case 7	+	9,32,251
Case 8	+	96,000
Case 9	+	3,84,136
Case 10	+	13,14,815
Case 11	+	6,30,533
Case 12	+	11,10,310
Case 13	+	32,16,320
Case 14	-	44,10,685
Case 15	+	3,37,450
Case 16	+	2,64,605
Case 17	+	2,13,156
Case 18	+	32,56,015
Case 19	+	15,64,715
Case 20	+	4,75,321
Control 1	-	LD*
Control 2	-	LD
Control 3	-	LD
Control 4	-	LD
Contro 5	-	LD
Control 6	-	LD
Control 7	-	LD
Control 8	-	LD
Control 9	-	LD
Control 10	-	LD
Control 11	-	LD
Control 12	-	LD
Control 13	-	LD
Control 14	-	LD
Control 15	-	LD
Control 16	-	LD
Control 17	-	LD
Control 18	-	LD
Control l19	-	LD

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Control l20	-	LD

Table 3. Comparison of the two cases which were negative with Rapid test And positive with CIA and RT-PCR

Patient ID	Rapid Test	CIA ARCHITECT	RT-PCR
Case 2	-	2.17	96,000
Case 14	-	40.02	44,10,685

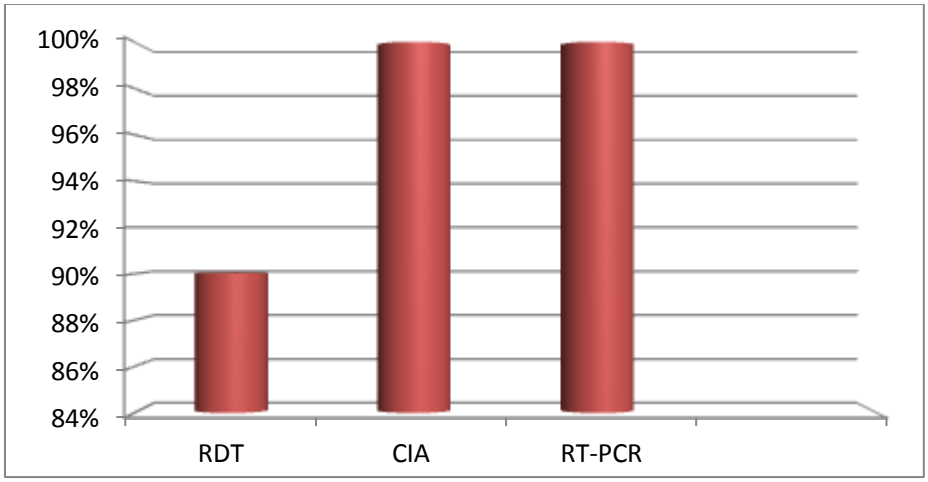

Figure 1. CIA and RT-PCR showed 100 % sensitivity while Rapid tridot test showed only 90% sensitivity

4. Discussion

New fourth-generation assays that detect both HIV antibodies and the p24 antigen are now available in some labs. These tests would be beneficial during the window period of acute HIV infection. Additional diagnostic tests are available for use in specific circumstances. The diagnosis of HIV-2 infection requires an ELISA finding that will detect HIV-2 antibodies, followed by an HIV-2 specific Western blot test. Many currently available HIV ELISA will detect antibodies to

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HIV-1 or HIV-2, and HIV-2 specific ELISAs are available. It is important to understand the test characteristics and a patient's risk of HIV-2 infection highest among immigrants from West Africa and in people with exposure to an individual at risk before drawing conclusions based on test results.

Rapid testing systems allow the HIV assays to be run in 5 to 20 minutes. Negative rapid test assays can be considered negative unless there concern of acute HIV infection; however, all positive rapid assays must be confirmed by standard ELISA and Western Blot assays. Currently available U.S. Food and Drug Administration (FDA)-approved kits are available for blood, plasma, serum, and saliva specimens. These tests are particularly beneficial in the delivery room, emergency room, and after occupational exposures. Additionally, the availability of Clinical Laboratories Improvement Act-waived testing allows these assays to be run in the community, expanding access to testing. Home tests are available that allow patients to collect a blood sample after a finger stick, which is then sent anonymously for testing and a recently FDA approved Over-the-counter, in-home test kits can detect HIV-1 and HIV-2 infection, some can distinguish between the 2 viruses, and others can detect only samples positive for HIV-1 antibodies (Lien et al 2000, Louie Wong, 2008)

As with all diagnostic tests, the positive predictive value depends on the rate of disease in the population being screened. In low-prevalence populations, the likelihood that a positive ELISA result represents a false-positive result may exceed the likelihood of the test's indicating true HIV infection [(11)]

1. Current HIV screening tests (ELISA IgM or ELISA/Ag combinations) are *much* more sensitive than the Western blot.
2. *Every* case with a positive screening test but a negative Western blot must have an HIV viral load.
3. Every case in which HIV acquisition might have been recent — symptoms of acute HIV, or HIV testing in the context of a recent STI (especially syphilis), or known recent negative HIV test — should have an HIV viral load sent along with HIV antibody testing (or at the very least, be tested using a 4th Generation combined antigen/antibody test).

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In order to maximize the detection of all infected individuals, especially during the period following exposure or in the early stages of infection before antibody is detectable, antigen and virus RNA tests should be used. As virus RNA tests are expensive, time-consuming, and not available in many laboratories, fourth-generation ELISAs have been designed to detect anti-HIV immunoglobulin and HIV core protein p24 antigen simultaneously in order to screen for both the early and late phases of infection. These new ELISAs have the advantage of decreasing the diagnostic window (mean reduction of 4–7 days), personnel and costs in comparison with the requirements for performing each assay individually [(12)]. The detection of early infection has been shown to be beneficial for the initiation of treatment and counselling of infected individuals, and for the institution of interventions to reduce the risk of further transmission.

With the introduction in India of the concept of point-of-care testing and Voluntary Counselling and Testing Centres, several rapid tests for HIV have been developed that are low-cost and easy to perform. Several previous reports have compared fourth- and third-generation ELISAs or two or more rapid HIV tests , but there are few reports that compare the performance of rapid HIV tests with fourth-generation ELISAs for the detection of HIV in patients' sera.

Early detection of infection is vital for patient management and the prevention of HIV transmission. The fourth-generation assays can detect HIV nearly 6 days before the third-generation assays and sometimes before p24 antigen-only assays, and less than 3 days after nucleic acid tests.

In a previous report by Kwon et al., the ARCHITECT combo assay detected infection 4–26 days earlier than third-generation assays.

The Elecsys HIV combi PT assay has been standard-ized to the WHO reference standard for p24 antigen and has an overall analytical sensitivity of 1.05 IU/mL at a cut-off index of 1.0.

Previous studies investigating laboratory-based (as opposed to point-of-care based) HIV assays have documented that HIV-1 O strains and HIV-1 subtypes F and C have not been detected by some assays, and the difficulty in diagnosing HIV-1 group O has also been highlighted by the case

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described by Henquell et al. Genetic variability, particularly of gp41, may affect the ability of enzyme assays to detect HIV antibodies [13].

Real time PCR-Viral load testing should be performed at a patient's initiation of HIV care. Viral load testing targets any one gene of HIV genome . the most commonly used region is the LTR region . Subsequent viral load testing is used as a marker for HIV viremia and should be tested every three to six months among those on treatment. New guidelines also recommend viral load testing two to eight weeks after the initiation of HAART to determine early response to therapy. Viral load can also be used to determine non-adherence or treatment failure as defined by a viral load of >200 copies/mL.

Thus, drawbacks to currently available rapid tests include a lower sensitivity than third- and fourth-generation EIA tests and, RT-PCR depending on the prevalence of infection in the population being tested, a low (< 90%) positive predictive value. RT-PCR has better positive pridictive value and in comparison to rapid tests and 3rd And 4th generation ELISA assays.

5. Conclusion

To summarize, our study shows that chemiluminescence based ELISA is 100 % sensitive and is comparable to RT-PCR method. In our view, Rapid tests can often give false positives or false negatives. Each test has its own drawbacks. In some instances even the viral load tests give false results, the limitation of this study has been the small sample size.

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