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COMPARISION BETWEEN AVIDITY TEST AND DETUNED ELISA TEST IN DETECTING THE RECENT AND ESTABLISHED HIV-1 INFECTION

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ABSTRACT

Identifying patients with early human immunodeficiency virus infection is an important public health goal. Those individuals who have acquired human immunodeficiency virus infection recently have high viral loads both in their blood and genital secretions, thus facilitating transmission. Identifying and intervening these individuals early in the course of their disease, helps to reduce onward transmission.The current study is undertaken, comparing the Avidity test and Detuned ELISA which are modified immunoassays to detect recent and established HIV infection. The study was conducted in 261 HIV-1 seropositive patients aged more than 15 years of age. Data was analyzed using SPSS 16.0 andvariables are compared by using chi square test. There were a total of 33 cases detected by detuned immunoassay with value ≤1 and 26 cases detected by Avidity test with value ≤0.9 as recent cases. 96.55% of concordance was found between Avidity test and Detuned ELISA.

KEYWORDS: Human Immunodeficiency Virus, Viral load, Avidity test, Detuned ELISA

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INTRODUCTION

Recently infected HIV patients have high viral loads both in their blood and genital secretions, thus facilitating transmission. Identifying and intervening these individuals early in the course of their disease, helps to reduce onward transmission, provides an opportunity for these individuals to engage in HIV care and prevention. Sexual contacts of patients with recent HIV are easy to track for assistance in prevention policies for target intervention. Monitoring of high risk groups for recent seroconversion is also helpful in early initiation of antiretroviral treatment and in determining the prognosis of the disease. Detecting HIV early in the course helps to identify individuals, which are most likely to be benefited from preventive vaccines and also in the enrollment of these individuals in anti HIV drug trials studies[1].

Several approaches are available to estimate the duration of HIV-1 infection but all have some pitfalls and limitations, for example in large-scale prospective cohort studies, individuals who are at-risk of acquiring HIV are followed for a long period of time to determine the number of patients with recent infection. These epidemiological studies are cumbersome, time consuming, expensive and require long term follow up of individuals[2]. Time period for seroconversion can also be demonstrated as early as 2 weeks by detecting p24 antigen and HIV RNA in serum, but sensitivity of these tests are as low as 60%[3.] Other methods include CD4 lymphocyte count and to a lesser extent, HIV proviral DNA load have shown to be correlated with the amount of time elapsed from infection, but they are not proven to be reliable markers of recent infection[4].

Recently, much interest has been focused on modified enzyme immune assays that can distinguish recent HIV infections from established infections in a single serum sample. For this purpose, a number of modified enzyme linked immune assays have been developed[5,6,7,8,9].These methods are primarily based on the response of evolving antibodies during early HIV infection like avidity, the binding strength of antibodies to antigen and antibody titers.

Antibodies produced in the early phase of infection shows lower avidity, or in other words lower binding strength for the antigen. The strength of the interaction between antigen and the antibody is weak because of presence of large numbers of low-avidity HIV-1 antibodies in the serum. The relative avidity of antibody is stronger in established infection and weaker in recent HIV infection which can easily be estimated serologically by modifying standard ELISA procedure. This modification is achieved by using certain chemical agents called chaotropic agents, which breaks down weak bonds between antigen and antibody. This modified method is also called as avidity index method[6,10,11,12].

The other approach used in estimating the recent infection, is to determine quantity of antibodies present in serum. In case of early HIV infection, amount of antibodies present are low and in chronic infection numbers are high.These parameters are exploited as a tool in order to estimate the relative time of HIV infection. For example, if antibody titers are low, it is likely that infection has occurred within past 6 months; conversely, high-titre signals an established infection that has been present for longer than 6 months. To determine the duration of HIV illness, conventional ELISA procedure is modified. This modified ELISA assay is known by various names as "detuned" assays or "sensitive/less-sensitive" (S/LS) enzyme immuno assay Detuned ELISA or serological testing algorithm for recent HIV seroconversion(STARHS)[1,6,7,8,9].This modified assay is based on the principle that antibodies titer increases with time and recent infection can be assumed, if test results become nonreactive following dilution of the individual's serum. In

such a case, an initially reactive sample, when tested with the routine (sensitive), becomes nonreactive when diluted in a modified (less-sensitive) assay. Conversely, the serum of an individual with established HIV infection will remain reactive even after dilution in the less-sensitive assay indicating presence of high levels of antibodies. This system was first developed, validated and standardized in 1998 by Centers for Disease Control and Prevention (CDC)[1].

There are no studies available in India, based on detuned method and avidity method to determine recently infected HIV individuals[13].There are very few studies available in literature comparing avidity test and Detuned immunoassay[6,10,14]. Keeping in view the above point, the current study is undertaken, comparing the two methods for estimation of the recent and established HIV infection.

MATERIALS AND METHODS

Study population: We conducted a cross-sectional study using consecutive serum samples (*n*=261) obtained from HIV seropositive patients of ≥15 years of age. Written informed consent was taken from all participants. Epidemiological, clinical and laboratory details were obtained in a standard questionnaire. Blood samples submitted for HIV testing in the microbiology laboratory was taken for study procedures. All cases of HIV-1 infection was tested with Enzyme linked Immunosorbant Assay (ELISA) and confirmed by western blot test. These samples were further tested by following methods to distinguish recent and established HIV infection:

1. Avidity test
2. Detuned ELISA

Avidity test

Principle:Antibody avidity has long been used as a marker for recent infection with several pathogens, and is currently being used for Rubella virus, *Toxoplasma gondii*, and human Cytomegalovirus. Antibodies produced in the early phase of infection show a lower avidity, or in other words lower binding strength for the antigen. The strength of the interaction between antigen and the antibody present is weak in early infection because of presence of large numbers of low-avidity HIV-1 antibody in early infection. The relative avidity of antibody is stronger in established infection and can be estimated serologically by modifying standard ELISA method. This modified method is also called as Avidity index method.

The weak bond between antibodies and antigen can be disrupted using chaotropic agents. Chaotropic agents are dissociating reagents such as urea at concentration of 8 Molar (M), potassium thiocyanate (3 M), magnesium chloride (4 M), and guanidine HCl (1 M)[14].

Avidity Test procedure

All reagents were equilibrated to room temperature before commencing assay

Serum predilution**:** Two 10 µl of serum were subjected to pretest dilution of 1:10 with phosphate buffered saline and 1M guanidine HCL. To the first 10 µl serum, 100 µl phosphate buffer saline diluent was added and to other 10 µl serum 100 µl of 1Molar guanidine HCl was added.

ELISA procedure

On both the diluted samples, ELISA test was done without further modification of test procedure.

1. Required number of ELISA wells were removed from the kit.
2. 100 µl of negative and positive controls added to well A1 and A2
3. 100 µl of diluted serum samples were then added to required numbers of wells
4. Microwells were then incubated at 37°C for 30 minute
5. While the samples were incubating, wash solution and working conjugate was prepared as specified in preparation of reagents.
6. Microwells were then taken out of incubator after incubation and washed 5 times with

wash solution according to wash procedure.
7. 100 µl of working conjugate solution was then added in each well and incubated at 37°C for 30 minute
8. After incubating time was over again wells were washed 5 times with wash solution.
9. 100 µl of working substrate solution was then added into each well.
10. Microwells were then incubated at room temperature (20-30°C) for 30 minutes in dark.
11. 50 µl of stop solution was then added.
12. Read absorbance at 450 nm with in 30 minutes in ELISA reader.

Calculation

Sample/cutoff (S/CO) ratios were calculated for Guanidine aliquot and PBS aliquot.

Avidity index of antibodies against HIV was calculated with following formula

$$\text{Avidity index} = \frac{\text{Sample /cutoff for guanidine HCL}}{\text{Sample /cutoff for PBS treated serum}}$$

Interpretation of results

Avidity index ≤0.9 were taken as cut off value for recent HIV infection with duration of HIV for less than 6 months.

Avidity index>0.9 were taken as cutoff value for established infection with duration of HIV for more than 6 months.

Detuned ELISA

Principle

In this method, the procedures of conventional ELISA (Vironostika HIV Ag/Ab, Microelisa system, Biomeriux) have been modified to allow discrimination of antibody titer. This modified assay has been called "detuned" assays or "sensitive/less-sensitive" (S/LS) enzyme immune assay. It is based on the principle that antibody titer increases with time and that recent infection can be assumed if test results become nonreactive following dilution of the individual's serum. In such a case, an initially reactive sample, when tested with the routine (sensitive), becomes nonreactive when diluted in a modified (less-sensitive) assay. Conversely, the serum of an individual with established HIV infection would remain reactive following dilution in the less-sensitive assay due to high levels of antibody. This system is also known as the Serologic Testing Algorithm for Determining Recent HIV Seroconversion (STARHS) method[1].

Detuned test procedure

The Vironostika HIV Uni-form II Ag/Ab System kit (BioMerieux) is a 96-well format EIA kit that uses an HIV virus lysate as the solid phase HIV antigen source. The assay is used for the diagnosis of HIV-1 and HIV-2 infection by testing human serum, plasma, and dried blood spots on filter paper. This assay was modified in accordance with the CDC vironostika-LS EIA protocol. To construct the less-sensitive test, the sample dilution was increased to 1:20,000, the sample incubation time was reduced to 30 minutes, and the conjugate incubation time was reduced to 30 minutes.

This modification of the above ELISA test was done by following steps:

1. 100 µl of 1:20000 diluted serum was added into required numbers of microwell
2. 100 µl of diluted controls were pipette into assigned wells
3. Microwells were incubated at 37°C for 30 minutes instead of original 60 minute incubation time
4. After incubating period was over, then each well was washed with wash solution

prepared as specified in preparation of reagents.

5.100 µl TMB substrate was added into each well.

6. Microwells were incubated at 15-30°C for 30 minutes.
7. 100 µl of stop solution was added.
8. Absorbance of the solution in each well was read at 450 nm.

Interpretation of results:

Standardized optical density ≤1 were taken as cut off value for recent HIV infection with duration of HIV for less than 6 months.

Standardized optical density >1 were taken as cut off value for established HIV infection with duration of HIV for more than 6 months.

Data analysis

Data analysis was done using SPSS 16.0 for windows and variables were compared by using chi square test.

RESULTS

TABLE 1
Distribution of HIV cases according to Avidity test

Avidity Test	Results
Value≤0.9	26
Value>0.9	235

26 Patients with avidity test value of ≤0.9 were termed as recent seroconvertors with antibodies appeared in serum for ≤6months and 235 patients with avidity value of >0.9 were termed as established cases with antibodies present for >6months duration.

TABLE 2
Distribution of HIV cases according to Detuned ELISA

Detuned ELISA	Results
Value≤1	33
Value>1	228

33 Patients with Detuned ELISA value of ≤1 were termed as recent seroconvertors with antibodies appeared in serum for ≤6months and 228 patients with Detuned ELISA value of >1 were termed as established cases with antibodies present for >6months duration.

Table 3
Established HIV infection misclassified as recent HIV infection

	Avidity test	Detuned ELISA
Total Recent cases detected	26	33
Established HIV cases misclassified as recent infection	1(0.4%)	4(1.75%)

There were total 26 cases detected by avidity test with value ≤0.9 as recent cases. Out of 26 cases, one case had long-term infection based on the history, thus classified wrongly as a case of recent infection.

Out of 33 cases detected by Detuned ELISA 4 cases had history of long term infection but value obtained was ≤1,thus wrongly classified as recent HIV infection

TABLE 4
Percentage agreement between Avidity test and Detuned ELISA

Detuned ELISA				
Avidity test		**>1**	**≤1**	Total
	>0.9	227 99.5%	8 24.2%	235 90%
	≤0.9	1 0.43%	25 75.7%	26 10%
	Total	228 100%	33 100%	261 100%

Concordance between Avidity test and Detuned ELISA - 96.55%
With Kappa coefficient of 0.828
Disconcordance between Avidity test and Detuned ELISA - 3.45%
Sensitivity - 99.5%
Specificity -75.7%
Positive predictive value -96.5%
Negative predictive value -96.2%

DISCUSSIONS

The present study was undertaken to evaluate avidity method and detuned ELISA method Detuned ELISA to determine the duration of HIV infection. The patients were considered as recent seroconvertors when duration of HIV acquisition was less than 6 months and established infection when infection was present for more than 6 months. To say patient has acquired HIV infection recently, optical density (O.D) cut off value of avidity test was taken as ≤0.9 and for Detuned ELISA it was taken as ≤1. For established infection optical density (O.D) cut off value of avidity test was >0.9 and for Detuned ELISA, value of >1 was taken.

In this study, the overall percentage agreement between avidity test and Detuned ELISA in detecting recent infection was found to be 96.5% with p value <0.001 while the percentage disagreement between the two tests was found to be 3.45%. Ausina et al[14] found overall agreement between both techniques as good with p value<0.001. Both techniques were concordant in classifying recent infection correctly. Similarly Murphy et al[14] found concordance of 98%(425/432) and disconcordance of 2%(7/432) between the two tests.

In the present study, out of the total 261 cases, Detuned ELISA has detected 33 cases of recent infection while avidity test detected only 26 cases of recent infection whereas seven cases of recent infection were missed. These missed recent infection cases 21.2% (7/33) were misclassified as established infection by avidity test.

Of the 33 recent cases detected using Detuned ELISA, 4 cases had long term opportunistic infection and these 1.7% (4/228) cases were wrongly classified into recent infection and out of 26 cases of recent infection detected by avidity test, 1 case had opportunistic infection which was also a misclassified case of recent infection in 0.4% (1/228) of overall cases.

Sensitivity and specificity of avidity test in the present study was roughly similar to many other studies[14,16,11]. In our study Detuned ELISA was considered gold standard to determine recent and established cases. When avidity test was compared with Detuned ELISA, almost equal sensitivity (99.6%) was obtained, moreover avidity test is inexpensive, easy to perform on routine ELISA kit and least affected by antiretroviral therapy, thus chances of misclassification of cases is also lowered compared to Detuned ELISA. These findings suggest that avidity test is good alternative to Detuned ELISA in determining duration of HIV illness.

The misclassification of cases in the present study and various other studies are due to the fact that tests used to establish HIV infection as recent or long term are based on quantitative measurement of the antibody response to viral antigen, so the factors that affects patient's immune response to viral antigen may lead to wrong results. For example, antiretroviral therapy can reduce the antibody levels by decreasing the total viral load in patients sera[17], test results can also be affected by the virus subtype and the patient's genetic background[18], and there are also observational studies showing that individuals with low CD4 counts with high viral loads are much more likely to be misclassified than infected individuals with high CD4 counts with low viral loads[19,20].These misclassified cases should be tested further with P24 Ag but due to limitation of resources, it was not possible in this study.

CONCLUSION

The present study has shown that both avidity test and Detuned immunoassays are simple, effective and convenient technique in determining recent HIV infection and estimating rate of new infection. Comparable results were obtained using these two methods to differentiate recent from long-standing HIV infection. Avidity test showed 96.5% agreement and comparable sensitivity with Detuned ELISA but specificity was found to be little less. The determination of early HIV infection using these techniques can be helpful in tracking sexual contacts. Further, because the high viral loads of early infection are associated with increased transmission risk, identification of high-incidence populations is helpful in assisting prevention policies for target intervention. The above techniques can be used in field setting for monitoring recent infection in high risk areas for early initiation of antiretroviral treatment, and also can provide information for prognosis, identify communities most likely to benefit from preventive vaccines, and assist in the enrollment of recently infected individuals in studies of anti HIV drug trials.

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