ORIGINAL ARTICLE

COMPARISON OF THE BED-IgG CAPTURED EIA HIV-1 INCIDENCE ASSAY WITH THE WHO-CRITERIA FOR IDENTIFYING RECENTLY HIV INFECTED INDIVIDUALS FOR ENROLLMENT IN THE HIVDR-TS

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ABSTRACT

Background: *With the advent of antiretroviral therapy (ART) in several developing countries, WHO has proposed that countries should monitor HIV drug resistance (HIVDR) based on a population-based threshold survey. The guideline uses age-criteria to identify newly infected individuals. The accuracy of such guideline has not been validated, however.*

Objective*: To compare the WHO criteria for identifying recently infected individuals with a biological marker HIV-1 BED incidence assay.*

Methods: *Individuals were enrolled from the VCT center, Addis Ababa, Ethiopia. Initial HIV screening was done based on current national Rapid HIV screening algorithm. Based on the WHO criteria, HIV-positive was classified as recently infected if less than 25years of age. Moreover the HIV-positive specimens were retested by two conventional EIA tests and discrepant results were confirmed by Western blot test. Then using the bio-marker HIV-1 BED incidence assay to classify the recent infection for comparison with the WHO-criteria.*

Results*: A total of 4492 individuals underwent VCT and screened for HIV. Of these 360 (8.0 %) were found to be HIV-positive; 64 (1.4%) were identified as recently infected based on the WHO criteria. Forty-two (0.93%) were identified as recently infected by the HIV-1 BED incidence assay, giving an estimated incidence of 2.36 % per year [95% CI, 1.7-3.1]. However, the strength of agreement between the WHO criteria and BED assay is considered to be poor to fair K=0.29 (95% CI, 0.11 – 0.47).*

Conclusion: *The concordance between WHO criteria and BED assay is low. This shows that whether the WHO criteria over estimate or the BED assay under estimates recent infection (or vice versa) is difficult to identify. In the absence of current gold -standard to decide which of the above criteria is more accurate, the need for identifying recent HIV infection for the purpose of population based studies is urgent.*

Key words: BED IgG CEIA, HIV recent infection, HIVDR-TS.

INTRODUCTION

Estimates of HIV incidence at the population level are of prime importance for understanding the dynamics of the HIV epidemic and for targeting and evaluating interventions to prevent HIV infection (1). Early surveillance systems were based either on case reporting of persons presenting with symptoms of HIV-1 infection or AIDS or on back-calculation models using AIDS incidence data, such data provided general clues as to the mode of transmission and which populations were more severely affected (2, 3, 4). It is apparent that the use of highly active antiretroviral therapy (HAART) has dramatically reduced the morbidity and mortality among patients infected with HIV-1. But the success of antiretroviral treatment (ART) on the other end is frequently limited by the pre-existence and the emergence of HIV drug resistance (HIVDR) (5, 6, 7).

The World Health Organization (WHO) has recommended that the identification of primary drug resistance mutation (pDRM) is significant among untreated individuals that may impact future therapeutic options (8). Although the duration of HIV infection in newly diagnosed individuals is usually unknown and can be highly variable, the ability to differentiate the recent from the chronic HIV-1 infection among newly diagnosed individuals would greatly improve estimates of incidence (9). As drug resistant viruses can be transmitted to newly infected individuals, monitoring the development and prevalence of drug resistance, especially in newly infected patients with pDRM, has remained a public health priority (10, 11, 12). Moreover development of HIV drug resistance is inevitable among patients under antiretroviral therapy. The key element of the HIVDR surveillance is to estimate the frequency of resistance in untreated

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persons recently infected with HIV in specific geographic settings using a standard surveillance protocol. In surveillance for transmission of HIVDR, the WHO has proposed the use of so called HIVDR threshold surveys (HIVDR-TS) to evaluate whether HIVDR remains less than 5% or above 15% in areas/ sites where transmitted HIVDR is likely to be seen first (13).

For HIVDR threshold surveys from antenatal care (ANC)-based HIV surveillance programs, HIV-positive women aged below 25 years and who present with first pregnancy (Age <25 years, primi gravida) are assumed as recently infected with HIV. This in a general context describes young ages of ≥15 to <25 years, and no previous pregnancy if female are assumed as proxy to recent infection (13).

However, in several developing countries, such as Ethiopia, early pregnancy is not an uncommon phenomenon (14), and recall for earlier pregnancy may not be accurate. Thus, especially those women above 20 years may not be necessarily recently infected. These pose some of the limitations of the WHO criteria for inclusion for HIVDR threshold surveys.

On the other hand, recent emphases on laboratory methods that can be used to detect and distinguish recent human immunodeficiency virus type 1 (HIV-1) infections from long-term infections has resulted in the exploration of a variety of approaches for estimation of the incidence of HIV-1 infection (15, 16, 17, 18, 19, 20).

Here we propose to employ the BED HIV-1 incidence assay (based on the competitive capture format of the assay: proportion of specific HIV-IgG to total non-HIV-IgG, the branched peptide antigen (BED peptide) include immunodominant sequences from the conserved gp41 region of subtypes B, E, and D, linked via lysine). The aim of the study is the BED assay with the WHO criteria for identifying individuals infected with HIV recently.
Therefore, the current study focused on the assessment of a useful tool for the differentiation of recent HIV infection from a long term infection. Hence, it will be helpful to better characterize the local and national epidemic through the investigation of recent infection.

MATERIALS AND METHODS

Study design and Setting:

A six month prospective cross-sectional study was conducted at the Voluntary Counseling and Testing (VCT) Center, Organization for Social Services for AIDS (OSSA), which is located in Addis Ababa metropolitan near the biggest open market in Africa "Merkato", Addis Ababa, Ethiopia. The estimated annual VCT clients at the free standing center for the year 2007 were 10,703 individuals with 7.1% HIV prevalence (21).

Laboratory:

All VCT attendees aged 15 years and above were included to the incidence study. The non-repeat samples and not tested before were collected at the free standing VCT Center during the study period. The samples were initially screened for HIV using the current national Rapid HIV screening algorithm which includes Step-1 Screening Determine™ HIV-1/2 (ABBOT JAPAN CO., LTD/ Inverness Medical Japan Co., Ltd), Step-2 Confirmatory CAPILLUS™ HIV-1/HIV-2 (TRINITY BIOTECH PLC, Ireland), Step-3 the Tie Breaker Uni-Gold™ HIV (TRINITY BIOTECH PLC, Ireland); the results were anonymously recorded in a separate sheet. All specimens found to be positive were stored frozen at -20° C until analysis.

All positive samples were verified with conventional EIA Vironostika® HIV Uni-Form II *plus O* (bioMerieux, Boxtel, The Netherlands) and Murex HIV-1.2.O. (Murex Biotech Limited UK) and then discrepant results were confirmed with Western blot GS HIV-1 (BIO-RAD Laboratories, USA). Those identified as HIV-positive were further undergoing assessment for recent infection using IgG-capture HIV-1 BED incidence EIA (Calypte Biomedical Corporation; Maryland, USA).

Statistical analysis:

The agreements between the two criteria were determined using *chi-square (x^2) test*. Data entered in excel and statistical analysis was done using the *SPSS* statistical package. The incidence (rate of new infections/100 persons/year) was calculated using the following formula: $I = [(365/W) \times (R)] / [N + (365/W \times R/2) \times 100$, where I is incidence rate, W is mean window of detection (155 days), R is number of subjects found to be recently infected by the BED incidence assay and N is the total number of HIV-seronegative subjects. The 95% confidence intervals (CIs) for estimated BED incidence were calculated by the following formula: 95% CI = I ± 1.96 (I/Square-root of R) (22).

Ethical considerations:

Enrollments of VCT clients for the study were going on underscoring the standard national guidelines for HIV counseling and testing. All subjects were asked

for written consent to be included in the study. Confidentiality throughout the testing was maintained. In addition, individuals identified as HIV-infected were referred for further care, treatment, and support. The study protocol was reviewed by the Institutional Review Committee, Faculty of Medicine, Addis Ababa University (AAU) and further approved by the Addis Ababa City Administration Health Bureau and the National Ethical Review Committee.

RESULTS

A total of 4551 individuals attended the free standing VCT center during the study period, between n=26 repeated tests during the study period). Eligible VCT attendees who fulfilled the criteria for assessing the recent HIV infection (RHI), age ≥15 years, and consented to test with no repeat visit were 4492, mean 26.8 years old (range=60, [15-75] years). Females were 46.2% (2076) and males 53.8% (2416). The most frequent VCT attendees were in the age group of 20-24 followed by 25-29.

Regarding the characteristics of the study subjects at the pre-counseling session, 63% had previous test elsewhere with a seronegative result, and 78.2% had sex. Self-reported history of STI was noted in 2.7%, and 1.01% were pregnant women. (Table 1)

Table 1: Distribution of self reported pre-counseling socio-behavioral characteristics of the VCT attendees, August/2008 to September/2008

Characteristics	Frequency	%
Pretested	n (4492)	% (100)
No	1596	35.5
Yes, Pos	49	1.1
Yes, Neg	2829	63.0
Yes, inconclusive	12	0.3
Result not give	2	0.04
Didn't take result	4	0.09
Ever had sex	n (4492)	% (100)
No	981	21.8
Yes	3511	78.2
History of STI	n (4492)	% (100)
No	3379	75.2
yes	123	2.7
Don't know	9	0.2
N/A*	981	21.8
Current pregnancy	n (2076)	% (100)
No	1682	81.0
Yes	21	1.01
Don't know	6	0.3
N/A*	367	17.7

*N/A = Not applicable

The overall HIV-1 seroprevalence during the study period was 8.0% (360). The peak prevalence of HIV infection among the VCT attendees was between 25-29 years of age group.

Females were at higher risk than males for HIV infection, with the overall female-to-male ratio of 1.5. (Table 2)

Table 2: Age-group and Gender with HIV status and recent infections in Addis Ababa, August/2008 to September/2008.

Age group	Male			Female			Total		
	Tested n	HIV+ n (%)	Recent n (%)	Tested N	HIV+ n (%)	Recent n (%)	Tested n	HIV+ n (%)	Recent n (%)
15-19	216	1 (0.04)	0 (-)	468	10 (0.48)	2 (0.1)	684	11 (0.24)	2 (0.04)
20-24	648	11 (0.46)	6 (0.25)	723	42 (2.02)	5 (0.24)	1371	53 (1.18)	11 (0.24)
25-29	646	16 (0.66)	3 (0.12)	534	87 (4.19)	8 (0.39)	1180	103 (2.29)	11 (0.24)
30-34	412	31 (1.28)	3 (0.12)	169	32 (1.54)	5 (0.24)	581	63 (1.40)	8 (0.18)
35-39	218	35 (1.45)	2 (0.08)	106	25 (1.20)	3 (0.14)	324	60 (1.34)	5 (0.11)
40-44	114	26 (1.08)	1 (0.04)	28	7 (0.34)	1 (0.05)	142	33 (0.73)	2 (0.04)
45-49	64	11 (0.46)	0 (-)	25	5 (0.24)	1 (0.05)	89	16 (0.36)	1 (0.02)
>50	98	15 (0.62)	2 (0.08)	23	6 (0.29)	0 (-)	121	21 (0.47)	2 (0.04)
Total	2416	146 (6.04)	17 (0.7)	2076	214 (10.31)	25 (1.2)	4492	360 (8.01)	42 (0.93)

Recent HIV infection using the IgG-capture BED-EIA was 0.93%, and the overall calculated HIV incidence rate was 2.36% per year (95% CI, 1.7 - 3.1). HIV incidence rate in the age group of $\geq$ 25 years is 2 times higher than the younger (<25 years) age group, with the annual incidence rates of 3.13% per year (95% CI, 1.9 – 4.3) and 1.52 per year (95% CI, 0.7 – 2.3), respectively. The annual HIV incidence is shown to be higher almost by two-folds in females than males. (Table 3)

HIV prevalence is peak among 25-29 years of age, but the HIV incidence proportion based on the BED assay is consistent with a rise on the age group of 20-24 years, (Figure 1) According to the WHO criteria, The concordance of the WHO criteria with the BED incidence assay was very low; only 13 cases coincided to be defined as recent HIV infection with both criteria. Results obtained as recent infection using the WHO criteria and the BED-EIA are shown in Table 4.

The performance of the BED assays of the mean initial plot and confirmatory was within 99% limits each. The level of concordance between initial and confirmatory test results was $R^2 = 0.993$, almost all specimens retaining the same classification as recent or long term infection. (Figure 2)

Table 3: **Age group and gender annual HIV incidence with BED-EIA in Addis Ababa, August/2008 to September/2008**

Age group	HIV incidence with IgG-capture BED-EIA*		HIV incidence (% per year) (95% CI)
	Gender	n (%)	
15 – 24	M	6 (14.3)	1.64% per year [95% CI, 0.3 – 2.3]
	F	7 (16.7)	1.44% per year [95% CI, 0.4 – 2.5]
	Total	13 (31.0)	1.52% per year [95% CI, 0.7 – 2.3]
$\geq$25	M	11 (26.2)	1.80% per year [95% CI, 0.7 – 2.9]
	F	18 (42.8)	5.68% per year [95% IC, 3.1 – 8.3]
	Total	29 (69.0)	3.13% per year [95% CI, 1.9 – 4.3]
Total	M	17 (40.0)	1.74% per year [95% CI, 0.9 – 2.6]
	F	25 (60.0)	3.10% per year [95% CI, 1.9 – 4.3]
	Total	42 (100.0)	2.36% per year [95% CI, 1.7 – 3.1]

Table 4: Concordance of the WHO criteria and the BED incidence assay to classify recently HIV infection (RHI) in Addis Ababa, August/2008 to September/2008

	WHO recent infection	WHO long term infection	Total
BED recent infection	13	29	42
BED long term infection	51	267	318
Total	64	296	360

K = 0.29 (95% CI, 0.11- 0.47)

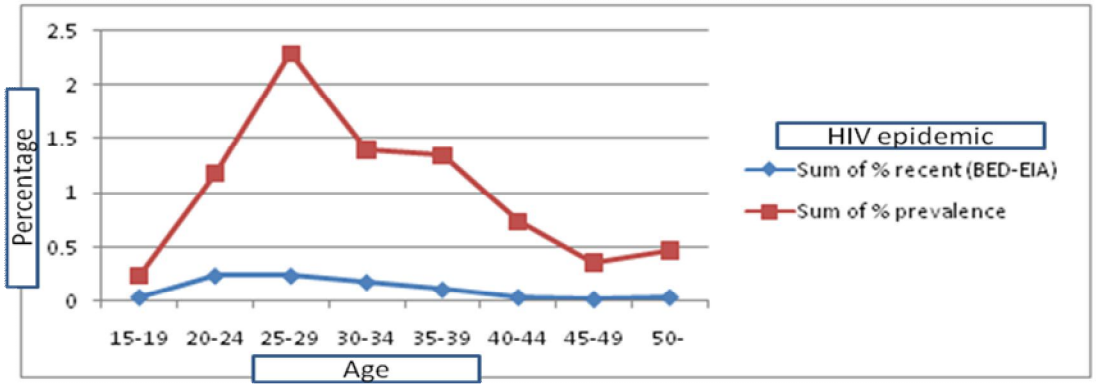

Figure 1: HIV epidemic by age category among VCT attendees in Addis Ababa, August/2008 to September/2008

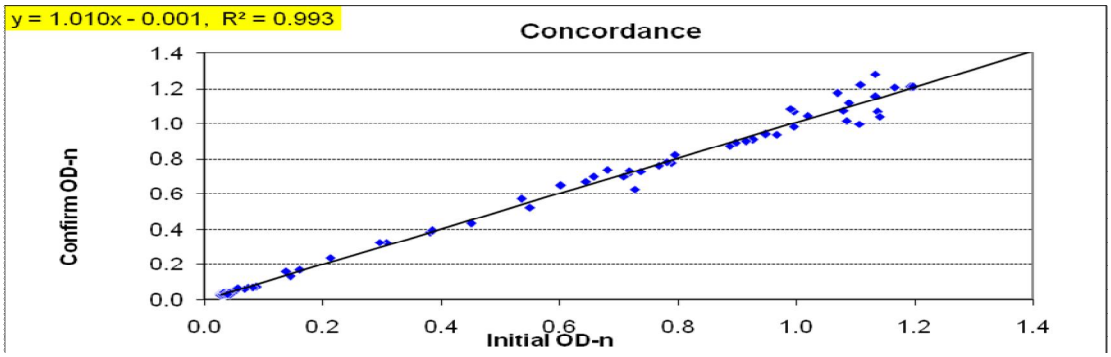

Figure 2: Concordance between the results of initial and confirmatory test plates for the 360 specimens (R^2 = 0.993) using the BED IgG captured incidence assay

DISCUSSION

In this study, comparison of the bio-marker-based BED-CEIA incidence assay (mean window of detection is 155 days) which measures the antibody proportion of HIV specific and total antibody and the WHO criteria using age < 25 years (prima gravida, if female) assumed as general context that describes young ages of ≥15 to <25 years, and no previous pregnancy if female are assumed as proxy to recent infection, for identification of recent HIV-1 infection were evaluated.

The peak prevalence of HIV infection among the VCT attendees remained among the age group of 25-29 years. The higher risk for HIV infection remains among women, and this has been noted in other studies as well (23, 24, 25). Moreover, in the current study, the HIV prevalence and incidence maintain the downward trend.

HIV prevalence has been the main measure used in monitoring HIV epidemics, but it is neither timely nor easily interpreted, especially with the advent of antiretroviral treatment that may increase prevalence with concomitant increase in the spread of the virus (26, 27).

A critical component in the effort to reduce transmission rates is the ability to identify where the highest infection rates are occurring, so that prevention programs can be deployed most effectively (28). For instance, incidence study using a prospective cohort conducted in Ethiopia in 1997 showed an HIV incidence rate of 0.4 per 100 person-years (95%

CI, 0.3-0.6) (29). However, this is protracted and difficult to manage the follow up and too costly to implement. To understand recent changes in HIV epidemic, it is necessary to estimate the incidence, i.e., the number of newly infected subjects in a defined period.

On the other hand, in a study conducted to determine the temporal trends in HIV incidence based on IgG-capture HIV-1 BED incidence assay, the incidence rates turned out to be 7.7%, 6.3%, 5.3%, 2.7%, 5.4%, 2.7%, and 2.0% per year, for the years 1995 through 2003 (30). In the current study, using the IgG-capture HIV-1 BED incidence assay, the annual incidence rate is 2.36% per year [95% CI, 1.7-3.1]. This apparently keeps the downward trend of the incidence as does the prevalence.

The performance characteristics of the IgG-capture BED-EIA have been shown to be outstanding in a number of studies. The comparison of the assay with other similar assays that would differentiate recent HIV infections (RHI) were comparable and suggest a wide application of the assay for monitoring epidemic and targeting prevention programs. Moreover, the specificity for measuring HIV incidence assays increased from 89.8% using only the standard BED assay to 99.2% using a multi-assay algorithm, without compromising sensitivity (31, 32, 33, 34, 35).

However, the agreement of the BED incidence assay with the WHO criteria for identifying recent infection was very low; only 13 cases were defined as recent HIV infection using the two criteria. In recent reports, BED- derived estimates of HIV incidence errors may vary by place, time, and age. Further variation, such as sex and pregnancy status could be considered as well. The overestimation of incidence using the BED incidence assay might be due to misclassifying the proportion of prevalent infection as recent infection (30, 36).

CONCLUSION

The concordance proportion between the WHO criteria and the BED assay was much below the expected. Plausible reasons might be the overestimation of using the WHO criteria (more crude way of selection of 25 years as cut-off is higher), underestimation of using the BED assay, or vice versa. In the absence of current gold-standard to decide which of the above criteria is more accurate, the need for identifying recent HIV infection for the purpose of population based studies is urgent.

The estimation of HIV-1 incidence can be made by using different approaches which help to look at the targets more accurately and precisely. It provides a better picture of the epidemic and may serve as surveillance data if it is scaled up to a larger population or subpopulation segment. It is important to consider a multi-assay algorism using the different approaches based on serological tests.

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