Prevalence of recent and long-established HIV-1 infections among adults newly enrolled for HIV care

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Abstract

Background: The ability to differentiate recent human immunodeficiency virus infections (infection within few months) from long-standing (established) infection is valuable for accurate measurement of the changing patterns of HIV transmission and disease management. We determined rates of recent and long-established HIV infection using anti-HIV avidity index (AI) immunoassay.
Methods: We conducted the study using sequential serum samples from 100 HIV-1 positive patients. The time since infection was estimated using the automated third generation anti-HIV enzyme immunoassay [AxSYM HIV1/2gO Avidity Index (AI) assay] to discriminate between recent and long established. AI of ≤0.80 indicated recent infection.
Results: The prevalence of recent infection was 11%. Age and sex were not associated with recent infection. The prevalence of those who were married with recent infection was (72.7%). The median CD4 of those recently infected was higher (364: IQR 31-600) cell count compared to those with long established infection was (134: 12-639) cell count. The median viral load of the study participants was higher among recent infection 99892 copies/ml while long established infection was lower 62971 copies/ml.
Conclusion: The use of AI clearly has a potentially important role in early diagnosis of HIV infection, discriminate recent from long-established infection among individuals and understanding of HIV/AIDS epidemic for public health interventions in resource-limited settings where the disease burden is highest.

Keywords: Avidity Index, HIV Infection, Late, Recent

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Introduction

The ability to differentiate recent human immunodeficiency virus infection (infections within few months) from long-standing (established) infection is valuable for accurate measurement of the changing pattern of HIV transmission and disease management. The advent of serological assays for detecting recent HIV infection is primarily for epidemiological and prevention purposes. However, it also has a potential in individual diagnosis[1, 2]. These assays help in estimation of prevalence of recent and log established infection in cross-sectional surveys. They are known to be simpler conducted, inexpensive, shorter turnaround time, and require fewer resources than the usual longitudinal studies in investigating incidence. The anti-HIV avidity index (AI), using third-generation immunoassays to detect anti-HIV antibody, is based on evidence that the antibody avidity/affinity for the antigen is low in the early phase of infection (0 to 12 months from infection) and increases with time until complete antibody maturation[3, 4]. Avidity index immunoassay has been shown to be an accurate tool for discriminating recent HIV infection (<6 months) from long-established infection (≥6 months). Since the early identification of newly acquired HIV-1 is crucial to yield information on the dynamics of the epidemic, transmission networks, patterns of transmitted drug resistance, and to obtain virologic, immunologic and clinical benefits, the need to introduce an assay that will distinguish recent from long-established infection is high. Despite the burden of HIV cases in Nigeria, no study known to the authors has examined the discriminating of recent from long established infection. This information is important; first, for predicting time of infection which could be used for prevention strategies in the fight against the HIV spread. Second, it may be possible to intervene during early infection to limit viral replication for better disease management[5]. The objective of this study was to determine rates of recent and long-established HIV infection using anti-HIV avidity index immunoassay among newly enrolled HIV-1 infected patients attending the Jos University Teaching Hospital.

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Materials and Methods

Study population

We conducted the study using serum samples from 100 HIV-positive persons who had been diagnosed with HIV infection and present for care at the adult ARV clinic of the Jos University Teaching Hospital (JUTH). The entry point for all patients was either through HIV counseling and testing (HCT) or referred HIV positive patients from other services within the hospital, around the community and from other neighboring states. The patients were recruited sequentially after obtaining informed consent between October 2010 and April 2011. A questionnaire was used to collect basic demographic data from each study participant. Criteria for inclusion in this study were patients who presented with a positive HIV test and were aged 18 years and above and antiretroviral therapy naive. Blood samples were collected in EDTA lined containers and plasma was extracted and cryopreserved. The samples were subsequently shipped in ice parked containers to the Kenya Medical Research Institute HIV-Resistance Laboratory, Kisian Kisumu, where the avidity assay automated third generation immunoassay was carried out.

IgG avidity assay using third generation AxSYM HIV 1/2 gO

To estimate time since infection, the IgG Avidity Index (AI) test was employed using automated anti-HIV enzyme immunoassay (EIA) as previously described[6]. The method was based on the rationale that antibodies produced in early phase of an infection show a low avidity increase progressively with time after exposure to an immunogen. Thus the low avidity indicates a recent infection and this was based on previous reports that a cut off of 0.80 for the AI corresponded to mean sero-conversion duration of 180 days using AxSYM HIV 1/2 gO[7]. Serum samples with an AI of $\leq$0.80 were classified as "recent infection" and those with an AI of >0.80 were classified as "established infection" (long-established infection).

The samples which were stored at −80°C, each sample were thawed, and two aliquots of 0.2µl each were subjected to a pre-analytic dilution with phosphate-buffered (1:10) saline. After incubation at room temperature for 5 minutes, the aliquots were assayed using the automated AxSYM HIV1/2gO assay (Abbott Diagnostics Division, Delkenheim, Germany) without modifying the recommended protocol by the manufacturer, and the AI results were obtained for each specimen. All specimens were tested in parallel under routine conditions.

$CD4^+$ T-lymphocytes enumeration

The $CD4^+$T-lymphocyte cell was measured by flow cytometry using Partec Cyflow Counter® (Partec GmbH, Munster Germany) using the "$CD4^+$ Easy Count kit" according to manufacturer's instructions. Twenty microliters of EDTA-ant coagulated blood was added to 20µl of monoclonal antibodies and mixed thoroughly for 5 seconds. The reactants were incubated for 15 minutes at room temperature in the dark, after which 800 µl of no lyse dilution buffer was added to the tube and was gently mixed for 5 seconds. The prepared specimen was then analyzed using the Partec CyFlow Counter for enumeration of $CD4^+$ T-lymphocytes[8]. Results were available in 2 minutes and recorded in cells/mm^3. All blood samples were processed on the same day that the blood was drawn

HIV-RNA (viral load) determination

The quantification of HIV-RNA levels from cryopreserved plasma samples was done with the commercial Roche Amplicor Monitor, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany) for amplification and quantification of HIV-1 RNA.

Data analysis

Data was collected on a predesigned paper form and subsequently transferred to Microsoft excel spread sheet. All the entries were checked for possible keyboard error(s) at the entry level. The electronic data was exported into the Epi Info software version 3.5.3 (CDC, Atlanta, Georgia) for data analysis. Baseline clinical and biological characteristics of the study subjects were summarized as percentages for categorical variables; and mean ± standard deviation (SD) or median (interquartile range-IQR) for quantitative variables. Occurrence of recent infection was noted and comparisons of variables between subjects with recent versus long-established infection were made using appropriate statistical tests. P-values <0.05 were considered statistically significant. The study was approved by the ethics committee of the Jos University Teaching Hospital

Results

Out of 100 individuals tested 11 (11.0%) turned out to be positive for recent HIV infection and 89 (89.0%) of patients had long established infection. The mean age of subjects with was long-established infection was 40±9 while it was 36±6 for subjects with recent infection. The rates of recent infection was higher among female 6 (54.5%), and 5 (56.2%) had long-established infection. There was slight predominance of married individuals: the prevalence of those who married among recent infection was (72.7%) while those with long-established infection (68.0%). The proportion of those whose spouses were positive among the recently infected was 27.0%, and those with long-established infection were (9.5%). The percentage of spouses on ARV among recently infected individuals was 54.5%, while for long-established infection it was 5.7%. The WHO disease staging categorization of recent infection was 63.6% and

42.7% was the long-established infection. The median CD4 cells count of those with long-established infection was lower 134 cell/mm^3 with an IQR:12-639) vs. 364 cell/mm^3 (IQR:31-600) for recent infection; p =0.0003. Median viral load was higher in recent infection 99892 (9942-305128) copies/ml compared to long-established infections 62971 (2188-2,059,850) copies/ml.

Table 1. Characteristics of Recent and Long-established Infection among patients enrolled for HIV care in Jos, Nigeria

Characteristics	Recent Infection (RI) n=11	Long-established (LI) Infection n=89	p- value
Mean Age	40±9	36±8	0.1
Sex, Male	5 (45.5)	39 (43.8)	0.9
Married	8 (72.7)	60 (68.0)	0.72
HIV positive spouse	3 (27.3)	8 (9.5)	0.24
Spouse on ARV	6 (54.5)	5 (10.2)	0.8
Early disease*	7 (63.6)	38 (42.7)	0.18
HIV-RNA copies/ml**	99892 (9942-305128)	62971 (2188-2,059,580)	0.92
Cd4+ Cells/mm^3**	364 (31-600)	134 (12-639)	0.0003

*WHO disease categorization early clinical (stage I & II); ** Median values with IQR

Discussion

The discrimination of recent and long-established infection is critical to both prevention and treatment interventions. The study used third generation enzyme immune assay (EIA) to discriminate between recent and long-established infection. The results of the study showed that the majority of patients infected with HIV present at the health facility are long established infection (89.0%) and are either at WHO stage 3 or 4[9]. This suggests that many individuals are unaware of their HIV status until advanced stage of the disease. The overall low CD4 count of the recent and long established infected participants supports this finding which is consistent with earlier studies[10, 11]. The clinical implications of failure diagnose HIV and access treatment on time contributes to poor treatment outcomes on ART initiation and gives the opportunity for further spread of the virus. There was slight predominance of females with recent infection and this is an agreement with reports that females tend to know their HIV status and access treatment earlier than males. The higher proportion of females having recent infection may be due to referrals where pregnant women are routinely tested for HIV at designated antenatal clinics. The proportions of married individuals were higher in both recent and long-established infection, and this confirmed high proportion of heterosexual transmission among the study population. The proportion of discordant couples was low among the recent infections compared to long-established infections which suggest the characteristics of patients who are mostly referrals with AIDS defining disease or advanced disease. Predominantly, the individuals with recent infections had their spouses on ARV, and this may suggest the reason for possible transmission of drug resistant variants among ARV treatment-naive individuals. Most of the patients receiving care were those who have their HIV status known through partner disclosure notification procedure. This showed that partner disclosure initiated program in management of HIV infection is key to early diagnosis, permits patient education as well as treatment that may delay disease progression.

In this study, the prevalence of recent infection was low (11.0%) and this suggest that the advantage of early initiation of therapy for improved survival may be maximized with concomitant reduction in co-morbidities and mortalities among the people. Overall, there was a significant association of CD4+ T-lymphocytes cell count in the study participants. This difference showed that most patients that attend tertiary institution are late presenters with AIDS defining diseases and suggests that poor immunologic rebound on drug initiation could be attributed to the fact that majority of these patients access health care late. These finding showed the importance of seeking antiretroviral therapy earlier stages which leads to reduced transmission, morbidity, mortality and better treatment outcomes on drug initiation.

Despite the limitations of small sample size and possible selection bias in relation to groups preferentially selected using viral load of those referred to access antiretroviral which may have underestimated rates of recent infection among the population, the study reflects the healthcare-seeking behaviors of many participants. This means that early access to ARV is still a challenge and preventive actions should aim at educating the populace on the importance of early access to available HIV care and treatment. The use of AI clearly has a potentially important role both in public health monitoring and individual diagnosis of HIV infection and understanding of HIV/AIDS epidemic in the region.

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References

1. Guy R, Gold J, Calleja JM, Kim AA, et al. WHO Working Group on HIV Incidence Assays. Accuracy of serological assays for detection of recent infection with HIV and estimation of population incidence: a systematic review. Lancet Infect Dis 2009;9:747-759.
2. Murphy G, Parry VJ. Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro Surveill 2008;13:4-10.
3. Cole KS, Murphey-Corb M, Narayan O, Joag SV, Shaw GM, Montelaro RC. Common themes of antibody maturation to simian immunodeficiency virus, simian-human immunodeficiency virus and human immunodeficiency virus type 1 infections. J Virol 1998;72:7852-7859.
4. Thomas HI, Wilson S, Morgan-Capner P, et al. Different maturation of avidity of IgG antibodies to gp41, p24 and p17 following infection with HIV-1. Clin Exp Immunol 1996;103:185-191.
5. Gianella S, von Wyl V, Fischer M, et al and the Swiss HIV Cohort Study (SHCS). Early antiretroviral therapy during primary HIV-1 infection results in a transient reduction of the viral setpoint upon treatment interruption. PLoS One. 2011; 6(11): e27463.
6. Gianella S, Wyl V, Fischer M et al and the Swiss HIV Cohort Study. Effect of early antiretroviral therapy during primary HIV-1 infection on cell-associated HIV-1 DNA and plasma HIV-1 RNA. Antivir Ther 2011;16:535-545
7. Suligoi B, Massi M, Rezza G, et al. Identifying recent HIV infections using the avidity index and an automated enzyme immunoassay. J Acquir Immunodefic Syndr 2003;32:424-428.
8. Zijenah LS, Kadzirange G, Madzime S, et al. Affordable flow cytometry for enumeration of absolute CD4+ T-lymphocytes to identify subtype C HIV-1 infected adults requiring antiretroviral therapy (ART) and monitoring response to ART in a resource-limited setting. J Transl Med 2006; 4:33.
9. WHO (2005) Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance, African region. http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf. (Accessed 20 October 2010)
10. Porter K, Wall PG, Evans BG. Factors associated with lack of awareness of HIV infection before diagnosis of AIDS. BMJ 1993. 307: 20-3.
11. Forbi CJ, Forbi DT, Agwale MS. Estimating the time period between infection and diagnosis based on CD4+ counts at first diagnosis among HIV-1 antiretroviral naïve patients in Nigeria. J Infect Dev Ctries 2010;4:662-667.