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2014; 1(1): 11-17
Published online April 10, 2014 (http://www.openscienceonline.com/journal/ijma)

Factors associated with long-standing HIV-1 infection among HAART treatment-naïve adults in Jos, Nigeria

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To cite this article

Anejo-Okopi JA, Agbaji OO, Ebonyi AO, Agaba PA, Onywera H, Oguche S, Mbaawuaga EM, Audu O, Abba JO, Nimzing L, Isa SE, Olonitola SO, Idoko JA, Okonkwo P. Factors Associated with Long-Standing HIV-1 Infection among HAART Treatment-Naïve Adults in Jos, Nigeria, *International Journal of Microbiology and Application.* Vol. 1, No. 1, 2014, pp. 11-17

Abstract

The estimation of the duration of HIV infection among newly enrolled HIV infected patients has become important because of clinical implications. Since many patients with HIV-1 infection are not usually diagnosed until they present with symptoms, the need to determine the factors associated with long-standing infection is becoming increasingly important for the epidemiologic purposes and of early access to HAART. We determine the factors associated with long-standing HIV infection in HAART naïve patients. One hundred and five cryopreserved plasma samples randomly selected from sample frame of 230 HIV-1 infected treatment-naïve patients at the adult ART clinic of the Jos University Teaching Hospital, were tested using enzyme immunoassay (EIA)-avidity index assay. Of the 105 samples, 100 were successfully tested. Basic demographic (age, sex, residence, education level, marital status), spouse on ARV, spouse HIV status), mode of transmission, WHO clinical staging, co-infections, HIV-1 viral load, and $CD4^+$cell count results were obtained and analyzed using Stata software version 10.1. Majority of the patients had long-standing HIV-1 infection and were late presenters to the ART treatment Centre. Both the univariate and multivariate analyses showed that low $CD4^+$ cell count was associated with the long-standing HIV infection at 95% CI 5.34 (1.30-21.92). Early detection of HIV-1 infection and access to ART is necessary to avoid rapid decline of $CD4^+$ cell count resulting in accelerated HIV disease progression and consequent development of opportunistic infections.

Keywords

HIV-1, EIA, Avidity Index, Factors, Long-Standing HIV Infection, Nigeria

1. Introduction

Over the last decade there has been significant progress in the treatment of Human Immunodeficiency Virus Type 1 (HIV-1) infection using combination antiretroviral therapy (cART), but many individuals infected continue to initiate cART late in both developed and developing countries [1,2]. Many of these individuals have long-standing HIV infection at presentation for ART [3]. Late presentation increase chances of the development of opportunistic infections as a result of increased viral load and decreased $CD4^+$ count leading to rapid HIV disease progression [4]. One of the important reasons for this late presentation is lack of available diagnostic test to estimate duration of infection at HIV testing centers.

HIV-1 prevention strategies in most resource-limited settings have been mainly focused on the use of rapid serological tests that do not distinguish between recent and long-standing infection. The ability to differentiate recent HIV infection (≤6 months) from long-standing (≥12 months) is a valuable tool for accurate measurement of duration of HIV infection and the need for optimal timing of cART initiation [5]. The early identification of newly acquired HIV-1 is crucial in yielding information on the dynamics of the epidemic; transmission networks, patterns of transmitted drug resistance, and the need to obtain virologic and immunologic data for clinical benefits.

In developed countries, 30-35% of patients have $CD4^+$ count below 200 cells/mm^3 at the time of presentation [1] as compared to those in resource-limited settings such as Nigeria where more than 50-65% have $CD4^+$ counts below 200 cells/mm^3 [7]. The study is aimed at predicting factors associated with long-standing infection which is useful for early intervention strategies in the fight against the HIV spread. The study was aimed at identifying factors associated with long-standing HIV-1 infection among HIV-1 infected adults newly enrolled for HAART in a Nigeria urban setting.

2. Methods

2.1. Study Setting

The study was carried out at the HIV treatment Centre of the Jos University Teaching Hospital (JUTH) in collaboration with AIDS Prevention Initiative in Nigeria (APIN) program. The care and treatment program has been supported by the United States President's Emergency Plan for AIDS Relief (PEFPAR) since 2004. This clinic provides comprehensive HIV care services for the city of Jos and its environs. All HIV-related clinic data for enrolled patients are maintained in electronic databases. Laboratory bench work was carried at CDC supported HIV-Research Laboratory, Kenya Medical Research Institute, Kisumu, Kenya.

2.2. Study Subjects

These were adult patients aged at least 18 years and were diagnosed as HIV-1 positive at the adult HIV clinic of JUTH but were yet to initiate cART.

2.3. Study Design

This was a cross-sectional study where 230 adult patients accessing care were consecutively enrolled at the adult ART clinic from October 2010 – April 2011. All patients included in the study provided written informed consent for the use of their data for research as approved by the Institutional Review Board "JUTH Ethic Committee". Of the 230 samples, 105 were randomly selected using computer generated numbers and shipped to the Kenya Medical Research Institute, Kisumu in Kenya, where 100 samples were successfully tested using avidity assay while the five remaining samples (4.8%) never met the required volume for the assay. The study utilized data that were captured using standardized questionnaire. Demographic data including; age, sex, residence, educational level, marital status, occupation and mode of HIV transmission, clinical data; spouse HIV status, spouse ARV treatment status, WHO HIV clinical stage [8], laboratory data; hepatitis B virus (HBV) status, viral load and $CD4^+$ cell count variables.

2.4. Laboratory Procedures

2.4.1. 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 [9]. 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 immunogens. 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 correspond to mean sero-conversion duration of 180 days using AXSYM HIV 1/2 gO. Serum samples with an AI of ≤0.80 were classified as "recent infection" while those with an AI of >0.80 were classified as "established infection" (long-standing infection).

Each of the samples stored at −80°C 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.

2.4.2. CD4+ T-Lymphocytes Enumeration

The $CD4^+$ T-lymphocyte cell was measured by flow cytometry by the Partec Cyflow Counter® (Partec GmbH, Munster Germany) using the "$CD4^+$ Easy Count kit" according to manufacturer's instructions. Twenty microliters of EDTA-anticoagulated 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 [10]. 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.

2.4.3. HIV-RNA (Viral Load) and HBsAg Determination

The quantification of HIV-1 RNA levels from cryopreserved plasma samples was done with the commercial Roche CobasAmplicor HIV-1 Monitor, version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany) for amplification and quantification of HIV-1 RNA. HBsAg was determined using Enzyme immunoassay (EIA) (MonolisaHBsAg Ultra3, Bio-Rad).

2.5. Statistical Methods

Analyses were performed using Stata software version 10.1 (Stata Corporation, College Station, Texas, USA). Univariate associations of each independent variable with the outcome (long-standing/recent infection) was examined using the Fisher's exact test or chi-squared test for categorical variables and the Wilcoxon signed rank test for continuous variables that were not normally distributed. For the univariate analysis, some continuous variables were categorized as follows: age into $\leq$35 and >35 years, viral load into $\leq$65218 and > 65218 copies/mL using the median cut-off values; and CD4+ cell count into $\leq$200 and >200cells/mm^3 using the WHO cut-off value of 200 cells/mm^3 [8]. All tests were two-sided and a p-value of 0.05 was considered significant.

Univariate analysis (logistic regression) was carried out to determine the crude association between long-standing/recent infections with each independent variable and expressed as odds ratio. Only those variables significantly associated with long-standing/recent infection at $p < 0.05$ were considered for inclusion in the multivariate model. Since sex and age influence many disease processes including HIV, they were included *a priori* in the modeling process. Forward stepwise modeling was used to build the multivariate model. The area under the receiver operating characteristic (ROC) curve was determined to assess the accuracy of the model.

3. Results

Eighty nine (89%) of the 100 HIV-1 infected subjects had long-standing HIV infection. The median age of the subjects was 35 years. Majority were females (56%), 55% were either in WHO clinical stage 3 or 4 at presentation, 63% had $CD4^+$ cell count <200 cell/mm^3, 66% were resident in Plateau state, 68% had secondary or tertiary education, 68% were married. The subjects whose spouses were not on ARV 84% and only 16% of the spouses were on ARVs, 87% tested negative for HBsAg, 88% did not have pulmonary tuberculosis (PTB) co-infection. Majority of the subject did not have Kaposi sarcoma 98%. Amongst the subjects, 87% did not have chronic diarrhea and 84% did not have oropharyngeal candidiasis. The major mode of HIV transmission in the subjects was by heterosexual sex 98%, and 49% of the spouses tested positive for HIV-1. The median viral load of the subjects was 62971 copies/mL (IQR 22628-152258)

Table 1. The median $CD4^+$ cell count was significantly lower in those with long-standing infection (134 cell/mm^3, IQR (67-251) compared to those with recent infection (369 cell/mm^3 (IQR, 72-600). In the univariate analysis, only $CD4^+$ cell count <200 cell/mm^3 was significantly associated with long-standing/recent infection with the odds of having a long-standing infection being about 5½ times more in those with $CD4^+$ count <200 cell/mm^3, p = 0.02. Though the odds of long-standing infection was higher in those aged $\leq$35 years versus >35 years (OR, 1.87), the other statistics were as follows: those with secondary/tertiary education versus illiterate/primary education (OR, 2.91), widowed/divorced separated versus married (OR, 1.60), those in WHO clinical stage 3/4 versus ½ (OR, 2.35), those with PTB co-infection versus those without PTB co-infection (OR, 1.41) and reduced by 54% (OR, 0.46) in those whose spouse were on ARVs compared to those not on ARVs, these associations were not statistically significant.

Table 1. *Characteristics of adults at presentation to JUTH according to duration of infection (long-standing/recent infection) status.*

Characteristics	Total	Duration of infection		P value*
		Long-standing infection	Recent infection	
	N (%)	N (%)	N (%)	
Age (yrs)				
≤35	50 (50)	46 (51.7)	4 (36.4)	0.53
>35	50 (50)	43 (48.3)	7 (63.6)	0.16**
Median	35.5	35	37	
Sex				
Male	44 (44)	39 (43.8)	5 (45.5)	

Characteristics	Total	Duration of infection		P value*
		Long-standing infection	Recent infection	
	N (%)	N (%)	N (%)	
Female	56 (56)	50 (56.2)	6 (54.5)	
Residence				
Plateau	66 (66)	57 (64.0)	9 (81.8)	0.32
Others	34 (34)	32 (36.0)	2 (18.2)	
Education level				
Illiterate/Primary	32 (32)	26 (29.2)	6 (54.5)	0.09
Secondary/Tertiary	68 (68)	63 (70.8)	5 (45.5)	
Marital status				
Married	68 (68)	60 (67.4)	8 (72.7)	
Widowed/Divorced/	13 (13)	12 (13.5)	1 (9.1)	1.00
Separated	19 (19)	17 (19.1)	2 (18.2)	
Single				
Spouse HIV status				
Positive	49 (49)	44 (49.4)	5 (45.5)	0.80
Negative	51 (51)	45 (50.6)	6 (54.5)	
Spouse on ARV drugs				
On ARV	16 (16)	13 (14.6)	3 (27.3)	0.38
Not on ARV	84 (84)	76 (85.4)	8 (72.7)	
Mode of HIV transmission				
Heterosexual	98 (98)	87 (97.8)	11 (100)	1.00
Blood transfusion	2 (2)	2 (2.2)	0 (0)	
WHO clinical stage				
Stages 3/4	55 (55)	51 (57.3)	4 (36.4)	0.21
Stages 1/2	45 (45)	38 (42.7)	7 (63.6)	
HBV status				
Positive	13 (13)	11 (12.4)	2 (18.2)	0.63
Negative	87 (87)	78 (87.6)	9 (81.8)	
PTB co-infection				
Present	12 (12)	11 (12.4)	1 (9.1)	1.00
Absent	88 (88)	78 (87.6)	10 (90.9)	
Oropharyngeal candidiasis				
Present	16 (16)	14 (15.7)	2 (18.2)	1.00
Absent	84 (84)	75 (84.3)	9 (81.8)	
Chronic diarrhoea				
Present	13 (13)	13 (14.6)	0 (0.00)	0.35
Absent	87 (87)	76 (85.4)	11 (100.00)	
Kaposi sarcoma				
Present	2 (2)	2 (2.5)	0 (0.00)	1.00
Absent	98 (98)	87 (97.5)	11 (100.00)	
HIV RNA viral load (copies /ml)				
>65218	50 (50)	44 (49.4)	6 (54.5)	
≤65218	50 (50)	45 (50.6)	5 (45.5)	0.75
Median (IQR)	65218 (22202-153725)	62971 (22628-152258)	99892 (11371-155192)	0.98**
Mean (SD) Log_{10} HIV RNA viral load	0.53 (0.50)	0.53 (0.50)	0.54 (0.52)	
CD4 count (per mm^3)				
<200	63 (63)	60 (67.4)	3 (27.3)	0.01
≥200	37 (37)	29 (32.6)	8 (72.7)	
Median (IQR)	141 (68-263)	134 (67-251)	369 (72-600)	0.02**

*Fisher's exact test or chi-squared test for the association between categorical variables and recent/ long-standing infection
**Wilcoxon rank sum test for comparison of median values.

Table 2 shows the multivariate analysis where, $CD4^+$ cell count still remained the only variable significantly associated with a long-standing HIV-1 infection with the odds of having a long-standing infection being about 5½ times more in those with $CD4^+$ count <200 cell/mm^3 compared to those with a count ≥200 cell/mm^3, p = 0.02 (Table 3).

4. Discussion

This study showed that eighty nine percent (89%) of patients enrolled for HAART were diagnosed with long-standing HIV-1 infection and majority had $CD4^+$ cell count below 200 cell/mm3. The study revealed that the majority of patients infected with HIV-1 presenting at an urban

health facility were in WHO stage 3 or 4 (Table 1). This may suggest that most of the HIV-1 infected individuals in Nigeria are diagnosed late and could not access cART until late in the course of the HIV-1 infection.

Table 2. *Crude associations of variables with duration of infection (long-standing/recent infection) in adults at presentation to JUTH.*

Variable	Crude odds ratio (95% CI)	P value
Age (yrs) >35 ≤35	1.00 (Ref) 1.87 (0.51 – 6.84)	0.34
Sex Female Male	1.00 (Ref) 0.94 (0.27 – 3.30)	0.92
Residence Others Plateau	1.00 (Ref) 0.40 (0.08 – 1.95)	0.25
Education level Illiterate/ Primary Secondary/ Tertiary	1.00 (Ref) 2.91 (0.82 – 10.37)	1.00
Marital status Married Widowed/Divorced/Separated Single	1.00 (Ref) 1.60 (0.18 – 14.00) 1.13 (0.22 – 5.84)	0.67 0.88
Spouse HIV status Negative Positive	1.00 (Ref) 1.12 (0.33 – 4.13)	0.80
Spouse on ARV drugs Not on ARV On ARV	1.00 (Ref) 0.46 (0.11 – 1.95)	0.29
Mode of HIV transmission Heterosexual Blood transfusion	1.00 (Ref) 0.00 (-- - --)	0.62
WHO clinical stage Stages 1/2 Stages 3/4	1.00 (Ref) 2.35 (0.64 – 8.6)	0.20
HBV status Negative Positive	1.00 (Ref) 0.63 (0.12 – 3.33)	0.95
PTB co-infection Absent Present	1.00 (Ref) 1.41 (0.16 – 12.11)	0.75
Oropharyngeal candidiasis Present Absent	1.00 (Ref) 0.84 (0.16 – 4.31)	0.83
Chronic diarrhoea Present Absent	1.00 (Ref) Predicts success perfectly	
Kaposi sarcoma Absent Present	1.00 (Ref) Predicts success perfectly	
HIV RNA viral load (copies/ml) ≤65218 >65218	1.00 (Ref) 0.81 (0.23 – 2.87)	0.75
$CD4^+$ cell count (cells/mm^3) ≥200 <200	1.00 (Ref) 5.52 (1.36 – 22.35)	0.02

Table 3. *Factors independently associated with duration of infection (long-standing/recent infection) in adults at presentation to JUTH.*

Variable	Adjusted odds ratio (95% CI)	P value
Age (yrs) >35 ≤35	1.00 (Ref) 1.61 (0.42 – 6.21)	0.49
Sex Female Male	1.00 (Ref) 0.90 (0.24 – 3.38)	0.87
$CD4^+$ count (cells/mm^3) ≥200 <200	1.00 (Ref) 5.34 (1.30 – 21.92)	0.02

The results also revealed that women have higher proportion than men and this collaborates the earlier reported case of improved intake of women in antenatal clinics at tertiary settings in Nigeria which has also reduced the rate of discordant couples *[9]*. Other factors for this disproportionality may be awareness, stigmatization and willingness to access treatment by women compared to men.

In this study, we also observed that only a few individuals presented for care had recent HIV-1 infection (Table 1). Amongst the advantages of diagnosing recent HIV infection is early initiation of cART leading to improved survival and quality of life. Further evidence suggests that routine HIV screening is a cost-effective prevention and management strategy in the era of cART [10, 11].

This finding is consistent with the report that HIV infected Nigerians were mostly diagnosed late with low $CD4^+$ cell count [12]. This underscores the need for early diagnosis to increase life expectancy, reduced cost of medication and better quality of life. A recent study carried out in Jos, Nigeria, also reported the association of low $CD4^+$ cell count with TB in newly enrolled HIV infected patients co-infected with TB [13]. This may imply late presentation by HIV-1 infected patients suggesting a long duration of HIV-1 infection in our urban settings. Our patients had lower $CD4^+$ cell counts than that reported in an earlier cohort study in India [2]. This underscores the need for early diagnosis to increase life expectancy, reduced cost of medication and better quality of life. It is important to note that increasing the threshold of $CD4^+$ cell count for starting cART is important, however, this should be accompanied with avidity assays result for achieving early detection of HIV-1 recent infection. Assessing HIV-1 infected patients at the point of enrolment for cART with long-standing HIV-1 infection may have multiplier effect as patients found to have long-standing infection will be triaged for immediate commencement of cART thereby reducing the likelihood of rapid disease progression and prevent complications. We also found that the prevalence of long-standing HIV-1 infection was higher in women than men; this corroborates the earlier reported improved uptake of women in antenatal clinics at the tertiary settings in Nigeria [9]. Other factors that may account for this higher

prevalence of long-standing HIV-1 infection in women may be increased awareness and willingness to access treatment by women when compared with men. In addition, the results although not statistically significant, showed that odd ratio (OR) in those whose spouses was far less on ART than those whose are not.

It was observed that using univariate and multivariate analysis, the low $CD4^+$ cell count remained the factor associated with long-standing HIV-1 infection, which showed that late presentation have much clinical implications and could impact largely on cART efficacy and $CD4^+$ cell recovery even after therapy initiation [2, 4]. This observation is important for designing public health epidemiological studies and development of intervention strategies to achieve early detection and proper estimation of HIV infection duration. It is also important to generalize that routine serological testing should be encouraged both in urban and rural settings, but avidity assays that estimate duration of infection be provided to enhance HIV disease classifications for early detection and efficient management. With the low $CD4^+$ cell count observed, the authors hypothesize that the high number of AIDS deaths and the short survival time for individuals after HIV-1 infection in Nigeria could be attributed to the fact that the majority of these patients were diagnosed late. The study has limitations of small sample size and information on the mode of transmission was obtained through direct questioning which may have underrepresentation of homosexuals because of perceived stigmatization.

5. Conclusion

We found the prevalence of long-standing HIV-1 infection to be high in this Nigerian cohort. Also, long-standing HIV-1 infection was associated with low $CD4^+$ cell count. This finding may have important implications for public health intervention strategies in developing countries. We recommend extensive education on the need for early diagnosis of HIV-1 infection to inform prompt initiation of cART. This is necessary to prevent a rapid decline of CD4+ cell count. These actions would contribute to public health initiative that would lead to a better understanding of the HIV epidemic in this region, encourage early HIV testing and access to cART to improve better management of HIV disease and reduce the development of opportunistic infections.

Conflict of Interest

None

Funding

This work was funded in part by the US Department of Health and Human Services, Health Resources and Services Administration (U51HA02522). The contents are solely the responsibility of the authors.

Acknowledgments

We are deeply indebted to the patients who agreed to participate in this study. We thank the Director and staff of HIV-R laboratory, Kenya Medical Research Institute Kisian Kisumu, Dr. Clement Zeh who permitted the training and analysis of the samples. Ramyil Seljul, Fumilayo Moulton and Titus Obadiah of APIN center JUTH were instrumental in overseeing sample collection, transport and processing. We also acknowledge the support of APIN Ltd GTE for the care of the patients.

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