HIV-1 Subtypes and treatment outcome among adults on Anti-retroviral therapy at tertiary hospital in Moshi, Tanzania

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Abstract

Background: Human Immunodeficiency virus (HIV) is characterized by great genetic diversity due to its high mutations that occur during replication. Its infection also characterized by high rates of viral turnover and extensive viral diversity. This diverse has implication on disease progression, diagnostic strategies, vaccine development as well as treatment response to antiretroviral drugs.

Methods: 63 HIV positive adults infected by different HIV-1 Subtypes at Kilimanjaro Christian Medical Centre (KCMC) in Moshi, Tanzania were studied. HIV-1 Subtypes were characterized using peptide ELISA representing HIV-1 subtypes A, B, C, D and E derived from consensus gp 120 V3 sequences. The CD4+T-lymphocyte cell counts were measured at baseline, six and twelve months using FACS Calliber (Becton Dickinson, San Jose, CA, USA).

Results: HIV-1 Subtype A was the most prevalent (47.62%), followed by Subtype C (36.51%) and Subtype D (15.87%). Subtype D showed higher immunological and clinical failures as compared to subtype A and C with Hazard Ratio (H.R, 5.6) and 95% CI=1.3-5.2, P=0.02). After adjustment for sex, baseline CD4+ T Lymphocyte and clinical stage, the association remained the same.

Conclusions: HIV-1 A was the most predominant followed by C and D was less predominant. HIV-1 D showed rapid progression of diseases with poor treatment outcomes relative to others subtypes.

Keywords: HIV-1 diversity, immunological failure, ELISA, Peptides and immunodominant region.

Introduction

Acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) ranks among the world's most devastating disease because it has spread rapidly particularly the sub Sahara African countries. The main modes of transmission are sexual intercourse, needle stick injuries and transmission from mother to child. It was estimated that HIV infection affected 33.4 million by 2008; 2.7 million being new infections and 430,000 of these being in infants and children (UNAIDS 2009; WHO 2011). With an estimate 22.4 million prevalent cases, 1.9 million new cases and 1.4 million deaths due to AIDS in the year 2008 , Sub Saharan Africa, one of the poorest regions in the world appears to be hardest hit by the pandemic (UNAIDS 2009). United Republic of Tanzania with an estimated population of 41,139,813 in 2009 had an estimated 2.2 million people living with HIV. The prevalence of HIV in the Tanzanian population in 2009 was estimated to be 5.7%. (Cohen et al. 2008).

HIV is characterized by a great genetic diversity due to its high mutations that occur during replication. HIV is classified into HIV-1 and HIV-2.HIV-1 is responsible for the AIDS pandemic and HIV-2 a less aggressive virus is localized in west-Africa. Phylogenetic analysis of the full-length or regions of the HIV-1 genome demonstrate a genetic variability which result into classifying the virus into groups M, N, O and P (Coffin et al,1998, Plantiel et al, 2008). The pandemic group M is further classified into nine subtypes A, B, C, D, F, G, H, J and K. Co-infections of these HIV-1 subtypes combine during their replication and result into recombinant virus (Buonaguro et al. 2007). Globally, different subtypes of HIV-1 circulate unevenly in different geographical

regions. HIV-1 subtype C is predominant in Sub Sahara particularly in the southern, central and eastern part of Africa. Variability of HIV-1 subtypes may implicate diagnostic strategies, vaccine development as well as treatment response to antiretroviral drugs (Geretti et al. 2006). Rolling out ARV has increased life expectancy for people leaving with HIV/AIDS in resource poor settings where multiple HIV-1 subtypes are circulating. It is therefore, imperative to investigate immunological response among patients infected with diverse HIV-1 subtypes receiving antiretroviral drugs.

Assessing and managing an ARV-experienced patient experiencing failure of ART is complex. Expert advice is critical and should be sought**.** Immunologic failure is defined as the failure to achieve and maintain an adequate CD4 response despite virologic suppression. (Geretti et al. 2009) Increases in CD4 counts in ARV-naive patients with initial ARV regimens are approximately 150 cells/mm^3 over the first year while virologic failure is the inability to achieve or maintain suppression of viral replication (to an HIV RNA level <400 copies/mL) (Bartlett et al. 2001;Ramadhani et al. 2007). The proportion of patients experiencing immunologic failure depends on how failure is defined, the observation period, and the CD4 count when treatment was started. In the longest study conducted to date, the percentage of patients with suppressed viremia who reached a CD4 count >500 cells/mm^3 through 6 years of treatment was 42% in those starting treatment with a CD4 count <200 cells/mm^3, 66% in those starting with a CD4 count 200-350 cells/mm^3, and 85% in those starting with a CD4 count >350 cells/mm3 (Moore and Keruly et al. 2007). Once virologic failure is confirmed, generally the

regimen should be changed as soon as possible to avoid progressive accumulation of resistance mutations (Hosseinipour et al. 2009).

PATIENTS AND METHODS

Study design and participants. HIV-1 subtypes and treatment outcome study was cross -sectional longitudinal study to determine HIV-1 Subtypes, immunological and clinical failure among adults (>18years of age) HIV infected patients attending Infectious disease clinic at Kilimanjaro Christian Medical Centre teaching and referral Hospital Moshi, Tanzania. Subjects were required to be HIV- positive individuals who attended at KCMC clinics for twelve months during follow up. These individuals were selected from the previous adherence study, and only those with adherence to ARV of >95% determined by pill counts and self reporting.

Sample collection and processing. Four milliliters of whole blood were collected from each of the 63 study participants. The whole blood samples were centrifuged and separated, serum aliquots were frozen at -80 centigrade ready for processing. Panel of gp120 V3 synthetic peptides derived from a consensus of V3 sequences representing HIV-1 subtypes A to E was given by the Centralized Facility for AIDS Reagents, UK Medical Research Council. The amino acid sequences are shown below:

Peptide A consensus (K S V H I G P G Q A F Y A T), Peptide B consensus(K S I H I G P G R A F Y T T), Peptide C consensus(K S I R I G P G Q T F Y A T), Peptide D consensus (R Q R T H I G P G Q A L Y T T) and Peptides E consensus (RTSITIGPGQVVYRT). Subtype-specific antibodies in the serum samples

will bind to the respective peptides. An anti-human immunoglobin enzyme conjugates 24 was added followed by the appropriate substrate. The result of the reaction is a colored product that was measured spectrophotometrically. We also used gp41 peptides representing HIV-1 subtypes A and D was donated Dr. Chou Pau of the Centers for Disease Control in Atlanta, Georgia, USA. These peptides were derived from the immunodominant region (IDR) of gp41 from HIV-1 LAI, a French subtype B isolate and HIV-1 ELI, a Zairean subtype D isolates (http://www.nibsc.ac.uk/catalog/aids-reagent) The CD4+T-lymphocyte cell counts were measured at baseline, six and twelve months using FACS Calliber (Becton Dickinson, San Jose, CA, USA).

Statistical analyses. Statistical analysis was done using Statistical Package for Social Science (SPSS) version 19.0 and then transferred to STATA version 12 for advanced model of analysis. Data were summarized using frequency distribution tables, cross tabulations and pie charts. Relationship was tested using Fishers test and P-value <0.05 was considered to be statistically significance.

Ethical consideration:

This study was approved by research ethics committee, of Kilimanjaro Christian Medical University College.

RESULTS

From August 2009 through July 2010 we successfully enrolled 63 patients in the present study whereby 42 were females and 21 were males with a mean age of 43 years (Range 17 - 74). Of 63 patients 30 (47.6%) were infected by HIV-1 subtype A while 23 (36.5%) by subtype C and 10 (15.9%) by subtype D. (Table 1). Out of the 63 subjects, 22% had CD4 T cells below 200cells/ul while 92% of study participants were HIV/AIDS stage two or more according to WHO clinical staging. (Table1).

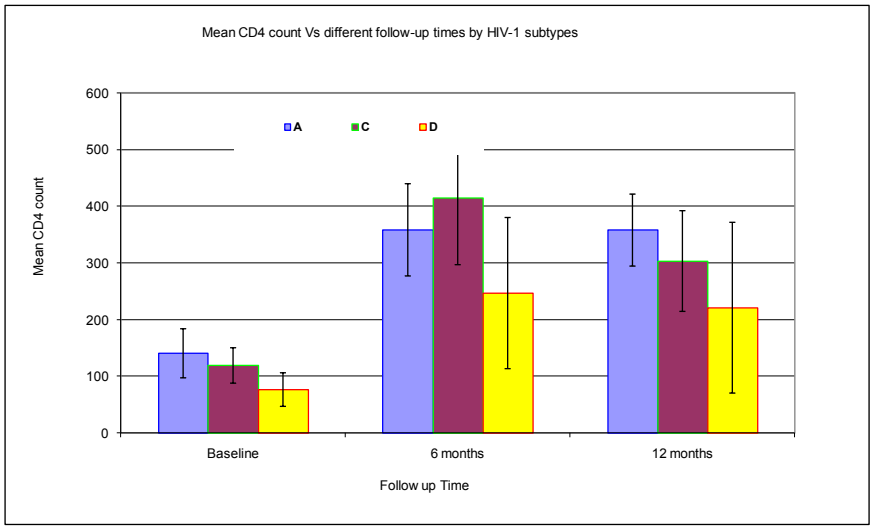
HIV-1 subtype D showed significant different on CD4 T cells across the three visits, at baseline, six months and twelve months. This was followed by HIV-1 subtype C while subtype A was the least. (Table2). When we analyzed the mean CD4 T cells across the three HIV-1 subtypes adjust for the three visits, there was statistical different among the HIV-1 subtypes C and D with P value = 0.02 and hazard ratio of 5.3 and 6.1 for subtype C and D respectively. (Figure1). The most regimen which was been used at that time were Lamivudine and Zidovudine (CBV) and Efavirenz (EFV), CBV/ Nevirapine (NVP) and stavudine (D4T)/ Lamivudine (3TC)/ Efavirenz (EFV), Triomune 30 (T30) or Stavudine (D4T)/ Lamivudine (3TC) and Nevirapine (NVP). There were no different outcomes among different regimen users (Table 1).

Table 1: Characteristics of 63 study participants infected with different HIV-1 subtypes on ART attending clinics at KCMC in Moshi, Northern Tanzania 2009-2010.

HIV-1 subtypes					
Variables	A	C	D	Total	Fisher's Exact Test p-value
Number (%)	30 (47.6)	23 (36.5)	10 (15.9)	63	
Sex					
Male n (%)	14 (66.7)	4(19.1)	3(14.3)	21	0.07
Female n (%)	16(38.1)	19(45.2)	7(16.7)	42	
CD4+ T lymphocyte cells count at 12 months (cells/mm3)					
<200	3(21.4)	6(42.9)	5(35.7)	14	0.08
201-249	10(66.7)	4(26.7)	1(6.6)	15	
350 or more	12(54.6)	8(36.4)	2(9.1)	22	
Age in years					
≤49	24 (47.1)	19(37.3)	8 (15.7)	51	1.00
>49	6(50.0)	4(33.3)	2(16.7)	12	
Death					
Yes	29(48.3)	23(38.3)	8(13.3)	60	0.06
No	1(33.3)	0(0.0)	2(66.7)	3	
Distribution of clinical stages					
Stage 1	0(0.0)	2 (40.0)	3(60.0)	5	0.006
Stage 2 or above	30 (51.7)	21(36.2)	7(12.1)	58	
ARV regimen					
CBV/EFV	8(44.4)	8(44.4)	2(11.1)	18	0.17
CBV/NVP	13 (46.4)	11 (39.3)	4(14.3)	27	
Others(T30,D4T/3TC/EFV,D4T/3TC/NVP	9(52.9)	4(23.5)	4(14.3)	17	

Table 2: The mean CD4+ T lymphocyte cells among individuals on ART infected with different HIV-1 subtypes attending clinics at KCMC in Northern Tanzania during twelve months follow up (2009-2010).

HIV-1 Subtypes	Mean (Range) CD4+ T lymphocyte cells at baseline	Mean (Range) CD4+ T lymphocyte cells at 6 months	Mean (Range) CD4+ T lymphocyte cells at 12months
A	140 (7-513)	358 (100-744)	357 (129-730)
C	119 (2-252)	414 (108-721)	303 (79-710)
D	76 (6-149)	246 (82-610)	221 (20-670)



Adjusted values P=0.02 (C&D) while Hazard ratio=5.3&6.1 for C& D respectively

Figure 1: The mean CD4+ T lymphocyte cells from different HIV-1 subtypes in Northern Tanzania in relation to different follow up time of twelve months duration (at baseline, at six month and twelve month) from 63 study participants as presented above: (2009-2010)

DISCUSSION

The present study demonstrated that, the predominant circulating HIV-1 subtype was subtype A followed by subtypes C and D among patients on ART attending clinics at KCMC in Moshi. In previous studies conducted in Moshi showed similar pattern, however, this study showed that HIV-1 subtype A is the predominant and stable, while the prevalence of subtypes C is increasing and subtype D is decreasing, respectively (Kiwelu et al. 2005:Nyombi et al. 2008). This phenomenon may be explained by the characteristic of HIV-1 subtype A being less virulent where by infected individuals survival for long time, while subtype C is characterized by high viral load in vaginal secretions promoting heterosexual transmissions in many regions of sub-Saharan. Furthermore, HIV-1 subtype C is characterized by high viral loads during acute phase of the infection leads to high transmission rate when compared to subtype A or D (Novitsky et al. 2009). HIV-1 subtype C has shown as well to be favored by mother to child transmission mode as compared to subtypes A and D hence increased prevalence of subtype (Renjifo et al. 2004).

The decrease of HIV-1 subtype D might be explained by two factors, first its dual tropic nature of using both CXCR4 and CCR5 co-receptors during acute as well as chronic infections enable the virus to infect many cells at once hence rapid disease progression to death. Dual or mixed use of co-receptor which is favoring subtype D contributes on poor clinical outcome of the patients (Wei et al. 2009). Secondly its ability to form syncytia where by single infected CD4 T-lymphocyte cluster with uninfected cells and form cluster of cells which will be destroyed by natural killer cells. The combination of these factors enhances the ability

of HIV-1 subtype D to infect more cells at once when compared to A and C (Kaleebu et al. 2001). Among 63 participants who were followed for twelve months the immunological failure, individuals presented with CD4+ T-lymphocyte count <200cells/mm3 were 24 (38%) and out of those HIV-1 subtype D contributed 71% while subtype A and C contributed 29%. At six months on ARV HIV-1 subtype C showed higher CD4+ lymphocyte cells recovery which later on dropped significantly due to viral escape from immune recognition hence co-infection with super-infection. Host involvement can as well explain this through alteration of frequency and even number of new virus produced. In HIV-1 subtype C infection CCR5+CD4+ T cells remained relatively stable during the first months of infection after seroconversion compared to high fluctuations in the percentage of CXCR4+CD4+T cells between patients which is accompanied by a gradual decrease over the first year of infection (Vladimir et al. 2009). Since both of them had similar access to HIV care and monitoring and antiretroviral therapy with adherence of >92%, HIV-1 subtype D and C showed statistically significant difference in the rate of CD4 T+ lymphocyte rebound compared to subtype A. Further more patients infected with HIV-1 subtype D had six times probability of maintaining CD4+ T lymphocyte below 200 cells/mm3 after twelve months follow-up compared to other two subtypes. Although we found no statistically significant differences in the rate of CD4+ T lymphocyte rebound to different antiretroviral therapy across three HIV-1 subtypes.

We assessed whether the more rapid CD4+T lymphocyte in HIV-1 subtype D and C infected patients than in patients with HIV-1 subtypes A could be explained by the shorter follow up, fewer CD4 cell count measurements at the baseline and other differences in demographic

characteristics. That was not the case because patients infected with HIV-1 subtypes C and D showed the faster rate of CD4+ count decline which remained statistically significant even after adjustment for all potential confounders including gender, TB disease, baseline CD4+cells, ART regimen and age. Geretti et .al. showed no difference in CD4+ T lymphocyte cells among HIV-1 subtypes A, C and D after six months on ART (Geretti et al. 2009).

During the course of therapy, some of the participants progressed to HIV WHO clinical stage 4, while others contracted TB infections and others died. These outcomes were also considered and there was significant difference across the subtypes. The crude mortality rate was very high in HIV-1 subtypes D when compared to C and A, individuals infected with subtypes D has six chance increase of dying from the disease when compared with the counterpart. If you are infected with HIV-1 subtype D, after controlling other confounders such as CD4+ T cells at the baseline, OIs still you have more than 90 percent more likely to be WHO clinical stage 4 when compared with other subtypes in our study which was supported by other studies (Mellors JW et al. 1997, Michael NL et al.1992, Loveday C et al. 1995). This study demonstrated that HIV-1 genetic diversity may have an influence on efficacy of antiretroviral therapy. This effect was more demonstrated in HIV-1 subtype C and D compared to HIV-1 subtypes A. Furthermore HIV-1 subtype D demonstrated poor disease outcome across three subtypes. Further studies are needed to assess the interplay between HIV-1 subtypes and the host genetic variation in relation to disease response to ART.

One of the limitations of our study was lack of viral load measurements due to high cost which most patients cannot afford to have viral load done to measure treatment outcome. Viral load data is very important

parameter for assessing treatment failure together with WHO clinical stage and CD4+ T cells.

In summary, this study we had found that HIV-1 subtypes A to be the predominant and HIV-1 subtype C-is increasing where compared to previous study in the region. HIV-1 subtype D is decreasing relative to other Subtypes. Furthermore we assessed disease response toward prescribed ART, whereby those patients infected with HIV-1 subtype D and C experienced immunological and clinical failure as compared to HIV-1 subtype A. Also patients infected with HIV-1 Subtype D experienced high mortality rate as compared to HIV-1 subtype A and C. We recommend that people should ART when CD4 is <350cells/mm3 according to WHO guidelines. Further studies to be done to address natural and acquired mutations associated with poor outcome to the given ART among people infected with HIV-1 subtype C and D.

Competing interest

The authors declare that they have no competing interest.

Author's contribution

EGK, BMN, JS and JM contributed on data acquisition from the laboratory information system and cleaned the data. ERS and JK- contributed on conception, design and analyzed the data and edited the first draft. ERS- wrote the first draft and final version and compiled figures and tables which were later on agreed upon by each author.

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