HIV-1 Subtype D Infection Is Associated with Faster Disease Progression than Subtype A in Spite of Similar Plasma HIV-1 Loads

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We investigated the effect of human immunodeficiency virus type 1 (HIV-1) subtype on disease progression among 145 Kenyan women followed from the time of HIV-1 acquisition. Compared with those infected with subtype A, women infected with subtype D had higher mortality (hazard ratio, 2.3 [95% confidence interval, 1.0–5.6]) and a faster rate of CD4 cell count decline (P = .003). The mortality risk persisted after adjustment for plasma HIV-1 load. There were no differences in plasma viral load by HIV-1 subtype during follow-up. HIV-1 subtype D infection is associated with a >2-fold higher risk of death than subtype A infection, in spite of similar plasma HIV-1 loads.

One of the defining features of the global spread of HIV-1 has been the rapid genetic diversification of the virus. HIV-1 group M viruses, representing the majority of infections worldwide, have been classified into subtypes, A through K, that differ by as much as 35% in the sequences that code for their viral envelope proteins [1]. Although there is general agreement that HIV-1 genetic diversity constitutes an important challenge in the development of an HIV-1 vaccine, there remains debate as to whether strain differences in HIV-1, particularly subtype differences, influence HIV-1 disease progression [2].

Only a small number of studies have examined the effect of HIV-1 subtype on HIV-1 disease progression [3–8]. Although some found that individuals infected with non-A subtypes, particularly subtype D, appeared to have faster progression than those infected with subtype A [3, 6–8], others have not shown significant differences [4, 5]. Importantly, most studies enrolled only seroprevalent cases of HIV-1 [4, 6, 7] or a mixture of incident and prevalent cases [5]. Thus, the date of HIV-1 acquisition was unknown for the majority of study participants, and the rates of HIV-1 disease progression could not be determined with precision. In the present study, we examined the effect of HIV-1 subtype on HIV-1 disease progression among Kenyan women followed from HIV-1 acquisition.

Participants and methods. In 1993, an open cohort of HIV-1–seronegative commercial sex workers attending a municipal clinic in Mombasa, Kenya, was established [9]. Women were followed monthly. Those who seroconverted to HIV-1 continued follow-up, and blood samples were collected quarterly thereafter. Participants who missed consecutive clinic appointments were traced at their workplaces. For women who died, the date of death was collected from colleagues. Participants provided informed consent. The study was approved by the ethics review committees of the University of Nairobi, the University of Washington, and the Fred Hutchinson Cancer Research Center.

HIV-1 seroconversion was detected using ELISA (Detect-HIV [Biochem ImmunoSystem]; confirmed by Recombigen [Cambridge Biotech]). Beginning in 1998, CD4 cell counts were measured quarterly (Cytosphere [Coulter] or Zymmune [Bar-tels]). Plasma HIV-1 RNA load was determined using archived plasma samples from all quarterly visits after HIV-1 seroconversion, as well as from the 2 visits before seroconversion, using the Gen-Probe HIV-1 load assay, which is sensitive to the HIV-1 subtypes found in Kenya [10].

The methods used for HIV-1 subtype analyses have been described elsewhere [11]. Briefly, an ~800-bp fragment spanning sequences coding for the V1–V3 loops of envelope was amplified from peripheral blood mononuclear cell (PBMC) DNA. The subtype of the fragment was assigned using the heteroduplex mobility assay (HMA) or by sequence and phylogenetic analysis. There was concordance between the results of HMA and sequence analyses for cases in which both were applied [11]. Analyses were performed using SPSS (version 10.0; SPSS) and SPLUS 2000 (MathSoft). Dates of HIV-1 infection were

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estimated using a combination of HIV-1 serology and plasma RNA results [9, 12]. Women were included in this analysis if they had detectable HIV-1 plasma RNA before seroconversion or <1 year between their last HIV-1–seronegative visit and their first HIV-1–seropositive visit [9]. Comparisons of demographic and behavioral characteristics were made by Mann-Whitney U tests of medians or by χ² tests.

Follow-up data through March 2004, when antiretroviral drug therapy was introduced into the cohort, were analyzed. Women who were not known to have died were censored at their last follow-up visit or last successful tracing attempt [12]. Cox proportional hazards models and Kaplan-Meier analyses, with log-rank tests, were used to analyze time to death after HIV-1 infection.

Comparisons of set-point plasma HIV-1 load (defined as the first measurement occurring 4–24 months after infection [12]) by HIV-1 subtype were performed using Mann-Whitney U tests of medians. Linear mixed-effects analyses were used to model the effect of HIV-1 subtype on plasma HIV-1 load rise and CD4 cell count decline over time [9]. All data for women contributing ≥2 measurements from ≥4 months after HIV-1 acquisition were used. Data from <4 months after infection were excluded to avoid transient effects of primary HIV-1 infection. The analyses accounted for multiple measurements from each individual; an autoregressive correlation structure was used. Models calculated an intercept (equivalent to an average plasma HIV-1 load or CD4 cell count at 4 months after HIV-1 infection) and a slope (equivalent to the average change per month in plasma HIV-1 load or CD4 cell count during subsequent follow-up).

Results. Between 1993 and 2004, 218 women seroconverted to HIV-1 and had dates of infection that could be estimated. PMBC samples were available for HIV-1 subtyping for 147 women (67%)—127 (86%) determined by HMA and 20 (14%) by sequence analyses. One hundred fourteen women (78%) had subtype A, 10 (7%) had subtype C, and 21 (14%) had subtype D. Two (1%) were infected with recombinant viruses (1 A/C and 1 A/D) and were excluded from the analyses. The median time from HIV-1 infection to the first HIV-1–seropositive visit was 55 days (interquartile range [IQR], 40–89 days). The median time from HIV-1 infection to the date of collection of the PBMC sample used for determining HIV-1 subtype was 76 days (IQR, 53–129 days), and 140 women (97%) had HIV-1 subtype determined from a sample collected within 1 year of HIV-1 acquisition.

At the time of HIV-1 infection, the median age was 28 years (IQR, 25–34 years), and the median duration of sex work was 3 years (IQR, 2–7 years). Women had a median of 7 years of education (IQR, 6–9 years). Ninety percent were employed as barmaids. During the 6 months before HIV-1 acquisition, the median number of sex partners per week was 1 (IQR, 0.7–1.3). There were no statistically significant differences by HIV-1 subtype in these demographic and behavioral measures. Women who had HIV-1 subtyping performed had slightly less education (median, 7 vs. 8 years; P = .04) but were otherwise similar to those without subtype information.

Women were followed for a median of 5.4 years (IQR, 2.8–7.1 years) after HIV-1 acquisition. Because participants acquired HIV-1 at different points throughout the study period, the maximum potential follow-up time differed for each woman. Vital status was known for 90% of women at 1 year after HIV-1 acquisition, 77% at 3 years, 71% at 5 years, 60% at 7 years, and 51% at 9 years. Loss to follow-up did not differ significantly by HIV-1 subtype.

Thirty women died—20 infected with subtype A, 3 with subtype C, and 7 with subtype D. HIV-1 subtype was related to mortality (figure 1). For women infected with subtype A, median survival was not achieved; survival at 8.7 years was 55% by Kaplan-Meier analysis. For women infected with subtypes C and D, median survival was 6.9 and 7.7 years, respectively, with the comparison between subtype D and subtype A achieving statistical significance (P = .05).

By Cox proportional hazards analysis, subtype D was associated with a >2-fold increased risk of death, compared with
Table 1. Linear mixed-effects model analyses of the effect of HIV-1 subtype on plasma HIV-1 load and CD4 cell count early in infection and over time

<table>
<thead>
<tr>
<th>Measure</th>
<th>Intercept (95% CI)</th>
<th>( P^a )</th>
<th>Slope (95% CI)</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma HIV-1 load,(^b) ( \log_{10} ) copies/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Reference                | 4.53 (4.36 to 4.69) | ...      | 0.0065 (0.0032 to 0.0099) | ...
| Additional effect of HIV-1 subtype | Reference |          |                |          |
| Subtype A                | 0.14 (−0.51 to 0.80) | .7       | −0.0043 (−0.0154 to 0.0068) | .4       |
| Subtype C                | −0.15 (−0.56 to 0.26) | .5       | 0.0006 (−0.0073 to 0.0084) | .9       |
| Subtype D                |                     |          |                |          |
| CD4 cell count,\(^c\) cells/\(\mu\)L |                    |          | −0.79 (−1.70 to 0.12) | ...
| Reference                | 433 (390 to 477)    | ...      |                |          |
| Additional effect of HIV-1 subtype | Reference |          |                |          |
| Subtype A                | −81 (−237 to 74)    | .3       | −0.29 (−3.25 to 2.68) | .8       |
| Subtype C                | 104 (−3 to 211)     | .06      | −3.44 (−5.70 to −1.18) | .003     |

\( \log_{10} \): \( \log \) to the base 10.

\( ^b \) One hundred seventeen women contributed 1073 viral load measurements.

\( ^c \) Eighty-seven women contributed 672 CD4 cell measurements. CD4 cell counts were not initiated in the cohort until 1998.

\( ^a \) Comparison vs. subtype A.

\( ^P \) \( P \) value.

\( ^m \) \( m \) stands for months.

\( ^n \) 81 women contributed CD4 cell measurements.

\( ^p \) p values.

\( ^1 \) Eighty-seven women contributed 672 CD4 cell measurements.

\( ^2 \) One hundred seventeen women contributed 1073 viral load measurements.

\( ^3 \) All data for women contributing \( \geq 2 \) measurements from \( \geq 4 \) months after HIV-1 acquisition were used. Models calculate an intercept equivalent to the average value of the plasma viral load or CD4 cell count at 4 months after HIV-1 infection; for plasma viral load, this approximates the set point and a slope equivalent to the average change per month in the plasma viral load or CD4 cell count during subsequent follow-up. Thus, for example, for women with subtype A infection, plasma HIV-1 load was estimated to be 4.53 \( \log_{10} \) copies/mL at 4 months after infection and increased by 0.0065 \( \log_{10} \) copies/mL/month thereafter, and for women with subtype D infection, plasma viral load was estimated at 4.38 \( \log_{10} \) copies/mL (= 4.53 − 0.15) at 4 months after infection, with a change over time of 0.0071 \( \log_{10} \) copies/mL/month (= 0.0065 + 0.0006). CI, confidence interval.

In this model, there were no statistically significant differences by subtype in plasma HIV-1 load at 4 months after infection or in the change in viral load over time thereafter (table 1).

Linear mixed-effects modeling was also used to examine the effect of subtype on CD4 cell count (table 1). There were no differences in the modeled CD4 cell count at 4 months after infection or in the change in CD4 cell count over time for women with subtype C, compared with those with subtype A. However, compared with women with subtype A, those with subtype D were calculated to have higher CD4 cell counts at 4 months after infection (difference, 104 cells/\( \mu \)L; \( P = .06 \)) but a faster decline in CD4 cell count over time (difference, −3.44 cells/\( \mu \)L/month; \( P = .003 \)). By this model, average CD4 cell counts equalized for subtypes D and A at \( \sim 34 \) months after infection, when the CD4 cell count would be 409 cells/\( \mu \)L. At 72 months after infection, the model estimated that an average woman with subtype D would have a CD4 cell count of 249 cells/\( \mu \)L, compared with 379 cells/\( \mu \)L for an average woman with subtype A.

**Discussion.** In this cohort of Kenyan women followed from before HIV-1 acquisition, infection with HIV-1 subtype D was associated with a faster rate of CD4 cell count decline and a >2-fold higher risk of death, compared with those with subtype A. These results add to a small but growing body of evidence suggesting that HIV-1 subtype D progresses more rapidly than subtype A (hazard ratio [HR], 2.3 [95% confidence interval [CI], 1.0–5.6]; \( P = .06 \)). Subtype C was also associated with higher mortality, compared with subtype A, although this did not achieve statistical significance (HR, 2.1 [95% CI, 0.6–7.2]; \( P = .2 \)).

We performed Cox proportional hazards analyses adjusting for set-point plasma HIV-1 load, which was available for 127 women (88%). The effect of HIV-1 subtype on mortality risk was maintained (for subtype C: adjusted HR, 1.8 [95% CI, 0.5–6.3] \( P = .3 \); and for subtype D: adjusted HR, 2.7 [95% CI, 1.0–7.2] \( P = .04 \); each compared with subtype A). In this analysis, plasma HIV-1 load was also independently related to mortality (for each \( \log_{10} \) copies/mL increase: HR, 2.1 [95% CI, 1.0–7.2]; each compared with subtype A). In this model, there were no statistically significant differences by subtype in plasma HIV-1 load at 4 months after infection or in the change in viral load over time thereafter (table 1).
subtype A [6–8]. Our study is unique in that our population consisted solely of incident cases of HIV-1 and in that we demonstrated that the greater mortality risk among those infected with subtype D appeared not to be a result of higher plasma HIV-1 load, either at set point or over time.

We did not find a statistically significant effect of subtype C on HIV-1 disease progression, although our number of cases of subtype C was small. One limitation of our analysis, as well as of others of this issue, is that subtype information was derived from partial envelope sequences and did not capture the subtype of the full genome. We chose to focus on those sequences encoding the surface unit because of the central role this protein plays in viral entry and cell tropism, likely reflecting effects on viral replication and virulence, although other viral proteins may also be relevant for HIV-1 pathogenesis.

Loss to follow-up in this highly mobile, socially marginalized population of commercial sex workers may have affected our results, and our findings may not be fully generalizable to all HIV-1–infected populations in sub-Saharan Africa. However, we have previously shown that survival in this cohort was similar to that among general populations of HIV-1–infected individuals in Africa and the United States before the introduction of antiretroviral therapy [12]. Moreover, our results may be particularly applicable to HIV-1–infected women, who make up the majority of infections in Africa.

The present study provides further evidence that subtype D may be more pathogenic than subtype A. Interestingly, recent studies have suggested that subtype D may be decreasing in prevalence [11], consistent with the notion that more pathogenic variants may be less fit for transmission [13]. The biological mechanisms that underlie such differences between subtypes are unknown. Our study suggests that increased pathogenesis of subtype D is not simply the result of increased replication because the differences in disease progression were independent of plasma HIV-1 load. It has been reported that subtype D is more likely to utilize the coreceptor CXCR4 than other subtypes [14] and to develop CXCR4 tropism early after infection [8]. A more facile coreceptor switch provides an attractive model for increased virulence of subtype D, because the emergence of CXCR4 variants has been linked to disease progression in subtype B infection [13]. Such differences in coreceptor use could also explain differences in transmissibility. More detailed studies of subtype D variants may help elucidate the biological properties that contribute to HIV-1 virulence.

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