Survival of Ugandan Infants with Subtype A and D HIV-1 Infection (HIVNET 012)


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Summary: Virologic factors may influence survival of HIV-1–infected infants. We compared survival of Ugandan infants with subtype A and subtype D HIV-1 infection. This study was performed in the context of the Ugandan clinical trial HIVNET 012, which compared the efficacy of single-dose nevirapine (NVP) and short-course zidovudine (AZT) for prevention of HIV-1 mother-to-child transmission. HIV-1 subtypes were determined by phylogenetic analysis of HIV-1 protease and reverse transcriptase sequences from 32 women in the NVP arm and 54 women in the AZT arm of HIVNET 012 whose infants were HIV-1 infected by 6 to 8 weeks of age. We found no association between HIV-1 subtype (A vs. D) and infant survival in this cohort. Further studies are needed to evaluate whether HIV-1 subtype influences clinical outcome in pediatric HIV-1 infection. Key Words: HIV-1—Uganda—Subtype—Nevirapine—Zidovudine—Infant.

Mother-to-child transmission (MTCT) is responsible for most cases of pediatric HIV-1 infection. Since the beginning of the AIDS epidemic, more than 4 million children have died of HIV-1 infection, most in the developing world (1). Disease progression in HIV-1–infected children is variable (2–6). Factors associated with disease progression in HIV-1–infected children include advanced maternal HIV disease, timing of HIV-1 infection, high HIV-1 RNA levels in infants at birth and during primary viremia, HIV accumulation in CD4 cells, cellular and humoral immune responses, and viral phenotype (e.g., SI vs. NSI) (7–13). Further evaluation of factors that influence the survival of HIV-1–infected infants is important for development of effective strategies to prevent and treat pediatric HIV-1 infection.

HIV-1 viruses can be categorized into different subtypes (clades), which vary in prevalence from one geographic region to another. Studies comparing the transmissibility or pathogenesis of different HIV-1 subtypes are limited. We recently analyzed the impact of HIV-1 subtype on MTCT in the Ugandan clinical trial HIVNET 012. HIVNET 012 was a two-armed, NIH-sponsored, randomized trial comparing the efficacy of single-dose nevirapine (NVP) with a short course of zidovudine (AZT) for prevention of HIV-1 MTCT (14,15). Each arm...
enrolled 313 women; nearly all infants (99%) were breast-fed. Women did not receive antiretroviral therapy before or after receiving NVP or AZT prophylaxis, which is consistent with the standard of care in Uganda. Both regimens were well tolerated, and the nature and frequency of adverse events were similar for the two groups. The NVP regimen was significantly more effective than the AZT regimen (14,15). Among 102 women in the NVP arm of HIVNET 012, we previously identified 50 with subtype A, 35 with subtype D, 4 with subtype C, and 13 with intersubtype recombinant HIV-1 (16).

Interestingly, women with subtype D had a higher rate of NVP resistance 6 to 8 weeks after NVP prophylaxis than women with subtype A (16). This did not appear to reflect more advanced disease in women with subtype D, because baseline viral loads and baseline CD4 cell counts were similar in women with subtype A versus subtype D among the women analyzed. We found no association between subtype (A vs. D) and the rate of MTCT among those women (16). In this report, we extended our analysis to include infants in the AZT arm of HIVNET 012 who were HIV-1 infected by 6 to 8 weeks of age and analyzed the survival of infants with subtype A and subtype D HIV-1 infection.

METHODS

The HIVNET 012 study protocol was reviewed and approved by institutional review boards in Uganda and the United States, and informed consent was obtained from all women prior to enrollment. Detailed methods and results of HIVNET 012 are presented elsewhere (14,15).

Laboratory Data from HIVNET 012

HIV-1 infection was diagnosed in infants prior to the age of 18 months using HIV-1 RNA PCR confirmed by an additional HIV-1 RNA PCR or HIV-1 culture. At 18 months of age, HIV-1 infection was diagnosed by enzyme immunoassay (EIA) and, if reactive, by confirmatory Western blot analysis. Viral loads were determined with the Roche Amplicor MONITOR Test Kit (Branchburg, NJ, U.S.A.). Methods for determination of CD4 cell counts have been described previously (14).

Genotyping and Phylogenetic Analysis of HIV-1 Sequences

HIV-1 sequences corresponding to protease amino acids 1 through 99 and reverse transcriptase (RT) amino acids 1 through 324 (297 and 972 nucleotides, respectively) were obtained from plasma HIV-1 using the Applied Biosystems ViroSeq HIV-1 Genotyping System (Applied Biosystems, Foster City, CA, U.S.A.) as previously described (17). Methods for phylogenetic analysis and subtype determination have been described in detail (16).

GenBank Accession Numbers

GenBank accession numbers for HIV-1 sequences from women in the NVP and AZT arms are AF388135-AF388166 and AF410203-AF410256, respectively.

Statistical Methods

To determine the differences in death rates between infants whose mothers had subtype A versus subtype D, we used the Kaplan-Meier method to estimate the binary outcome—death at 18 months. Death rates for these two groups of infants were then compared using a z test, with standard errors obtained using the Greenwood formula. For overall survival, a Cox proportional hazards model was used to determine the association between HIV-1 subtype (A vs. D) and infant death, adjusting for baseline covariates, maternal viral load, and maternal CD4 cell count. All statistical analyses were done using the SAS system (version 8.2).

RESULTS

HIV-1 Subtype Analysis

In HIVNET 012, 37 infants in the NVP arm (including one pair of twins) and 59 infants in the AZT arm were HIV-1 infected by 6 to 8 weeks of age (15). Because plasma samples from these infants were limited in availability, we performed HIV-1 subtyping using samples from the corresponding mothers. Plasma samples collected at 6 to 8 weeks postpartum were available from 33 of the women in the NVP arm and 59 of the women in the AZT arm whose infants were infected. HIV-1 pol region sequences (protease and RT) were used for subtype determination. Subtypes were successfully determined for 32 women in the NVP arm and 54 women in the AZT arm. Subtypes identified included A, C, and D as well as intersubtype recombinant HIV-1 (A/D recombinants). The distribution of subtypes was similar for women in the NVP and AZT study arms (Table 1). In 19 cases where infant subtypes were determined directly from infant plasma samples, the subtype of the infant matched the subtype of the corresponding mother (data not shown). For this study, we assumed that the HIV-1 subtype of each infant was the same as the HIV-1 subtype of its mother.

Relation Between HIV-1 Subtype and Infant Survival

Infant death was monitored in HIVNET 012 through 18 months of age. We compared the survival of infants with subtype A versus subtype D infection. This analysis was restricted to infants who were diagnosed with HIV-1 infection by 6 to 8 weeks of age. A total of 72 infants were included in this analysis. One infant infected with
subtype A (1.4%) was lost to follow-up at 12 months and was censored at that time in the survival analysis. Among infants in the NVP arm, the proportion of HIV-1–infected infants who died by 18 months of age was 60% for those with subtype A infection versus 18% for those with subtype D infection (see Table 1). The difference was not statistically significant, however. The proportion of infants who died within 18 months in the AZT arm and in the NVP and AZT arms combined was similar for infants with subtype A versus subtype D (see Table 1; \( p = .352 \) for the combined arms). We found no evidence for more rapid disease progression among the infants infected with intersubtype recombinant HIV-1 compared with subtype A or D. The number of infants with recombinant HIV-1 was too small for meaningful statistical analysis, however.

Similar results were obtained when assessing the association of HIV-1 subtype with the continuous outcome of survival. In a multivariate analysis, the estimated hazard ratio for death comparing infants with subtype A versus subtype D in a Cox model adjusting for randomization (AZT vs. NVP), maternal baseline CD4 cell count, and maternal baseline viral load was 0.92 (95% CI: 0.61–1.40). The number of infants with recombinant or subtype C HIV-1 was too small for meaningful statistical analysis. When data from the NVP and AZT arms were combined, there was no apparent association between HIV-1 subtype and infant survival (Fig. 1).

**DISCUSSION**

In our cohort, HIV-1 subtype (A vs. D) did not appear to influence infant survival. To our knowledge, this is the

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**TABLE 1. Proportion of infants who died by 18 months of age**

<table>
<thead>
<tr>
<th>Study arm</th>
<th>HIV-1 subtype</th>
<th>A (%)</th>
<th>D (%)</th>
<th>C (%)</th>
<th>R (%)</th>
<th>Not determined</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevirapine</td>
<td>Infants</td>
<td>15 (47%)</td>
<td>11 (34%)</td>
<td>3 (9%)</td>
<td>3 (9%)</td>
<td>5</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Deaths</td>
<td>9 (60%)</td>
<td>2 (18%)</td>
<td>2 (67%)</td>
<td>1 (33%)</td>
<td>0</td>
<td>14 (38%)</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Infants</td>
<td>28 (52%)</td>
<td>18 (33%)</td>
<td>1 (2%)</td>
<td>7 (13%)</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Deaths</td>
<td>9 (32%)</td>
<td>7 (39%)</td>
<td>0 (0%)</td>
<td>3 (33%)</td>
<td>3</td>
<td>22 (37%)</td>
</tr>
<tr>
<td>Total</td>
<td>Infants</td>
<td>43 (50%)</td>
<td>29 (34%)</td>
<td>4 (5%)</td>
<td>10 (12%)</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Deaths</td>
<td>18 (42%)</td>
<td>9 (31%)</td>
<td>2 (50%)</td>
<td>4 (40%)</td>
<td>3</td>
<td>36 (38%)</td>
</tr>
</tbody>
</table>

* Infants with evidence of HIV-1 intersubtype recombination in the region analyzed.
* Either no sample was available or genotyping did not provide a sequence sufficient for subtyping.
* Indicates the number and percentage (%) of infants with subtype A, C, or D or intersubtype recombinant HIV-1.
* Indicates the number and percentage (%) of infants with each subtype who died by 18 months of age.

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**FIG. 1.** Kaplan-Meier plot of infant survival by HIV-1 subtype (A vs. D). Survival of infants with subtype A (closed circles) or subtype D (open circles) who were infected by 6 to 8 weeks in HIVNET 012. Data from the nevirapine and zidovudine arms were combined for this analysis.
first study comparing the survival of infants infected with different HIV-1 subtypes. Studies of the impact of subtype on disease progression in adults have found varied results. In a study of female sex workers from Senegal, women with non-A subtypes (C, D, or G analyzed as a single group) were more likely to develop AIDS in the first 5 years of infection than those with subtype A (18). In contrast, a study of 164 Ugandan adults suggested that disease progression was slower for women with subtype A than for women with subtype D, although this was not a major effect (19). Other studies have not found an association between subtype and disease progression (20,21).

Our inability to demonstrate an association between HIV-1 subtype and infant survival may reflect the relatively small sample size in our study. It is also possible that subtype has a more significant impact on clinical outcome of infected infants in the absence of antiretroviral prophylaxis. Further studies are needed to evaluate this was not a major effect (19). Other studies have not suggested that disease progression was slower for women with subtype D, although this was not a major effect (19). Other studies have not found an association between subtype and disease progression (20,21).

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REFERENCES