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Subtype C Is Associated with Increased Vaginal Shedding of HIV-1

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Abstract

The prevalence of human immunodeficiency virus (HIV)–1–infected cells and HIV-1 RNA levels in genital secretions and breast milk and the risk of mother-to-child transmission of HIV-1 were compared among subtypes A, C, and D in a Kenyan cohort. Pregnant women infected with subtype C were significantly more likely to shed HIV-1-infected vaginal cells than were those infected with subtype A or D (odds ratio [OR], 3.6 [95% confidence interval {CI}, 1.4–8.8]; $P = .006$). This relationship held after adjusting for age, CD4 cell count, and plasma HIV-1 RNA load (OR, 3.1 [95% CI, 1.1–8.6]; $P = .03$). These observations suggest that HIV-1 subtype influences mucosal shedding of HIV-1.

HIV-1 is divided into types and subtypes on the basis of sequence relatedness, and typically only small regions of the viral genome are compared. There are conflicting data on the role played by HIV-1 subtype in HIV-1 disease progression [1, 2]. There is also speculation that HIV-1 subtype is responsible for differences in the epidemic spread of HIV-1. Specifically, subtype C has been associated with what is perceived to be the most rapid epidemic spread of HIV-1. However, it is difficult to discern whether the more rapid spread of subtype C epidemics is due to subtype or to other coexisting factors in populations affected by this subtype. It is possible that sociobehavioral factors preexistent in settings with newly introduced subtype C virus are responsible for the rapid spread of infection within the population, rather than the properties of subtype C virus.

It has been challenging to determine the clinical and epidemiologic consequences of genetic differences in HIV-1 subtypes, because many geographic regions have an overwhelming predominance of 1 subtype and insufficient numbers of other subtypes to enable comparisons between them. In some regions that do have >1 frequently circulating HIV-1 subtype, such as Thailand, specific subtypes are associated with specific HIV-1 risk factors (i.e., parenteral versus sexual transmission), which makes it difficult to ascribe transmission differences between subtypes solely to subtype [3].

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Perinatal HIV-1 transmission cohorts in sub-Saharan Africa often include women infected with different HIV-1 subtypes. The relative homogeneity of risk behaviors of the mothers in these cohorts may minimize bias and enable comparisons of HIV-1 shedding and transmission between different subtypes. Studies of perinatal transmission have yielded mixed evidence that subtype influences transmission [4, 5].

Mucosal shedding is a frequently used surrogate marker of infectivity. Thus, identification of differences in shedding between HIV-1 subtypes may be an important first step in understanding the role played by HIV-1 subtype in transmission. To determine whether HIV-1 subtype influences shedding in mucosal surfaces or secretions that are responsible for transmission, we compared HIV-1 shedding in genital secretions and breast milk and the risk of mother-to-child transmission of HIV-1 in mothers infected with subtype A, C, or D in Nairobi, Kenya.

Subjects, materials, and methods

HIV-1-seropositive women were enrolled during pregnancy, after informed consent was obtained, between 1992 and 1998. The study was approved by the University of Washington Institutional Review Board and the Kenyatta National Hospital Ethical Review Committee. Methods of recruitment and follow-up have been described elsewhere [6, 7]. Cervical and vaginal swab specimens were collected from the women at ~32 weeks gestation; breast milk specimens were collected at 2, 6, and 10 weeks postpartum [7, 8]. Genital specimens were tested for HIV-1 DNA by a qualitative polymerase chain reaction (PCR) assay that detects the presence of HIV-1-infected cells in a fixed amount of swab specimen. Plasma, genital, and breast milk HIV-1 RNA levels were quantified by the Gen-Probe HIV-1 viral load assay, which is sensitive for all Kenyan subtypes [9, 10]. Maternal peripheral-blood mononuclear cells (PBMCs) were used to determine HIV-1 envelope subtype by a heteroduplex mobility assay or by viral sequencing [11]. This analysis was limited to a 0.8-kb region of the envelope gene; this region was chosen because it encodes domains within the surface unit protein that determine cell entry and tropism and, thus, is likely to be most relevant to the outcome measures examined. Infant HIV-1–infection status was determined by a PBMC DNA PCR assay [12].

Analyses were restricted to women infected with subtype A, C, or D; women infected with undefinable or recombinant subtypes were excluded. The prevalences of cervical and vaginal HIV-1 DNA in women infected with different subtypes were compared by the χ^2 test and Fisher's exact test. To determine whether the relationship between vaginal shedding and infection with subtype C was independent of disease status, multivariate logistic regression models were created that adjusted for age, CD4 cell count, and plasma HIV-1 RNA load as continuous variables individually and simultaneously. Mean HIV-1 RNA loads in cervical secretions, vaginal secretions, colostrum, and breast milk for the different subtypes were compared by Student's *t* test. For infants with defined HIV-1–infection status, overall transmission risk, early infection (≤2 months old), and late infection (>2 months old) were compared for the different subtypes by Cox regression.

Results

Of 365 women whose infecting subtype was determined, 25 (7%) were infected with subtype C, 259 (71%) were infected with subtype A, and 71 (20%) were infected with subtype D. One woman was infected with subtype G, and 9 (2%) were infected with recombinant subtypes.

Median age was 23 years (interquartile range [IQR], 20–26 years), median parity was 2 (IQR, 1–3), and median prenatal CD4 cell count was 422 cells/μL (IQR, 270–523 cells/μL).

Women infected with subtype C had a higher median age and lower CD4 cell counts than did women infected with non-C subtypes (table 1). Median plasma HIV-1 RNA load was 4.6 $\log_{10}$ copies/mL (IQR, 4.0–5.2 $\log_{10}$ copies/mL) for all women and did not differ significantly between women infected with different subtypes.

Detection of HIV-1–infected cells in genital specimens correlated with plasma HIV-1 RNA levels (P<.001, for cervical secretions; P<.001, for vaginal secretions). HIV-1 DNA was detected in cervical secretions from 10 (44%) of 23 women infected with subtype C, 84 (36%) of 233 women infected with subtype A, and 21 (34%) of 61 women infected with subtype D, and detection did not differ significantly between subtypes (table 1). HIV-1 DNA was detected in vaginal secretions from 9 (41%) of 22 women infected with subtype C, 43 (19%) of 232 women infected with subtype A, and 5 (8%) of 63 women infected with subtype D (for subtype C vs. non-C infection, P = .006). Infection with subtype C was associated with a 3.6-fold (95% confidence interval [CI], 1.4–8.8-fold) increased odds of vaginal shedding of HIV-1–infected cells, compared with infection with non-C subtypes; a 3.0-fold (95% CI, 1.2–7.6-fold) increased odds of vaginal shedding, compared with infection with subtype A; and an 8.0-fold (95% CI, 2.3–28.0-fold) increased odds of vaginal shedding, compared with infection with subtype D. In all models, infection with subtype C remained independently associated with vaginal HIV-1 proviral shedding compared with non–subtype C, whether adjusting for age, CD4 cell count, and plasma HIV-1 RNA load individually or simultaneously (odds ratio, 3.1 [95% CI, 1.1–8.6], for the model that simultaneously adjusted for all 3 variables).

Cervical and vaginal HIV-1 RNA loads were assessed in a subset of women for whom additional genital specimens in freezing medium were available [8]. In cervical and vaginal secretions, HIV-1 RNA loads were higher in women infected with subtype A than in women infected with subtype D, but the differences were not significant (table 1). Only 3 women infected with subtype C provided cervical and vaginal specimens for quantitation of HIV-1 RNA, making it impossible to assess the effect of subtype C on vaginal HIV-1 RNA load.

Median colostrum HIV-1 RNA loads were 3.2 $\log_{10}$ copies/mL in women infected with subtype C, 2.9 $\log_{10}$ copies/mL in women infected with subtype A, and 2.2 $\log_{10}$ copies/mL in women infected subtype D. Colostrum and breast milk HIV-1 RNA loads were significantly lower in women infected with subtype D (for subtype D vs. non-D infection, P = .02 for colostrum and P = .04 for breast milk).

Among women with infants whose HIV-1–infection status was known, 7 (28%) of 25 women infected with subtype C transmitted infection, compared with 58 (24%) of 245 women infected with subtype A and 14 (21%) of 67 women infected with subtype D. Transmission risk did not differ significantly between the subtypes. There were no significant differences in the rates of early or late transmission between the subtypes (data not shown).

Discussion

In this maternal cohort from Nairobi, Kenya, women infected with subtype C had 3.0- and 8.0-fold increased odds of vaginal shedding of HIV-1–infected cells than did women with subtypes A and D, respectively. It is possible that disease duration differed between women infected with different subtypes in this cross-sectional evaluation and that this resulted in differences in immune status and plasma viral load between subtypes, which, in turn, affected genital shedding of HIV-1. However, in multivariate logistic regression models that adjusted for age, CD4 cell count, and plasma HIV-1 RNA load, infection with subtype C independently predicted vaginal shedding of HIV-1–infected cells; this suggests that subtype

influences genital shedding of HIV-1. Thus, to the extent that genital shedding of HIV-1 influences transmission, we would predict that transmission to infants or partners from women infected with subtype C would be more frequent than that from women infected with subtype A, and we would predict that subtype D would be the least transmissible. Consistent with this hypothesis, the risk of mother-to-child transmission in the present cohort was highest in women infected with subtype C, was next highest in women infected with subtype A, and was lowest in women infected with subtype D, although the differences did not reach statistical significance.

In light of observations of the relatively rapid epidemic spread of HIV-1 in countries such as South Africa, where subtype C is predominant, our observation of increased vaginal shedding of HIV-1 in women infected with subtype C is particularly intriguing. Interestingly, however, during limited serial cross-sectional surveys, we have not observed an increase in the prevalence of subtype C over the last decade in Kenya (J. Overbaugh, unpublished data). It may be that other cofactors, in combination with increased genital shedding, accelerate regional expansion of subtype C epidemics. Cofactors such as partner networks, host factors, and concomitant sexually transmitted diseases in regions with subtype C epidemics may act synergistically with increased genital shedding to fuel rapid sexual spread of HIV-1.

We found that colostrum HIV-1 RNA loads were also highest in women infected with subtype C (for subtype C vs. D, ~1 log higher) and that colostrum and breast milk HIV-1 RNA loads were significantly lower in women infected with subtype D than in women infected with non-D subtypes. However, we did not observe significant differences in risks of mother-to-child transmission. The present study had >80% power to detect at least a 2.8-fold difference in transmission risk between subtypes A and D, but it was underpowered to evaluate a comparable difference in transmission risk for subtype C. Our results are consistent with those of a Ugandan study of vertical transmission, in which there was no difference in transmission risk between subtypes A and D [4]. The lack of a difference in transmission risk between subtypes A and D is notable, given our observation of significantly increased HIV-1 shedding in genital secretions and breast milk in women infected with subtype A. This observation suggests that the effect of subtype on mucosal shedding is more pronounced than the effect of subtype on transmission risk. It may be optimal to separately analyze the effect of subtype on in utero, intrapartum, and late postnatal transmission. However, such stratified analyses further diminish power to observe associations.

Our study had several limitations. First, because we evaluated shedding in a cross-sectional cohort, confounding effects due to differences in disease duration could not be completely excluded. We adjusted for age, CD4 cell count, and plasma HIV-1 RNA load—all of which reflect disease duration—but a prospective study in which women had defined timing for the beginning of infection would enable better adjustment for disease duration. Second, we limited our evaluation of subtype to the *env* region because this viral region is crucial for initial cell binding and infection and, thus, is most likely to influence levels of viral replication and transmission. Because our study was limited to the *env* region, we did not comprehensively exclude subtype recombinants that may have unique properties. This is of particular relevance, because our studies suggest that ~20%–50% of the viral genomes circulating in Kenya are unique intersubtype recombinants [13]. Third, the relative roles of cervical versus vaginal shedding and of HIV-1 DNA versus RNA shedding in transmission have not been well defined, making it difficult to extrapolate the effect of subtype on shedding to its effect on sexual transmission. Finally, perhaps our biggest limitation was a lack of statistical power to assess the effect of subtype C on the risk of mother-to-child transmission. This limitation derives from the low population prevalence of subtype C in

Nairobi and reflects the most common obstacle to comparisons of subtypes in population studies.

In conclusion, we found significant differences in mucosal shedding of HIV-1 between subtypes. To our knowledge, this is the first report comparing mucosal shedding between HIV-1 subtypes, although differences in genital shedding have been noted between the more distantly related HIV-1 and HIV-2, with shedding being lower for the latter [14]. Of note, HIV-2 also causes a more indolent disease course than does HIV-1 and is less efficiently transmitted [15]. The present study suggests that the magnitude of the effects of the different HIV-1 subtypes on shedding and transmission are more modest than the effects of different HIV types, mirroring their relative genetic divergence. Thus, although the different subtypes may influence viral shedding, transmission, and pathogenesis, it seems unlikely that this alone accounts for the rapid spread of particular subtypes, such as subtype C, in certain parts of the world.

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Table 1

Associations between HIV-1 subtype and cervical, vaginal, colostrum, and breast milk HIV-1 DNA and RNA shedding.

	Subtype			Subtype comparison, *P* value (OR [95% CI])				
Variable	**C**	**A**	**D**	**C vs. A or D**	**C vs. A**	**C vs. D**	**A vs. D**	**D vs. A or C**
Age, median (IQR), years	24 (23–28)	23 (20–26)	23 (20–26)	.04	.06	.03	.5	.4
Prenatal cell counts, median (IQR), cells/μL								
CD4	333 (181–461)	410 (286–541)	410 (253–523)	.02	.02	.1	.5	.7
CD8	885 (604–1104)	833 (632–1117)	847 (615–1149)	.9	.9	.8	.7	.7
HIV-1 DNA, percentage (no. positive/no. tested)								
Cervical	44 (10/23)	36 (84/233)	34 (21/61)	.5 (1.4 [0.6–3.3])	.6 (1.4 [0.6–3.2])	.4 (1.5 [0.6–3.9])	.8 (1.1 [0.6–1.9])	.7 (0.9 [0.5–1.6])
Vaginal	41 (9/22)	19 (43/232)	8 (5/63)	.006 (3.6 [1.4–8.8])	.02 (3.0 [1.2–7.6])	.001 (8.0 [2.3–28.0])	.05 (2.6 [1.0–7.0])	.02 (0.3 [0.1–0.9])
HIV-1 RNA load, median (IQR), $\log_{10}$ copies/mL								
Plasma	4.9 (4.1–5.4) [*n* = 21]	4.6 (4.0–5.3) [*n* = 229]	4.6 (3.9–5.0) [*n* = 65]	.5	.6	.2	.2	.2
Cervical	NA	2.8 (1.0–3.7) [*n* = 33]	2.2 (1.0–4.1) [*n* = 11]	NA	NA	NA	.8	.7
Vaginal	NA	2.8 (1.0–3.3) [*n* = 32]	1.3 (1.0–3.3) [*n* = 13]	NA	NA	NA	.2	.3
Colostrum[a]	3.2 (2.2–3.7) [*n* = 17]	2.9 (2.1–3.6) [*n* = 126]	2.2 (1.4–3.2) [*n* = 36]	.4	.6	.06	.03	.02
Breast milk[b]	3.3 (2.4–3.6) [*n* = 21]	2.8 (2.2–3.6) [*n* = 168]	2.6 (1.9–3.1) [*n* = 47]	.2	.3	.03	.07	.04

NOTE. CI, confidence interval; IQR, interquartile range; NA, not applicable; OR, odds ratio.

[a] Obtained from mothers with infants ≤10 days old.

[b] Maximum level during infant's first 3 months of life.