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Nevirapine Resistance Viral Mutations After Repeat Use of Nevirapine for Prevention of Perinatal HIV Transmission

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To the Editors:

Single-dose nevirapine (sdNVP) for prevention of perinatal HIV transmission when used for the first time selects viral mutations associated with resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs).¹ Selection of genotypic resistance is more common if maternal viral load is high, CD4 count is low, tends to be related to viral subtype, and can generally be detected even if other short-course prophylaxis drug regimens are given as well.^{2,3} However, the in vivo consequence of repeat use of sdNVP for selection of genotypic resistance has not been described. Repeat use may occur in a single pregnancy in the case of false labor or, more importantly, in subsequent pregnancy among women who previously received nevirapine (NVP) prophylaxis in a prior pregnancy. In either case the development of genotypic resistance may compromise the efficacy of sdNVP prophylaxis to reduce transmission, and it has important policy implications for this widely implemented public health practice.⁴ In the setting of a prospective study, we assessed whether repeat use of sdNVP is associated with increased detection of NNRTI resistance mutations among HIV-positive women after delivery.

Blood samples collected 6 weeks after delivery were selected from 47 HIV-positive women followed as part of the Zambia Exclusive Breast-feeding Study⁵ conducted in Lusaka, Zambia. Twenty-three women were identified as repeat users. Fifteen of these were known to have taken sdNVP in their prior pregnancy and received it for a second time in the index pregnancy (the prior pregnancy for 12/15 women occurred while enrolled in the Zambia Exclusive Breast-feeding Study, the others occurred in an earlier study at the same site). The mean time between the prior delivery and the index delivery was 18 months (median 16 months, range 11–27). Eight women were identified who consumed two 200-mg NVP tablets before delivery, in all cases due to false labor. The median interval between the 2 doses was 2 days. A similar number (n = 24) of women who took NVP only once for the first time in the index pregnancy were selected for comparison with the repeat users. These women were randomly selected from the cohort matched as closely as possible to the repeat users by baseline viral load.

HIV RNA was extracted and amplified from stored plasma samples (2/49 selected samples could not be amplified). Codons 1 through 335 of the reverse transcriptase (RT) gene were sequenced using the ViroSeq HIV-1 Genotyping system version 2.0 (Applied Biosystems, Foster City, CA), analyzed on a genetic analyzer (model 3100, ABI PRISM), and assembled

with the use of version 2.5 of the ViroSeq HIV-1 Genotyping system for the mutations associated with resistance to NNRTI. All samples were subtype C except one that was subtype G based on *pol* sequence. CD4 counts (FACSCount system, BD Immunocytometry Systems, San Jose, CA) and viral load (Roche Amplicor version 1.5, Roche, Branchburg, NJ) were measured at baseline during the index pregnancy (median 10 weeks before delivery).

RT mutations associated with high- or intermediate-level of NVP resistance were detected among 14 (29.8%) of 47 maternal samples tested. The prevalence of resistance mutations was similar regardless of prior history of NVP exposure. Genotypic resistance was detected among 33.3% of women taking sdNVP for the first time, 25.0% of women taking 2 doses in a single pregnancy, and 26.7% of women taking it for a second time within a subsequent pregnancy ($P = .86$, Table 1). The most common resistance mutations were K103N(8/47) and V106A/M (6/47, a mutation that seems to be commonly selected among subtype C-infected populations⁶), and the distribution of specific mutations was similar between the groups. There were no significant differences between the groups in time of blood sampling for resistance testing (mean 43 days post-delivery among first time users, 46 days among repeat users in the same pregnancy, and 40 days among repeat users in a second pregnancy).

Baseline viral load and CD4 count measurements were not significantly different between the groups. The mean HIV RNA copy number (CD4 count) was 4.50 (340) among first-time users, 4.70 (418) among repeat users in the same pregnancy, and 4.18 (316) among repeat users in a second pregnancy. After adjustment for baseline viral load, CD4 count, and time of blood sampling in a multivariable logistic regression model, there continued to be no increased detection of intermediate- or high-level NVP resistance in second pregnancies relative to first pregnancies [odds ratio (OR) = 1.06; 95% confidence interval (CI) 0.2-5.2].

The mutations associated with intermediate- and high-level resistance to NVP were strongly correlated with baseline viral load, and there was a trend, albeit weaker, in the same direction with low CD4 count (Table 1). Each log increase of baseline viral load was associated with increased likelihood of genotypic NVP resistance (OR 4.00; 95% CI 1.1-15.2). A relatively common variant was K166R (8/47), which has not been associated with NNRTI resistance and may not be related to sdNVP exposure; its detection was not correlated with baseline viral load or CD4 count.

Our preliminary data suggest that there is no increase in the detection of viral mutations associated with NNRTI resistance when sdNVP is taken for a second time in a subsequent pregnancy. Although we were able to assemble a unique population of repeat users to compare to first-time users matched by viral load, sample size is small, and the results should be confirmed in larger studies. Because our data are based on population sequencing of detectable resistance mutations at 6 weeks post-delivery, we may have missed other possible changes in the dynamics of viral resistance. Sequential measurements over time and quantification of low-level mutants may provide a more sensitive indicator of the development of resistance mutations with repeat use of NVP. The strong correlation we observed between viral load and genotypic resistance also cautions against uncritical comparisons across studies because the proportions of women with high viral loads may differ substantially between study populations.

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TABLE 1

HIV-1 RNA RT Mutations in Samples Collected 6 Weeks After Delivery From 47 HIV-Positive Women in Lusaka, Zambia Taking sdNVP for Prevention of Perinatal HIV Transmission

	N	n (%) of Samples With any RT Mutation [*]	n (%) of Samples With High/Intermediate NVP Resistance Mutations [*]	n (%) of Samples With K103N Mutations
Repeat use	—	—	—	—
sdNVP used twice in prior pregnancy and in index pregnancy	15	7(46.7%) K166R (3), K103N (2), V106A (2), Y181C , Y188C , K101Q, K101E	4 (26.7)	2 (13.3)
Used twice in index pregnancy (but not before)	8	3(37.5%) K103N (2), G196E	2 (25.0)	2 (25.0)
Nonrepeat use	—	—	—	—
sdNVP used once for the first time in the index pregnancy	24	13 (54.2%) K166R (5), K103N (4), V106A (2), V106M (2), Y181C (2), V179D, G190A	8 (33.3)	4 (16.7)
Maternal baseline HIV RNA <10,000 copies/mL V179D, G196E	11	5 (45.5) K166R (2), V106A , Y181C , K101E, K101Q,	2 (18.2)	0
10,000-50,000	20	8 (40.0) K166R (5), K103N , V106A (3), Y188C	3 (15.0)	1 (5.0)
>50,000	16	10 (62.5) K166R, K103N (7), V106M (2), Y181C (2), G190A	9 (56.3) **	7 (43.8) **
Maternal baseline CD4 count	—	—	—	—
<200	10	7(70.0) K166R (2), K103N (5), Y181C (2), G190A	5 (50.0)	5 (50.0)
≥200	37	16(43.2) K166R (6), K103N (3), V106A (4), V106M (2), Y181C , Y188C , V179D, K101E, K101Q, G196E	9 (24.3)	3 (8.1) **

* The specific mutations detected in each group are shown. The number in parentheses indicated the number of samples in which the mutation was detected (if there is no number, it was detected only once). Eight samples had 2 mutations detected. Mutations associated with intermediate- and high-level NVP resistance defined based on Stanford HIV Drug Resistance Database are in bold.

** $\chi^2 P < .05$