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Short Communication: *In Utero* HIV Infection Is Associated with an Increased Risk of Nevirapine Resistance in Ugandan Infants Who Were Exposed to Perinatal Single Dose Nevirapine

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Abstract

Use of single dose nevirapine (sdNVP) to prevent HIV mother-to-child transmission is associated with the emergence of NVP resistance in many infants who are HIV infected despite prophylaxis. We combined results from four clinical trials to analyze predictors of NVP resistance in sdNVP-exposed Ugandan infants. Samples were tested with the ViroSeq HIV Genotyping System and a sensitive point mutation assay (LigAmp, for detection of K103N, Y181C, and G190A). NVP resistance was detected at 6–8 weeks in 36 (45.0%) of 80 infants using ViroSeq and 33 (45.8%) of 72 infants using LigAmp. NVP resistance was more frequent among infants who were infected *in utero* than among infants who were diagnosed with HIV infection after birth by 6–8 weeks of age. Detection of NVP resistance at 6–8 weeks was not associated with HIV subtype (A vs. D), pre-NVP maternal viral load or CD4 cell count, infant viral load at 6–8 weeks, or infant sex. NVP resistance was still detected in some infants 6–12 months after sdNVP exposure. In this study, *in utero* HIV infection was the only factor associated with detection of NVP resistance in infants 6–8 weeks after sdNVP exposure.

Single-dose nevirapine (sdNVP) is used to prevent mother-to-child transmission (MTCT) of HIV in resource-limited settings.[1] Unfortunately, NVP resistance emerges in many infants who are HIV infected despite sdNVP prophylaxis.[2–5] In women, emergence of NVP resistance after sdNVP has been associated with high viral load, low CD4 cell count, HIV subtype (C > D > A), and increased NVP exposure (e.g., decreased oral clearance).[4] In previous studies, emergence of NVP resistance in infants was associated with high maternal viral load[3] and HIV subtype (C > A and D combined).[4] There are limited data on persistence of NVP resistance in infants after sdNVP.[2,3,5] In one study, NVP resistance persisted in 13/19 infants at 6 months, 4/8 infants at 12 months, and 1/2 infants tested 18 months after sdNVP.[3]

Analysis of factors associated with NVP resistance in sdNVP-exposed infants is often limited by the small number of infants who are HIV infected in a single study. In this report, we pooled data from four clinical studies conducted in Kampala, Uganda to analyze emergence and persistence of NVP resistance in sdNVP-exposed infants who were HIV infected by 6–8 weeks of age (Table 1). Guidelines of the U.S. Department of Health and Human Services and the authors' institutions were followed in the conduct of this research. Informed consent was obtained from all subjects for

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Table 1. Detection of NVP Resistance at 6–8 Weeks among Infants Who Were HIV Infected *In Utero* vs. Infants Who Were Diagnosed with HIV Infection at 6 Weeks: Four Studies, Uganda[a]

	HIVNET 012[b]	*Repeat pregnancy*	*Breast feeding*	*SWEN*	*Total*[c]
Number infected by 6–8 weeks	36	19	18	36	109
Number with samples available	24	17	17	24	82
ViroSeq results[d]					
Number with resistance/number with results (%)	11/24 (45.8)	6/17 (35.3)	7/15 (46.7)	12/24 (50.0)	36/80 (45.0)[e]
Number with resistance/number infected at birth (%)	10/19 (52.6)	5/11 (45.5)	6/10 (60.0)	7/10 (70.0)	28/50 (56.0)
Number with resistance/number uninfected at birth (%)	1/5 (20.0)	1/6 (16.7)	1/5 (20.0)	5/14 (35.7)	8/30 (26.7)
p value[f]	0.327	0.333	0.282	0.214	0.006
LigAmp results[g]					
Number with resistance/number with results (%)	15/23 (65.2)	3/15 (20.0)	8/14 (57.1)	7/20 (35.0)	33/72 (45.8)
Number with resistance/number infected at birth (%)	13/18 (72.2)	3/11 (27.3)	6/10 (60.0)	5/9 (55.6)	27/48 (56.3)
Number with resistance/number uninfected at birth (%)	2/5 (40.0)	0/4 (0.0)	2/4 (50.0)	2/11 (18.2)	6/24 (25.0)
p value[f]	0.297	0.517	1.0	0.16	0.022

[a]Infants were enrolled in four studies; dates of enrollment are shown: HIVNET 012 (1997–1999, sdNVP arm only),[1] the Repeat Pregnancy study (2004–2006, prospective portion only),[13] the Pathophysiology of Breast Milk study (2003–2004), and the Ugandan component of the SWEN study (2004–2006, sdNVP arm only).[14]

[b]This includes one set of twins; one infant had Y181C detected at 6–8 weeks; the other infant did not have any NVP resistance mutations detected at 6–8 weeks.

[c]Seventy-two infants had test results from both ViroSeq and LigAmp; 39 of those 72 infants had at least one NVP resistance mutation (six infants had resistance mutations detected by ViroSeq only, seven infants had resistance detected by LigAmp only, and 26 infants had resistance detected by both assays).

[d]Resistance detected by ViroSeq indicates detection of one or more NVP resistance mutation (A98G, L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, Y188C/H/L, G190A/S/C/E/Q/T/V, M230L, K103R + V179D). ViroSeq may not detect mutations present in minor virus subpopulations.

[e]There was no significant difference in the proportion of infants with NVP resistance detected by ViroSeq in the four individual studies ($p = 0.822$).

[f]p values for the association of resistance and timing of infection in individual studies were determined using Fisher's exact test. The overall p values for the association between HIV infection timing and resistance in the four studies (total) were determined using the Mantel–Haenszel test, stratified by study. The common OR for ViroSeq was 4.6 (95% CI: 1.5–13.6) and the common OR for LigAmp was 4.0 (95% CI: 1.2–13.1).

[g]Resistance detected by LigAmp indicates detection of one or more of the following mutations: K103N (AAC), Y181C (TGT), and G190A (GCA).

participation in the studies. Each study was approved by the Institutional Review Boards (IRB) in Uganda. In addition, IRB approval was obtained from the Johns Hopkins University School of Medicine (JHU) and/or the U.S. Centers for Disease Control and Prevention IRB.

In the four studies analyzed, 109 infants who received sdNVP were diagnosed with HIV infection by 6–8 weeks of age (Table 1); none of the mothers or infants received any other antiretroviral (ARV) drugs. Eighty-two of the 109 infants had a plasma sample available for resistance studies. HIV genotyping was performed with the ViroSeq HIV Genotyping System v2.6,[2] and HIV subtypes were determined by phylogenetic analysis of HIV *pol* sequences.

Independent sample chi-square tests and Fisher's exact tests were used to evaluate the association of NVP resistance at 6–8 weeks with *in utero* HIV infection (diagnosis of HIV infection at birth), infant viral load at 6–8 weeks, infant sex, HIV subtype, pre-NVP maternal viral load, and CD4 cell count. Odds ratio (OR) estimates and 95% confidence intervals (CI) for these variables were obtained using logistic regression. A Mantel–Haenszel OR for timing of HIV infection as a predictor for resistance was also estimated, treating each study as a stratum. McNemar's test for matched pairs was used to compare the difference in detection of NVP resistance between ViroSeq and LigAmp.

ViroSeq results were obtained for 80 (97.5%) of the 82 infants who had plasma samples available from 6 to 8 weeks of age. Thirty-six (45.0%) of the 80 infants had at least one NVP resistance mutation detected; the mutations identified were Y181C ($n = 28$), K103N ($n = 9$), Y188C ($n = 3$), G190A ($n = 30$), V106A ($n = 2$), V106M ($n = 2$), and K101E ($n = 1$); 10 infants had two or more NVP resistance mutations detected. The HIV subtypes of the infants were A ($n = 41$), C ($n = 4$), D ($n = 24$), and intersubtype recombinant HIV ($n = 11$). The mean pre-NVP maternal $\log_{10}$ viral load and mean pre-NVP maternal CD4 cell count were similar for the 80 women whose infants had ViroSeq resistance results vs. the 29 women whose infants did not ($p = 0.45$ and $p = 0.96$, respectively; 11/109 women were missing viral load data, 5/109 women were missing CD4 cell count data).

Table 2. Analysis of Factors Associated with Detection of NVP Resistance in Infants at 6–8 Weeks of Age[a]: Four Studies, Uganda

	Resistance detected by ViroSeq[b]			*Resistance detected by LigAmp*[c]		
Predictor variable	N	*Odds ratio (95% CI)*	p value	N	*Odds ratio (95% CI)*	p value
Maternal pre-NVP viral load (per $\log_{10}$ increase in HIV RNA)	71	1.06 (0.5–2.3)	0.89	64	0.98 (0.4–2.2)	0.95
Maternal pre-NVP CD4 cell count (per decrease of 100 cells/μl)	77	1.15 (0.9–1.4)	0.20	69	1.15 (0.9–1.4)	0.21
Infant viral load at 6–8 weeks (per $\log_{10}$ increase in HIV RNA)[d]	41	1.65 (0.7–4.1)	0.28	37	1.49 (0.6–4.7)	0.21
HIV subtype (D vs. A)[e]	65	1.51 (0.6–4.2)	0.42	62	1.15 (0.4–3.2)	0.79
HIV subtype (D vs. non-D)[e]	80	1.70 (0.7–4.4)	0.28	72	1.45 (0.5–3.9)	0.46
Infant sex (male vs. female)	80	0.50 (0.2–1.2)	0.13	72	0.65 (0.3–1.7)	0.37
Diagnosed with HIV infection at birth (yes/no)	80	3.50 (1.3–9.4)	0.013	72	3.90 (1.3–11.4)	0.015

[a]Univariate logistic regression models were used for analysis. Infants were enrolled in four studies (see Table 1).[b]Resistance detected by ViroSeq indicates detection of one or more NVP resistance mutation (A98G, L100I, K101E/P, K103N/S, V106A/M, Y181C/I/V, Y188C/H/L, G190A/S/C/E/Q/T/V, M230L, K103R + V179D).

[c]LigAmp testing was performed to detect K103N, Y181C, and G190A; resistance detected by LigAmp indicates detection of one or more of these three mutations.

[d]Viral load testing was performed at 6–8 weeks for infants in HIVNET 012, the Breast Feeding study, and the SWEN study; viral load testing was not performed at 6–8 weeks for infants in the Repeat Pregnancy study.

[e]Among the 80 infants who had HIV subtype data, 41 had subtype A, 4 had subtype C, 24 had subtype D, and 11 had intersubtype recombinant HIV.

For samples with subtype A or D HIV, PCR products produced in the ViroSeq system were also tested using the LigAmp assay (assay cutoffs for mutation detection: 0.5% for K103N, 1.0% for Y181C, 0.5% for G190A).[2,5,6] LigAmp results were obtained for 72 (90%) of the 80 infants who had ViroSeq results; the remaining infants either had subtypes other than A or D (not tested) or did not have PCR products remaining for testing. The proportion of infants who had K103N, Y181C, or G190A detected by LigAmp (33/72 = 45.8%) was similar to the proportion of infants who had resistance detected by ViroSeq (36/80 = 45.0%, $p = 0.563$). The two assays detected Y181C in a similar proportion of infants (LigAmp: 40.3%, ViroSeq: 35.0%, $p = 0.157$). In contrast, LigAmp detected K103N and G190A in a higher proportion of infants than ViroSeq (K103N: 23.6% vs. 11.3%, $p = 0.021$; G190A: 20.8% vs. 3.8%, $p = 0.0003$). The median levels of the mutations (% of the viral population) were Y181C: 19.8% (range: 1.4–90.6%), K103N: 3.5% (range: 0.5–100%), and G190A: 2.2% (range: 0.7–19.6%). In 4 of 72 samples, mutations were detected by ViroSeq, but not by LigAmp, due to alternate codon use or mismatches in the oligonucleotide binding region. The proportion of infants who had more than one mutation detected was higher when LigAmp was used for testing (LigAmp: 26.4%, ViroSeq: 12.5%, $p = 0.0016$).

Infants who were infected *in utero* were more likely to have resistance detected at 6–8 weeks, compared to infants who were diagnosed with HIV infection after birth by 6–8 weeks of age (Tables 1 and 2). A similar trend (association of *in utero* infection with resistance) was observed in each of the four individual studies, but the association was not statistically significant, most likely due to the small number of HIV-infected infants in each study (Table 1). We did not see an association of NVP resistance with maternal pre-NVP viral load or pre-NVP CD4 cell count, infant viral load at 6–8 weeks, HIV subtype (for A vs. D or D vs. non-D), or infant sex (Table 2); there was also no association of HIV subtype with resistance among the subsets of infants who were HIV infected *in utero* or were diagnosed at 6 weeks of age (data not shown). However, when the infants were stratified by both time of infection and HIV subtype, the number of infants in each subset was small. Among infants who were HIV uninfected at birth, but were infected by 6–8 weeks of age, about one in four had NVP resistance detected at 6–8 weeks of age (26.7% with ViroSeq, 25% with LigAmp, Table 1). These infants could have acquired NVP resistance through transmission of NVP-resistant HIV during breast-feeding, or through selection of NVP-resistant HIV in infants after infection with an NVP-susceptible strain; emergence of NVP resistance by either mechanism would be facilitated by the long half-life of NVP in infants.[7]

Overall, 43 infants who were diagnosed with HIV infection by 6–8 weeks of age had NVP resistance detected by ViroSeq and/or LigAmp at 6–8 weeks. Thirty-four of those infants had a plasma sample collected at either 6 months of age (in the Repeat Pregnancy study, Breast Feeding study, and the SWEN study) or at 12 months of age (in HIVNET 012). We analyzed persistence of NVP resistance in 27 of the 34 infants (19 infants at 6 months and eight additional infants at 12 months); seven infants were excluded from this analysis because they were started on ARV therapy before the 6–month study visit. At 6 months, NVP resistance was detected by either ViroSeq or LigAmp in 12 (63.2%) of 19 infants tested. Eight infants had mutations detected by ViroSeq [Y181C ($n = 4$), K103N ($n = 1$), V106M ($n = 1$), Y188C ($n = 1$), and V179D + K103R ($n = 1$)], and four infants had resistance detected by LigAmp only (all with Y181C, at 1.2%, 1.4%, 3.5%, and 7.8%; one infant also had G190A at 1.9%). At 12 months, NVP resistance was detected in four (50%) of eight

infants tested. Two (25%) infants had resistance detected by both ViroSeq and LigAmp (one with G190A and one with Y181C) and two infants had resistance mutations detected by LigAmp only (both with Y181C, at 1.7% and 4.7%). The proportion of infants with resistance at 6 or 12 months was higher among those with subtype D HIV than among those with subtype A HIV ($9/10 = 90\%$ vs. $6/11 = 54.5\%$) and was higher among those with *in utero* than among infants who were HIV uninfected at birth ($11/15 = 73.3\%$ vs. $5/12 = 41.7\%$); however, those differences were not statistically significant ($p = 0.15$ for subtype A vs. D, $p = 0.13$ for *in utero* vs. postnatal infection), possibly because of the small number of 6–12 month samples available for analysis. Most of the infants who had resistance detected at 6 or 12 months had Y181C. This is surprising, since Y181C fades from detection rapidly in Ugandan women after sdNVP, particularly among those with subtype A infection.[4]

In other studies, *in utero* HIV infection was associated with high maternal viral load, infant sex (female), and low birth weight.[8,9] High maternal HIV viral load was also associated with NVP resistance after sdNVP in a study of 42 HIV-infected infants in South Africa ($p = 0.04$).[3] In this study, we did not find an association of maternal viral load or infant sex with NVP resistance. We also found no association of NVP resistance with maternal CD4 cell count or HIV subtype (A vs. D); both of those factors, as well as maternal viral load, have been shown to influence the emergence of NVP resistance in women after sdNVP exposure.[4] Even though this study included a large number of HIV-infected infants ($n = 80$), it is still smaller than many individual studies of HIV-infected women; this may have limited our power to detect an association of resistance with these factors.

In most resource-poor settings, first-line regimens for treatment of HIV-infected children include a nonnucleoside reverse transcriptase inhibitor (NNRTI). In the CHER study, initiation of ARV treatment by 3 months of age reduced infant mortality by 75%.[10] Therefore, many sdNVP-exposed infants may begin ARV therapy before NVP-resistant variants have time to fade. In one study, when treatment with an NVP-containing regimen was initiated at a median of 8–9 months of age, 76.9% of sdNVP-exposed infants had virologic failure compared to only 9.1% of sdNVP-unexposed infants.[11] However, in another study, the virologic response to an NVP-containing regimen was similar among sdNVP-exposed children (median age 1.7 years) versus sdNVP-unexposed children (median age 7.8 years).[12] Further studies are needed to evaluate the relationship between the timing of HIV MTCT, the emergence and persistence of NVP resistance, and ARV treatment response.

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Disclosure Statement

None of the authors has a commercial or other association that might pose a conflict of interest with the following exception: Dr. Susan Eshleman is a co-inventor of the LigAmp assay and Johns Hopkins University has filed a patent application with the U.S.-Patent and Trademark Office. The inventors may receive royalty payments if the patent is awarded and licensed.

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