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Antiretroviral Resistance among HIV Type 1-Infected Women First Exposed to Antiretrovirals during Pregnancy: Plasma versus PBMCs

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Abstract

Resistance-associated mutations (RAMs) in plasma samples from HIV-1-infected women who received antiretroviral (ARV) prophylaxis during pregnancy was assessed and correlated with the detection of RAMs in peripheral blood mononuclear cells (PMBCs). The study population was composed of HIV-1-infected women enrolled in a prospective cohort study in Latin America and the Caribbean (NISDI Perinatal Study) as of March 1, 2005, who were diagnosed with HIV-1 infection during the current pregnancy, who received ARVs during pregnancy for prevention of mother-to-child transmission of HIV-1, and who were followed through at least the 6–12 week postpartum visit. Plasma samples collected at enrollment during pregnancy and at 6–12 weeks postpartum were assayed for RAMs. Plasma results were compared to previously described PBMC results from the same study population. Of 819 enrolled subjects, 197 met the eligibility criteria. Nucleic acid amplification was accomplished in 123 plasma samples at enrollment or 6–12 weeks postpartum, and RAMs were detected in 22 (17.9%; 95%CI: 11.7–25.9%). Previous analyses had demonstrated detection of RAMs in PBMCs in 19 (16.1%). There was high concordance between RAMs detected in plasma and PBMC samples, with only eight discordant pairs. The prevalence of RAMs among these pregnant, HIV-1-infected women is high (>15%). Rates of detection of RAMs in plasma and PBMC samples were similar.

Introduction

One of the most successful strategies to prevent transmission of HIV-1 is the prevention of mother-to-child transmission (MTCT) through the use of antiretrovirals (ARVs). ARV prophylaxis of MTCT of HIV-1 has proven efficacy,[1–3] and has become a routine part of the management of HIV-1-infected women in many countries.[4,5] However, the increase in the prevalence of HIV-1 resistance to ARVs and the transmission of this resistance to new hosts could limit the clinical effectiveness of both ARV prophylaxis, during the index and future pregnancies, and future ARV therapy.[6]

Resistance testing is usually performed with plasma specimens to detect resistance-associated mutations (RAMs) in circulating viruses. However, the presence of archived RAMs in proviral DNA from peripheral blood mononuclear cells (PBMCs) also has been proposed.[7]

We previously described RAMs detected in PBMC samples[8] from HIV-1-infected women receiving ARVs for prevention of MTCT who were enrolled in the National Institute of Child Health and Human Development (NICHD) International Site Development Initiative (NISDI) Perinatal Study at multiple sites in Latin America and the Caribbean.[9] In this analysis, we describe RAMs detected in plasma samples from these women, and compare these results to those obtained from PBMC samples in the same study population.[8]

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Materials and Methods

NISDI perinatal protocol

The NISDI Perinatal Study is a prospective cohort study being conducted in Latin American and Caribbean countries.[9] Enrollment began in September 2002 and is ongoing. Maternal study visits are conducted during pregnancy, at delivery, at hospital discharge following delivery, and at 6–12 weeks and 6 months postpartum. During each of these study visits, a medical history is obtained, a physical examination is conducted, and laboratory samples are obtained (except at the delivery and the 6 month postpartum visits). Therefore, clinical, immunologic, and virologic characteristics of the women are assessed during pregnancy, at the time of hospital discharge following delivery, and at the 6–12 week postpartum visit. Signed informed consent is obtained for all subjects prior to enrollment into the study. The protocol was approved by the ethical review board at each clinical site where subjects were enrolled, as well as by institutional review boards at the sponsoring institution (NICHD) and at the data management center (Westat).

Definitions and study population for this analysis

Subjects enrolled in the NISDI Perinatal Study were classified as having received ARV prophylaxis if they were not receiving ARVs when they became pregnant, but then initiated one or more ARV drugs during pregnancy and discontinued these drugs at or before the 6–12 week postpartum visit. Conversely, women were classified as receiving ARV treatment if they initiated ARVs prior to the index pregnancy and/or continued ARV drugs after the 6–12 week postpartum visit. The most complex ARV regimen received during pregnancy for 28 days or more was categorized as follows: none, one nucleoside/nucleotide analogue reverse transcriptase inhibitor (NRTI) only; two NRTIs, two NRTIs with one nonnucleoside reverse transcriptase inhibitor (NNRTI); two NRTIs with one protease inhibitor (PI); or other. The inclusion criteria for this analysis were enrollment in the NISDI Perinatal Study as of March 1, 2005, known to have been diagnosed with HIV-1 infection during the current pregnancy, received ARV prophylaxis during pregnancy, and were followed through at least the 6–12 week postpartum visit.

Laboratory analyses

All available plasma samples collected at baseline and/or at 6–12 weeks postpartum for subjects eligible for inclusion in these analyses were assayed for the presence of RAMs. Plasma samples were assayed with the ViroSeq HIV-1 genotyping system v 2.6, Celera Diagnostics, at the Molecular Virology Laboratory of the Instituto Nacional de Ciencias Médicas y Nutricion Salvador Zubiran in México City, according to the manufacturer's specifications.

The identification of RAMs was based on recommendations from the International AIDS Society-USA (IAS-USA) Drug Resistance Mutations Group, a panel of experts focused on identifying key HIV-1 drug resistance mutations.[10] Only those RAMs described as major mutations were assessed in this analysis.

Statistical analysis

Genotypic resistance was assessed at baseline and at the 6–12 week postpartum visit, with the number and percentage of subjects with RAMs identified singly or in combination reported overall and according to subject characteristics. The association of RAMs with subject characteristics was evaluated using the Fisher–Freeman–Halton[11]exact test. Because the number of RAMs detected was relatively small, there was no attempt to model the risk of RAMs as a function of subject characteristics. The kappa statistic, an index that compares the observed agreement against that expected by chance, was used to compare results obtained from PBMCs to plasma specimens.[12] Kappa takes on a value of +1 if there is perfect agreement, with values below 0 indicating observed agreement is less than chance agreement; a value of 0 indicates no agreement above that expected by chance alone.

Results

Study population

Of 819 women enrolled in the NISDI Perinatal Study at clinical sites in Argentina, the Bahamas, Brazil, and Mexico as of March 1, 2005, 197 met inclusion criteria for this analysis. Forty-three (21.7%) were not receiving ARVs at the time of enrollment and 154 (78.3%) initiated ARVs during the current pregnancy, before enrollment into the study. Among those who were ARV-exposed at enrollment, the median duration of receipt of ARVs (from the date of initiation of ARVs through the date of enrollment into the study) was 7.1 weeks.

Characteristics of the study population, overall and according to the timing of initiation of ARVs, have been described previously.[9] Briefly, 62% had plasma HIV-1 RNA concentrations below 1000 copies/ml at enrollment. By 6–12 weeks postpartum, 27% had plasma viral loads <1000 copies/ml. Most (53%) had $CD4^+$ counts ≥ 500 cells/mm^3 at enrollment compared to 64% at 6–12 weeks. A majority (81%) of the women received a three-drug combination ARV regimen during pregnancy (two NRTIs + one PI, or two NRTIs + one NNRTI), and most (76%) received only one ARV regimen during pregnancy. Women received ARVs for a mean of 4.2 days following delivery (standard deviation $\pm$ 8.7 days). The range of duration of receipt of ARVs following delivery was 0–47 days.

RAMs in plasma samples

Among the 197 eligible subjects, plasma samples were available at enrollment from 191 and at 6–12 weeks postpartum from 186 (Table 1). Samples from 74 subjects could not be amplified from plasma at either time point (136 at enrollment and 89 at 6–12 weeks postpartum) and, among the 74, most (83%, 60/74) had plasma viral load values at enrollment below 1000 copies/ml. The association between viral load and whether or not resistance testing could be performed was statistically significant ($p < 0.0001$) (data not shown). Of the 123 plasma samples where amplification was possible at either time point, RAMs were observed in 22 [17.9%; 95% confidence interval (CI) 11.7–25.9%] subjects [seven (12.7%; 95% CI 5.2–24.6%) at enrollment and 16 (16.5%; 95% CI 9.8–25.5%) at 6–12 weeks postpartum] (Table 1).

Table 2 shows the specific RAMS detected in plasma specimens at enrollment and at 6–12 weeks postpartum. The most common mutations found were K70R, M184V, K103N, and

Table 1. Drug Resistance-Associated Mutations in Plasma Specimens According to Study Visit ($N = 198$)

	Subjects		
Samples and RAMs	*Enrollment*	*6–12 weeks postpartum*	*Either time point*
Available samples	191	186	197
Could not be amplified	136	89	74
Could be amplified	55	97	123
RAMs detected: *n* (%)	7 (12.7)[a]	16 (16.5)	22 (17.9)
(95% CI)	(5.2–24.6)	(9.8–25.5)	(11.7–25.9)
RAMs not detected: *n* (%)	48 (87.3)[b]	81 (83.5)	101 (82.1)

[a]Three specimens obtained before receipt of ARVs, four after initiating ARVs.
[b]Twenty-one specimens obtained before ARVs, 27 after initiating ARVs.

M46I. Table 3 describes individual subjects with RAMs identified at enrollment and/or at 6–12 weeks postpartum in plasma or PBMC specimens. Information is provided for each subject regarding ARV regimens received during pregnancy, $CD4^+$ and viral load testing, and RAMs in plasma and in PBMCs. Of the 16 subjects with RAMs detected in plasma samples at 6–12 weeks postpartum, only one (Subject 14) had a plasma sample from enrollment that could be amplified. In this subject, the same RAMs were detected at each of the two time points.

RAMs and clinical characteristics

The occurrence of RAMs was not associated with clinical or immunological disease stage, or with plasma viral load, at either time point ($p > 0.1$). The occurrence of RAMs varied according to ARV regimen [no ARVs, 0/10 (0%) had RAMs detected; one NRTI only, 2/15 (13.3%); two NRTIs, 5/15 (33.3%); two NRTIs with one NNRTI, 8/31 (25.8%); two NRTIs with one PI, 7/52 (13.5%)], but the association between ARV regimen and RAMs was not statistically significant ($p = 0.15$).

Comparison of RAMS from plasma versus PBMC samples

RAMs were observed in PBMC samples from 19 of 198 eligible subjects (16.1%) at either enrollment or 6–12 weeks postpartum, from 11 (14.5%) subjects at enrollment, and from 14 subjects (14.4%) at 6–12 weeks postpartum[8] (Table 3). Of 25 samples with successful amplification in both plasma and PBMCs at enrollment, 23 sample pairs were concordant (21 without RAMs and 2 with RAMs) while 2 were discordant (one with RAMs in plasma only and one with RAMs in PBMCs only) (kappa = 0.62; 95% CI: 0.23–1.00) (Table 4). At 6–12 weeks postpartum, 57 samples could be amplified in both plasma and PBMC specimens; 51 sample pairs were

Table 2. Resistance-Associated Mutations by Gene Location and Study Visit for Plasma Specimens

	Patients with RAMs, n *(%)*	
Resistance-associated mutation	*Enrollment (N = 55)*	*6–12 weeks (N = 97)*
Reverse transcriptase		
M184V	1 (1.8)	5 (5.2)
M41L	1 (1.8)	2 (2.1)
D67N	0	1 (1.0)
K70R	2 (3.6)	5 (5.2)
L210W	1 (1.8)	0
K219Q	0	1 (1.0)
V75I	0	0
K103N	1 (1.8)	3 (3.1)
V118I	2 (3.6)	1 (1.0)
G190A	0	0
E44D	1 (1.8)	0
V118I	1 (1.8)	0
V179D	0	2 (2.1)
Protease		
D30N	1 (1.8)	1 (1.0)
L33F	0	0
M46I	2 (3.6)	1 (1.0)
V82A	0	1 (1.0)

Table 3. Individual Subjects with Resistance-Associated Mutations (RAMs) Identified at Enrollment and/or at 6–12 weeks Postpartum in Plasma or PBMC Specimens

		ARV regimens during pregnancy				*CD4 and viral load testing*			*RAMs—plasma specimens*		*RAMs—PBMC specimens*	
Subject	*Delivery*[a]	*ARV regimen*[b]	*Start*[a]	*Stop*[a]	*Duration (days)*	*Visit (timing)*[c]	*CD4*$^+$ *cnt/%*	*Viral load (copies/mL)*	*Enroll*[d]	*6–12 weeks*[d]	*Enroll*[d]	*6–12 weeks*[d]
1	38.4	None	0.1	13.7	96	Enrl (37.4)	418/40	4,685	NT	K70R	NT	NT
		3TC, NVP, ZDV	13.9	39.3	179	HD (38.6)	846/45					
		None	39.3	48.7	67	6–12 (48.7)	729/34	3,140				
2	38.0	None	0.1	25.7	180	Enrl (32.0)	348/30	646	NT	K70R	NT	K70R
		3TC, NVP, ZDV	25.9	38.9	92	HD (38.1)	503/24	228				
		None	38.9	44.0	37	6–12 (44.0)	446/18	61,115				
3	39.1	None	0.1	23.7	166	Enrl (33.0)	261/51	82	NT	K70R	K70R	NT
		3TC, NVP, ZDV	23.9	39.1	108	HD (40.0)	238/50					
		None	39.1	47.0	56	6–12 (47.0)	156/22					
4	41.0	None	0.1	27.3	191	Enrl (34.0)	554/41	400	NT	M184V	*None*	*None*
		3TC, ZDV	27.4	31.0	26	HD (41.3)	516/40	400				
		3TC, NVP, ZDV	31.1	43.0	84	6–12 (47.9)	473/29	11,400				
		3TC, ZDV[e]	43.1	43.4	3							
		None	43.4	47.9	32							
5	38.3	None	0.1	20.1	141	Enrl (36.0)	430/38	6,760	K70R	*None*	NT	*None*
		3TC, ZDV	20.3	35.0	104	HD (38.7)	677/41	508				
		3TC, NVP, ZDV	35.1	41.0	42	6–12 (45.9)	535/36	11,800				
		3TC, ZDV[e]	41.1	41.4	3							
		None	41.4	45.9	32							
6	40.3	None	0.1	31.3	219	Enrl (34.0)	507/31	478	NT	K103N	NT	*None*
		ZDV	31.4	32.1	6	HD (40.7)	623/35					
		3TC, NVP, ZDV	32.3	43.1	77	6–12 (49.9)	616/34	16,400				
		None	43.1	49.9	48							
7	37.7	None	0.1	27.0	189	Enrl (36.0)	327/25	3,670	NT	K103N	K70R	*None*
		ZDV	27.1	37.1	71	HD (38.1)	447/25					
		3TC, NVP, ZDV	37.3	40.0	20	6–12 (43.9)	524/32	4,710				
		3TC, ZDV[e]	40.1	40.4	3							
		None	40.4	43.9	25							
8	40.0	None	0.1	26.0	182	Enrl (23.0)	858/42	198,000	K70R	NT	K70R, M46I	K70R, D30N
		3TC, NVP, ZDV	26.1	43.0	119	Ante (34.0)	772/59	950				
		NVP[e]	43.1	43.4	3	HD (40.3)	879/55					
		None	43.4	45.9	18	6–12 (45–9)	684/51	11,300				
9	39.0	None	0.1	25.0	175	Enrl (26.0)	465/35	554	NT	M184V	K70R	M184V
		3TC, NVP, ZDV	25.1	30.1	36	Ante (34.0)	458/39					
		None	30.3	32.0	13	HD (39.3)	721/36					
		3TC, NVP, ZDV	32.0	44.9	91	6–12 (44.9)	709/34	2,010				
10	38.0	None	0.1	20.4	143	Enrl (35.7)	613/41	50	NT	K70R	NT	NT
		3TC, NVP, ZDV	20.6	30.9	73	HD (39.4)	854/45	50				
		3TC, NVP, d4T	31.0	38.1	51	6–12 (44.7)	650/38	11,557				

		NVP[e]	38.3	38.9	5							
		None	38.9	44.7	42							
11	35.4	None	0.1	23.6	165	Enrl (25.1)	330/30	7,792	NT	D67N,	NT	NT
		3TC, NFV, ZDV	23.7	35.6	84	Ante (35.1)	207/25			K70R,		
		None	35.6	43.1	54	HD (35.9)	326/30	106		K219Q,		
						6–12 (43.1)	311/26	21,255		M41L,		
										V82A		
12	40.1	None	0.1	18.0	126	Enrl (28.0)	462/39	155	M46I	*None*	NT	NT
		ZDV	18.1	24.0	42	Ante (34.0)	535/44					
		3TC, NFV, ZDV	24.1	40.3	114	HD (40.3)	317/22					
		None	40.3	49.0	62	6–12 (49.0)	448/21	14,213				
13	40.1	None	0.1	22.0	154	Enrl (25.0)	469/NA		NT	V179D	V179D	NT
		3TC, NVP, ZDV	22.1	25.1	22	Ante (34.0)	697/58					
		3TC, ZDV	25.3	25.3	1	HD (40.4)	715/51	60				
		ZDV	25.4	40.6	107	6–12 (47.7)	585/32	3,443				
		None	40.6	47.7	51							
14	37.9	None	0.1	13.7	96	Enrl (36.0)	649/NA	6,167	D30N,	D30N,	*None*	D30N,
		3TC, NFV, ZDV	13.9	40.9	190	HD (38.0)	457/NA	11,290	K103N,	K103N,		K103N,
		None	40.9	48.0	51	6–12 (48.0)	635/40	5,961	M184V,	M184V,		M184V,
									V108I	V108I		V108I,
												V75I
15	39.4	None	0.1	26.7	187	Enrl (31.0)	551/39	227	L210W,	NT	L210W,	L210W,
		3TC, NFV, ZDV	26.9	39.4	89	HD (39.7)	896/NA		M41L,		M41L,	M41L,
		None	39.4	48.0	61	6–12 (48.0)	538/36		(E44D and		(E44D and	(E44D
									V118I)		V118I)	V118I),
												D67N
16	38.7	None	0.1	24.6	172	Enrl (30.0)	165/24	5,620	NT	M184V	*None*	M184V
		3TC, ZDV	24.7	44.3	138	HD (39.0)	167/23	44,000				
		None	44.3	48.0	27	6–12 (48.0)	105/21	75,000				
17	40.7	None	0.1	29.9	209	Enrl (33.0)	806/45		NT	M41L	NT	NT
		3TC, ZDV	30.0	40.9	77	HD (41.0)	398/44					
		None	40.9	49.0	58	6–12 (49.0)	655/36	6,810				
18	37.7	None	0.1	23.9	167	Enrl (33.0)	436/29	5,790	NT	M184V	*None*	NT
		3TC, ZDV	24.0	43.3	136	HD (38.0)	348/26	14,100				
		None	43.3	50.0	48	6–12 (50.0)	389/24	7,910				
19	39.7	None	0.1	25.9	181	Enrl (19.0)	311/26	2,050	NT	M46I	NT	*None*
		3TC, NVP, ZDV	26.0	40.0	99	Ante (33.0)	360/30					
		None	40.0	47.7	55	HD (40.0)	402/29	61				
						6–12 (47.7)	445/28	1,001				
20	39.1	None	0.1	30.1	211	Enrl (30.0)	1119/38	1,610	V108I	NT	NT	NT
		3TC, ZDV	30.3	39.7	67	HD (40.0)	1170/43					
		None	39.7	52.0	87	6–12 (52.0)	979/38	1,371				
21	43.1	None	0.1	22.9	160	Enrl (12.0)	336/20	31,967	M46I	*None*	NT	NT
		3TC, ZDV	23.0	33.3	73	Ante (21.9)	336/21	47,806				
		3TC, NFV, ZDV	33.4	43.4	71	Ante (32.0)	490/31	3,711				
		None	43.4	52.0	61	HD (43.9)	866/37	1,886				
						6–12 (52.0)	407/18	95,623				
22	37.7	None	0.1	12.7	89	Enrl (22.0)	747/37		NT	V179D	NT	NT

(*continued*)

TABLE 3. INDIVIDUAL SUBJECTS WITH RESISTANCE-ASSOCIATED MUTATIONS (RAMs) IDENTIFIED AT ENROLLMENT AND/OR AT 6–12 WEEKS POSTPARTUM IN PLASMA OR PBMC SPECIMENS (*CONT'D*)

		ARV regimens during pregnancy				*CD4 and viral load testing*			*RAMs—plasma specimens*		*RAMs—PBMC specimens*	
Subject	*Delivery*[a]	*ARV regimen*[b]	*Start*[a]	*Stop*[a]	*Duration (days)*	*Visit (timing)*[c]	*$CD4^+$ cnt/%*	*Viral load (copies/mL)*	*Enroll*[d]	*6–12 weeks*[d]	*Enroll*[d]	*6–12 weeks*[d]
		3TC, NFV, ZDV	12.9	37.7	175	Ante (32.7)	736/46					
		None	37.7	46.7	64	HD (38.0)	656/40					
						6–12 (46.7)	806/36	6,460				
23	40.0	None	0.1	28.0	196	Enrl (35.0)	926/33	83	NT	NT	NT	L33F
		3TC, NVP, ZDV	28.1	40.1	85	HD (40.3)	573/36	54,340				
		None	40.1	47.0	49	6–12 (47.0)	501/23	8,908				
24	37.1	None	0.1	19.3	135	Enrl (37.0)	232/30		NT	NT	K70R	K70R
		3TC, NVP, ZDV	19.4	37.1	125	HD (37.3)	240/33					
		None	37.1	44.1	50	6–12 (44.1)	288/34					
25	36.3	None	0.1	18.0	126	Enrl (27.0)	343/25	44,900	NT	NT	NT	K70R
		ZDV	18.1	30.1	85	Ante (34.0)	428/38	597				
		3TC, NVP, ZDV	30.3	36.9	47	HD (37.3)	925/42	400				
		3TC, ZDV[e]	37.0	37.3	3	6–12 (42.9)	494/25	37,000				
		None	37.3	42.9	40							
26	37.6	None	0.1	26.0	182	Enrl (27.0)	403/31		NT	NT	K70R	K70R
		3TC, NVP, ZDV	26.1	40.0	98	Ante (33.0)	351/36					
		3TC, ZDV[e]	40.1	40.4	3	HD (37.9)	717/32					
		None	40.4	43.9	25	6–12 (43.9)	490/35	556				
27	38.0	None	0.1	33.6	235	Enrl (38.0)	556/29	2,280	NT	NT	NT	K70R
		3TC, ZDV	33.7	38.1	32	HD (38.3)	597/22	13,500				
		None	38.1	47.1	64	6–12 (47.1)	3100/59	23,700				
28	29.9	None	0.1	28.0	196	Enrl (27.0)	245/21	379,193	NT	NT	G190A	*None*
		ZDV	28.1	29.9	13	HD (30.6)	441/22	7,651				
		None	29.9	40.0	72	6–12 (40.0)	468/30	175,785				
29	37.7	None	0.1	18.9	132	Enrl (18.0)	196/23	3,524	NT	NT	M46I	*None*
		3TC, ZDV	19.0	37.9	133	Ante (34.0)	380/24	101				
		None	37.9	45.9	57	HD (38.0)	372/35	162				
						6–12 (45.9)	299/20	3,823				
30	40.7	None	0.1	18.4	129	Enrl (26.0)	301/35	5,900	NT	NT	K103N,	K103N
		3TC, NFV, ZDV	18.6	33.9	108	Ante (34.3)	373/33	496			M184V,	
		IDV, TDF, d4T	34.0	42.1	58	HD (40.9)	446/36	418			V108I	
		None	42.1	47.3	37	6–12 (47.3)	288/32	28,300				
31	39.1	None	0.1	18.7	131	Enrl (20.0)	440/16	3,400	NT	NT	NT	K70R
		3TC, NFV, ZDV	18.9	39.3	144	Ante (32.0)	292/24					
		None	39.3	45.7	46	HD (39.3)	588/26					
						6–12 (45.7)	398/14	54,900				
32	31.0	None	0.1	21.7	152	Enrl (31.0)	587/33		NT	NT	NT	K103N
		3TC, NFV, ZDV	21.9	31.1	66	HD (31.7)	323/34	4,320				
		None	31.1	42.7	82	6–12 (42.7)	309/26	60,000				

[a]Delivery date and start and stop of ARV use during pregnancy are given in weeks of gestation.

[b]"None" used to denote periods during pregnancy (through 6–12 week postpartum visit) when no ARVs were received.

[c]Visit and timing (in weeks of gestation) corresponding to available $CD4^+$count, $CD4^+$ percent and viral load (HIV-1 RNA) measures. Visits: Enrl = enrollment; Ante = antepartum visit; HD = hospital discharge; and 6–12 = 6–12 week postpartum visit.

[d]NT = sample not tested for resistance; None = no resistance associated mutations were detected. Where resistance associated mutations were detected, the specific codon substitutions are identified.

[e]ARVs started after delivery.

Table 4. Corrrelation of Genotypic Resistance Results between Plasma and PBMC Samples According to Study Visit

	Enrollment			*6–12 weeks postpartum*		
	RAMs detected in PBMCs	*RAMs not detected in PBMCs*	*Total*	*RAMs detected in PBMCs*	*RAMs not detected in PBMCs*	*Total*
RAMS detected in plasma	2	1	3	4	4	8
RAMs not detected in plasma	1	21	22	2	47	49
Total	3	22	25	6	51	57

concordant (47 without RAMs and 4 with RAMs) while six were discordant (four with RAMs in plasma only and two with RAMs in PBMCs only) (kappa = 0.51; 95% CI: 0.26–0.77) (Table 4).

Discussion

Among a population of HIV-1-infected mothers from four Latin American and Caribbean countries who received ARVs during pregnancy for MTCT prophylaxis, RAMs were detected in 17.9% of plasma samples at either enrollment (antepartum) or postpartum. The frequency of detection of M184V was 1.8% at enrollment and 5.2% at 6–12 weeks postpartum, and of K103N was 1.8% at enrollment and 3.1% at 6–12 weeks postpartum. These frequencies could be underestimates, since the subjects discontinued their ARV regimens by the time of the 6–12 week visit (some discontinued ARVs shortly after delivery), and the resistance assays would not be able to detect those RAMs, as some disappear quickly and/or some are not detected by genotypic resistance assays that have poor sensitivity for RAMs present in less than 25% of the prevailing viral population.[13]

Of seven subjects with RAMs at enrollment, four had already received ARVs during pregnancy but before enrollment (albeit only for a median duration of 7 weeks). Three of the seven women had not received ARVs prior to enrollment, and thus the RAMs detected in these women's samples represent transmitted resistance. RAMs were detected among 16 plasma samples at 6–12 weeks postpartum, but, of these 16 samples, the enrollment plasma sample was able to be amplified in only one of the 16 (and the RAMs detected at 6–12 weeks were the same as those detected at enrollment). Thus, although the proportion of the study population with RAMs could be determined, it is not possible to assess development of resistance mutations in relation to ARV prophylaxis regimens received by the women in this cohort. However, possible risk factors for the development of resistance, such as viral load, CD4 count, and clinical stage, did not differ between subjects with and without RAMs in our study population. Poor adherence may lead to subtherapeutic levels of ARVs, thereby increasing the risk for development of drug resistance mutations. Although adherence currently is not assessed in the NISDI Perinatal Study, the protocol is being revised to incorporate assessments of adherence.

ARV resistance among HIV-1-infected women receiving ARVs for prevention of MTCT of HIV-1 has been described previously, including women receiving a two-drug regimen (zidovudine/lamivudine)[14–16] and women receiving three-drug regimens.[17] The latter report is most comparable to our study, in which 81% of women received a three-drug regimen. The overall proportion of RAMs detected in our study is not statistically significantly different from that described by Lyons *et al*.,[17] in which RAMs were detected in 13% of 39 samples obtained postpartum (none was detected prior to receipt of ARVs).

There is limited information available regarding genotypic resistance testing in PBMCs. It is known that PBMC samples could offer information about archived mutations, while plasma samples provide information on replicating viruses. Plasma samples are recommended for decision making in the clinical care setting,[7,18,19] although PBMC samples are acceptable when plasma viral loads are low in extensively treated patients. Recently Bon *et al*.[20] described 31 ARV-naive patients whose plasma and PBMC specimens were tested for transmitted resistance. They found that RAMs in the reverse transcriptase were found more frequently in PBMCs and that primary protease mutations were found only in PBMCs, suggesting that the detection of archived mutations could be better for the study of transmitted resistance.

A strength of this analysis is the large size of the study cohort. However, since most of the women received a three-drug ARV regimen, viral loads were relatively low in this population, limiting the number of samples that could be amplified.

Our results indicate genotypic resistance among women receiving ARVs for prophylaxis of MTCT of HIV-1 occurs at a rate similar to or even higher than that reported in other studies. The association of resistance to ARVs given for MTCT prophylaxis on subsequent disease progression and response to future ARV treatment should be evaluated, as this information is very limited.[21]

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