

Emergence of genotypic resistance in HIV-1 infected pregnant women taking triple antiretroviral to reduce mother-to-child transmission

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EMERGENCE OF GENOTYPIC RESISTANCE IN HIV-1 INFECTED PREGNANT WOMEN TAKING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) TO REDUCE MOTHER-TO-CHILD TRANSMISSION OF HIV-1 (MTCT).

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Background: Antenatal antiretroviral therapy (ART) reduces MTCT. Concerns for emergence of resistance have prompted a change from mono and dual therapy to HAART. In women not requiring HAART for their own health the impact of antenatal HAART on emergence of viral resistance is unknown.

Methods: Pregnant HIV-1 infected women with a pre-treatment CD4 >500/ $10^6/L$ were prescribed HAART from the third trimester and discontinued therapy postpartum (n=40). All women were counselled on the importance of adherence to therapy. Those taking regimens including NVP stopped HAART cessation for pharmacokinetic reasons. Genotypic resistance testing (RT) (HIV-1 TruGene®) was performed after HAART cessation and on pretreatment samples when postpartum samples showed primary mutations. Samples with viral load (VL) >500cpm were included in analysis. Statistical analysis was performed using SPSS 12.0.

Results: 30 (60%) women were from Sub-Saharan Africa. 32 (80%) had sequences available for analysis; 8 were excluded (6=VL<500; 2=no sequence). 2/32(6.2%) took ART in a previous pregnancy (1=ZDV, 1=combivir), 30/32(94%) were ART naïve. Pre-treatment parameters: median CD4=419/ $10^6/L$ (300-1082), median VL 2851cpm (50-34753). HAART regimens = combivir/NVP (27), combivir/NLF (4), DDI/ZDV/NLF (1). Median duration of HAART = 66days (3-110). VL around the time of delivery: 20/32(62%) <50cpm, 25/32(78%) <1000cpm, 6/32(16%) no result as delivered early (5) or failed to attend (1); 1/32(3%) >1000cpm (12.7%). Median time from HAART cessation to RT = 48days (13-198). Of the 32 postpartum sequences 22/32 (69%) = nonB subtype, 10/32 (31%) = B subtype. 7 primary mutations (V106A(I), Y181C(2), G190A(I), K101E(I), M184V(I), T215S(I)) were detected in 5/32(16%) women. 5/5 were on regimens including NVP. 5/5 ART were naïve. 4/5 had no mutations detectable pre-treatment (1 pre-treatment RT not available, VL=83cpm). Baseline VL($p=0.48$), baseline CD4($p=0.55$), duration of therapy($p=0.67$) and delivery VL($p=0.08$) were similar in those that developed resistance and those that did not.

Conclusion: In this cohort 16% of women demonstrated primary genotypic resistance postpartum despite HAART. Pretreatment parameters, duration of therapy and response to therapy did not predict emergence of mutations. The impact of these mutations on future ART response and management of future pregnancies remains to be observed.

Background

Maternal antenatal antiretroviral therapy is an integral part of any programme to reduce MTCT. The pivotal PACTG 076 study demonstrates the clear benefits of ART in pregnancy (1). Since then combination ART in pregnancy has been associated with lower MTCT versus ZDV monotherapy (OR = 0.27, 95% CI 0.08-0.94) (2).

Background (continued)

Prevention and management of HIV viral resistance is a growing challenge for HIV physicians. Genetic resistance to antiretroviral therapy can evolve spontaneously but is more likely in the setting of a non-suppressive regimen. In the context of pregnancy, short term use of ART if associated with poor virological response can lead to significant mutations conferring genetic resistance to antiretroviral agents.

This could compromise future antiretroviral options for women and result in transmission of resistant virus.

Since January 2002 all HIV-1 infected women attending the GUIDE Clinic, St. James's Hospital are offered triple antiretroviral therapy in pregnancy regardless of maternal need for therapy.

Methods

In line with the Irish Guidelines for the management of HIV-1 infection in pregnancy all women with a pre-treatment CD4 >300/ $10^6/L$ were prescribed triple antiretroviral therapy from 28 weeks of pregnancy and discontinued therapy postpartum. All women were counselled on the importance of adherence to therapy.

In recognition of the different pharmacokinetic profiles of NRTITs and NNRTITs women on regimens that included nevirapine were instructed to stop the nevirapine immediately following delivery and continue the Combivir® for 5 days. To facilitate this process women were given a 5 day post-partum pack of Combivir® at their last visit pre-delivery.

Pre and post treatment plasma samples were collected. Genotypic resistance testing (TruGene HIV-1 Genotyping Kit, Visible Genetics) was performed after HAART cessation and on pretreatment samples when postpartum samples showed primary mutations. Samples with viral load >500cpm were included in analysis. The entire protease gene and codons 1-246 of the RT gene were sequenced and each resistance associated codon was examined. The genetic HIV-1 subtypes were determined using the web based BLAST subtyping algorithm

(www.ncbi.nlm.nih.gov/genotools/HIV-1/) with sequence data from the *pol* gene.

Statistical analysis was performed using SPSS Version 12.0.

Results

40 women received triple ART in pregnancy and discontinued therapy following delivery. 30/40 (75%) women were from Sub-Saharan Africa. 32/40 (80%) had sequences available for analysis. 8 of the 40 were excluded: 6 because the viral load was <500cpm and 2 because it was not possible to obtain a sequence. Of the 32 with available sequences 2/32 (6.2%) had taken ART in a previous pregnancy (1 zidovudine monotherapy, 1 dual NRTI with zidovudine and lamivudine). The remaining 30 (94%) were ART naïve. Baseline characteristics are shown in Table 1. Median duration of HAART was 66 days (3-110).

Table 1 – baseline CD4, Viral load and ART regimens

Median CD4	419 x $10^6/L$	(300-1082)
Median VL	2851cpm	(50-34753)
ZDV/3TC/NVP	27	
ZDV/3TC/NLF	4	
ZDV/DDI/NLF	1	

Median time from HAART cessation to genotypic resistance testing was 48 days (13-198). Of the 32 postpartum sequences 22/32 (69%) were nonB subtype, 10/32 (31%) were B subtype. 7 primary mutations (V106A(I), Y181C(2), G190A(I), K101E(I), M184V(I), T215S(I)) were detected in 5/32(16%) women. All of these women were antiretroviral naïve before this pregnancy. The baseline characteristics and ART regimens in these 5 women are shown in Table 2.

Table 2 - baseline characteristic of those with mutations postpartum

	baseline CD4	baseline VL	ART	days of ART
1.	428 x $10^6/L$	33,372cpm	ZDV/3TC/NVP	84
2.	300 x $10^6/L$	3708cpm	ZDV/3TC/NVP	56
3.	557 x $10^6/L$	83cpm	ZDV/3TC/NVP	26
4.	306 x $10^6/L$	1335cpm	ZDV/3TC/NVP*	95
5.	542 x $10^6/L$	1056cpm	ZDV/3TC/NVP	13

*nevirapine was interchanged to neftirnavir after 59 days as there was a change in protocol for nevirapine use in light of concerns for excess maternal hepatotoxicity (3). She had documented viral suppression on ZDV/3TC/NVP.

The mutations detected and pre-delivery viral loads are shown in Table 3.

Table 3 – delivery viral loads and mutations

	Delivery VL	Days to RT*	Mutations detected	Subtype
1.	73cpm	28	V106A	b
2.	<400cpm	42	Y181C, G190A, T215S	b/g
3.	na**	56	M184V	f2
4.	<50cpm	73	K101E	f2
5.	na**	18	Y181C	g

*RT, resistance testing, ** na, not available (both had presented late in pregnancy and delivered before follow-up visit)

In 4 of the 5 with mutations detected postpartum no mutations were detected on the pretreatment samples. For 1 woman it was not possible to sequence virus as the pretreatment viral load was 83cpm. Adherence issues were not identified, on questioning, in any of these 5 women and for all women taking nevirapine at the time of delivery it was stopped immediately postpartum and continued Combivir® for a further 5 days.

Women with mutations detected postpartum did not differ significantly from those who did not have mutations detected postpartum when comparing median baseline viral load ($p=0.48$), median baseline CD4 ($p=0.55$), median duration of therapy ($p=0.67$) and median delivery viral load ($p=0.08$).

Conclusion

In this cohort 16% of women demonstrated primary genotypic resistance postpartum. Use of triple combination antiretroviral therapy did not protect against emergence of significant mutations. Pretreatment parameters, duration of therapy and response to therapy did not predict emergence of mutations. The impact of these mutations on future ART response and management of future pregnancies remains to be observed

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