Full Viral Suppression, Low-Level Viremia, and Quantifiable Plasma HIV-RNA at the End of Pregnancy in HIV-Infected Women on Antiretroviral Treatment

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Abstract

There is limited information on full viral suppression and low-level HIV-RNA viremia in HIV-infected women at the end of pregnancy. We investigated HIV-RNA levels close to delivery in women on antiretroviral treatment in order to define rates of complete suppression, low-level viremia, and quantifiable HIV-RNA, exploring as potential determinants some clinical and viroimmunological variables. Plasma samples from a national study in Italy, collected between 2003 and 2012, were used. According to plasma HIV-RNA levels, three groups were defined: full suppression (target not detected), low-level viremia (target detected but <37 copies/ml), and quantifiable HIV-RNA (≥ 37 copies/ml). Multivariable logistic regression was used to define determinants of full viral suppression and of quantifiable HIV-RNA. Among 107 women evaluated at a median gestational age of 35 weeks, 90 (84.1%) had HIV-RNA <37 copies/ml. Most of them (59/90, 65.6%) had full suppression, with the remaining (31/90, 34.4%) showing low-level viremia (median: 11.9 copies/ml; IQR 7.4–16.3). Among the 17 women with quantifiable viral load, median HIV-RNA was 109 copies/ml (IQR 46–251), with only one case showing resistance (mutation M184V; rate: 9.1%). In multivariable analyses, women with higher baseline HIV-RNA levels and with hepatitis C virus (HCV) coinfection were significantly more likely to have quantifiable HIV-RNA in late pregnancy. Full viral suppression was significantly more likely with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens and significantly less likely with higher HIV-RNA in early pregnancy. No cases of HIV transmission occurred. In conclusion, HIV-infected pregnant women showed a high rate of viral suppression and a low resistance rate before delivery. In most cases no target HIV-RNA was detected in plasma, suggesting a low risk of subsequent virological rebound and development of resistance. Women with high levels of HIV-RNA in early pregnancy and those who have concomitant HCV infection should be considered at higher risk of having quantifiable HIV-RNA at the end of pregnancy.

Introduction

SEVERAL STUDIES OF PREGNANT women with HIV have shown that combination antiretroviral (ARV) therapy significantly reduces the risk of mother-to-child transmission of HIV.¹ Effective ARV regimens are therefore recommended for all HIV-infected pregnant women, regardless of virological, immunological, or clinical parameters.^{2,3} For women not on treatment at conception and needing ARV only to prevent

perinatal HIV transmission, current U.S. and European guidelines suggest starting ARV between 12 and 28 weeks of gestation,^{2–6} with the goal of achieving maximal viral suppression at delivery and reducing the risk of transmission associated with high maternal viral load. Reducing HIV viral load in late pregnancy to undetectable or minimal levels may also allow eligibility for vaginal delivery (avoiding cesarean section)^{2,3,7,8} and prevent or delay the development of resistance and treatment failure.

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The recent availability of assays with enhanced sensitivity, able to detect and quantify HIV-RNA at lower levels compared to standard assays, has made it possible to investigate the rate and significance of low-level viremia in patients receiving ARV. Levels of HIV-RNA not detected by common assays (low-level or "residual" viremia) have been found to be associated with increased risk of virological failure, virological rebound, and the development of resistance.⁹⁻¹¹

Despite growing information on the clinical relevance of residual viremia and on its impact on immunological impairment in people with HIV receiving ARV treatment,¹² there is currently no information on this issue in HIV-infected women receiving treatment in pregnancy. We therefore studied HIV-infected pregnant women receiving ARV therapy, assessing the levels of HIV-RNA below the common threshold in late pregnancy. The aim of the study was to define rates of complete suppression (target not detected), low-level viremia, and quantifiable HIV-RNA, and to explore as potential determinants some clinical and viroimmunological variables.

Materials and Methods

We used data from the National Program on Surveillance on Antiretroviral Treatment in Pregnancy, an ongoing observational study on HIV pregnant women established in Italy in 2001.¹³ Only HIV-positive pregnant women are included, and no specific guidance is given for treatment of HIV infection or prophylaxis for mother-to-child transmission, which are decided by the treating physician. Laboratory and clinical data are collected from hospital records, following the women's consent based on a patient information sheet approved by the competent Ethics Committee (of the Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani in Rome, ref. deliberation no. 578).

Information and measurements are collected at routine pregnancy visits, at delivery, and during a follow-up of mothers and newborns for up to 18 months. Gestational age at birth is determined on the basis of the last menstrual period, ultrasound biometry, or both. Only a few selected sites had collected and stored samples and then sent them to the central laboratory for virological evaluation. We considered eligible for this study all pregnant women with HIV from these sites, diagnosed before or during pregnancy, who had plasma samples collected during the third trimester of pregnancy and available for analysis. Women had to be on ARV treatment at the time of plasma sampling.

Viral load in plasma samples collected at 36 weeks of gestation was measured at the central laboratory of the Istituto Superiore di Sanità, Rome, using the kPCR Versant assay, in accordance with the manufacturer's instructions (Siemens Healthcare Diagnostics, Deerfield, IL), with a lower limit of 37 copies/ml. After collection, plasma samples were stored at -80°C and thawed only at the time of testing. According to the three possible results of the kPCR assay output, and as already reported by Gianotti *et al.*,¹⁴ the patients were classified into three groups: (1) patients with strictly undetectable viral load (output: "target not detected"), classified as "Full Viral Suppression"; (2) viral load detectable but below the threshold of 37 copies/ml, classified as "Low-Level Viremia"; and (3) patients with viral load detectable but ≥ 37 copies/ml, classified as "Quantifiable HIV-RNA."

Information on the presence or history of resistance mutations at the beginning of pregnancy was collected from hospital records. Moreover, all end-of-pregnancy plasma samples with HIV-RNA levels ≥ 37 copies/ml were tested at the central laboratory for the presence of HIV reverse transcriptase and protease resistance mutations using the TruGene HIV-1 Genotyping kit (Siemens Healthcare Diagnostics). Resistance mutations were classified according to the International AIDS Society–USA Panel.¹⁵ Hepatitis C virus (HCV) coinfection was defined by the presence of detectable HCV-RNA in plasma.

Demographic and laboratory data were summarized with descriptive statistics. The three groups were compared using the χ^2 test in 3×2 contingency tables for categorical variables and the Kruskal–Wallis test for independent samples for quantitative variables. The variables with different distributions in the above tests were evaluated in univariate and multivariable logistic regression models to define covariate-adjusted determinants of full viral suppression and quantifiable HIV-RNA. The final multivariable models were selected based on best fitting characteristics, as defined by Nagelkerke R^2 values and model accuracy (percentage of correctly predicted outcome). Significance levels were set at the threshold of 0.05. All the analyses were performed using SPSS software, version 22 (IBM Inc., Chicago, IL).

Results

Plasma samples were collected at a median gestational age of 35 weeks (IQR: 35–36) between March 2003 and December 2012. Among the 107 women with plasma samples available, 90 (84.1%) had HIV-RNA levels below 37 copies/ml, and 17 (15.9%) had quantifiable (≥ 37 copies/ml) HIV-RNA levels (median HIV-RNA level when quantifiable: 109 copies/ml; IQR 46–251). Most of the women showing viral load below 37 copies/ml had full HIV suppression (59/90, 65.6%), with the remaining 31 women (29.0% overall, 34.4% of those with less than 37 copies/ml) showing low-level viremia (median 11.9 copies/ml; IQR 7.4–16.3).

The main characteristics for the entire population and for each group are shown in Table 1. Overall, a large proportion of women (43, 40.2%) was antiretroviral naive at the start of pregnancy, and most of them (34/43, 79.1%) were diagnosed with HIV during their current pregnancy and had no ARV exposure in the periconception phase. Among the remaining 64 women, 50 were already on ARV treatment at conception, and 14 started ARV during the first trimester. These 14 women were not on treatment at conception but had previous ARV treatment exposure (median: three drugs). Eight of them had received ARV therapy during previous pregnancies.

No differences were observed among the three groups regarding age, ethnicity, diagnosis of HIV in the current pregnancy, route of infection, CDC stage of HIV disease, and HBV coinfection. Compared to women with full viral suppression and low-level viremia, women with quantifiable viral load were more likely to be coinfecting with HCV (25.0% vs. 1.8% and 3.2%, respectively, $p=0.002$), and had lower CD4 cell levels (median values: 338.5/mm³ vs. 488/mm³ and 560.5/mm³, respectively, $p=0.030$), with trends for less frequent ARV exposure at conception (29.4% vs. 50.8% and 48.4%, respectively, $p=0.289$, Fig. 1), and later start of ARV in pregnancy (median week of start: 13.5 versus 4 and

TABLE 1. GENERAL CHARACTERISTICS FOR THE ENTIRE GROUP AND FOR THE THREE HIV-RNA SUBGROUPS

	All patients	Full suppression	Low-level viremia	Quantifiable HIV-RNA	p value
Number (%)	107	59 (55.1%)	31 (29.0%)	17 (15.9%)	
Age (years) (<i>n</i> = 107)	32 (29–36)	32 (30–36)	32 (28–36)	32 (26–34.5)	0.810
Ethnicity (African) (%) (<i>n</i> = 107)	46.7	45.8	54.8	35.3	0.420
Diagnosis of HIV in current pregnancy (%) (<i>n</i> = 106)	32.1	27.6	38.7	35.3	0.537
Transmission: sexual/drug use/other (%) (<i>n</i> = 107)	87.9/3.7/8.4	93.2/3.4/3.4	87.1/6.5/6.5	70.6/11.8/17.7	0.113
HIV-CDC disease stage: A/B/C (%) (<i>n</i> = 106)	84.9/7.5/7.5	87.9/8.6/3.4	77.4/9.7/12.9	88.2/0.0/11.8	0.318
HCV-antibody positive (%) (<i>n</i> = 106)	10.4	5.2	6.5	35.3	0.001
HCV-RNA positive (%) (<i>n</i> = 103)	5.8	1.8	3.2	25.0	0.002
HBV coinfection (%) (<i>n</i> = 103)	30.1	28.1	33.3	31.3	0.873
CD4 (cells/ μ l) (<i>n</i> = 81)	486 (335.5–685)	488 (333–720)	560.5 (404.5–679)	338.5 (162–430.5)	0.030
HIV-RNA at start of pregnancy (log copies/ml) (<i>n</i> = 81)	1.70 (1.70–3.81)	1.70 (1.70–3.62)	1.70 (1.70–3.62)	3.82 (3.23–4.55)	0.002
HIV-RNA detectable at start of pregnancy (<i>n</i> = 81)	48.1	42.6	36.4	91.7	0.004
On ARV at conception (%) (<i>n</i> = 107)	46.7	50.8	48.4	29.4	0.289
Antiretroviral naive (%) (<i>n</i> = 107)	40.2	39.0	41.9	41.2	0.960
Weeks from start of ARV to sampling (<i>n</i> = 105)	25 (17–35)	31 (19–35)	29.5 (15.5–36)	21.5 (8.25–34.75)	0.287
Pregnancy week of start of ARV (<i>n</i> = 105)	12 (1–18)	4 (1–16)	7.5 (1.0–19.25)	13.5 (1–26.5)	0.169
ARV modified in pregnancy (%) (<i>n</i> = 104)	27.9	29.3	23.3	31.3	0.795
History/presence of resistance (%) (<i>n</i> = 71)	19.7	18.8	17.4	25.0	0.827
ARV regimen: ZDV-3TC/TDF-FTC/other (%) (<i>n</i> = 104)	58.7/29.8/11.5	60.3/27.6/12.1	53.3/33.3/13.3	62.5/31.3/6.3	0.938
ARV regimen: NNRTI (%) (<i>n</i> = 104)	14.4	22.4	3.3	6.3	0.032
ARV regimen: PI (%) (<i>n</i> = 104)	85.6	74.1	100.0	100.0	0.001

Values are expressed as median and interquartile range (IQR) or percentage.

p values indicate overall significance of chi-square test (in 3×2 contingency tables) for categorical variables and of the Kruskal–Wallis test for independent samples for quantitative variables. HCV, hepatitis C virus; HBV, hepatitis B virus; IQR, interquartile range; ARV, antiretroviral treatment; ZDV, zidovudine; 3TC, lamivudine; TDF, tenofovir; FTC, emtricitabine; NNRTI, nonnucleoside reverse transcriptase inhibitors; PI, HIV protease inhibitor.

FIG. 1. Antiretroviral (ARV) treatment status at conception and viral load status at the end of pregnancy. Group definition: full suppression: target HIV-RNA not detected in plasma sample; low-level viremia: plasma HIV-RNA detected, <37 copies/ml; quantifiable HIV-RNA: plasma HIV-RNA detected, ≥37 copies/ml. Bar composition: *dark gray*: on ARV treatment at conception; *white*: not on ARV at conception, treatment experienced; *light gray*: not on ARV at conception, treatment naive.

7.5, respectively, $p=0.169$). This group also had a significantly higher HIV-RNA viral load at first evaluation in pregnancy (usually the first trimester): 3.82 log copies/ml vs. 1.70 log copies/ml in the other two groups ($p=0.002$) and a much higher rate of detectable (<50 copies/ml) HIV-RNA at first evaluation in pregnancy (91.7% vs. 42.6% and 36.4%, respectively, $p=0.004$).

Treatment modifications during pregnancy overall involved 27.9% of the women, with no significant differences by group ($p=0.795$). A history of resistance was also similar in the three groups. All the current regimens were based on a dual nucleoside reverse transcriptase inhibitor (NRTI) backbone (mostly zidovudine–lamivudine or tenofovir–emtricitabine) plus either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Two women on PI-based regimens also received integrase inhibitors (raltegravir). The NRTI backbone was similar in the three groups. Conversely, NNRTI use was more common among women with full viral suppression ($p=0.032$) and PI use more common in the other two groups ($p=0.001$).

Analysis of resistance in samples with HIV-RNA ≥37 copies/ml at the end of pregnancy was conducted on all 17 samples. Among these, six could not be analyzed because of failure in nucleic acid amplification, while in the remaining 11 samples only one (9.1%) was positive for resistance-associated mutations, showing the reverse transcriptase M184V mutation. Such a mutation was not previously present in the patient.

The main outcomes associated with full viral suppression and quantifiable HIV-RNA in univariate and multivariable analyses are summarized in Table 2.

TABLE 2. FACTORS ASSOCIATED WITH FULL VIRAL SUPPRESSION AND WITH QUANTIFIABLE HIV-RNA AT END OF PREGNANCY IN UNIVARIATE AND MULTIVARIATE LOGISTIC REGRESSION MODELS

	Univariate analysis			Multivariate analysis		
	UOR	95% CI	p value	AOR	95% CI	p value
Full viral suppression (HIV-RNA target not detectable)						
NNRTI in the regimen received in pregnancy	6.356	1.355–29.81	0.019	20.58	1.92–274.3	0.016
HIV-RNA at start of pregnancy (per additional log in copies/ml)	0.645	0.432–0.962	0.032	0.411	0.20–0.84	0.015
HCV-RNA positive	0.153	0.02–1.36	0.092	0.073	0.01–1.43	0.085
On ARV at conception	1.448	0.672–3.122	0.345	0.171	0.03–0.96	0.045
Pregnancy week of start of ARV (per additional week increase)	0.964	0.925–1.005	0.081	n.c. ^a	n.c. ^a	0.709
CD4 cell count at start of pregnancy (per 100 unit increase)	1.081	0.906–1.291	0.387	n.c. ^a	n.c. ^a	0.362
Quantifiable HIV-RNA (≥37 copies/ml)						
HCV-RNA positive	14.17	2.34–85.86	0.004	113.7	5.16–2504.3	0.003
HIV-RNA at start of pregnancy (per additional log in copies/ml)	2.838	1.497–5.379	0.001	8.819	2.25–34.52	0.002
On ARV at conception	0.417	0.136–1.280	0.126	11.21	1.02–123.1	0.048
NNRTI in the regimen received in pregnancy	0.352	0.043–2.87	0.331	n.c. ^a	n.c. ^a	0.232
Pregnancy week of start of ARV (per additional week increase)	1.058	1.00–1.19	0.049	n.c. ^a	n.c. ^a	0.553
CD4 cell count at start of pregnancy (per 100 unit increase)	0.679	0.484–0.951	0.024	n.c. ^a	n.c. ^a	0.605

^an.c., not calculated: variables included in the multivariable model but AOR, 95% CI, and p values not calculated by the model for variables not significantly associated with the outcome.

UOR, unadjusted odds ratio; AOR, adjusted odds ratio; ARV, antiretroviral treatment; NNRTI, nonnucleoside reverse transcriptase inhibitors.

Full viral suppression was significantly more likely with NNRTI-based regimens and significantly less likely with higher values of HIV-RNA at the start of pregnancy. HCV coinfection and being on ARV at conception showed a negative association with full viral suppression of a borderline level of significance. The multivariable model showed no significant association between week of start of ARV in pregnancy and CD4 levels at start of pregnancy.

The analysis of determinants of quantifiable HIV-RNA (≥ 37 copies/ml) showed a significant role of HCV coinfection and higher entry levels of HIV-RNA as independent predictors of quantifiable HIV-RNA at the end of pregnancy (Table 2).

Discussion

We evaluated end-of-pregnancy plasma HIV-RNA levels in pregnant women with HIV on ARV treatment, with particular reference to rate and determinants of full viral suppression. A first positive finding of our study was the low rate of quantifiable viral load at the end of pregnancy, despite the adoption of a low threshold for detectability: at a median gestational age of 35 weeks, only 15.9% of the women had HIV-RNA levels ≥ 37 copies/ml. This rate compares favorably with other case series, where rates of detectable HIV-RNA at the end of pregnancy were between 38% and 93%, also depending on treatment status and viral load levels at the start of pregnancy.^{5,6,16,17} This positive finding should, however, be interpreted taking into account that our study, based on plasma samples taken at a scheduled visit, may have selected a sample with regular follow-up, better adherence, absence of complications, and no obstetric emergencies.

The clinical significance of viral load at the end of pregnancy includes the risk of vertical transmission, the risk of developing resistance, and eligibility for vaginal delivery. In this case series, only one woman had a viral load above 1,000 copies, and no cases of vertical transmission were observed. This indicates an overall good control of viral load in pregnancy, a very limited risk of transmission for women adequately followed, and a high rate of eligibility for vaginal delivery, which may reduce the risk of complications related to cesarean delivery.^{7,17}

In terms of resistance rate and patterns, only a limited number of cases had a detectable viral load at the end of pregnancy, and usually at low viral load levels. In such a situation, the possibility of detecting resistance and the clinical significance of the findings are limited. Despite such limitations, the low rate of resistance observed and the absence of multiclass resistance can be considered reassuring.

Quantifiable HIV-RNA at the end of pregnancy was associated in the multivariable analysis with higher HIV-RNA levels at the start of pregnancy and HCV coinfection. The significant link between HIV-RNA levels at the start of pregnancy and HIV-RNA at the end of pregnancy or time to viral suppression, already shown in other studies,^{16,18–20} reinforces the importance of adequate treatment of women with high viral load in early pregnancy.

Our findings on the role of HCV coinfection as a predictor of quantifiable viral load are consistent with other studies that showed lower efficacy or attenuated response to HAART in coinfecting subjects, possibly because of poorer tolerance, lower adherence, or adverse events leading to treatment

discontinuation.^{21–23} For other factors, particularly for low CD4, which was a significant predictor of quantifiable viral load in univariate analyses, the limited sample size may have reduced the power to maintain statistical significance in the multivariable analysis.

Our study showed that in almost two-thirds (59/90, 65.6%) of the samples below 37 copies/ml, no target nucleic acid was detected. The infrequent occurrence of low-level viremia compared to full viral suppression can be considered favorably, because it indicates that only a minority of women is exposed to the risk of virological rebound and the development of resistance.^{9–11} The analysis of predictors of full viral suppression also showed that the presence of an NNRTI in the regimen significantly increased the probability of having target HIV-RNA not detected in plasma. This finding is consistent with other studies showing with such regimens a significantly earlier achievement of viral suppression,^{16–20} a better virological outcome, and a less frequent occurrence of low-level viremia.^{24–26}

In conclusion, despite a relatively limited sample size, which may have precluded the identification of additional relevant associations and a better assessment of drug resistance, our study provided reassuring results for the care of pregnant women with HIV, showing that the majority of pregnant women with undetectable plasma viral load have full HIV suppression, a condition that translates into a lower risk of virological rebound or failure and the development of resistance.

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The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Author Disclosure Statement

No competing financial interests exist.

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