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Levels and patterns of HIV RNA viral load in untreated pregnant women

European Collaborative Study[a]

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KEYWORDS
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Summary
Objective: To assess pregnancy levels and patterns of HIV RNA in the absence of antiretroviral therapy, while appropriately adjusting for potential confounders, including maternal immune status and race.
Methods: Data on ≥1 antenatal HIV RNA measurements were available for 333 untreated HIV-infected pregnant women enrolled in the European Collaborative Study. CD4 counts and HIV RNA measurements were routinely collected from 1992 and 1998, respectively. Linear mixed effects models based on 246 women for whom complete data were available examined changes in HIV RNA levels over pregnancy, with a nested random effects term accounting for measurement variability within women and period of sample collection.
Results: The change in HIV RNA over pregnancy varied significantly by race ($p = 0.005$): from the second trimester until delivery, HIV RNA decreased significantly by an estimated 0.019 $\log_{10}$ copies/ml/week in white women (95% CI −0.03, −0.007); in black women the estimated 0.016 $\log_{10}$ copies/ml/week increase (95% CI −0.005, 0.037) was not statistically significant. At delivery, HIV RNA levels in black women were 0.45 $\log_{10}$ copies/ml higher (95% CI 0.08, 0.83) than in white women.
Conclusions: Our findings suggest that HIV RNA dynamics over pregnancy differ by race, although other interpretations cannot be excluded, due to potential for unmeasured confounding.

Introduction

Pregnancy requires a relative immunosuppression, particularly cell-mediated, to protect the fetus from maternal rejection.[1] This pregnancy-associated decline in cellular

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immune response has also been associated with an increased frequency of infectious diseases, including viral infections such as influenza and varicella.[2–4] Data available on whether pregnancy may also impact on HIV RNA levels — the pre-eminent risk factor for mother-to-child transmission (MTCT)[5,6] — are limited, and may have implications for the management of HIV-infected pregnant women. Most studies have reported relatively stable levels during pregnancy,[7–11] with some also reporting increases in viral load in the early postpartum period.[7,9] However, these studies included only small numbers of untreated women, or included women who were on monotherapy. Additionally these studies either did not adjust for confounders in adjusted analyses, such as maternal CD4 counts, or did not use appropriate methods for repeated measures data.

Using data on over 300 untreated HIV-infected women enrolled in the European Collaborative Study (ECS), a large prospective cohort study, this analysis was carried out to assess pregnancy levels and patterns of HIV RNA viral load in the absence of antiretroviral therapy, while appropriately adjusting for potential confounders, including maternal immune status and race.

Methods

The ECS is an ongoing cohort study established in 1985, in which HIV-infected pregnant women are enrolled and followed in pregnancy and their infants prospectively observed according to standard protocols.[12] Informed consent was obtained and local ethics committee approval granted. Information collected included timing and type of antiretroviral treatment, maternal CD4 count, HIV RNA viral load, and socio-demographic characteristics.

CD4 cell counts and HIV RNA viral load measurements were routinely collected from 1992 and 1998, respectively. Maternal plasma and serum samples for the 263 women delivering before 1998 were frozen locally and shipped in dry ice to either of two laboratories in Padua and Stockholm, where they were stored at −70 °C until testing in 1997/1998, with the assays used reported; testing of samples for women delivering post-1998 was performed locally in laboratories based in tertiary care/university hospitals. For HIV RNA quantification, the Amplicor HIV-1 monitor tests (standard and version 1.5, Roche Diagnostic Systems Inc., Branchburg, NJ, USA), NASBA/Nuclisens assay (Organon Teknika, Oss, the Netherlands), or Quantiplex HIV-1 RNA (b-DNA) assay (version 3.0; Chiron Diagnostics, Emeryville, CA, USA) were used. Midpoints were imputed for measurements, which were recorded at the assay lower limit of quantification.

We restricted the analysis to women who were reported as untreated prior to and throughout pregnancy, with at least one HIV RNA measurement during pregnancy. Women receiving intrapartum only zidovudine for prevention of MTCT were not excluded. As women delivered at different gestational ages, HIV RNA viral load was modeled with respect to weeks before the time of delivery and therefore ranged from −35 to 0 weeks.

Statistical methods

The pattern of $\log_{10}$ transformed HIV RNA viral load over gestational age was explored using supersmoothers (a running mean in which the sampling window size varies according to the local density of measurements); we added a small amount of random noise to the plot in order to improve data visualization. Linear mixed effects (LME) regression models were used to examine changes in pregnancy, while adjusting for potential confounders.[13] Change-point, cubic spline, and orthogonal polynomial models to describe the pattern of HIV RNA over pregnancy were examined. The most appropriate model was chosen on the basis of model log-likelihood and Akaike's information criteria (AIC); the latter is a goodness of fit criterion that allows comparison of non-nested models.[13,14] The following variables were considered in the model in a forward stepwise selection procedure and retained if inclusion resulted in an improved log-likelihood ($p \leq 0.10$): race (white or black), first CD4 cell count measured in pregnancy ($\geq$500, 200–499, or $<$200 cells/mm^3), maternal age ($\leq$25, 26–31, or $>$31 years), time of HIV RNA measurement (weeks before delivery), type of assay (NASBA/Nuclisens or Roche), blood material (plasma or serum), timing of HIV diagnosis (before or during pregnancy), and history of injecting drug use (IDU; yes/no). The final model included a random effect term for the intercept nested within the period of sample collection (i.e., before and during routine collection of HIV RNA measurements from 1998). Residual plots were used to examine non-normality of within-group residuals, with a variance power function added to the final model in an attempt to achieve normality.[13] Skewed continuous variables were compared using the Mann–Whitney test and categorical variables with the Chi-square test. Analyses were carried out using R version 2.3.1[15]).

Results

The characteristics of the 333 pregnant women with $\geq$1 HIV RNA measurement available are given in Table 1. A fifth of women were black, with the majority born in sub-Saharan Africa. Between black and white women there were no differences in the first CD4 cell count measured (median 460 (IQR 20, 657) cells/mm^3 vs. 460 (IQR 70, 550) cells/mm^3; $p = 0.18$) or in the first HIV RNA measurement (median 3.64 (IQR 3.30, 4.11) $\log_{10}$ copies/ml vs. 3.46 (IQR 3.12, 4.19) $\log_{10}$ copies/ml; $p = 0.46$), but black women were more likely to have been diagnosed as HIV-infected during pregnancy (45/66 vs. 93/247; Chi-square = 15.5; $p < 0.0001$). The overall median gestational age at delivery was 38 weeks (IQR 37, 40), not significantly different for black and white women (median 38 (IQR 38, 39) vs. 38 (IQR 37, 40); $p = 0.67$).

Over a third (35%) of women had at least two HIV RNA measurements, with a median of three measurements per woman (IQR 3, 4) and the remainder of women had only one measurement in pregnancy. Nineteen women had measurements taken in the first trimester, 83 in the second trimester, 120 in the third trimester, and 241 at delivery; the median HIV RNA levels were, respectively, 3.50 (IQR 3.35, 4.01), 3.51 (IQR 3.10, 4.20), 3.52 (IQR 2.70, 4.34), and 2.76 (IQR 2.00, 3.75) $\log_{10}$ copies/ml. The overall proportion of undetectable HIV RNA measurements was high (31%) in this untreated group of women.

Of the 152 undetectable values of HIV RNA, 13 (9%) were $<$50 copies/ml, 91 (60%) were $<$200 copies/ml, 45 (29%) were $<$400 copies/ml and the remaining three (2%) were measured with detection limits of $<$500 and $<$1000 copies/ml. The proportion of undetectable measurements among samples

Table 1 Baseline characteristics

Characteristic	Women[a] (*N* = 333)	Measurements on women[a] (*N* = 490)
Viral load values (copies/ml)		
Median (IQR)	-	2000 (IQR 350, 11 000)
Viral load measurement		
Above detection level	-	338 (69)
Below detection level	-	152 (31)
First CD4 cell count (cells/mm^3)		
Median (IQR)	437 (IQR 287, 633)	-
Timing of CD4 measurement (weeks from delivery)	−13 (IQR −19, −3)	-
Missing	45 (14)	-
Race		
White	249 (77)	356 (76)
Black	66 (21)	101 (21)
Asian	6 (2)	14 (3)
Unknown	12	19
Age at delivery (years)		
Median (IQR)	28 (25, 31)	
15–26	108 (33)	158 (33)
26–31	140 (43)	198 (41)
≥32	78 (24)	127 (26)
Unknown	7	7
History of IDU		
Non-IDU	156 (49)	248 (53)
IDU	162 (51)	224 (47)
Unknown	15	18
Timing of HIV diagnosis		
Pre-pregnancy	182 (55)	287 (59)
Antenatal	151 (45)	203 (41)
HIV RNA assay		
NASBA/Nuclisens	-	188 (38)
Roche	-	283 (58)
Other	-	19 (4)
Sampling period		
Routine collection	70 (21)	98 (20)
Before routine collection	263 (79)	392 (80)
Year of delivery		
1987–1993	228 (68)	350 (71)
1994–1998	40 (12)	52 (11)
1998–2005	65 (20)	88 (18)

IQR, interquartile range; IDU, injecting drug use.
[a] *n* (%) unless otherwise stated.

taken before 1998 was considerably higher than among those from the later period (135/392 (34%) vs. 17/98 (17%); Chi-square = 5.54; $p = 0.02$), reflecting the lower sensitivity of the earlier generation of these assays, and almost all of the later samples were taken from plasma only (data not shown). HIV RNA levels were measured in plasma for 58% ($n = 285$) of samples and were significantly lower than those measured with serum (median 3.15 (IQR 2.30, 3.95) vs. median 3.48 (IQR 3.00, 4.19), $p < 0.001$).

Figure 1 reveals the structure of the HIV RNA data ($n = 467$) with respect to the time to delivery, with measurements from white and black women marked separately. Overall, there was a slight linear increase until −13 weeks, followed by a linear decrease up to the time of delivery.

To improve the regression estimates of mean levels of HIV RNA viral load over pregnancy and to examine interactions between race and gestational age (only white women had measurements in early pregnancy), the 23 measurements available between −35 and −25 weeks (roughly corresponding to 5 and 15 weeks gestation, respectively) and the few measurements ($n = 14$) available on Asian women were excluded leaving 453 measurements. For 86 of the remaining measurements available, information was missing on at least one of the variables considered for inclusion in the model; in

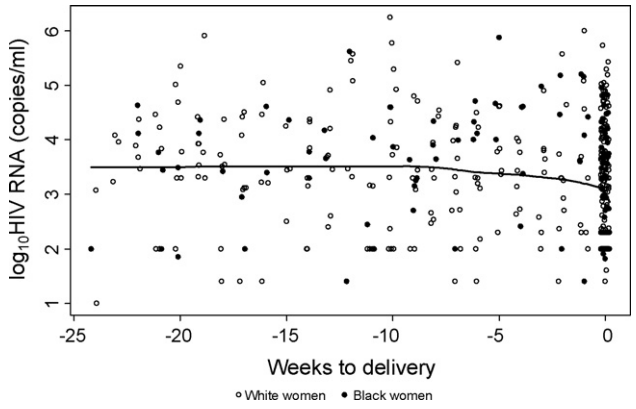

Figure 1 Scatter plot of HIV RNA over pregnancy with super-smoother by race.

most cases information was missing on CD4 counts (45/86) and race (19/86). The 367 HIV RNA viral load measurements included in subsequent LME models were overall similar to the 100 not included with respect to the distribution of HIV RNA (median 3.30 (IQR 2.56, 4.08) vs. 3.30 (IQR 2.43, 3.94) $\log_{10}$ copies/ml; $p = 0.41$), proportion of censored values (110/367 (30%) vs. 38/100 (38%); $p = 0.16$), and crude pattern of HIV RNA over gestational age (data not shown).

An LME model with a linear term in gestational weeks best described the pattern of HIV RNA viral load during pregnancy. Of additional variables considered for inclusion in the model, race, assay, sample type, IDU, and CD4 count were required. The presence of interaction effects between the linear term in gestational weeks and race (Chi-square = 7.12; $p < 0.01$) and assay and blood sample type (Chi-square = 14.84; $p < 0.001$) improved the model fit significantly. An interaction effect between gestational age and CD4 measurements in pregnancy was not tested, as measurements were taken at different times for all women and an interaction between IDU and ethnicity could not be tested as there were no black IDUs. There was no evidence of an interaction effect between any other covariates included in the model, including between CD4 count and ethnicity (Chi-square = 0.35; $p = 0.84$) or IDU and time from delivery (Chi-square = 1.73; $p = 0.19$).

The estimated coefficients of change for mean $\log_{10}$ HIV RNA viral load and the standard deviations for the random effect parameters from the final model are given in Table 2. From around the start of the second trimester (approximately 13 gestational weeks), HIV RNA viral load was estimated to decrease by 0.019 $\log_{10}$ copies/ml per week up to delivery for white women. This corresponds to a 4.3% weekly decrease in HIV RNA copies/ml over pregnancy (95% CI −6.7%, −1.6%). The slope of the change in viral load over pregnancy for black women was positive and significantly different to that for white women (Table 2); the weekly increase in HIV RNA viral load over pregnancy for black women was estimated to be 0.016 $\log_{10}$ copies/ml per week (−0.005, 0.037), equating to a 3.8% copies/ml (95% CI −1.1%, 8.9%) weekly increase, but this was not significantly different from zero, possibly due to the limited number of measurements and

Table 2 Adjusted coefficients of change for $\log_{10}$ HIV RNA viral load in pregnancy

	Multivariate coefficient[a]	95% CI	*p*-Value
HIV RNA at delivery	3.41	2.70, 4.12	<0.001
Weeks from delivery increase (WD)	−0.019	−0.03, −0.007	0.002
Race			
White	0.00		
Black	0.45	0.08, 0.83	0.02
CD4 count (cells/mm^3)			
≥500	0.00		
200–499	0.29	0.05, 0.53	0.02
<200	0.78	0.44, 1.11	<0.001
History of IDU			
No	0.00		
Yes	−0.20	−0.47, 0.06	0.14
WD and race interaction			
WD for white women	0.00		
WD for black women	0.035	0.01, 0.06	0.005
Random effects parameters			
σ intercept between period of sample collection	0.44	0.15, 1.29	-
σ intercept between women	0.69	0.60, 0.80	-

CI, confidence interval; IDU, injecting drug use.
[a] Also adjusted for blood and assay type, with an interaction effect between these terms.

women. The intercept estimate in this model corresponds to the mean HIV RNA level at delivery; the estimated mean at delivery was 3.41 $\log_{10}$ copies/ml (95% CI 2.70, 3.41) for white women and 3.86 $\log_{10}$ copies/ml (95% CI 3.16, 4.57) for black women.

The inclusion of gestational weeks (13—43 weeks gestation) as the time of HIV RNA measurement instead of weeks to delivery was examined in the final model and resulted in near identical estimates and standard errors for the mean change of HIV RNA over pregnancy for white and black women (data not shown).

In order to estimate the within-woman correlation and to examine the effect on the regression estimates of excluding the large number of women who had only one measurement in pregnancy (65%), a sensitivity analysis including only the 80 women with at least two HIV RNA measurements in pregnancy and with available information on all variables was carried out. The model included the same covariate and interaction terms as the previous model but without a random effect term for the sampling period, as its inclusion did not significantly improve the model fit here. The mean HIV RNA at delivery for white women was estimated to be 3.86 $\log_{10}$ copies/ml (95% CI 3.44, 4.28), higher than the 3.41 $\log_{10}$ estimated in the model that included all women, while the magnitude of the estimated change in HIV RNA over pregnancy was smaller, but still statistically significant, at −0.013 $\log_{10}$ copies/ml per week (95% CI −0.025, −0.0004). The between- and within-women standard deviations were estimated to be 0.58 and 0.49, respectively, and the estimated correlation of $\log_{10}$ HIV RNA within a woman over the 24 weeks of pregnancy observed was 0.66 (95% CI 0.50, 0.79).

Discussion

In this analysis of untreated HIV-infected pregnant women enrolled within the large ECS mostly before 1997, levels of HIV RNA did not remain constant over pregnancy. However, HIV RNA patterns differed significantly by race, with decreases over gestational age for white women and generally stable levels for black women. The level of HIV RNA in pregnancy was also significantly associated with immune status.

The few studies that have addressed HIV RNA dynamics in pregnancy in untreated HIV-infected women, have reported levels to be relatively stable over pregnancy,[7,8,10] or described only small and non-significant decreases in viral load.[9,11,16] However, only two of these five studies used appropriate statistical methods for correlated longitudinal data,[7,11] and most did not report adjustment for maternal immune status,[8—11,16] which together with HIV RNA levels is an important prognostic indicator for progression to symptomatic disease.[17] Here, a linear mixed effects model was used, which was able to account for variability in measurements within and between women and also allowed for women with only one measurement to contribute to estimation of the model. The adjusted analysis here included 246 women with viral load measurements available from the start of the second trimester of pregnancy, a larger number of exclusively untreated women than included in most existing studies. After adjusting for CD4 count and other covariates related to viral load, white women were estimated to have a 4.3% weekly decrease in viral load from the start of the second trimester up to delivery, while HIV RNA levels for black women increased over pregnancy, although this was not statistically significant possibly due to limited sample size.

These results stand in contrast to published studies of untreated women in pregnancy that report no significant association between gestation and viral load in untreated HIV-infected pregnant women, or for those receiving mono or dual therapy.[8—11,16] In a study of 204 women in the Ariel Project, viral load decreased by a non-significant 2 copies/ml/day for untreated women or those receiving zidovudine monotherapy, but rose significantly post-pregnancy.[9] An analysis by the SEROGEST cohort involving 254 pregnant women, 49% of whom were from sub-Saharan Africa and without adjustment for CD4 count, revealed no significant variations during pregnancy, although postpartum values were significantly lower.[8] The statistical methods used, adjustment for CD4 count and race, and other factors such as sample size, unobserved population differences, antepartum timing of measurements, and the number of untreated women included in our analysis could explain why a significant decrease in pregnancy was seen here and not in any of the other studies examining viral load in pregnancy.[7—11,16]

Significant and substantial differences between white and black women (the latter mostly born in sub-Saharan Africa) were observed in both levels and patterns of HIV RNA over pregnancy. In black women, the change in viral load over pregnancy was estimated to be significantly different to that of white women, but this increase was not significantly different to zero. Although there was no significant difference in the first HIV RNA level observed in pregnancy by ethnic group, the estimated mean viral load at delivery for black women was 3.86 $\log_{10}$ copies/ml, significantly higher than white women who had a mean of 3.41 $\log_{10}$ copies/ml and is in line with the estimated decrease in white women and increase in black women over pregnancy. Although carried out at an earlier time and using qualitative HIV methods, a virological study carried out in Sweden found that African women had higher frequencies of positive plasma isolations than European women, and significantly so at delivery.[18] A different pattern of HIV viral load during pregnancy among white and black women could possibly be explained by host biological or genetic differences, or varying viral characteristics, such as sub-type, between these groups.

We did not collect data on co-infections during pregnancy, the presence of which could possibly explain the difference between white and black women in viral load dynamics in pregnancy; a recent ECS sub-study analysis of 1050 women found that women born in Africa were at a significantly increased risk of having a viral sexually transmitted infection (STI) diagnosed in pregnancy.[19] Concurrent co-infection with viral STIs, particularly herpes viruses, have been shown to increase the rate of viral replication, purportedly through increased levels of certain cytokines or through modulation of immune responses that control HIV viremia.[20] Pregnancy may be a time of re-activation of chronic viral STI infection due to relative immunosuppression[21] and may help to explain the finding of a significant difference between black and white women among viral load levels measured at delivery, but not at first measurement. Fewer black women in our study knew their HIV status at the start of pregnancy than

white women; there is a widespread policy of screening pregnant women identified as HIV-infected for STIs throughout Europe,[19] but we were unable to assess whether there was a differential risk of undiagnosed and untreated STI by ethnic group.

Plasma HIV RNA levels have been reported to be relatively stable on a week-to-week or month-to-month basis in clinically stable non-pregnant patients, as long as antiretroviral therapy is not initiated or changed, and a change in viral load of >0.5 $\log_{10}$ is generally considered to reflect a biologically relevant change in the level of viral replication.[22] Additionally, hemodilution occurring in pregnancy results in increases in plasma volume of up to 50%, with most of the increase occurring after the first trimester[23] and complicates the interpretation of measurements of immune markers in pregnancy, including lymphocytes, with declining values over pregnancy associated with hemodilution.[24–26] In this analysis, mean HIV RNA levels in white women were estimated to decrease by approximately 0.46 $\log_{10}$ copies/ml in the 24 weeks of follow-up over the second and third trimesters of pregnancy; this equates to an approximate decrease of 65% and may be explained by changes in plasma volume in pregnancy and intra-assay sample and biologic variability alone.

Several study limitations should be acknowledged when interpreting these results. As our objective was to model HIV RNA patterns in pregnancy in untreated women, most (80%) viral load measurements were from women enrolled in the earlier years of the ECS, when such measurements were not routinely collected. In this period, availability of viral load and CD4 measurements was dependent on the individual center and therefore, although representative of the HIV-infected population in each center, the extent to which these women are representative of other untreated pregnant women in the ECS during the same period is unknown. The number of undetectable HIV RNA measurements (152; 31%) among this untreated group of women was also high, and the proportion of undetectable measurements among samples taken before 1998, where some had been stored for a longer duration, was considerably higher than among those from the later period. These early HIV RNA measurements were obtained from serum and plasma samples (stored at -70 °C) for several years and the extent of the effect of duration of storage on the accuracy of quantification of test results is uncertain.[27,28] The analyses were adjusted for sample and assay type and a nested random effects structure was used to account for any unobserved differences between measurements taken from this earlier time period, which should have reduced some of the bias in the estimates from these models. However we were unable to adjust for the lower sensitivity of earlier generations of assays, which partly explains the finding of a large number of undetectable measurements in the earlier period of the study and the results here should be interpreted in this light. The large number of women with only one measurement may also limit inferences on the shape of viral load over pregnancy; however a sensitivity analysis including women with at least two measurements also revealed a decreasing slope in pregnancy for white women, albeit of smaller magnitude.

In summary, we found a significant association between HIV RNA levels and maternal immune status in HIV-infected women who remained untreated throughout pregnancy, with significant race-specific variations in viral load with increasing gestation. Although we acknowledge possible limitations of our study, the proven success of antiretroviral therapy means that it is unlikely that additional data on untreated pregnant women will become available and our findings may thus be among the last to shed light on the complicated relationship between pregnancy and virological markers of HIV infection.

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