Leukocyte Telomere Length in HIV-Infected Pregnant Women Treated With Antiretroviral Drugs During Pregnancy and Their Uninfected Infants

Tuhina Imam, MSc, Marissa H. J. Jitratkosol, MSc,* Hugo Soudeyns, PhD,†‡§ Beheroze Sattha, BSc,* Izabelle Gadawski, BSc,* Evelyn Maan, RN,‖ John C. Forbes, MD,† Ariane Alimenti, MD,† Normand Lapointe, MD,§¶ Valerie Lamarre, MD,# Deborah M. Money, MD,‖** Hélène C. F. Côté, PhD,*†† and the CIHR Emerging Team Grant on HIV Therapy and Aging: CARMA*

Objectives: HIV disease can lead to accelerated telomere attrition, although certain drugs used as part of antiretroviral therapy (ART) can inhibit telomerase reverse transcriptase activity. This could in turn lead to shorter telomeres. We hypothesized that HIV and ART exposure would be associated with shorter leukocyte telomere length (TL) in exposed mother/infant pairs compared with controls.

Methods: In these retrospective and prospective observational cohort studies, TL was evaluated in peripheral blood leukocytes obtained from HIV-infected pregnant women treated with ART and their uninfected infants, and compared with HIV^+ untreated (retrospective cohort) or HIV mothers and their infants (prospective cohort).

Results: In HIV-infected ART-exposed mothers, leukocyte TL was not significantly shorter than that in HIV^+ untreated mothers or HIV^- controls, nor was their infants' TL significantly different. Cord blood of ART-exposed infants exhibited TL shorter than that from infants born to HIV-negative mothers. Placenta also showed evidence of shorter TL after adjustment for relevant covariates. Factors associated with shorter maternal and infant TL included smoking and the use of drugs of addiction in pregnancy.

Conclusions: These results suggest that maternal HIV infection or exposure to ART has minimal effect on infant leukocyte TL, a reassuring finding. In contrast, tissues that express higher telomerase activity such as umbilical cord blood and placenta appear comparatively more affected by ART. Smoking and the use of drugs of addiction have a negative impact on maternal and infant leukocyte TL, possibly through oxidative telomere damage.

Key Words: leukocyte telomere length, antiretroviral therapy, blood, HIV-infected pregnant women, HIV-exposed uninfected infants, HIV pregnancy

(*J Acquir Immune Defic Syndr* 2012;60:495–502)

INTRODUCTION

It is recommended that HIV-infected pregnant women receive nucleoside reverse transcriptase inhibitor (NRTI)–containing antiretroviral therapy (ART) for their own health and to prevent transmission of HIV to their child.[1,2] Most NRTIs studied, including zidovudine (AZT), stavudine (d4T), and lamivudine (3TC), can cross the placenta and accumulate in the amniotic fluid,[3] leading to drug exposure to the developing fetus. Although toxicities have been reported in HIV- and ART-exposed infants, there are limited data on long-term effects of in utero ART exposure on uninfected children, and currently, the benefits of

Received for publication January 11, 2012; accepted April 10, 2012.

From the *Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; †Unité d'immunopathologie virale, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada; ‡Department of Microbiology & Immunology, Faculty of Medicine, Université de Montréal, Montreal, Canada; §Department of Pediatrics, Faculty of Medicine, Université de Montréal, Montreal, Canada; ‖Children's and Women's Health Centres of British Columbia, Vancouver, Canada; ¶Centre maternel et infantile sur le SIDA, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada; #Service des Maladies Infectieuses, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada; **Women's Health Research Institute, Vancouver, Canada; and ††Centre for Blood Research, Vancouver, Canada.

Member of the CIHR Team grant on HIV therapy and aging (CARMA): Judy Wong, Ari Bitnun, Lindy Samson, Jason Brophy, Julio Montaner, Marianne Harris, David Burdge, Neora Pick, Julie van Schalkwyk, Patti Janssen, and Fatima Kakkar.

Supported by a Canadian Foundation for AIDS Research grant (H.C.F.C. and D.M.M.) as well as the Canadian Institutes of Health Research (CIHR) emerging team grant in HIV therapy and aging (H.C.F.C., J.C.F., A.A., and D.M.M., HET-85515).

H.C.F. Côté was partially supported by a Michael Smith Foundation for Health Research Scholar award [CI-SCH-50(02-1)], CIHR New Investigator award (YSH-80511), and a Canadian Foundation for Innovation-New Opportunity and Infrastructure operating fund (10427). H. Soudeyns and N. Lapointe were supported in part by an infrastructure grant from the Réseau SIDA et maladies infectieuses, Fonds de la recherche en santé du Québec.

The authors have no conflicts of interest to disclose.

Ethical approval for this study was obtained from the Research Ethics Boards of the University of British Columbia and from the Hospital Research Review Committee of the Children's and Women's Health Centre of British Columbia (H03-70356 and H04-70540) and le Comité d'éthique de la recherche du Centre Hospitalier Universitaire Sainte-Justine (#2872). All study participants (mothers) provided informed consent.

T. Imam and M. H.J. Jitratkosol contributed equally.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

Corresponding to: Hélène C. F. Côté, PhD, Department of Pathology & Laboratory Medicine, University of British Columbia, G227-2211 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 2B5 (e-mail: helene.cote@ubc.ca).

ART in pregnancy greatly outweigh potential risks to the infant.[4] Nevertheless, it remains vitally important to minimize toxicity.

NRTIs are known inhibitors of the telomerase reverse transcriptase (TERT), an enzyme that shares both mechanistic[5] and structural[6] homology with HIV reverse transcriptase. NRTIs can also cause telomere shortening,[7,8] and AZT's ability to inhibit TERT is central to its chemotherapeutic action in acute T-cell leukemia.[9] In animal studies, shorter telomeres were detected in various tissues from newborn mice exposed to AZT transplacentally[10] and from monkeys exposed to AZT + 3TC.[11] Shorter telomeres are associated with reduced life span and a variety of age-associated diseases including cardiovascular,[12] neurodegenerative, liver, and kidney diseases.[13] Although newer antiretrovirals exist that may not affect TERT, the lack of clinical trial safety data currently precludes their recommendation for widespread use in pregnancy.

The aim of the present study was to address 2 fundamental questions: Do infants exposed to ART in utero and peripartum show shorter telomeres than those born to (a) HIV-infected untreated mothers? or (b) HIV-negative mothers? To address the first question, leukocyte telomere length (TL) was measured retrospectively in a group of HIV-infected women who were either untreated or treated with ART, as well as in their HIV-exposed uninfected (HEU) newborn infants (SJ cohort). To address the second question, a group of HIV-infected women treated with combination ART and their infants, as well as control HIV-uninfected women/infant pairs, were prospectively studied (PR/CARMA cohort) (see **Table 1, Supplemental Digital Content**, http://links.lww.com/QAI/A329). Placental tissue and progenitor cells present in higher abundance in umbilical cord blood both express telomerase activity.[14,15] Hence, TL was also examined in these tissues.

MATERIALS AND METHODS

Study Subjects

Retrospective SJ Cohort

Stored samples from participants (N = 120) of the Centre Maternel et Infantile sur le SIDA Mother–Child Cohort, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada, were used. HIV-infected women who were either ART untreated (n = 39) or treated with mono-, dual-, or triple-ART (n = 81) during pregnancy, as per standard of care at the time, and their HEU infants born between 1990 and 2000 were included. These women were primarily newcomers from countries where HIV is endemic. Dried blood spot samples, collected from the women near delivery (<4 months before delivery to <1 month after) and from infants <6 weeks old, were stored at −80°C. Data collected for all subjects included maternal age, gestational age at birth, maternal ethnicity, clinical and pregnancy history, ART history, where applicable, smoking ever during pregnancy, use of drugs of addiction during pregnancy, duration of HIV infection at delivery, and $CD4^+$ T-cell counts and HIV plasma viral load (pVL) at the visit closest to, but before, delivery (Table 1). For the infants, birth information and perinatal ART exposures were recorded.

Prospective PR/CARMA Cohort

HIV-infected women (n = 59) and HIV-uninfected controls (n = 44) were enrolled during pregnancy in a mother/child HIV cohort study at British Columbia Women's Hospital, Vancouver, Canada, from 2005 to 2009. All HIV-infected subjects received combination ART, as per time-specific clinical guidelines. Maternal venous blood was collected between 32 and 36 weeks of gestation, and heel prick blood was collected from uninfected infants within 7 days of birth and stored at −80°C. Placental tissue was taken from both sides (maternal and foetal) of the organ, usually within 2 hours of delivery and either frozen at −80°C or in liquid nitrogen. Cord blood was collected at birth and stored at −80°C. Most PR/CARMA cohort participants had all 4 types of samples collected. Sociodemographic and clinical data were collected as above, except that $CD4^+$ T-cell counts and HIV pVL were measured 1–4 weeks before delivery (Table 2). History of hepatitis C virus (HCV) infection was based on a positive HCV polymerase chain reaction (PCR) and/or HCV antibody test ever. In both cohorts, repeat pregnancies were excluded.

Measurement of TL

See **Supplemental Digital Content** (http://links.lww.com/QAI/A329).

Statistical Analysis

Analysis of covariance was used to examine the differences in TL between exposed and unexposed infants (SJ cohort) or exposed and control infants (PR/CARMA cohort) and their mothers. Relevant covariates, including maternal age at delivery, gestational age at birth (for infants), maternal smoking ever in pregnancy, and use of drugs of addiction and/or methadone ever in pregnancy, were included in the statistical model. Linear regression analyses were used to investigate additional HIV-specific predictors of TL, including maternal HIV pVL, $CD4^+$ cell count, duration of HIV infection, and duration of maternal prepregnancy ART exposure. Both Pearson and Spearman correlations were used to examine the relationships between TL of samples.

RESULTS

Demographics, clinical characteristics, and laboratory values for the SJ and PR/CARMA cohorts are shown in Table 1 and Table 2, respectively. The comparison between average leukocyte TL measured by quantitative PCR and median absolute lymphocyte TL measured by flow fluorescence in situ hybridization on a subset of cord blood samples showed the that 2 methods yielded highly correlated TL measures (n = 26; Pearson: $r = 0.91$; $P < 0.0001$) (see **Figure 1, Supplemental Digital Content**, http://links.lww.com/QAI/A329).

Influence of ART Exposure on TL in HIV-Infected Women and Their HEU Infants (SJ Cohort)

Uninfected infants born to ART-treated and untreated mothers had similar gestational age at delivery, birth weight,

TABLE 1. Demographics, Clinical Characteristics and Laboratory Values of Mothers and Infants of the SJ Cohort

	HIV+/ART Exposed, n = 81	HIV+/ART Unexposed, n = 39	*P*
Infant characteristics			
Male gender	42 (52)	25 (64)	0.21
Birth weight, kg	3.2 [2.9–3.5] (1.9–4.2)	3.1 [2.8–3.4] (1.9–4.3) (n = 37)	0.64
Gestational age at delivery, wk	39.0 [38.0–40.0] (34.9–41.1)	39.0 [37.0–40.0] (32.0–41.0)	0.17
Apgar score at 5 min	9 [9–9] (7–10)	9 [9–9] (6–10) (n = 36)	0.91
Maternal characteristics			
Maternal age, yr	28.6 [25.2–31.6] (17.3–43.9)	26.1 [24.3–31.0] (17.8–37.4)	0.12
Maternal ethnicity			0.74
Caucasian	30 (37)	14 (36)	
Black	48 (59)	24 (62)	
Hispanic	1 (1)	1 (3)	
Asian + Other	2 (2)	0 (0)	
Maternal use ever during pregnancy			
Smoking	19 (23)	12 (33) (n = 36)	0.27
Drugs of addiction and/or methadone*	10 (12)	3 (9) (n = 35)	0.55
ART exposure			
Duration of maternal ART in pregnancy, wk			—
AZT	17.7 [14.0–23.1] (1.1–41.1) (n = 45)	N/A	
AZT/3TC	22.4 [14.5–25.1] (5.1–39.7) (n = 20)	N/A	
AZT/ddI	22.7 (n = 1)	N/A	
Triple therapy	21.4 [15.4–37.3] (3.1–39.4) (n = 15)	N/A	
HIV clinical data			
Detectable pVL near delivery†	12 (27) (n = 45)	2 (33) (n = 6)	0.66‡
HIV pVL near delivery (copies/mL)	1455 [645–3209] (205–33863)	N/A	—
CD4+ count near delivery (cells/μL)	430 [290–575] (33–1794)	464 [324–576] (75–713)	0.51

Data are number (%) of subjects or median [interquartile range] (range) and n, number of subjects with available data (if not available for entire group) unless indicated otherwise. χ^2 test was used for categorical data.

*Drugs of addiction included all "social" drugs as per data collection form.

†For most study subjects, HIV pVL before delivery was routinely used.

‡Fisher exact test used if <5 subjects in a given category.

ddI, didanosine; N/A, not applicable; NA, not available; pVL, plasma viral load.

gender, and Apgar score at 5 minutes. There was also no significant difference in age or ethnicity between the mothers. The majority of subjects in both the groups were blacks, followed by whites (Table 1). Smoking and use of drugs of addiction in pregnancy were also similar between the 2 groups. Thirteen (16%) HIV+/ART-exposed pregnant women conceived on ART, 2 (2%) started ART in the first trimester, 55 (68%) in the second trimester, and 11 (14%) in the third trimester, for a median in utero exposure of 20 weeks. After ART initiation, all subjects remained on therapy for the duration of pregnancy. Of 81 HIV+/ART-exposed pregnant women in the SJ cohort, 45 (56%) received AZT monotherapy, 21 (26%) received dual therapy, and the remaining 15 (19%) were treated with triple therapy. Of those treated with triple therapy, 14 received AZT + 3TC + nevirapine.

Leukocyte TL was compared between mothers and infants within and between the ART-exposed and the ART-unexposed groups of the SJ cohort. Maternal leukocyte TL was significantly shorter than that of their infants ($P < 0.001$) (Fig. 1A). Maternal/infant TL were weakly correlated (see **Table 2, Supplemental Digital Content**, http://links.lww.com/QAI/A329). However, there were no significant difference in TL between ART-exposed and ART-unexposed mothers ($P = 0.19$), nor was there a difference in TL between their infants ($P = 0.68$). There was also no difference between maternal and infant TL whether AZT monotherapy, or dual or triple therapy was used during pregnancy (see **Figure 2, Supplemental Digital Content**, http://links.lww.com/QAI/A329). There was no change in statistical significance between the 2 groups after adjustment for the following covariates: maternal age at delivery, gestational age at delivery, smoking ever in pregnancy, and use of drugs of addiction ever in pregnancy (Table 3; Fig. 1A). Older maternal age was associated with longer infant telomeres (n = 120; Pearson: $r = 0.232$; $P = 0.01$). Smoking during pregnancy was independently associated with shorter infant TL (n = 117; Pearson: *r*

TABLE 2. Demographics, Clinical Characteristics, and Laboratory Values of Mothers and Infants of the PR/CARMA Cohort

	HIV+/ART Exposed, n = 59	Unexposed Controls, n = 44	*P*
Infant characteristics			
Male gender	33 (56)	25 (57)	0.93
Birth weight, kg	3.1 [2.8–3.4] (1.6–4.1)	3.1 [2.7–3.5] (1.4–5.2)	0.39
Gestational age at delivery, wk	38.7 [37.7–39.9] (31.3–41.3)	39.0 [38.1–39.9] (28.9–41.9)	0.63
Apgar score at 5 min	9 [9–9] (7–10)	9 [9–9] (5–9)	0.55
Vaginal birth*	34 (58)	31 (70)	0.18
Maternal characteristics			
Maternal age, yr	30.6 [25.6–34.7] (18.0–42.4)	31.5 [27.2–35.4] (22.2–43.0)	0.40
HCV coinfection†	20 (36) (n = 56)	5 (19) (n = 27)	0.11
Maternal ethnicity			**<0.001**
Aboriginal	17 (29)	6 (14)	
Caucasian	23 (39)	28 (64)	
Black	9 (15)	0 (0)	
Hispanic	1 (2)	0 (0)	
Asian + Other	9 (15)	10 (23)	
Maternal use ever during pregnancy			
Smoking	36 (63) (n = 57)	22 (50)	0.18
Drugs of addiction and/or methadone‡	22 (40) (n = 55)	18 (43) (n = 42)	0.78
ART exposure			
Duration of maternal ART in pregnancy, wk	19.3 [14.8–29.1] (1.9–41.1)	N/A	—
Duration of pre-pregnancy ART, wk	58.9 [23.3–101.5] (0.1–603.9) (n = 30§)	N/A	—
ART combination			
AZT + 3TC + PI (±ritonavir)	41 (69)	N/A	—
AZT + 3TC + non-NRTI	1 (2)	N/A	—
Other	17 (29)	N/A	—
HIV clinical data			
Duration of HIV infection at delivery, yr‖	3.9 [1.3–6.4] (0.1–15.3)	N/A	—
Detectable pVL near delivery¶	10 (17)	N/A	—
HIV pVL near delivery (copies/mL)	321 [81–440] (46–1280)	N/A	—
$CD4^+$ count near delivery (cells/μL)	450 [315–665] (90–1200)	N/A	—

Data are presented as median [interquartile range] (range) unless otherwise indicated. If data was not available for all, n is indicated. χ^2 test was used for categorical data. Not all samples types (maternal/infant blood, cord blood, placenta) were collected for all study subjects. Significant *P* values are bolded.

*The remainders were Cesarean sections.

†History of HCV infection based on HCV RNA PCR or HCV antibody.

‡Drugs of addiction included but were not limited to heroin, cocaine, methamphetamine, ecstasy [3,4-methylenedioxymethamphetamine], and benzodiazepines.

§Other subjects had no prior exposure to ART.

‖Based on HIV clinical diagnosis date.

¶Near delivery refers to 1–4 weeks before delivery.

PI, protease inhibitor; N/A, not applicable.

= −0.197; $P = 0.03$) and had a similar influence on maternal TL (n = 117; Pearson: $r = -0.18$; $P = 0.06$). Use of drugs of addiction in pregnancy was associated with shorter TL in both infants (n = 117; Pearson: $r = -0.20$; $P = 0.03$) and mothers (n = 116; Pearson: $r = -0.20$; $P = 0.04$).

Influence of Exposure to ART and HIV Infection on TL in HIV-Infected Mothers and Their HEU Children Compared With Uninfected, Unexposed Healthy Controls (PR/CARMA Cohort)

HEU infants born to HIV-infected, ART-treated mothers and uninfected children born to HIV-uninfected control mothers were similar in terms of gestational age and weight at birth, gender, Apgar score at 5 minutes, delivery method, and maternal age, although there were differences with respect to ethnicity. The HIV-infected, ART-exposed group included more aboriginal people and fewer Asians than the control group and no black or Hispanic women (Table 2). A history of HCV infection was more common in HIV-infected, ART-exposed mothers than in controls. As data on HCV infection was not available for many control mothers (because it is not part of routine screening in HIV-negative pregnancies), it was not included in the statistical model. Of note, when available, HCV infection was highly correlated with the use of drugs of addiction. Smoking and use of drugs of addiction and/or methadone ever during pregnancy were well balanced between the 2 groups. All the mothers who reported smoking marijuana during pregnancy also smoked cigarettes,

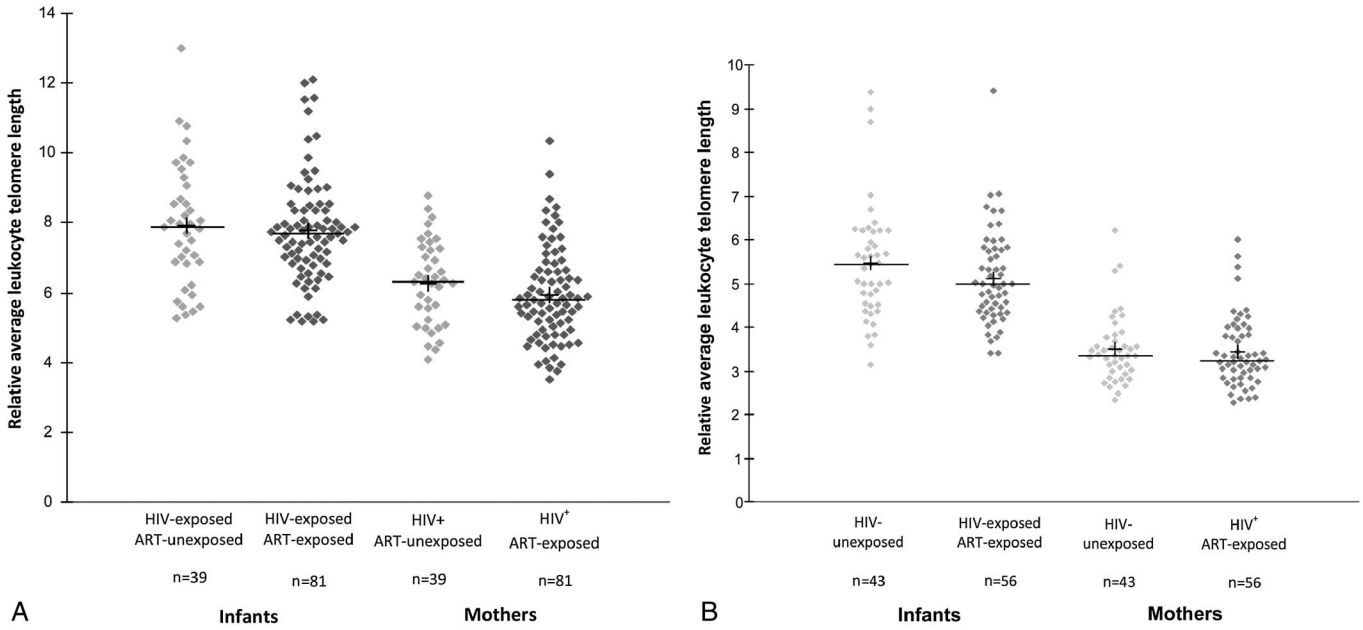

FIGURE 1. Scatter plot of peripheral blood leukocyte TL in infants and mothers in the SJ cohort (A) and the PR/CARMA cohort (B). The horizontal bars represent the median and the crosses the mean.

and of the mothers who received methadone during pregnancy, 81% (n = 21 of 26 in both groups) also used drugs of addiction. In the PR/CARMA cohort, 11 (19%) HIV-infected women conceived while on ART and remained on treatment, whereas 9 (15%) started ART in the first, 33 (56%) in the second, and 6 (10%) in the third trimester, for a median in utero ART exposure of 19.3 weeks, which was not statistically different in the SJ cohort ($P = 0.66$). The majority of the women in the PR/CARMA cohort were treated with AZT + 3TC + protease inhibitor (±ritonavir) (n = 41, 69%) and the women who conceived on ART were more likely to be on alternate regimens (n = 6, 55%). Half (30 of 59) of the HIV-infected women in this cohort were ART exposed before the studied pregnancy. Near delivery, median $CD4^+$ T-cell count was 450 cells per microliter and 17% of women had a detectable pVL, with a median of 321 HIV RNA copies per milliliter plasma.

Peripheral blood leukocyte TL was compared between mothers and infants within and between the HIV-infected ART-exposed group and the control group. Again, maternal TL was significantly shorter than that of their infants ($P < 0.001$). However, no statistically significant TL differences were observed between HIV-infected and control mothers, or between their infants, before or after adjusting for covariates (Table 3; Fig. 1B). In the PR/CARMA cohort, infant TL correlated with maternal TL (Fig. 2) and more strongly correlated with cord blood and placenta TL than maternal TL was, whether considering all samples or each group separately (for detailed correlations

TABLE 3. Summary of the Relative TL (mean ± SD) Measured in Various Samples, the Difference Between Means and the *P* Values of Unadjusted and Adjusted Comparisons

Cohort Sample Type	n	Unexposed TL	n	Exposed TL	% Difference	Unadjusted *P*	Adjusted *P*
SJ							
Infant DBS	39	7.90 ± 1.76	81	7.77 ± 1.54	−1.7	0.68	0.57
Mother DBS	39	6.27 ± 1.21	81	5.93 ± 1.36	−5.4	0.19	0.15
PR/CARMA							
Infant blood	43	5.46 ± 1.33	56	5.11 ± 1.07	−6.4	0.16	0.13
Maternal blood	43	3.49 ± 0.78	56	3.27 ± 0.74	−6.3	0.70	0.08
Cord blood	35	6.23 ± 1.54	51	5.65 ± 1.11	−9.3	**0.04**	0.06
Placenta (foetal side)	36	7.15 ± 1.75	41	7.09 ± 1.58	−0.1	0.88	0.06
Placenta (maternal side)	36	7.21 ± 1.65	41	6.96 ± 1.65	−3.8	0.50	**0.03**

All comparisons were adjusted for the following covariates: maternal age and smoking ever in pregnancy. For the PR/CARMA cohort, maternal ethnicity, use of drugs of addiction, or methadone ever in pregnancy was also included. In addition, for infant blood, gestational age at delivery was included; for placenta, mode of delivery (vaginal vs. Cesarean section) was included. Significant *P* values are bolded.

DBS, dried blood spot.

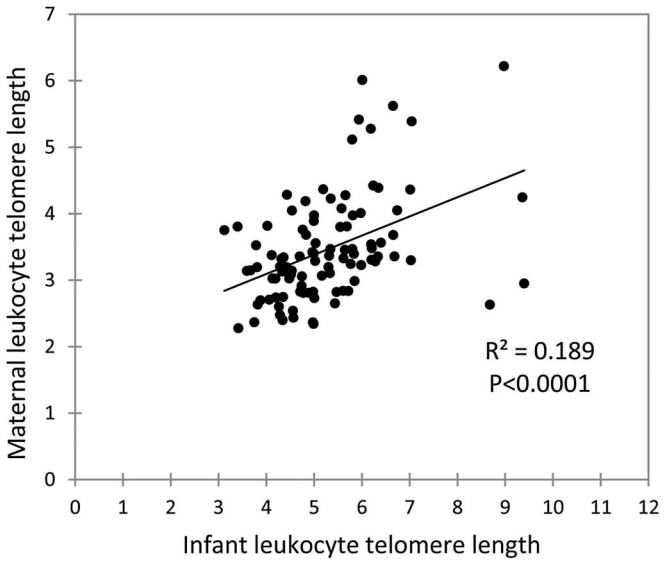

FIGURE 2. Pearson correlation between PR/CARMA cohort maternal and infant peripheral blood leukocyte TL (n = 99).

between samples, see **Table 3, Supplemental Digital Content,** http://links.lww.com/QAI/A329).

Cord blood leukocyte TL, as measured by quantitative PCR, was significantly shorter in the HIV-infected, ART-exposed group than in the control group ($P = 0.04$). After controlling for maternal age, maternal ethnicity, gestational age at delivery, smoking ever, and use of drugs of addiction/methadone ever in pregnancy, this difference became more marginally significant ($P = 0.06$). None of the covariates examined were independently associated with TL in peripheral or cord blood.

In placental tissue, TL strongly correlated between the maternal and foetal sides of the organ in both groups (see **Table 3, Supplemental Digital Content** http://links.lww.com/QAI/A329), and there were no statistically significant relationships between gestational age at delivery or maternal age and TL. No differences in TL were observed between the 2 groups in unadjusted comparisons (Table 3). However, after adjusting for covariates, placenta from HIV-infected, ART-exposed pregnancies showed shorter TL on the maternal side than controls ($P = 0.032$) and a similar trend was noted in samples obtained from the foetal side of the organ ($P = 0.08$). Smoking in pregnancy was independently associated with shorter placental TL on the maternal side ($P = 0.041$) and tended similarly on the foetal side ($P = 0.055$).

Among HIV-infected, ART-exposed subjects from the PR/CARMA cohort, the following variables were also considered as potential predictors of TL in linear regression models: duration of maternal HIV infection, duration of maternal prepregnancy ART exposure, length of infant in utero ART exposure, and pVL and $CD4^+$ T-cell counts near delivery. None of these variables was associated with infant TL or placental TL on the maternal side. However, higher $CD4^+$ T-cell counts ($P = 0.047$) and longer duration of HIV infection at delivery ($P = 0.016$) were associated with shorter maternal TL (n = 52). In similar regression models, higher $CD4^+$ T-cell counts ($P = 0.018$), higher pVL ($P = 0.009$), and older maternal age ($P = 0.043$) were associated with longer cord blood TL (n = 47). Finally, higher HIV pVL was associated with longer placental TL on the foetal side ($P = 0.028$).

DISCUSSION

Exposure to ART or HIV/ART and TL

Results obtained in our retrospective cohort study (SJ cohort) comparing TL in HIV-infected mothers treated or untreated with ART in pregnancy and in their HEU infants did not reveal significantly different TLs in maternal or infant peripheral blood leukocytes. This suggests that in the context of pregnancy, perinatal exposure to ART itself is not associated with shorter TL in infants, a reassuring finding. Further comparison of HIV-infected ART-treated mother/infant pairs, this time with uninfected control mothers and their infants as part of the prospective PR/CARMA cohort study, also did not detect any significant TL difference between the groups, suggesting that maternal HIV infection does not exert an important effect on TL. In the PR/CARMA cohort, maternal TL positively correlated with both infant blood and cord blood TL, in accordance with other studies.[16,17] Some differences emerged in cord blood and placenta, whereby shorter TL were seen in the HIV/ART-exposed group compared with uninfected controls. Interestingly, the mean TL value for each of the 7 sample types investigated within the 2 cohorts was consistently lower in the exposed group than in the unexposed one (Table 3). Although the difference in TL reached significance in a few instances only, such unidirectional change in TL between the groups is unlikely to be coincidental. It is noteworthy that the greatest decrease in TL was seen in cord blood, a tissue that contains telomerase-expressing hematopoietic, mesenchymal, and endothelial stem cells in higher abundance than peripheral blood.[18] As hematopoietic stem cells are the precursors of other cord blood nucleated cells, inhibition of telomerase activity by ART could exert the most impact on TL within this compartment. The larger effect seen in cord blood compared with infant peripheral blood could also reflect its distinct rather than linear origin, as recently suggested.[19] Nevertheless, this observation raises concern on the potential effect of NRTI on stem cells and requires further study.

Importantly, although ART exposure itself did not emerge as an important predictor of shorter TL in mothers or infants, some well-recognized health risk factors did. Smoking and use of drugs of addiction ever in pregnancy was associated with shorter TL in mothers and in their infants in the SJ cohort. Both are known sources of oxidative stress,[20–22] a major cause of telomere attrition through decreased TERT expression, which is regulated by redox-sensitive transcription factors,[23–26] and/or telomeric DNA oxidative damage.[23] In a mouse model, cigarette smoke exposure was shown to induce oxidative stress, telomere shortening, and apoptosis.[27] In tissues that have higher expression of telomerase, namely, cord blood and placenta, the association observed between higher pVL and longer placenta TL among HIV/ART-treated PR/CARMA

subjects could suggest some placental telomerase inhibition in the presence of effective ART. However, although foetal growth retardation has previously been associated with reduced placental telomerase activity[28] and accelerated placenta telomere shortening,[29] we did not observe any difference in infant birth weight in the ART-exposed groups compared with both the untreated (SJ cohort) or uninfected groups (PR/CARMA cohort). Among HIV+/ART-treated mothers in the PR/CARMA cohort, duration of HIV infection, which may be a surrogate indicator of exposure to inflammation and oxidative stress, was also associated with shorter TL. Taken together, our results suggest that the negative effects of known risk factors, such as smoking, use of drugs of addiction, and inflammation, likely exert greater influence than ART on the leukocyte TL of mothers and their infants.

Age and TL

As expected,[30,31] mothers had shorter TL than their infants in both cohorts. Furthermore, in agreement with previous studies, TL was longest in placental tissue,[32] followed by cord blood, infant, and maternal peripheral blood leukocytes.[33] In both cohorts, maternal and infant TL positively, albeit weakly, correlated, but maternal age and maternal TL showed no correlation, likely because the age of the mothers (interquartile range = 26–35 years) spans a time of high interindividual variability and relative leveling of telomere attrition rate compared with rates seen at younger or older ages.[34] In regression analyses, older maternal age was associated with longer TL in both the infants' peripheral blood and cord blood. This seemingly counterintuitive observation is in agreement with previous cohort studies that demonstrated a clear relationship between older parental age and longer TL in progeny,[35,36] presumably a result of telomerase activity in germ line cells.[36] Although this relationship is strongest with paternal age, it has also been reported on the maternal side in a large cohort.[17] Paternal age or paternal ethnicity data were not available for this study, but as there was no difference in the maternal ages between the groups, it is plausible that there would be little difference in paternal ages.

Other limitations of this study include the higher interindividual coefficients of variation we observed for control infant peripheral blood leukocyte TL and cord blood TL (both 20%–25%), compared with those reported in the literature[16,37] when this study was designed. This challenged the power of our study to detect small differences in TL. However, the cord blood TL variability we observed is in agreement with a more recent study, published after we completed data collection.[38] Given that in the prospective observational cohort, there were no women who were HIV infected and not treated with ART, it was not possible to ascertain whether any TL differences observed were related to HIV, ART, or both. Finally, peripheral blood leukocyte TLs were generally longer in the SJ cohort than in the PR/CARMA cohort, and some of the associations with TL were not reproduced in both cohorts. Potential explanations include, but are not restricted to, the fact that different samples were collected (dried blood spot vs. whole blood) and stored for different lengths of time. The ethnic makeup of the 2 cohorts also differed considerably, and the threshold for categorizing a subject as smoking/using drugs of addiction may have differed slightly between the 2 cohorts.

In conclusion, our results on TL in mother and infant peripheral blood suggest that oxidative stress related to smoking or use of drugs of addiction is a stronger factor affecting TL than HIV and/or ART exposure. Advocating a healthy lifestyle for HIV-infected women could play an important role in counteracting any negative effect of HIV or ART on their child's future health. In tissues that express higher telomerase activity, such as cord blood and placenta, reduced exposure to ART, as reflected by higher pVL, may better preserve telomeres. Maternal HIV infection itself also seems to have minimal effect on infant TL, although in HIV-infected mothers, longer duration of HIV infection, which could reflect a greater exposure to inflammation and oxidative stress, may explain the association seen with shorter maternal TL.

At a time when programs involving prevention of mother-to-child HIV transmission with ART are being extended to large numbers of mothers and infants in the developed and developing countries, our observations suggesting that ART does not significantly affect TL in the peripheral blood of ART-exposed mothers and their infants are reassuring. However, the trend observed toward shorter TL in exposed samples in general, and in cord blood in particular, warrants further study. This is especially true considering several reports of possible long-term side effects associated with ART exposure.[39–42] These studies at the cellular level represent an important complement to long-term clinical studies on ART in the context of pregnancy.

ACKNOWLEDGMENTS

The authors acknowledge Laura Oliveira, Silvie Valois, Martine Caty, Johanne Samson, Tessa Chaworth-Musters, Daljeet Mahal, Judy Wong, Katherine Heath, and Michael Papsdorf for their contribution to this study, and Fatima Kakkar for critical reading of the manuscript.

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