Chronic Placental Inflammation Among Women Living With HIV in Uganda

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Background: HIV-exposed, uninfected (HEU) children have poorer early-life outcomes than HIV-unexposed children. The determinants of adverse health outcomes among HEU children are poorly understood but may result from chronic placental inflammation (CPI).

Setting and methods: We enrolled 176 pregnant women living with HIV (WLWH) taking antiretroviral therapy in southwestern Uganda and 176 HIV-uninfected women to compare CPI prevalence by maternal HIV serostatus. Placentas were evaluated histologically by an expert pathologist for presence of CPI, defined as chronic chorioamnionitis, plasma cell deciduitis, villitis of unknown etiology, or chronic histiocytic intervillositis. Placentas with CPI were additionally immunostained with CD3 (T cell), CD20 (B cell), and CD68 (macrophage) markers to characterize inflammatory cell profiles.

Results: WLWH and HIV-uninfected women had similar age, parity, and gestational age. Among WLWH, the mean CD4 count was 480 cells/μL, and 74% had an undetectable HIV viral load. We detected CPI in 16 (9%) placentas from WLWH and 24 (14%) from HIV-uninfected women ($P = 0.18$). Among WLWH, CPI was not associated with the CD4 count or HIV viral load. Villitis of unknown etiology was twice as common among HIV-uninfected women than WLWH (10 vs. 5%, $P = 0.04$). Among placentas with CPI, more villous inflammatory cells stained for CD3 or CD68 among HIV-uninfected women than WLWH (79% vs. 46%, $P = 0.07$).

Conclusions: CPI prevalence did not differ by HIV serostatus. T-cell (CD3) and macrophage (CD68) markers were more prevalent in placental inflammatory cells from HIV-uninfected women. Our results do not support CPI as a leading mechanism for poor outcomes among HEU children in the antiretroviral therapy era.

Key Words: placenta, HIV, resource-limited, Africa, pathology, pregnancy

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INTRODUCTION

Due to the success of prevention of mother to child transmission programs, most children born to women living with HIV (WLWH) are now HIV-exposed but uninfected (HEU). In 2018, there were an estimated 14.8 million HEU children worldwide, with 13.2 in sub-Saharan Africa and 1.1 million in Uganda alone.[1] HEU children have poorer early-life outcomes than their HIV-unexposed peers, with increased mortality, higher risk of severe infection, and poorer neurodevelopment.[2,3] These phenomena are observed even in infants born to WLWH with CD4 count >800 cells/mm^3.[2,3] The pathophysiology of poor health outcomes in HEU infants is poorly understood, although mounting evidence demonstrates higher levels of inflammation in HEU infants than their HIV-unexposed counterparts.[4] Inflammation among HEU children may begin in utero, and potential sources include exposure to maternal HIV, comorbid infections and maternal antiretroviral therapy (ART). We hypothesized that these unique exposures may drive chronic placental inflammation (CPI), which could be linked to immune activation and poor outcomes in HEU children.

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CPI is found in 5%–30% of all placentas[5] and characterized histologically as chronic chorioamnionitis, plasma cell deciduitis, villitis of unknown etiology (VUE), or chronic histiocytic intervillositis. Among WLWH in the pre-ART era, CPI was found in 37%–47% of placentas and was significantly more common among WLWH than HIV-uninfected women.[6,7] CPI may contribute to poor HEU child health outcomes by affecting transplacental antibody transfer, producing inflammatory cytokines, and breaking down pathogen barrier functions.[2,5,8] However, it is not known whether CPI is more common in WLWH taking ART. Understanding whether CPI is a potential source of inflammation in HEU children born in the ART era has important public health implications including interventions to prevent CPI such as further optimizing prenatal ART regimens to minimize HIV-related and ART-related placental inflammation and avoid fetal toxicities.

We enrolled a cohort of women presenting for delivery in Uganda to compare the prevalence of CPI by maternal HIV serostatus. We hypothesized that WLWH would have higher CPI prevalence than HIV-uninfected women. Placentas with CPI were additionally immunostained with CD3 (T cell), CD20 (B cell), and CD68 (macrophage) markers to characterize inflammatory cell profiles.

METHODS

Participant Recruitment

Participants were recruited from Mbarara Regional Referral Hospital (MRRH) in Mbarara, Uganda between September 2017 and February 2018. MRRH serves a largely rural catchment population of 9 million people and reports approximately 10,000 deliveries annually. Consecutive pregnant women presenting to MRRH for delivery were screened for enrollment and considered eligible if they were aged ≥18 years, in early-stage labor, reachable by phone after discharge for follow-up, spoke English or Runyankole (the local language), and had single-gestation pregnancy. WLWH meeting these criteria were eligible only if they also reported taking ART within the last 30 days. Eligible WLWH were enrolled consecutively, and HIV-uninfected comparators were selected as the next eligible laboring woman presenting after each enrolled WLWH. After 150 total participants were enrolled, HIV-uninfected women were selectively enrolled to balance parity and gestational age by the HIV serostatus, as these variables are associated with CPI. The study was approved by the Institutional Ethics Review Boards at Mbarara University of Science and Technology (MUST, 11/03-17), Partners Healthcare (2017P001319/MGH), and the Uganda National Council of Science and Technology (HS/2255). All participants gave written consent to participate.

Sample Collection and Placental Histology

After enrollment, maternal whole blood was tested for HIV (Determine HIV 1/2, Abbott, HIV-uninfected women only); CD4 count and HIV viral load were also measured for WLWH if these test results were not available in their clinic records within the last 6 months. After birth, the placenta was delivered into a clean plastic bucket and transferred to a clean field. A full gross pathologic examination was performed, and samples of membrane roll, full-thickness placental parenchyma (1 from the placental center and 1 from the periphery), umbilical cord, and grossly identified placental lesions were formalin-fixed, routinely processed, paraffin-embedded, and sectioned to 5 μm at the MUST Pathology Laboratory. An expert perinatal pathologist (D.J.R.) blinded to maternal HIV serostatus performed all histopathologic analysis. Membrane rolls and umbilical cords were scored for acute chorioamnionitis (maternal and fetal inflammatory response) using the Amsterdam consensus[9] nosology for histologic inflammation. The composite CPI variable was defined as histologic diagnosis of one or more of chronic chorioamnionitis, plasma cell deciduitis, VUE, or chronic histiocytic intervillositis, using published criteria.[5,9] CPI of the infectious origin was excluded.

Data Collection

A structured face-to-face interview was conducted with each participant to gather sociodemographic information, medical, and obstetric history. Data from all 352 participants were included in the final analysis. The gestational age was defined by the participant report of the last normal menstrual period or chart documentation if the participant report was missing. Data were entered into a Research Electronic Capture (REDCap) database.[10]

Sample Size and Data Analysis

Based on the previous studies, we estimated 5% of HIV-uninfected women would have CPI.[5] We calculated 176 women would need to be enrolled in each group to detect a clinically meaningful 3-fold difference in CPI prevalence by the HIV serostatus. Demographic characteristics and outcomes were compared by the maternal HIV serostatus using the Student t test or Wilcoxon rank-sum test for continuous variables and χ^2 analysis for categorical variables, with P values <0.05 considered statistically significant. Descriptive statistics were used to characterize placental pathology findings. Differences in histopathology characteristics between placentas by maternal HIV serostatus were assessed using a χ^2 test or Fisher exact test for cell sizes less than 5. All analyses were performed using STATA software (Version 15.0, StataCorp, College Station, TX).

RESULTS

Enrollment and Cohort Characteristics

Over a 6-month period, 1940 women were screened, 451 were ineligible, and 176 WLWH and 176 HIV-uninfected women were enrolled (see Supplemental Figure, Supplemental Digital Content, http://links.lww.com/QAI/B514). The mean age was 27 years (SD 6 years) for WLWH and 26 years (SD 6 years) for HIV-uninfected women. Compared with their HIV-uninfected counterparts, WLWH

TABLE 1. Demographic, Medical, and Placental Characteristics of a Cohort of Women Presenting in Labor to Mbarara Regional Referral Hospital in Uganda, by HIV Serostatus

Characteristic	Living with HIV (n = 176)	HIV Uninfected (n = 176)	P*
Demographics			
Age category			0.38
≤19	11 (6)	18 (10)	
20–34	139 (79)	135 (77)	
≥35	26 (15)	23 (13)	
Resides in Mbarara District	121 (69)	106 (60)	0.10
Married	153 (87)	164 (93)	0.05
Monthly income (median USD, IQR)	$28 (17–56)	$56 (28–83)	0.01
Formal employment outside the home	55 (31)	72 (41)	0.06
Obstetric and medical			
Gestational age in wks (mean, SD)	39 (2.1)	39 (1.7)	0.47
Parity before current delivery			0.07
0 (primiparous)	30 (17)	46 (26)	
1–3 (multiparous)	109 (62)	90 (51)	
>4 (grand multiparous)	37 (21)	40 (23)	
Attended ≥4 antenatal care visits this pregnancy	112 (64)	111 (63)	0.91
Malaria prophylaxis with IPTp or TMP/SMX	174 (99)	162 (92)	0.002
Hours in labor (median hours, IQR)	14 (8–24)	15 (9–24)	0.33
Cesarean delivery	66 (38)	48 (27)	0.04
Referred to MRRH for care	24 (14)	32 (18)	0.25
5-min Apgar score <7	2 (1)	6 (4)	0.16
Days hospitalized for delivery (mean, SD)	2.2 (2)	2.2 (3)	0.73
Birthweight category (in kg)			0.27
<2.5	9 (5)	7 (4)	
2.5–3.5	139 (79)	126 (72)	
3.6–4.0	24 (14)	35 (20)	
>4.0	3 (2)	6 (3)	
Gross pathology			
Trimmed placental weight in grams (mean, SD)	450 (93)	465 (107)	0.16
Greatest placental diameter in cm (mean, SD)	20 (2)	20 (2)	0.24
Greatest placental thickness in cm (mean, SD)	1.6 (0.6)	1.7 (0.5)	0.12
Umbilical cord length in centimeters (mean, SD)	38 (16)	41 (17)	0.06
No. of umbilical cord coils	6.9 (4.3)	7.1 (3.7)	0.67
Histopathology and immunohistochemistry			
Presence of CPI	16 (9)	12 (14)	0.18
VUE	8 (5)	18 (10)	0.04
Plasma cell deciduitis	7 (4)	5 (3)	0.82
Chronic chorioamnionitis	1 (0.6)	3 (2)	0.37
Chronic histiocytic intervillositis	0 (0)	0 (0)	—
Acute chorioamnionitis	45 (26)	54 (31)	0.29
IHC markers among placentas with CPI			
Any CD3 staining	3 (23)	11 (61)	0.07
Any CD20 staining	0 (0)	4 (25)	0.11
Any CD68 staining	7 (50)	15 (75)	0.16
CD3, CD20, or CD68 decidual staining	6 (44)	6 (25)	0.30
CD3, CD20, or CD68 villous staining	6 (46)	19 (79)	0.07
CD3, CD20, or CD68 chorionic plate staining	1 (7)	3 (13)	1.0
CD3 predominance	0 (0)	4 (29)	0.10
CD20 predominance	0 (0)	0 (0)	1.0
CD68 predominance	7 (50)	12 (60)	0.73
Mixed predominance	2 (15)	6 (26)	0.68

*Tests of association between cohort characteristics and HIV serostatus were performed using χ^2, Wilcoxon rank-sum, and *t* tests.

Reported by participants in Ugandan Shillings, converted to USD (United States Dollar) using the exchange rate for the study start date (March 1, 2017: 1 USD = 3600 Ugandan Shillings); IQR, interquartile range; IPTp, intermittent preventative treatment in pregnancy with either sulfadoxine-pyrimethamine or dihydroartemisinin–piperaquine or by routine prophylactic trimethoprim/sulfamethoxazole in participants living with HIV; TMP/SMX, trimethoprim/sulfamethoxazole; MRRH, Mbarara Regional Referral Hospital. Results listed as "n (%)" unless otherwise noted.

were less likely to be married (87 vs. 93%, $P = 0.05$, Table 1) and had a lower monthly income ($P = 0.01$, Table 1). Among WLWH, the median CD4 count was 440 cells/mm^3 and 74% had an undetectable VL within the past 6 months. The most commonly used antiretroviral regimen was TDF/3TC/EFV (n = 139, 73%), followed by AZT/3TC/EFV (n = 13,7%). WLWH were more likely to have taken malaria prophylaxis in pregnancy (99 vs. 92%, $P = 0.002$). Maternal age, parity, and gestational age did not differ significantly by HIV serostatus, although WLWH were more likely to deliver by cesarean than HIV-uninfected women (38% vs. 27%, $P = 0.04$, Table 1).

Outcomes, Placental Gross Pathology, Histopathology, and Immunohistochemistry

The proportion of low birth weight babies <2.5 kg was similar between WLWH and HIV-uninfected comparators (5% vs. 4%, $P = 0.62$). There were no differences in placental weight, diameter, and thickness between WLWH and HIV-uninfected women ($P = 0.12$–0.67, Table 1). CPI was seen in 16 (9%) placentas from WLWH and 24 (14%) placentas from HIV-uninfected women ($P = 0.18$, Table 1). Among WLWH, CPI was not associated with the CD4 count or detectable HIV viral load ($P = 0.41$, 0.70). Because only 1 woman with CPI had a viral load >1000 copies/mL, we could not investigate the impact of ART adherence on CPI. Although the prevalence of CPI was similar by maternal HIV serostatus,

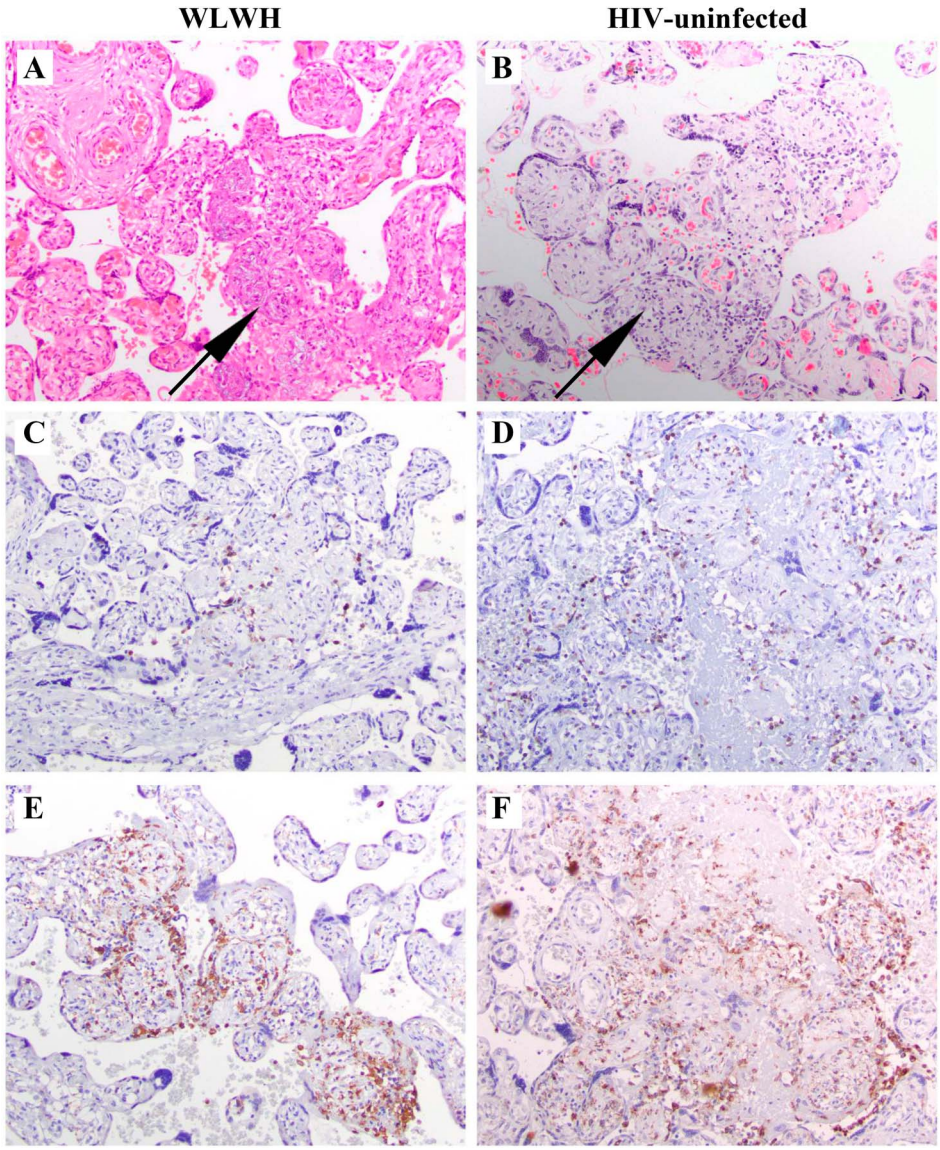

FIGURE 1. Representative histologic images of placentas with VUE from a WLWH (A, C, and E at ×20) and an HIV-uninfected comparator (B, D, and F at ×20). Top panels (A and B) are images of hematoxylin and eosin (H&E)-stained slides with arrows indicating VUE. Middle (C and D) and bottom (E and F) panels are images of IHC staining for CD3 (T cell; C and D) and CD68 (macrophage; E and F) brown nuclear stain indicating the presence of these markers in areas where VUE is present. CD20 staining not shown.

VUE was twice as common among HIV-uninfected women than WLWH (10% vs. 5%, $P = 0.04$). Among 40 placentas with CPI, immunohistochemistry (IHC) was performed and interpretable for 38 (95%). HIV-uninfected women were more likely to have large numbers of CD3 and CD68 stained villous inflammatory cells than WLWH (79% vs. 46%, $P = 0.07$), decidual and chorionic plate inflammatory cell staining was similar by the HIV serostatus. CD3 (61% vs. 23%, $P = 0.07$) and CD68 (75% vs. 50%, $P = 0.16$)-stained cells were more prevalent in decidua, villi, or chorionic plate in HIV-uninfected women (Table 1 and Fig. 1). CD20 staining was rare in both groups. No cases of infectious villitis were seen.

DISCUSSIONS

In a cohort comprised of WLWH on ART and HIV-uninfected comparators in Uganda, we found no association between maternal HIV serostatus and CPI prevalence. Our findings differ from previous studies conducted in the pre-ART era, which demonstrated a higher prevalence of CPI in placentas from WLWH.[6,7] CPI is believed to be caused by a chronic rejection-like immune host vs. graft phenomenon or a yet-unknown infectious etiology.[5] In our study, 74% of WLWH had recent undetectable HIV viral load. Thus, lower CPI prevalence in this cohort may reflect effective HIV control with ART, resulting in reduced systemic and placental inflammation. Another potential mechanism for the lower than expected prevalence of CPI in WLWH could be use of TMP/SMX use during pregnancy. Although intermittent preventative treatment in pregnancy (IPTp) given to HIV-uninfected pregnant women for malaria and TMP/SMX both have anti-inflammatory properties,[11,12] daily TMP/SMX use by WLWH potentially leads to more consistent anti-inflammatory properties than IPTp given 2–4 times in pregnancy achieves in HIV-uninfected women. Furthermore, WLWH were significantly more likely to take TMP/SMX than HIV-uninfected women were to take

IPTp (P = 0.002, Table 1), suggesting TMP/SMX may contribute to lower CPI prevalence among WLWH.

In addition to slightly higher CPI prevalence (14% vs. 9%, P = 0.18), VUE was twice as common among HIV-uninfected than WLWH participants (10% vs. 5%, P = 0.04). This finding is in contrast to high VUE prevalence among South African WLWH with low CD4 counts in the absence of ART and undescribed TMP/SMX use.[13] Like CPI, lower VUE prevalence in WLWH may reflect the anti-inflammatory effects of ART and/or TMP-SMX prophylaxis. Although VUE is associated with fetal growth restriction,[5] the prevalence of VUE was low at 7% (n = 26 cases) overall, and its contribution to neonatal outcomes remains unclear. Finally, we also identified greater T-cell (CD3) and macrophage (CD68) staining in placentas from HIV-uninfected women than WLWH. Because inflammatory cells associated with VUE are CD3 and CD68 positive, this finding aligns with higher VUE prevalence in HIV-uninfected women, and may reflect dysregulated immune inflammatory responses in WLWH, and should be explored as a possible determinant of immune development in WLWH offspring.[4] Determining the cause of differences in neonatal inflammation could lead to optimization of maternal and infant vaccination strategies to reduce early life infection risk among HEU children.

Our study has several strengths, including a relatively large and well-characterized study population with few missing data and a well-matched HIV uninfected comparator group by maternal parity and gestational age. We comprehensively characterized placentas through gross examination, histopathology, and immunohistochemistry reviewed by an expert perinatal pathologist. However, some studies have suggested that at least 3 parenchymal slides are necessary to optimally diagnose VUE.[14] Because of cost constraints in our study, only 2 routine parenchymal slides were sampled, with additional sections taken from gross parenchymal lesions and firm areas. Some cases of VUE may have been missed because of undersampling, despite examining an average of 2.4 parenchymal slides. However, proportion of cases with VUE was the same for cases with <3 compared with cases with ≥ 3 slides (7% vs. 8%, P = 0.70). In addition, we did not collect data on outcomes in children, and the generalizability of our findings is limited by enrollment at a single center referral hospital, which might not be representative of other settings. We also had limited power to perform multivariable analyses because of the rarer-than-expected outcome. Finally, we only examined CD3, CD20, and CD68 staining. Further research with a more diverse array of cellular markers of immune activation and their downstream effects on cytokine production and transplacental antibody transfer are warranted.

In conclusion, we found no association between CPI and maternal HIV serostatus among women presenting for delivery in Uganda. These results suggest that, in the ART era, CPI seems to be less common than previously reported and not a differentiating factor between WLWH and HIV-uninfected women. Although further study is needed of interactions between CPI and child outcomes, our data do not support CPI as a major determinant of poor child health outcomes among HEU children. Despite the lack of association between CPI and HIV serostatus, other placental abnormalities, such as vascular changes and malperfusion, seem to be associated with maternal HIV infection and could putatively contribute to the poor health outcomes in HEU children.[15] Given the growing number of HEU children, discerning the causes of impaired growth and neurodevelopment in this population is a public health imperative. Therefore, studies evaluating the in utero contributions of treated HIV infection and ART on HEU child health outcomes and long-term development are needed.

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