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MAJOR ARTICLE

Intestinal Barrier Dysfunction and Microbial Translocation in Human Immunodeficiency Virus–Infected Pregnant Women Are Associated With Preterm Birth

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Background. Preterm birth (PTB) rates are high in human immunodeficiency virus (HIV)–infected populations, even when on treatment. Still, only a subset of all births in HIV-infected pregnant women result in PTB, suggesting that risk factors other than HIV infection itself are also important. Inflammation is a known risk factor in uninfected populations, but its role in HIV-infected population have not been studied; in addition, the immune pathways involved are not clear and noninvasive immune markers with predictive value are lacking. Our objective was to determine the association of select markers of inflammation with PTB in HIV-1–infected pregnant women.

Methods. Within a randomized trial of pregnant women receiving nevirapine (Six-Week Extended-Dose Nevirapine [SWEN] trial), we nested a case-control study (n = 107; 26 cases, 81 controls) to determine the association of maternal inflammation with PTB. Cases were defined as PTB (<37 weeks' gestational age). We assessed inflammation by measuring plasma levels of markers of general inflammation (C-reactive protein [CRP]), intestinal barrier dysfunction (intestinal fatty acid binding protein [I-FABP]), and microbial translocation/monocyte activation (soluble CD14 [sCD14] and CD163 [sCD163]). Multivariable logistic regression was used to determine the odds of PTB per $\log_2$ increase of each marker.

Results. In multivariable models, there was increased odds of PTB per unit increase of $\log_2$ sCD14 (adjusted odds ratio [aOR], 2.45; 95% confidence interval [CI], 1.24–4.86), $\log_2$ sCD163 (aOR, 3.87; 95% CI, 1.43–10.49), and $\log_2$ I-FABP (aOR, 2.28; 95% CI, 1.18–4.41) but not $\log_2$ CRP (aOR, 0.72; 95% CI, .48–1.09).

Conclusions. Our results show that select immune markers can identify women at higher risk for PTB in HIV-1–infected populations and suggest that modulating gut barrier integrity and microbial translocation may affect PTB.

Clinical Trials Registration. NCT00061321.

Keywords. preterm birth; HIV; microbial translocation; inflammation; intestinal integrity.

Preterm birth (PTB) is the leading cause of childhood mortality worldwide, accounting for 35% of neonatal mortality and 15% of under-5 deaths [1]. Both antiretroviral therapy (ART)–naive and ART-experienced human immunodeficiency virus (HIV)–infected pregnant women have higher incidence of PTB compared with HIV-uninfected women [2–8]. For example, the global prevalence of PTB is around 8% [9], but the rates in HIV-infected populations are as high as 25% [10]. These rates are unacceptably high; however, HIV infection still only results in PTB among a subset of all HIV-infected pregnant women. Recent studies of HIV-infected pregnant women have identified low maternal CD4 count, high viral load, comorbidities, and treatment regimen as some of the risk factors for PTB [11–13], but the role of inflammation has not been examined.

Studies in HIV-uninfected populations show that high maternal inflammation during pregnancy is associated with an increased incidence of PTB [14–17]. Although inflammation is known to be a major contributor to PTB, noninvasive immune markers in pregnant women that can identify women at higher risk for PTB are lacking [18]. HIV-infected pregnant women and their infants are an especially important population to study the role of inflammation and birth outcomes, yet studies are lacking. In addition, studies have shown that higher inflammation in HIV-infected maternal–child populations is associated with mortality and HIV mother-to-child transmission [19, 20], but further studies are needed to address the association with PTB.

Importantly, HIV infection disrupts the host immune profile and it is only partially corrected after ART initiation. HIV infects a disproportionate amount of $CD4^+$ T cells from the gastrointestinal tract [21]. Even after suppressive ART, there is irreversible damage to the gastrointestinal tract affecting mucosal

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immunity (including incomplete reconstitution of $CD4^+$ T cells) and gut dysbiosis, resulting in microbial translocation and inflammation [21, 22]. Thus, when assessing the relationship of inflammation with PTB in HIV-infected populations, it is valuable to also measure markers of intestinal epithelial integrity and microbial translocation in addition to the more general markers of systemic inflammation.

To address these questions, we conducted a case-control study in HIV-1–infected pregnant women to determine the association of maternal inflammation (assessed by select markers of acute phase response, intestinal integrity, and microbial translocation) during pregnancy with PTB.

METHODS

Study Population and Design

We conducted a case-control study nested within a randomized trial of single-dose vs extended-dose nevirapine to prevent HIV-1 transmission via breastfeeding [23]. In the parent Six-Week Extended-Dose Nevirapine (SWEN) study (NCT00061321), which took place from 2002 to 2007, HIV-1–infected women received intrapartum nevirapaine, while the newborns were randomized to receive either a single dose of nevirapine after birth or extended doses through 6 weeks after birth [23]. Eligible HIV-1–infected pregnant women who provided informed consent were enrolled in sites from India, Ethiopia, and Uganda.

For this nested study, we focused on the participants and samples from India. The objective of this study was to determine the association of maternal inflammation with preterm birth. We utilized a case-control approach where cases were defined as preterm birth (<37 weeks' gestational age) and controls as term birth. The following methods, listed in the order of preference depending on data availability and as described before [24, 25], were used to estimate gestational age: ultrasound in the first trimester, first day of last menstrual period (provided the participant is confident of the dates), first available ultrasound, and fundal height assessment. Cases were chosen based on sample availability while controls were randomly chosen to provide an approximate 1:3 case:control ratio to improve the power of the study [26]. As parturition itself is considered a proinflammatory process [27], in this study we only included women (both cases and controls) with samples collected well before labor (21–33 weeks' gestational age) to assess inflammation during midpregnancy (rather than at delivery). In addition, a small subset of women (3/107) included in this study were enrolled in SWEN but were never randomized because their infants did not meet the study drug administration criteria (ie, birthweight <2000 g).

The parent study only enrolled women ≥32 weeks; as a result, our analysis excludes very preterm birth (<32 weeks). As the SWEN study was completed before universal Option B+ rollout (recommendation to provide ART to all HIV-infected pregnant women), a significant proportion of women in this study did not receive any HIV drugs during pregnancy, whereas others received either azidothymidine (AZT) alone or combination ART (cART). Among the 12 women taking ART, there was some variability in their regimen. However, more than half of them were taking a combination of azidothymidine, lamivudine, and nevirapine. For the purposes of this substudy, women receiving nevirapine intrapartum was not taken into account as our exposure variable (inflammation) was assessed prior to that while our outcome variable (PTB or term birth) would not be affected by intrapartum nevirapine administration.

Laboratory Assessments

We used enzyme-linked immunosorbent assays (ELISAs) to measure maternal plasma concentrations of select immune markers that are known to have a role in other adverse outcomes in HIV-infected adults [20, 28–30]: C-reactive protein (CRP), intestinal fatty acid-binding protein (I-FABP), soluble CD14 (sCD14), and soluble CD163 (sCD163). Quantikine ELISA kits were purchased from R&D Systems (Minneapolis, Minnesota), and samples were run in duplicates at the National Institutes of Health International Centre for Excellence in Research laboratory in Chennai, India. Results from prior studies that have frozen samples to a similar time range as SWEN show that these immune markers in plasma are stable over time [31]; in addition, case and control samples in this study were frozen for a similar amount of time.

Statistical Analyses

To assess differences in covariates by case or control status (PTB or not), Fisher exact test for categorical variables and Wilcoxon rank-sum test for continuous variables were used to calculate *P* values. Covariates such as parity, history of previous preterm birth, maternal body mass index (BMI), age, and education were chosen because they are known risk factors for PTB in the general population [32, 33]. Other HIV-specific risk factors such as CD4 count, viral load, and treatment regimen were chosen based on prior studies [12, 34]. For this study, we used CD4 count and viral load values (collected by the parent study) corresponding to the visit when plasma that was used for immune assessment was collected. The treatment regimen for this analysis refers to treatment received prior to labor (not intrapartum); women who only received nevirapine intrapartum and no treatment before are categorized as "none".

Maternal plasma concentrations of the inflammation markers were log transformed to base 2, and differences in median concentrations by cases (PTB) and controls (term birth) were assessed using the Wilcoxon rank-sum test. Data transformed to $\log_2$ was more appropriate to present and interpret the odds ratio compared to using untransformed linear values. The odds of PTB per $\log_2$ increase of each inflammation marker was determined using univariable and multivariable logistic regression. Multivariable models adjust for known risk factors

including maternal age, BMI, education, parity, history of previous PTB, anemia (<11.0 g/dL), $CD4^+$ T-cell count, viral load, and maternal treatment during pregnancy.

RESULTS

Study Population Characteristics

Pregnant women in this study had a median age of 23 years (interquartile range [IQR], 21–26 years) and a median BMI of 21.3 kg/m^2 (IQR, 19.8–23.4 kg/m^2). Median CD4 T-cell count was 425 (IQR, 294–560) cells/μL and median viral load was 4.32 (IQR, 3.51–4.68) $\log_{10}$ copies/mL (Table 1). Around 40% of women had primary level education or less. Parity was ≥2 for 23% of the women and 1 for 41% the women. Eighteen percent of these women had a history of previous PTB. Only 11% of the women had received cART (with or without AZT) during pregnancy, whereas 47% received AZT alone and 42% received no treatment (Table 1). Forty percent of the women were anemic. The median hemoglobin level was 11.6 g/dL; cases had median hemoglobin levels of 10.9 g/dL vs 11.6 g/dL for controls. Cases and controls were significantly different by HIV treatment status during pregnancy (P = .004) whereas there were no significant differences by age (P = .81), CD4 count (P = .82), viral load (P = .90), BMI (P = .25), education (P = .50), parity (P = .84), anemia (P = .08), and history of previous PTB (P = .78) (Table 1).

In the overall study population, median gestational weeks at delivery was 38 (IQR, 37–39): cases with 36 weeks (IQR, 35–36) and controls with 39 weeks (IQR, 38–40). Median birthweight in the overall study population was 2600 g (IQR, 2450–3000 g); those with PTB had a birthweight of 2325 g (IQR, 2100–2500 g) compared with 2800 g (IQR, 2500–3100 g) for controls. Fifty-one percent of the infants were female (with similar distribution among those with and without PTB) and 6-month mortality in these infants was 0.9% (1 infant who was born preterm).

Association of Maternal Inflammation With PTB

Cases (PTB) and controls (term birth) had significantly different median plasma levels of $\log_2$ sCD163 (9.97 vs 9.73 ng/mL; P = .03) and $\log_2$ I-FABP (10.43 vs 9.99 pg/mL; P = .02) but not $\log_2$ CRP (2.14 vs 2.46; P = .25) or $\log_2$ sCD14 (20.58 vs 20.61; P = .49) (Figure 1).

In univariable models, increased levels of $\log_2$ sCD163 (P = .02), $\log_2$ sCD14 (P = .04), and $\log_2$ I-FABP (P = .03) but not $\log_2$ CRP (P = .33) were associated with higher odds of PTB (Table 2). Similar associations were observed in multivariable models adjusting for maternal age, BMI, education, parity, history of previous PTB, CD4 count, viral load, anemia, and maternal HIV treatment during pregnancy. There was an increased odds of PTB per $\log_2$ increase of sCD163 (adjusted odds ratio [aOR], 3.87; 95% confidence interval [CI], 1.43–10.49;

Table 1. Maternal Characteristics by Case and Control Status

Characteristic	All (N = 107)	Cases (n = 26 [24%])	Controls (n = 81 [76%])	*P* Value[a]
Age, y, median (IQR)	23 (21–26)	23 (22–25)	23.0 (21–26)	.81
CD4 count, cells/mL, median (IQR)	425 (294–560)	445 (275–560)	417 (300–558)	.82
Viral load, $\log_{10}$ copies/mL, median (IQR)	4.32 (3.51–4.68)	4.38 (3.29–4.70)	4.27 (3.60–4.67)	.90
BMI, kg/m^2, median (IQR)	21.3 (19.8–23.4)	21.0 (19.6–22.8)	21.5 (19.8–23.8)	.25
Education				
Primary level or less	43 (40)	12 (28)	31 (72)	.50
Secondary or higher	64 (60)	14 (22)	50 (78)	
Parity				.84
0	38 (36)	8 (21)	30 (79)	
1	44 (41)	12 (27)	32 (73)	
≥2	25 (23)	6 (24)	19 (76)	
Previous preterm birth				
No	88 (82)	21 (24)	67 (76)	.78
Yes	19 (18)	5 (26)	14 (74)	
Anemia				
No	63 (60)	12 (19)	51 (81)	.08
Yes	42 (40)	14 (33)	28 (67)	
Treatment during pregnancy[b]				
None	45 (42)	17 (38)	28 (62)	.004
AZT only	50 (47)	5 (10)	45 (90)	
cART ± AZT	12 (11)	4 (33)	8 (67)	

Data are presented as No. (%) of subjects unless otherwise indicated. Cases were defined as human immunodeficiency virus (HIV)–infected pregnant women who had a preterm birth (delivery <37 weeks' gestation), and controls were HIV-infected pregnant women who had a normal delivery >37 weeks' gestation.

Abbreviations: AZT, azidothymidine; BMI, body mass index; cART, combination antiretroviral therapy; IQR, interquartile range.

[a] *P* values were calculated using the Fisher exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables to determine the difference between cases and controls.

[b] While women in the parent Six-Week Extended-Dose Nevirapine (SWEN) study received nevirapine during labor as part of the randomized trial, this is referring to treatment received during pregnancy (ie, prior to labor).

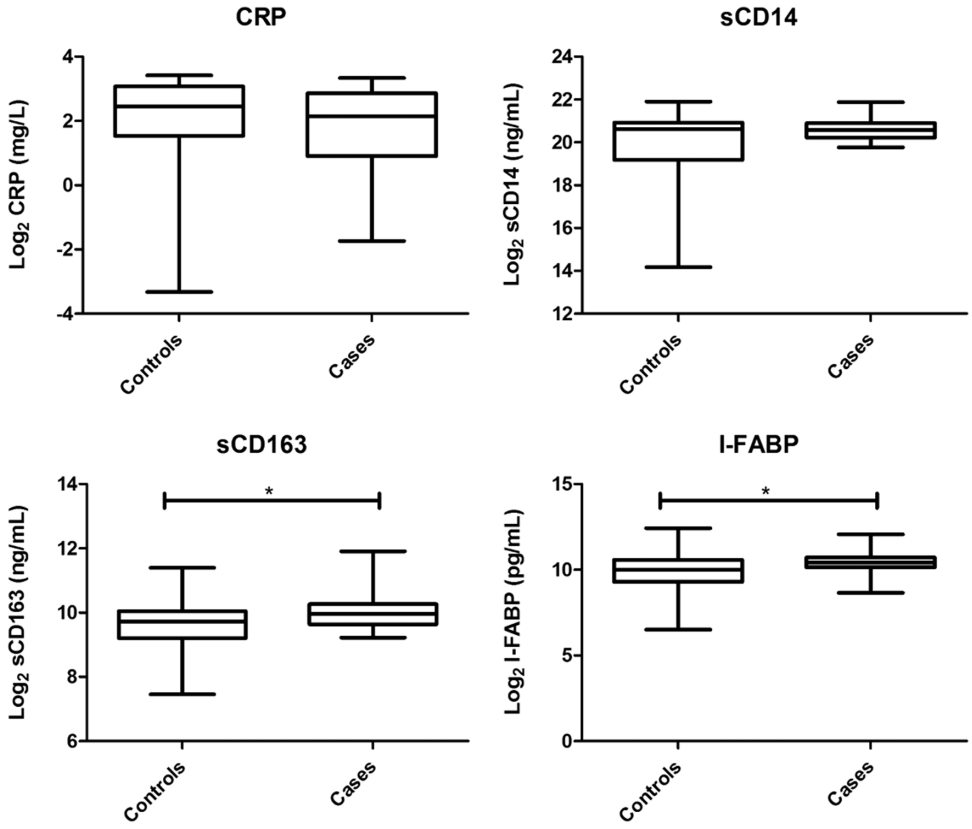

Figure 1. Levels of inflammation markers in overall study population and by case (preterm birth) and control (term birth) groups. Shown as median (interquartile range). Wilcoxon rank-sum test used to calculate *P* values to show the difference by cases and controls. *$P < .05$. Abbreviations: CRP, C-reactive protein; I-FABP, intestinal fatty acid binding protein; sCD14, soluble CD14; sCD163, soluble CD163.

$P = .008$), sCD14 (aOR, 2.45; 95% CI, 1.24–4.86; $P = .01$), and I-FABP (aOR, 2.28; 95% CI, 1.18–4.41; $P = .01$) but not CRP (aOR, 0.72; 95% CI, .48–1.09; $P = .12$) (Table 2).

DISCUSSION

In our study of HIV-1–infected pregnant women from India, higher levels of markers for intestinal barrier dysfunction and microbial translocation were independently associated with increased odds of PTB. Prior studies in HIV-infected individuals have shown that intestinal barrier dysfunction and microbial translocation were associated with various adverse outcomes including mortality and mother-to-child HIV transmission [20, 29]. Our results suggest that intestinal integrity and microbial translocation can also affect birth outcomes such as PTB and potential interventions to modulate intestinal integrity and microbial translocation may reduce PTB. In addition, future studies are needed to determine whether these markers are also associated with PTB in HIV-uninfected pregnant women.

Inflammation is a known risk factor for PTB [16], but studies are lacking in HIV-infected populations. It is important to note that although pregnancy used to be considered an immunosuppressive state, new studies have suggested there are different

Table 2. Association of Pregnancy Inflammation Markers With Preterm Birth

	Univariable Model		Multivariable Model[a]	
Marker	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Log_2 CRP	0.86 (.63–1.17)	.33	0.72 (.48–1.09)	.12
Log_2 sCD14	1.70 (1.01–2.86)	.04	2.45 (1.24–4.86)	.01
Log_2 sCD163	2.32 (1.15–4.69)	.02	3.87 (1.43–10.49)	.008
Log_2 I-FABP	1.85 (1.07–3.22)	.03	2.28 (1.18–4.41)	.01

Abbreviations: CI, confidence interval; CRP, C-reactive protein; I-FABP, intestinal fatty acid binding protein; OR, odds ratio; sCD14, soluble CD14; sCD163, soluble CD163.

[a]Multivariable logistic regression models study the association of maternal inflammation with preterm birth (cases), and are adjusted for maternal age, body mass index, education, parity, history of previous preterm birth, anemia, CD4 T-cell count, and viral load (at time of inflammation assessment) and maternal human immunodeficiency virus treatment during pregnancy.

immune phases during pregnancy [27, 35, 36]. In fact, based on the immune profile, pregnancy can be divided into 3 phases. The first (implantation and early pregnancy) and last (parturition) phases are proinflammatory, while the second phase (fetal growth and development) is anti-inflammatory [27]. As a result, high inflammation during the anti-inflammatory second phase is associated with adverse birth outcomes including PTB. However, the immune pathways involved in PTB are not well delineated. In this analysis, we studied the relationship of plasma markers of general inflammation (an acute phase protein) measured during this second phase (second and early third trimester) with PTB. In addition, given that HIV impairs gut integrity, we also determined the association of plasma markers of intestinal barrier dysfunction and microbial translocation with PTB.

Our results show that a plasma marker of acute phase response (CRP) was not associated with PTB. Results from other studies in uninfected populations have been inconsistent when plasma CRP has been used as a marker [18]. Some potential reasons for this inconsistency include CRP having a short half-life and being nonspecific [37]. Future studies with serial measurements of plasma CRP during pregnancy could help clarify this relationship as it could help distinguish between acute and persistent inflammation [38, 39]. Another proposed reason for the inconsistency is that plasma levels might not clearly reflect what is happening in maternofetal interface. In fact, studies have shown that CRP in amniotic fluid but not plasma is associated with preterm birth [18]. However, obtaining amniotic fluid is an invasive procedure and not practical for wide use in clinical settings. To study other noninvasive immune markers that can identify women at higher risk for PTB, we focused on plasma markers of intestinal barrier dysfunction and microbial translocation. We chose these markers based on the pathophysiology of HIV-1 infection, which results in depletion of mucosal $CD4^+$ T cells followed by impaired gut function, gut dysbiosis, and microbial translocation [21, 22]. Microbial translocation, which is the translocation of microbial products from the gastrointestinal tract to circulation, leads to monocyte activation and immune activation [40].

Our results show that maternal plasma I-FABP, sCD14, and sCD163 were associated with PTB. I-FABP has been recently shown to be a marker for gut barrier dysfunction, and studies have assessed the relationship of I-FABP with various outcomes in HIV-infected adults and infants [28, 29]. To our knowledge, this is the first study to show that higher levels of plasma I-FABP are associated with PTB in HIV-infected pregnant women. In addition, it is not yet known whether this relationship also holds true in HIV-uninfected populations and whether other conditions (eg, malnutrition) that affect gut integrity and microbial translocation can also increase the risk of PTB.

Because sCD14 and sCD163 correlate with plasma lipopolysaccharide (LPS) levels, they are also widely used as a marker for microbial translocation [40]. However, they are more specifically a marker of monocyte/macrophage activation, with sCD163 considered a more specific marker (as sCD14 can also be produced by other cells) [41]. In our study, both of these markers were associated with PTB. Although results for sCD14 in Figure 1 and Table 2 may seem inconsistent, these results are best explained through the different statistical methods used, where the Wilcoxon rank-sum test (Figure 1) is only comparing the medians while Table 2 is comparing the odds of PTB per unit increase of $\log_2$ sCD14 levels, Our result on the association of increased sCD14 with PTB is in agreement with a prior study on PTB [42], whereas previous studies have not assessed the role of sCD163. Although we did not directly measure LPS, our data on I-FABP, taken together with the data on sCD14 and sCD163, provide support to the model of gut barrier dysfunction, microbial translocation, and monocyte/macrophage activation playing a significant role in adverse outcomes in HIV-infected adults including PTB.

Our study has a few limitations. The sample size of this study is small and limited to only the Indian participants from SWEN. While we adjusted for potential confounders, there might be unmeasured or unknown confounders that we have not accounted for, such as nutritional status, although we did observe a trend of PTB associated with anemia. In addition, this study did not identify the potential reasons why some HIV-infected pregnant women had more intestinal barrier dysfunction and monocyte activation compared to others. While future studies should address potential causes such as micronutrient deficiencies or intestinal infections (eg, diarrhea or parasitic diseases of the gut), our results suggests that, regardless of the cause, compromised intestinal integrity and subsequent microbial translocation/monocyte activation is associated with PTB. Another limitation is that only 11% of our study participants received cART. While cART was not the standard of care during 2002–2007, the time period that SWEN was conducted, further studies of inflammation and PTB will need to be conducted to determine the association in the era of Option B+. While we adjusted for both treatment status and viral load in our analysis, we would also like to acknowledge a potential limitation in the uneven distribution of women on cART between cases and controls in this study population. Another point to note is that future studies should assess a more comprehensive list of immune markers, as we only studied a select few markers that were predictive of adverse outcomes in nonpregnant HIV-infected populations.

In conclusion, our analysis of a convenience sample of archived plasma from HIV-1–infected pregnant women remote from delivery showed that markers of intestinal barrier dysfunction and monocyte activation were associated with PTB. We propose that in the future, these and other biomarkers could serve to identify women at increased risk for PTB. In addition, therapeutics targeting intestinal integrity and microbial

translocation could prove valuable in the prevention or treatment of preterm labor. Finally, further studies are needed to assess whether these markers could also be useful in HIV-uninfected women, and in HIV-infected women receiving optimized combination ART.

Notes

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