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Elevated systemic microbial translocation in pregnant HIV-infected women compared to HIV-uninfected women, and its inverse correlations with plasma progesterone levels

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Abstract

In HIV infection, increased adverse perinatal outcomes reported among HIV-associated pregnancies are not fully understood. Currently, microbial product translocation (MT) from a permeable mucosa is demonstrated as a driver of inflammation, and may contribute to preterm delivery in HIV. Here, our results showed that plasma LPS levels (a representative marker of MT) were increased in HIV-infected women in the first and second trimester. Progesterone levels were significantly decreased in HIV-infected subjects in the first trimester and second trimester. There were significant inverse correlations between plasma LPS and progesterone in the first and second trimester. These results suggested heightened systemic MT and decreased plasma progesterone levels in HIV-infected pregnant women may play a role in increased incidence of preterm delivery.

Keywords

Microbial translocation; HIV; Progesterone; Pregnancy

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Conflict of interest

The authors declare that there are no conflicts of interest.

Introduction

Microbial translocation (MT), the subclinical systemic circulation of bacterial products derived from a permeable GI tract, induces a chronic inflammatory response in HIV-infected individuals (Jiang et al. 2009). MT can be measured directly by lipopolysaccharide (LPS) and bacterial 16s rDNA or indirectly through soluble CD14 (sCD14) from plasma or serum samples. Soluble CD14, an acute phase reactant, binds LPS and activates NF- κ B signaling pathway, resulting the induction of an inflammatory response (Jiang et al. 2009). This signaling pathway activation has important implications for HIV-infected individuals, including an association with an increased risk of HIV progression (Jiang et al. 2009).

Infection and inflammation are thought to be important etiologic pathways for at least 25–49% of spontaneous preterm birth (PTB) (Goldenberg et al. 2008); however, the exact mechanisms are poorly described. Increased incidence of PTB has also been reported in HIV-infected women (18–29%) (Goldenberg et al. 2008). Elevated plasma levels of sCD14 in the first trimester have been observed in HIV-infection pregnant women experiencing PTB (Martinez-Lopez et al. 2014, Lopez et al. 2016). In healthy term neonates, normal spontaneous delivery is not associated with systemic activation of inflammatory mediators, whereas increased levels of LPS and sCD14 in cord blood are found in the setting of prematurity and chorioamnionitis (Martinez-Lopez et al. 2014)., As sCD14 is not a specific marker for LPS responsiveness (Shive et al. 2015), we were interested in characterizing LPS levels during pregnancy and investigating any correlation with HIV-infection status.

Low progesterone level is a known risk factor for preterm birth in pregnancy (Goldenberg et al. 2008). Previous studies have demonstrated the protective effect of progesterone on pregnancy after an immune challenge with LPS in animal models, which may be via Th-2 mediated immunity (Johnson et al. 1975). Moreover, progesterone has been shown to play a role in the adaptive immune response to stress during a gestation in mice *in vivo*; progesterone prevent NF- κ B activation in response to LPS stimulation *in vitro* by decreasing leukocyte adhesion molecule receptor expression on human epithelial cells (Johnson et al. 1975).

With this background, we hypothesized that the decreased levels of progesterone during pregnancy may play a role in systemic inflammation and preterm delivery in HIV subjects. The purpose of the present study was therefore to investigate the microbial translocation and plasma levels of sex hormones in HIV infected and uninfected participants during pregnancy.

Materials and Methods

Study design and participants

This study was approved by the IRB from the Medical University of South Carolina (Pro00020570). Participants were excluded if they were not 18–45 years old, more than 13 weeks of gestation, or unable to provide informed consent. Blood draws were collected at enrollment of 8–13 weeks gestation (visit 1), 22–24 weeks (visit 2) and 34–36 weeks

gestation (visit 3). 15 mL of plasma was collected from study participants at these time points, processed for plasma separation, then stored in -80°C freezer until ready for use.

HIV RNA Viral Load

We obtained stored plasma samples from the available 5 HIV-infected study participants and age- and race-matched 5 HIV uninfected controls. HIV RNA viral load (copies/mL) are typically collected on a monthly basis until <40 copies/mL then every 3 months until 34–36 weeks when a level is drawn as part of routine obstetric care for women living with HIV. The closest viral load corresponding to each patient visit was used for analysis.

Measurement of plasma LPS

Stored plasma samples were thawed, and then diluted to 10% with endotoxin-free water. Samples were then heated to 85°C for 12 minutes to inactivate inhibitory plasma proteins. We quantified plasma LPS with a commercially available limulus amebocyte assay (Cambrex, East Rutherford, NJ, USA), according to manufacturer's protocol. We ran samples in duplicated, and background attributable turbidity of the diluted plasma was subtracted (Jiang et al. 2009).

Measurement of plasma sex hormones

Total plasma 17 beta estradiol, progesterone and testosterone levels were determined using ELISA Kits from Abcam (ab108667, ab108670, ab174569; Abcam, Cambridge, UK) according to the manufacturer's instructions. Results are reported as ng/mL and were calculated from a standard density curve based on optical density spectrometry readings.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad, San Diego, USA). LPS, 17 beta estradiol, progesterone, testosterone between HIV-infected and uninfected controls were compared using non-parametric Mann-Whitney test. $P = 0.05$ was considered statistical significance.

Results and discussion

Clinical characteristics

No significant racial or ethnicity differences were observed between the HIV-infected versus uninfected patients. There were no significant differences in tobacco use, history of recreational drug use, depression, threatened pre-term labor, sexually transmitted infection history or diagnosis in pregnancy, or chorioamnionitis (Table S1). HIV-infected subjects had a median gravidity of 2.5 compared to 3 for non-HIV infected subjects. Mean age was similar between the two groups (Table S1). Regarding HIV care parameters, all of the HIV-infected subjects were taking antiretroviral therapy throughout pregnancy.

Plasma level of LPS

Recently, higher levels of sCD14 and LBP at first trimester and higher levels of sCD14 at third trimester were found in the HIV-infected women during pregnancy (Lopez et al. 2016).

In the present study, we found that plasma LPS levels were increased or marginally increased in HIV-infected women compared to HIV-uninfected controls at visit 1 ($P = 0.06$) and 2 ($P = 0.03$), but not at visit 3 ($P = 0.23$) (Figure 1A). In addition, LPS levels were consistently lowest in the second trimester in both patients and controls. Little has been published about LPS or other bacterial product level longitudinally over the course of pregnancy. The significance of the LPS nadir in the second trimester is unclear, however, may be partially explained by a more immunologically restrictive state associated with the second trimester of pregnancy compared to first and third trimesters, which are characterized by the pro-inflammatory events of implantation and labor, respectively (Lopez et al. 2016). While the sample size is an obvious study limitation, this study was not powered to detect differences in birth timing or complications, and we hope to investigate this question with a follow-up study.

Plasma levels of sex hormones

17 beta estradiol levels were not significantly different at any time point between HIV-uninfected controls and HIV-infected participants (visit 1: $P = 0.53$, visit 2: $P = 0.40$, visit 3: $P = 0.39$; Figure 1B). However, progesterone levels were significantly decreased in HIV-infected patients compared to HIV-uninfected controls at visits 1 ($P = 0.008$), and marginally decreased at visit 2 ($P = 0.06$) but not at visit 3 ($P = 0.36$) (Figure 1C). No significant intra- and inter-group differences were noted in testosterone levels (Figure 1D). There is sufficient evidence supporting use of supplemental progesterone for the prevention of preterm birth in women at risk (Johnson et al. 1975). Moreover, Papp et al. suggest that HIV-infected women exposed to protease inhibitors have decreased progesterone levels which put them at higher risk of adverse birth outcomes including preterm birth (Papp et al. 2016).

Inverse correlation between plasma LPS and progesterone in HIV-infected women

Gestational age, plasma 17 beta estradiol and testosterone levels were not significantly correlated with LPS at any time point (Table S2). However, there were significant inverse correlations between plasma LPS and progesterone in the first ($P = 0.003$) and second trimester ($P = 0.05$) (Table S2). The associations between plasma LPS levels and progesterone suggest that female sex hormones may play a role in regulating mucosal permeability. In addition, estradiol and human chorionic gonadotropin (hCG) may have an effect on mucosal permeability, and subsequently ignite an elevated microbial translocation and systemic inflammation (Herr et al. 2013, Kuruca et al. 2017, Zhou et al. 2017). However, our results imply that progesterone may have the opposite effect as estrogen and hCG on mucosal permeability and microbial translocation during pregnancy, but need further investigation.

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Figure 1.

Plasma levels of LPS and sex hormones in HIV-infected subjects and healthy controls during pregnancy. Plasma levels of LPS at 8–13 weeks gestation (visit 1), 22–24 weeks (visit 2) and 34–36 weeks gestation (visit 3) were analyzed using the limulus amoebocyte lysate QCL-1000 kit (A). Plasma levels of 17 beta estradiol (B), progesterone (C) and testosterone (D) at visit 1, visit 2 and visit 3 were analyzed using ELISA kits according to the manufacturer's instructions. Mann Whitney U test.