Markers of Preterm Delivery in HIV+ Women; Role of Protease Inhibitors and Vitamin D

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INTRODUCTION

Premature delivery (PD is an important cause of maternal and infant morbidity and mortality. Pregnant women living with HIV (WLWH) in the US have an incidence of PD of up to 37%, which is considerably higher than the 13% average in the general population. Rates of PD in the general population vary by geographic area from 4% in Central and Eastern Asia to 18% in Southern Africa. Corresponding numbers in WLWH are 11% and 25%, demonstrating the world-wide correlation between HIV infection and PD.

Systemic and local inflammatory changes are thought to play an important role in the development of PD. Parturition is normally triggered by inflammation, both in the context of term delivery (TD) and spontaneous PD (SPD). However, women with SPD exhibit earlier and exacerbated inflammatory signals during pregnancy compared with women with TD. These signals include multiple inflammatory cytokines, chemokines and other factors that are also increased by HIV infection, suggesting a potential link between HIV infection and SPD.

Several studies have shown associations between the use of protease inhibitors (PI) and increased incidence of SPD, particularly when the PI-containing antiretroviral treatment (ART) is initiated before or during the 1st trimester of pregnancy. The mechanism whereby PIs may increase the risk of SPD is unclear and warrants further investigation.

Vitamin D is an important regulator of the immune response. Vitamin D deficiency, which has been commonly reported in individuals living with HIV, has also been associated with increased activity of inflammatory diseases. Vitamin D potentiates the immunoregulatory effect of estradiol. A study in women without HIV showed that Vitamin D supplementation during pregnancy decreased the incidence of adverse pregnancy outcomes.

This study investigated the association of inflammatory and anti-inflammatory marker plasma concentrations with the risk of SPD in WLWH and with the use of PI-containing ART and vitamin D levels.

PARTICIPANTS AND METHODS

STUDY DESIGN

This was a case-control study of WLWH enrolled in IMPAACT P1025 who had a live singleton birth. Women with delivery occurring at ≤ 35 weeks gestation constituted the cases, and women with delivery occurring ≥ 37 weeks gestation the controls. Cases and controls who had ≥ 2 ml archived plasma, did not take immunosuppressive medication, did not have blood transfusion ≤ 2 weeks before plasma sample collection, and did not have infants with congenital anomalies associated with polyhydramnios were included in the study. For women who had multiple pregnancies in P1025, the most recent eligible pregnancy was retained in the analysis. Controls were matched to cases at a 2:1 ratio on race (Black vs. Non-black) and gestational age at the time of plasma specimen collection (14-20 vs. 21-27 vs. 28-34 weeks gestation).

LABORATORY ASSAYS

Cytokines and chemokines, including TNF alpha, IL-6, IL-8, IL-1 beta, IL-18, G-CSF, MCP-1, IP-10, sIL-2R alpha, sCD14, MMP9, GMC-SF, GRO alpha, IFN gamma, IL-17, VEGF-A, IL-10 and TGF beta were measured with chemoluminescent multiple arrays or ELISA using commercial kits as per manufacturers' instructions.
Prostaglandins, including PG D2, PG E2, PG F2, PG J2, PG Delta-12, PG 15-deoxy D2, PG 8-Isoprostane, 9-HODE, 13-HODE, 5-HEPE, 8-HEPE, 9-HETE, 11-HETE, 12-HETE and 15-HETE were measured by liquid chromatography -tandem mass spectrometry (LC-MS/MS).
250H-Vitamin D was measured by antibody competitive immunoassay using the ADVIA Centaur kit as per manufacturer's instructions.

STATISTICAL ANALYSIS

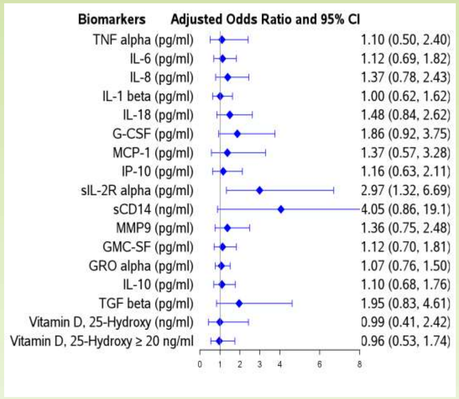
Log10-based transformation was performed for all biomarkers to approximate a normal distribution. Multivariable conditional logistic regression models taking into account the matching effect between cases and controls were built to evaluate the associations of each biomarker concentration with SPD. Weighted linear regression models accounting for the sampling fraction of cases and controls from the underlying eligible P1025 study population were used to evaluate the associations of HIV viral suppression status, time of initiating PI-containing HAART regimen during pregnancy, and the vitamin D plasma concentration with biomarkers which were associated with preterm delivery at an overall p-value ≤ 0.1. Pre-selected covariates were included in each of the multivariable models.

RESULTS

TABLE 1. DEMOGRAPHIC AND HIV DISEASE CHARACTERISTICS OF THE STUDY POPULATION

Characteristica		Total (N=308)	Preterm delivery (N=103)	Term delivery (N=205)
Age (years) at delivery	N	308	103	205
	Mean (s.d.)	28.8 (6.1)	29.0 (6.0)	28.7 (6.1)
	Median (Q1, Q3)	28.4 (23.8, 33.6)	29.1 (24.1, 33.4)	28.4 (23.6, 33.9)
Race: Black	Non-Black	128 (42%)	43 (42%)	85 (41%)
	Black	180 (58%)	60 (58%)	120 (59%)
Hispanic	No	205 (67%)	67 (65%)	138 (67%)
	Yes	101 (33%)	34 (33%)	67 (33%)
	Unknown	2 (1%)	2 (2%)	0 (0%)
Last maternal CDC classification during pregnancy: category C	No	257 (83%)	77 (75%)	180 (88%)
	Yes	51 (17%)	26 (25%)	25 (12%)
Last maternal CD4 at/prior to plasma sample collection: < 350 (cells/mm³)	No	193 (63%)	59 (57%)	134 (65%)
	Yes	115 (37%)	44 (43%)	71 (35%)
Last maternal RNA at/prior to plasma sample collection > 400 (copies/mL)	No	235 (76%)	67 (65%)	168 (82%)
	Yes	73 (24%)	36 (35%)	37 (18%)
Time of initiating PI-containing ARV	1st Trimester or earlier	102 (33%)	38 (37%)	64 (31%)
	2nd/3rd Trimester	145 (47%)	49 (48%)	96 (47%)
	No PI-containing HAART exposure before plasma collection	60 (19%)	15 (15%)	45 (22%)
	Unknown	1 (0%)	1 (1%)	0 (0%)
Gestational age (weeks) at plasma sample collection	14 - 20	8 (3%)	3 (3%)	5 (2%)
	21 - 27	90 (29%)	30 (29%)	60 (29%)
	28 - 34	210 (68%)	70 (68%)	140 (68%)

FIGURE 1: ADJUSTED ASSOCIATIONS OF CYTOKINES, CHEMOKINES BIOMARKERS AND VITAMIN D PLASMA CONCENTRATION LEVELS WITH PRETERM DELIVERY



Separate multivariable models were fit for each of the biomarkers, adjusting for alcohol and illicit drugs use during pregnancy, maternal obesity, maternal sexually transmitted diseases (STD) and other genital infections during pregnancy, maternal Hepatitis B or C during pregnancy, maternal infections other than STD or Hepatitis B/C during pregnancy, last maternal CD4 counts and RNA at/prior to plasma sample collection; missing indicator was included in the model for covariates with >5% - <20% missing data (including alcohol, illicit drug use during pregnancy, and maternal obesity); cigarette use during pregnancy was excluded from the model due to high volume of missing data (20%).
Other non-significant biomarkers which were not shown in plot: IFN gamma, IL-17, VEGF-A.

TABLE 2: ADJUSTED ASSOCIATIONS OF SELECTED BIOMARKERS WITH VIRAL SUPPRESSION, TIME OF INITIATING PI-CONTAINING HAART, AND VITAMIN D

		G-CSF		sIL-2R alpha		sCD14		PG F2 alpha		5-HEPE	
Covariate		Estimated Difference[1] (95% CI)	P-value	Estimated Difference[1] (95% CI)	P-value	Estimated Difference[1] (95% CI)	P-value	Estimated Difference[1] (95% CI)	P-value	Estimated Difference[1] (95% CI)	P-value
Last maternal RNA at/prior to plasma sample collection > 400 (copies/mL)	Yes	-0.12 (-0.24, 0.00)	0.06	0.05 (-0.08, 0.17)	0.46	-0.02 (-0.09, 0.05)	0.57	-0.10 (-0.26, 0.06)	0.23	-0.11 (-0.29, 0.06)	0.20
Time of initiating PI-containing HAART (Ref: 1st Trimester or earlier)	2nd/3rd Trimester	-0.01 (-0.12, 0.10)	0.84	-0.01 (-0.12, 0.10)	0.82	0.02 (-0.04, 0.08)	0.54	-0.11 (-0.25, 0.04)	0.15	-0.23 (-0.39, -0.08)	0.004
	No PI-containing HAART exposure before plasma collection	-0.15 (-0.29, -0.02)	0.03	-0.04 (-0.18, 0.09)	0.51	0.03 (-0.05, 0.10)	0.45	-0.05 (-0.23, 0.12)	0.55	-0.26 (-0.44, -0.07)	0.01
Vitamin D plasma concentration		0.04 (-0.13, 0.21)	0.66	-0.02 (-0.19, 0.15)	0.85	-0.08 (-0.17, 0.01)	0.08	-0.37 (-0.59, -0.15)	< 0.001	0.22 (-0.02, 0.47)	0.07
Vitamin D plasma concentration ≥ 20 ng/ml	Yes	-0.01 (-0.12, 0.10)	0.85	0.07 (-0.04, 0.18)	0.19	-0.06 (-0.12, -0.00)	0.04	-0.16 (-0.30, -0.02)	0.02	0.24 (0.09, 0.40)	0.002

[1] Estimated difference in adjusted mean biomarker concentration level per one unit increase in continuous covariate measures; or estimated difference in adjusted mean biomarker concentration level between participants with vs. without a specific characteristic for categorical covariate.
Weighted linear regression models were used, accounting for the sampling fraction of cases and controls from the underlying P1025 study population.
Biomarker associated with preterm delivery with an overall p-value <= 0.1 were included in above analyses; Log10-based transformation was performed for all biomarkers.
Models for viral suppression included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, last maternal CD4 count at/prior to plasma sample collection, last maternal CDC classification during pregnancy.
Models for time of initiating PI-containing HAART included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, last maternal CD4 count at/prior to plasma sample collection, last maternal CDC classification during pregnancy.
Models for vitamin D concentration included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, last maternal CD4 count at/prior to plasma sample collection, last maternal RNA at/prior to plasma sample collection, last maternal CDC classification during pregnancy.
missing indicator was included in the model for covariates with >5% - <20% missing data (including alcohol, illicit drug use during pregnancy, and maternal obesity); cigarette use during pregnancy was excluded from the model due to high volume of missing data (20%) .
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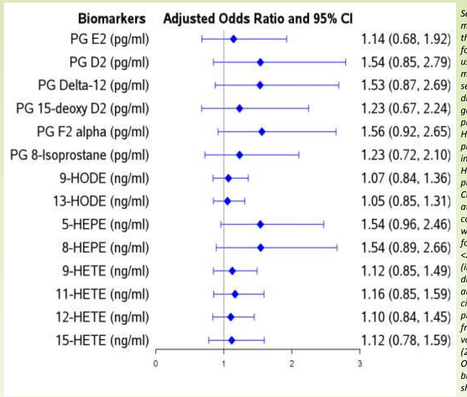
FIGURE 2: ADJUSTED ASSOCIATIONS OF PROSTAGLANDINS BIOMARKER PLASMA CONCENTRATION LEVELS WITH PRETERM DELIVERY



Separate multivariable models were fit for each of the biomarkers, adjusting for alcohol and illicit drugs use during pregnancy, maternal obesity, maternal sexually transmitted diseases (STD) and other genital infections during pregnancy, maternal Hepatitis B or C during pregnancy, maternal infections other than STD or Hepatitis B/C during pregnancy, last maternal CD4 counts and RNA at/prior to plasma sample collection; missing indicator was included in the model for covariates with >5% - <20% missing data (including alcohol, illicit drug use during pregnancy, and maternal obesity); cigarette use during pregnancy was excluded from the model due to high volume of missing data (20%).
Other non-significant biomarkers which were not shown in plot: PG J2.

TABLE 3. ADJUSTED ASSOCIATIONS OF LIPID BIOMARKERS WITH THE TIME OF INITIATING PI-CONTAINING ARV AND 25OH-VITAMIN D

	Time initiating PI-containing ARV (Ref: 1st trimester or earlier)			Vitamin D plasma concentration (continuous)		Vitamin D plasma concentration ≥ 20 ng/ml (Ref: no)		
Biomarker		Estimated Difference[1] (95% CI)	P-value	Estimated Difference (95% CI)	P-value		Estimated Difference[1] (95% CI)	P-value
PG E2	2nd/3rd Trimester	-0.107 (-0.252, 0.037)	0.15	-0.271 (-0.488, -0.054)	0.01	Yes	-0.133 (-0.271, 0.005)	0.06
	No PI-containing HAART exposure before plasma collection	-0.106 (-0.281, 0.068)	0.23					
PG D2	2nd/3rd Trimester	-0.053 (-0.177, 0.072)	0.41	-0.312 (-0.499, -0.124)	0.001	Yes	-0.256 (-0.373, -0.139)	< 0.001
	No PI-containing HAART exposure before plasma collection	-0.109 (-0.260, 0.041)	0.16					
PG Delta-12	2nd/3rd Trimester	-0.046 (-0.175, 0.083)	0.48	-0.212 (-0.407, -0.016)	0.03	Yes	-0.180 (-0.302, -0.057)	0.004
	No PI-containing HAART exposure before plasma collection	-0.128 (-0.283, 0.028)	0.11					
PG 15-deoxy D2	2nd/3rd Trimester	-0.027 (-0.145, 0.091)	0.65	0.016 (-0.167, 0.199)	0.87	Yes	0.022 (-0.094, 0.137)	0.71
	No PI-containing HAART exposure before plasma collection	-0.081 (-0.222, 0.061)	0.27					
PG F2 alpha	2nd/3rd Trimester	-0.105 (-0.249, 0.039)	0.15	-0.369 (-0.587, -0.150)	< 0.001	Yes	-0.163 (-0.302, -0.023)	0.02
	No PI-containing HAART exposure before plasma collection	-0.053 (-0.227, 0.121)	0.55					
PG 8-isoprostane	2nd/3rd Trimester	-0.064 (-0.199, 0.071)	0.35	-0.287 (-0.491, -0.082)	0.01	Yes	-0.191 (-0.320, -0.061)	0.004
	No PI-containing HAART exposure before plasma collection	0.015 (-0.148, 0.177)	0.86					
9-HODE	2nd/3rd Trimester	-0.318 (-0.610, -0.027)	0.03	0.206 (-0.245, 0.657)	0.37	Yes	0.233 (-0.051, 0.518)	0.11
	No PI-containing HAART exposure before plasma collection	-0.217 (-0.569, 0.134)	0.23					
13-HODE	2nd/3rd Trimester	-0.345 (-0.665, -0.025)	0.03	0.226 (-0.269, 0.720)	0.37	Yes	0.268 (-0.044, 0.579)	0.09
	No PI-containing HAART exposure before plasma collection	-0.204 (-0.590, 0.182)	0.30					
5-HEPE	2nd/3rd Trimester	-0.233 (-0.390, -0.076)	0.004	0.224 (-0.020, 0.468)	0.07	Yes	0.245 (0.092, 0.397)	0.002
	No PI-containing HAART exposure before plasma collection	-0.255 (-0.444, -0.066)	0.01					
8-HEPE	2nd/3rd Trimester	-0.141 (-0.268, -0.013)	0.03	0.186 (-0.010, 0.382)	0.06	Yes	0.157 (0.033, 0.280)	0.01
	No PI-containing HAART exposure before plasma collection	-0.149 (-0.303, 0.004)	0.06					
9-HETE	2nd/3rd Trimester	-0.262 (-0.509, -0.015)	0.04	0.162 (-0.220, 0.544)	0.41	Yes	0.165 (-0.076, 0.406)	0.18
	No PI-containing HAART exposure before plasma collection	-0.172 (-0.470, 0.126)	0.26					
11-HETE	2nd/3rd Trimester	-0.238 (-0.459, -0.017)	0.03	0.089 (-0.254, 0.431)	0.61	Yes	0.091 (-0.126, 0.307)	0.41
	No PI-containing HAART exposure before plasma collection	-0.144 (-0.410, 0.123)	0.29					
12-HETE	2nd/3rd Trimester	-0.324 (-0.571, -0.078)	0.01	0.086 (-0.296, 0.468)	0.66	Yes	0.191 (-0.050, 0.432)	0.12
	No PI-containing HAART exposure before plasma collection	-0.187 (-0.484, 0.111)	0.22					
15-HETE	2nd/3rd Trimester	-0.223 (-0.416, -0.029)	0.02	0.130 (-0.170, 0.431)	0.39	Yes	0.147 (-0.042, 0.337)	0.13
	No PI-containing HAART exposure before plasma collection	-0.167 (-0.401, 0.067)	0.16					

[1] Estimated difference in adjusted mean biomarker concentration level per one unit increase in continuous exposure measurements; or estimated difference in adjusted mean biomarker concentration level between participants with vs. without a specific characteristic for categorical exposure measurements
Weighted linear regression models were used, accounting for the sampling fraction of cases and controls from the underlying P1025 study population.
Log10-based transformation was performed for all biomarkers.
Models for time of initiating PI-containing HAART included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, last maternal CD4 count at/prior to plasma sample collection, last maternal CDC classification during pregnancy
Models for vitamin D concentration included race, age at delivery, alcohol/illicit drug use during pregnancy, maternal BMI, maternal acute or chronic infections at the time of plasma collection, maternal vaccination received within 14 days before plasma collection, maternal STDs and other genital infections during pregnancy, last maternal RNA at/prior to plasma sample collection, last maternal CDC classification during pregnancy.
Bold font indicates significant or marginally significant associations with markers of spontaneous preterm delivery.

CONCLUSIONS

- sIL2Ralpha plasma concentration during pregnancy was the strongest predictor of SPD in WLWH.
- With the exception of having detectable HIV VL, which was higher in cases compared with controls, HIV disease characteristics, including CD4 cell numbers, did not differ between cases and controls.
- This study had a relatively low number of participants, which may have accounted for our inability to find any differences in the use of PI-containing ART or 25-OH Vitamin D levels in this study.
- Use of PI and 25OH-Vitamin D levels were associated with the levels of prostaglandins, suggesting a potential mechanism to affect the risk of SPD by interfering with inflammation and direct stimulation of uterine contractions.

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