No association between early antiretroviral therapy during pregnancy and plasma levels of angiogenic factors: a cohort study

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Abstract

Background: Early antiretroviral therapy (ART) during pregnancy has dramatically reduced the risk of perinatal HIV transmission. However, studies have shown an association between premature delivery and the use of ART during pregnancy (particularly protease inhibitor (PI)-based therapies), which could be explained by placental dysfunction. The objective of this study was to evaluate the association of ART (class, duration of exposure and time of initiation) with placental function by using angiogenic factors placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) as biomarkers. Methods: Clinical and biological data from 159 pregnant women living with HIV were analyzed. Levels of each biomarker were measured in the first and second trimester of pregnancy. After logarithmic transformation, we compared these using generalized estimating equations according to (a) the type of ART; (b) the duration of exposure to ART; and (c) the time of initiation of ART. Results: After adjusting for variables such as ethnicity, maternal age, gestational age, body mass index, parity, smoking status, and sex of the fetus, we found no significant association between the class of ART (PI-based or not) and serum concentrations of PlGF or sFlt-1. Furthermore, no significant association was found between biomarker levels and the duration of ART exposure or the timing of ART initiation (pre- or post-conception). Conclusions: This study suggests that first and second trimester angiogenic factor levels are not significantly associated with ART, regardless of the duration or type (with or without PI). These observations seem reassuring when considering the use of ART during early pregnancy.

Background

An estimated 17.8 million of the 36.7 million people living with HIV in 2016 (48.5%) were women of childbearing age (15 years and older).(1) Despite their HIV status, many of these women want to have children.(2) Antiretroviral therapy (ART) reduces the risk of HIV transmission from mother to child to 1-2%, compared to 15-40% without intervention.(3) ART also improves the health status of the mothers, raises their quality of life and prolongs their life expectancy, leading more and more women living with HIV to conceive.(2) According to current recommendations, all adults living with HIV should be treated with ART.(4-7) As a result, most women living with HIV are now already on ART at the time of conception and early pregnancy, whereas in the past, ART was initiated only in the second trimester of pregnancy.(8)

Although ART has significantly reduced rates of adverse perinatal outcomes such as stillbirth, intrauterine growth restriction and preterm birth associated with maternal HIV infection,(9, 10) these rates are still higher for women living with HIV while receiving ART compared to HIV-negative women.(11-14) These adverse pregnancy outcomes may be related to the class of ART used during pregnancy, notably to protease inhibitor (PI) regimens, (14-18) which have been associated with placental vascular changes(19).

Anti-angiogenic effects have been reported following the use of PIs in oncology.(20, 21) Placental growth factor (PlGF) and the soluble receptor, soluble fms-like tyrosine kinase-1 (sFlt-1) are respectively pro- and anti-angiogenic factors. PlGF is synthesized in several organs (heart, skeletal muscle, lungs, adipose

tissue, platelets). sFlt-1 is released by vascular endothelial cells and circulating cells (monocytes, macrophages, platelets). The placenta (trophoblast and endothelial cells of the placental villi) is the main source of PlGF and sFlt-1 during pregnancy; they participate in vasculogenesis and feto-placental angiogenesis. Increased sFlt-1 and decreased free PlGF levels in maternal blood, or increased sFlt-1/ PlGF ratio are directly implicated in the pathophysiology of preeclampsia, including maternal endothelial dysfunction.(22-29) A change in these factors is also associated with other complications during pregnancy, such as intrauterine growth restriction,(27, 30) preterm delivery,(31) spontaneous abortion and stillbirth,(26, 32, 33), confirming the association between impaired placental perfusion and systemic changes in angiogenic factors.(34-36) These observations have been noted in cohorts of women living with HIV.(37)

We hypothesized that pregnant women living with HIV who receive PI-based ART may have impaired placental function compared to untreated women living with HIV, altering the plasma concentration of the angiogenic factors placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). These disturbances could be influenced by the duration of ART exposure or by the timing of ART initiation. The objective of this study was to evaluate the association of the class, duration and timing of initiation of ART with serum concentrations of PlGF and sFlt-1 biomarkers, in the first and second trimesters of pregnancy.

Methods

This study used data from the database of pregnant women living with HIV who were consented and enrolled at their first antenatal visit in the prospective cohort of Centre maternel et infantile sur le sida (CMIS), CHU Sainte-Justine, Montreal, QC, Canada. The CMIS database and biobank contain information and biologic samples from more than 900 mother-child pairs and enrolls approximately 40 additional pairs annually. De-identified data are collected and managed using REDCap electronic data capture tools. (38)

This study included data from pregnant women who were enrolled between January 2003 and December 2016, and for whom serum samples were available during both the first trimester (5-14 weeks) and the second trimester (15-28 weeks) of pregnancy. Maternal serum samples were collected at the time of clinically indicated blood tests and stored at -80°C for research purpose. Levels of PlGF and sFlt-1 were measured using DuoSet Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D systems, Minneapolis, MN). Lower limits of detection were 15.6 pg/ml (sFlt-1) and 62.5 pg/ml (PlGF). There was no dilution for PlGF and it is a factor of 5 for sFLT-1. In all cases, both biomarkers were assayed using the same serum aliquot.

Gestational age was defined based on the crown-rump length from the first-trimester ultrasound if available and if not, from the date of the last menstrual period. Preterm birth was defined as delivery *before* 37 weeks of gestation. For gestational age, *small* (SGA) was defined as a birth weight below the 10th percentile for the gestational age.

Women living with HIV were categorized according to ART exposure at first and second trimester (PI-based ART, other ART, or no treatment). The duration of ART exposure during pregnancy was expressed in *weeks since conception*. Time of initiation of ART was defined relative to *conception* (either before conception or during pregnancy).

Statistical analysis

Descriptive analyses were conducted on the socio-demographic, clinical and biological data of the participants. For each categorical and continuous variable, data are reported as proportions or mean (with standard deviation) or median with interquartile range (IQR) respectively. The Wilcoxon test for matched samples was used to compare serum marker concentrations in the first and second trimesters and Mann-Withney U test to compare angiogenic factor levels in the two groups with undetectable viral load or not.

Linear regression evaluated the association between angiogenic factor concentrations and birth outcome groups (preterm birth and SGA) at the first and second trimester. To account for repeated measurements from the same individuals in the first and second trimesters of pregnancy, linear generalized estimating equations (GEE) were used to analyze the association between ART (class, duration of exposure and initiation time) and plasma concentration of the two biomarkers. A first-order autoregressive (AR1) correlation matrix was used. Models were adjusted for potential confounding factors previously identified in a review of the literature, including ethnicity, parity, maternal age, gestational age, body mass index (BMI), smoking status and sex of the fetus.(39-43) Confounding variables that resulted in a +/- 10% variation of the regression coefficient when introduced into the bivariate model were retained in the final model. All variables with a $p<0.05$ were also included in the final model. As suggested by residual analysis, a logarithmic transformation of biomarkers levels was performed. Using sensitivity analyses, we considered models with different unstructured or exchangeable correlation matrices and compared these models using Quasi-likelihood under Independence Model Criterion (QIC). A value of $p<0.05$ was considered statistically significant. 95% confidence intervals are shown. Considering the values of PlGF and sFlt-1 (mean and standard deviation) obtained in the reference group (group without ART), we calculated that with the available sample size (n = 159), we could have been able to detect respective mean (delta) differences of 33.67 and 1905.15 pg / ml using an alpha error of 0.05 and a statistical power (beta) of 80%. Statistical analyses were performed using IBM SPSS Statistics for Windows version 24 (IBM Corp, Armonk, NY).

This study was approved by the Research Ethics Committee of CHU Sainte-Justine. All participants provided written informed consent.

Results

A total of 318 paired serum samples from 159 pregnant women living with HIV were analyzed. Demographic, clinical and biological characteristics of the participants are presented in Table 1. In the first trimester, nearly 69% of women were receiving ART, 82% of whom were receiving PI-based regimens.

In the second trimester, more than 96% were receiving ART, 86% of which were PI-based. The mean duration of ART exposure from the start of pregnancy to first-trimester and second-trimester biomarker testing was 9.5 ± 2.9 weeks and 14.8 ± 7.1 weeks, respectively.

Table 1. Population characteristics

cteristics	1st trimester n=159			2nd trimester n=159			Total n=159
	No ART n=50	PI n=89	Other regimens n=20	No ART n=6	PI n=131	Other regimens n= 22	
nal age at y, years, mean	31.7± 5.3	33.1± 4.9	31.3± 5.0	29.7± 3.0	32.5± 5.1	32.3± 5.4	32.4 ± 5.1
ity, n (%)							
o-Caribbean	43 (86.0)	69 (77.5)	18 (90.0)	6 (100.0)	103 (78.6)	21 (95.5)	130 (81.8)
ıcasian	7 (14.0)	17 (19.1)	1 (5.0)	0 (0.0)	24 (18.3)	1 (4.5)	25 (15.7)
ıer	0 (0.0)	3 (3.4)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.5)
:g/m^2, median	27.2 [24.9-31.0]	25.8 [22.8-29.9]	26.5 [22.9-30.3]	28.2 [24.4-34.9]	26.4 [23.6-30.4]	24.9 [23.2-28.7]	26.3 [23.5-30.2]
, n (%)							
	15 (30.0)	31 (34.8)	6 (30.0)	2 (33.3)	41 (31.3)	9 (40.9)	52 (32.7)
	18 (36.0)	31 (34.8)	10 (50.0)	4 (66.7)	45 (34.4)	10 (45.5)	59 (37.1)
	12 (24.0)	14 (15.7)	1 (5.0)	0 (0.0)	26 (19.8)	1 (4.5)	27 (17.0)
d more	5 (10)	13 (14.7)	3 (15.0)	0 (0.0)	19 (14.5)	2 (9.1)	21 (13.2)
er, n (%)	6 (12.0)	8 (9.0)	1(5.0)	0 (0.0)	6 (4.6)	0 (0.0)	
ic hypertension,	2 (4.0)	1 (1.1)	0 (0.0)	0 (0.0)	3 (2.3)	0 (0.0)	3 (1.9)
ional ension or ampsia, n (%)	4 (8.0)	9 (10.1)	2 (10.0)	1 (16.7)	11 (7.2)	3 (13.6)	15 (9.4)
fetus, n (%)							
le	25 (50.0)	53 (59.6)	4 (20.0)	1 (16.7)	76 (58.0)	5 (22.7)	82 (51.6)
ount[a], median							
:al/mm^3	495.5 [270.0-679.5]	573.5 [404.25-751.5]	580.5 [372.5-733.5]	493.5 [193.5-859.5]	561.0 [378.5-722.5]	645.0 [470.75-1056.0]	
·centage	25.0 [16.0-33.0]	32.0 [25.0-38.5]	33.5 [23.0-43.5]	26.0 [17.0-37.0]	31.0 [24.0-39.0]	40.0 [27.0-44.0]	
ectable viral n (%)	4 (8.0)	72 (80.9)	16 (80.0)	2 (33.3)	94 (71.8)	19 (86.4)	
ancy outcome, n							
A	8 (16.0)	19 (21.3)	2 (10.0)	1 (16.7)	26 (19.8)	2 (9.1)	29 (18.6)

term birth	6 (12.0)	17 (19.1)	2 (10.0)	0 (0.0)	22 (16.8)	3 (13.6)	25 (15.7)
ionnal age* , median [IQR]	9.7 [7.9-11.9]	10.7 [8.9-11.9]	10.7 [7.9-11.8]	18.3 [15.8-19.1]	19.6 [17.1-21.3]	19.3 [17.1-23.0]	

andard deviation; BMI: body mass index; IQR: interquartile range; ART: roviral therapy; SGA: small for gestational age
sampling

The median concentration of PlGF in the first trimester was 93.5 pg/ml [IQR = 74.2-129.0] compared to 229.0 pg/ml [IQR = 154.8-329.0] in the 2nd trimester (p <0.0001). The median concentration of sFlt-1 in the first trimester was 3372.7 pg/ml [IQR = 1736.7-5781.8] compared to 4009.1 pg/ml [IQR = 2600.0 - 8236.4] in the second trimester (p = 0.006). The angiogenic factors levels are similar in the two groups with undetectable viral load or not in the first trimester (respectively 93.5 pg/ml [IQR = 74.2-125.0] compared to 91.9 pg/ml [IQR = 77.4-145.2]; p= 0.752 for PlGF and 3668.2 pg/ml [IQR = 1452.3-7509.1] compared to 2850.0 pg/ml [IQR = 1963.6 - 5554.5]; p= 0.194 for sFlt-1) and in the second trimester (respectively 229.0 pg/ml [IQR = 148.4-322.6] compared to 240.3 pg/ml [IQR = 177.4-392.7]; p= 0.328 for PlGF and 4145.5 pg/ml [IQR = 2600.0-10190.9] compared to 3918.2 pg/ml [IQR = 2611.4 - 6725.0]; p= 0.659 for sFlt-1).

Figure 1 shows the distribution of the different biomarkers according to the class of ART and figure 2 the distribution by time of ART initiation.

Bivariate comparisons are presented in Table 2. After adjustment, no significant association was found between the class of ART (whether PI-based or not) and the level of PlGF, sFlt-1 or the PlGF/SFlt-1 ratio (Table 2). Furthermore, no significant association was found between serum angiogenic factors and duration of ART exposure (including all PI-containing ART) or initiation time (pre-conception versus during pregnancy) (Table 2).

Table 2. Association between biomarkers and ART (type, duration and initiation time)

rkers	Crude coefficient	CI (95%)	p-value	Adjusted coefficient*	Adjusted CI (95%)	p-value
ased ART[a]	0.238	0.166 - 0.310	0.000	0.018[b]	-0.051 - 0.088	0.611
r ART[a]	0.201	0.095 - 0.307	0.000	0.028[b]	-0.064 - 0.121	0.552
exposure on	0.020	0.014 - 0.026	0.000	0.001[c]	-0.006 - 0.007	0.873
xposure on	0.018	0.011 - 0.025	0.000	0.000[c]	-0.007 - 0.006	0.936
e of ART on[d]	-0.019	-0.089 - 0.051	0.592	0.000[c]	-0.066 - 0.066	0.997
ased ART[a]	0.306	0.109 - 0.503	0.002	0.177[e]	-0.032 - 0.386	0.097
r ART[a]	0.233	-0.108 - 0.573	0.180	0.091[e]	-0.246 - 0.428	0.597
exposure on	0.003	-0.008 - 0.015	0.584	-0.001[f]	-0.014 - 0.012	0.872
xposure on	0.007	-0.004 - 0.019	0.218	0.002[f]	-0.010 - 0.015	0.722
e of ART on[d]	0.085	-0.074 - 0.244	0.294	0.024 [g]	-0.139 - 0.188	0.772
PlGF Ratio						
ased ART[a]	0.033	-0.179 - 0.245	0.759	0.145[h]	-0.076 - 0.366	0.199
r ART[a]	-0.036	-0.386 - 0.314	0.840	0.063[h]	-0.319 - 0.445	0.747
exposure on	-0.014	-0.028 - 0.000	0.044	0.000[e]	-0.018 - 0.017	0.956
xposure on	-0.007	-0.021 - 0.006	0.299	0.003[g]	-0.013 - 0.019	0.683
e of ART on[d]	0.104	-0.071 - 0.279	0.243	0.028[g]	-0.152 - 0.208	0.761

tiretroviral therapy; PI: protease inhibitor; CI: confidence interval

r Generalized Estimating Equations

ol: no ART

ed for gestational age at the date of test and ethnicity.

ed for gestational age at the date of test, body mass index and ethnicity

ol: ART initiated during pregnancy

ed for gestational age at the date of test and body mass index.

ed for gestational age at the date of test and maternal age.

ed for gestational age at the date of test, maternal age and ethnicity

ed for gestational age at the date of test, maternal age, body mass index, parity, and sex of the fetus.

The association between angiogenic factor concentrations and birth outcomes (preterm birth and SGA) was further evaluated (Table 3). After adjustment, significantly lower concentrations of sFlt-1 and sFlt/PlGF ratio at the first trimester and significantly lower concentrations of PlGF at the second trimester were seen in SGA cases than in normal weight cases. No significant association between angiogenic factor concentration and preterm birth was observed.

Table 3. Association between biomarkers and adverse birth outcomes

Biomarkers	Crude coefficient	CI (95%)	p-value	Adjusted coefficient*	Adjusted CI (95%)	p-value
First trimester						
PlGF						
SGA	-0.006	-0.109 - 0.097	0.904	-0.023[a]	-0.130 - 0.085	0.980
Preterm birth	0.054	-0.046 - 0.154	0.290	0.056[b]	-0.043 - 0.155	0.266
sFlt1						
SGA	-0.192	-0.384 - 0.001	0.051	-0.260[c]	-0.432 - -0.088	0.003
Preterm birth	0.090	-0.100 - 0.280	0.350	0.064[c]	-0.106 - 0.233	0.458
sFlt1/PlGF Ratio						
SGA	-0.184	-0.415 - 0.048	0.119	-0.227[c]	-0.437 - -0.016	0.035
Preterm birth	0.035	-0.193 - 0.264	0.760	0.002[d]	-0.207 - 0.211	0.986
Second trimester						
PlGF						
SGA	-0.096	-0.220 - 0.028	0.127	-0.117[e]	-0.225 - -0.010	0.033
Preterm birth	-0.071	-0.193 - 0.050	0.248	-0.006[f]	-0.113 - 0.100	0.909
sFlt1						
SGA	0.007	-0.147 - 0.161	0.930	0.048[g]	-0.121 - 0.216	0.576
Preterm birth	0.072	-0.082 - 0.226	0.920	-0.077[h]	-0.083 - 0.237	0.342
sFlt1/PlGF Ratio						
SGA	0.107	-0.097 - 0.306	0.291	0.162[i]	-0.038 - 0.362	0.111
Preterm birth	0.132	-0.067 - 0.331	0.193	0.013[j]	-0.193 - 0.218	0.903

ART: antiretroviral therapy; CI: confidence interval; SGA: small for gestational age

*Linear regression

[a] Adjusted for gestational age at the date of test, maternal age, body mass index, parity, sex of the fetus and ART.

[b] Adjusted for gestational age at the date of test and ART.

[c] Adjusted for gestational age at the date of test, body mass index, sex of the fetus and ART.

[d] Adjusted for gestational age at the date of test, body mass index, ethnicity, parity, sex of the fetus and ART.

[e] Adjusted for gestational age at the date of test, maternal age, body mass index, ethnicity, parity, and ART.

[f] Adjusted for gestational age at the date of test, body mass index, ethnicity, parity, and ART.

[g]Adjusted for gestational age at the date of test, maternal age, body mass index, ethnicity, parity, smoking, sex of the fetus and ART.
[h]Adjusted for gestational age at the date of test, maternal age, smoking, sex of the fetus and ART.
[i]Adjusted for gestational age at the date of test, maternal age, body mass index, ethnicity, parity, smoking and ART.
[j]Adjusted for gestational age at the date of test, maternal age, body mass index, smoking, sex of the fetus and ART.

Discussion

In this cohort study, first and second trimester angiogenic factor concentrations are not significantly associated with ART exposure of any type and duration, nor are they associated with the timing of treatment initiation (pre-conception or during pregnancy). The biomarker levels observed among women living with HIV appear to be similar to those reported in HIV-negative women in other studies.(44-48) The strong association of these angiogenic factor concentrations with gestational age and other baseline data (BMI, ethnicity) is consistent with data from HIV-negative pregnant women.(48, 49) This explains why the significant differences between ART exposure groups in the unadjusted analyses were not confirmed in the multivariate analyses (Table 2). The association between angiogenic factor concentrations and adverse pregnancy outcomes is consistent with reports in HIV-negative women (27, 30) and in women living with HIV.(37)

The absence of an HIV-negative control group in this study is a limitation that prevents us from assessing the impact of HIV infection itself on angiogenic factors and whether the infection itself can lead to dysregulation of angiogenesis. However, it seems unlikely that viral activity has much of an influence on angiogenic factor concentrations when we consider the similarity of levels in the untreated group (with a detectable viral load) compared to the treated one. The heterogeneity of ART regimens received by women is another limitation of our study (see Table, Additional file 1, which illustrates the different nucleoside reverse transcriptase inhibitors in the ART). As drug use was very rare in our cohort (<1%), we could not evaluate the impact of this factor on placental angiogenic factors. However, repeated measurements in the first and second trimester increased the number of observations, giving us a greater statistical power. Measurements of the levels of angiogenic factors were all performed on the same day to minimize inter-assay variability. We also attempted to eliminate bias with a conservative method of adjustment for potential confounding factors.

Very few studies have explored the relationship between ART and the serum concentration of these angiogenic factors during pregnancy. Studies in oncology, however, have suggested anti-angiogenic effects for some PIs.(20, 21) The effects of PIs on placental vascular system formation and fetal development have to date only been examined in a mouse model.(50) Mice exposed to ART had significantly smaller fetuses and placentas compared to controls. Litter size and fetal viability were negatively impacted by exposure to two nucleoside analogs and two PIs at doses equivalent to human therapeutic doses. Although PlGF levels were unchanged, significantly lower levels of placental sFlt-1 were noted.(19, 50)

Lower PlGF levels were reported in South Africa amongst pregnant women living with HIV, compared to uninfected pregnant women, whether or not they were preeclamptic.(51) However, the sample size in that study was small (27 HIV-negative women and 31 women living with HIV) and the authors did not specify if the subjects were receiving ART. By contrast, Govender et al. reported no relationship between HIV infection and angiogenic factors measured in the third trimester of pregnancy, but provided no details concerning ART exposure.(25)

Even though our study is the first to report data on periconceptionnal and first trimester ART exposure, our results are consistent with those of a recent study in Uganda of 326 pregnant women living with HIV who began receiving ART in the second trimester. That study reported no significant difference in the levels of angiogenic factors according to the class of ART (PI vs non-nucleoside reverse transcriptase inhibitor (NNRTI).(37) Similarly, another study of a cohort of 71 women whose ART was initiated after 26 weeks of pregnancy also found no association between the duration of ART exposure or changes in angiogenic factor concentrations.(52) The authors reported no significant changes in angiogenic marker levels after one month of ART, and no significant differences in serum concentration of sFlt-1 or PlGF relating to the type of ART (NNRTI versus PI).

To our knowledge, our study constitutes the first report on first-trimester angiogenic marker levels in a cohort of women who received early exposure to ART during pregnancy. Our results are consistent with reports on later ART initiation in pregnancy.(37) These finding needs to be confirmed by the study of circulating levels of other angiogenic biomarkers as well as direct study of the early placenta vasculature. If confirmed, even though angiogenic processes in the placenta are critical regulators of fetal growth and impact birth outcomes, the pathophysiological mechanism of the association between ART and preterm birth would unlikely be through an early and direct effect on placental angiogenesis. Immune restoration as a result of ART initiation is a hypothesis that needs to be explored.(53) Indeed, a study from the United Kingdom suggests that ART-induced immune reconstitution plays a central role in the pathogenesis of pre-eclampsia in pregnant, women living with HIV receiving ART. HIV infection could be associated with a low risk of preeclampsia and this risk restored to the expected values in women treated with ART.(53)

Conclusions

This study suggests that ART, whether PI-based or not, is not associated with the serum concentration of angiogenic factors PlGF and sFlt-1 in the first and second trimesters of pregnancy. There is also no significant association with duration of treatment or timing of treatment initiation (before conception or during pregnancy). These observations seem generally reassuring for the potential consequences of early ART use during pregnancy. Further studies are needed to confirm the safety of early ART exposure regarding placental angiogenesis and its implications for adverse pregnancy outcomes, especially considering the rapid evolution of ART guidelines.

Abbreviations

ART: antiretroviral therapy

PlGF: placental growth factor

sFlt-1: soluble fms-like tyrosine kinase-1

PI: protease inhibitor

CMIS: Centre maternel et infantile sur le sida

ELISA: Enzyme-Linked Immunosorbent Assay

SGA: small for gestational age

IQR : interquartile range

GEE: generalized estimating equations

BMI: body mass index

NNRTI: non-nucleoside reverse transcriptase inhibitor

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of CHU Sainte-Justine. All participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due to restrictions associated with anonymity of participants but are available from the corresponding author on reasonable request.

Competing interests

IB, HT and FK were the recipients of salary awards (chercheur-boursier) from FRQ-S. HT was the recipient of a New Investigator salary award from the Canadian Institutes of Health Research (CIHR). The authors declare that they have no competing interests.

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Authors' contributions

AD performed the quantitative analysis of the data and was a major contributor in writing the manuscript. SG performed the PlGF and Sflt-1measurements and provided expertise on placental inflammation in the interpretation of results. HT contributed to the development of methods and the statistical analysis of data. IB designed the research protocol, supervised the analysis and interpretation of the results. All co-authors provided suggestions and comments throughout the project, reviewed the manuscript and approved the final version.

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References

1. UNAIDS. Fact sheet - Latest global and regional statistics on the status of the AIDS epidemic. 2017 [Available from: http://www.unaids.org/en/resources/documents/2017/UNAIDS_FactSheet.
2. Loutfy M, Kennedy VL, Poliquin V, Dzineku F, Dean NL, Margolese S, et al. No. 354-Canadian HIV Pregnancy Planning Guidelines. Journal of Obstetrics and Gynaecology Canada. 2018;40(1):94-114.
3. Siegfried N, van der Merwe L, Brocklehurst P, Sint TT. Antiretrovirals for reducing the risk of mother-to-child transmission of HIV infection. Cochrane Database of Systematic Reviews 2011(7):CD003510.
4. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents: Department of Health and Human Services; 2019 [updated July 10, 2019. Available from: https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.
5. World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV: World Health Organization; 2015 [Available from: https://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/
6. Lundgren J, Gatell J, Rockstroh J, Furrer H. EACS Guidelines, version 8.2 2015 [Available from: http://www.eacsociety.org/guidelines/guidelines-archive/archive.html.
7. Waters L, Ahmed N, Angus B, Boffito M, Bower M, Churchill D, et al. BHIVA guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2015 (2016 interim update) 2016

[Available from: https://www.bhiva.org/file/RVYKzFwyxpgiI/treatment-guidelines-2016-interim-update.pdf

8. Burdge DR, Money DM, Forbes JC, Walmsley SL, Smaill FM, Boucher M, et al. Canadian consensus guidelines for the management of pregnant HIV-positive women and their offspring. CMAJ. 2003;168(13):1671-4.
9. Brocklehurst P, French R. The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 1998;105(8):836-48.
10. Wedi CO, Kirtley S, Hopewell S, Corrigan R, Kennedy SH, Hemelaar J. Perinatal outcomes associated with maternal HIV infection: a systematic review and meta-analysis. The Lancet HIV. 2016;3(1):e33-e48.
11. Ekouevi DK, Coffie PA, Becquet R, Tonwe-Gold B, Horo A, Thiebaut R, et al. Antiretroviral therapy in pregnant women with advanced HIV disease and pregnancy outcomes in Abidjan, Cote d'Ivoire. Aids. 2008;22(14):1815-20.
12. Townsend CL, Cortina-Borja M, Peckham CS, Tookey PA. Antiretroviral therapy and premature delivery in diagnosed HIV-infected women in the United Kingdom and Ireland. Aids. 2007;21(8):1019-26.
13. Newell M-L, Bunders MJ. Safety of antiretroviral drugs in pregnancy and breastfeeding for mother and child. Current Opinion in HIV and AIDS. 2013;8(5):504-10.
14. Zash R, Jacobson DL, Diseko M, et al. Comparative safety of antiretroviral treatment regimens in pregnancy. JAMA Pediatrics. 2017;171(10):e172222.
15. Lopez M, Figueras F, Hernandez S, Lonca M, Garcia R, Palacio M, et al. Association of HIV infection with spontaneous and iatrogenic preterm delivery: effect of HAART. Aids. 2012;26(1):37-43.
16. Li N, Sando MM, Spiegelman D, Hertzmark E, Liu E, Sando D, et al. Antiretroviral therapy in relation to birth outcomes among HIV-infected women: a cohort study. Journal of Infectious Diseases. 2015;213(7):1057-64.
17. Alemu FM, Yalew AW, Fantahun M, Ashu EE. Antiretroviral Therapy and Pregnancy Outcomes in Developing Countries: A Systematic Review. International journal of MCH and AIDS. 2015;3(1):31-43.
18. Powis KM, Kitch D, Ogwu A, Hughes MD, Lockman S, Leidner J, et al. Increased Risk of Preterm Delivery Among HIV-Infected Women Randomized to Protease Versus Nucleoside Reverse Transcriptase Inhibitor-Based HAART During Pregnancy. The Journal of Infectious Diseases. 2011;204(4):506-14.
19. Mohammadi H, Papp E, Cahill L, Rennie M, Banko N, Pinnaduwage L, et al. HIV antiretroviral exposure in pregnancy induces detrimental placenta vascular changes that are rescued by progesterone supplementation.
20. Pore N, Gupta AK, Cerniglia GJ, Maity A. HIV Protease Inhibitors Decrease VEGF/HIF-1α Expression and Angiogenesis in Glioblastoma Cells. Neoplasia. 2006;8(11):889-95.

21. Sgadari C, Barillari G, Toschi E, Carlei D, Bacigalupo I, Baccarini S, et al. HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi sarcoma. Nature Medicine. 2002;8:225.
22. Lecarpentier E, Vieillefosse S, Haddad B, Fournier T, Leguy M-C, Guibourdenche J, et al. Le facteur de croissance placentaire (PlGF) et son récepteur soluble (sFlt-1) au cours de la grossesse: physiologie, dosage et intérêt dans la préeclampsie. Annales de Biologie Clinique. 2016;74(3):259-67.
23. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. New England Journal of Medicine. 2004;350(7):672-83.
24. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble Endoglin and Other Circulating Antiangiogenic Factors in Preeclampsia. New England Journal of Medicine. 2006;355(10):992-1005.
25. Govender N, Naicker T, Rajakumar A, Moodley J. Soluble fms-like tyrosine kinase-1 and soluble endoglin in HIV-associated preeclampsia. European Journal of Obstetrics, Gynecology, and Reproductive Biology 2013;170(1):100-5.
26. Chaiworapongsa T, Romero R, Korzeniewski SJ, Kusanovic JP, Soto E, Lam J, et al. Maternal plasma concentrations of angiogenic/antiangiogenic factors in the third trimester of pregnancy to identify the patient at risk for stillbirth at or near term and severe late preeclampsia. American Journal of Obstetrics & Gynecology. 2013;208(4):287.e1-.e15.
27. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, et al. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. The Journal of Maternal-Fetal & Neonatal Medicine. 2008;21(1):9-23.
28. Baltajian K, Bajracharya S, Salahuddin S, Berg AH, Geahchan C, Wenger JB, et al. Sequential plasma angiogenic factors levels in women with suspected preeclampsia. American Journal of Obstetrics & Gynecology. 2016;215(1):89.e1-.e10.
29. Holme AM, Roland MCP, Henriksen T, Michelsen TM. In vivo uteroplacental release of placental growth factor and soluble Fms-like tyrosine kinase-1 in normal and preeclamptic pregnancies. American Journal of Obstetrics & Gynecology. 2016;215(6):782.e1-.e9.
30. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: Evidence for abnormal placental angiogenesis in pathologic pregnancies. American Journal of Obstetrics & Gynecology. 2003;188(1):177-82.
31. Mijal RS, Holzman CB, Rana S, Karumanchi SA, Wang J, Sikorskii A. Mid-pregnancy levels of angiogenic markers as indicators of pathways to preterm delivery. Journal of Maternal-Fetal & Neonatal Medicine. 2012;25(7):1135-41.
32. Romero R, Chaiworapongsa T, Erez O, Tarca AL, Gervasi MT, Kusanovic JP, et al. An imbalance between angiogenic and anti-angiogenic factors precedes fetal death in a subset of patients: results of a longitudinal study. The Journal of Maternal-Fetal & Neonatal Medicine. 2010;23(12):1384-99.

33. Andersen LB, Dechend R, Karumanchi SA, Nielsen J, Joergensen JS, Jensen TK, et al. Early pregnancy angiogenic markers and spontaneous abortion: an Odense Child Cohort study. American Journal of Obstetrics & Gynecology. 2016;215(5):594.e1-.e11.
34. Korzeniewski SJ, Romero R, Chaiworapongsa T, Chaemsaithong P, Kim CJ, Kim YM, et al. Maternal plasma angiogenic index-1 (placental growth factor/soluble vascular endothelial growth factor receptor-1) is a biomarker for the burden of placental lesions consistent with uteroplacental underperfusion: a longitudinal case-cohort study. American Journal of Obstetrics & Gynecology. 2016;214(5):629.e1-.e17.
35. Redman CWG, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. American Journal of Obstetrics & Gynecology. 2015;213(4):S9.e1-S9.e4.
36. Fisher SJ. Why is placentation abnormal in preeclampsia? American Journal of Obstetrics & Gynecology. 2015;213(4):S115-S22.
37. Conroy AL, McDonald CR, Gamble JL, Olwoch P, Natureeba P, Cohan D, et al. Altered angiogenesis as a common mechanism underlying preterm birth, small for gestational age, and stillbirth in women living with HIV. American Journal of Obstetrics & Gynecology. 2017;217(6):684.e1-.e17.
38. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.
39. Yang J, Pearl M, DeLorenze GN, Romero R, Dong Z, Jelliffe-Pawlowski L, et al. Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. American Journal of Obstetrics & Gynecology. 2016;215(3):359.e1-.e9.
40. Browne JL, Klipstein-Grobusch K, Koster MP, Ramamoorthy D, Antwi E, Belmouden I, et al. Pregnancy associated plasma protein-A and placental growth factor in a sub-Saharan African population: a nested cross-sectional study. PloS one. 2016;11(8):e0159592.
41. Andersen LB, Jørgensen JS, Herse F, Andersen MS, Christesen HT, Dechend R. The association between angiogenic markers and fetal sex: Implications for preeclampsia research. Journal of Reproductive Immunology. 2016;117:24-9.
42. Zera CA, Seely EW, Wilkins-Haug LE, Lim K-H, Parry SI, McElrath TF. The association of body mass index with serum angiogenic markers in normal and abnormal pregnancies. American Journal of Obstetrics and Gynecology. 2014;211(3):247.e1-.e7.
43. Mijal RS, Holzman CB, Rana S, Karumanchi SA, Wang J, Sikorskii A. Midpregnancy levels of angiogenic markers in relation to maternal characteristics. American Journal of Obstetrics and Gynecology. 2011;204(3):244.e1-.e12.
44. Kasdaglis T, Aberdeen G, Turan O, Kopelman J, Atlas R, Jenkins C, et al. Placental growth factor in the first trimester: relationship with maternal factors and placental Doppler studies. 2010;35(3):280-5.
45. Krauss T, Pauer HU, Augustin HG. Prospective Analysis of Placenta Growth Factor (PlGF) Concentrations in the Plasma of Women with Normal Pregnancy and Pregnancies Complicated by Preeclampsia. Hypertension in Pregnancy. 2004;23(1):101-11.

46. Portelli M, Baron B. Clinical Presentation of Preeclampsia and the Diagnostic Value of Proteins and Their Methylation Products as Biomarkers in Pregnant Women with Preeclampsia and Their Newborns. Journal of pregnancy. 2018;2018:2632637.
47. Saffer C, Olson G, Boggess KA, Beyerlein R, Eubank C, Sibai BM. Determination of placental growth factor (PlGF) levels in healthy pregnant women without signs or symptoms of preeclampsia. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health. 2013;3(2):124-32.
48. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides K, Gynecology. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. J Ultrasound in Obstetrics 2015;45(5):591-8.
49. Vatten LJ, Eskild A, Nilsen TI, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. American Journal of Obstetrics & Gynecology. 2007;196(3):239. e1-. e6.
50. Papp E, Mohammadi H, Serghides L. Changes in placental vasculature and pregnancy outcomes in HIV-antiretroviral drug exposed mice. Placenta. 2014;35(9):A17.
51. Govender N, Naicker T, Moodley J. Maternal imbalance between pro-angiogenic and anti-angiogenic factors in HIV-infected women with pre-eclampsia: cardiovascular topics. Cardiovascular journal of Africa. 2013;24(5):174-9.
52. Powis KM, McElrath TF, Hughes MD, Ogwu A, Souda S, Datwyler SA, et al. High viral load and elevated angiogenic markers associated with increased risk of preeclampsia among Women Initiating Highly Active Antiretroviral Therapy (HAART) in Pregnancy in the Mma Bana Study, Botswana. Journal of acquired immune deficiency syndromes. 2013;62(5):517.
53. Wimalasundera R, Larbalestier N, Smith J, De Ruiter A, Thom SM, Hughes A, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. The Lancet. 2002;360(9340):1152-4.

Figures

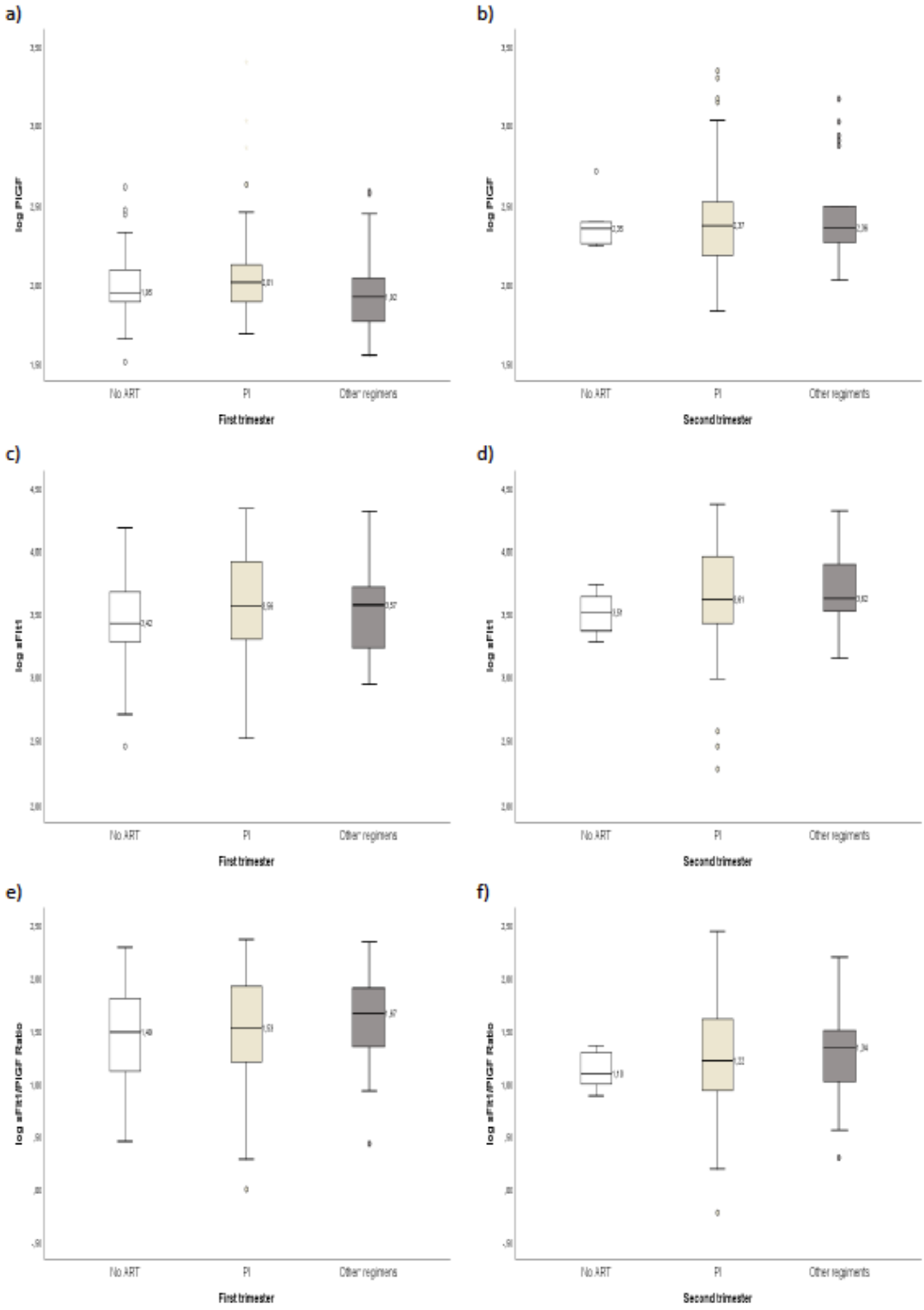
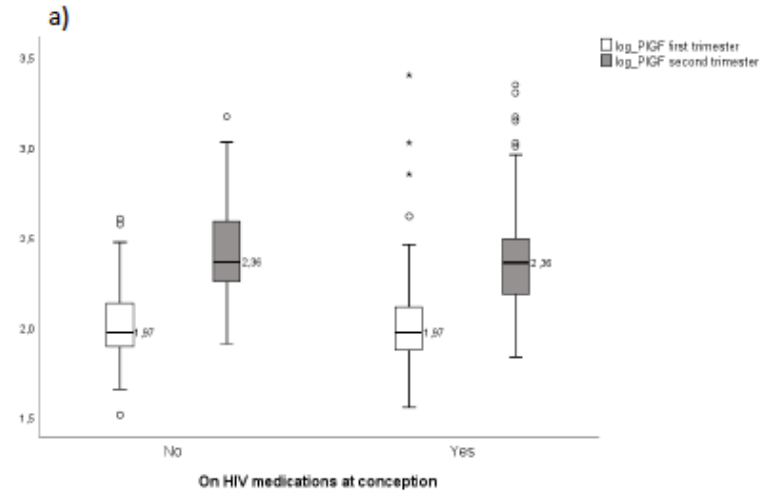
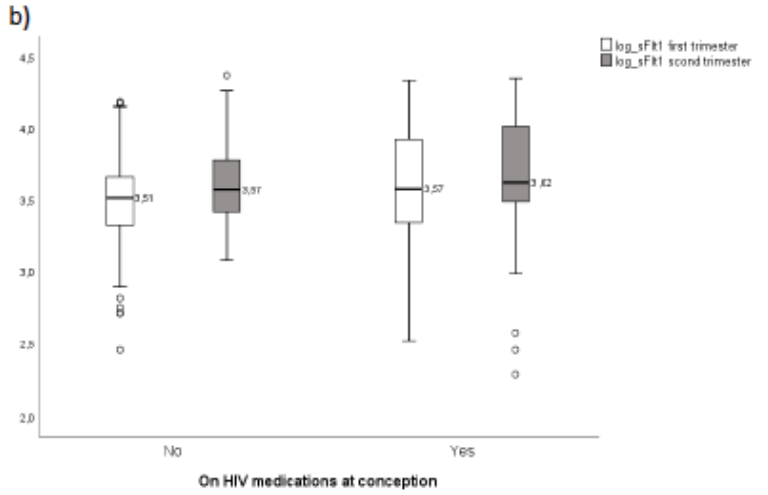


Figure 1

Biomarker distribution by class of antiretroviral therapy (Box-and-whiskers representation). (a) Placental growth factor level (PlGF) in 1st trimester; (b) PlGF level in 2nd trimester; (c) soluble fms-like tyrosine kinase-1 (sFlt-1) level in 1st trimester; (d) sFlt-1 level in 2nd trimester (e) sFlt-1/PlGF ratio in 1st trimester; (f) sFlt-1/PlGF ratio in 2nd trimester. First trimester: no ART, n = 50; PI, n = 89, other regimens, n = 20; Second trimester: no ART, n = 6; PI, n = 131, other regimens, n = 22. The boxes extend from the 25th

percentile to the 75th percentile (i.e., the interquartile range); lines inside boxes represent median values. Lines emerging from boxes (i.e., the whiskers) extend to the upper and lower adjacent values. The lower adjacent values provide an estimate of the lower limit of the array and represents the first quartile value less 1.5 times the difference between the first and third quartiles. The upper adjacent value provides an estimate of the upper limit of the array and represents the third quartile value plus 1.5 times the difference between the first and third quartiles. Values outside these limits are outliers. ART: antiretroviral therapy; PI: protease inhibitor





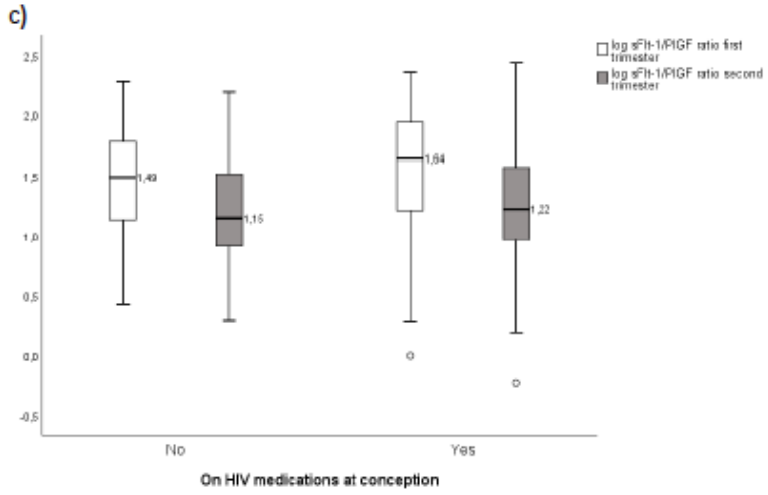


Figure 2

Biomarker distribution by time of ART initiation (Box-and-whiskers representation). (a) Placental growth factor level (PlGF) in 1st trimester and 2nd trimester; (b) soluble fms-like tyrosine kinase-1 (sFlt-1) level in 1st trimester and 2nd trimester (c) sFlt-1/PlGF ratio in 1st trimester and 2nd trimester. By time of ART initiation (before or after conception). On HIV medication at conception: No, n = 62; Yes, n = 97. The boxes extend from the 25th percentile to the 75th percentile (i.e., the interquartile range); lines inside boxes represent median values. Lines emerging from boxes (i.e., the whiskers) extend to the upper and lower adjacent values. The lower adjacent values provide an estimate of the lower limit of the array and represents the first quartile value less 1.5 times the difference between the first and third quartiles. The upper adjacent value provides an estimate of the upper limit of the array and represents the third quartile value plus 1.5 times the difference between the first and third quartiles. Values outside these limits are outliers. ART: antiretroviral therapy; PI: protease inhibitor

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