Placental decidual arteriopathy and vascular endothelial growth factor A (VEGF-A) expression among women with and without HIV

Lisa M. Bebell[1,2,3,], Kalynn Parks[4], Mylinh H. Le[3], Joseph Ngonzi[5], Julian Adong[5], Adeline A. Boatin[2,6], Ingrid V. Bassett[1,3], Mark J. Siedner[1,2,3], Alison D. Gernand[4], Drucilla J. Roberts[7]

[1]Massachusetts General Hospital Division of Infectious Diseases, Boston, MA, USA

[2]MassGeneral Global Health, Massachusetts General Hospital, Boston, MA, USA

[3]Medical Practice Evaluation Center of Massachusetts General Hospital, Boston, MA, USA

[4]Department of Nutritional Sciences, Pennsylvania State University, University Park, PA, USA

[5]Mbarara University of Science and Technology, Mbarara, Uganda

[6]Massachusetts General Hospital Department of Obstetrics and Gynecology, Boston, MA, USA

[7]Massachusetts General Hospital Department of Pathology, Boston, MA, USA

ABSTRACT

Background

Women with HIV (WHIV) are at higher risk of adverse birth outcomes. Proposed mechanisms for the increased risk include placental arteriopathy (vasculopathy) and maternal vascular malperfusion (MVM) due to antiretroviral therapy (ART) and medical comorbidities. However, these features and their underlying pathophysiologic mechanisms have not been well characterized in WHIV.

Methods

We performed gross and histologic examination and immunohistochemistry staining for vascular endothelial growth factor A (VEGF-A), a key angiogenic factor, on placentas from women with one or more MVM risk factors including: weight <5th percentile, histologic infarct or distal villous hypoplasia, nevirapine-based ART, hypertension, and pre-eclampsia/eclampsia during pregnancy. We compared pathologic characteristics by maternal HIV serostatus.

Results

A total of 27/41 (66%) placentas assessed for VEGF-A were from WHIV. Mean maternal age was 27 years. Among WHIV, median CD4 T-cell count was 440 cells/mm^3 and HIV viral load was undetectable in 74%. Of VEGF-A stained placentas, both decidua and villous endothelium tissue layers were present in 36 (88%). VEGF-A was detected in 31/36 (86%) with decidua present, and 39/40 (98%) with villous endothelium present. There were no differences in VEGF-A presence in any tissue type by maternal HIV serostatus (P=0.28-1.0). MVM was more common in placentas selected for VEGF-A staining (51 versus 8%, P<0.001).

Conclusions

VEGF-A immunostaining was highly prevalent, and staining pattern did not differ by maternal HIV serostatus among those with MVM risk factors, indicating the role of VEGF-A in placental vasculopathy may not differ by maternal HIV serostatus.

Key words

malperfusion, small for gestational age, intra-uterine growth restriction, Africa, resource-limited, pregnancy, pregnant, immunohistochemistry, histology, pathology

BACKGROUND

A well-developed placental vascular network is crucial to ensure efficient transport of nutrients, oxygenated blood, and waste between maternal and fetal circulation. Despite the critical importance of normal blood flow at the maternal-fetal interface, placental decidual arteriopathy (placental vasculopathy) has been incompletely characterized [1-4]. Decidual arteriopathy is a pathologic change occurring in decidual spiral arteries that has important potential consequences for fetal development. Decidual arteriopathy is associated with maternal metabolic and autoimmune disease, placental insufficiency, fetal growth restriction, and low birthweight, and shares some histologic features with coronary arterial atheromatous lesions [5]. Decidual arteriopathy is one abnormality seen in maternal vascular malperfusion (MVM), a placental injury pattern of the maternal decidual vessels related to altered uterine and intervillous blood flow [6].

Women with HIV (WHIV) are at higher risk of placental vascular abnormalities including arteriopathy and MVM due to side effects of specific classes of antiretroviral therapy (ART), and co-existing medical conditions [7]. However, few studies have focused on placental arteriopathy among WHIV, and data from women living in resource-limited settings are even more scarce. WHIV are also at increased risk of adverse birth outcomes including miscarriage, preterm labor, intrauterine growth restriction and small-for-gestational-age neonates [8-10], all of which could result from, or be complicated by, decidual arteriopathy and MVM [6].

Angiogenic factors, including vascular endothelial growth factor A (VEGF-A), play important roles in placental vascular development. One of several VEGF isoforms, VEGF-A is produced by placental cytotrophoblasts (the stem cell of the trophoblast lineage) and Hofbauer cells (villous histiocytes), and promotes vasculogenesis during early pregnancy and angiogenesis throughout pregnancy [11]. VEGF-A exerts pro-angiogenic effects on associated transmembrane tyrosine kinase receptors, and its effects are inhibited when bound to soluble tyrosine kinase receptors, as occurs in hypertensive disorders of pregnancy including pre-eclampsia and eclampsia [12]. Angiogenic factor imbalance can also lead to MVM [13], intrauterine hypoxia, miscarriage [14, 15], and adverse birth outcomes including preterm delivery, low birthweight, and small-for-gestational age birth [10, 16]. However, patterns of VEGF-A expression and association with placental arteriopathy and MVM have not been described in WHIV or women living in sub-Saharan Africa.

To address this gap in knowledge, we carried out a secondary study of decidual arteriopathy among a large cohort of WHIV and HIV-uninfected women presenting for delivery in Uganda. Our goal was to compare prevalence of MVM by maternal HIV serostatus and VEGF-A expression patterns in a subset of 44 placentas with risk factors for or histopathologic signs of decidual arteriopathy. We hypothesized that VEGF-A expression and MVM would be more prevalent in placentas from WHIV.

METHODS

Participant recruitment

Between September 2017 and February 2018, pregnant participants were recruited from Mbarara Regional Referral Hospital (MRRH) in southwestern Uganda into a prospective cohort study of chronic placental inflammation among [17]. MRRH serves a semi-rural catchment population of approximately nine million people and reports nearly 10,000 deliveries annually. All women presenting to MRRH for delivery were screened for enrollment and were eligible if they were in early-stage labor, age ≥18 years, reachable by phone after discharge for follow-up, spoke English or the local language Runyankole, and had a single-gestation pregnancy. WHIV were eligible only if they met these criteria and also reported taking ART within the last 30 days. Eligible WHIV were enrolled consecutively, and HIV-uninfected comparators were selected as the next eligible woman presenting for delivery after each enrolled WHIV. After enrolling 150 total participants, HIV-uninfected women were enrolled selectively to balance gestational age and parity by HIV serostatus. The study was approved by the institutional ethics review boards at Mbarara University of Science and Technology (MUST, 11/03-17), Partners Healthcare (2017P001319/MGH), and the Uganda National Council of Science and Technology (HS/2255). All participants gave written informed consent to participate.

Data collection

After enrollment, a structured face-to-face questionnaire was administered to collect sociodemographic information, and obstetric and medical history. Gestational age was calculated using participant report of last normal menstrual period, or chart documentation if participant report was missing. Data were entered into a Research Electronic Capture (REDCap) database [18].

Sample collection and placental gross pathology and histology

Maternal whole blood was collected and tested for HIV (Determine HIV 1/2, Abbott, HIV-uninfected women only). CD4 count and HIV viral load were measured for WHIV if results within the last six months were not available. The placenta was delivered after birth into a clean plastic bucket and transferred to a sterile field. Complete gross pathologic examination was performed by research assistants trained by an experienced perinatal pathologist (DJR), and samples of membrane roll, full-thickness placental parenchyma (at least two, one from the placental center and one from the periphery), umbilical cord, and grossly identified placental lesions were formalin-fixed. Placental samples were transferred to the MUST Pathology Laboratory adjacent to MRRH for routine processing, paraffin-embedding, sectioning at 5μm, and H&E staining. Slides were mounted with DPX and sent to an expert perinatal pathologist (DJR), who performed all histopathologic analysis, blinded to maternal HIV serostatus. Membrane rolls, umbilical cords, and parenchyma were scored for abruption, infarcts, MVM, fetal vascular malperfusion, and arteriopathy, using the Amsterdam consensus [1] nosology. Due to funding constraints, a subset of 44/352 (13%) placentas was selected for VEGF-A immunostaining based on having one or more features of or risk factors for MVM: placental weight less than 5th percentile (<300g at term, *n*=16), placental infarct (*n*=13), taking nevirapine for HIV treatment (*n*=9), diagnosis of pre-eclampsia or eclampsia during this pregnancy

(*n*=2), placental histology with diffuse distal villous hypoplasia (*n*=3), abruption (*n*=1), or hypertension during this pregnancy (*n*=2) [19-21]. Two of 44 (0.5%) cases met more than one selection criteria.

Placental VEGF-A immunostaining

In the MUST pathology laboratory, new sections were cut from the selected placental paraffin blocks and were de-paraffinized with xylene and alcohol and hydrated in distilled water using a manual protocol. Sections were heated in the Bio SB TintoRetriever Pressure Cooker (Santa Barbara, USA) using 1X citrate buffer (Bio SB), cooled, and then blocked for 10 minutes with Dako peroxidase (Agilent, Santa Clara, USA). Slides were then buffered in Tris solution for three minutes before incubating with monoclonal anti-rabbit VEGF-A diluted 1:100 (Abcam, Cambridge, UK) in Dako Primary Antibody Diluent (Agilent) for 60 minutes at room temperature. Slides were rinsed with Tris buffer and incubated with Dako HRP-labelled anti-rabbit secondary antibody (Agilent) for 30 minutes. Slides were rinsed with Tris buffer and incubated with Dako 3,3-diaminobenzidine tetrahydrochloride hydrate (DAB) chromogen for 10 minutes (Agilent), rinsed in distilled water, and counterstained with Hematoxylin for one minute followed by tap water and alcohol dehydration using an autostainer. Slides were mounted with DPX and sent to DJR for interpretation. VEGF-A staining intensity was graded separately for maternal decidua, maternal endothelium, and fetal tissues on a semiquantitative scale of absent (0) weak (1+, 5% stained cells), moderate (1+ to 2+, 10-25% stained cells), strong (2+, 25-50% stained cells), or very strong (3+, >50% stained cells). Transmitted light images were taken using a 10x/0.25 or 40x/0.65 N Plan objective of an Olympus BX41 microscope and Olympus DP27 microscope-mounted camera. Images were acquired using Olympus CellSens software and optimized with Adobe Photoshop.

Sample size and data analysis

Sample size for the parent study was calculated to determine the difference in prevalence of chronic placental inflammation by maternal HIV serostatus (primary parent study aim). No sample size calculation was performed for this secondary analysis, and all placentas having one or more risk factors for or features of MVM were selected for staining. Demographics, outcomes, and placental characteristics were compared between women whose placentas were selected for VEGF-A immunostaining and those not stained using student's t-test or Wilcoxon rank sum for continuous variables and Chi-squared or Fisher's exact test for categorical variables as appropriate, with *P*-values <0.05 considered statistically significant. Summary statistics were used to characterize placental pathology findings, and differences in placental immunostaining characteristics by maternal HIV serostatus were assessed using Fisher's exact test. All analyses were performed using Stata software (Version 16.0, StataCorp, College Station, USA).

RESULTS

Enrollment and cohort characteristics

Over six months, 1,940 women were screened for the parent cohort, 1,489 were eligible, and 176 WHIV and 176 HIV-uninfected women were enrolled (Supplemental Figure). Mean cohort age was 27 (standard deviation [SD] 6) years and was the same for women whose placentas were VEGF-A immunostained and those not (*P*=0.83). Parity was similar in both immunostained and unstained groups (2.9 *versus* 2.7, *P*=0.49), but gestational age was significantly lower in the VEGF-A immunostained group (38 *versus* 39 weeks, *P*<0.001, Table). Median CD4 T-cell count among WHIV was 440 cells/mm^3 and 74% had an undetectable HIV viral load within the last six months. The most common ART regimens among WHIV whose placentas were not VEGF-A immunostained were tenofovir/lamivudine/efavirenz (113, 76%) and zidovudine/lamivudine/efavirenz (11, 7%) and among WHIV whose placentas were VEGF-A immunostained tenofovir/lamivudine/efavirenz (15, 56%) and lamivudine/stavudine/nevirapine (2, 7%).

Outcomes, placental gross pathology, histopathology

Birthweight was significantly lower in the VEGF-A group (2.9 *versus* 3.2 kilograms, *P*<0.001), as was trimmed placental weight (394 *versus* 466 grams, *P*<0.001) and diameter (19 *versus* 20 centimeters, *P*<0.001, Table). Prevalence of MVM was significantly higher in placentas meeting criteria for VEGF-A staining (51 *versus* 8%, *P*<0.001) but did not differ by maternal HIV serostatus (19 *versus* 17%, *P*=0.68).

VEGF-A immunostaining

Due to insufficient reagent, 3/44 (1%, abruption *n*=1, nevirapine use *n*=2) placentas initially selected for immunostaining were not stained, for a final stained sample size of *n*=41. VEGF-A staining quality was fully interpretable for 34/41 (83%) cases. Due to damaged or incomplete tissue planes, maternal endothelium was not assessable for 7/41 (17%), and maternal decidua was not assessable 5/41 (12%) but fetal villous endothelium was assessable for all 41/41 (100%). VEGF-A was seen in the maternal decidua in all assessable cases (*n*=36/36, 100%), and in the fetal villous endothelium in 40/41 cases (98%, Figure 1). VEGF-A was seen in the maternal endothelium of 9 (43%) placentas from of WHIV and 8 (67%) placentas from HIV-uninfected women (*P*=0.28, Figures 1 and 2). Placental tissue staining location did not differ by HIV status for any of the tissue regions examined (Figure 2).

DISCUSSION

In this subset of VEGF-A immunostained placentas selected from a cohort comprised of WHIV and HIV-uninfected women in Uganda, VEGF-A expression was highly prevalent in the maternal decidua and fetal endothelium and less prevalent in the maternal endothelium. We found no association between maternal HIV serostatus and presence or grade of VEGF-A in any placental tissue location,

which was highly expressed overall. We had hypothesized that VEGF-A expression would be more prevalent in placentas from WHIV, based on previously published associations between ART use and adverse birth outcomes among WHIV in resource-limited settings that could be mediated by increased VEGF-A expression [22, 23]. Furthermore, VEGF-A has been demonstrated to be a biomarker for placental abnormalities in hypertensive disorders including preeclampsia or eclampsia [24-27] and fetal growth restriction [28]. However, in this cohort of WHIV taking ART, VEGF-A expression (location and staining intensity) was similar by maternal HIV serostatus. The similarity in VEGF-A expression by maternal HIV serostatus could be due to relatively good immune reconstitution and high proportion with suppressed HIV viral load (74%) among WHIV in this cohort, or to similarities in the pathophysiology of MVM and VEGF-A expression between WHIV and HIV-uninfected women. To our knowledge, no other published studies have reported on placental VEGF-A immunostaining, but among a group of pregnant WHIV in eastern Uganda there were no differences in plasma angiogenic factor levels was seen by maternal ART group, though there was no HIV-uninfected comparison group and VEGF-A was largely undetectable [16]. Our findings should be confirmed in other populations, but suggest that among women on ART, imbalances in placental VEGF-A expression may not be an important contributor to adverse birth outcomes among WHIV. In our prior work, we demonstrated that chronic placental inflammation was also similar by maternal HIV serostatus, and is also unlikely to be a main contributor to adverse birth outcomes among WHIV [17]. Thus, the placental origins of adverse pregnancy and birth outcomes among WHIV remain unclear and further research examining a more diverse array of maternal cellular markers and their downstream effects on placental angiogenesis, arteriopathy, function, antibody transfer, placental arteriopathy, and childbirth outcomes are needed.

Our study has several strengths, including comprehensive characterization of placentas, including gross and histologic placental examination, and immunohistochemistry. Our descriptive study adds to the limited understanding of the placental effects on outcomes, especially among WHIV and women living in resource-limited settings. However, the small sample size (n=41) limits our ability to detect differences between groups, and cohort enrollment at a single Ugandan referral hospital limits the generalizability of our findings, as the women enrolled may not be representative of most women. Furthermore, due to resource constraints we did not stain placentas without MVM evidence or risk factors, measure soluble VEGF decay receptors such as sFLT1, or other placental growth factors. Future studies on placental arteriopathy should investigate other vascular markers and include a comparison group without arteriopathy risk factors.

ACKNOWLEDGMENTS

The authors are grateful to the cohort participants and to the Mbarara Regional Referral Hospital Maternity Staff, Mbarara University of Science and Technology, MUST Pathology Laboratory, and MRRH ISS Clinic for their partnership in this research.

FOOTNOTES

Conflicts of interest

None of the authors have a commercial or other association that might pose a conflict of interest.

Source of funding

This work was supported by the Harvard University Center for AIDS Research National Institutes of Health/National Institute of Allergy and Infectious Diseases [grant number P30AI060354 to LMB] and supported by a KL2/Catalyst Medical Research Investigator Training award from Harvard Catalyst | The Harvard Clinical and Translational Science Center [grant number KL2TR002542 to LMB], and the Charles H. Hood Foundation (to LMB), a career development award from the National Institute of Allergy and Infectious Diseases [grant number K23AI138856 to LMB] and midcareer mentoring award [grant number K24AI141036 to IVB], and the American Society of Tropical Medicine and Hygiene Burroughs Wellcome Fellowship (to LMB). AAB is supported by the career development awards from the Eunice Kennedy Schriver National Institute of Child Health and Human Development [grant number K23HD097300] and Massachusetts General Hospital Executive Committee on Research through the Center for Diversity and Inclusion. The sponsors had no role in study design, data collection, analysis or interpretation, writing the report, or decision to submit the article for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, the National Institutes of Health, or other funders.

Presentations at meetings

None

Corresponding author contact information

Lisa M. Bebell

Infectious Diseases Division, GRJ-504

55 Fruit St

Boston, MA 02114

lbebell@mgh.harvard.edu

Tel: (617) 726-3812

Fax: (617) 726-7416

Email: lbebell@mgh.harvard.edu

REFERENCES

1. Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Archives of pathology & laboratory medicine **2016**; 140:698-713.

2. Redline RW, Patterson P. Patterns of placental injury. Correlations with gestational age, placental weight, and clinical diagnoses. Archives of pathology & laboratory medicine **1994**; 118:698-701.

3. Hecht JL, Zsengeller ZK, Spiel M, Karumanchi SA, Rosen S. Revisiting decidual vasculopathy. Placenta **2016**; 42:37-43.

4. Chan JS, Heller DS, Baergen RN. Decidual Vasculopathy: Placental Location and Association With Ischemic Lesions. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society **2017**; 20:44-8.

5. Avagliano L, Monari F, Po G, et al. The Burden of Placental Histopathology in Stillbirths Associated With Maternal Obesity. American journal of clinical pathology **2020**; 154:225-35.

6. Ernst LM. Maternal vascular malperfusion of the placental bed. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica **2018**; 126:551-60.

7. Kalk E, Schubert P, Bettinger JA, et al. Placental pathology in HIV infection at term: a comparison with HIV-uninfected women. Trop Med Int Health **2017**; 22:604-13.

8. Evans C, Humphrey JH, Ntozini R, Prendergast AJ. HIV-Exposed Uninfected Infants in Zimbabwe: Insights into Health Outcomes in the Pre-Antiretroviral Therapy Era. Frontiers in immunology **2016**; 7:190.

9. Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A. Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections. Lancet Infect Dis **2012**; 12:330-40.

10. Obimbo MM, Zhou Y, McMaster MT, et al. Placental Structure in Preterm Birth Among HIV-Positive Versus HIV-Negative Women in Kenya. J Acquir Immune Defic Syndr **2019**; 80:94-102.

11. Y W, S Z. Vascular Biology of the Placenta. San Rafael (CA): Morgan & Claypool Life Sciences, **2010**.

12. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. The Journal of clinical investigation **2003**; 111:649-58.

13. Chehroudi C, Kim H, Wright TE, Collier AC. Dysregulation of inflammatory cytokines and inhibition of VEGFA in the human umbilical cord are associated with negative pregnancy outcomes. Placenta **2019**; 87:16-22.

14. Krukier, II, Pogorelova TN. Production of vascular endothelial growth factor and endothelin in the placenta and umbilical cord during normal and complicated pregnancy. Bull Exp Biol Med **2006**; 141:216-8.

15. Sajjadi MS, Ghandil P, Shahbazian N, Saberi A. Association of vascular endothelial growth factor A polymorphisms and aberrant expression of connexin 43 and VEGFA with idiopathic recurrent spontaneous miscarriage. The journal of obstetrics and gynaecology research **2020**; 46:369-75.

16. Conroy AL, McDonald CR, Gamble JL, 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 and gynecology **2017**; 217:684.e1-.e17.

17. Bebell LM, Siedner MJ, Ngonzi J, et al. Brief Report: Chronic Placental Inflammation Among Women Living With HIV in Uganda. J Acquir Immune Defic Syndr **2020**; 85:320-4.

18. 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. Journal of biomedical informatics **2009**; 42:377-81.

19. Zash RJ, Morgan Roberts, Drucilla J Souda, Sajini Diseko, Modiegi Morgan, Shally Jacobson, Denise Mayondi, Gloria Kayembe, Mukendi Bebell, Lisa M Mmalane, Mompati Essex, Max Lockman, Shahin Makhema, Joseph Shapiro, Roger L. Abstract 834: Placental evidence of maternal vascular malperfusion among HIV-infected women: Conference on Retroviruses and Opportunistic Infections, Boston, MA, 2018, **2018**.

20. Rotshenker-Olshinka K, Michaeli J, Srebnik N, et al. Recurrent intrauterine growth restriction: characteristic placental histopathological features and association with prenatal vascular Doppler. Arch Gynecol Obstet **2019**; 300:1583-9.

21. Wright E, Audette MC, Ye XY, et al. Maternal Vascular Malperfusion and Adverse Perinatal Outcomes in Low-Risk Nulliparous Women. Obstet Gynecol **2017**; 130:1112-20.

22. Chen JY, Ribaudo HJ, Souda S, et al. Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana. The Journal of infectious diseases **2012**; 206:1695-705.

23. Ejigu Y, Magnus JH, Sundby J, Magnus MC. Pregnancy outcome among HIV-infected women on different antiretroviral therapies in Ethiopia: a cohort study. BMJ open **2019**; 9:e027344.

24. Bhavina K, Radhika J, Pandian SS. VEGF and eNOS expression in umbilical cord from pregnancy complicated by hypertensive disorder with different severity. BioMed research international **2014**; 2014:982159.

25. Bustamante Helfrich B, Chilukuri N, He H, et al. Maternal vascular malperfusion of the placental bed associated with hypertensive disorders in the Boston Birth Cohort. Placenta **2017**; 52:106-13.

26. Wathén KA, Tuutti E, Stenman UH, et al. Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation. J Clin Endocrinol Metab **2006**; 91:180-4.

27. Johnson KM, Zash R, Haviland MJ, et al. Hypertensive disease in pregnancy in Botswana: Prevalence and impact on perinatal outcomes. Pregnancy hypertension **2016**; 6:418-22.

28. Spinillo A, Gardella B, Adamo L, Muscettola G, Fiandrino G, Cesari S. Pathologic placental lesions in early and late fetal growth restriction. Acta Obstet Gynecol Scand **2019**; 98:1585-94.

Table. Demographic, medical, and placental characteristics of a cohort of women presenting in labor to Mbarara Regional Referral Hospital in Uganda, by vascular endothelial growth factor A (VEGF-A) staining.

Characteristic	VEGF-A Stained *n* = 41 (12%)	Not VEGF-A Stained *n* = 311 (88%)	*P*-value*
DEMOGRAPHICS			
Age category			0.29
≤19	3 (7)	26 (8)	
20-34	29 (70)	245 (79)	
≥35	9 (22)	40 (13)	
Resides in Mbarara District	24 (59)	203 (65)	0.40
Married	35 (85)	282 (91)	0.29
Completed more than primary school education	24 (59)	183 (59)	0.97
Monthly income[a] (median USD[b], IQR[c])	$25 (14, 53)	$42 (28, 83)	0.04
Formal employment outside the home	15 (37)	112 (36)	0.94
OBSTETRIC AND MEDICAL			
HIV seropositive	27 (66)	149 (48)	0.03
Gestational at delivery in weeks (mean, SD)	38 (2.6)	39 (1.7)	<0.001
Preterm delivery <37 weeks	2 (5)	3 (1)	0.1
Parity including current delivery			0.49
1 (primiparous)	10 (24)	77 (25)	
2-4 (multiparous)	21 (51)	132 (42)	
≥4 (grand multiparous)	10 (24)	102 (33)	
Attended ≥4 antenatal care visits this pregnancy	25 (61)	198 (64)	0.74
Received iron supplementation in pregnancy	29 (71)	184 (59)	0.15
Received folic acid supplementation in pregnancy	14 (34)	137 (44)	0.23

Received combination iron/folic acid in pregnancy	32 (78)	210 (68)	0.17
Received malaria prophylaxis with IPTp[d] or TMP/SMX[e]	39 (95)	297 (96)	0.91
Hours in labor (median hours, IQR)	12 (9, 18)	14 (8, 24)	0.33
Cesarean delivery	13 (32)	101 (32)	0.92
Referred to MRRH[f] for care	7 (17)	49 (16)	0.84
5-minute Apgar score <7	0 (0)	8 (3)	0.32
Days hospitalized for delivery (mean, SD[g])	2.2 (2)	2.2 (3)	0.99
Birthweight category (in kilograms)			0.003
<2.5	6 (15)	10 (3)	
2.5-3.5	32 (78)	233 (76)	
3.6-4.0	2 (5)	57 (19)	
>4.0	1 (2)	8 (3)	
PATHOLOGY			
Trimmed placental weight in grams (mean, SD)	394 (116)	466 (96)	<0.001
Trimmed weight ≤300 grams	10 (6)	6 (3)	0.31
Greatest placental diameter in cm[h] (mean, SD)	19 (2)	20 (2)	<0.001
Greatest placental thickness in cm (mean, SD)	1.6 (0.5)	1.7 (0.6)	0.90
Umbilical cord length in centimeters (mean, SD)	41 (14)	40 (17)	0.72
Cord coiling index (number of coils/length in cm; mean, SD)	0.16 (0.08)	0.18 (0.07)	0.04
Histologic abruption	0 (0)	1 (0.3)	1.0
Placental infarct(s)	13 (32)	0 (0)	<0.001
Diffuse distal villous hypoplasia	3 (7)	0 (0)	0.001
Decidual arteriopathy	0 (0)	1 (0.3)	0.89

Fetal vascular malperfusion	4 (13)	37 (11)	0.76
Maternal vascular malperfusion (MVM)	20 (51)	23 (8)	<0.001
Chronic villitis	2 (7)	39 (12)	0.48
Villitis of unknown etiology	2 (8)	24 (8)	0.51
Acute chorioamnionitis	7 (17)	92 (30)	0.09

Table legend: Of 352 participants, 44 (13%) placentas were selected for VEGF-A immunostaining based on having one or more features of or risk factors for maternal vascular malperfusion: placental weight less than 5th percentile, placental infarct, taking nevirapine for HIV treatment, pre-eclampsia or eclampsia during this pregnancy, placental histology with diffuse distal villous hypoplasia, or hypertension during this pregnancy. Two of 44 cases met more than one selection criteria. *Tests of association between cohort characteristics and HIV serostatus were performed using Chi-square, Wilcoxon rank sum, and t-tests. [a]Reported by participant in Ugandan Shillings, converted to [b]USD (United States Dollar) using exchange rate for study start date (March 1, 2017: 1 USD = 3600 Ugandan Shillings); [c]IQR: interquartile range; [d]IPTp (intermittent preventative treatment in pregnancy) with either sulfadoxine-pyrimethamine or dihydroartemisinin-piperaquine, or by routine prophylactic trimethoprim/sulfamethoxazole in participants living with HIV; [e]TMP/SMX: trimethoprim/sulfamethoxazole; [f]MRRH: Mbarara Regional Referral Hospital; [g]SD: standard deviation; [h]cm: centimeters; Results listed as "*n* (%)" unless otherwise noted.

Figure 1. Vascular endothelial growth factor A (VEGF-A) immunohistochemistry staining of 41 placentas by maternal HIV serostatus (*n*=27 HIV-positive [HIV+], *n*=14 HIV-negative [HIV-]), selected from a cohort of women presenting in labor in Uganda.

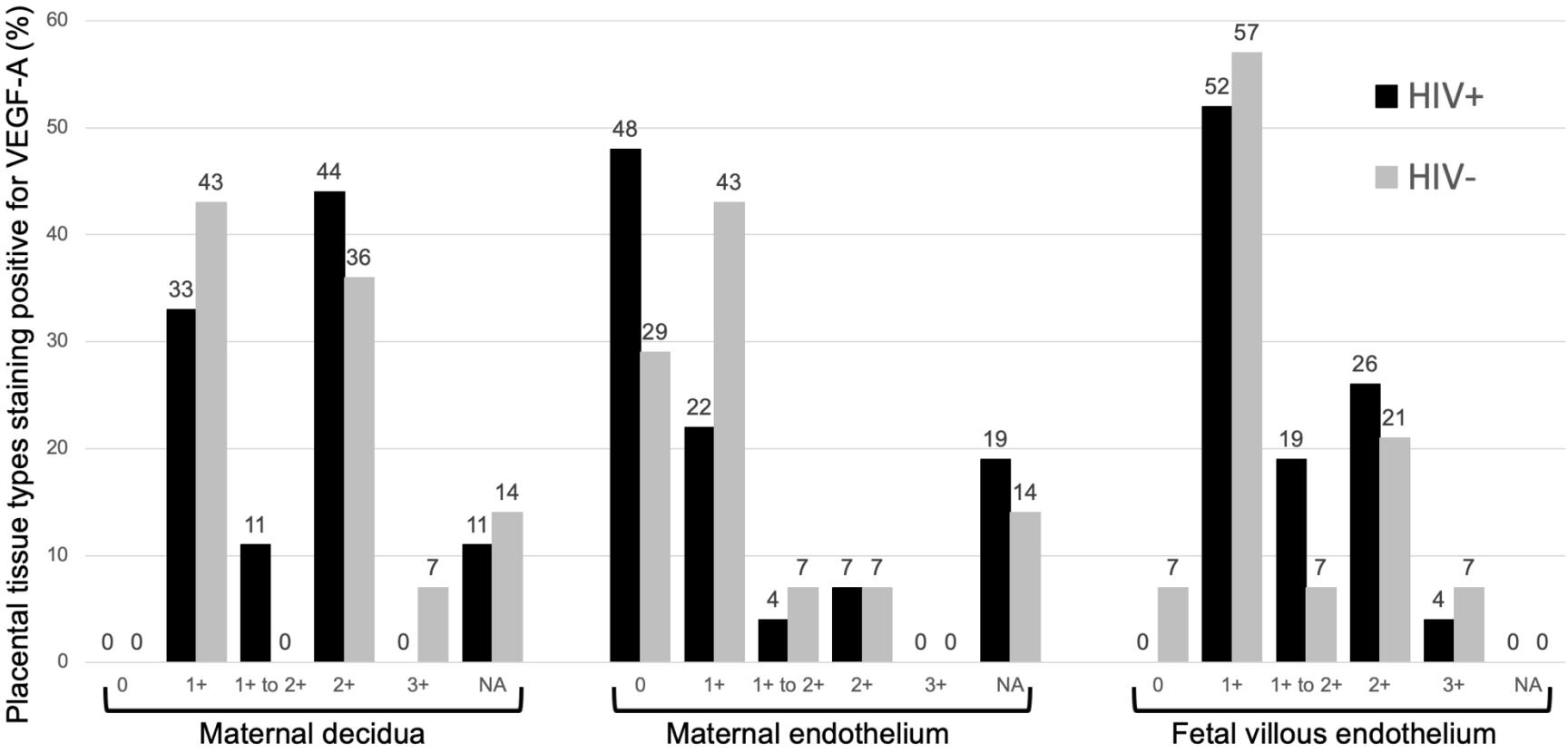

Figure 1 legend: Tissue VEGF-A staining is rated as 0 = absent, 1+ = weak (5% stained cells), 1+ to 2+ = moderate (10-25% stained cells), 2+ = strong (25-50% stained cells), 3+ = very strong (>50% stained cells), NA = not assessable (tissue damaged or not present in sample).

Figure 2. Representative 40X histologic images of vascular endothelial growth factor A (VEGF-A) immunostained placental samples from women living with HIV in Uganda. Panel A is a 40X magnification of placental membrane roll demonstrating positive VEGF-A staining in maternal endothelium (arrowhead) and decidua (large arrow) and negative staining in the extra-villous trophoblast of the *chorion laeve* (small arrow). Panel B is a 40X magnification of placental parenchyma demonstrating positive VEGF-A staining in chorionic villi and villous (fetal) endothelium (arrowheads).