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Placental soluble fms-like tyrosine kinase expression in small for gestational age infants and risk for adverse outcomes

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ABSTRACT

Introduction: Soluble fms-like tyrosine kinase 1 (sFLT-1) is an anti-angiogenic factor implicated in the pathogenesis of preterm preeclampsia. We evaluated sFLT-1 expression and placental pathology in pregnancies complicated by small for gestational age (SGA) infants (<10th percentile), without evidence of preeclampsia.

Methods: Clinical and histologic data were compared between groups with high or low sFLT-1 expression determined by immunohistochemistry on archived placentas.

Results: Nineteen of 69 placentas showed high sFLT-1 expression. The high sFLT-1 group had higher predelivery median systolic blood pressure (BP); 140 (interquartile range (IQR) 133–152) vs. 126 (118–139) mm Hg ($p = 0.003$), and median diastolic BP; 87 (78–94) vs. 77.5 (71–86) mm Hg ($p = 0.02$). Abnormal umbilical Doppler abnormalities were more prevalent; 89.5% vs. 46% ($p = 0.001$). These pregnancies delivered earlier; 31.9 weeks (28.3–34.7 weeks) vs. 37.1 weeks (33.7–38.7 weeks) ($p < 0.001$), and infants had lower birthweight; 980 grams (520–1545 grams) vs. 2087.5 grams (1455–2340 grams) ($p < 0.001$). Placental-weight to fetal-weight ratios, a marker of vascular insufficiency, was increased in the high sFlt-1 group: 0.18 (0.14–0.28) vs 0.15 (0.13–0.18), $p = 0.03$. Placentas with high sFLT-1 showed more decidual vasculopathy; 42.1% vs. 10.0% ($p = 0.005$), infarction; 36.8% vs. 14.0% ($p = 0.048$), distal villous hypoplasia; 78.9% vs. 36.0% ($p = 0.001$), and fetal thrombotic vasculopathy; 47.4% vs. 16.0% ($p = 0.01$).

Discussion: Placental sFLT-1 expression is upregulated in approximately 28% of non-preeclamptic pregnancies complicated by SGA infants. These pregnancies showed increased placental vascular pathology, more umbilical Doppler abnormalities, and earlier delivery with lower birthweight. A subgroup of non-preeclamptic fetal growth restriction with upregulated sFlt-1 expression may share a common pathogenic pathway with preterm preeclampsia. This subgroup is worthy of additional study.

1. Introduction

Fetal growth restriction (FGR) is associated with increased neonatal morbidity and mortality [1]. In pregnancies complicated by growth restriction, the estimated rate of fetal demise is 1.5% when fetal weight is < 10th percentile and 2.5% when <5th percentile [2–4]. Tools to triage risk of poor outcome with FGR are a major research focus [5,6]. Differentiating FGR into subtypes based on pathogenesis would improve risk stratification for early delivery, thereby minimizing the risk of iatrogenic prematurity.

FGR may be caused by maternal, fetal, placental, genetic factors [7]. Preeclampsia is an important placental cause of fetal growth restriction. Fetal growth is most dramatically affected in patients with severe preterm preeclampsia (usually <32 weeks gestation). Severe cases also show the highest levels of the anti-angiogenic factor soluble fms-like tyrosine kinase 1 (sFLT-1) produced by the placenta; sFLT-1 is both a mediator, serum and placental marker of the disease [8,9]. Umbilical artery Doppler are often abnormal with

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late manifestations of placental vascular insufficiency characterized by decreased, absent, or reversed end-diastolic flow. Placental histology often shows infarction, decidual vasculopathy, accelerated villous maturation (distal villous hypoplasia), fetal thrombotic vasculopathy, and endoplasmic reticulum stress [10,11]. These same placental lesions, as well as abnormal Doppler studies are seen in a subset of pregnancies complicated by idiopathic FGR without preeclampsia, suggesting they may share a common final placental dysfunction pathway [12,13].

The focus of this study was to evaluate if placental protein sFlt-1 expression is dysregulated in non-preeclamptic pregnancies with fetal growth restriction.

2. Methods

2.1. Ethics statement

The study was approved by the Beth Israel Deaconess Medical Center (BIDMC) institutional review board (Protocol # 2015P-000151).

2.2. Study population

We identified archived placental tissue obtained from women who were monitored for fetal growth restriction and delivered small for gestational age (SGA) infants between January 2008 and December 2015 at BIDMC in Boston, MA. These women were identified from an antenatal ultrasound database based on growth restriction. Neonatal birth weights were confirmed to be small for gestational age by Fenton growth curves, see below. At BIDMC, placentas belonging to pregnancies with suspected growth restriction are sent for pathologic analysis as a general institutional practice. We excluded placentas belonging to women with a clinical diagnosis of preeclampsia from our analysis. One mother in our cohort had two pregnancies complicated by SGA and both placentas were included in our analysis.

2.3. Clinical definition of small for gestational age (SGA) infants

We included placentas if the birth weight was less than 10th percentile when plotted on Fenton growth curves [14]. From the medical record, we collected data on maternal age, race, height, weight, smoking status, gestational age at delivery, blood pressure, laboratory tests, birth weight, and fetal ultrasounds. With regard to fetal ultrasounds, umbilical Doppler velocimetry was reviewed. At our institution, we perform Doppler studies as part of clinical practice using a 5–8 MHz curvilinear transabdominal probe and a General Electric Voluson E8 machine (GE Medical Systems). Criteria for abnormal umbilical artery Doppler velocimetry were based on the clinical guideline published by the Society of Maternal Fetal Medicine in 2012: increased systolic to diastolic (S/D) ratio above two standard deviations for gestational age, absent end-diastolic flow, and reversed end-diastolic flow [15].

2.4. Pathologic examination of the placenta

We performed a blinded histopathologic analysis for lesions associated with placental hypoxia and injury, including decidual vasculopathy, infarction, abruption, distal villous hypoplasia, fetal thrombotic vasculopathy, hematoma, laminar decidual necrosis, chorionic cysts, villitis, and umbilical vessel abnormalities [11]. An experienced placental pathologist (JLH) performed the histopathologic analysis. The pathologist was blinded to clinical characteristics with the exception of gestational age at delivery since gestational age is needed to assess the villous maturation. All of the placentas were processed according to routine clinical procedures which included 1–2 representative section of the free membranes (membrane roll), one section of umbilical cord, two full thickness sections of grossly normal placental parenchyma, and additional sections from gross lesions such as infarctions.

Based on review of the hematoxylin and eosin (H&E) stained sections, uteroplacental vascular lesions were evaluated based on morphologic characteristics described by Redline [16], and scored in a semiquantitative manner using criteria adapted from Ghidini [17]. These included: A) infarcts (0 = absent, 1 = single, 2 = multiple); B) abruption, as defined by gross retroplacental hematoma or decidual and parabasal hemorrhage with subjacent placental infarct or foci of villous stromal hemorrhage (0 = absent, 1 = present); C) Distal villous hypoplasia, defined as alternating areas of agglutinated villi with increased syncytial knots and intervillous fibrin, and villous paucity due to decreased villous branching, present in more than 30% of villi in five 200× fields (0 = absent, 1 = present). Generally, the finding was present on multiple sections from the placental disc. D) fetal thrombotic vasculopathy defined by either clusters of more than 15 sclerotic villi or organizing thrombi in the wall of a stem vessel (0 = absent, 1 = single focus, 2 = more than one focus); E) parenchymal hematoma (0 = absent, 1 = present); F) laminar decidual necrosis in membrane sections (0 = absent, 1 = present); G) chorionic pseudocysts (0 = absent, 1 = present); H) non-specific chronic villitis (0 = absent, 1 = present on one slide, 2 = present on more than one slide).

2.5. Immunohistochemistry

As part of clinical routine, sections from fresh placentas were fixed in 10% neutral buffered formalin for 4–6 h prior to processing to paraffin for histology. From these blocks, paraffin sections (5 μm) were cut onto polysine-coated slides (Fisher, Atlanta, Georgia) were deparaffinized and rehydrated. Optimal staining was achieved with an antigen retrieval method that was performed in 10 mmol/l citric acid, pH 6.00, for 15 min. Endogenous peroxidase was quenched with 3% H_2O_2 in ddH_2O for 15 min. Sections were blocked with 2.5% normal horse serum at room temperature for 40 min. Then primary anti-human Flt-1 antibody that recognizes the N-terminal region of sFLT-1 (catalog no. AF321; 1:800 dilution, R&D Systems, Minneapolis, MN) was used and an ImmPRESS anti-goat staining kit (catalog no. MP-7405; Vector Laboratories, Burlingame, CA) according to published protocols [18]. Sections with no primary antibody were used as negative control slides. sFLT-1 staining was validated using known positive control placentas from women with preeclampsia [19]. sFLT-1 was evaluated in both villous syncitiotrophoblasts and villous trophoblasts, using intermediate implantation site trophoblast as a positive internal reference.

Evaluation of the sFLT-1 staining was performed in a blinded fashion by two of the authors (JLH and MS) at a double headed microscope. Scoring scale for immunohistochemical staining intensity of sFlt-1 in villous trophoblasts was based on high or low/absent staining intensity as compared to intermediate trophoblasts at the implantation site adjacent to the decidua as an internal control (Fig. 1).

2.6. Statistical analysis

Categorical data were compared using the chi-square or Fisher's exact tests, and continuous data were compared using the Mann-Whitney *U* test. The data is shown as median (interquartile range) or n (%). All p values were 2 sided, and values of $p < 0.05$ were considered statistically significant. Birth weight and placental weight percentiles were adjusted for gestational age at delivery