Early Human Development 90 (2014) 195–200

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Cord blood biomarkers of vascular endothelial growth (VEGF and sFlt-1) and postnatal growth: A preterm birth cohort study

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ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form 17 December 2013
Accepted 8 January 2014

Keywords:
Birth weight
Cord blood
Postnatal growth
Preterm infants
Vascular endothelial growth factor (VEGF)
Fetal growth restriction
Fetal origins

ABSTRACT

Background: Preterm infants are at risk for postnatal growth failure (PGF). Identification of biomarkers that are associated with neonatal growth may help reduce PGF and associated long-term morbidity.
Objective: To investigate the associations between cord blood vascular endothelial growth factor (VEGF) and its soluble receptor (sFlt-1) with birth weight (BW) and postnatal growth in premature infants.
Study design and methods: From an ongoing birth cohort, 123 premature infants from 23 to 36 weeks gestational age (GA) were studied. Cord blood plasma VEGF and sFlt-1 were measured via enzyme-linked immunoassay. Growth parameters and nutritional information were evaluated. Multivariate logistic regression models were constructed to evaluate the associations of VEGF and sFlt-1 on PGF, defined as weight <10th percentile at 36 weeks corrected age or discharge.
Results: VEGF was positively correlated, and sFlt-1 was negatively correlated with BW and BW-for-GA percentiles. Higher cord blood VEGF levels were associated with reduced risk of PGF (OR = 0.7; 95% CI = 0.5–0.9), while higher sFlt-1 levels appeared to increase the risk of PGF (OR = 1.6; 95% CI = 1.1–2.4). The above biomarker associations were attenuated after adjustment for maternal preeclampsia, fetal growth restriction and related neonatal characteristics, and when taking into account placental vascular pathologies. Longitudinal growth patterns by mean weight and length percentiles were consistently lower among infants with low VEGF/sFlt-1 ratios.
Conclusions: Our data support that intrauterine regulation of angiogenesis is an important mechanism of fetal and postnatal growth. Cord blood VEGF and sFlt-1 are useful in elucidating how intrauterine processes may have long-standing effects on developing premature infants.

1. Introduction

Preterm infants are at considerable risk for postnatal growth failure (PGF), which has been defined as body weight <10th percentile at 36 weeks corrected gestational age (GA) [1,2]. Preterm infants who are small-for-gestational age (SGA) at birth are at greatest risk for poor postnatal growth [3]. Failure to gain weight during the neonatal period may have a significant negative effect on both short- and long-term health. In SGA infants, the outcome of intellectual performance was found to be worse among those who do not catch-up in height, weight, and head circumference, compared to those with catch-up growth [4,5]. In addition, epidemiological studies have linked poor neonatal growth to obesity, insulin resistance, and cardiovascular disease [6,7].

Although fetal growth restriction and resultant SGA birth weight (<10th percentile) are strongly associated with poor postnatal growth, even appropriate for GA (AGA) premature infants are at risk. The mechanisms for PGF are poorly understood, but angiogenesis, the formation of new blood vessels from preexisting ones, appears to be a critical process for intra- and extra-uterine growth [8]. Pro-angiogenic vascular endothelial growth factor (VEGF) and its soluble antagonist, fms-like tyrosine kinase 1 (sFlt-1), are promising biomarkers for understanding and predicting PGF, since they function as a regulatory system for angiogenesis. Excess sFlt-1 and decreased VEGF in the placenta, maternal serum, and umbilical cord blood may have a pathological role in preeclampsia, which is an important risk factor for fetal growth and perhaps early postnatal growth [9–14].

In this study, we evaluated the relationship between cord blood VEGF and sFlt-1, birth weight (BW), postnatal growth patterns and placental

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http://dx.doi.org/10.1016/j.earlhumdev.2014.01.003

histopathology in preterm infants enrolled in an ongoing birth cohort study. We hypothesized that cord blood VEGF and sFlt-1 are associated with PGF, independent of BW status and other factors related to preterm birth. These findings will enhance our understanding of the pathophysiology of neonatal growth in preterm infants who are at risk for PGF.

2. Methods

2.1. Study design and patients

Study patients (mothers and their infants) were part of an ongoing longitudinal prospective cohort study being conducted at the Northwestern Prentice Women's Hospital in Chicago, IL. Inclusion criteria for this larger cohort are all infants live-born at <37 weeks GA with available cord blood at delivery. Infants with a prenatal diagnosis of congenital anomalies or syndromes, or those for whom GA cannot be reliably assessed were excluded. For this present study, a subset of mother–infant pairs was selected from the larger cohort to include the following BW status groups among preterm infants: 1) ≤1000 g; 2) 1001–1500 g; 3) 1501–2000 g; 4) >2000 g. Parental consent was obtained prior to enrollment. The study was approved by the institutional review board of the Northwestern University.

2.2. Maternal and infant covariates

Maternal and infant clinical information were recorded using a standardized abstraction form that included data on prenatal care, intrapartum management, pregnancy complications and birth outcomes. Standardized definitions included the following: 1) GA was determined based on last normal menstrual period (LNMP) confirmed by ultrasounds or averaged ultrasound parameters obtained prior to 20 weeks gestation [15]. LNMP estimate was used whenever the estimated date of confinement correlated with first trimester ultrasound within 1 week or second trimester ultrasound within 2 weeks. Otherwise, ultrasound estimate was used. Only births that could be accurately dated by this algorithm were included in the study; 2) Maternal preeclampsia was defined according to American College of Obstetrics and Gynecology Committee criteria for clinical practice diagnosis that included gestational hypertension (new onset after 20 weeks' gestation) with documented proteinuria, and included eclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome [16]; 3) Placental weight and histopathology information were recorded via standardized pathology review by the study's perinatal pathologist. Placental vascular pathology included evidence of maternal or fetal vascular lesions according to the criteria by Redline et al. [17,18]; 4) Neonatal complications were identified according to the widely used benchmarks: bronchopulmonary dysplasia (BPD) [19]; intraventricular hemorrhage (IVH) [20]; necrotizing enterocolitis (NEC) [21]; and diagnoses of retinopathy of prematurity (ROP) were obtained from NICU records for each infant.

2.3. Parameters of growth

Longitudinal growth parameters of weight (g), length (cm), and head circumference (cm) were measured at birth and at weekly intervals up to hospital discharge. Continuous variable weight, length, and head circumference percentiles were determined using the most recent Fenton growth curves for premature infants [22]. Values were calculated using the formula provided by this reference, in which growth parameters were entered for the completed weeks of gestation for each infant. Postnatal growth failure (PGF) was defined as weight-for-corrected GA <10th percentile at 36 weeks or NICU discharge, whichever came first.

2.4. Cord blood VEGF and sFlt-1

Mixed arterial–venous cord blood was collected at delivery into EDTA tubes, stored temporarily on ice, and spun at 2500 rpm for 10 min in a refrigerated tabletop centrifuge. Plasma was separated into aliquots and stored at −80 °C until assay. Plasma VEGF and sFlt-1 were simultaneously measured in duplicate using commercially available EIA kits (R&D Systems, Minneapolis, MN). EIA results were read using a Bio-Rad iMark (Hercules, CA) automated plate reader at 450 nm with background correction at 540 nm. For VEGF, the limit of detection (LOD) was non-detectable, and intra- and interassay coefficients of variation (CV) were 6.7% and 8.8%, respectively. For sFlt-1, LOD was 1.5 pg/ml, and intra- and interassay CV were 3.8% and 9.8%, respectively. The ratio of VEGF to sFlt-1 level (VEGF/sFlt-1) for each patient was calculated to indicate relative amount of circulating VEGF to its soluble receptor [23]. For purposes of plotting longitudinal growth (Fig. 1), low VEGF/sFlt-1 was defined as a ratio in the 1st quartile (<0.03). High VEGF/sFlt-1 was defined as a ratio in the 4th quartile (>0.5).

2.5. Statistical analysis

The chi-square or Fisher's exact tests and Student's *t*-tests were used to compare categorical and continuous variables, respectively.

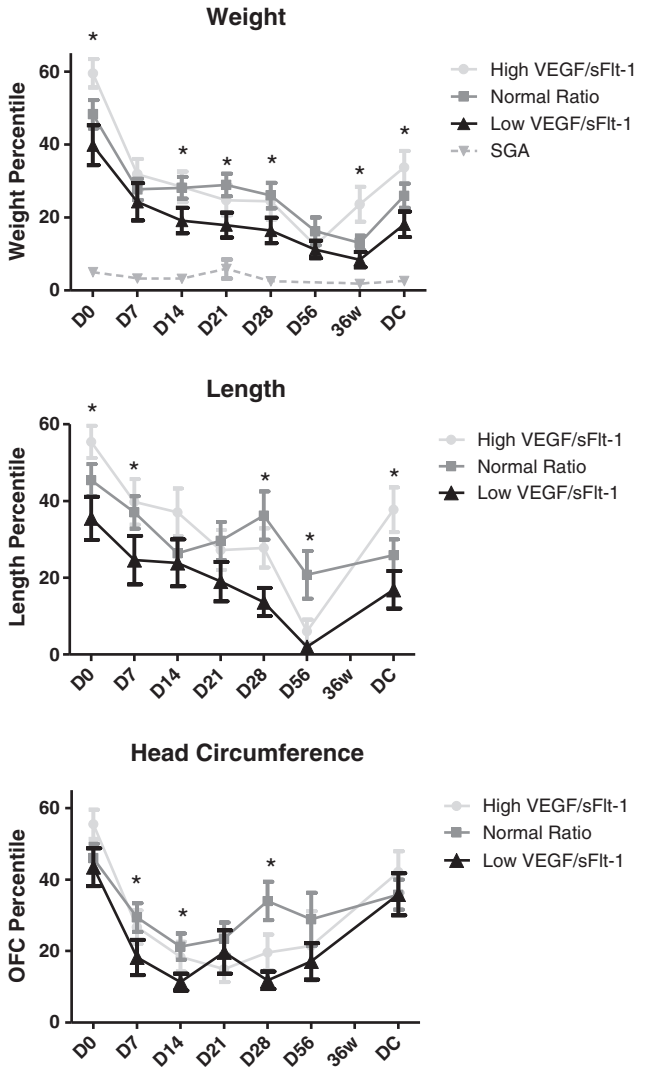

Fig. 1. Postnatal growth percentiles of weight, length, and head circumference (occipital–frontal circumference, OFC) according to cord blood VEGF/sFlt-1 ratio. Low VEGF/sFlt-1 (1st quartile), normal ratio VEGF/sFlt-1 (2nd–3rd quartile), and high VEGF/sFlt-1 (4th quartile) shown. *$P < 0.05$, low ratio vs. normal to high ratio percentile.