Placenta 36 (2015) 1078–1086

Contents lists available at ScienceDirect

Placenta

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

Hypoplastic left heart syndrome is associated with structural and vascular placental abnormalities and leptin dysregulation

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ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form
29 July 2015
Accepted 4 August 2015

Keywords:
Angiogenesis
Vascular biology
Congenital heart disease

ABSTRACT

Introduction: Hypoplastic left heart syndrome (HLHS) is a severe cardiovascular malformation (CVM) associated with fetal growth abnormalities. Genetic and environmental factors have been identified that contribute to pathogenesis, but the role of the placenta is unknown. The purpose of this study was to systematically examine the placenta in HLHS with and without growth abnormalities.
Methods: HLHS term singleton births were identified from a larger cohort when placenta tissue was available. Clinical data were collected from maternal and neonatal medical records, including anthropometrics and placental pathology reports. Placental tissues from cases and controls were analyzed to assess parenchymal morphology, vascular architecture and leptin signaling.
Results: HLHS cases ($n = 16$) and gestational age-matched controls ($n = 18$) were analyzed. Among cases, the average birth weight was 2993 g, including 31% that were small for gestational age. When compared with controls, gross pathology of HLHS cases demonstrated significantly reduced placental weight and increased fibrin deposition, while micropathology showed increased syncytial nuclear aggregates, decreased terminal villi, reduced vasculature and increased leptin expression in syncytiotrophoblast and endothelial cells.
Discussion: Placentas from pregnancies complicated by fetal HLHS are characterized by abnormal parenchymal morphology, suggesting immature structure may be due to vascular abnormalities. Increased leptin expression may indicate an attempt to compensate for these vascular abnormalities. Further investigation into the regulation of angiogenesis in the fetus and placenta may elucidate the causes of HLHS and associated growth abnormalities in some cases.

1. Introduction

Hypoplastic left heart syndrome (HLHS) continues to be one of the most challenging cardiovascular malformations (CVM) to manage. Rapidly evolving treatment paradigms have resulted in both increased survival and improved long-term outcomes, but the underlying cause(s) remain poorly understood. The etiology of HLHS is thought to be multifactorial, attributed to a combination of complex inheritance and environmental factors [1]. Research has identified different influences that contribute to the manifestation of HLHS, including genetic predisposing factors, environmental risk factors and physiologic perturbations in the developing and growing heart [2,3], but it remains unclear how these factors interact to impact pathogenesis.

HLHS infants are at increased risk of being small for gestational age (SGA), and both SGA and low birth weight (<2500 g) are

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http://dx.doi.org/10.1016/j.placenta.2015.08.003
0143-4004/

associated with adverse clinical outcomes [4,5]. Growth patterns have been characterized in HLHS, and about 50% of fetuses are affected by growth abnormalities [6]. Recently, we described fetal growth in HLHS, and in a significant proportion of cases, weight and/or head circumference demonstrate diminished growth trajectories late in gestation [7–9]. Whether growth abnormalities in HLHS represent primary and/or secondary insults to the fetus is unknown, and the potential role of impaired placental development and/or function in the manifestation of these growth abnormalities remains unclear.

The placenta plays a central role in fetal growth, and abnormal placental implantation, growth, development and function can all negatively impact fetal growth and development [10,11]. Gross placental pathology has not been identified in large studies examining all CVM collectively [12]. Several studies have shown pregnancies complicated by fetuses with HLHS have normal umbilical artery Doppler by ultrasound assessment [7,13,14], suggesting appropriate feto-placental blood flow, but these studies have not assessed placental structure, vascularization or function. The significantly increased incidence of any growth abnormality in newborns with HLHS, approximately 40%, implicates placental dysfunction. As recently highlighted, placental insufficiency can underlie a significant proportion of late-onset fetal growth restriction cases without the presence of abnormal umbilical artery Doppler [15]. The role of the placenta in the manifestation of HLHS and associated growth abnormalities is unknown. One emerging concept proposes that primary placental abnormalities may be associated etiologically with primary CVMs [16,17], suggesting the placenta may contribute to or be impacted by primary cause. This so-called heart–placental axis proposes that the heart, placental vasculature and villous tree develop concurrently in early pregnancy [17]. Recent studies in mice have shown that common factors exist, such as PPARgamma, that lead to CVM, reduced placental vasculature, impaired placental function and reduced somatic growth [18,19]. The causal factors for HLHS may impact placental vasculature and further investigation into the regulation of vasculogenesis, as well as angiogenesis, as it applies to the cardiovascular and placenta systems may help elucidate these factors.

Altered placental development or function and resulting aberrant fetal growth has previously been associated with dysregulation of several growth factors and adipokines such as IGF-1 and leptin [20,21]. Leptin is an angiogenic and mitogenic hormone produced by the placenta that has both paracrine and autocrine effects [22,23]. In a healthy pregnancy, leptin levels are positively correlated with placental weight and a number of specific indices of fetal growth, including body weight and length, head circumference, ponderal index, adiposity and bone mineral density [24]. Furthermore, it has been demonstrated that leptin has a pro-angiogenic effect in placental tissue [25] and an anti-apoptotic effect on trophoblast cells [22]. Several placental pathologies display altered leptin levels, including lower fetal serum concentrations in growth restricted fetuses and higher maternal serum levels in fetal macrosomia, diabetes mellitus, and recurrent fetal demise [26–30]. The adipokine, metabolic and angiogenic functions of placental leptin have not been examined in the context of CVM.

The objective of this study was to characterize placental micropathology in HLHS, correlate changes with associated fetal growth abnormalities, and identify mechanisms that contribute to pathogenesis. We hypothesized that placental tissue in HLHS is immature and characterized by morphological and vascular abnormalities in some cases, and these findings are more severe in those cases with growth abnormalities.

2. Methods

2.1. Study population

This cohort was assembled retrospectively as a single center case series. Maternal, fetal and neonatal clinical data were collected for term (≥37 weeks gestation) HLHS cases from Cincinnati Children's Hospital Medical Center (CCHMC) and Good Samaritan Hospital (GSH, Cincinnati, Ohio) from 2003 to 2010. Prematurity, multiple gestation pregnancies, and fetuses with genetic abnormalities or additional CVMs were excluded. In addition, cases with a history of maternal diabetes, preeclampsia or hypertension were excluded. HLHS was strictly defined as atresia or stenosis of the aortic and mitral valves, and hypoplasia of the left ventricle and ascending aorta [31], excluding other single ventricle lesions. Placental tissue was available in a subset of cases. Control placental tissue was obtained from uncomplicated term pregnancies with documented normal fetal cardiac structure and function. This study was approved by the Institutional Review Boards of CCHMC and GSH.

2.2. Maternal health

Clinical records were reviewed and the following data was collected: maternal age, pre-pregnancy body mass index (BMI, kg/m^2), smoking habits and weight gain during pregnancy. Pre-pregnancy BMI and smoking habits were ascertained by self-reporting. Smoking was defined as any history of smoking during pregnancy, excluding cessation prior to pregnancy. In addition, mode of delivery and the presence of chorioamnionitis were noted.

2.3. Fetal and neonatal health

The diagnosis of HLHS was determined by neonatal echocardiogram.

Estimated fetal weight and gestational age data were collected at the in utero stage. Anthropometrics, including birth weight, gender and gestational age at the time of delivery were collected at birth. Fetal and newborn percentiles were derived from the Olsen standard, based on growth curves derived from a large contemporary US sample adjusted for gestational age and gender [32]. Fetal Growth Restriction (FGR) was defined as an estimated fetal weight less than or equal to the tenth percentile. Small for gestational age (SGA) was defined as a birth weight less than or equal to the tenth percentile. Low birth weight (LBW) was considered a birth weight less than 2500 g.

2.4. Placenta

Gross placental evaluation was based on routine clinical pathology reports generated at the time of delivery for all cases and controls. This evaluation included measures of stripped weight and dimensions, site of cord insertion, the presence of chorioamnionitis, inflammation, thrombi, infarction or fibrin deposition. Placentas from both Control and HLHS cohorts were formalin fixed immediately following delivery, and sent for gross pathological assessment. Placental tissue blocks were obtained subsequently for additional research analyses as described below.

2.5. Histochemistry and immunohistochemistry

Serial sections were stained with Hematoxylin and Eosin (H&E) and assessed for evidence of inflammation and alterations in villous architecture as described below. For antibody staining,