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Hemoglobin Bart hydrops fetalis: A model for studying vascular changes in placental hypoxia

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ABSTRACT

Introduction: Placental ischemia can be pre-placental (maternal), placental or post-placental (fetal), with corresponding changes in villous vasculature. Hydrops fetalis (HF) resulting from hemoglobin (Hb) Bart disease can serve as a model for intrauterine hypoxia, and placentas from such cases show a distinctive peripheral villous stromal myofibroblastic hypercellularity (PVSH). We hypothesized that Hb Bart disease, which results in profound fetal hypoxia, would lead to placental hypoxia on a post-placental basis.
Methods: We assessed villous vasculature using computerized morphometry, comparing placentas in 14 Hb Bart HF cases to 18 non-Hb Bart HF cases. Morphometric parameters were matched as closely as possible to those reported in the literature for comparison purposes.
Results: Villous vessels of Hb Bart HF showed significantly increased numbers of vessels ($p = 0.001$), longer vascular perimeter ($p = 0.002$), thickening of vascular endothelial layer ($p = 0.038$) and higher shape coefficient ($p = 0.042$) indicating a more branching pattern of vessels. In addition, placental villi of Hb Bart HF containing PVSH showed a longer vascular perimeter ($p = 0.008$) and narrower lumen ($p = 0.002$), with a higher shape coefficient ($p = 0.03$), in comparison to villi lacking PVSH.
Discussion: Contrary to expectations, the overall pattern of vascular changes in Hb Bart HF suggested multifactorial hypoxia: pre-placental, on the basis of the marked placentomegaly, compromising blood flow from uterine distention; placental, from hydropic villi causing a generalized diminished intervillous space; and post-placental from the greatly reduced capacity of Hb Bart to extract oxygen from the intervillous space. Standardized vascular morphometry will facilitate comparison between different conditions, for a better understanding of placental hypoxia.

1. Introduction

Diffuse chronic hypoxic placental injury can be divided into pre-placental, placental and post-placental categories [1–3]. The placenta responds to chronic hypoxia in more than one morphologic pattern [1–4] and villous vascular patterns often reflect the etiology of hypoxia [1,4,5]. For example, hypervascularity with a branching pattern of vascularization is more often related to pre-placental hypoxia or placental hypoxia, whereas a hypovascularized, non-branching pattern is more often associated with post-placental hypoxia [1–3,5–7]. An accurate assessment of vascularity by routine light microscopy is difficult and subjective, but with computerized morphometry, a variety of vascular parameters can be accurately measured. Studies that have followed this approach have shown a correlation between vascular morphometry and the type of placental hypoxia [1,6–10].

We have previously shown that hydrops fetalis (HF) in cases of hemoglobin (Hb) Bart can serve as a model for intrauterine fetal hypoxia [11,12]. Fetuses with Hb Bart suffer from hypoxia very early in gestation since Hb Bart has an extremely high oxygen affinity, preventing oxygen transportation to the body tissues. We previously reported a distinctive morphologic feature termed peripheral villous stromal hypercellularity (PVSH) that was observed multifocally throughout placental tissue in most cases of Hb Bart HF, but not in placentas from HF attributed by other causes. Such hyperplastic stromal cells were myofibroblasts and associated with complex vascular networks [13].

The vascular changes in post-placental hypoxia have not been well characterized by morphometry to the same degree as pre-placental and placental causes [1,6]. We reasoned that Hb Bart HF

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could be used as a model to study placental hypoxia, and that this clinical situation would correspond to post-placental hypoxia. We therefore performed morphometry on placentas from a series of Hb Bart HF cases to document vasculature changes.

2. Materials and methods

2.1. Study group

The study utilized placentas from stillborn fetuses with HF examined from 2001 to 2012 in the Pathology Department at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. The study was approved by the hospital's institutional review board (Certificate of Approval #614/2015). Thirty-two placentas with available blocks were recruited in the study, comprising 14 cases of Hb Bart HF (proven by cord blood electrophoresis) and 18 cases of HF by other causes including 7 cases related to anemia (non-Hb Bart) and 11 cases due to causes other than anemia. The details of these cases have been previously published [13]. Placentas were weighed before fixation and following removal of adherent blood, umbilical cord, and extraplacental membranes. Placental weight percentiles were based on published means and standard deviations (SDs) [14].

2.2. Placental immunohistochemistry

Hematoxylin and eosin-stained slides of placental tissues were reviewed. Blocks from Hb Bart HF cases were selected for morphometry to include both villi showing PVSH and villi lacking this change. PVSH was identified as an increase in the number of stromal cells at the periphery of the intermediate placental villi beneath the trophoblastic cells, confirmed in each case by immunopositivity for α-smooth muscle actin (BioGenex; dilution 1:700) [13]. Hb Bart HF placentas with absent PVSH or PVSH present only in one area on a single slide were not included in the study. Villous vessels were identified by CD34 immunostaining (DAKO; ready to use). 3-μm formalin-fixed, paraffin-embedded tissue sections were mounted on positively charged slides and baked overnight at 60 °C prior to immunostaining. Immunostaining was performed on the Ventana Benchmark auto-immunostainer (Tucson, AZ, USA) using the ultraVIEW Universal DAB (diaminobenzidine) Detection Kit (Ventana). Slides were counterstained with hematoxylin.

2.3. Vessel morphometry

Vessel morphometry was undertaken on digitalized images of CD34-immunostained slides. Whole slide images of each case were viewed on a computer screen. Villi from three different areas were analyzed, including the layer immediately below the chorionic plate, the basal plate, and the area between these two. The measurements were taken using digital image analysis software (Aperio ScanScope™ system) that includes the following parameters: total area of villous stroma (mm^2), number of vessels (n), vessel density ($n/\mu m^2$), perimeter (cross-sectional vascular boundary) (μm), vascular area (μm^2), luminal area (μm^2) and endothelial thickness (μm). To reflect the villous vessel growth and remodeling during maturation, shape coefficient as a second variable was calculated as $perimeter^2/area$ [15,16]. Shape coefficient is a value independent of vascular calibre and related to shape. A higher value of shape coefficient correlates with a greater branching pattern of vasculature [6,16].

Using an on-screen magnification of ×400, villous contours and vessels were manually outlined on the screen with a mouse-controlled cursor. Intermediate villi were considered to be villi with a diameter of >80 μm and terminal villi, with a diameter <80 μm [6,7,17]. Villi with stromal connective tissue were excluded to rule out stem villi. For placentas from HF cases other than Hb Bart, vessel analysis was done on 15 intermediate villi and 15 terminal villi, randomly selected. All placentas in Hb Bart HF cases showed generalized delayed villous maturation with predominantly intermediate villi and rare to absent terminal villi [18,19], and therefore vessel analysis in these cases did not include terminal villi. For each placenta belonging Hb Bart HF, vessel analysis was done from 15 intermediate villi containing PVSH and 15 intermediate villi lacking PVSH, randomly selected. The number of 15 villi of each subtype was chosen based on previous studies showing an inter-observer and intra-observer variability of <15%, with randomly selected villi [7,17,20].

2.4. Statistical analysis

Data were analyzed using Statistical Package for the Social Science (SPSS) for window. The variables were compared by one-way analysis of variance (ANOVA) with the Scheffe or Tamhane's test, as appropriate. The relationships between Hb levels and morphometric data were tested by the non-parametric Spearman rank correlation method. All tests were performed at a 2-sided significance level of 5%.

3. Results

In the Hb Bart HF group, there were 6 male and 8 female, with a mean (±SD) gestational age of 29 (±4.4) weeks. In the group of HF by other causes, there were 8 male and 10 female, with a mean (±SD) gestational age of 30 (±3.3) weeks. There was no statistically significant difference between the mean gestational ages of 2 groups ($p = 0.89$). The mean (±SD) Hb levels of the Hb Bart HF and anemic HF of non-Bart groups were 6.1 (±1.39) g/dL and 6.2 (±4.16) g/dL, respectively, this difference being not statistically significant ($p = 0.98$). Placental weights of >95th percentile were observed in all 14 cases (100%) in the Hb Bart HF group, 6/7 cases (86%) in anemic HF of non-Bart etiology group, and 6/11 cases (55%) in the non-anemic HF group. Using the value of mean ± 1 SD for gestational age as a cut-off point, the placental weights of the Hb Bart HF group were significantly heavier than those in other 2 groups ($p = 0.031$), and independent of gestational age ($p = 0.08$) or Hb level ($p = 0.22$).

Table 1
Vascular parameters of the placental intermediate villi containing peripheral villous hypercellularity (PVSH) and those lacking PVSH in hemoglobin Bart hydrops fetalis.

Variables	Villi containing PVSH	Villi lacking PVSH	*p* value
Total villous area of analysis (mm^2)	21.5 ± 14.18	27.8 ± 13.40	0.47
Number of vessels (n)	169 ± 100.9	242 ± 102.9	0.34
Vessel density ($n/\mu m^2$)	0.0009 ± 0.00051	0.0009 ± 0.00045	0.97
Vascular perimeter (μm)	202.0 ± 74.09	127.2 ± 65.96	0.008*
Luminal area (μm^2)	52.5 ± 26.21	137.9 ± 64.53	0.002*
Endothelial thickness (μm)	2.2 ± 0.71	2.1 ± 0.45	0.97
Shape coefficient	110.7 ± 87.20	54.8 ± 30.80	0.03*