BASIC SCIENCE: OBSTETRICS

Angiogenic dysfunction in molar pregnancy

David Kanter, MD; Marshall D. Lindheimer, MD; Eileen Wang, MD; Romana G. Borromeo, MD; Elizabeth Bousfield, MD; S. Ananth Karumanchi, MD; Isaac E. Stillman, MD

OBJECTIVE: Molar pregnancy is associated with very early-onset preeclampsia. Since excessive circulating antiangiogenic factors may play a pathogenic role in preeclampsia, we hypothesized that molar placentas produce more antiangiogenic proteins than normal placentas.

STUDY DESIGN: This retrospective case-control study used a semiquantitative immunohistochemical technique to compare histologic sections of molar placentas to normal controls. Tissue slides were treated with 2 antisera: one recognized the antiangiogenic markers fms-like tyrosine kinase receptor 1 (Flt1) and its soluble form (sFlt1), while the other recognized vascular endothelial marker CD31. Stain intensity was graded from 1+ (strong focal staining) to 4+ (91-100% staining).

RESULTS: Molar placentas (n = 19) showed significantly more staining than controls (n = 16) for Flt/sFlt1 ($P < .0001$).

CONCLUSION: There was a significant difference in Flt1/sFlt1 immunostaining intensity when molar placentas were compared to controls. This supports a hypothesis that the phenotype of preeclampsia in molar pregnancy may result from trophoblasts overproducing at least 1 antiangiogenic protein.

Key words: antiangiogenic factors, fms-like tyrosine kinase receptor, hydatidiform mole, molar pregnancy, soluble fms-like tyrosine kinase receptor 1

Cite this article as: Kanter D, Lindheimer MD, Wang E, et al. Angiogenic dysfunction in molar pregnancy. Am J Obstet Gynecol 2010;202:184.e1-5.

Hydatidiform mole and preeclampsia are 2 disorders unique to pregnancy. Both have dysfunctional placentas that are integral to each disease process. Hydatidiform mole, or molar pregnancy, is a group of disorders of genomic imprinting characterized by varying degrees of trophoblastic proliferation and hydropic change of the chorionic villi.

The forms of molar pregnancy are termed *complete* and *partial*.[1] *Complete moles* are characterized by a 46XX karyotype, with both sets of chromosomes typically of paternal origin. Villi are voluminous and show diffuse hydropic (edematous) changes, with cytologically atypical hyperplastic trophoblasts. In contrast, *partial moles* are usually triploid. Villi show focal hydropic changes, with minimal cytologic atypia. Compared to complete moles, partial moles are less likely to evolve into choriocarcinoma. Edematous or hydropic villi, regardless of molar form, typically display cistern formation, namely a central acellular space. Such villi are often avascular or display markedly reduced vessel density.

The incidence of molar pregnancy varies greatly geographically, and appears related to ethnicity,[2] being more frequent in Southeast Asia (1/1000-2/1000 in Japan and China) than North America (0.5/1000-1/1000).[2] However, population-based studies have shown variable incidence within a geographic location. One example is North America, where the incidence of molar pregnancy for American Indians in New Mexico is 1/486 pregnancies. Similarly, Alaskan natives have an incidence rate 3-fold to 4-fold higher than non-Hispanic whites.[3] Molar pregnancy is a risk factor for very early-onset preeclampsia,[4] a disorder in which increased soluble fms-like tyrosine kinase receptor 1 (sFlt1) has been implicated in its pathogenesis.[5-8]

In the past, molar pregnancies were suspected when vaginal bleeding, increased uterine size for gestational age, and elevated β-hCG (human chorionic gonadotropin) were observed in early pregnancy. Hyperemesis and pre-

From the Departments of Obstetrics and Gynecology (Drs Kanter, Lindheimer, and Wang) and Medicine (Dr Lindheimer), The Pritzker School of Medicine, University of Chicago, Chicago, IL; the Departments of Obstetrics and Gynecology (Dr Borromeo) and Pathology (Dr Bousfield), Makati Medical Center, Makati City, Republic of the Philippines; the Departments of Medicine (Dr Karumanchi) and Pathology (Dr Stillman), Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; and Howard Hughes Medical Institute, Chevy Chase, MD (Dr Karumanchi).

Presented at the 16th World Congress of the International Society for the Study of Hypertension in Pregnancy, Washington, DC, Sept. 21-24, 2008; the 28th Annual Meeting of the Society for Maternal-Fetal Medicine, Dallas, TX, Jan. 28-Feb. 2, 2008; and the Annual Meeting of the North American Society for the Study of Hypertension in Pregnancy, San Diego, CA, June 29-July 1, 2007.

Received April 24, 2009; revised July 11, 2009; accepted Sept. 10, 2009.

Reprints not available from the authors.

Dr Karumanchi is supported by a Clinical Scientist Award from the Burroughs Wellcome Fund and an Established Investigator Award from the American Heart Association and an investigator at the Howard Hughes Medical Institute. Dr Karumanchi is a coinventor on patents for the use of angiogenic proteins for the diagnosis/therapy of preeclampsia. Dr Karumanchi is a consultant to Abbott (Abbott Park, IL), Beckman Coulter (Fullerton, CA), Roche (Basel, Switzerland), and Johnson & Johnson (New Brunswick, NJ).

Dr Kanter is currently with the Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Women's Hospital, Pittsburgh, PA.

0002-9378/$36.00 • © 2010 Published by Mosby, Inc. • doi: 10.1016/j.ajog.2009.09.005

eclampsia before midgestation raised suspicion further.[4] This clinical picture has changed, as widespread use of β-hCG measurement and ultrasound have led to the earlier diagnosis of molar pregnancy. Treatment by evacuation of the uterus often occurs prior to the presentation of many of the previous hallmark signs and symptoms. Consequently, while vaginal bleeding remains the most common presenting symptom, others, such as increased uterine size, hyperemesis, and very early-onset preeclampsia, are significantly less common.[4] However, in developing nations, where access to health care is limited, the classic symptoms described above may be more prevalent.

Preeclampsia is customarily diagnosed when new-onset hypertension and proteinuria occur after midgestation. While the etiology of preeclampsia remains unclear, 2 of its phenotypes, hypertension and proteinuria, and its characteristic renal lesion, glomerular endotheliosis, are believed to be caused by an excess of circulating sFlt1, an endogenous antiangiogenic protein that enters the maternal circulation after being overproduced in the placenta. Specifically, the soluble factor sFlt1 antagonizes, or decreases, free maternal circulating levels of angiogenic proteins such as free vascular endothelial growth factor (VEGF) and free placental growth factor (PlGF).[5,6,9] The free circulating angiogenic factors VEGF and PlGF are critical for endothelial growth, differentiation, and vascular integrity.[10] They also decrease vascular resistance and blood pressure.

Given the association between molar pregnancy and very early-onset preeclampsia, we hypothesized that placentas from molar pregnancies would produce more antiangiogenic proteins than normal controls.

MATERIALS AND METHODS

Subject selection

This was a retrospective case-control study examining archived tissue and patient records. Cases (partial, complete, and invasive moles) were from Makati Medical Center, Makati City, Republic of the Philippines, while controls (first-trimester normal placentas) were from the University of Chicago Hospitals. The institutional review board at the University of Chicago approved this study. Institutional review board approval at Makati Medical Center was not necessary because no therapeutic treatment was rendered. The following clinical data were obtained from each subject's medical record: subject age, estimated gestational age, medical history, history of gestational trophoblastic disease, presenting signs and symptoms (including signs and symptoms of preeclampsia), entry β-hCG, blood pressure, and qualitative measurement of proteinuria. The pathologic diagnosis was available for all samples, with approximately 50% of cases being complete molar pregnancies. Preeclampsia was defined as new-onset hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) plus de novo proteinuria (qualitative, 1+; or, quantitative, ≥300 mg/day).[9]

Placentas from subjects undergoing elective pregnancy termination during the first trimester were used as controls. Control subjects with known risk factors for preeclampsia were excluded. This included diagnoses of end-stage renal disease, vasculitis, poorly controlled hypertension, or poorly controlled diabetes mellitus.

Placental weight was unavailable for both cases and controls.

Morphologic evaluation

Formalin-fixed, paraffin-embedded tissue was processed and stained with hematoxylin and eosin using routine methods. Two sets of immunoperoxidase studies were then performed. Villous vascular density was documented using the endothelial marker CD31 (antibody against clone 1A10, together with the Bond Polymer Detection system currently supplied by Leica Microsystems Inc, Bannockburn, IL). Fms-like tyrosine kinase receptor 1 (Flt1) and sFlt1 were identified using a goat antihuman VEGFR-1/Flt1 antibody (catalog no. AF321; R & D Systems, Minneapolis, MN) and an antigoat cell and tissue staining kit (catalog no. CTS 008; R & D Systems). Antigen retrieval was performed using citrate buffer and microwave heating. Two preparations were made for all samples, 1 with a primary antibody dilution of 1:80, and the other 1:200. Initial evaluation of the Flt1 preparations was done using the 1:80 antibody dilution. In an attempt to increase the discriminating power of the technique, Flt1 immunoperoxidase preparations were repeated using a primary antibody concentration of 1:200. The remainder of the protocol was identical.

Evaluation of the histologic and immunoperoxidase sections was performed by a single pathologist (I.E.S.) in a blinded fashion. Grading of villous trophoblast Flt1 staining (1:200 dilution) was done using a semiquantitative ordinal scale as follows: 1+ (strong focal trophoblast staining), 2+ (<50% of the villous trophoblast showing staining), 3+ (51-90% staining), and 4+ (91-100% staining). Weak staining was considered to be negative.

A 2-sided *t* test was used to determine statistical significance. Statistical analysis was performed with the use of software (Stata 10 SE; StataCorp LP, College Station, TX).

RESULTS

There were 20 cases and 16 controls selected for analysis. One case was excluded because pathologic review showed invasive disease. The remainder of the diagnoses were confirmed by hematoxylin and eosin review. One control had well-managed hypertension and was included in the analysis. Final analysis was performed on 19 cases and 16 controls.

As expected, hydropic villi showed markedly reduced vessel density by CD31 staining (Figure 1, A and B). Semiquantitative analysis revealed that the molar tissue showed significantly more staining for Flt1/sFlt1 than controls (Figure 1, C and D). The spikeplot (Figure 2) shows the distribution of stain intensity as a function of disease state.

Data on gestational age were unavailable for 1 case and 2 controls. With the available data, the mean gestational age of subjects comprising the cases was