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Twin-to-twin transfusion syndrome: an anti-angiogenic state?

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Abstract

Objective—An imbalanced chronic blood flow between the donor and recipient twin through placental vascular anastomoses is the accepted pathophysiology of twin-to-twin transfusion syndrome (TTTS). Vascular endothelial growth factor receptor-1 (VEGFR-1) mRNA is overexpressed only in the syncytiotrophoblast of the donor twin in some cases of TTTS. This study was conducted to determine maternal plasma concentrations of placental growth factor (PIGF), soluble VEGFR-1, and soluble endoglin (s-Eng) in monochorionic-diamniotic pregnancies with and without TTTS.

Study design—This case-control study included monochorionic-diamniotic pregnancies between 16–26 weeks with and without TTTS. Maternal plasma concentrations of PIGF, sVEGFR-1 and s-Eng were determined with ELISA. A p-value <.05 was considered statistically significant.

Results—Patients with TTTS had higher median plasma concentrations of s-Eng [14.8 ng/ml vs. 7.8 ng/ml; p<0.001] and sVEGFR-1 [6383.1 pg/ml vs. 3220.1 pg/ml; p<0.001]; and lower median plasma concentrations of PIGF [115.5 pg/ml vs. 359.3 pg/ml; p=0.002] than those without TTTS.

Conclusions—We propose that an anti-angiogenic state may be present in some cases of TTTS.

Keywords

monochorionic; twin pregnancy; TTTS; angiogenesis; angiogenic factors; endoglin; sVEGFR-1; sFlt1; placental growth factor; PIGF; birthweight discordancy

INTRODUCTION

Chorionicity, rather than zygosity, is the main determinant of pregnancy outcome in twin gestation.^{1–3} Indeed, monochorionic (MC) twins have a higher risk of miscarriage, fetal death, preterm delivery, intrauterine growth restriction, birthweight discordancy,^{2;4–7} as well as a higher rate of cerebral palsy and neurologic morbidity^{8;9} than dichorionic (DC) twins. These differences have been attributed to abnormalities in the placental angioarchitecture, including abnormal umbilical cord insertion,^{10–12} unequal placental sharing,¹³ and to the presence of placental vascular anastomoses that lead to the twin-to-twin transfusion syndrome (TTTS) and its consequences.^{3;14}

The conventional view is that a chronic blood flow imbalance from the donor to the recipient twin due to a net unidirectional blood flow through placental vascular anastomoses^{15–17} is responsible for the development of TTTS. However, the presence of vascular anastomosis is necessary but not sufficient to induce TTTS. Indeed, almost all monochorionic twin placentas have vascular anastomoses;^{16–18} however, TTTS is present only in 5 to 15% of these pregnancies,^{19–21} and no more than 25% of the MC twin pairs with TTTS have >15% of hemoglobin discordance.²² Thus, although placental vascular anastomoses are a *sine qua non* requirement for the development of TTTS, the pathophysiology of TTTS may not be explained only on the basis of placental vascular anastomoses.

Angiogenesis plays a central role in normal placental development,^{23–25} and accumulating evidence indicates that angiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and anti-angiogenic factors such as soluble VEGF receptor-1 (sVEGFR-1, also referred to as sFlt1) and the soluble form of Endoglin (s-Eng) are involved in the pathophysiology of preeclampsia,^{26–54} small for gestational age (SGA),^{31; 54–59} placental abruption,⁶⁰ “mirror syndrome,”^{61–63} preeclampsia with parvovirus-induced hydrops,⁶⁴ and unexplained fetal death.⁶⁵ Recently, it has been reported that VEGFR-1 mRNA is overexpressed in the syncytiotrophoblast of the donor but not in that of the recipient twin in some cases of TTTS,⁶⁶ suggesting that this anti-angiogenic factor may play a role in TTTS. The objective of this study was to determine if there are changes in the maternal plasma concentrations of angiogenic (PlGF), and anti-angiogenic factors (sVEGFR-1 and s-Eng) in monochorionic-diamniotic twin pregnancies with and without TTTS.

MATERIALS AND METHODS

Study design and population

A case-control study was designed to examine the maternal plasma concentration of angiogenic and anti-angiogenic factors in monochorionic twins with and without TTTS. We searched our clinical database, bank of biological samples, and digital library of ultrasound images to identify patients with monochorionic-diamniotic twin pregnancies between 16 and 26 weeks of gestation with and without TTTS. Patients with the diagnosis of preeclampsia at the time of venipuncture or fetal congenital anomalies were excluded.

Definitions

Monochorionic placentation was diagnosed by ultrasonography before 20 weeks of gestation based on the presence of a single placental mass, same fetal gender, absence of the twin-peak sign and dividing membrane thickness <2 mm,^{67–70} confirmed with placental histopathology. TTTS was defined as oligohydramnios [maximum vertical pocket (MVP) of amniotic fluid <2 cm] in the donor twin and polyhydramnios (MVP >8 cm) in the recipient twin. Preterm delivery was defined as delivery before 37 completed weeks of gestation. Preeclampsia was diagnosed in the presence of systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg

on at least two occasions 4 hours to 1 week apart, after the 20th week of gestation, and proteinuria >300 mg in a 24-hour urine collection, or two random urine specimens obtained 4 hours to 1 week apart containing $\geq 1+$ protein by dipstick^{71;72} or one dipstick measurement $\geq 2+$ protein.⁷³ Fetal death was considered to have occurred in the absence of fetal heart activity after 20 weeks of gestation. Intrauterine growth restriction was defined as a birthweight below the 10th percentile.^{74;75}

All patients provided written informed consent before participating in the study. The collection and utilization of samples, and the use of clinical and ultrasound data for research purposes was approved by the Institutional Review Boards of the Sotero del Rio Hospital (a major affiliate of the Catholic University, Santiago, Chile), Wayne State University, and the National Institute of Child Health and Human Development (NICHD/NIH/DHHS).

Sample collection and human PIGF, sVEGFR-1, and s-Eng immunoassays

Samples of peripheral blood were obtained by venipuncture and collected in tubes containing EDTA. The samples were centrifuged at 4°C for 10 minutes and stored at -70°C until assay. Maternal plasma concentrations of sVEGFR-1, PIGF and soluble endoglin were determined by specific and sensitive enzyme-linked immunoassays (R&D Systems, Minneapolis, MN). Briefly, plasma samples were incubated in duplicate wells of the micro titer plates, which had been pre-coated with antigen specific (PIGF, sVEGFR-1, or s-Eng) monoclonal antibodies. During this incubation, PIGF, sVEGFR-1 or s-Eng present in the standards or maternal plasma samples was immobilized by their specific pre-coated antibodies (form antigen-antibody complexes). Repeated washing and aspiration removed all other unbound materials from the assay plate. This step was followed by incubation with a specific antibody-enzyme reagent for a specified amount of time. Following a wash to remove excess and unbound materials, a substrate solution was added to the wells of the micro titer plate and color developed in proportion to the amount of antigen bound in the initial step of the individual assays. The color development was stopped with the addition of an acid solution and the intensity of color was read using a programmable micro titer plate spectrophotometer (SpectraMax M2 micro plate workstation, Molecular Devices Corporation, Sunnyvale, CA). The concentrations of PIGF, sVEGFR-1 or s-Eng in maternal plasma samples were determined by interpolation from individual standard curves composed of purified human PIGF, sVEGFR-1 or s-Eng. The calculated inter-assay coefficients of variation for s-Eng, sVEGFR-1 and PIGF in our laboratory were 3.3%, 5.3%, and 7%, respectively. The calculated intra-assay coefficients of variation for s-Eng, sVEGFR-1, and PIGF were 2.7%, 2.2%, and 5.8%, respectively. The sensitivity was calculated to be 0.04 ng/ml for s-Eng, 13.1 pg/ml for sVEGFR-1 and 5.5 pg/ml for PIGF assays.

Statistical analysis

The Kolmogorov–Smirnov test was used to test for normal distribution of the data. Comparisons among groups were performed using Mann-Whitney U test for continuous variables, and Chi-square or Fisher's exact test for categorical variables. A p-value <.05 was considered statistically significant. The statistical package used was SPSS v.14.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Sixty-nine monochorionic-diamniotic twin pregnancies with (n=16) and without (n=53) TTTS at 16–26 weeks of gestation were included in this study. In the TTTS group, all maternal blood samples were collected at the time of the clinical presentation and before treatment. Staging of TTTS was scored according to a previously described system.⁷⁶ There were two patients

in stage I, six in stage II, seven in stage III and one in stage V. There were no patients with stage IV.

Table 1 displays the demographic and clinical characteristics of the study population. Patients with TTTS had a significantly lower proportion of primigravidae [12.5% (2/16) vs. 39.6% (21/53); $p=0.04$] and delivered earlier [31 weeks (interquartile range (IQR): 31.9–37.5) vs. 35.6 weeks (IQR: 22.7–34.2); $p=0.009$] than those without TTTS.

Figures 1, 2, and 3 display the median maternal plasma concentrations of s-Eng sVEGFR-1, and PlGF, respectively, in patients with monochorionic-diamniotic pregnancies with and without TTTS between 16 and 26 weeks of gestation.

Patients with TTTS had a significantly higher median plasma concentration of s-Eng [14.8 ng/ml (IQR: 9.6–33.5) vs. 7.8 ng/ml (IQR: 6.7–9.5); $p<0.001$] and sVEGFR-1 [6383.1 pg/ml (IQR: 4874.5–18047.8) vs. 3220.1 pg/ml (IQR: 2310.1–5172.1); $p<0.001$], and a significantly lower median maternal plasma concentration of PlGF [115.5 pg/ml (IQR: 51.9–357.4) vs. 359.3 pg/ml (IQR: 224.6–693.9); $p=0.002$] than those without TTTS.

Among patients without TTTS, a subanalysis was performed to determine the maternal plasma concentrations of PlGF, sVEGFR-1 and s-Eng at the time of delivery between those with ($n=18$) and without ($n=24$) IUGR. Among patients with IUGR, eleven had one IUGR fetus, whereas in 7 cases both fetuses were IUGR. No significant differences were observed in the median maternal plasma concentrations of PlGF [IUGR: 162.5 pg/ml (IQR: 105.8–226.2) vs. no IUGR: 184.5 pg/ml (IQR: 134.5–290.2); $p=0.3$], sVEGFR-1 [IUGR: 13543.5 pg/ml (IQR: 6909.5–23448) vs. no IUGR: 16140.7 pg/ml (IQR: 9105.7–22785.7); $p=0.4$], and s-Eng [IUGR: 35 ng/ml (IQR: 18.8–41.2) vs. no IUGR: 30.2 ng/ml (IQR: 23.1–44.7); $p=0.98$].

COMMENT

Principal findings of this study

Patients with monochorionic twin pregnancies complicated by TTTS between 16 to 26 weeks of gestation have significantly higher median maternal plasma concentrations of s-ENG and sVEGFR-1, and a significantly lower median maternal plasma concentration of PlGF than those without TTTS.

Placental vascular anastomoses and TTTS

Almost all monochorionic placentas have vascular anastomoses, which are either superficial or deep.^{16–18} The traditional view of the pathophysiology of TTTS is that there is an imbalance of blood volume exchange from the donor to the recipient twin through these placental vascular anastomoses.^{15–17} Although almost all monochorionic twin placentas have vascular anastomoses,^{16–18} TTTS is present only in a small proportion of these pregnancies.^{19–21} It has been proposed that the number, type, and size of the anastomoses are the key factors that allow the hemodynamic stability among monochorionic twins.⁷⁷ Virtually all placentas from patients with TTTS have at least one arteriovenous anastomosis from the donor to the recipient, and approximately 96% have an arteriovenous anastomosis from recipient to donor. In addition, approximately 20% of placentas of patients with TTTS have a superficial anastomosis.⁷⁸ Interestingly, TTTS can also develop through superficial anastomoses in the absence of deep vascular communications, suggesting that superficial anastomoses may be the cause of TTTS in a subset of patients.⁷⁹

It has been proposed that the pathophysiology of TTTS is due to multiple pathologic processes^{80–82} and a growing body of evidence suggests that other mechanisms may play a role in the pathophysiology and clinical presentation of TTTS: 1) recipient twins have higher

concentrations of atrial natriuretic peptide and endothelin-1 than donor twins, which has been associated with cardiac dysfunction in the recipient twin;⁸³ 2) overexpression of renin in the kidney of the donor twin and severe arterial and glomerular lesions like hypertension-induced microangiopathy in the kidney of the recipient have been reported in pregnancies complicated by TTTS;⁸⁴ and 3) donor twins have lower cord blood concentrations of leptin⁸⁵ and insulin-like growth factor-II⁸⁶ than the recipient twins, suggesting that discordant placental metabolic function may be the cause of the growth restriction in those twins and, because placental dysfunction is related with increased fetoplacental resistance, that could be an explanation for transfusion from the growth-retarded donor to the recipient twin.⁸⁰ Whether these conditions are causative or an epiphenomenon is not clear.

Twin-to-twin transfusion syndrome: an anti-angiogenic state?

There is a paucity of data regarding maternal plasma or serum concentrations of pro- and anti-angiogenic factors in twin pregnancies. The study reported herein demonstrated that women with monochorionic twin pregnancies complicated by TTTS between 16 to 26 weeks of gestation have significantly higher median maternal plasma concentrations of the anti-angiogenic factors soluble Endoglin and sVEGFR-1, and a significantly lower median maternal plasma concentration of the angiogenic factor PlGF than those without TTTS. This finding is novel and it is in keeping with a study reporting that VEGFR-1 mRNA is overexpressed in the villi of the donor, but not in that of the recipient twin in some cases of TTTS.⁶⁶ The villi of the donor showed increased syncytiotrophoblastic knots, shrinkage of villi, increased perivillous fibrin deposition, villous infarction, and villous hypercapillarization. The authors suggest that, in some cases of TTTS, this may represent a hypoxic/ischemic state in the donor villi due to fetal villous hypoperfusion rather than an abnormal uteroplacental circulation.⁶⁶

In this study, among monochorionic-diamniotic twin pregnancies without TTTS, the prevalence of preeclampsia was 11.8%. In contrast to what was expected, no patients in the TTTS group developed preeclampsia. It is possible that these patients did not have enough time to develop preeclampsia, since the median gestational age at delivery in the TTTS group was 31 weeks of gestation and almost 70% of patients with TTTS delivered before 34 weeks.

Since we do not have longitudinal maternal blood samples available in the TTTS group, it is not possible to determine if the maternal plasma concentrations of angiogenic (PlGF) and anti-angiogenic factors (sVEGFR-1 and s-Eng) in the third trimester were low and high enough, respectively, to be associated with the development of preeclampsia.

Recently, Nevo et al.⁸⁷ examined the mRNA and protein expression of sFlt-1 in placentae of twin pregnancies complicated by IUGR of one twin or preeclampsia. In dichorionic twins complicated by twin birthweight discordancy (defined as inter-twin birthweight difference >25%), the sFlt-1 mRNA expression was significantly higher in the IUGR twin placenta compared to the normal twin pair placenta. Similar findings were observed in monochorionic twin pregnancies when the sFlt-1 mRNA placental expression was compared to the normal twin pair placenta as well as to normal control twins without

IUGR. Control twins did not have changes in sFlt-1 mRNA expression. Of interest, the authors demonstrated that the sFlt-1 mRNA expression is higher in the dichorionic than the monochorionic IUGR placenta, and postulated that findings in dichorionic placentae are probably associated with impaired placental development as occurs in singleton pregnancies with IUGR, while in monochorionic twins the presence of vascular anastomoses and unequal placental sharing further complicate the placental findings. In addition, sFlt-1 mRNA expression was significantly higher in the placentae of dichorionic and monochorionic IUGR twins than that of their normal twin pairs and control twins without IUGR.⁸⁷ Among dichorionic twin pregnancies with preeclampsia, but without birthweight discordancy, sFlt-1

mRNA expression was higher in one placenta compared to the other, suggesting discordancy in sFlt-1 expression between twin pairs. The same results were found for sFlt-1 protein expression. The authors proposed that one placenta may elicit the onset of preeclampsia in these patients.⁸⁷ This interpretation is consistent with previous reports of clinical resolution of preeclampsia following the death of the IUGR or hydropic fetus in twin pregnancies.^{88–92}

In conclusion, the study reported herein demonstrates that patients with TTTS had a significantly higher median maternal plasma concentration of s-Eng and sVEGFR-1 (both anti-angiogenic factors), and a significantly lower median maternal plasma concentration of PlGF (an angiogenic factor) than those without TTTS. We propose that an anti-angiogenic state may be present in some cases of TTTS. Further longitudinal studies⁹³ are required to define the subset of TTTS patients in which this occurs and to determine whether this is a cause or consequence of TTTS.

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Glossary

TTTS	twin-to-twin transfusion syndrome
BMI	body mass index
SBP	systolic blood pressure
DBP	diastolic blood pressure
IUGR	intrauterine growth restriction
NS	not significant

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Figure 1.

Median maternal plasma concentrations of soluble Endoglin (s-Eng) among patients with monochorionic-diamniotic pregnancies with and without TTTS between 16 and 26 weeks of gestation. Patients with TTTS had a significantly higher median plasma concentration of s-Eng [14.8 ng/ml (IQR: 9.6–33.5) vs. 7.8 ng/ml (IQR: 6.7–9.5); $p < 0.001$] than those without TTTS.

Figure 2.

Median maternal plasma concentrations of soluble Vascular Endothelial Growth Factor (sVEGFR-1) among patients with monochorionic-diamniotic pregnancies with and without TTTS between 16 and 26 weeks of gestation. Patients with TTTS had a significantly higher median plasma concentration of sVEGFR-1 [6383.1 pg/ml (IQR: 4874.5–18047.8) vs. 3220.1 pg/ml (IQR: 2310.1–5172.1); $p < 0.001$] than those without TTTS.

Figure 3.

Median maternal plasma concentrations of Placental Growth Factor (PlGF) among patients with monochorionic-diamniotic pregnancies with and without TTTS between 16 and 26 weeks of gestation. Patients with TTTS had a significantly lower median maternal plasma concentration of PlGF [115.5 pg/ml (IQR: 51.9–357.4) vs. 359.3 pg/ml (IQR: 224.6–693.9); $p=0.002$] than those without TTTS.

Table 1

Demographic and clinical characteristics of patients with monochorionic-diamniotic twin pregnancies between 16 and 26 weeks of gestation

	No TTTS(n=53)	TTTS(n=16)	p
Maternal age (yrs)	27 (22 – 34)	28.5 (23 – 33)	NS
Primigravida	39.6 (21/53)	12.5 (2/16)	0.04
Height (cm)	155 (152 – 160)	155.5 (152 – 162)	NS
Weight (Kg)	58 (55 – 68)	59.5 (53 – 68)	NS
Pre-pregnancy BMI (Kg/m ²)	24.0 (21.9 – 27)	23.8 (22.5 – 28.9)	NS
Smoking	9.4 (5/53)	12.5 (2/16)	NS
Gestational age at blood draw (wks)	21.7 (19.8 – 23.3)	21.3 (19.5 – 23.5)	NS
SBP (mm Hg) [†]	110 (104 – 125)	110 (110 – 120)	NS
DBP (mm Hg) [†]	70 (65 – 80)	70 (60 – 72)	NS
Preeclampsia	11.8 (6/45)	0 (0/15)	NS
Gestational age at delivery (wks)	35.6 (31.9 – 37.5)	31 (22.7 – 34.2)	0.009
Preterm delivery			
<37 weeks	64.2 (34/53)	84.6 (11/13)	NS
<34 weeks	30.2 (16/53)	69.2 (9/13)	0.009
<32 weeks	24.5 (13/53)	61.5 (8/13)	0.01
IUGR	39.6 (21/53)	60 (6/10)	NS
Fetal death [*]	7.5 (8/106)	10 (3/30)	NS
Sample storage time (yrs)	4.1 (2.4 – 6)	2.7 (1.3 – 5.8)	NS

Values are expressed as percentage (number) or median (interquartile range).

[†] Systolic and diastolic blood pressures at the time of blood draw.

^{*} Values are expressed as percentage (number/total number of fetuses).