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Changes in sVEGFR-1 and sVEGFR-2 Levels Following Fetoscopic Laser Photocoagulation in Twin-to-Twin Transfusion Syndrome: Implications for Fetal demise Prediction

ABSTRACT

Background: Twin-to-twin transfusion syndrome (TTTS) is a severe complication in monochorionic twin pregnancies, leading to high perinatal morbidity and mortality. Fetoscopic laser photocoagulation (FLP) is the gold standard treatment; however, fetal demiseremains a concern. The soluble vascular endothelial growth factor receptors, sVEGFR-1 and sVEGFR-2, play a crucial role in regulating angiogenesis and vascular function. This study evaluates changes in sVEGFR-1 and sVEGFR-2 levels before and after FLP and explores their role in predicting fetal demise post-surgery. **Objective:** Therefore, this study aims to evaluate pre- and post-surgical changes in sVEGFR-1 and sVEGFR-2 levels in TTTS cases treated with FLP and determine their predictive value for fetal demiseafter surgery. **Methods:** A prospective longitudinal study was done with 27 pregnant women with TTTS stage II-IV according to Quintero classification from 16 to 26 weeks of gestation undergoing FLS. Among them, 11 cases were carried out coagulation the placental vascular anastomoses, 16 cases were done ablation umbilical cord for the selective fetal reduction because of TTTS stage IV, selective intrauterine growth restriction (sIUGR) or proximate cord insertions. All the studies subject investigated the soluble levels of biomarkers. We quantified plasma levels of VEGF-R1, VEGF- R2 in twin pregnant with TTTS before and one week after surgery by ELISA. Many factors included maternal age, gestational age at surgery, stage of TTTS, placental location, level of polyhydramnios, FLS methods, sIUGR, amount of amniotic fluid drawn, duration of surgery, change of maternal circulating biomarker levels were analyzed to find out the association with fetal demise after FLS. **Results:** Statistics showed that VEGF-R1 levels were significantly decreased after surgery and change in soluble VEGF-R1 levels after surgery had a difference between the group of fetal demise and non-fetal demise. ROC curve showed that degree of VEGF-R1 levels reduction after surgery were higher, the risk of fetal demise was bigger (AUC: 0.8472), in which, cut-off point of degree of VEGF-R1 levels reduction after surgery was 36.5% (sensitivity: 66.67%, specificity: 95.83%). **Conclusion:** Our data suggest that change in VEGF-R1 after surgery could play a prognostic role of fetal demise after fetoscopic laser surgery.

Keywords: Twin-to-twin transfusion syndrome, laser photocoagulation, sVEGFR-1, sVEGFR-2, fetal loss, prognosis.

1. BACKGROUND

Twin-to-twin transfusion syndrome (TTTS) is a severe complication affecting 10-15% of monochorionic diamniotic (MCDA) twin pregnancies, with an incidence of approximately 1 in 1,000 pregnancies (1). This syndrome results from abnormal vascular anastomoses in the shared placenta, leading to an imbalanced blood exchange between twins. The donor twin experiences hypovolemia, oligohydramnios,

and growth restriction, whereas the recipient twin develops hypervolemia, polyhydramnios, and cardiac overload, significantly increasing the risk of fetal heart failure, preterm birth, and intrauterine demise (2, 3).

Fetoscopic laser photocoagulation (FLP) is the gold-standard treatment for TTTS, as it selectively coagulates abnormal vascular connections, restoring hemodynamic balance between the twins (4). Despite its effectiveness in reducing perinatal mortality, fetal demiseremains a major concern, occurring in 15-30% of cases post-FLP (5, 6). The early identification of biomarkers predictive of fetal demise post-FLP is crucial for improving post-surgical monitoring and neonatal outcomes.

Angiogenesis plays a central role in placental vascular function and fetal development. The soluble vascular endothelial growth factor receptors sVEGFR-1 (sFLT-1) and sVEGFR-2 are key regulators of the VEGF signaling pathway, which maintains endothelial homeostasis and ensures optimal placental perfusion (7). sVEGFR-1 functions as an anti-angiogenic factor by sequestering VEGF, thus reducing its availability for endothelial cell proliferation and vascular repair, whereas sVEGFR-2 facilitates VEGF-mediated angiogenesis (8, 9). In TTTS, an imbalance in sVEGFR-1 and sVEGFR-2 levels may contribute to placental dysfunction and fetal compromise (10).

Previous studies suggest that elevated sVEGFR-1 and reduced sVEGFR-2 levels post-FLP may lead to placental hypoxia, endothelial dysfunction, and increased fetal distress (10). However, limited research has quantified the magnitude of sVEGFR-1 increase and sVEGFR-2 decrease post-FLP, and few studies have directly investigated the relationship between these biomarkers and fetal demiserisk.

2. OBJECTIVE

Therefore, this study aims to evaluate pre- and post-surgical changes in sVEGFR-1 and sVEGFR-2 levels in TTTS cases treated with FLP and determine their predictive value for fetal demiseafter surgery.

3. MATERIAL AND METHODS

Study populations

A prospective study examined a series of 27 consecutive pregnant women affected by TTTS who underwent FLS at HOGH from September 2019 to July 2021. Inclusion critera included pregnant women with TTTS gestational age from 16 to 26 weeks diagnosed stage II, III, IV and treated with FLS in HOGH. Among them, 11 cases were carried out coagulation the placental vascular anastomoses, 16 cases were done ablation umbilical cord for the selective fetal reduction because of TTTS stage IV, selective intrauterine growth restriction (sIUGR) or proximate cord insertions. After the surgery, there were three cases of fetal demise. Blood samples for VEGF-R1 and VEGF-R2 were collected at two points of time pre-FLP and one week post-FLP treatment.

We excluded those who have more than 2 fetuses, severe conditions who have contra-indication to surgeries, fetuses with severe abnormalities, stillbirth pre-operetion, rupture of the membranes, current threaten miscarriage and preterm birth. All patients gave written informed consent for the procedure and consented to their clinical data being used for research purposes. This study was approved by the Institutional Review Board (IRB) of Hanoi Medical University (IRB No NCS25/HMU-IRB).

The diagnosis of TTTS was made according to ISOUG criteria, with polyhydramnios of the recipient fetus and oligodramnios of the stuck donor fetus, and staging was according to that of Quintero et al.. Chorionicity was established by first-trimester sonographic examination at the referring center and confirmed during the detailed sonographic examination at our center (6, 11).

Quantification of plasma VEGF-R1 and VEGF-R2 concentrations by ELISA

The concentrations of VEGF-R1 and VEGF-R2 were quantified in the plasma samples from study subjects by ELISA method using the VEGF Receptor 1 (Soluble) Human ELISA Kit (Invitrogen, Waltham, Massachusetts, USA, Catalog Numbers: BMS2019) and the VEGF Receptor 2 / KDR Human ELISA Kit (Invitrogen, Waltham, Massachusetts, USA, Catalog Numbers: BMS268-3), respectively. The procedures were followed according to the manufacturer's.

Outcomes

The pre-operative, intra-operative, and postoperative outcomes were noted. The main variables were VEGF-R1 and VEGF-R2 level and their changes before and after surgery survival rate postoperation. Many factors included maternal age, gestational age at surgery, stage of TTTS, placental location, level of polyhydramnios, FLS methods, sIUGR, amount of amniotic fluid drawn, duration of surgery, change of maternal circulating biomarker levels were analyzed to find out the association with fetal demise after FLS.

Data were collected and managed by Redcap software and analyzed by STATA 16.0 software. Continuous variables were described as mean with standard deviation and compared

Factors	Time	n	χ SD (Min - Max)	p
VEGF-R1 (ng/mL)	pre-FLP	27	1046,78±707,63 (376,88 - 3147,69)	p<0,05*
	post-FLP	27	825,09±394.47 (359,53 - 1848,11)	
VEGF-R2 (ng/mL)	pre-FLP	27	5280,63±1264,42 (3012,34 - 8608,3	p>0,05*
	post-FLP	27	5390,46±1949,75 (1189 - 3111,33)	

Table 1. Marker Levels pre-FLP and post-FLP. Mean values are expressed as ± SD (Min – Max); (*) t-test

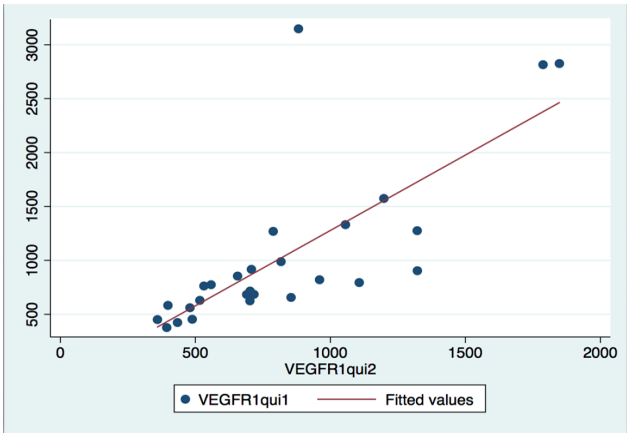


Figure 1: Correlation of Preoperative and Postoperative VEGF-R1 Levels

using the Mann-Whitney U test. Fisher's exact test analyzed categorical variables, and a p-value < 0.05 was considered a statistically significant difference.

4. RESULTS

There was a statistically significant change in VEGF-R1 levels before and after surgery, with post-surgical VEGF-R1 levels being lower than pre-surgical levels.

Preoperative and postoperative VEGF-R1 levels are strongly correlated (r=0.85)

Preoperative and postoperative VEGF-R1 levels showed no statistically significant association with maternal characteristics or TTTS-related factors. Additionally, postoperative VEGF-R1 levels were not influenced by intraoperative factors.

The association between the decrease in VEGF-R1 levels before and after surgery (ΔVEGF-R1) and two key postoperative outcomes—preterm birth and fetal demise—was analyzed. The change in VEGF-R1 levels was significantly associated with fetal demise ($p<0.05$), indicating that a greater reduction in VEGF-R1 levels corresponded to an increased risk of fetal demise.

Changes in VEGF-R1 levels before and after surgery have the potential to predict the risk of fetal demise (AUC: 0.8472; Se 66.67%; Sp 95.83%). A greater reduction in ΔVEGF-R1 before and after surgery is associated with a higher risk of fetal demise. When the ΔVEGF-R1 levels exceeds 36.5%, the risk of fetal demise increases 16-fold (RR = 16; 95% CI: 2.00 - 127.92; p = 0.0012.

preVEGF-R1 Factors	preVEGF-R1 r	p
Mother's age (years)	0,0026	p>0,05*
Gestational age at surgery (weeks)	-0,03	p>0,05*
MVP of polyhydramnios fetus (ml)	0,08	p>0,05*
MVP of oligohydramnios fetus (ml)	0,3	p>0,05*
	χ± SD	p
Vaginitis		
No (24)	1108,96 ± 752,72	p>0,05**
Yes (4)	698,63 54,13	
TTTS stage		
Stage II (20)	1014,97 ± 691,48	p>0,05**
Stage III (4)	1438,29 ± 1201,68	
Stage IV (3)	941,61 ± 303,15	
Placental position		
Posterior placenta (17)	899,68 ± 430,16	p>0,05**
Anterior placenta (10)	1304,21 ±1003,31	
sIUGR		
no (15)	1126,83 ± 812,67	p>0,05**
yes (12)	950,72 ± 569,62	
postVEGF-R1 Factors	postVEGF-R1 SD	p
Surgical method		
Laser selective umbilical cord ablation	929,35 ± 566,15	p>0,05**
Laser selective vascular anastomoses coagulation	1252,29 ± 895,29	
	r	p
Surgery duration (minute)	0,06	p>0,05*
Amniotic fluid drainage volume (ml)	-0,2	p>0,05*

Table 2. The relationship between pre-FLP and post FLP VEGF-R1 levels with various factors . (*) Spearman test; (**) Mann -Whitney test

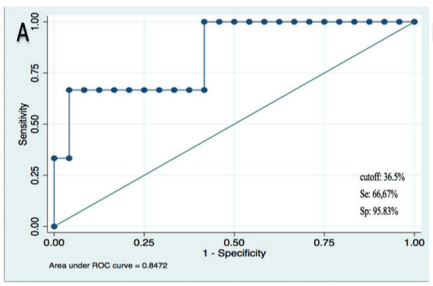
ΔVEGF-R1 Factors	ΔVEGF-R1 χ± SD	p
Fetal demise		
No	5,73 ± 23,48	p<0,05**
Yes	42,13 ± 27,48	
Preterm birth before 37 weeks		
No	0,0058 ± 10,74	p>0,05**
Yes	13,19 ± 29,14	
Preterm birth before 37 weeks		
No	11,71 ± 16,26	p>0,05**
Yes	6,9 ± 36,89	

Table 3. Relationship Between Changes in VEGF-R1 Levels Before and After Surgery (ΔVEGF-R1) and Various Factors . ΔVEGF-R1= (preVEGF-R1 - postVEGF-R1)/preVEGF-R1*100%; (**) Mann -Whitney test

ulation (FLP) in pregnancies complicated by twin-to-twin transfusion syndrome (TTTS). The results demonstrated a significant reduction in sVEGFR-1 levels post-FLP, with a statistically significant difference between the stillbirth and non-stillbirth groups. Notably, a sharp decline in sVEGFR-1 following surgery was associated with an increased risk of stillbirth, with a cutoff value of 36.5% (AUC: 0.8472; Se: 66.67%; Sp: 95.83%). In contrast, sVEGFR-2 levels remained largely unchanged, suggesting distinct roles for these two receptors in the pathophysiology of TTTS and the biological response to FLP. sVEGFR-1 plays a crucial role in maintaining placental homeostasis and regulating angiogenesis. In TTTS, an imbalance between sVEGFR-1 and VEGF may contribute

5. DISCUSSION

This study evaluated changes in the biomarkers sVEGFR-1 and sVEGFR-2 before and after fetoscopic laser photocoag-



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Fetal demise	ΔVEGF-R1<36,5%	ΔVEGF-R1 ≥ 36,5%	n	p	RR (95% CI)
No	23 (95,83%)	1 (33,33%)	24 (88,89%)	p<0,05*	16 (2,00 - 127,92)
Yes	1 (4,17%)	2 (66,67%)	3 (11,11%)		
	24 (100%)	3 (100%)	27 (100%)		

(*) Fisher test

Figure 2. Association Between ΔVEGF-R1 Levels Before and After Surgery and the Prognosis of Fetal Demise

to impaired placental perfusion and an increased risk of pregnancy complications (8).

A study reported a tendency for sVEGFR-1 levels to decrease post-FLP, suggesting that this change reflects adjustments in placental vascular hemodynamics following the removal of abnormal vascular connections (12). Similarly, it was observed that a reduction in sVEGFR-1 levels after FLP may facilitate the restoration of placental circulation, thereby supporting improved fetal development (13). However, some studies did not identify a significant difference in sVEGFR-1 levels between the stillbirth and non-stillbirth groups, indicating that other factors such as postoperative inflammation or coagulation abnormalities may play a more critical role (14).

Beyond TTTS, several studies have explored the role of VEGF and its soluble receptors in the pathophysiology of other pregnancy complications. In preeclampsia, elevated sVEGFR-1 levels have been associated with the inhibition of angiogenesis and reduced placental perfusion (15). Additionally, research on pregnancies complicated by intrauterine growth restriction (IUGR) found that an imbalance in sVEGFR-1 can exacerbate vascular dysfunction, increasing the risk of adverse fetal outcomes (16). Furthermore, a study on pregnancies affected by placental dysfunction identified elevated sVEGFR-1 levels as a significant biomarker for predicting stillbirth, which aligns with the findings of our study (15,16).

Our study highlights the potential clinical value of monitoring sVEGFR-1 as a prognostic biomarker for stillbirth risk. Given its predictive utility, post-FLP surveillance of sVEGFR-1 levels could aid in identifying high-risk pregnancies, thereby guiding tailored monitoring strategies and early intervention. Moreover, our findings suggest that sVEGFR-1 may serve as an important biomarker for evaluating postoperative hemodynamic adjustments. This insight paves the way for future research into therapeutic approaches that target angiogenic factor modulation to optimize pregnancy outcomes. During follow-up, if sVEGFR-1 levels decline excessively after FLP, closer monitoring or adjustments in treatment strategies may help mitigate complications.

Despite its clinical significance, this study has several limitations. First, the small sample size (n=27) may impact the reliability of the findings, necessitating validation in larger cohorts. Second, the short follow-up period, with sVEGFR-1 measurements taken at only two time points (preoperative and one week postoperatively), limits the ability to assess long-term trends. Third, potential confounding factors such as inflammation, coagulation disorders, or environmental influences were not extensively analyzed, which may have affected the results.

Nevertheless, our study provides a foundation for future multicenter research with larger sample sizes to confirm the prognostic value of sVEGFR-1. Long-term monitoring of sVEGFR-1 levels after FLP, extending over several weeks or until delivery, may further clarify its relationship with pregnancy outcomes. Additionally, integrating sVEGFR-1 with other prognostic indicators, such as umbilical artery and middle cerebral artery Doppler indices or inflammatory markers, could enhance the accuracy of clinical models for predicting stillbirth risk.

6. CONCLUSION

FLP significantly alters angiogenesis-related biomarkers, particularly sVEGFR-1 and VEGF, which influence placental function. Increased sVEGFR-1 is a key predictor of fetal mortality and should be closely monitored. Biomarker-based risk assessment may improve TTTS management by guiding early interventions in high-risk cases. However, further research is needed to explore therapeutic strategies for optimizing pregnancy outcomes following FLP.

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- **Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms.
- **Author's contribution:** Nguyen Thi Thu Ha and Phan-Thi Huyen Thuong gave a substantial contribution in acquisition, analysis, and data interpretation. Phan Thi Huyen Thuong and Nguyen Thi Thu Ha prepared, drafted, and revised manuscript critically for important intellectual content. Each author gave the nal approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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