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ORIGINAL ARTICLE

Circulating Vascular Growth Factor (VEGF) Angiopoietin-1 (Angi-1) and Soluble Tie-2 Receptor in Pregnancy Complicated with Pre-eclampsia: A Prospective Study

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Abstract

Background Preeclampsia is a leading cause of maternal and fetal/neonatal mortality and morbidity worldwide. Although the etiology of preeclampsia (PE) is still unclear, recent studies suggest that its major phenotypes, high blood pressure and proteinuria, are due in part to the disturbed angiogenic process.

Study Design This study included the following groups: (1) women with normal pregnancies ($n = 150$), (2) patients with PE ($n = 88$), and (3) patients who delivered small growth for date (SGA) neonate ($n = 50$). Maternal serum concentrations of VEGF, Angi-1, and sTie-2 were measured by a sensitive immunoassay. Non-parametric statistics were used for analysis.

Results The median maternal serum concentration of sVEGF and sAngi-1 was lower in normal pregnant women as compared to that in PE and SGA and the differences were statistically significant ($P < 0.01$). In contrast, there is a significant reduction in sTie-2 levels in PE and SGA groups as compared to that in normal pregnancy group ($P < 0.01$). Serum VEGF and Angi-1 were significantly higher in the late onset PE subgroup as compared to that in the early onset PE ($P < 0.01$), but sTie-2 was not significantly different in the 2 subgroups ($P > 0.05$). Serum VEGF, sAngi-1, and sTie-2 were significantly higher and Tie-2 was significantly lower in the severe PE subgroup as compared to that of the milder PE subgroup ($P < 0.01$ for all).

Conclusion Patients with PE and those with SGA fetuses have lower median serum concentrations of sTie-2 and higher sVEGF and sAngi-1 than women with normal pregnancies. These findings lend support to the hypothesis that circulating angiogenic proteins may have an important biologic role in PE.

Keywords Preeclampsia · Angiopoietin-1 · VEGF · Tie-2

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Introduction

Preeclampsia(PE) affects about 2–7 % of pregnancies and is the most common cause of maternal and fetal/neonatal morbidity and mortality worldwide [1]. Although PE has been recognized as a disease for a long time, its etiology and pathogenesis are still not fully elucidated. Several observations about the failure of normal physiologic angiogenesis were reported [2].

The "Angiopoietins/Tie system" contribution to vascular development/angiogenesis operates through a complementary, coordinated and sequential activity with members of the vascular endothelial growth factors (VEGFs) family.

In this model of angiogenesis, VEGFs accomplish their major role during the first stages of vessel development [3–5], whereas the "Angiopoietins/Tie system" is subsequently involved in the promotion of vessel stabilization and remodeling [6–8].

Indeed, the binding of Angiopoietin-1 to Tie-2 is followed by a weak endothelial cell mitogenic activity, but plays a relevant role in regulating the interactions between endothelial cells and the surrounding support cells and matrix [6, 8, 9] after VEGF-A has initiated vascular formation [3, 4] to guarantee endothelial maturation and stabilization, which are central events for vascular stability [10–12]. In contrast, endothelial Tie-2 receptor binding by Angiopoietin-2 blocks its constitutive effect on vascular stabilization and maturation, conferring to the vessels a more plastic state and possibly a greater response to the signals provided by VEGF-A, favoring remodeling and sprouting of the vascular network. Of interest, the expression of Tie-2 receptor and its ligands has been detected in placental tissue and trophoblast, suggesting an involvement in the context of placenta angiogenesis [13–15].

Soluble Tie-2 (sTie-2) is the soluble form of the Tie-2 receptor, which is released in the circulation through mechanisms that are still under investigation. Similar to the soluble form of the vascular endothelial growth factor receptor-1 (sVEGF-R1), sTie-2 may participate in the regulation of angiogenesis. It is well established that changes in the concentration of circulating angiogenic and anti-angiogenic proteins can precede the clinical recognition of preeclampsia and small for gestational age by several weeks [16, 17] and that their measurement in biologic fluids [16–18] has clinical potential [19].

Although the data about the levels of sVEGF and sAngi-1 in normal pregnancies, PE, and SGA are somewhat clear, the levels regarding sTie-2 receptors are controversial. Therefore, the aim of this study was to determine whether there are differences in the maternal serum concentrations of Angiopoietin-1, sTie-2, and VEGF in the presence of pregnancy as compared to that in PE and SGA.

Study Design

This study was designed to include the following groups: (1) women with normal pregnancies ($n = 150$), (2) patients with PE ($n = 88$), and 3) of the included preeclampsia group, 50 gave birth to an infant SGA. The PE group included 38 women with severe and 50 with a mild degree. Forty women were earlier onset PE and 48 were of late onset.

PE was diagnosed in the presence of hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on at least two occasions, 4 h to 1 week apart, after the 20th week of gestation) and proteinuria (≥300 mg in a 24-h urine collection or one dipstick measurement ≥2+). Severe preeclampsia was defined as either severe hypertension (diastolic blood pressure ≥110 mm Hg) and mild proteinuria or mild hypertension and severe proteinuria (a 24-h urine sample containing 3.5 g protein or urine specimen ≥3+ protein by dipstick measurement). Patients with an abnormal liver function test (aspartate aminotransferase >70 IU/L) and thrombocytopenia (platelet count <100,000/cm^3), as well as those with eclampsia, were also classified as having severe preeclampsia. In addition, patients with preeclampsia were sub-classified as having either early onset (≤34 weeks) or late onset (>34 weeks) disease, according to the gestational age at diagnosis. A neonate was defined as SGA if the birth weight was below the 10th percentile for the gestational age [20, 21]. Patients were considered to have a normal pregnancy if they did not have any obstetrical, medical, or surgical complication of pregnancy and delivered a term (≥37 weeks) neonate with a birth weight above the 10th percentile for the gestational age [20, 21]. All patients were enrolled at Mansoura University Hospital, Mansoura, Egypt, and provided written informed consent for the inclusion in the present study and collection of clinical data and blood samples.

Sample collection and Angiopoietin, VEGF, and Tie-2 assay

Samples of blood were collected in tubes free from anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation, and aliquots of serum were stored at −80 °C until analyses. sAngi-1, sVEGF, and sTie-2 concentrations were determined in duplicate by ELISA according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN).

Statistical Analysis

Kolmogorov–Smirnov was used to determine whether the data were normally distributed. Kruskal–Wallis with post hoc tests was utilized to determine the differences of the median among groups. The Mann–Whitney *U* test was used to compare between two groups. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL, USA). A P value of <0.05 was considered significant.

Results

The highest concentrations of sVEGF and sAngi-1 were in the SGA followed by the PE group and then the normal

Table 1 Serum VEGF, Angi-1, and sTie-2 in normal and complicated pregnancies

	Normal pregnancy ($n = 150$)	Preeclampsia ($n = 88$)	Small birth for date ($n = 50$)
sVEGF (ug/L)	1.5	6.2	9.5
Median range	2.2–3.6	3.2–8.1	4.9–13.5
sTie-2 (ng/mL)	19.5	6.6	7.0
Median range	11.6–62.0	4.8–47.3	4.2–31.0
Angi-1 (ng/mL)	3,380	4,390	5,200
Median range	2,650–5,100	3,850–5,400	4,200–5,390
	$P < 0.01$	$P < 0.01$	$P < 0.01$

Table 2 sVEGF, sTie-2, and sAngi-1 in mild vs severe preeclampsia

	Mild preeclampsia ($n = 50$)	Severe preeclampsia ($n = 38$)	
sVEGF (ug/L)	4.4	7.7	$P < 0.01$
Median range	3.2–5.4	6.8–8.1	
sTie-2 (ng/mL)	35.9	15.7	$P < 0.01$
Median range	7.0–77.3	6.8–75.0	
sAngi-1 (ng/mL)	3,950	4,500	$P < 0.01$
Median range	3,850–4,200	4,390–5,400	

pregnancy group, and the differences were statistically significant ($P < 0.01$). In contrast, the serum Tie-2 levels were the highest in the normal pregnancy group followed by the PE and then the SGA group, and the differences were statistically significant ($P < 0.01$) (Table 1).

Evaluation of the studied parameters in relation to the degree of severity of PE revealed that the 2 parameters (sVEGF, sAngi-1) were significantly higher and Tie-2 was significantly lower in severe PE as compared to the milder one ($P < 0.01$ for all) (Table 2).

Comparing the serum levels of VEGF, Angi-1, and Tie-2 in early vs. late onset PE revealed that the sVEGF and sAngi-1 were significantly higher in the late vs. the earlier one. On the other hand, sTie-2 levels were not significantly different in the two studied subgroups ($P > 0.05$) (Table 3).

Table 3 Serum VEGF, sTie-2, and Angi-1 in early and late preeclampsia

	Early preeclampsia ($n = 40$)	Late preeclampsia ($n = 48$)	
VEGF (ug/L)	4.8	6.9	$P < 0.01$
Median range	3.2–6.0	4.88–8.1	
Tie-2 (ng/mL)	17.0	18.5	$P > 0.05$
Median range	7.8–66.1	6.8–77.3	
sAngi-1 (ng/mL)	4,580	5,320	$P < 0.01$
Median range	3,850–4,690	4,850–5,400	

Discussion

Serum VEGF and Angi-1 concentrations were significantly higher in the SGA and PE group as compared to normal pregnancy ($P < 0.01$). In contrast, the serum Tie-2 levels were the highest in the normal pregnancy group followed by PE and then SGA group, and the differences were statistically significant. These findings are in agreement with previous studies regarding sVEGF and sAngi-1 levels [2, 22–25]. However, the results are controversial regarding sTie-2 levels in PE. Two previous studies reported that sTie-2 maternal serum/plasma concentrations in patients with PE were either no different [26] or significantly higher than those of normotensive pregnant women [24].

In parallel with the results in the current study, Gotsch et al. [25] stated that circulating Tie-2 concentrations were reduced in the PE group as well as SGA as compared to normal pregnancy. These findings imply that impaired placental vascular development and maternal vascular function are associated with an excess of anti-angiogenic factors in PE women.

There is emerging evidence that Tie-2 can be proteolytically cleaved, resulting in the production of a 75-kDa soluble Tie-2 receptor (sTie-2) fragment, which binds to both Angi-1 and Angi-2 and inhibits angiopoietin-mediated Tie-2 phosphorylation [27].

In accordance with our finding, Sung et al. [23] stated that the lowering levels of sTie-2 receptor were attributed to an interaction between VEGF and Tie-2 in uterine endothelial cells and a potential mechanism for the decrease in circulating sTie-2 levels in PE, likely through inhibition of VEGF signaling. Further studies on VEGF-Tie-2 interactions during pregnancy should provide new insights into the mechanisms underlying the failure of vascular remodeling in PE and other pregnancy complications.

The 2 parameters (sVEGF, sAngi-1) were significantly higher and Tie-2 was significantly lower in severe PE as compared to the milder one. Moreover, mothers who deliver a small for gestational age neonate and have PE have lower serum concentrations of sTie-2 than normal pregnant women. A possible explanation is that both small for gestational age and PE have an anti-angiogenic state. This interpretation is consistent with previous reports [28, 29]. Further evidence in support of the involvement of the Tie-2/Angiopoietin system in pregnancy complicated by SGA comes from the study of placentas of affected

individuals. Dunk et al. [30] reported that even though ribonucleotide protection assays did not show significant changes in the expression of Angiopoietin-2 mRNA between placentas from normal pregnancies and pregnancies with SGA, this may contribute to the abnormal development of the villous vasculature. Hagen et al. [31] using real time PCR and Western immunoblot analysis observed a differential expression of Angi-1 and Tie-2 in ovine placental tissue obtained in a model of fetal growth restriction [31]. The changes observed in Tie-2 expression (Tie-2 mRNA concentrations in the presence of fetal growth restriction: increased in fetal cotyledons at 55 dGA, decreased in fetal cotyledons, and maternal caruncles at 135 dGA), in addition to changes in Angi-1 expression observed during early to mid gestation, may result in increased branching angiogenesis, but may also set the stage for increased non-branching angiogenesis during late gestation, abnormal placental architecture, and placental insufficiency [31].

sVEGF and sAngi-1 were significantly higher in late onset PE as compared to the earlier one. On the other hand, serum Tie-2 levels were not significantly different in the two groups. Sung et al. [23] reported that sTie-2 levels were significantly lower in PE subjects starting at 24–48 week of gestation and continued to be lower through the time of delivery.

The results of Sung et al. [23] on human uterine microvascular endothelial cell (UtMVEC) culture studies suggest that VEGF may antagonize Angi1-Tie-2 signaling, which normally acts to stabilize the vessels, and promote a proangiogenic state in the placental bed during pregnancy through proteolytic shedding of Tie-2 (MMP dependent) and not by regulation of expression of Angi-1 or Tie-2.

In conclusion, we have demonstrated an increase in the circulating Angi-1 and VEGF level concentrations in PE and SGA, accompanied by decreases in the circulating sTie-2 levels. These findings lend support to the hypothesis that circulating angiogenic proteins may have an important biologic role in preeclampsia.

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