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PREECLAMPSIA AND SMALL FOR GESTATIONAL AGE ARE ASSOCIATED WITH DECREASED CONCENTRATIONS OF A FACTOR INVOLVED IN ANGIOGENESIS: SOLUBLE TIE-2

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Abstract

OBJECTIVE—An anti-angiogenic state has been described in patients with preeclampsia, small for gestational age (SGA) fetuses and fetal death, and changes in the concentration of circulating angiogenic and anti-angiogenic factors can precede the clinical recognition of preeclampsia and small for gestational age by several weeks. Gene deletion studies demonstrate that a selective group of endothelial growth factors are required for vascular development, including members of the vascular endothelial growth factor (VEGF) family, as well as Angiopoietin-1 and Angiopoietin-2, both ligands for the tyrosine kinase endothelial cell receptor Tie-2. These angiogenic factors have been proposed to promote angiogenesis in a coordinated and complementary fashion.

Soluble Tie-2 (sTie-2) is the soluble form of the Tie-2 receptor which is detectable in biological fluids. The purpose of this study was to determine whether patients with preeclampsia and mothers who deliver a small for gestational age neonate have changes in the plasma concentrations of sTie-2.

STUDY DESIGN—This cross-sectional study included patients in the following groups: 1) non-pregnant women (n=40); 2) women with normal pregnancies (n=135); 3) patients with preeclampsia (n=112); and 4) patients who delivered a small for gestational age (SGA) neonate (n=53). Maternal plasma concentrations of sTie-2 were measured by a sensitive immunoassay. Parametric statistics were used for analysis.

RESULTS—1) The median maternal plasma concentration of sTie-2 was lower in normal pregnant women than in non-pregnant women [median 16.0 ng/ml (range 5.0–71.6) vs. median 20.7 ng/ml (range 10.8–52.4), respectively; p=0.01)]; 2) Plasma sTie-2 concentrations in normal pregnancy changed significantly as a function of gestational age; 3) Patients with preeclampsia and those who delivered SGA neonates had a lower median maternal plasma concentration of sTie-2 than those with a normal pregnancy [Preeclampsia: median 14.9 ng/ml (range 4.9–67.3); SGA: median 10.9 ng/ml (range 5.1–29.1); Normal pregnancy: median 16.0 ng/ml (range 5.0–71.6); p=0.048 and p<0.001, respectively]; 4) Patients with SGA neonates had a lower median plasma concentration of sTie-2 than that of those with preeclampsia [median 10.9 ng/ml (range 5.1–29.1) vs. median 14.9 ng/ml

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(range 4.9–67.3), respectively; p<0.001)]; and 5) Patients with early-onset preeclampsia (≤34 weeks) had lower concentrations of sTie-2 than women with late-onset preeclampsia (>34 weeks) [median of delta values: −0.13 ng/ml (range −0.47–0.58) vs. median of delta values: −0.09 ng/ml (range: −0.60–0.58), respectively; p=0.043]. In contrast, there were no significant differences in the maternal plasma sTie-2 concentration between women with severe and mild preeclampsia (p=0.6).

CONCLUSION—Patients with preeclampsia and those with SGA fetuses have lower median plasma concentrations of soluble Tie-2 than women with normal pregnancies.

Keywords

Small for gestational age; intra-uterine growth restriction; Tie2; pregnancy; angiogenesis; angiopoietin

INTRODUCTION

Vasculogenesis (establishment of a primitive vascular network) and angiogenesis (remodeling of an existing vascular network) are crucial for placentation. Physiologic transformation of the spiral arteries is key in normal human placentation [1–6], but can be abnormal in cases of obstetrical syndromes such as preeclampsia [2,4,7–19], small for gestational age (SGA)[9, 13–15,20,21], fetal death [22–24], preterm labor [25] and preterm prelabor rupture of membranes (PPROM) [26,27].

Gene deletion studies have confirmed that only a small number of endothelial growth factors are required for vascular development during the embryonic period, including members of the vascular endothelial growth factor (VEGF) family [28–30], as well as Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) [31], which are ligands for the tyrosine kinase endothelial cell receptors Tie-1 and Tie-2 [32–34]. These angiogenic factors have been proposed to promote angiogenesis in a coordinate and complementary fashion.

The biology of Tie-2 receptor and its ligands is complex because Tie-2 mediates distinct and opposite biological activities depending to which ligand it binds to, either Angiopoietin-1 or Angiopoietin-2. Of note, Tie-2 is the only known tyrosine kinase receptor with a natural agonist (Angiopoietin-1) and a natural antagonist (Angiopoietin-2), and this fine regulation has been interpreted as an indicator of the importance of a well titrated activation of this system during angiogenesis. Indeed, 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 [35–37] after VEGF-A has initiated vascular formation [28,38] to guarantee endothelial maturation and stabilization, which are central events for vascular stability [29,37,39–42]. 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 [29,39,42,43], 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 [44–46].

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. Similarly 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 [47,48], and that their measurement in biological fluids [49] has clinical potential [50].

There is limited data available on the role of Angiopoietins/Tie receptors in normal pregnancy, preeclampsia and SGA. The aim of this study was to determine whether there are differences in the maternal plasma concentrations of sTie-2 in the presence of pregnancy complications characterized by a disturbance in angiogenesis, such as preeclampsia and SGA.

METHODS

Study Design

A cross-sectional study was designed to include patients divided into the following groups: 1) non-pregnant women (n=40); 2) women with normal pregnancies (n=135); 3) patients with preeclampsia (n=112); and 4) patients who delivered an SGA neonate (n=53). Preeclampsia was diagnosed in the presence of hypertension (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 one dipstick measurement ≥2+) [51]. Severe preeclampsia was defined as either severe hypertension (diastolic blood pressure ≥110 mmHg) 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) [51]. 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 [51]. 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 birthweight was below the 10th percentile for the gestational age [52]. 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 gestational age [52]. Non-pregnant women included healthy volunteers not taking oral contraceptives who donated blood in the secretory phase of their cycle. All patients were enrolled at Hutzel Hospital, Detroit MI, USA and provided written informed consent for the collection of clinical data and biological materials under protocols approved by the Institutional Review Boards of both Wayne State University and the National Institute of Child Health and Human Development of the National Institute of Health (NIH/DHHS). Many of these samples have been employed to study the biology of inflammation, hemostasis, angiogenesis regulation, cytokine biology, and growth factor concentrations in non-pregnant women, normal pregnant women, and those with complications.

Sample collection and human Tie-2 immunoassay

Samples of blood were collected in EDTA containing tubes, centrifuged and stored at −70° C. Concentrations of human Tie-2 in maternal plasma were determined by a sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN, USA). The calculated inter- and intra-assay coefficients of variation (CVs) in our laboratory were 5.2% and 1.1%, respectively. The calculated sensitivity of the Tie-2 immunoassay was 0.109 ng/ml.

Statistical analysis

Kolmogorov-Smirnov or Shapiro-Wilk tests were used to determine whether the data was normally distributed. Kruskal-Wallis with post-hoc tests was utilized to determine the differences of the median among groups. Since maternal plasma sTie-2 concentration varies as a function of gestational age, the delta value (difference between the observed and the expected plasma sTie-2 concentration, the latter derived from the regression equation of plasma sTie-2 concentration of normal pregnancy) was calculated for each patient. The delta value was then used to examine the differences of plasma sTie-2 concentration between women with early- and late-onset preeclampsia. Contingency tables and Chi-square tests were employed for comparisons of proportions. Regression analysis and Spearman correlation were used to

assess the relationship between two continuous variables. Analysis was conducted with SPSS V.12 (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered significant.

RESULTS

Three-hundred and forty patients were included in this study. The demographic and clinical characteristics of the study groups are displayed in Table I. Soluble Tie-2 was detectable in the serum of all subjects.

Maternal plasma concentration of sTie-2 in pregnancy

Patients with normal pregnancies had a significantly lower median plasma concentration of sTie-2 than non-pregnant women (normal pregnancy: median 16.0 ng/ml (range 5.0–71.6 ng/ml) vs. non-pregnant: median 20.7 ng/ml (range 10.8–52.4 ng/ml; p=0.01; Figure 1).

Maternal plasma sTie-2 concentrations in normal pregnancy changed as a function of gestational age according to the equation Log (sTie-2+1) = −0.0031 (gestational age in weeks)^2 + 0.1894 (gestational age in weeks) −1.4779 (r^2=0.17; p< 0.001).

Maternal plasma sTie-2 concentrations in SGA and preeclampsia

Patients who delivered an SGA neonate and those with preeclampsia had significantly lower median plasma concentrations of sTie-2 than normal pregnant women (normal pregnancy: median 16.0 ng/ml, (range 5.0–71.6 ng/ml) preeclampsia: median 14.9 ng/ml, (range 4.9–67.3 ng/ml) SGA, median 10.9 ng/ml, range 5.1–29.1 ng/ml; p=0.048 and p<0.001, respectively; Figure 2). Similar findings were observed after adjusting for parity (nulliparous status), gestational age at blood sampling and sample storage interval. In addition, the median plasma concentration of sTie-2 was lower in mothers delivering an SGA neonate than in those with preeclampsia (SGA, median 10.9 ng/ml, (range 5.1–29.1 ng/ml) vs. preeclampsia, median 14.9 ng/ml, (range 4.9–67.3 ng/ml) p<0.001; Figure 2).

Among patients with preeclampsia, 40% (45/112) were classified as early-onset and 79% (89/112) as having severe preeclampsia. Patients with early-onset preeclampsia had lower concentrations of sTie-2 than women with late-onset preeclampsia, after adjusting for gestational age using the delta value (median of delta values for early-onset preeclampsia (median delta: −0.13 ng/ml (range −0.47–0.58) vs. median of delta values for late-onset preeclampsia: −0.09 ng/ml (range: −0.60–0.58); p=0.043; Figure 3). In contrast, there were no significant differences in the maternal plasma sTie-2 concentration between women with severe and mild preeclampsia, after gestational age at blood draw adjustment was performed (p=0.6).

Within the group of patients with preeclampsia, 52% (58/112) delivered a neonate of appropriate birthweight (“only-preeclampsia group”), whereas the remaining delivered an SGA neonate. We conducted a sub-analysis to compare maternal plasma sTie-2 concentrations between patients with preeclampsia without SGA and those with SGA alone. This sub-analysis confirmed that patients with SGA (n=53) had a significantly lower median plasma concentration of sTie-2 than that of patients with preeclampsia without SGA (only-preeclampsia group”) (n=58) [median 10.9 ng/ml (range 5.1–29.1) vs median 16.9 ng/ml (range 5.9–67.3), respectively; p<0.001].

The birthweight was below the 5th percentile in 79% (42/53) of the patients who delivered an SGA neonate. No significant differences in the median maternal plasma concentration of sTie2 were observed between women in this group and those who delivered a neonate with a birthweight between 5th–9th percentile.

DISCUSSION

Principal findings of this study

1) Maternal plasma sTie-2 concentrations in normal pregnancy are significantly lower than those of non-pregnant women, and change as a function of gestational age; 2) Patients with preeclampsia and those who deliver an SGA neonate have a significantly lower median plasma concentration of sTie-2 than normal pregnant women; and 3) Mothers who deliver an SGA neonate have a significantly lower median maternal plasma concentration of sTie-2 than those with preeclampsia.

The biology of Tie receptors

Cloning and characterization of Tie, as a novel type of human endothelial cell surface tyrosine kinase receptor, was first reported in the early 1990s [32–34]. The name "Tie" was assigned because the ~ 1100 amino acid chain contains both an Immunoglobulin (Ig) and an Epidermal growth Factor (EGF) -like domain, a feature which is unique among known tyrosine kinases. Indeed, the extracellular domain, whose highly conserved structure from zebrafish to human has been considered to be a predictor of important biological function [53], contains a combination of motifs belonging to three different gene superfamilies including Ig-like, EGF-like and fibronectin-like, and this is viewed as an example of how evolution combines already existing motifs to create new molecules [32–34,54]. The intracellular segment of the Tie receptor includes two conserved tyrosine kinase domains, interrupted by a small insert [32–34,54]. Two Tie receptor sub-types have been identified: Tie-1 and Tie-2 [29,32,33], and their four known ligands are the vascular endothelial specific growth factors designated Angiopoietins (Ang-1 through Ang-4) [40,43,55].

Tie-2 receptor

We elected to focus this study on Tie-2 because its expression has been detected on both embryonic [33,34,54,56] and adult [43,57,58] endothelial cells. The observation that Tie-2 is present in the endothelium of all adult tissues examined and most, if not all, blood vessels (including arteries, veins and capillaries) suggests a role both in the maintenance of a normal adult endothelium as well as in the process of development, remodeling and stabilization of new vessels [58]. Tie-2 is also involved in solid tumors angiogenesis [59–67] as well as in other cases of pathological angiogenesis including hypoxia, inflammation (expression of Tie-2 can be induced by pro-inflammatory cytokines, such as TNF-α and IL-1β) [68], experimental ischaemia/reperfusion [69–71], atherosclerosis and atherogenesis [72], coronary disease [72, 73], and ischemic-necrotic myocardium damage [73].

Of interest, mRNA and protein expression of Tie-2 and its ligands has been detected in endothelial and trophoblast cells from both animal and human placentas, and recent evidence suggests that Tie-2/Angiopoietins participate in maternal vessels remodeling during human placentation, as well as in fetal vasculature development [74]. In addition, Angiopoietin-1 and Angiopoietin-2 can bind to Tie-2 receptors in the trophoblast and promote cell growth and migration. The following observations support a role for this system in normal placentation: 1) Tie-2 receptor mRNA expression has been localized (by in situ hybridization) on endothelial cells of fetal and maternal (decidual) vessels [74]; 2) Tie-2 mRNA and protein are expressed by the endothelium of fetal capillaries and maternal blood vessels [44]; 3) Tie-2 protein expression (by immunocytochemistry) is abundant in the fetal vessels within the villi throughout early, mid and late gestation in the baboon [45]; 4) Tie-2 expression (by real time reverse transcriptase PCR) has been demonstrated in normal human placentas at term [46]; 5) Tie-2 mRNA expression (demonstrated by in situ hybridization) in the marmoset placenta has been localized in the mesenchymal cells of the cytotrophoblast cones during early placentation (7 weeks). Of interest, the site and intensity of expression change with gestational age; it is

high in the chorionic vessels and mesenchymal cells of the chorionic plate from 14 weeks onward, and evenly distributed in the chorion plate at term [44]; 6) In addition to the villous endothelial cells, Tie-2 receptor mRNA and peptide have also been shown to be localized in cytotrophoblast and syncityotrophoblast cells [74–76]; 7) In first trimester trophoblast cell lines expressing either cytotrophoblast-like (ED_{27}) or extravillous trophoblast-like (ED_{77}) properties, Angiopoetin-2 stimulates DNA synthesis and NO release from ED_{27} cells, whereas Angiopoietin-1 stimulates ED_{77} cells migration. These effects are dose dependent and inhibited by recombinant Tie-2 [75]; 8) Tie-2 receptor is expressed on endovascular invasive trophoblasts (trophoblast invading the spiral arteries) [74], and it is the first marker that can distinguish between invading interstitial trophoblasts and those invading blood vessels [74]. This suggests that trophoblasts expressing Tie-2 have acquired an endothelial phenotype [77].

Other cells expressing Tie-2 include: lens epithelial cells [34], Schwann cells [78] and neurons [78–80]. In the nervous system, Angiopoietin-1 mediated Tie-2 activation has neuroprotective effects in primary neuronal cultures [80] while stimulating neurite outgrowth in cultured dorsal root ganglion cells [79]. In addition, Tie receptors are expressed on hematopoietic cell progenitors [81,82] on hematopoietic stem cells [83,84], as well as on leukaemic cells [32, 54,85]. The hematopoietic impairment observed in Tie-2 deficient mice suggests a role for Tie receptors in this process [81], which includes the mediation of stem cells adhesion to the matrix protein fibronectin [83] and the enhancement of hematopoietic progenitor cells proliferation [81]. Changes in the expression of the Tie-2 receptor have been reported to occur in several diseases including psoriasis [86], pulmonary hypertension [87,88], infantile hemangiomas [89], and different tumors [90], nevertheless the exact mechanisms through which Tie signaling contributes to disease are not yet known [90,91].

The involvement of Tie-1 in angiogenesis, particularly through a modulation of Tie-2 signaling [92], is supported by several observations: 1) The full length and a truncated endodomain (result of regulated endoproteolytic cleavage) of the Tie-1 receptor have been detected within endothelial cells in association with the intracellular domain of Tie-2, forming a Tie-1:Tie-2 complex [92]; 2) Inflammatory cytokines [93] and VEGF [93,94] stimulate the proteolytic cleavage and release of soluble Tie-1 receptor from human endothelial cells through metalloprotease activation [93]. Loss of the ectodomain would prevent binding of any Tie-1 ligands and may be a mechanism for downregulating the receptor. The endodomain can influence Tie-2 signaling by modifying the Tie-1:Tie-2 complex [94]; and 3) Tie-2 enhances Tie-1 phosphorylation experimentally which can be induced by a soluble Angiopoietin-1 chimeric protein in doubly transfected cells [95]. We elected to focus this study on the Tie-2 receptor, rather than on the Tie-1 subtype, because although Tie-1 phosphorylation is experimentally inducible by multiple angiopoietin proteins [95], a specific ligand has not yet been identified, and little is known about the signaling pathways following its activation [92].

The “Angiopoietins/Tie system” in angiogenesis

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 [28,38,96], whereas the “Angiopoietins/Tie system” is subsequently involved in the promotion of vessel stabilization and remodeling [36,97,98].

Four ligands (Angiopoietin-1 to 4) [40,43,55] can interact with Tie-2 and activate a phosphatidylinositol 3’ kinase (PI3-K) as a major pathway of signaling [99–103]. The most well characterized ligands for Tie-2 receptor are Angiopoietin-1 [37,40] and Angiopoietin-2 [43], which are small glycoproteins (~ 500 amino acids) encoded by genes localized on

chromosome 8 [104], and characterized by an homologous (60%) structure with N-terminal coiled-coil domains, promoting homo-oligomerization patterns and a C-terminal fibrinogen-like domain mediating ligand activity [105,106]. Of interest, the biological consequences of Tie-2 receptor activation are dictated by the specific angiopoietin. Angiopoietin-1 acts as an agonist [40] for the Tie-2 receptor, while Angiopoietin-2 acts preferentially as natural antagonist [43] of the Tie-2/Angiopoietin-1 complex, (blockage of endothelial Tie-2 activation mediated by Angiopoietin-1). Since Angiopoietin-2 is the unique natural antagonist up to date described for a tyrosine kinase vertebrate receptor, in vivo activation of Tie-2 must be particularly well titrated [55]. Less is known about Angiopoietin-3 and Angiopoietin-4 and the consequences of their interaction with Tie-2. Nevertheless, a context dependent action as antagonistic and agonist ligands has been proposed [55,107].

Tie-2/Angiopoietin-1 signaling—Angiopoietin-1 is considered the primary physiologic Tie-2 receptor agonist. Indeed, its binding to the extracellular domain of Tie-2 results in receptor dimerization, kinase activation, and autophosphorylation of specific tyrosine residues [37,40], followed by activation of cytoplasmatic signaling pathways [108]. Tie-2 activation mediated by Angiopoietin-1 interaction is involved in vascular angiogenesis, lymphangiogenesis [109–111], and inflammation [112–115]. Evidence from knock-out experiments supports the concept that the interaction between Tie-2/Angiopoietin-1 is essential for developmental angiogenesis. In mouse embryos lacking either Angiopoietin-1 or Tie-2, the early stages of VEGF dependent vascular development occur normally, resulting in the formation of a primitive vasculature [35–37]. However, sprouting, remodeling (conversion of primary capillary plexus to larger and/or smaller vessels), as well as stabilization of this primitive vasculature are severely perturbed, leading to embryonic lethality. Some of the major abnormalities recognized in Tie-2 deficient mice include vascular network malformations, absence of both capillary sprouting in the neuroectoderm and vessels branching in the myocardial circulation, as well as growth retardation [36]. However, transgenic mice over-expressing Angiopoietin-1 seem generally healthy, although they exhibit larger, more copious and remarkably branched vessels [98], as in cases of VEGF overexpression [116], but with no evidence of plasma leakage, edema or erythrocytes extravasation [117,118].

Two of the major functions of Angiopoietin-1 are: stabilization/maturation of neoformed vessels and an anti-permeability effect. Evidence for a role of Tie-2/Angiopoietin-1 in stabilization/maturation of neoforming vessels includes: 1) synergistic effects on neovascularization when Angiopoietin-1 is co-administrated with VEGF-A [119–121] or other angiogenic growth factors [122]; 2) promotion of survival of differentiated endothelial cells through an anti-apoptotic mechanism [122–124]; 3) promotion of endothelial cell migration [36,42]; and 4) induction/enhancement of angiogenic sprouting [41,102,119–121,125], resulting in the development of a more complex vascular network with vessels of increased luminal size [126]. In contrast to what is described herein, there are few reports attributing an anti-angiogenic activity to Angiopoietin-1 [127–129]. Similarly, there are conflicting observations on whether or not Angiopoietin-1 can be mitogenic for endothelial cells [40,41], and capable of inducing capillary-like tubule formation [40,130].

There is a large body of evidence that Tie-2/Angiopoietin-1 interaction protects vessels from plasma leakage [112,117,131–133]. In vitro and in vivo studies supporting this conclusion include: 1) Angiopoietin-1 inhibits endothelial monolayer permeability induced by thrombin [112,113], VEGF [112], bradykinin [113] and histamine [113]; and 2) transgenic overexpression [117] or adenovirus-mediated gene transfer [131] of Angiopoietin-1 protects blood vessels against plasma leakage in mice challenged with serotonin, platelet-activating factor (PAF) or vascular endothelial growth factor (VEGF).

The molecular mechanisms through which Tie-2/Angiopoietin-1 accomplish its anti-permeability role include: 1) strengthening of the junctions between endothelial cells, by enhancing the localization of adhesion proteins such as platelet endothelial cell adhesion molecule-1 (PECAM-1), and decreasing the phosphorylation of PECAM-1 and vascular endothelial cadherin [112]; 2) enhancement of endothelization (chemotactic activity of Angiopoietin-1 on endothelial cells) [42]; 3) stimulation of endothelial cells interaction with the surrounding matrix, mesenchyme and periendothelial cells [37] also through pericyte and smooth muscle cell recruitment [37,134,135]; and 4) reduction of the permeability effects of different pro-inflammatory mediators, possibly by suppression of a common pathway utilized by these edemagenic agents [113]. Other anti-inflammatory properties of the Tie-2/Angiopoietin-1 interaction include: 1) down-regulation of E-selectin (marker of endothelial activation, expressed in inflammation, proliferation and angiogenesis) [112,114], tissue factor [136], ICAM-1 [114] and V-CAM [114] endothelial cell expression; 2) reduction of the production of IL-8 (polymorph nucleate cells chemotactic factor) from endothelial cells challenged with thrombin [113]; and 3) contrasting reports on whether it can inhibit the TNF-alfa induced leukocytes adhesion and transmigration [112,113].

Tie-2/Angiopoietin-2 signaling—Angiopoietin-2 has the potential to activate or block the Tie-2 receptor in a context dependent fashion, depending on the cell type and culture conditions [40,43,137,138]. Consistent with the notion that Angiopoietin-2 is a natural antagonist for the Tie-2/Ang-1 interaction on vascular endothelial cells is the observation that transgenic embryos over-expressing Angiopoietin-2 exhibit a lethal myocardial and vascular phenotype mimicking that of knockouts embryos either for Angiopoietin-1 or Tie-2 [43]. A gene targeting approach in mice has shown that while Angiopoietin-2, unlike Angiopoietin-1 and VEGF, is not a requisite during vascular development, it is necessary for subsequent postnatal vascular remodeling, when angiogenic sprouting and vascular regression normally occur in a coupled fashion [31]. Evidence that such role is specific for Angiopoietin-2 is the lack of rescue of the vascular remodeling defect upon replacement of Angiopoietin-2 gene with cDNA encoding Angiopoietin-1 [31].

The angiogenic activity of Angiopoietin-2 consists in providing the key destabilizing signal which is a requisite for initiating angiogenic remodeling. This is accomplished through the natural Angiopoietin-2 antagonism to the constitutive stabilizing effect mediated by Angiopoietin-1 signaling, which allows the endothelium to revert to a more plastic state, reminiscent of developing vessels [29]. Detachment of smooth muscle cells as well as loosening of the matrix surrounding vessels [43,139] are some of the Angiopoietin-2 biological effects which may be operating in this context.

Tie-2/Angiopoietin-2 signaling also participates in the development of lymphatic vessels. Engineered mice lacking Angiopoietin-2 display extensive lymphatic defects encompassing anatomical (irregular and disorganized pattern, loss of relationship with blood vessels, absence of central lacteals in the intestinal villi), as well as morphologic anomalies (poor association with smooth muscular cells) leading to severe and generalized lymphatic dysfunction, chylous ascitis and subcutaneous edema [31]. Therefore, it has been proposed that the role of Tie-2/Angiopoietin-2 signaling, within the lymphatic vasculature, is to promote and optimize the interactions between endothelial cells and the surrounding smooth muscle cells, similarly to the effect of Tie-2/Angiopoietin-1 on blood vasculature [31]. Since replacement of Angiopoietin-2 with Angiopoietin-1 can rescue completely the lymphatic defects, in contrast to the angiogenic defects, it has been suggested that Angiopoietin-2 acts as a Tie-2 agonist in the former setting but as an antagonist in the latter setting [31].

Soluble Tie-2

Compelling evidence suggests that soluble Tie-2 has anti-angiogenic properties. Soluble Tie-2 blocks: 1) the Angiopoietin-1 induced capillary-like tubule formation from bovine endothelial cells [130]; 2) the stabilization effect of Angiopoietin-1 on HUVEC network organization [122]; 3) the anti-apoptotic effect of Angiopoietin-1 on endothelial cells [124]; 4) the angiogenesis stimulated by tumor cell conditioned media in the rat cornea [140]; 5) adenovirus-directed systemic expression of sTie-2 inhibits and regresses rat corneal [141], retinal and choroidal [142] neovascularization in murine models of chemical-mechanical trauma [141], and ischemia induced neovascularization [142]; 6) adenovirus-directed administration of sTie-2 to mice with primary murine mammary carcinoma or murine melanoma inhibits the growth of both tumors, the formation of metastasis and if any, their histologic examination revealed only small avascular clusters of tumor cells [143]; and 7) administration of sTie-2, in a rat xenograft model of a mammary tumor, resulted in inhibition of tumor growth as well as vascular density [140].

Normal pregnant women have a significantly lower median plasma sTie-2 concentration than non-pregnant women

Our finding that pregnancy is associated with lower median plasma concentration of sTie-2 is consistent with a previous report [144]. The lower concentrations of sTie-2 in during gestation may be part of the adaptations that promote an angiogenic state in normal pregnancy [144]. It is noteworthy that some reports indicate that during normal pregnancy sTie-2 serum [145] or plasma [146] concentrations either do not change [146] or are higher [145] when compared to non-pregnant controls.

Maternal plasma sTie-2 concentrations in normal pregnancy change as a function of gestational age

Vuorela et al. [145] reported that maternal serum concentrations of sTie-2 decreased with advancing gestation from the 14^{th} week of gestation onwards ($r=0.4$, $p<0.05$) and that the association between maternal serum Tie-2 concentrations and gestational age was significant after 26 weeks ($r=0.6$, $p<0.001$). The authors proposed that this reduction may be due to hemodilution [145]. We found a curvilinear change in sTie-2 in maternal plasma concentrations. The explanation for this pattern requires further investigation.

Patients with preeclampsia have a significantly lower median plasma concentration of sTie-2 than normal pregnant women

Our results are in agreement with a previous study [145] reporting that women with preeclampsia had a median serum sTie-2 concentration lower than that of healthy pregnant women of corresponding gestational age [145]. However, two previous studies reported that sTie-2 maternal serum/plasma concentrations in patients with preeclampsia were either no different [144] or significantly higher [146] than those of normotensive pregnant women.

When compared to non-pregnant controls, normal pregnant women have: 1) significantly higher maternal plasma [146] and serum [144] concentrations of Angiopoietin-2 [144,146]; 2) significantly higher concentrations of Angiopoietin-1 [146]; and 3) a similar ratio of plasma Ang-1:Ang-2, which is approximately 1:2 [146]. These absolute and relative changes in plasma/serum concentrations of the different components of the Tie-2/Angiopoietin system reflect changes in the angiogenic status in normal gestation. In particular, evidence from cancer research supports that high Angiopoietin-1 relative to Angiopoietin-2 and VEGF concentrations, would sustain vessel integrity and quiescence while high Angiopoietin-2 relative to Angiopoietin-1 and VEGF concentrations favor angiogenesis and responsiveness to hypoxic and inflammatory stimuli [67].

In contrast, a dysregulation of the Tie-2/Angiopoietin system may contribute to the anti-angiogenic state described in pregnancies complicated by preeclampsia. Indeed, when compared to normotensive pregnant women, mothers with preeclampsia or gestational hypertension have: 1) a significantly lower serum [144]/plasma [146] concentration of Angiopoietin-2 [144,146]; 2) a significantly higher plasma concentration of Angiopoietin-1 [146]; and 3) differences in the ratio between Ang-1 and Ang-2 with a ratio in the preeclampsia group which is reversed up to 2.5:1, suggesting a shift of the balance away from Angiopoietin-2 toward Angiopoietin-1 [146]. In addition, in normal pregnancies there is a correlation between maternal plasma concentrations of sTie-2 and: 1) Ang-2 [($r=0.402$, $p<0.001$)[146] and ($r=0.366$, $p<0.0718$) [144]]; 2) Flt-1 ($r=0.407$, $p<0.001$) [146] and 3) VEGF ($r=0.248$, $p=0.003$) [146]. Such correlations may not be significant in preeclampsia and a possible explanation is the loss of physiologic regulation of Angiopoietin-2 and the Tie-2 system in the setting of this disorder [144,146].

Mothers who deliver a small for gestational age neonate have lower plasma concentrations of sTie-2 than normal pregnant women and than patients with preeclampsia

Our study is the first to report that the maternal plasma concentrations of sTie-2 are lower in women who delivered a small for gestational age neonate than in normal pregnancy. A possible explanation is that both small for gestational age and preeclampsia have an anti-angiogenic state. This interpretation is consistent with previous reports [147–154].

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. [75] reported that, even though ribonucleotide protection assays did no show significant changes in the expression of Angiopoietin-2 mRNA between placentas from normal pregnancies and pregnancies with SGA, immunoblot analysis demonstrated a significantly lower Angiopoietin-2 protein expression in SGA placentas. The authors proposed that lower angiopoietin-2 may contribute to the abnormal development of the villous vasculature [75]. Hagen et al.[155] (using Real time PCR and Western immunoblot analysis) observed a differential expression of Ang1, Ang2 and Tie-2 in ovine placental tissue obtained in a model of fetal growth restriction [155]. The changes observed in Tie-2 expression (Tie2 mRNA concentrations in presence of fetal growth restriction: increased in fetal cotyledons at 55dGA, decreased in fetal cotyledons and maternal caruncles at 135dGA), in addition to changes in Ang-1 and Ang-2 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 [155].

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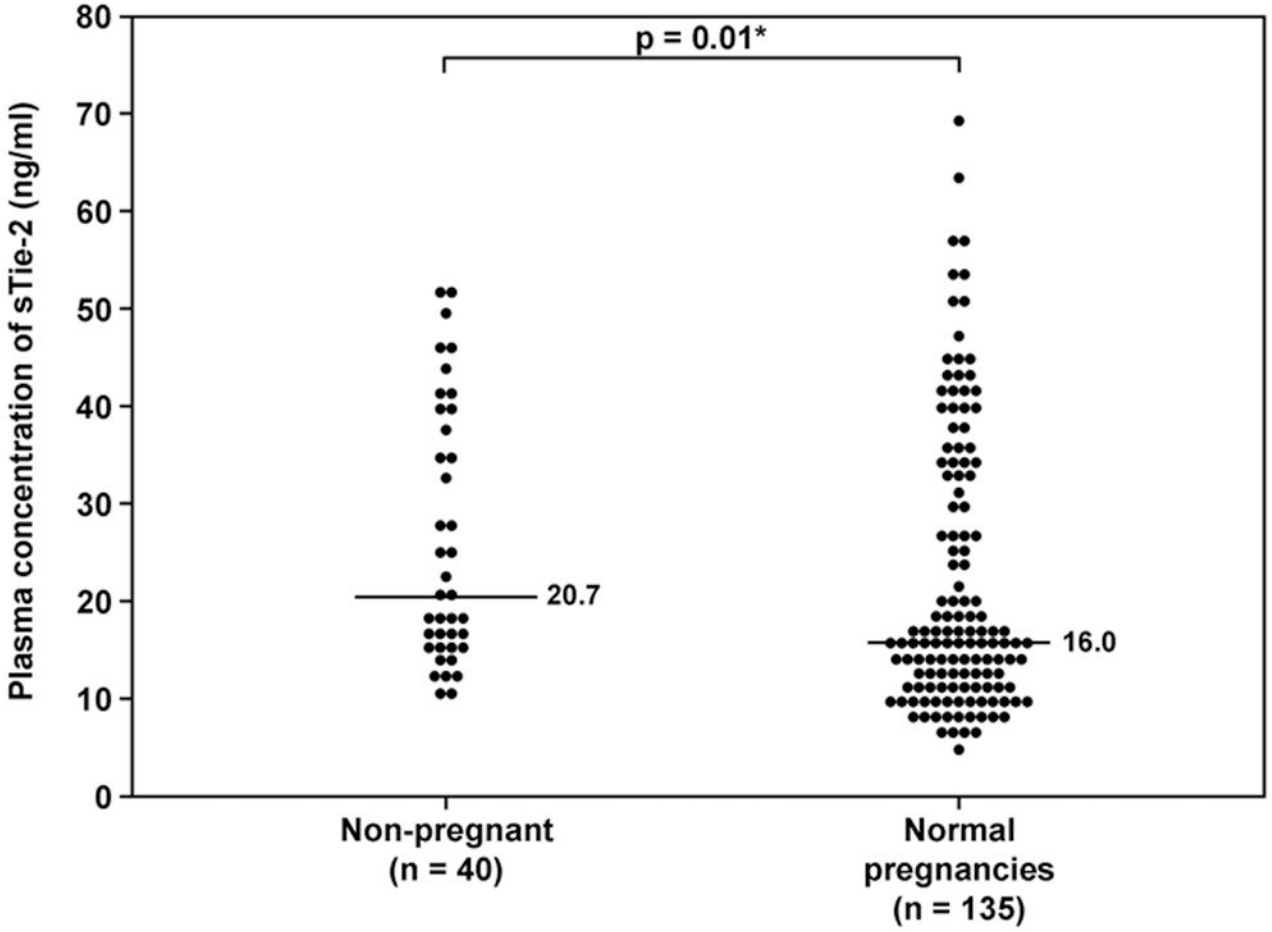

Figure 1.

Patients with normal pregnancies had a significantly lower median plasma concentration of sTie-2 than non-pregnant women (normal pregnancy: median 16.0 ng/ml (range 5.0–71.6 ng/ml) vs. non-pregnant: median 20.7 ng/ml (range 10.8–52.4 ng/ml; p=0.01).

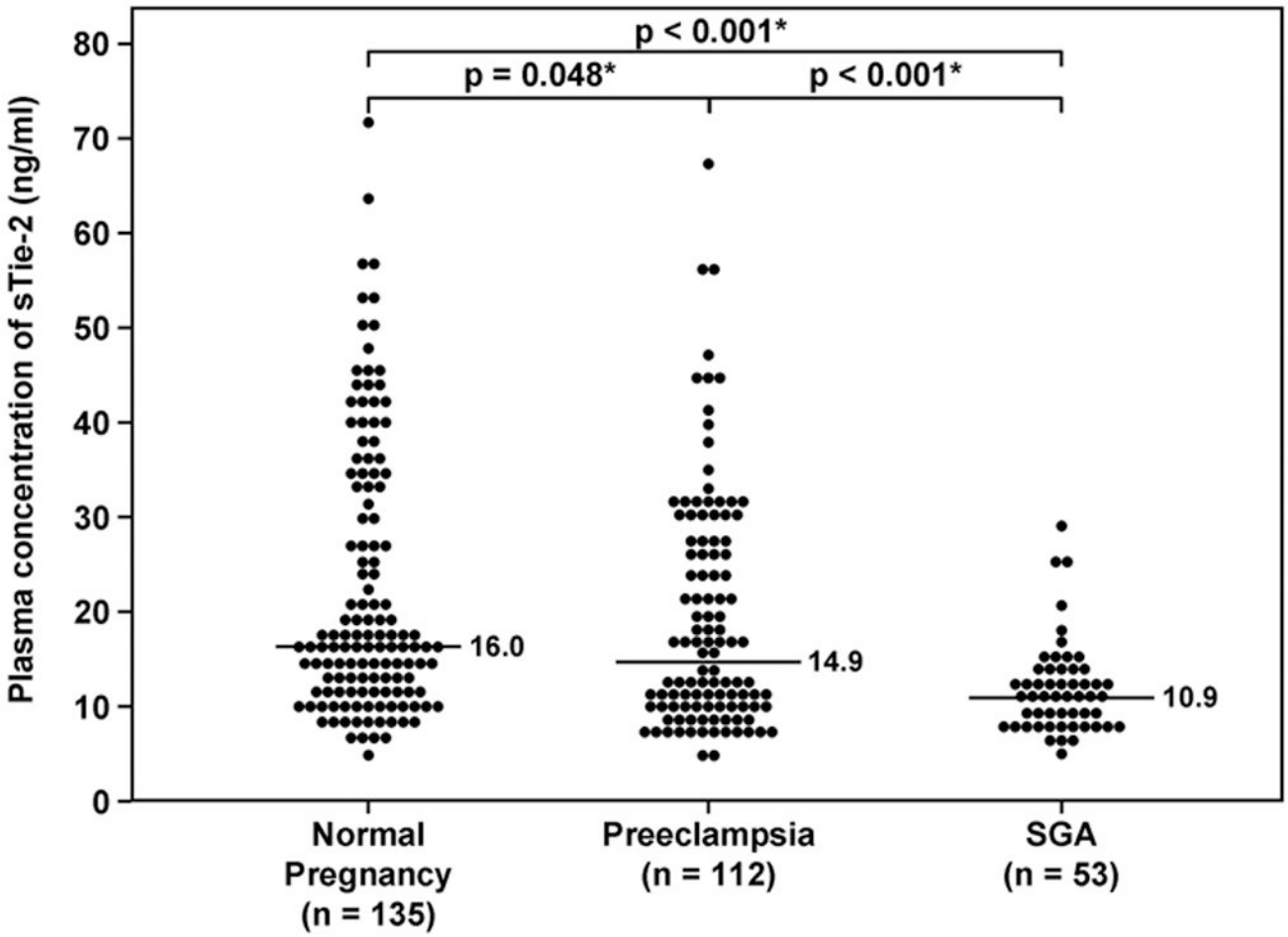

Figure 2.

Patients who delivered a small for gestational age neonate and mothers with preeclampsia had lower median plasma concentrations of sTie-2 than normal pregnant women (normal pregnancy: median 16.0 ng/ml, range 5.0–71.6 ng/ml; preeclampsia: median 14.9 ng/ml, range 4.9–67.3 ng/ml; SGA, median 10.9 ng/ml, range 5.1–29.1 ng/ml; p=0.048 and p<0.001 respectively). In addition, the median plasma concentration of sTie-2 was lower in mothers delivering a small for gestational age neonate than in those with preeclampsia (SGA, median 10.9 ng/ml, range 5.1–29.1 ng/ml, vs. preeclampsia, median 14.9 ng/ml, range 4.9–67.3 ng/ml; p<0.001).

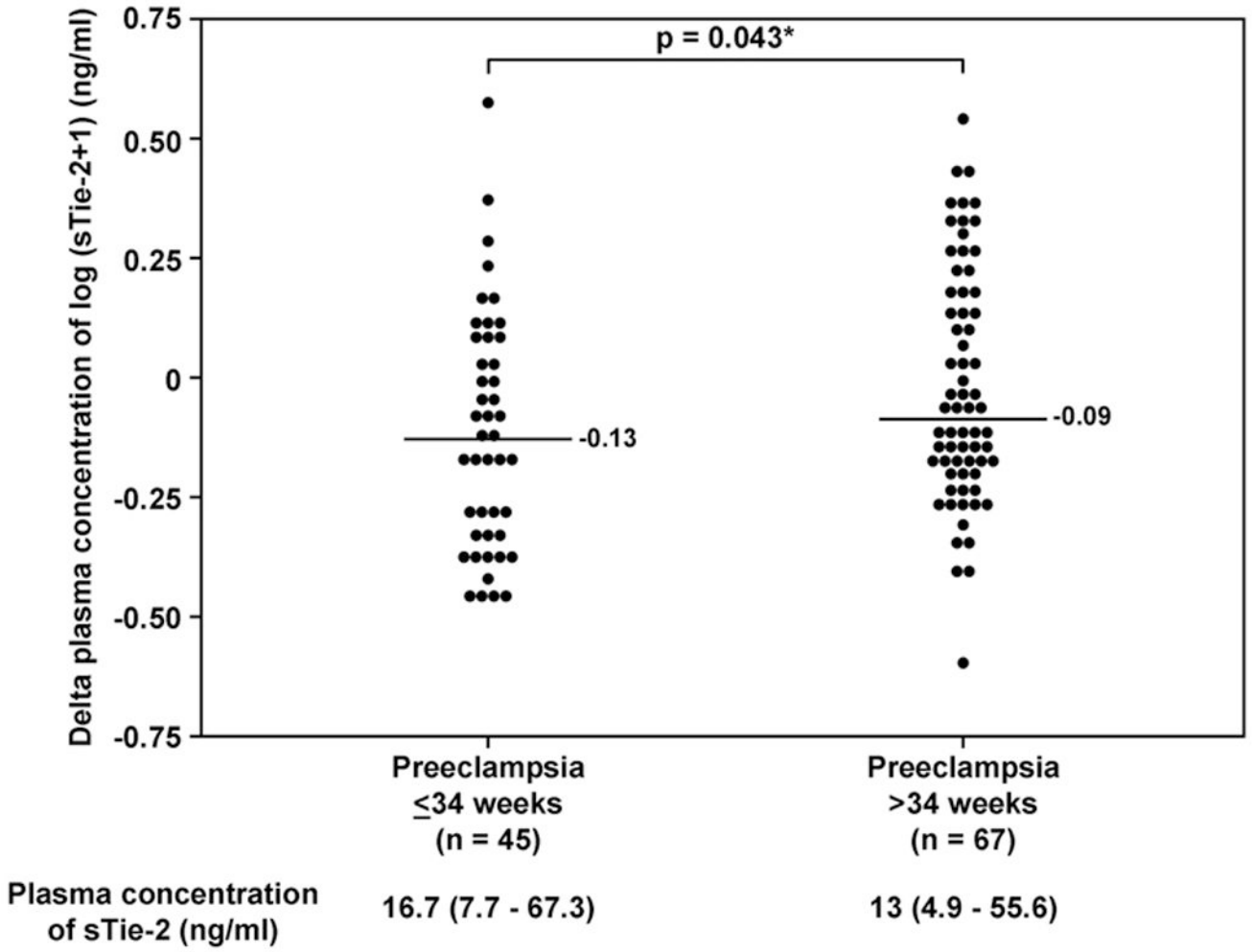

Figure 3.

Among patients with preeclampsia, 40% (45/112) were classified as early-onset. Patients with early-onset preeclampsia (≤34 weeks) had lower concentrations of sTie-2 than women with late onset preeclampsia (>34 weeks), after adjusting for gestational age using the delta value (median of delta values for early-onset preeclampsia (median delta: −0.13 ng/ml (range −0.47–0.58) vs. median of delta values for late-onset preeclampsia: −0.09 ng/ml (range: −0.60–0.58); p=0.043).

Table I

Clinical characteristics of the study population

		Normal pregnancy n = 135	P_1	SGA n=53	P_2	Preeclampsia n = 112	P_3
Age (y) $^{\delta}$		25 (17–40)	0.5	24 (15–43)	0.6	23 (13–43)	0.2
Race:	- African American	107 (79.3%)		46 (86.8%)		91 (81.3%)	
	- Caucasian	15 (11.1%)		4 (7.5%)		13 (11.6%)	
	- Hispanic	7 (5.2%)	0.3	1 (1.9%)	0.8	5 (4.5%)	0.6
	- Asian	0		1 (1.9%)		1 (0.9%)	
	- Other	6 (4.4%)		1 (1.9%)		2 (1.8%)	
Nulliparity		37 (27.4%)	0.005*	26 (49.1%)	0.1	69 (61.6%)	<0.001*
GA at blood sampling (weeks) $^{\delta}$		37.6 (20–41.7)	0.3	36.9 (25–39.7)	0.5	35.6 (23.4–42.4)	0.3
GA at delivery (weeks) $^{\delta}$		39.3 (37–42.4)	<0.001*	37.1 (25–39.7)	0.3	35.7 (23.7–42.4)	<0.001*
Birthweight (grams) $^{\delta}$		3345 (2610–4080)	<0.001*	2050 (300–2880)	0.03*	2195 (530–4460)	<0.001*
Adjusted birthweight for GA (MOM) $^{\phi}$		−0.01 ± 0.08	<0.001*	−0.35 ± 0.10	<0.001*	−0.22 ± 0.17	<0.001*
Birthweight <5th percentile		0	<0.001*	42 (79.2%)	<0.001*	32 (28.6%)	<0.001*

Value expressed as median (range) or number (percent) except adjusted birthweight for GA as mean ± sd

GA: gestational age; MOM: multiple of the median

$^{\delta}$Kruskal-Wallis with post-hoc tests

$^{\phi}$ANOVA with post-hoc Dunnett T3 test; p<0.001

P_1 = Normal Pregnancy vs. SGA

P_2 = SGA vs. Preeclampsia

P_3 = Normal Pregnancy vs. Preeclampsia