Evaluation of first trimester serum soluble endothelial cell-specific tyrosine kinase receptor in normal and affected pregnancies

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ABSTRACT

Aims: To assess soluble endothelial cell-specific tyrosine kinase receptor (sTie-2) levels in the first trimester of pregnancy and its association with adverse pregnancy outcomes; and examine the predictive accuracy.

Study Design: In this nested case-control study, serum sTie-2 levels were measured in 2,616 women with singleton pregnancies attending first trimester screening in New South Wales, Australia. Multivariate logistic regression models were used to assess the association and predictive accuracy of serum sTie-2 with subsequent adverse pregnancy outcomes.

Results: Median (interquartile range) sTie-2 for the total population was 19.6 ng/ml (13.6-26.4). Maternal age, weight, and smoking status significantly affected sTie-2 levels. There was no difference in serum sTie-2 between unaffected and women with adverse pregnancy outcomes. After adjusting maternal and clinical risk factors, low sTie-2 (<25th centile) was associated with preeclampsia (Adjusted odds ratio: 1.61; 95%CI: 1.01-2.57), however, the accuracy of sTie-2 in predicting preeclampsia was not different from chance (AUC=0.54; P=0.08) and does not add valuable predictive information to maternal and clinical risk factors.

Conclusions: Our findings suggest that low sTie-2 levels are associated with preeclampsia, however, it does not add valuable information to clinical and maternal risk factor information in predicting preeclampsia or any other adverse pregnancy outcomes.

INTRODUCTION

Endothelial cell-specific tyrosine kinase receptor (Tie-2) is expressed by maternal and fetal vascular endothelial cells that mediate several physiological functions including placental development[1]. Tie2 receptor signalling controls vascular stability and is therefore crucial for angiogenesis and placental growth. This process is enhanced by the presence of other angiogenic factors such as the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Moreover, Tie2 signalling on endovascular invasive trophoblasts promotes vascular remodelling during pregnancy resulting in the widening of spiral arteries through the loss of arterial vascular smooth muscle which is required to establish a high capacity low resistance environment to facilitate uteroplacental perfusion[2]. The tyrosine kinase activity of Tie-2 is activated by angiopoietin (Ang)-1which promotes vascular stability and maintains cellular contact between endothelial cells and pericytes[3]. This control is antagonized by Ang-2, which binds but does not activate Tie2, through competition for the ligand binding site on Tie2. In the presence of pro-angiogenic factors, such as VEGF receptor ligands, this promotes angiogenesis and placental formation.

Recent studies have raised the profile of anti-angiogenic agents such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin as pathogenic molecules during gestation. Soluble Flt-1 is an anti-angiogenic factor that may play a role in the clinical syndrome of preeclampsia[4] and other complications such as small for gestational age[5], preterm birth and fetal death[6]. Abnormal high serum levels promote poor placentation by sequestering VEGF and PlGF, preventing VEGFR1 activation on the endothelium and diminishing angiogenesis[7]. Similarly, an alternate

path to attenuate Tie-2 signalling on endothelial cells is for the soluble form of Tie2 receptor (sTie-2) to bind and sequester Ang-1. Similar to the effects of Ang-2 this mechanism prevents the tyrosine kinase activity of full length Tie2 on endothelial cells from being activated and deprives cells the stabilising influence of Ang-1. This results in the promotion of angiogenesis by other factors to proceed unchecked as well as the development of adverse events (such as vascular permeability and tissue oedema, inflammation of the vessel wall and often endothelial cell dysfunction). The extracellular domain of Tie2 is released into the circulation by a shedding mechanism, similar to the soluble form of the VEGF receptor-1, and may participate in the regulation of angiogenesis by binding to free Ang-1 and Ang-2[8].

Many of these processes feature in pathological pregnancies. We have previously reported an association between low first trimester serum levels of Ang-2 with the subsequent development of preeclampsia, small for gestational age infant, preterm birth and miscarriage[9]. We hypothesise that the Ang-2/Tie-2 binding mechanism would be altered in compromised pregnancies and therefore be expressed in abnormal first trimester sTie-2 serum levels. Previous studies have reported no association between serum sTie-2 levels in first and second trimester [10] and decreased serum sTie-2 levels at term in women with preeclampsia or that had a SGA infant [11,12]. However, these studies were small, not representative of a general maternity population and inadequate to determine normative levels in pregnancy. Additionally, there is no information about plausible valuable predictive information of first trimester sTie-2 added to risks assessment that can be determined from an antenatal booking history. Identification of women at risk of developing adverse pregnancy

outcomes early in pregnancy would allow ample time for monitoring and implementing preventive strategies.

There were three aims of this study: (i) to determine normative serum levels of soluble Tie2 in the first trimester of pregnancy; (ii) assess the association of maternal serum soluble Tie2 in first trimester with the risk of adverse pregnancy outcomes; and (iii) to assess the clinical utility of sTie2 in the prediction of adverse pregnancy outcomes.

METHODS

Study population and sample testing

This case-cohort study was conducted from a cohort of 11,130 women attending first trimester Down syndrome screening between January and October 2007 in New South Wales (NSW), Australia. Serum samples were collected by the Pacific Laboratory Medicine Services (PaLMs), and then archived and stored at -80°C. During this period, this was the state's only public screening service and received samples from throughout NSW.

Serum samples for this study were thawed and serum levels of sTie-2 measured using a Quantikine ELISA immunoassay (R&D Systems, Minneapolis, MN). The reported analytic sensitivity of the immunoassays was $0.156 - 10.00$ ng/ml and the intra-assay and inter-assay coefficient of variation were <17%. Serum samples were routinely diluted 1 in 10 prior to measurement. Serum samples were stored according to time of collection in boxes containing 81 samples in 9x9 rows. Cases of each study outcome of interest (small for gestational age (SGA), preterm birth,

preeclampsia, gestational diabetes, miscarriage and stillbirth) were selected using a computerized random-number function, with the specific box and row identified. Laboratory scientists then located and analysed the full row of the storage box (9 samples) containing the case but were unaware its position within the row. The remaining eight samples in each row were used as controls, regardless of their outcome status. Inevitably, the pregnancy outcome for some of these eight samples was subsequently identified to be pregnancy complications and these were allocated to the relevant case status, accordingly. This allowed laboratory scientists to remain blinded to pregnancy outcomes and increase the efficiency of processing.

Data sources

Maternal information for archived serum samples was derived from the laboratory database and corresponding pregnancy and birth outcomes were ascertained via record linkage from the Perinatal Data Collection (PDC) and the Admitted Patient Data Collection (APDC). The PDC includes records of all births in NSW of at least 400 grams birth weight, or at least 20 weeks' gestation and includes demographic, medical and obstetric information on the mother, labour, delivery and birth outcome. The APDC includes records for both mothers and liveborn infants from hospital admissions in NSW public and private hospitals. It contains demographic, clinical and health services information for each admission. Relevant diagnoses and procedures are recorded for each hospital admission and coded according to the International Classification of Diseases version 10 – Australian Modification (ICD10-AM) and Australian Classification of Healthcare Interventions, respectively. In Australia unique identifiers are not available for record linkage of unit record data from multiple datasets [13]. Consequently probabilistic linkage methods

were utilised. This involves a complex process of blocking and matching combinations of personal identifiers (such as name, date of birth, address and hospital) using record-linkage software[14]. Probability weights are calculated, adjusted for incomplete and missing data, and used to establish correct matches. The validity of the probabilistic record linkage is extremely high with less than 1% of records having an incorrect match[13-16]. The NSW Centre for Health Record Linkage conducted the record linkage and identifying information was removed prior to the release of data for analysis. The CHeReL assesses the linkage quality for each study, and for this study reported <5/1000 missed links and <2/1000 false positive links. The study was approved by the NSW Population and Health Services Research Ethics Committee.

Study outcomes assessed included: small for gestational age (SGA), preterm birth, preeclampsia, gestational diabetes, miscarriage and stillbirth. SGA was defined as birthweight less than the 10th centile and less than the 3rd centile (severe SGA) of the distribution for gestational age and infant sex [17]. Gestational age is reported in the birth data in completed weeks of gestation and determined by the best clinical estimate including early ultrasound (>97%) and last menstrual period. Preterm birth was defined as delivery at less than 37 weeks and very preterm birth less than 34 weeks gestation. During the study period, preeclampsia was defined as onset of hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) from 20 weeks' gestation accompanied by proteinuria [18]. Information on preeclampsia was obtained from both the APDC and PDC data, to maximize ascertainment[19,20]. Early onset preeclampsia was defined as women with preeclampsia delivered at ≤34 weeks gestation. Miscarriage was defined as a spontaneous pregnancy loss between 10-20 weeks gestation and identified from APDC data, while

stillbirth was defined as a spontaneous pregnancy loss after 20 weeks gestation and was identified from PDC data.

The key explanatory variable for this study was sTie-2. Information on serum Ang-1 and Ang-2 concentrations were available for 1,058 women and were obtained from data from a previous study[9]. Additional covariate information available for the analysis included maternal age and weight (kilograms) ascertained at the time of first trimester screening, parity (nulliparous/multiparous), smoking during pregnancy, previously diagnosed hypertension (chronic or pregnancy), previously diagnosed diabetes (pre gestational or gestational), previous miscarriage, country of birth and socio-economic disadvantage. Socio-economic disadvantage was defined according to the Socio-Economic Indexes for Areas (SEIFA) relative disadvantage scores developed by the Australian Bureau of Statistics (ABS) [21] and categorized into quintiles. Maternal weight was missing in 380 (14.5%) of the records and multiple imputation was used to substitute this [22]. Other missing data were infrequent and were excluded from the analyses: smoking was missing in 24 (1.2%) and country of birth in 3 (0.1%) of the records.

Statistical analysis

As the study population was selected from a population based-cohort, we weighted sTie-2 to account for sampling probabilities to determine normative concentrations for a pregnancy cohort. Weight calculation involved multiplying each case and control by the inverse proportion of the probability of selection. The sTie-2 distribution was then calculated applying individual weights. Similarly, to ensure comparability with the NSW state maternity population, we weighted all observations

for the population rates of age, parity, smoking and socio-economic disadvantage using SEIFA quintiles [23]. Descriptive statistics were calculated and spearman coefficient was used to determine the correlation between sTie-2, Ang-1 and Ang-2 after logarithmic transformation. Kruskall-Wallis test was used to compare concentrations of sTie-2 by maternal characteristics and between women that subsequently had an adverse pregnancy outcome with women with unaffected pregnancies. Multiple of the Medians (MoM) were calculated for sTie-2, Ang-1 and Ang-2 levels, to account for differences in values attributable to gestational week of the test, maternal age or maternal weight. Soluble Tie-2 MoM levels were then dichotomized by the <25th and the >75th centile.

Conditional multivariate logistic regression was used to assess the association between low and high sTie-2 MoM with adverse pregnancy outcomes, taking into account maternal and clinical risk factors. A backward elimination method retaining only significant explanatory variables was used to fit models for each outcome. We then assessed the predictive accuracy of sTie-2 by examining the area under the Receiver Operating Characteristic (ROC) curve (AUC), derived from logistic regression analysis. AUC results were also examined to determine whether models performed better than chance (0.5). Models for serum sTie-2 alone, those including maternal and clinical risk factors only and with sTie-2 and risk factors combined were compared. This method was applied to assess whether serum levels of sTie-2 provided any additional information to maternal and clinical risk factors in predicting severe adverse pregnancy outcomes by evaluating the maximum likelihood estimates using the likelihood ratio (X^2) test. A P-value of <0.05 was considered statistically

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significant and analyses were performed using SAS software 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 2,987 samples were tested with health information of the relevant pregnancy available for 2,774 (92.9%) samples. Excluded were 179 women whose blood sample were taken before 10 or after 14 weeks gestation, had a twin pregnancy, a medical abortion, or an infant with a major congenital anomaly. Soluble Tie-2 was undetectable in 240 samples and these women were assigned a value equal to half the limit of detection [24]. A total of 2,595 women were included in the analysis. The mean (SD) maternal age and weight were 32.9 (4.9) years and 66.8 (14.9) kilos, respectively. 1,261 (48.2%) women where nulliparous, 167 (6.4%) smoked during pregnancy and 136 (5.2%) women had previously diagnosed hypertension. Table 1 presents the median interquartile range (IQR) serum levels of sTie-2 by maternal characteristics. The median (IQR) serum levels of sTie-2 for total population of were 9.0 ng/ml (3.3 – 14.7). Compared with women that did not smoke (median 8.5 ng/ml; IQR: 2.8 - 14.2), median sTie-2 was higher in women who smoked during pregnancy (median 12.2 ng/ml IQR: 7.2 – 16.1). Serum Tie-2 concentrations were also higher among women <25 years and weight >85 kilograms, but lower among women aged over 40 years. Serum sTie-2 increased with increasing maternal weight (r =0.09, P<0.001) and decreased with increasing maternal age (r = -0.08, P<0.001) (Table 1). Levels of sTie-2 did not change by gestational age between 10 and 14 weeks. No correlation between sTie-2 with Ang-1 (r = -0.008; P=0.79) or Ang-2 (r = -0.031; P=0.31), was observed.

Figure 1 presents median (IQR) serum levels of serum sTie-2 by adverse pregnancy outcome. There was no difference in sTie-2 levels between unaffected women with woman who subsequently developed adverse pregnancy outcomes. The association of low sTie-2 MoM (<25th centile) and high sTie-2 MoM (>75th centile) with adverse pregnancy outcomes is presented in Table 2. Low sTie-2 (<0.31 MoM) was associated with 60% increased risk of preeclampsia. There was a tendency for women with low Tie-2 to be at increased risk of early onset preeclampsia or miscarriage and high sTie-2 of stillbirth, but these associations did not reach statistical significance due to small number of cases.

Figure 2 illustrates the receiver operating characteristic curves (ROC) of sTie-2 in predicting preeclampsia, early-onset preeclampsia miscarriage and stillbirth in comparison with maternal and clinical risk factors. The predictive accuracy of low or high sTie-2 alone was not different from chance for preeclampsia (AUC=0.54; P=0.08), early-onset preeclampsia (AUC=0.65; P=0.08), miscarriage (AUC=0.58; P=0.15) and stillbirth (AUC=0.58; P=0.18). Sensitivity, specificity and positive likelihood ratios were: for preeclampsia (33%, 75% and 1.32), early-onset preeclampsia (44%, 75% and 1.75), miscarriage (41%, 75% and 1.62) and for stillbirth (40%, 75% and 1.61), respectively. Maternal risk factors alone had better predictive accuracy compared with sTie-2 and adding sTie-2 to maternal risk factors did not improve the ability of the models to predict preeclampsia (X^2=1.12; P=0.29), early-onset preeclampsia (X^2=0.57; P=0.45), miscarriage (X^2=0.63; P=0.42) and stillbirth (X^2=0.53; P=0.46) (Figure 2).

DISCUSSION

This the largest population-based study to examine maternal soluble Tie-2 levels of women in first trimester, and assessed the association with adverse pregnancy outcomes. This is also the first study evaluating the predictive accuracy of serum sTie-2 in first trimester. Our study highlights that serum sTie-2 concentrations in pregnant women are affected by maternal age, weight and smoking. There was no difference in median sTie-2 between women who developed adverse pregnancy outcomes and those who did not. After adjusting for maternal and clinical risk factors, low first trimester sTie-2 in first trimester was associated with preeclampsia, however, it did not predict preeclampsia or any other adverse pregnancy outcome better than maternal and clinical risk factors.

Variation in serum sTie-2 by age and smoking has been reported in both pregnant and non-pregnant populations[10,25]. While weight has been reported to influence sTie-2 concentrations in non-pregnant populations[25], no correlation has been found between sTie-2 concentrations with BMI in pregnant women [10]. Adipose tissue, a highly vascular and active endocrine organ, may be a source of angiopoietins and their receptors; and may influence sTie-2 levels [26]. Despite variation by maternal characteristics, previous studies have also described constant serum sTie-2 levels across first trimester [27], mid pregnancy [10] and a slight increase after 26 weeks gestation [12].

Although there have been a number of studies examining sTie-2 in pregnancy. only two assessed sTie-2 in first trimester and the association with adverse pregnancy outcomes[10,28]. Our findings are consistent with these studies that reported no

difference in first trimester sTie-2 levels in women subsequently diagnosed with preeclampsia or had an infant diagnosed with IUGR, compared with unaffected women.

Soluble Tie2 is functional, binding both Ang1 and Ang2, and inhibits ligand-mediated receptor activation and downstream cellular responses[29]. Furthermore, abnormal sTie-2 levels are associated with other pathologies involving vascular remodelling, such as acute coronary syndromes[30]. Overall, our results indicate that impairment of placental angiogenesis may not be substantial enough to alter serum sTie-2 levels, at least in first trimester, to predict subsequent development of preeclampsia. Although we found a borderline association between low sTie-2 with preeclampsia, these may be chance findings and further studies and larger cases numbers are required to confirm these findings and the tendency towards increased odds of early-onset preeclampsia, miscarriage or stillbirth. These associations may suggest the role of angiogenic/anti-angiogenic factors in the pathogenesis of adverse pregnancy outcomes, however, their value in screening of women at-risk of these conditions may be not appropriate in a clinical setting. In a recent study, we found that despite a positive association between elevated first trimester Ang-1/Ang-2 ratio levels with SGA, preeclampsia, preterm birth and miscarriage in a large cohort of women[9], the overall predictive accuracy was poor.

Studies evaluating serum angiogenic/anti-angiogenic factors such as placental growth factor and soluble vascular endothelial growth factor receptor-1, have provided promising results for the diagnosis[4], prognosis[31], and assessment of severity of adverse pregnancy outcomes[32]. But this evaluation has focused later in pregnancy,

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when it may be too late for preventive interventions to be effective. Early pregnancy assessment provides an ideal opportunity to incorporate an additional test into existing, routine antenatal testing for identification of pregnancies at-risk of adverse pregnancy outcomes for closer surveillance. To date, first trimester serum levels of agiogenic/anti-angiogenic biomarkers, single or combined, have not provided adequate predictive accuracy for antenatal screening. Moreover, in a previous study we found that compared with serum biomarker information alone, routinely collected maternal and clinical risk factors, such as parity, previously diagnosed hypertension and maternal weight provide better accuracy in

predicting preeclampsia[33].

Strengths of the study were the evaluation of a large sample from a population-based cohort of pregnant women attending first trimester screening. Ascertainment and follow-up of pregnancy outcomes was possible for 92% of the samples using record linkage of laboratory to birth and hospital data with only minimal missing information. Missing health and pregnancy information was mostly attributable to women giving birth in hospitals out of state. Yet, women with missing information had similar characteristics compared with those included in the study. One of the limitations of the study was that maternal weight was missing in 14.5% of the women, which was addressed by applying multiple imputations, a technique shown to be robust and valid for dealing with missing data [22]. Our results for miscarriage must be interpreted with caution as miscarriage is under ascertained. First, it was restricted to women screened from 10 to 14 weeks and second, only miscarriages resulting in admission to hospital can be identified. Another potential weakness were the socio-demographic differences between our cohort compared with

the total state maternity population during the same period, which may be due to a healthier and more affluent group attending first trimester screening. We addressed this issue by weighting our data by population characteristics to ensure generalizability of the results.

In conclusion, our findings suggest that low sTie-2 concentrations in first trimester are associated with subsequent onset of preeclampsia, but do not predict preeclampsia or other adverse pregnancy outcomes any better than clinical and maternal risk factor information.

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Table 1: First trimester soluble Tie-2 serum levels by maternal characteristics

Maternal characteristics	sTie-2 (ng/ml)		
	n (%)	Median (IQR)	P-value
All women	2595	9.0 (3.3, 14.7)	
Maternal age			
<25	162 (6.2)	11.6 (4.9, 15.6)	0.01
25 - 29	532 (20.3)	9.9 (4.0, 15.2)	
30 - 34	983 (37.6)	8.3 (2.8, 13.7)	
35 - 39	789 (30.2)	8.4 (2.4, 14.2)	
40+	150 (5.7)	7.7 (2.5, 14.7)	
Parity			
Nulliparous	1261 (48.2)	8.7 (3.1, 14.0)	0.13
Parous	1355 (51.8)	9.4 (3.6, 15.1)	
Smoking during pregnancy			
Yes	167 (6.4)	12.2 (7.2, 16.1)	<0.001
No	2425 (93.6)	8.5 (2.8, 14.2)	
Maternal weight (kg)			
<55	428 (19.1)	8.5 (4.2, 13.5)	<0.001
55 - 64	452 (20.2)	7.4 (1.9, 14.0)	
65 - 74	403 (18.0)	8.1 (2.7, 13.0)	
75 - 84	484 (21.7)	9.1 (3.2, 15.4)	
85+	469 (21.0)	11.4 (5.5, 15.9)	
Gestational week at sampling			
10	256 (9.8)	10.2 (3.0, 14.2)	0.08
11	846 (32.3)	8.2 (2.7, 14.3)	
12	1072 (41.0)	9.5 (3.8, 15.2)	
13	442 (16.9)	8.8 (4.6, 14.5)	
Country of birth			
Australia & New Zealand	1683 (64.4)	9.2 (3.5, 14.8)	0.47
Pacific Islands	25 (1.0)	7.3 (3.3, 12.5)	
Europe, NA & SA	255 (9.8)	7.7 (2.0, 14.4)	
Middle east	63 (2.4)	10.6 (2.5, 15.0)	
South east Asia	176 (6.7)	9.2 (4.6, 13.6)	
China, Hong Kong & Taiwan	162 (6.2)	10.8 (4.6, 15.0)	
Japan and Koreas	70 (2.7)	8.8 (4.4, 15.0)	
India & surroundings	115 (4.4)	7.5 (1.6, 14.2)	
Central and South America	39 (1.5)	3.1 (1.5, 11.1)	
Africa and Caribbean	24 (0.9)	6.8 (2.0, 16.2)	

IQR: Interquartile range; NA: North America; SA: South Africa

Figure 1: First trimester serum soluble Tie-2 levels in women that subsequently developed adverse pregnancy outcomes compared with unaffected women. The line inside the boxes represents the median value and diamond represents the mean.
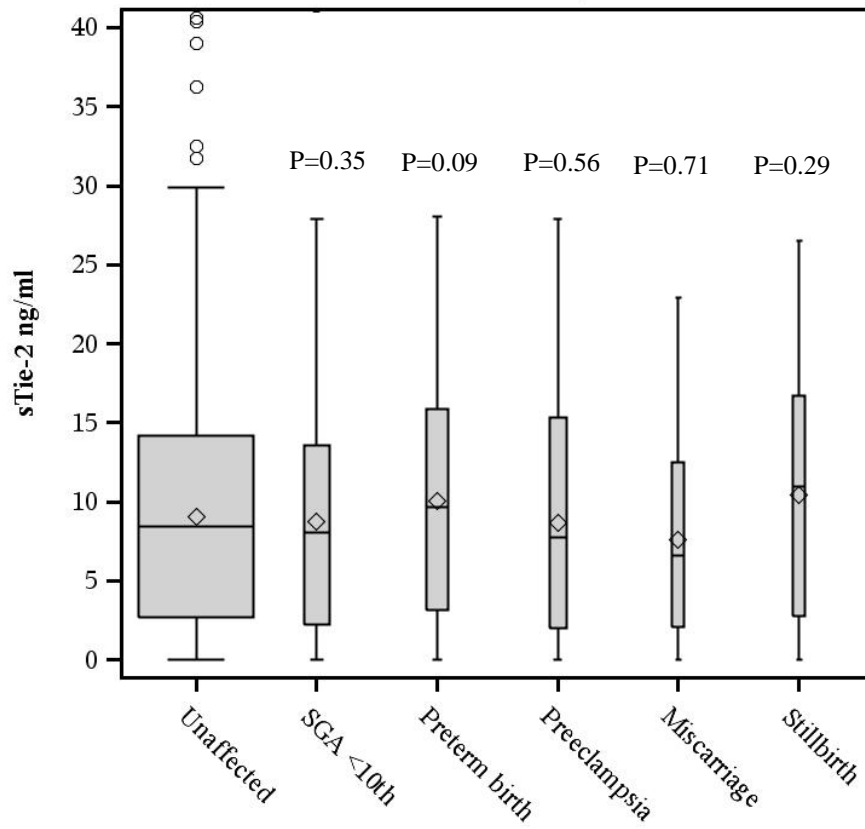
Table 2: Association of first trimester Tie-2 with adverse pregnancy outcomes

Pregnancy outcome	Affected with low sTie-2	Affected with high sTie-2	Univariate Odds ratio (95% CI)		Adjusted Odds ratio (95% CI)*	
			Low sTie-2 MoM <0.31 [a]	High sTie-2 MoM >1.68 [a]	Low sTie-2 MoM <0.31 [a]	High sTie-2 MoM >1.68 [a]
SGA<10th centile (n=298)	78	75	1.07 (0.80, 1.44)	1.04 (0.77, 1.40)	1.11 (0.82, 1.49)	1.06 (0.78, 1.44)
SGA<3rd centile (n=73)	20	18	1.13 (0.65, 1.98)	1.03 (0.58, 1.84)	1.27 (0.72, 2.24)	1.11 (0.62, 2.00)
Preterm birth <37 weeks (n=196)	45	55	0.94 (0.65, 1.36)	1.18 (0.83, 1.67)	0.97 (0.68, 1.40)	1.22 (0.86, 1.73)
Preterm birth <34 weeks (n=68)	18	20	1.19 (0.66, 2.15)	1.33 (0.75, 2.37)	1.24 (0.68, 2.25)	1.32 (0.73, 2.37)
All preeclampsia (n=110)	35	24	1.48 (0.95, 2.32)	1.02 (0.62, 1.68)	1.61 (1.01, 2.57)	1.13 (0.67, 1.92)
Early-onset preeclampsia (n=9)	4	3	3.93 (0.72, 21.48)	2.99 (0.50, 17.93)	4.13 (0.75, 22.79)	2.68 (0.43, 16.57)
Miscarriage (n=22)	9	4	1.97 (0.78, 4.98)	0.88 (0.27, 2.88)	2.08 (0.82, 5.28)	0.96 (0.29, 3.14)
Stillbirth (n=20)	5	8	1.40 (0.44, 4.43)	2.29 (0.83, 6.34)	1.48 (0.47, 4.70)	2.49 (0.89, 6.97)

*Adjusted for: maternal age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, previously diagnosed diabetes, country of birth or socio-economic disadvantage. [a] Reference sTie2 MoM: 0.31 – 1.68.

Figure 2: Receiver Operating Characteristic curves comparing first trimester serum Tie-2 with maternal and clinical risk factors predicting preeclampsia, early onset preeclampsia, miscarriage and stillbirth. AUC: Area under the curve. Risk factors are: maternal age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, previously diagnosed diabetes, country of birth or socio-economic disadvantage.