

Is There Any Association Between the Angiogenic Factors sFlt-1 / Plgf And Intrauterine Growth Restriction in Patients with Preeclampsia?

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Received Date: May 21, 2019 Accepted Date: June 17, 2019 Published Date: June 19, 2019

Citation: Al Naimi (2019) Is There Any Association Between the Angiogenic Factors sFlt-1 / Plgf And Intrauterine Growth Restriction in Patients with Preeclampsia? J Womens Health Gyn 6: 1-7.

Abstract

Purpose: To evaluate the association between the soluble FMS-like tyrosin kinase-1 (sFlt-1) and placental growth factor (PlGF) with intrauterine growth restriction (IUGR) in women with preeclampsia. The correlation between PlGF, sFlt-1 and IUGR (which is confirmed at delivery) and the value of adding the Doppler ultrasound findings of the uterine artery to this combination was evaluated.

Results: This single-center prospective observational cohort study included 728 participants diagnosed with clinical preeclampsia of which 80% (584 patients) had complete follow-up till delivery at our center while 19% (138) delivered a baby with birth weight below the 10th percentile. These patients had significantly higher sFlt-1 as well as lower PlGF concentrations in comparison to patients without IUGR. The delta in PlGF concentration was more sensitive than that of sFlt-1 (Wilcoxon-Mann-Whitney $r=0.46$, $r=0.28$). sFlt-1/PlGF ratio could predict IUGR with a 79.1% sensitivity and a 72.4% specificity. Logistic regression showed that the combination of sFlt-1/PlGF with uterine artery notching slightly improves the predictive value for IUGR from the isolated factors on their own with 79.5% sensitivity. PI of the uterine artery was not beneficial in improving the predictive power of this combination. Wald's p-values are 0.000012 for s-Flt-1/PlGF, 0.000285 for notching and 0.04945 for PI of the uterine artery.

Conclusions: Angiogenesis factors sFlt-1/PlGF are beneficial for predicting IUGR in high-risk groups such as a population with preeclampsia. Their combination with uterine artery notching could provide valid models for IUGR screening.

Keywords: IUGR, sFlt-1, PlGF, Angiogenic Factors, Uterine Artery Doppler

Introduction

The spiral arteries undergo remodeling throughout pregnancy that is essential for normal fetal growth. This remodeling increases the diameter of the end spiral arteries by 4-6 times as well as the blood flow from initial 50 ml/min to 600 ml/min [1]. Failed remodeling is associated with increased risk for intrauterine growth restriction (IUGR) [2]. IUGR is a failure of the fetus to reach its genetic growth potential and is confirmed as weight below the 10th percentile for the gestational age accompanied by an increased impedance of the umbilical artery [3]. It commonly complicates pregnancy and increases the risk for adverse outcome such as stillbirth, neonatal death and increases perinatal morbidity. Other cases of small for gestational age (SGA) are associated with congenital anomalies, infections and drugs [4].

The pathophysiology of IUGR, similar to preeclampsia, is based on the failure of the spiral arteries to physiologically transform during pregnancy from high pressure, low capacity vessels to low pressure high capacity ones [5]. Placental growth factor (PlGF) and soluble FMS-like tyrosine kinase-1 (sFlt-1) are two important factors in regulating this transformation. PlGF is a proangiogenic growth factor that stimulates cellular proliferation and is expressed in the syncytiotrophoblast [6], while sFlt-1 is a truncated splice of the vascular endothelial growth factor (VEGF) receptor that binds to both PlGF and VEGF and block them, thus antagonising their action. It is mainly produced in the syncytiotrophoblasts and monocytes [7].

Several publications show the importance of sFlt-1/PlGF in the development of preeclampsia, its prediction and confirming the diagnosis [8]. sFlt-1 is higher in isolated IUGR pregnancies than in normal pregnancies, and its level of correlates directly with the abnormality in the uterine and umbilical Doppler impedance [9]. Failed remodeling causes abnormalities in the uterine artery Doppler with increased impedance and diastolic notching. The uterine artery Doppler velocimetry can be used in screening for patients at risk for developing IUGR [10]. Antenatal diagnosis of IUGR has low sensitivity with a detection rate of 25% in a high-risk population [11] and only 15% in low-risk population [12]. Newer data report better detection rates, but they are still lacking with an overall detection rate of 36% generally and 56% in a high-risk population [13].

Evaluation of the uterine artery Doppler in the first and second trimester could help in screening for early-onset IUGR associated with preeclampsia. Reported detection rates vary from 30% [14] to about 75% which could also be improved to

reach 90% when combined with other maternal risk factors and biochemical markers [15].

This study assesses the value of the angiogenic factors for predicting IUGR in a population with manifested preeclampsia, and to assess the value of combining uterine artery Doppler with the angiogenic factors for improving the predictive power for IUGR.

Materials and Methods

This was a single center prospective observational cohort study and was conducted at Buergerhospital (Frankfurt am Main, Germany). We included 728 singleton pregnancies in this study after giving consent. They were selected from a high-risk collective with manifested preeclampsia. The collective was classified as high-risk according to the German antenatal care guidelines. Additional risk factors including maternal age, history of IUGR, increased Body Mass Index (BMI), chronic hypertension, autoimmune diseases etc. were separately recorded. The data from this collective was analyzed for assessing the value of the angiogenic factors in predicting IUGR knowing that these patients have manifested preeclampsia. Exclusion criteria included complications that would affect the course of the pregnancy, such as chromosomal or structural anomalies, premature preterm rupture of membranes, placenta praevia, and breech presentation. Medical history, obstetric history, ultrasound examination and laboratory results were recorded for each patient.

Ultrasound Doppler sonography was utilized for diagnosing notching of the uterine arteries, mean PI of the uterine arteries, and IUGR. Biparietal diameter, abdominal circumference, and femur length were used for estimating fetal weight. The ultrasound examinations and the Doppler investigations were performed using the Aplio 500 and Aplio 300 (Toshiba Medical System Europe) and GE Voluson E8 and GE Voluson 730 Expert (GE Healthcare) with 5.0-MHz convex transducers.

Blood samples were taken to assess PlGF, sFlt-1, and their ratio. The serum levels of sFlt-1 and PlGF were measured in Synlab Leinfelden, using the immunoassay kits (Elecsys® sFlt-1 and Elecsys® PlGF) manufactured by Roche Diagnostics GmbH.

Pregnancy outcome for the patients who were not lost to follow up was recorded with mode of delivery, gestational age at delivery, 5' minute APGAR score and fetal weight in gram (g). IUGR suspicion was recorded based on the ultrasound findings, but was only confirmed when birth weight was below the tenth percentile for the gestational age at delivery.

Statistical analysis was undertaken with the help of Biometrische Analyse von Stichproben, BiAS (biometric analysis of random samples) version 10.12 from Dr. Ackermann, Goethe University Frankfurt am Main, Germany. The Wilcoxon-Mann-Whitney-U, Chi square contingency table, Pearson's contingency coefficient (C), Receiver operating characteristic (ROC) curves, Logistic regression and the Kruskal-Wallis test were utilized in the analysis. A p-value of 0.05 was set as the cut-off point for significance.

Results

This study included 728 individual patients, which is a fraction of the 10600 deliveries in this hospital for this time period. The chi-square test was used to evaluate the prenatal diagnosis of IUGR with postnatally confirmed IUGR cases. The results showed good diagnostic power with 86.2% sensitivity and 93% specificity ($p < 10^{-6}$).

Follow-up data with delivery information at the study hospital were available for 584 patients, 138 of which (23.6 %) had IUGR at delivery. sFlt-1, PlGF and their ratio were separately tested against IUGR-related delivery. Table 1 summarizes the results of Wilcoxon-Mann-Whitney-U test for the angiogenic factors in relation to IUGR-related delivery. sFlt-1 showed a weak to moderate effect for predicting IUGR-related delivery with r of 0.29. PlGF and the ratio showed moderately good effect with high statistical significance.

Roc curve analyses were utilized for assessing the sensitivity and specificity of the angiogenic factors for predicting IUGR at delivery. The optimal cut-off point for sFlt-1 was 7077 pg/ml with a 63.2% sensitivity, 69.6% specificity and 33.6% misclassification rate. The optimal cut-off point for PlGF was 93.5 pg/ml with a 74.6% sensitivity, 76.5% specificity and 24.4% misclassification rate. The optimal cut-off point for the ratio was 69.39 with a 79.1% sensitivity, 72.4% specificity and 24.2% misclassification rate.

Spearman's test was used to assess the correlation between sFlt-1, PlGF and their ratio on the one side with PI of the uterine artery, diabetic status, birth weight, 5 minutes APGAR score, and gestational age at delivery on the other side. The statistically significant correlations ranged mainly from poor to moderate. Uterine artery PI showed a moderately good negative correlation with PlGF. Birth weight showed a moderately good positive correlation with PlGF and a negative correlation with the quotient. The results of these tests are summarized in table 2

	IUGR related	median	min.	max.	n.	r	P
sFlt-1	no	4217	22.97	69761	441	0.29	0.69
	yes	8239	85	45017	136		
PlGF	no	177.5	7	3604	441	0.46	0.19
	yes	56.11	3	528	136		
Ratio	no	24.92	0.45	723.4	441	0.45	0.81
	yes	154.4	2.55	1551.3	136		

Table 1. The results of the Wilcoxon-Mann-Whitney-U test for the angiogenesis factors and IUGR at delivery (min: minimum, max: maximum, n: number of patients, r: effect size, P: Mann-Whitney P value)

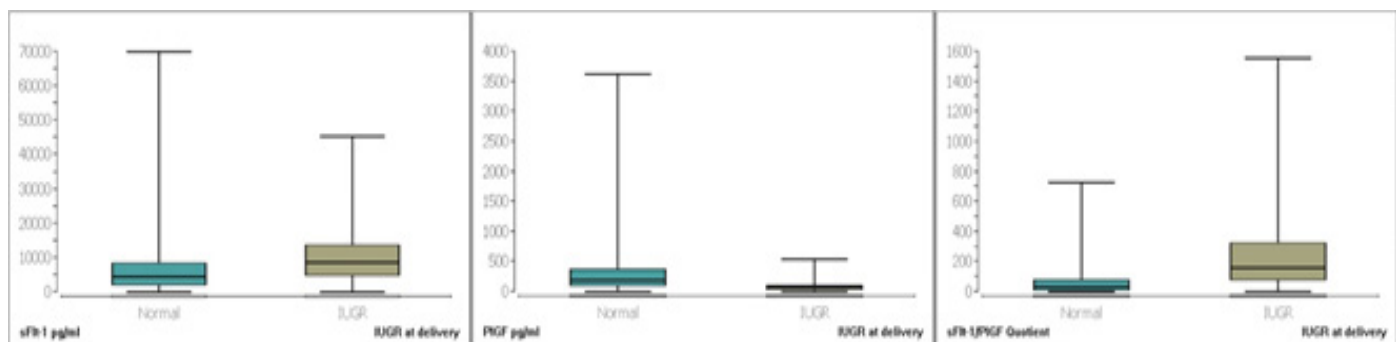


Figure 1. Box plot of sFlt-1, PlGF, and their ratio for patients who had IUGR-related delivery against isolated preeclampsia.

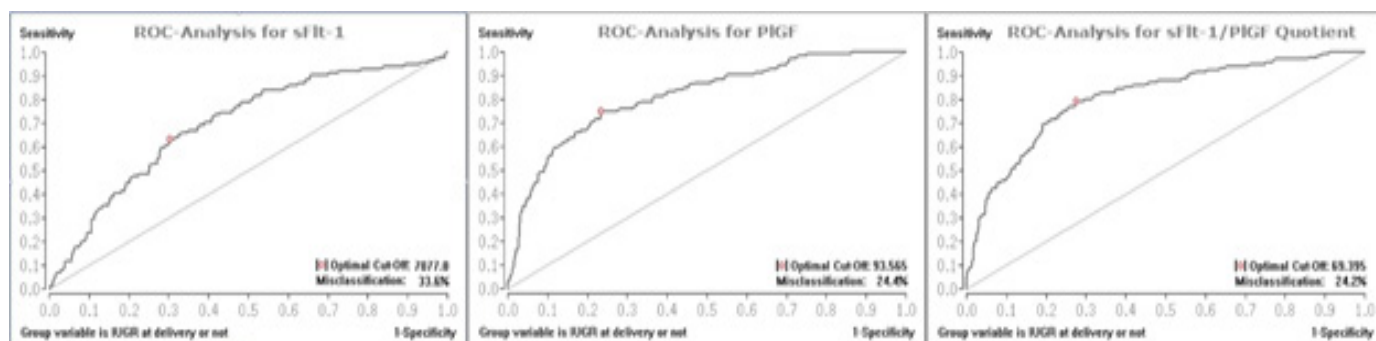


Figure 2. Roc curve analysis for sFlt-1, PlGF, and their ratio as a diagnostic value for predicting IUGR at delivery.

		sFlt-1	PlGF	Ratio
Uterine artery PI	ρ	0.14	-0.43	0.33
	p	<10-3	<10-6	<10-6
Diabetes	ρ	-0.07	-0.04	-0.03
	p	0.06	0.25	0.47
Gestational age at delivery	ρ	-0.32	0.3	-0.39
	p	<10-6	<10-6	<10-6
5 minutes APGAR	ρ	-0.15	0.23	-0.22
	p	<10-3	<10-6	<10-6
Birth weight	ρ	-0.37	0.41	-0.49
	p	<10-6	<10-6	<10-6

Table 2. The results of Spearman's correlation between the angiogenesis factors and Doppler results, diabetes and other delivery variables (ρ = Spearman's correlation Rho, p = value for statistical significance)

Table 3. shows the range, median and the quartiles of the uterine artery PI in patients who developed IUGR in comparison to those without. Wilcoxon-Mann-Whitney-U test showed a statistically significant ($p < 10^{-6}$) moderate to strong effect of the uterine artery PI for predicting IUGR with r of 0.4 and Mann-Whitney P of 0.75. This correlation is also shown as a box plot in figure 3.

Chi-square test for notching showed a positive correlation with IUGR-related delivery. Corrected Pearson's contingency coefficient for this correlation was 0.58 ($P < 10^{-6}$).

Uterine artery PI	median	Range	1. quartile	3. quartile
Normal	0.77	0.37-2.99	0.6	1.07
IUGR	1.23	0.33-3.33	0.94	1.66

Table 3. Comparison of the uterine artery PI values in patients who develop IUGR and normal patients

Logistic regression was used to test a model of using notching and PI of the uterine artery in combination with the sFlt-1/PlGF ratio for anticipating IUGR-related delivery. The results show that the addition of the uterine artery PI to the combination of notching and the ratio does not improve the diagnostic power. The diagnostic power of the combination of notching and sFlt-1/PlGF was 79.5%. Wald's P value for the quotient is 10^{-5} , for notching 3×10^{-4} .

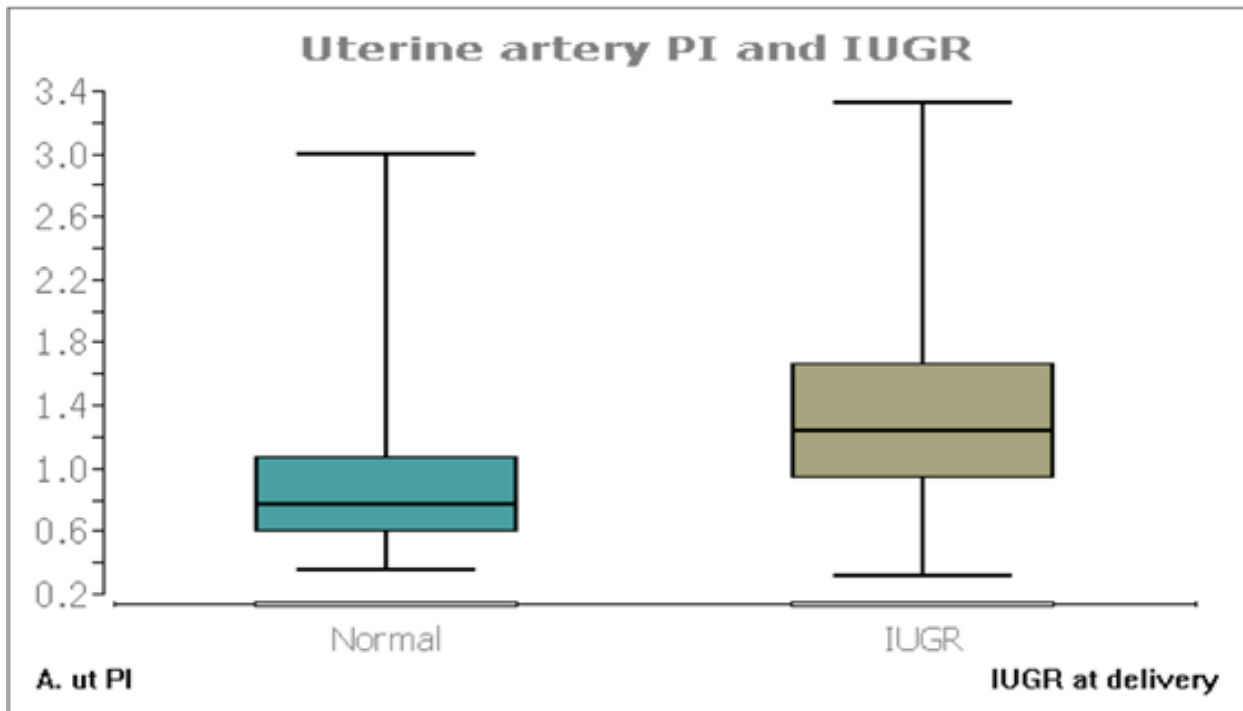


Figure 3. Box plot of the uterine artery PI for patients who eventually develop IUGR against those without.

Discussion

We made the observation that patients who eventually had IUGR had higher levels of sFlt-1, lower levels of PlGF and higher sFlt-1/PlGF ratios than in isolated preeclampsia patients. sFlt-1 in IUGR pregnancies versus isolated preeclampsia had medians of 8239 pg/ml and 4217 pg/ml respectively and a statistically significant weak to moderate Wilcoxon Mann Whitney-U effect of 0.29. Using an sFlt-1 cut-off value of 7077 pg/ml could be used for anticipating IUGR-related delivery with 63.2% sensitivity, 69.6% specificity and 33.6% misclassification rate (false positive + false negative). This weak performance might be the reason why some studies suggest that there was no sFlt-1 increase in IUGR pregnancies, especially publications with small sample size that might miss the weak to moderate increase [16,17]. The PlGF medians were 177.5 pg/ml and 56.11 pg/ml respectively with moderate Wilcoxon Mann Whitney-U effect of 0.46. The best cut-off value of 93.5 pg/ml for PlGF could be used for predicting IUGR in preeclampsia patients with 74.6% sensitivity, 76.5% specificity and 24.4% misclassification rate. This is a better performance than sFlt-1, which might provide a reason for using PlGF in screening for pregnancies with risk for IUGR. sFlt-1/PlGF ratios had medians of 154.4 vs 24.92 respectively and statistically significant moderately good Wilcoxon Mann Whitney-U effect of 0.45. Using a ratio cut-off value of 69.39 could be used

for anticipating IUGR with a 79.1% sensitivity, 72.4% specificity and 24.2% misclassification rate, which is a comparable performance to PlGF. We analyzed our population and correlated between severe and moderate preeclampsia among those with and without IUGR and found a difference with no statistical significance ($p=0.25$). This finding supports the theory that the difference of sFlt-1/PlGF between IUGR and non-IUGR cases was not caused by the severity of preeclampsia.

sFlt-1 correlates indirectly, PlGF directly, and sFlt-1/PlGF ratio indirectly, with gestational age at delivery, neonatal birth weight and very weakly with the 5 minutes APGAR. The correlation coefficients of the ratio are stronger than sFlt-1 and PlGF on their own, which renders it useful for anticipating adverse fetal outcome at delivery.

The incidence of IUGR in our population was 1.3%, which is lower than the quoted global IUGR incidence of 2.8% [18]. IUGR has high correlation with abnormal Doppler findings, stillbirths, iatrogenic prematurity, fetal distress and caesarean sections [19]. Our data mirrors this correlation.

IUGR patients delivered significantly earlier than those with isolated preeclampsia with average gestational ages of 34/1 and 37/4 weeks respectively, and had significantly smaller babies. The earlier delivery can definitely be attributed to iatrogenic causes. The combination of early delivery and lighter

birth weight might be a reason for adverse outcome. IUGR cases were associated with higher rates of cesarean section 73% in comparison to 50% in the isolated preeclampsia collective.

Intrauterine fetal demise (IUFD) had a significant association with extreme cases of IUGR, which is to be expected as reported in literature [20]. Identified IUGR cases have a 5- to 10-fold risk for developing IUFD. These need to be closely monitored with methods such as umbilical artery Doppler, which leads to a reduction of the IUFD rate and increases iatrogenic prematurity as in our data without increasing the neonatal mortality [21].

There is a moderate negative correlation between PlGF and the uterine artery PI with Spearman's ρ value of -0.43. Both notching and PI of the uterine artery correlate with predicting IUGR. Combining sFlt-1/PlGF with notching provides an effective model with a diagnostic power of 79.5% for anticipating IUGR without the need for uterine artery PI.

These results show that using the angiogenic factors in combination with the ultrasound findings could provide valid models for predicting IUGR in preeclampsia population. This is a reconfirmation of preexisting data regarding the relationship between sFlt-1, PlGF and IUGR in a normal population, and this data is specifically within the subpopulation of manifested preeclampsia.

Compliance with Ethical Standards

Conflict of interest disclosures: Al Naimi, Schmidt-Fittschen, Herzeg, Kyvernitakis, Louwen and Bahlmann declare that we have no conflict of interest

Ethical approval: This work has been approved by the internal Ethical Board of Buergerhospital, which is led by Professor Schwenn. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study according to the declaration of Helsinki and the German law.

Acknowledgments

This work was supported by the Dr. Senckenberg Foundation, Frankfurt am Main.

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