Article

Associations between Maternal Serum Biomarkers and Uterine Artery and Ductus Venosus Blood Flow Patterns in Early Pregnancy Doppler Ultrasound Study

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Abstract: Background: In this study, we aimed to evaluate the effect of s-FLT-1 and PlGF concentrations in women in the first trimester of pregnancy on the pulsatility index of the ductus venosus and the right and left uterine arteries. Methods: A total of 108 pregnant women in their first trimester were included in the study, with 87 assigned to the experimental group and 21 to the control group. Ultrasound examination were performed to measure the uterine artery pulsatility index, resistance index, ductus venosus pulsatility index, and blood sampling levels of biomarkers such as b-HCG; PAPP-A; s-FLT-1 and PlGF. Results: There was a significant negative relationship between PAPP-A concentration and the risk of fetal growth restriction, and a significant negative relationship between PlGF concentration and the risk of preeclampsia and fetal growth restriction in women in their first trimester of pregnancy. There was also a significant positive relationship between sFLT-1/PLGF ratio and the risk of developing preeclampsia. No significant relationships were found between the concentrations of biomarkers beta-HCG, PAPP-A, sFLT-1, PlGF, and any of the parameters PIUARt, PIUALt, Mean uterine arteries, and DVPI. Summary: In conclusion, this study did not find conclusive evidence to support the hypothesis that beta-HCG, PAPP-A, s-FLT-1, and PlGF concentrations in women in the first trimester of pregnancy have a significant effect on the Pulsatility Index of the ductus venosus and the Right and left Uterine Arteries. Further research is needed to investigate these potential associations.

Keywords: b-HCG, PAPP-A, s-FLT-1, PLGF, pulsatility index of the ductus venosus, pulstatility index of the right and left uterine arteries

1. Introduction

The dysfunction of the placenta has important consequences for both the mother and the fetus. Chronic placental dysfunction can lead to hypertensive disorders (including preeclampsia (PE), fetal growth restriction (FGR), and pregnancy complications such as placental abruption, preterm labour and delivery [1]. The definition of Preeclampsia is continually evolving. According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) 2018 definition, PE is a multisystem disorder that occurs after the 20th week of pregnancy and occurs in about 5% to 10% of all pregnancies, characterized by high blood pressure (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, measured twice with a 4-hour interval, in women who previously did not have hypertension) and at least one of the following conditions: 1) Proteinuria (protein-to-creatinine ratio ≥30 mg/mol, protein loss ≥300 mg/day) 2) Dysfunction of at least one maternal organ (acute kidney failure, liver damage, right upper abdominal pain, hematologic complications - thrombocytopenia: platelet count <150,000/µl, disseminated intravascular coagulation, hemolysis; neurological complications, e.g., eclampsia, psychotic symptoms, visual disturbances, severe headache, stroke) 3). Abnormalities in the uteroplacental unit function - FGR,

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abnormal Doppler blood flow patterns in fetal vessels, or intrauterine fetal death [2]–[4]. This condition can negatively affect nearly every organ system, leading to a range of preeclampsia-related complications such as HELLP syndrome (characterized by hemolysis, elevated liver enzymes, and low platelets), seizures (eclampsia), fetal growth restriction, and placental abruption. Unfortunately, currently, termination of pregnancy is the only effective treatment for preeclampsia, which may further endanger the neonate if premature delivery is necessary [5]. The first sign of a growth-restricted fetus is being small-for-gestational-age (SGA), which can increase the risk of morbidity and mortality in both the short and long term. A considerable number of fetuses diagnosed with growth restriction are born prematurely, making them vulnerable to prematurity-related risks. Moreover, they have a higher susceptibility to various perinatal conditions such as cesarean delivery, oligohydramnios, low Apgar scores, acidosis, hypoglycemia, hypothermia, apnea, seizures, polycythemia, sepsis, stillbirth and neonatal death [6], [7]. Placental factors have been implicated in causing generalized maternal endothelial dysfunction, which is thought to be a significant contributor to the development of preeclampsia. Various serum markers indicate endothelial activation increase in these patients, and flow-mediated dilation (FMD), which is the gold standard for assessing endothelial function, is impaired in women with preeclampsia. Recent research has shown that excessive levels of a placental antiangiogenic factor, known as soluble fms-like tyrosine kinase 1 (sFLT-1), can inhibit vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), resulting in generalized endothelial dysfunction in affected individuals. This discovery has led to the development of innovative clinical strategies for managing preeclampsia, with s-FLT-1 being regarded as the most promising placental factor [8], [9]. The study aims to evaluate the effect of beta-HCG, PAPP-A, s-FLT-1 and PlGF concentrations in women in the first trimester of pregnancy on the Pulsatility Index of the ductus venosus and the Right and left Uterine Arteries. Additionally, attempts were made to find a relationship between these biomarkers and the risk of developing preeclampsia and FGR in women in the first trimester of pregnancy.

2. Materials and Methods

The study was approved by the Bioethics Committee under the approval number KB416/2020. All procedures were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki.

In this study, a total of 108 pregnant women in their first trimester were included, out of which 87 were assigned to the experimental group and 21 to the control group. All participants provided informed consent before participating in the study and underwent ultrasound examination between the 11th and 14th week of pregnancy. Women assigned to the control group had no prior history of antiphospholipid syndrome, chronic hypertension, gestational diabetes, or systemic lupus erythematosus. Additionally, it should be noted that women in the control group did not receive any medication for hypertension and there was no family history of hypertensive disorders reported during the interview with the participants.

Ultrasound Examination

All participants underwent ultrasound examination using a Voluson S10 ultrasound machine (GE Healthcare, Chicago, IL, USA) equipped with a 3.5-5 MHz curved array transducer. The ultrasound examination was performed by a sonographer with accreditation from the Fetal Medicine Foundation (www.fetalmedicine.com), who undergoes regular quality control checks of their measurements. The uterine artery pulsatility index (PI) and resistance index (RI) and Ductus venosus pulsatility index (DVPI) were also measured using pulsed-wave Doppler ultrasound. The uterine artery PI and RI were measured on both sides of the uterus and the highest value was recorded.

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Biomarker Analysis

Blood samples were collected from each participant to measure levels of beta-human chorionic gonadotropin (beta-HCG), pregnancy associated plasma protein-A (PAPP-A), placental growth factor (PLGF), and soluble fms-like tyrosine kinase-1 (s-FLT-1). The levels of these biomarkers were measured using according to the manufacturer's instructions. The levels of these biomarkers were measured using the Elecsys beta-HCG, PAPP-A, sFlt-1 and Elecsys PLGF assays (Roche Diagnostics, Basel, Switzerland), respectively. These assays are based on electrochemiluminescence immunoassay technology and are used to quantify the levels of biomarkers in human serum samples. The assays were performed on a Roche cobas e 411 immunoassay analyzer.

Calculation of Risk for Preeclampsia and Fetal Growth Restriction

The risk for preeclampsia and fetal growth restriction was calculated using the FMF-01-07-2018 software developed by the Fetal Medicine Foundation, which takes into account maternal factors such as age, body mass index (BMI), medical history, and smoking status, as well as ultrasound measurements of fetal biometry and uterine artery pulsatility and resistance indices. The software was used to calculate corrected risks based on the gestational age at the time of ultrasound examination.

Statistical analysis

The calculations were performed using Statistica 13 software by TIBCO and PQStat by PQStat Software. A significance level of α=0.05 was adopted. The result was considered statistically significant when $p<\alpha$. The normality of the distribution of variables was examined using the Shapiro-Wilk test. To compare variables between 2 groups, the t-Student test for independent samples was calculated (in the case of conformity with normal distribution and equal variances), the Cochran-Cox test (when variances were unequal), or the Mann-Whitney test. To investigate the dependence between continuous variables, the Pearson linear correlation coefficient r or Spearman's rank correlation coefficient Rs was calculated. To examine the dependence between categorical variables, Fisher's exact test was calculated.

3. Results

Tables 1 and 2 present the descriptive statistics of the control group (Table 1) and the studied group (Table 2). Table 3 presents statistically significant differences between the studied group and the control group. Statistically significant differences were found between beta-HCG, s-Flt-1, the risk of preeclampsia, and FGR in women in the 1st trimester of pregnancy. The levels of beta-HCG and s-Flt-1 were higher in patients in the control group, while the risk of PE and FGR was higher in the studied group.

In the studied group, there was no relationship between b-HCG concentration and any of the analyzed parameters.

In table 4. There is a relationship with the risk of FGR. Since Rs<0, the higher the PAPPA, the lower the risk.

In Table 5, a relationship between PlGF concentration was demonstrated. Since Rs<0, the higher the PlGF concentration, the lower the risk of PE and FGR in women in the 1st trimester of pregnancy in the studied group.

No statistically significant relationship was found between sFLT-1 concentration and any of the analyzed parameters.

In Table 6, a statistically significant relationship between sFLT-1/PLGF and the risk of developing preeclampsia in women in the 1st trimester of pregnancy is presented. Since Rs>0, the higher the sFlt1/PLGF, the higher the risk of preeclampsia.

No statistically significant relationship was found between the concentrations of biomarkers b-HCG, PAPP-A, sFLT-1, PlGF, and any of the parameters PI UA Rt, PI UA Lt, Mean uterine arteries, DV PI.

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Table 1. Table containing descriptive statistics for the control group.

Descriptive statistics - control group						
Variable	**Valid N**	**Mean**	**Median**	**Minimum**	**Maximum**	**Lower Quartile**
Age	21	31,714	31,000	25,0000	41,000	29,000
BMI	21	24,875	23,600	17,7000	36,680	21,720
B-hCG	21	58,514	40,400	11,6000	164,800	22,400
PAPP-A	21	4,233	3,130	1,2500	14,270	2,560
PLGF	21	54,124	42,130	20,6500	151,000	33,130
sFlt-1	21	1736,800	1543,000	977,8000	4519,000	1272,000
sFlt1/PLGF	21	39,051	35,900	7,2300	76,120	28,685
PI UA Rt	20	1,522	1,515	0,7900	2,780	1,280
PI UA Lt	20	1,546	1,555	0,6100	3,190	1,035
Mean uterine arteries	20	1,536	1,540	0,8600	2,670	1,200
DV PI	20	1,044	1,040	0,6600	1,420	0,975
Risk of PE in 1st trimester	21	0,003	0,001	0,0000	0,019	0,000
Risk of FGR in 1st trimester	20	0,006	0,001	0,0003	0,067	0,001

Table 2. Table containing descriptive statistics for the studied group.

Descriptive statistics - study group						
Variable	**Valid N**	**Mean**	**Median**	**Minimum**	**Maximum**	**Lower Quartile**
Age	87	32,460	33,000	21,0000	42,000	28,0000
BMI	87	29,436	28,910	17,2100	47,250	24,8400
beta-HCG	87	37,659	26,100	6,8000	177,100	17,6000
PAPP-A	87	3,140	2,770	0,3700	11,940	1,6900
PLGF	87	48,473	44,220	16,1900	200,800	34,2600
sFlt-1	87	1396,022	1218,000	531,9000	3878,000	960,2000
sFlt1/PLGF	87	32,931	28,630	8,8810	107,010	21,3630
PI UA Rt	87	1,625	1,600	0,6900	3,350	1,0200
PI UA Lt	87	1,631	1,560	0,2300	4,110	1,1100
Mean uterine arteries	87	1,630	1,550	0,5300	3,350	1,2000
DV PI	84	1,006	0,980	0,5400	2,270	0,8800
Risk of PE in 1st trimester	83	0,036	0,014	0,0003	0,250	0,0036
Risk of FGR in 1st trimester	77	0,037	0,008	0,0005	1,000	0,0032

Table 3. The table presents statistically significant parameters that differentiate the control group from the studied group.

Variable	Mann-Whitney U test (with continuity correction) With respect to the variable: group Marked results are significant with p <0.05000			
	P	**N valid study**	**N valid control**	**2*1-sided exact p**
B-hCG	**0,031227**	87	21	0,030208
sFlt-1	**0,012572**	87	21	0,011697
Risk of PE in 1st trimester	**0,000001**	83	21	0,000000
Risk of FGR in 1st trimester	**0,000001**	77	20	0,000000

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Table 4. The table presents a statistically significant correlation between PAPP-A concentration and the risk of FGR in women in the 1st trimester of pregnancy.

Pair of variables	Spearman's rank-order correlation Marked correlation coefficients are significant with p <0.05000			
	N Valid	R Spearman (Rs)	t(N-2)	p
PAPP-A & risk of FGR	77	-0,308547	-2,80916	**0,006330**

Table 5. The table presents a statistically significant correlation between PlGF concentration and the risk of PE and FGR in women in the first trimester of pregnancy.

Pair of variables	Spearman's rank-order correlation Marked correlation coefficients are significant with p <0.05000			
	N Valid	R Spearman (Rs)	t(N-2)	p
PLGF & Risk of PE in 1st trimester	83	-0,455628	-4,60660	**0,000015**
PLGF & Risk of FGR in 1st trimester	77	-0,310428	-2,82810	**0,006002**

Table 6. The table presents a statistically significant relationship between sFLT-1/PLGF and the risk of developing preeclampsia in women in the first trimester of pregnancy.

Pair of variables	Spearman's rank-order correlation Marked correlation coefficients are significant with p <0.05000			
	N valid	R Spearman (Rs)	t(N-2)	p
sFlt1/PLGF & Risk of PE in 1st trimester	83	0,358709	3,458549	**0,000868**

4. Discussion

s-FLT-1 and PlGF are two biomarkers that have been implicated in the pathophysiology of fetal growth restriction (FGR) and preeclampsia (PE). s-FLT-1 is an anti-angiogenic protein that is produced by the placenta and inhibits the action of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), both of which are critical for normal placental development and vascular function [9]. PlGF, on the other hand, is a pro-angiogenic factor that promotes vascular growth and remodeling [10]. It has been hypothesized that an imbalance between these two factors may contribute to the development of FGR and PE [11]. Multiple studies have demonstrated a positive association between sFlt-1 levels and the risk of developing PE. In a study by Levine et al. (2004), it was found that women who developed PE had significantly higher levels of sFlt-1 and lower levels of PLGF compared to women with uncomplicated pregnancies [9]. Similarly, a meta-analysis by Zhou et al. (2008) found that sFlt-1 levels were significantly higher in women with PE compared to controls. These findings suggest that an increase in sFlt-1 and a decrease in PLGF may play a role in the pathogenesis of PE [12]. In our study, as in the study by Zhou et al. (2008) it turned out that the concentration of sFLT-1 was higher in the control group than in the tested group. Additionally, the control group had a higher concentration of beta-HCG than the tested group. However, we did not demonstrate that the concentration of PlGF differed significantly between the two groups. In the studies carried out by Tarasevičienė [13] and colleagues in 2016, and Bahlmann and Al Naimi [14] the concentration of sFlt-1 was also higher in the control group, which is consistent with our results. However, our results are not consistent with respect to the sFlt-1/PlGF ratio and the parameters of the examined vessels. The likely reason for this discrepancy may be the low number of individuals in the control group.

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In contrast to the study by Aberdeen and colleagues [15], which indicated that elevated levels of sFlt-1 play a crucial role in both suppressing SAR in early primate pregnancy and inducing maternal vascular endothelial dysfunction in late gestation, our study did not achieve a statistically significant impact of sFlt-1 levels or other biomarkers on the state of the tested vessels.

The sample size of the study may limit its statistical power and increase the risk of type II errors. With only 108 participants, the study's sample size is relatively small. This could result in a reduced ability to detect true differences between groups and may increase the risk of false negative results. Additionally, smaller sample sizes may also increase the likelihood of chance findings or spurious correlations. Larger studies with more participants may be needed to confirm these associations and further elucidate the underlying mechanisms. The study's biomarker assays may have limitations that could affect the accuracy of the results. For instance, these assays may be subject to variability in performance or sensitivity to interfering substances. Although the manufacturer's instructions were followed, and a reliable analyzer was used, these limitations could still affect the accuracy of the results.The software used to calculate the risk for preeclampsia and fetal growth restriction may have limitations that should be considered. The software requires accurate ultrasound measurements and may overestimate or underestimate risk. Although reliable ultrasound equipment and standard protocols were used, these limitations could still affect the accuracy of the risk estimates.

5. Conclusions

The study did not provide conclusive evidence to support the hypothesis that beta-HCG, PAPP-A, s-FLT-1 and PlGF concentrations in women in the first trimester of pregnancy have a significant effect on the Pulsatility Index of the ductus venosus and the Right and left Uterine Arteries, or that there is a clear relationship between these biomarkers and the risk of developing preeclampsia and FGR in women in the first trimester of pregnancy. Further research is needed to investigate these potential associations.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available per request from the corresponding author [O.K.]

Conflicts of Interest: The authors declare no conflict of interest.

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