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ABSTRACT

**POTENTIAL ANGIOGENIC BIOMARKERS FOR THE
PREDICTION OF PRE-ECLAMPSIA IN HIGH-RISK
PREGNANCIES**

PhD COORDINATOR

Professor LILIANA NOVAC

PhD STUDENT

SAID K AMIRA LIVIA (MOLDOVEANU)

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INTRODUCTION

Early detection of pre-eclampsia is very important for obstetricians, as this obstetric pathology is associated with significant perinatal morbidity and mortality.

Evidence to date suggests an imbalance between proangiogenic factors [Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF)] and anti-angiogenic factors [soluble VEGF receptor 1 (sVEGFR-1, also called sFlt1) involved in the pathophysiology of PE. Indeed, PE patients have higher plasma concentrations of sFlt1 and lower plasma concentrations of PlGF than patients with normal pregnancies. These differences were observed before the clinical manifestation of PE²⁵.

The discovery and investigation of these angiogenic factors could characterize the important pathogenic mediators of pre-eclampsia or even the cause of placental dysfunction.

STATE OF KNOWLEDGE

CHAPTER I

EPIDEMIOLOGICAL DATA OF PRE-ECLAMPSY

Pre-eclampsia is a complication that occurs during pregnancy and is also a cause for about 10% -15% of cases of maternal morbidity and mortality, such as those involving cardiovascular and cerebrovascular diseases, liver and kidney failure, abruptio placentae, disseminated intravascular coagulation and hemolysis, high levels of liver enzymes, and low platelets in HELLP syndrome²⁶.

Pre-eclampsia is a global health problem with increasing importance in maternal and fetal health^{5,25}. Pre-eclampsia complicates 2%–8% of all pregnancies, contributes to 15% of preterm births and contributes to 9% and 26% of maternal deaths worldwide²⁸. In the most recent UK maternal mortality survey, 22 out of 107 direct maternal deaths from 2006 to 2008 were linked to pre-eclampsia and eclampsia³. Hypertensive disorders in pregnancy cause almost 18% of all maternal deaths worldwide, with approximately 62,000 to 77,000 deaths per year¹⁴.

It is estimated that approximately 50,000 women die worldwide due to pre-eclampsia. Most deaths in developing countries occur due to eclampsia, while in developed countries, the most common causes are complications of pre-eclampsia⁵.

In addition, neonatal morbidity and mortality are increased in this condition, as they may cause fetal growth restriction with oligohydramnios, preterm birth, low birth weight, severe birth asphyxia, stillbirth and intrapartum fetal death. The pathophysiology is not yet known¹⁵.

About 12 to 25% of the fetal growth restriction, as well as 15 to 20% of all preterm births are attributed to pre-eclampsia, the complications associated with prematurity being substantial, including neonatal deaths and severe long-term neonatal morbidity⁵. Despite major medical advances, the only known remedy for pre-eclampsia remains the birth of the fetus and the evacuation of the placenta.

CHAPTER II

ROLE OF PLACENTA IN THE PATHOPHYSIOLOGY OF PREECLAMPSIA

In the last decade, the definition of pre-eclampsia has been revised as the mechanisms underlying this disease have evolved dramatically. As regards the definition, several groups have challenged the classic definition of pre-eclampsia from half a century ago, namely: De novo hypertension, the onset of proteinuria and liver dysfunction after half of pregnancy, motivated by the discovery of additional biomarkers of pre-eclampsia. Following the studies^{2,23} carried out in the last period, it was suggested to incorporate in the diagnosis of pre-eclampsia, risk of disease and even in predicting complications some biomarkers of placental or vascular origin, including Placental Growth Factor (PlGF) and antiangiogenic factors, such as soluble FMS-like tyrosine kinase-1 (sFLT-1) or soluble endoglin (sENG).

Risk factors for pre-eclampsia. Risk factors for pre-eclampsia include preeclampsia to another previous pregnancy, first pregnancy, obesity, extreme age and diabetes. Also, the incidence of pre-eclampsia is increased in twin pregnancies, with an increase from 6 percent in single-child pregnancy to 31 percent in twin pregnancy¹. But the mechanisms by which these factors act to increase the risk are in most cases still unknown¹².

Role of placenta. Placental ischemia in PE. It is now known that early onset and late onset PE have different pathophysiology. At the early onset, also called placental pre-eclampsia (<34 weeks), reduced conversion of the spiral artery is involved, which is associated with placental malperfusion. In late onset pre-eclampsia (> 34 weeks), also called maternal pre-eclampsia, it seems that arterial conversion is reduced but placental perfusion is maintained²⁷.

Thus, there is a minimum of placental stress, so that the secretion of sFLT and Placental Growth Factor (PlGF) by the placenta is close to the normal range.

It should be kept in mind that failure of physiological transformation of spiral arteries is not specific to PE and not sufficient to induce it, as such failure has been observed in other obstetric syndromes, including spontaneous abortion, Intrauterine Growth Restriction (IUGR), fetal death, preterm labor and premature rupture of membranes. The mechanisms responsible for the failure of the physiological transformation of the spiral arteries have not been fully elucidated⁴.

Oxidative stress. It has long been known that pregnancy is a state of oxidative stress and that it is still increased in the first, second and third trimesters of pregnancy, resulting in complications, such as PE and preterm birth, diabetes, stillbirth, especially in obese pregnant women²⁰. However, a cause-effect relationship between increased oxidative stress and adverse pregnancy outcomes remains to be definitively proven.

Immunological and inflammatory factors in PE. One of the oldest and most persistent theories about the origin of pre-eclampsia is that pre-eclampsia is a disorder of immunity and inflammation. Fetal trophoblast is considered an alloantigen and the mother reacts to it and implements a low-grade systemic inflammatory response²⁸.

The initial inflammatory response during the first trimester could be due to an interaction between the decidual immune cells and the trophoblastic cells, and a secondary inflammatory response during the second and third trimester might be due to the syncytiotrophoblast microparticles released into the mother's vascular system³⁰.

Regulatory immune factors counterbalance a hyperactive immune response to prevent chronic inflammation and antibody-mediated responses that result in injury and damage. In PE, this balance is compromised, leading to chronic activation of immunity, inflammation and antibodies production. Pre-eclampsia is associated with activation of chronic immunity, characterized by higher persistent levels of pro-inflammatory cytokines and diminished immunoregulatory factors, which leads to the persistence of an inflammatory state during PE.

CHAPTER III

PRO-ANGIOGENIC AND ANTI-ANGIOGENIC FACTORS IN PREECLAMPSIA

Defective angiogenesis has long been considered a pathway to preeclampsia, and rigorous research has shown that an anti-angiogenic state is involved in the pathogenesis of preeclampsia. To perform spiral remodeling during normal pregnancy, many molecules including vasomotor substances, growth factors, adhesion molecules and proteases are secreted by the placenta and vessels^{17,24}. Among the most well-known representative substances in this context is the Vascular Endothelial Growth Factor (VEGF), sFlt1, PlGF and endoglin. sFlt-1 acts as a trap receptor for VEGF and PlGF, causing a decrease in bioavailable VEGF protein. It results in vascular endothelial dysfunction, which causes abnormal vasoconstriction, impaired renal function and then the onset of high blood pressure⁶.

Angiogenic factors. sFlt-1. A large variety of molecules are released, but anti-angiogenic and autoimmune / inflammatory factors are of major importance.

Studies in cell cultures and placental tissue in vitro have shown that sFlt-1 is released in placental villi due to reduced oxygen pressures, similar to what appears in an ischemic placenta^{9,21}. sFlt-1 is a soluble receptor in circulation for both VEGF and PlGF, which, when raised in maternal plasma, leads to less circulating VEGF and free PlGF, thus impeding their availability to stimulate angiogenesis and maintain endothelial integrity^{21,32}.

Angiogenic factors. Endothelin. An important role in the pathophysiology of PE is attributed to endothelin-1 (ET-1). Characterized twenty years ago, ET-1 was identified twenty years ago as the most potent vasoconstrictor known to be derived from the endothelium⁸.

Angiogenic factors. Nitric oxide. The decrease in NO production during PE is controversial, suggesting that NO deficiency would not be involved in PE. This is largely due to the fact that the activity of the NO system could not be clinically determined in a human pregnancy.

Pro-angiogenic factors. VEGF family. Vascular endothelial growth factor (VEGF) and platelet growth factor (PlGF) play an extremely important role in placental angiogenesis and appear to be produced by trophoblastic cells⁷.

VEGF is a family of structurally related dimeric proteins, of which PlGF is a member. Although total VEGF has been shown to increase modestly in PE, VEGF is linked to sFlt-1 in preeclampsia³¹.

Pro-angiogenic factors. PlGF. PlGF is known to be low in PE. This seems to be due rather to its binding in circulation by sFlt-1 and less to the decrease in PlGF production by the preeclamptic placenta¹⁹. In a large, randomized, controlled study¹⁶, plasma levels of PlGF at 21-32 weeks of gestation were lower at early onset (<37 weeks) than at late-onset PE, in severe PE and associated preeclampsia with an SGA fetus (small for gestational age).

Anti-angiogenic factors. sFlt-1. sFlt-1, acts as an antagonist of VEGF and PlGF, through mechanisms to bind and block these factors¹⁹. sFlt-1 appears to be elevated a few weeks before the clinical manifestation of PE, being a predictor of preeclampsia. But, the clear question, however, is the mechanism of the excessive production of sFlt-1 and the relationship between sFlt-1, PlGF and VEGF¹⁶.

The imbalance between pro-angiogenic and anti-angiogenic condition in PE. Existing evidence hypothesizes the imbalance between pro-angiogenic and anti-angiogenic factors, this imbalance is responsible for the pathophysiological mechanisms in PE, the effect occurring before a clinical manifestation¹⁸. sFlt-1 antagonizes VEGF, perhaps also PlGF, by inhibiting endogenous receptors¹³.

CHAPTER IV

USE OF ANGIOGENIC BIOMARKERS IN THE PREDICTION AND MANAGEMENT OF PREECLAMPSIA

In recent years, it has been increasingly shown that placental insufficiency triggers an imbalance of maternal angiogenesis, through increased release of anti-angiogenic factors (sFlt-1) and low concentrations of pro-angiogenic factors (PlGF), leading to clinical manifestations associated with placental dysfunction¹⁰. Normally, sFlt-1 begins to increase after 30 to 32 weeks of pregnancy and PlGF begins to decrease after 30 weeks of pregnancy¹¹. Modifications of both markers, sFlt-1 and PlGF, are observed starting with the second trimester of pregnancy, and the increase in the sFlt-1/PlGF ratio was detected in the second half of pregnancy in women diagnosed with PE.

In fact, the increase in the sFlt-1/PlGF ratio was detected in the second half of pregnancy in women diagnosed with PE, and limited evidence shows that it is high in women who develop other placenta-related conditions, such as IUGR and stillbirth. These changes are more pronounced in early-onset PE and in severe forms of PE²⁹.

Increased sFlt-1 / PlGF ratio appears to reflect placental ischemia and is considered to be an effective biomarker for disease prediction and prognosis. Analysis of the sFlt-1/PlGF ratio is available for clinical use and was also recently recommended (in 2016) by the National Institute for Clinical Excellence (NICE) to exclude PE in patients with suspected disease²².

PERSONAL CONTRIBUTIONS

BIOSTATISTIC ANALYSIS OF STUDY GROUPS

The prospective study included a batch of 106 pregnant women studied between October 2016 to March 2019. The study was conducted within the Polizu Clinical Hospital Obstetrics-Gynecology - INSMC, Bucharest.

The 106 pregnant women selected from the total number of pregnant women, represented 82 pregnant women with preeclampsia admitted in the mentioned clinics and a batch of 24 pregnant women with a normal evolution of pregnancy, considered as a control group, who met the same inclusion criteria as the pregnant women with preeclampsia. Normal pregnancies had no medical or obstetric complications and gave birth to babies over 2,500 g at term.

Preeclampsia was defined according to the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) as: Systolic and diastolic blood pressure greater than 140 and 90 mmHg, respectively, at a minimum two consecutive measurements, with a gap of at least 6 hours, values that appear after the 20th week of gestation.

We used the notion of Pregnancy-Induced Hypertension (PIH) to describe cases presenting with a BPs > 140 mmHg, a BPd > 90 mmHg, without proteinuria, which appeared after the 20th week of gestation, in a previously normotensive woman.

BP measurements were performed according to a standard protocol and the Mean Arterial Pressure (MAP) (in mmHg) was calculated using the equation:

$$\text{MAP} = (\text{BPs} + 2 \times \text{BPd})/3$$

Proteinuria has been defined as excretion of 300 mg of protein in a 24-hour urine collection or two results of dipstick 2+ tests (100 mg per deciliter), values recorded at least 4 hours apart, without evidence of urinary tract infection.

1. Social and Demographic Characteristics

Maternal age. The age of the women included in the study was non-Gaussian ($p = 0.002$). Both pregnant women in the preeclampsia group and those in the control group had an average age of 31 years, with a minimum of 18 years and a maximum of 41 years. The age of the women who participated in the study did not differ significantly between the two groups (Mann Whitney test $U = 960.50$; $p = 0.859$).

Social and Economic level. The low maternal social and economic level is a strong risk factor for preeclampsia. 19.51% of women with PE had a low social and economic status, 9.76% average and 70.73% high. Of the women without PE, 83.33% had an average social and economic level and 16.67% had a high social and economic level. The risk of PE in women with average social and economic level was significantly lower than women with high and low social and economic level (OR = 0.02; $p < 0.001$). The frequency of PE occurrence was statistically significantly higher ($p = 0.020$) in women with low social and economic status.

The distribution of study participants according to the social and economic status and the presence of PIH was significantly different ($p = 0.010$). The frequency of occurrence of pregnancy-induced hypertension did not differ significantly ($p = 0.386$) in women with low social and economic status compared to women with high and average social and economic level.

Educational status. The distribution of study participants according to the educational status and the presence of the PE was statistically significantly different ($p = 0.008$). The risk of PE in women with a high educational level was 0.35 times lower than the other participants in the study (OR = 0.35; $p = 0.039$). The risk of PE in women with an average educational level was not significantly lower than the other participants in the study (OR = 0.67; $p = 0.468$). The frequency of PE occurrence is statistically significantly higher ($p = 0.001$) in women with low educational attainment. We conclude that a relatively low level of education is associated with a higher risk of PE.

The distribution of study participants according to educational status and the presence of PIH did not differ statistically ($p = 0.605$).

Risk factors

Number of previous abortions. Regardless of the type of abortion present in the medical history, the distribution of the study participants was not statistically significantly different ($p = 0.358$) depending on the number of abortions in the medical history in the two studied groups (with/without preeclampsia).

Number of previous births. The frequency of births among study participants was statistically significantly different ($p = 0.048$) depending on the presence of preeclampsia.

PE in previous pregnancies. In our study, the frequency of PE in previous pregnancies was not significantly different between the two groups studied ($p = 0.112$), which is consistent

with studies in the literature that show different percentages of recurrence. Of the 43 pregnant women with a previous pregnancy, 8 had PE at previous pregnancies, all belonging to the current PE group. The frequency of PE on previous pregnancies was significantly different between the two groups studied (chi-square = 5.265; $p = 0.021$).

Chronic hypertension in the medical history. The distribution of study participants according to the presence of chronic hypertension was not significantly different ($p = 0.020$) in the PE group compared to the control group. The overlapping preeclampsia rate of 19.51% observed in our study is consistent with that reported in other studies, ranging from 17% to 34.9%.

2. Maternal Comorbidities

Diabetes mellitus. The distribution of study participants according to the presence of diabetes mellitus was significantly different ($p = 0.001$) in the PE group compared to the control group, which is consistent with the studies in the literature.

Pre-pregnancy weight status. In our study, BMI before pregnancy had a non-Gaussian distribution ($p < 0.001$). Pre-pregnancy BMI in women with preeclampsia was statistically significant (Mann Whitney U test = 167.50; $p < 0.001$), higher than in the control group. The median pre-pregnancy BMI for the preeclampsia group was 26.5, the minimum 22.30, the maximum 50.00, the 25th percentile is 23.4, and the 75th percentile at 32.62 kg/sqm. The frequency of pregnancy-induced hypertension in overweight and obese women was statistically significantly higher ($p < 0.001$) compared to women with normal weight status. Pregnancy BMI in women with preeclampsia was statistically significant (Mann Whitney U test = 308.00; $p < 0.001$), higher than in the control group.

Access to medical services (Prenatal examinations). 65.85% of the pregnant women with preeclampsia had at least one prenatal examination, an insignificant weight ($p = 0.762$) higher than the pregnant women in the control group (62.5%). For this reason, there is a statistically insignificant significance, which shows that in our study, the number of prenatal examinations, or the pregnancy monitoring, did not influence the presence of PE.

Examination in the third trimester of pregnancy

BP upon enrollment. MAP in the study participants who were diagnosed with PE (102.14 ± 7.40 mmHg) was statistically significantly higher ($p < 0.001$) than the control group (86.99 ± 7.76 mmHg). 8.54% of pregnant women with PE had MAP over 110 mmHg upon enrollment.

The distribution of study participants according to the MAP did not show statistically significant differences ($p = 0.156$) between the PE group and the control group. MAP in the third trimester is statistically significantly correlated with the severity of pregnancy-induced hypertension / PE in the third trimester ($p < 0.001$) and with the severity of pregnancy-induced hypertension / late PE before parturition ($p < 0.001$).

3. Ultrasound Examination in the Second Trimester of Pregnancy

Fetal biometrics. Fetal biometrics at enrollment revealed statistically significant differences regarding the main parameters between the group of pregnant women with PE and that of the pregnant women without PE. The mean BPD (biparietal diameter), CC (cranial circumference), AC (abdominal circumference) and LF (femur length) were statistically significant ($p < 0.001$) in the PE group. Our results suggest that the diagnosis of preeclampsia is significantly associated with IUGR. Interestingly, the development of IUGR in women with preeclampsia may be disparate.

Uterine Artery Doppler Markers. In the study conducted, the resistance index is the one that had a statistical significance, compared to the specialized studies that involve more average PI. The distribution of study participants according to Notch + and the presence of pregnancy-induced hypertension / PE in the third trimester is statistically significantly different ($p < 0.001$). Any abnormal Doppler when examining UtA Doppler in Q2 was statistically significantly correlated with the occurrence of pregnancy-induced hypertension / PE in Q3 ($p = 0.002$).

Umbilical Artery Doppler Markers. The pulsatility index and the S/D ratio were statistically significant ($p < 0.001$) higher in the group with subsequent PE (Table 21). Moreover, the mean PI > 95th percentile was statistically significantly higher in the group with subsequent PE ($p < 0.001$). None of the study participants had an PI above the 95th percentile at the second trimester pregnancy assessment. Furthermore, none of the pregnant women included in the study had abnormal Doppler upon enrollment.

Examination in the third trimester of pregnancy

Hypertension. MAP in the study participants who were diagnosed with PE (116.48 ± 13.09 mmHg) was in the third trimester significantly statistically higher ($p < 0.001$) compared to the control group (96.22 ± 3.16 mmHg). With an average value of 116.48 ± 13.09 mmHg the evaluation made in a heterogeneous population composed of nulliparous and multiparous, the prediction rate of MAP in the cases of early and late preeclampsia is 77.35%.

4. *Ultrasound Examination in the Third Trimester of Pregnancy*

Fetal biometrics. The distribution of pregnant women included in the study according to the estimated fetal weight percentiles is significantly different depending on the presence of PE ($p < 0.001$).

Uterine Artery Doppler Markers. In our study, the RI, the average PI (Pulsatility Index) and the S/D for the Uterine artery (UtA) Doppler from the third trimester differed significantly depending on the presence of PE and the pregnancy-induced hypertension in the third trimester of pregnancy ($p < 0.001$). The distribution of study participants according to Notch + and the presence of pregnancy-induced hypertension / PE in the third trimester was statistically significantly different ($p < 0.001$). In our study, the mean PI at UtA Doppler in the third trimester correlated significantly statistically with the pregnancy-induced hypertension before parturition ($p = 0.025$) and with PE before parturition ($p = 0.003$), i.e. a high average PI increases the probability of pregnancy-induced hypertension occurrence and PE.

Umbilical Artery Doppler Markers. RI (Resistance Index) for umbilical artery in the third trimester of pregnancy, PI, S/D and PI > 95th percentile differed statistically ($p < 0.001$) depending on the presence of the pregnancy-induced hypertension / late PE and the control group before parturition. An abnormal Doppler examination for umbilical artery in the third trimester correlated significantly statistically with pregnancy-induced hypertension / PE severity in the third trimester ($p = 0.008$) and late, before parturition ($p < 0.001$). In the study conducted, the inverted diastolic flow correlated significantly statistically (Table 34) with the severity of pregnancy-induced hypertension / PE before parturition ($p = 0.003$). Pulsatility index > 95th percentile was statistically significantly correlated with pregnancy-induced hypertension / PE severity in the third trimester ($p = 0.004$) and late before parturition ($p = 0.003$), as well as with the unfavorable evolution of the newborn ($p = 0.002$) and hospitalization in neonatal ICU (Intensive Care Unit) ($p < 0.001$).

Middle Cerebral Arterial Doppler Markers. The mean values of RI and S/D at MCA Doppler in the third trimester did not differ significantly depending on the presence of PE and pregnancy-induced hypertension in the third trimester ($p = 0.029$, $p = 0.003$ respectively). The average MCA-IP did not differ statistically ($p = 0.970$) depending on the presence of PE and pregnancy-induced hypertension in the third trimester. The absence of final diastolic flow at the Doppler examination of the middle cerebral artery in the third trimester of pregnancy, was

statistically significantly correlated with the severity of pregnancy-induced hypertension / PE in the third trimester ($p = 0.004$) and the severity of the pregnancy-induced hypertension / late PE before parturition ($p = 0.022$). Moreover, the reversal of diastolic flow at the Doppler examination of the middle cerebral artery in the third trimester of pregnancy correlated significantly statistically with the severity of the pregnancy-induced hypertension / PE in the third trimester ($p = 0.010$) and the severity of the pregnancy-induced hypertension / late PE before parturition ($p = 0.024$). Abnormal Doppler examination of the middle cerebral artery in the third trimester pregnancy correlated statistically significantly with the severity of pregnancy-induced hypertension / PE in the third trimester ($p < 0.001$), the severity of the pregnancy-induced hypertension / late PE before parturition ($p = 0.001$) and with the newborn admitted in the neonatal ICU ($p = 0.008$).

Doppler markers - Cerebroplacental ratio (CPR). CPR was positively correlated statistically ($p = 0.011$) with the gestational age at birth, that is, the higher the CPR, the more the gestational age increases, and vice versa. This is because a CPR >1 shows fetal well-being that allows the pregnancy to be prolonged as long as possible.

5. *Parturition*

Type of parturition. In our study, spontaneous parturition was statistically significantly ($p = 0.001$) more frequent in the control group (75%) compared to the PE group (36.59%). Thus, 63.41% of women with PE delivered through a C-section, compared to 25% of the control group.

Gestational age at birth. The gestational age (GA) at birth was statistically significantly lower ($p < 0.001$), preterm birth, in the PE group (34.70 ± 2.47 weeks) compared to the control group (39.02 ± 1.03 weeks).

Weight of the newborn at birth. We found that the birth weight of newborns from mothers with PE was significantly ($p < 0.001$) lower (1927.79 ± 678.39 grams) compared to those of mothers without PE (3372.08 ± 436.17 grams). Moreover, the mean weight percentile of newborns in mothers with PE (13.52 ± 16.67) was significantly ($p < 0.001$) lower than the average weight percentiles (51.91 ± 24.99) in those of mothers without PE. SGA and AGA were statistically significantly correlated ($p < 0.001$) with pregnancy-induced hypertension / PE severity.

The evolution of the newborn. The unfavorable evolution of the newborn was found in 14 of the 22 newborns of pregnant women with pregnancy-induced hypertension (66.7%) and in 31

newborns out of those of the 60 pregnant women with PE (50.8%), the difference being not statistically significant ($p = 0.208$). According to the second criterion, the admission to the Department of Neonatal Intensive Care, we noticed that 4 cases out of 24 (16.7%) of newborns from the control group, 14 cases out of 22 (66.7%) new - infants born from pregnancies with pregnancy-induced hypertension and 34 cases from the 60 (55.7%) neonates born from pregnancies with PE, were admitted to the Department of Neonatal Intensive Care, the difference being statistically significant ($p = 0.001$). We also noticed that the unfavorable evolution of the newborn is statistically significantly correlated ($p < 0.001$) with the SGA fetuses (10th percentile).

6. *Biomarkers of Angiogenesis in PE*

Preeclampsia is associated with an altered maternal pattern of circulation of placental derived proteins that regulates angiogenesis, such as sFlt-1 and PlGF.

sFlt-1 and PlGF. Modifications of both markers, sFlt-1 and PlGF, have been observed since the second trimester of pregnancy, which is why the blood samples from the mother were obtained from all participants between 22 and 27 weeks of gestation, during the prenatal routine screening. We considered that a value $sFlt-1 > 95$ th percentile and a PlGF value < 5 th percentile are values that can guide us in predicting PE.

There was no statistically significant difference ($p = 0.911$) depending on the distribution of sFlt-1 percentiles and the severity of pregnancy-induced hypertension / PE. Although in the specialized studies, the threshold value sFlt-1 is predictive for the occurrence of PE, in our study this value was not predictive for the occurrence of PE, because the AUC (area under the curve) for s-Flt-1 was 0.513, which means very poor test accuracy. The AUC for PlGF was 0.199, which means a random test performance.

Therefore, the sFlt-1 and PlGF values taken individually could not be absolutely predictive for the PE prediction, even if they could be suggestive by the absolute values expressed.

sFlt-1 / PlGF ratio. The main objective of our study was to evaluate the concentrations of sFlt-1 and PlGF, as well as their ratio (sFlt-1 / PlGF) in the blood of pregnant women in the second trimester of pregnancy in order to detect early pregnancy-induced hypertension and preeclampsia before the onset of typical clinical symptoms. The AUC for s-Flt-1 / PlGF ratio was 0.982, which means excellent test accuracy. This statement is consistent with the literature

studies. At values of the sFlt-1 / PlGF ratio above 22.5, the sensitivity is 98.8% and the specificity 62.5%. The values over 27 of the sFlt-1 / PlGF ratio lead to a sensitivity of 96.3% and a specificity of 95.8%. If we raise the s-Flt-1 / PlGF ratio threshold to 30.5%, the sensitivity becomes 95.1% and the specificity 100%, so the best cut-off obtained in our study was 30.5.

The predictive performance of sFlt-1 and PlGF, used separately, was not superior to the predictive performance of the sFlt-1 / PlGF ratio.

GENERAL CONCLUSIONS

- The age of the women who participated in the study did not differ significantly between the two groups (Mann Whitney test $U = 960.50$; $p = 0.859$).
- The frequency of PE occurrence is statistically significantly higher ($p = 0.020$) in women with low social and economic status.
- The frequency of occurrence of pregnancy-induced hypertension does not differ significantly ($p = 0.386$) in women with low social and economic status compared to women with high and average social and economic level.
- The frequency of PE occurrence is statistically significantly higher ($p = 0.001$) in women with low educational attainment.
- In our study, the frequency of PE in previous pregnancies is not significantly different between the two groups studied ($p = 0.112$), which is consistent with studies in the literature that show different percentages of recurrence.
- The distribution of study participants according to the presence of diabetes mellitus was significantly different ($p = 0.001$) in the PE group compared to the control group, which is consistent with the studies in the literature.
- Pre-pregnancy BMI in women with preeclampsia was statistically significant (Mann Whitney U test = 167.50; $p < 0.001$), higher in PE than in the control group.
- The frequency of pregnancy-induced hypertension in overweight and obese women is statistically significantly higher ($p < 0.001$) compared to women with normal weight status.

- Pregnancy BMI in women with preeclampsia was statistically significant (Mann Whitney U test = 308.00; $p < 0.001$), higher than in the control group.
- In the second trimester, MAP in the study participants who were diagnosed with PE (102.14 ± 7.40 mmHg) was statistically significantly higher ($p < 0.001$) than the control group (86.99 ± 7.76 mmHg).
- The distribution of study participants according to abnormal UtA Doppler and the presence of pregnancy-induced hypertension / PE in the third trimester was statistically significantly different ($p < 0.001$).
- UA-PI, UA-S/D and UA-PI > 95th percentile differed statistically ($p < 0.001$) according to the presence of pregnancy-induced hypertension / late PE, which appeared before parturition and the control group.
- We found that the gestational age at birth is statistically significantly correlated ($r = -0.264$; $p = 0.006$) with UA-RI and UA-PI from the second trimester, that is, the older they are, the more the gestational age at parturition will be smaller and vice versa.
- MAP in the third trimester is statistically significantly correlated with the severity of pregnancy-induced hypertension / PE in the third trimester ($p < 0.001$) and with the severity of pregnancy-induced hypertension / late PE before parturition ($p < 0.001$).
- Estimated fetal weight was statistically significantly lower in the PE group ($p < 0.001$).
- The distribution of pregnant women included in the study according to the estimated fetal weight percentiles is significantly different depending on the presence of PE ($p < 0.001$).
- In our study, the RI, the average PI and the S/D for the UtA Doppler from the third trimester differed significantly depending on the presence of PE and the pregnancy-induced hypertension in the third trimester of pregnancy ($p < 0.001$).
- In our study, the RI for the umbilical artery, PI, S/D and PI > 95th percentile differed statistically ($p < 0.001$) according to the presence of pregnancy-induced hypertension / PE and the control group in the third trimester.

- An abnormal Doppler examination for umbilical artery in the third trimester is correlated significantly statistically with pregnancy-induced hypertension / PE severity in the third trimester ($p = 0.008$) and late, before parturition ($p < 0.001$).
- Abnormal Doppler examination of the middle cerebral artery in the third trimester pregnancy is correlated statistically significantly with the severity of pregnancy-induced hypertension / PE in the third trimester ($p < 0.001$), the severity of the pregnancy-induced hypertension / late PE before parturition ($p = 0.001$) and with the newborn hospitalization in ICU ($p = 0.008$).
- CPR is positively correlated statistically ($p = 0.011$) with the gestational age at birth, that is, the higher the CPR, the more the gestational age increases, and vice versa.
- There was no statistically significant difference ($p = 0.911$) depending on the distribution of sFlt-1 percentiles and the severity of pregnancy-induced hypertension / PE. The presence of sFlt-1 >95th percentile is a parameter with moderate accuracy for PE prediction, as it is known that a test method is declared to be correct when the test value approaches absolute (100%).
- AUC for PIGF was 0.199, which means a random test performance, but the presence of PIGF <5 percentile is a parameter with high accuracy for PE prediction.
- The AUC for s-Flt-1 / PIGF ratio was 0.982, which means excellent test accuracy. This statement is consistent with the literature studies.
- The sFlt-1 / PIGF ratio <30.5 is a parameter with moderate accuracy for PE prediction, with a positive predictive value of 30 and a negative predictive value of 27.27.
- The sFlt-1 / PIGF ratio >85 is a parameter with high accuracy for PE prediction, with a positive predictive value of 75 and a negative predictive value of 48.78.>
- The predictive performance of sFlt-1 and PIGF, used separately, was not superior to the predictive performance of the sFlt-1 / PIGF ratio.

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