

New Laboratory Biomarkers for Early Diagnosis of Preeclampsia

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Abstract: *The aim of the study was to examine whether increased serum sFlt1 levels are related to development of PE. Subjects were recruited from the Obstetrics and Gynecology Department at American Hospital in Tirana, Albania between January 2012 and December 2014. The study subjects included 95 women who subsequently developed preeclampsia and 140 women with normal singleton pregnancies who were matched for maternal age, and gestational age. sFlt-1 levels were significantly higher in the preeclamptic women, median-interquartile range 4011.2 (157.7) than in normal controls 679.2 (140.1) ($p < 0.001$), while the PIGF levels were significantly lower, 402 (14) as compared to controls 1128.6 (62.5) ($p < 0.001$) (fig. 2). In preeclamptic women, sFlt-1 levels were negatively correlated with the PIGF levels ($r = -0.25$, $p = 0.04$). The predictive accuracy of preeclampsia was higher as denoted by greater AUC (0.982). In future these biomarkers will be the first signals for preeclampsia and help prevent its severe forms.*

Keywords: preeclampsia, sFlt-1, PIGF, gestational age, hypertension

1. Introduction

Preeclampsia (PE), a pregnancy-specific syndrome affecting about 5% to 10% of pregnancies, is characterized by the presence of hypertension and proteinuria. This syndrome is one of the leading causes of maternal and fetal morbidity and mortality worldwide and occurs only in the presence of a placenta and remits dramatically after the placenta has been delivered. Although the exact pathogenesis of preeclampsia remains unclear, it has been suggested that endothelial dysfunction may play a central role in the development of preeclampsia. Impaired trophoblastic invasion of the maternal placental bed is considered to be the initial event of endothelial dysfunction in preeclampsia. Consequently, reduction of uteroplacental blood perfusion by shallow implantation results in local placental hypoxia. A hypoxic/ischemic placenta may then release placental factors into the maternal circulation eventually causing endothelial dysfunction, which leads to the main clinical symptoms of preeclampsia (including hypertension and proteinuria). A role of the balance between soluble fms-like tyrosine kinase (sFlt-1) and placental growth factor (PIGF) in the pathogenesis of preeclampsia has been proposed by several investigators (1-4). sFlt-1 is a secreted splice variant of Flt-1 that antagonizes vascular endothelial growth factor (VEGF) and PIGF by binding, and prevents their interaction with endothelial receptors on the cell surface (5). PIGF is a secreted dimeric glycoprotein that possesses potent angiogenic and mitogenic activities capable of inducing the proliferation, migration, and activation of endothelial cells (6, 7). It has been recently shown that increased sFlt-1 occurs approximately five to six weeks before the onset of clinical symptoms of preeclampsia (1, 2), whereas decreased PIGF levels are observed prior to 20 weeks of gestation in women who subsequently develop preeclampsia (3,4). Moreover, administration of exogenous sFlt-1 to pregnant rats leads to

reduced PIGF, hypertension, proteinuria, and glomerular endothelial injury (8). Therefore, the pathogenesis of preeclampsia may involve an imbalance of angiogenic factors, and the measurement of sFlt-1 in combination with PIGF may distinguish women who subsequently develop preeclampsia from women who remain normotensive during pregnancy (8-10). In this study we investigated whether the second trimester plasma levels of sFlt-1 and PIGF were altered in women who subsequently developed preeclampsia.

2. Materials and Methods

Subjects

Subjects were recruited from the Obstetrics and Gynecology Department at American Hospital in Tirana, Albania between January 2012 and December 2014. The study subjects included 95 women who subsequently developed preeclampsia and 140 women with normal singleton pregnancies who were matched for maternal age, and gestational age at the time of blood sampling. Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg after 20 weeks gestation) and proteinuria (≥ 300 mg in a 24 hr urine collection or $\geq 1+$ on dipstick testing). Exclusion criteria included major congenital anomalies, fetal chromosomal abnormalities, multiple pregnancies, chronic hypertension, diabetes mellitus, and renal disease. Control subjects were selected randomly from women who were normotensive and without proteinuria throughout pregnancy and who delivered a healthy neonate at term without significant medical or obstetric complications such as; chronic hypertension, diabetes, renal insufficiency, congenital anomalies or fetal demise. This study was approved by the Ethics Committee at the American Hospital, and written informed consent was obtained from all enrolled women. The

aim of the study was to examine whether increased serum sFlt1 levels are related to development of PE.

Statistical analysis

Data were analyzed using Medcalc statistical software. All data are presented as the median (range) and number (percentage). Data not normally distributed were analyzed by nonparametric tests. A comparison of continuous variables between the two groups was performed using the Student's t test or Mann-Whitney U test, and proportions were compared with the chi square test or Fisher's exact test. Spearman's rank correlation was used to assess the relationship between the two variables. Receiver operating characteristic (ROC) analysis was performed to assess the best cut-off value and discriminating capacity of the log[sFlt-1/PIGF] ratios in week 24-28 plasma for predicting women who subsequently developed preeclampsia. Sensitivity, specificity, with 95% confidence intervals (CI) were calculated in order to consider the predictive efficacy of the log[sFlt-1/PIGF] ratio.

3. Results and Discussion

Regarding the clinical characteristics of the study population there were no significant differences in maternal age, gestational age at blood sampling, or platelet count between the preeclamptic and normal pregnant women. As expected, systolic and diastolic blood pressures were significantly higher in the preeclamptic women than in normal controls. By contrast, nulliparity, gestational age at delivery, and birth weight were lower in the preeclamptic women than in the normal controls. Maternal plasma sFlt-1 levels were significantly higher in the preeclamptic women, median-interquartile range 4011.2 (157.7) than in normal controls 679.2 (140.1) ($p < 0.001$) (fig. 1), while the PIGF levels were significantly lower, 402 (14) as compared to controls 1128.6 (62.5) ($p < 0.001$) (fig. 2). In preeclamptic women, sFlt-1 levels were negatively correlated with the PIGF levels ($r = -0.25$, $p = 0.04$). The predictive accuracy of preeclampsia was higher as denoted by greater AUC (0.982). The plasma log[sFlt-1/PIGF] ratio was significantly higher in the preeclamptic women than in normal controls (median 9.9, range 0.1- 16.1 vs. median 0.6, range 0.4-0.7, $p < 0.001$). The maternal plasma log[sFlt-1/PIGF] ratio with the cut-off value of 0.7 provided the best combination with 98.2% sensitivity and 100% specificity (area under the curve [95% CI]: 0.982 [0.949 to 0.996], $p < 0.001$) (Fig. 3).

4. Conclusion

In summary, our results showed that sFlt-1 levels were increased and PIGF levels were decreased in second trimester plasma of women who subsequently developed preeclampsia compared to the normal pregnant women, and that a negative correlation existed between the circulating sFlt-1 and PIGF levels in the preeclamptic women. We also found that sFlt-1/PIGF ratios were significantly higher in women and PIGF have been shown to be associated with preeclampsia and may

be potential markers for the early prediction of preeclampsia before clinical manifestations are identified, further prospective, large-scale, longitudinal studies are essential to determine the usefulness of sFlt-1 and PIGF in predicting preeclampsia. Other studies in literature report similar findings with our study (11-14). This was the first attempt to establish periodic values for preeclampsia biomarkers sFlt-1 and PIGF levels in Albanian laboratory medicine. In future these biomarkers will be the first signals for preeclampsia development and help prevent its severe forms for a better outcome for the mother and baby health.

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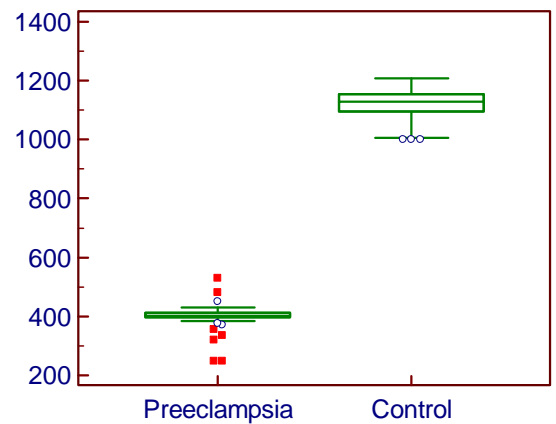


Figure 2: Maternal plasma PIGF levels (pg/mL)

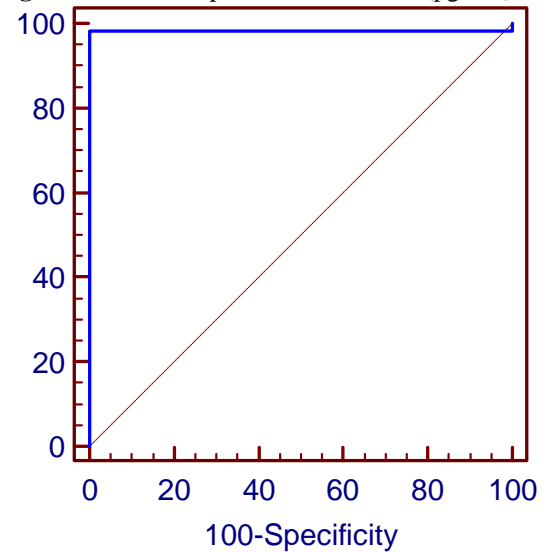


Figure 3: Receiver operating characteristic curve (ROC)

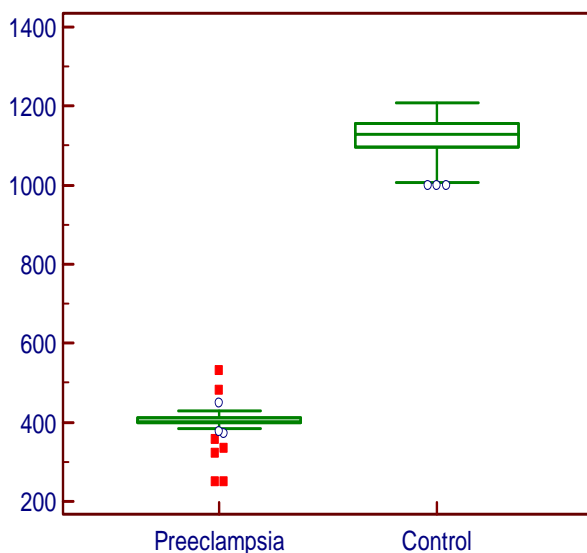


Figure 1: Maternal plasma sFlt-1 levels (pg/mL)