The comparison of selected clinical and sonographic features with concentrations of angiogenic factors and aneuploidy markers in the late 1st trimester of pregnancy

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Abstract

Background: Pregnant women suffering from preeclampsia (PE) present with an apparent imbalance between serum concentrations of angiogenic and antiangiogenic factors. Combined screening for fetal anomalies late in the 1st trimester has become widely used standard of perinatal care. AIM: to compare the selected clinical, sonographic (CRL, BPD, NT) and biochemical (PAPP-A, free betaHCG) factors with the expression of VEGFR-1 and EGFR. Methods. Clinical data (maternal age, BMI, previous pregnancy loss) were recorded in women who presented at our Department for prenatal screening between 11th and 13th weeks of gestation. Fetal measurements including NT, and serum concentrations of PAPP-A and f-beta HCG were measured according to Fetal Medicine Foundation standards. VEGF-R1 and EGFR serum levels were measured with ELISA. Results: The study group included 83 pregnant women, of whom 55 (66%) were multiparas and 28 were primiparas. Median concentration of VEGFR-1 was 17.5 pg/ml (range: 10.9-22.2 pg/ml) and median EGFR concentration was 2.05 pg/ml (range 1.3-2.8 pg/ml). VEGFR-1 levels increased with gestational age between 11th and 13th weeks of pregnancy. The medians and range were as follows: in the 11th week pg/ml 15.9 (range: 10.2-21.6 pg/ml) for VEGFR-1 and 1.87 pg/ml (range: 1.56-2.93 pg/ml) for EGFR in the 12th week; 18.6 pg/ml (range: 12.24-7 pg/ml) for VEGFR-1 and 2.5 pg/ml (range: 1.06-2.3) for EGFR; in the 13th week 17.6 pg/ml (range: 11-24.7 pg/ml) for VEGFR-1 and 2.5 pg/ml (range: 1.49-2.8) for EGFR. No statistically significant correlation was found between gestational age and both VEGFR-1 and EGFR median concentrations. However, a significant correlation was observed between VEGFR-1 concentration and BMI (R = 0.31; p = 0.003). Both studied angiogenic factors as well as placental proteins concentrations expressed as MoM’s were not correlated with each other (p < 0.05). Conclusion: We conclude that the assessment of selected angiogenesis markers in the blood of pregnant women late in the 1st trimester of gestation could potentially constitute new, additional markers for the early diagnosis of serious pregnancy complications including preeclampsia.

Key words: fetal growth, placental dysfunction, angiogenesis, VEGF-R1, EGFR concentrations

An imbalance between angiogenic and antiangiogenic factors has been proposed as one of the most important factors in the pathophysiology of preeclampsia (PE), a potentially detrimental multiorgan disease of pregnant women [1]. The so called “anti-angiogenic state” has been implicated not only as a mechanism of pathological changes related to PE, but also it was found in more severe pregnancy complications such as intrauterine growth restriction (IUGR) and HELLP syndrome [2-4]. One of the most important findings in pregnant women affected with PE is an apparent imbalance between serum concentrations of angiogenic factors such as placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) and antiangiogenic molecules, such as soluble VEGF receptor-1 (sVEGFR-1) and soluble endoglin (s-Eng) [5-7]. Elevated serum and plasma concentrations of sVEGFR-1 and s-Eng have been observed not only in women with symptoms of preeclampsia but also in patients before the recognition of clinical stage of the disease [3, 6]. Angiogenesis regulation is important to normal placental and fetal growth and development [5]. Blood vessels in the human placenta have two distinct phases of growth with peaks late in the 1st trimester and in the late second and early third trimesters of pregnancy. Mayhew et al. suggested that these processes result from predominant endothelial proliferation in early pregnancy followed by vascular remodeling [8]. Placental new blood vessel formation is controlled by several different growth factor families. One of the most important is signaling by vascular endothelial growth factors (VEGFs). These cytokines control a critical step in physiological placental angiogenesis [9, 10]. It has been proposed that vascular growth in the placenta is locally regulated by a soluble form of VEGF receptor, namely, sVEGFR-1.
which is produced by the trophoblast [8]. Expression of sVEGR-1 mRNA was found both in villous and extravillous trophoblast. Clark et al. [11] have detected sVEGFR-1 in the sera of women with uncomplicated pregnancies, but not in non-pregnant women. More recent reports indicate, however, that there are detectable levels of sVEGFR-1 both in plasma and serum of healthy non-pregnant individuals [12]. The cytokine may be derived from monocytes and endothelial cells, which suggests that sVEGFR-1 may contribute to the fine regulation of VEGF bioavailability in both pregnant and non-pregnant women [13]. These authors have postulated that the formation of heterodimers with the VEGF receptors in the cell surface could serve as an additional mechanism by which sVEGFR-1 regulates the bioavailability of VEGF. In such way their signal transduction would be cancelled. This precise control of VEGF concentration is extremely important as continuous low levels of the cytokine are required for endothelial cell proliferation and survival [13].

Epidermal growth factor receptor (EGFR) is a member of the ERBB family of receptor tyrosine kinases which also play an important physiologic role in many aspects of pregnancy development. However, the role of EGFR in early fetal growth is less clear. It has been shown that embryos lacking EGFR exhibit strain-specific defects in placental development that can result in mid-gestational fetal lethality [14, 15]. These authors observed significant differences between mouse placenta and embryo size as well as in the cellular composition and expression of trophoblast cell subtype markers. They proposed a hypothesis that differential expression in the placenta of glucose transporter essential for normal embryonic growth (Glut3) may contribute to significant differences in fetal intrauterine growth restriction caused by reduced EGFR activity [14, 15]. Currently, fetal growth in the late first trimester of gestation is assessed by sonographic crown-rump length (CRL) and biparietal (BPD) measurements and the risk of genetic and chromosomal disorders is calculated based on fetal nuchal translucency measurements combined with selected placental protein assessment in the serum of pregnant women [4, 16-18]. The aim of our study was to compare the selected clinical, sonographical (CRL, BPD, NT) with the expression of VEGF-R1 and EGFR and placental proteins PAPP-A and ß-beta hCG concentrations in women between 11th and 13th weeks of gestation. Maternal age and body-mass index (BMI) were recorded in each pregnant woman followed by ultrasound scan according to FMF guidelines. Parameters measured included: fetal crown-rump length (CRL), biparietal diameter (BPD), femur length (FL) and nuchal translucency (NT) as well as fetal heart rate (FHR). Following ultrasound examination maternal serum samples were collected. Venous blood samples were allowed to coagulate at room temperature for 30 min, centrifuged at 2000 g for 10 min, and serum was separated, aliquotted and stored at −70°C until further assays. Medical University in Lublin local Ethical Committee approval and patient’s consent were obtained for research to be carried out on the remaining excess serum. Serum PAPP-A and free beta HCG was measured using Delfia EXPRESS System (Perkin-Elmer, USA). The results were converted to Multiple of the Medians (MoM’s) for gestational age according to Fetal Medicine Foundation protocol using licensed ASTRAIA (Germany) software. For angiogenic cytokines levels measurements samples were analyzed using an enzyme-linked immunosorbent assay kit designed to quantitatively measure human soluble VEGF-R1 and sEGFR concentration in serum (Human sEGFR and sVEGFR-1 Immunoassay; R&D Systems, Minneapolis, USA) according to manufacturer’s instructions. The intensity of the developed color was measured by reading absorbance at 450 nm. Each measurement was made in duplicate and the serum levels of sEGFR and sVEGFR-1 were determined by extrapolation from a standard curve generated for each set of samples assayed. Statistical software package STATISTICA v.6.0 was used for all data analyses and p values of < 0.05 were considered statistically significant.

Results

In the studied group maternal age varied from 20 to 42 years (mean 31 years). Of 83 women, 55 (66%) were multiparas and 28 were primiparas. Twenty women had previously miscarried their pregnancy. In this group 15 women had one miscarriage and 5 had more than one pregnancy failure. Medians and range of selected clinical, sonographic and biochemical parameters of studied group of women are presented in Table 1. Table 2 presents medians and range of the serum VEGF-R and EGFR concentrations in the selected weeks of the 1st trimester of pregnancy. Median concentration of VEGFR-1 in the studied population was 17.5 pg/ml (range: 10.9-22.2 pg/ml) and median EGFR concentration was 2.05 pg/ml (range 1.3-2.8 pg/ml), data presented in Fig. 1 and 2, respectively.

Methods

The study group included 83 healthy women who presented at routine first trimester prenatal screening between 11th and 13th weeks of gestation.
Table 1. Medians and range of selected clinical, sonographic and biochemical parameters of women in the selected weeks of the 1st trimester of pregnancy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>11th week MD (range)</th>
<th>12th week MD (range)</th>
<th>13th week MD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>23.9 (21.5-25.9)</td>
<td>22.5 (20.4-25)</td>
<td>23.4 (22.4-24.9)</td>
</tr>
<tr>
<td>CRL</td>
<td>50.5 (49-53)</td>
<td>57.5 (52-62)</td>
<td>71 (67.5-78)</td>
</tr>
<tr>
<td>BPD</td>
<td>15 (14.7-17.7)</td>
<td>18.7 (17-19.7)</td>
<td>22 (20.8-23.5)</td>
</tr>
<tr>
<td>NT</td>
<td>1.56 (1.34-1.78)</td>
<td>1.79 (1.59-2.02)</td>
<td>2.13 (1.88-2.28)</td>
</tr>
<tr>
<td>FL</td>
<td>6.2 (5.35-6.4)</td>
<td>6.9 (6.1-7.6)</td>
<td>9.3 (8.35-10.4)</td>
</tr>
<tr>
<td>FHR</td>
<td>163 (162-176)</td>
<td>161 (154-171)</td>
<td>156 (150-161)</td>
</tr>
<tr>
<td>βHCG (MoM)</td>
<td>0.64 (0.49-1.28)</td>
<td>0.74 (0.52-0.95)</td>
<td>0.7 (0.5-1.23)</td>
</tr>
<tr>
<td>PAPP-A (MoM)</td>
<td>2.42 (1.34-4.08)</td>
<td>3.11 (2.04-4.88)</td>
<td>3.1 (1.73-4.78)</td>
</tr>
</tbody>
</table>

Table 2. Medians and range of the serum VEGF-R and EGFR concentrations of women in the selected weeks of the 1st trimester of pregnancy

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Number of patients</th>
<th>EGFR(pg/ml) Median (range)</th>
<th>VEGFR-1 (pg/ml) Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11th week</td>
<td>8</td>
<td>1.87 (1.56-2.93)</td>
<td>15.9 (10.2-21.6)</td>
</tr>
<tr>
<td>12th week</td>
<td>43</td>
<td>2.05 (1.06-2.3)</td>
<td>18.6 (12-24.7)</td>
</tr>
<tr>
<td>13th week</td>
<td>32</td>
<td>2.05 (1.49-2.8)</td>
<td>17.6 (11-21.5)</td>
</tr>
</tbody>
</table>

VEGFR-1 levels increased with gestational age between 11th and 13th weeks of pregnancy. The medians and range were as follows: in the 11th week 15.9 pg/ml (range: 10.2-21.6 pg/ml) for VEGFR-1 and 1.87 pg/ml (range: 1.56-2.93 pg/ml) for EGFR in the 12th week; 18.6 pg/ml (range: 12-24.7 pg/ml) for VEGFR-1 and 2.5 pg/ml (range: 1.06-2.3) for EGFR; in the 13th week 17.6 pg/ml (range: 11-21.5 pg/ml) for VEGFR-1 and 2.5 pg/ml (range: 1.49-2.8) for EGFR. No statistically significant correlation was found between gestational age and both VEGFR-1 and EGFR median concentrations.

However, a significant correlation was observed between VEGFR-1 concentration and BMI ($R = -0.31; p = 0.003$). Figure 3 presents an inverse correlation between these parameters. Both studied angiogenic factors as well as placental proteins concentrations expressed as MoM’s were not correlated with each other ($p < 0.05$).

A statistically significant correlation between fetal bi-parietal diameter ($R = 0.86; p = 0.006$), but not with CRL or FL was found in a subgroup of women examined during 11th week of their gestation. Additionally, we observed close to statistical significance relationship of
EGF-R concentration with beta HCG levels expressed as MoM’s \( (R = 0.65; p = 0.07) \) and with maternal BMI \( (R = -0.65; p = 0.07) \).

Correlation between serum VEGFR-1 concentration and body-mass index (BMI) of women in the 1st trimester of pregnancy

Correlation between serum VEGFR-1 concentration and body-mass index is presented in Fig. 1. Interestingly, in 12th week of gestation no statistical correlations between studied parameters were found except for VEGFR-R1 and BMI \( (R = 0.4; p = 0.007) \). VEGFR-1 concentrations and NT values were correlated \( (R = 0.42; p = 0.01) \) with each other in the subgroup of women in their 13th week of pregnancy. Both concentrations of cytokines receptors were moderately elevated (2.18 pg/ml vs 1.99 pg/ml for EGFR and 18 pg/ml vs 16.8 pg/ml for VEGFR-1, respectively) in women who had previously miscarried their pregnancies, but the differences did not reach statistical significance.

### Discussion

Abnormal placentation followed by generalized microvascular and endothelial cells dysfunction are key steps in the development of preeclampsia. For many years the syndrome of proteinuria and hypertension was regarded as a form of "toxemia of pregnancy" [1, 2]. This name suggested that an unknown "toxic factor" or multiple "toxic factors" produced by the ischemic placenta gain the access to maternal circulation [4, 5, 7]. The resulting systemic endothelial dysfunction produces clinically measurable symptoms, including hypertension, proteinuria and in severe cases also disseminated multiorgan damage. Effective function of endothelial cells in the human placenta is controlled by two angiogenic factors, vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) [9, 19]. Acting through two high-affinity tyrosine kinase receptors: VEGFR-1 (VEGF receptor-1 or flt-1 or fms-like tyrosine kinase-1) and VEGFR-2 (VEGF receptor-2 or KDR/Flik-1 or kinase domain receptor), VEGF exerts its biologic effects which include endothelial cell proliferation, migration and survival. Two isoforms of VEGF receptor exist, one of them is transmembranous form (VEGF-R1) and a soluble form (sVEGFR-1) [9, 11]. The latter isoform lacks the signaling tyrosine kinase domain, binds VEGF and inhibits its biological activities [9]. Another ligand for VEGF-R1 is PIGF, which potentiates the angiogenic effects of VEGF [2, 12]. Whereas VEGFR-2 is the major mediator of the mitogenic, angiogenic, permeability-enhancing, and endothelial survival effects of VEGF, the precise function of VEGFR-1 is still unclear. Recent data indicate that it is able to inhibit the activity of VEGF on vascular endothelium by preventing binding of VEGF to VEGFR-2. However, in some pathological conditions such as ischemia of retina or heart muscle, VEGFR-1 signaling can be functional [9, 19]. Erez et al. [3] found that in normal pregnancy, maternal plasma concentrations of sVEGFR-1 remain largely unchanged between the first and second trimesters, and increase modestly thereafter. The profile of maternal plasma concentrations of angiogenic (PIGF) and anti-angiogenic factors (s-Eng and sVEGFR-1) between the first and second trimesters is significantly different among patients who subsequently had a normal pregnancy and those destined to develop PE or to deliver SGA neonates [3]. In this study, an increase in the maternal plasma concentration of s-Eng and sVEGFR-1 between the first and second trimester increased chances for the development of preterm PE. Importantly, the combination of the three cytokines into a pro-angiogenic versus antiangiogenic ratio \( [\text{PIGF}/(\text{s-Eng x VEGFR-1})] \) also increases the risk for the subsequent development of preterm PE. A patient with a subnormal increase of PIGF, and increases of s-Eng and sVEGFR-1 from the first to the second trimester was at substantial risk for the development of preterm PE. A patient with a subnormal increase of PIGF, and increases of s-Eng and sVEGFR-1 from the first to the second trimester was at substantial risk for the development of preterm PE, and small for gestational age neonate [3]. It is also likely that changes in the levels of studied cytokines are caused through a mechanisms other than angiogenesis. The most probable explanation could be a modulating role of VEGF-A [2]. This cytokine plays a central role in the control of angiogenesis, but it could also stimulate endothelial growth because deletion of VEGF-A gene activity results in progressive endothelial degeneration [19]. Prior to the discovery of the relationship between angiogenic proteins and preeclampsia, endothelial dysfunction was one of the main topics studied to understand pathophysiology of preeclampsia [5, 20, 21]. Interestingly, recent-
ly published data on angiogenic proteins do not con-
tradict existing body of knowledge, but rather could ser-
ve to explain a potential mechanism coupling placental
hypoxia and ischemia to systemic maternal endothelial
dysfunction. Our results support this interpretation. We
conclude that the assessment of selected angiogenesis
markers in the blood of pregnant women late in the 1st
trimester of gestation could potentially constitute new,
additional markers for the early diagnosis of serious
pregnancy complications including preeclampsia.

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ceptor-1) factors in normal pregnancy and patients de-
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