The effect of antihypertensive therapy in preeclampsia on hormonal production of placenta

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Abstract

Objectives: Preeclampsia is a multisystem disorder which complicates 2-5% of all pregnancies and is the second major cause of maternal mortality. The latest researches indicated that antihypertensive therapy with alpha-methyldopa may have an effect on the concentration of placental hormones in pregnancies complicated by preeclampsia. The aim of this study was to determine the effect of α-methyldopa on levels of inhibin A, activin A in serum and estriol in saliva. Methods: We analyzed 62 pregnant women in third trimester of pregnancy. A venous blood and saliva sample were collected from control (29) and studied group (33). In all patient levels of activin A, inhibin A and estriol were measured by means of two-site enzyme-linked immunosorbent assays (ELISA). Results: The mean saliva estriol in control group was 3072.592 pg/ml and in PE group 2198.933 pg/ml (p = 0.4004). The mean activin A concentration in control cases was 568.558 pg/ml and studied cases 890.002 pg/ml (p = 0.0729). Inhibin A in normal pregnancies group was 259.078 pg/ml and in preeclampsia 283.92 pg/ml (p = 0.581). Conclusion: Levels of activin A and inhibin A in serum and estriol in saliva in preeclampsia with antihypertensive therapy did not significantly vary from normal pregnancy.

Key words: hormones, placenta, preeclampsia, antihypertensive therapy, activin A, inhibin A, estriol

Introduction

Preeclampsia (PE) is a multisystem disorder which complicates 2-5% of all pregnancies and is the second major cause of maternal mortality [1]. The potential lethal complications of preeclampsia are: premature abruption of placenta, acute renal failure, hemorrhage to the central nervous system, disseminated intravascular coagulation (DIC) and respiratory distress or circulation failure. Women with preeclampsia have raised risk of developing cardiovascular disorders or stroke [2].

The leading hypothesis of ethiopathogenesis of preeclampsia/eclampsia is the theory of dysfunction in fetal circulation (placental ischemia). Other mechanisms also occur, including: early inflammation response [3], toxic influence of peroxidated lipids [4], malfunction of immunologic adaptation (incorrect expression of HLA G in trophoblast) [5], increased immunopathological processes and associated gene polymorphisms [6]. Placental ischemia is thought to be developed by abnormal cytotrophoblastic invasion. Causing the release of different placental factors and imbalance of angiogenic factors and widespread endothelial dysfunction [7].

In recent decades there is much attention putted into research of activin A and inhibin A as hormones produced in placenta. Inhibin is a heterodimeric glycoprotein with an α- and β-subunit. Activin is a homodimer of two β-subunits. Their biological role as hormones in pregnancy is auto and/or paracrine. In the nineties of the XXth century Petralia and Silver, as one of the first researchers, discovered elevated concentration of activin A and inhibin A in preeclampsia [8, 9]. Nowadays these hormones are used to predict preeclampsia in low or high risk population. Their evaluation takes place in first and second trimester of pregnancy with proceeding of ultrasound of maternal uterine arteries [10]. Estriol is a hormone thought to be responsible for the competence of the fetoplacental unit in pathogenesis of preeclampsia [11].

The drugs used for therapy of preeclampsia are: alpha-methyldopa, beta-blockers (labetalol), calcium channel blockers (nifedipine) and magnesium sulfuricum. The latest researches indicated that antihypertensive therapy with alpha-methyldopa may have an effect on the synthesis and/or release of placental hormones in pregnancies complicated by PE [12].

The aim of this study was to determine the effect of antihypertensive therapy on levels of inhibin A, activin A in serum and estriol in saliva.

Material

This was a prospective study of women with preeclampsia in third trimester of pregnancy. We analyzed 62 pregnant women hospitalized in Maternity Ward and Pathology of Pregnancy Ward in Department of Obstet-
trics and Pathology of Pregnancy in Medical University in Lublin in years 2008-2010. Gestational age was established on the basis of menstrual dates and/or ultrasonographic examination prior to 14 weeks’ gestation. Preeclampsia was defined according to the guidelines of the International Society for the Study of Hypertension in Pregnancy [13]. The hypertension was diagnosed after the 20 weeks of gestation as elevated blood pressure (measured at two time points at least 6 hours apart) with systolic pressure of at least 140 mm Hg and diastolic pressure of at least 90 mm Hg. Also with new onset of proteinuria (at least 1+ in dipstick or ≥300 mg pre 24 hours urine collection). The subjects had: normal blood pressure before the pregnancy, no health aggravations (women with diabetes mellitus, thyroid gland diseases, chronic hypertension, renal failure, connective tissue diseases were excluded), no history of illness in present pregnancy (fetal growth retardation, premature labor, gestational diabetes mellitus and thrombocytopenia were excluded). There were no structural and genetic malformations in fetus during the prenatal ultrasound scan and confirmed after the labor. All studied patients had normal liver function (AlaT, AspaT, Bilirubin) and kidney function (creatinine, urea, uric acid). The platelets count was in normal range (150,000-450,000/mm³). During the pregnancy complications in fetus during the prenatal ultrasound scan and confirmed after the delivery. All studied patients had normal liver function (AlaT, AspaT, Bilirubin) and kidney function (creatinine, urea, uric acid). The platelets count was in normal range (150,000-450,000/mm³). During the hospitalization all patients with preeclampsia were administered antihypertensive treatment (alpha methyldopa or nifedypine).

This study was approved on 8th of May 2008 by the Bioethics Commission of Medical University in Lublin (No. KE-0254/93/2008). The informed consent and randomized questionnaire was obtained from the participants.

Methods

A venous blood and saliva sample were collected from control and studied group at the same time. Saliva was collected to salitubes by the patients by themselves. The blood samples were centrifuged at 5000 rpm for 10 min. Serum was separated and frozen at −80°C and kept until the analyze.

In all patient levels of activin A, inhibin A were measured by means of two-site enzyme-linked immunosorbent assays (ELISA) (USCNK, USA). The kit for evaluating inhibin A had polyclonal antibodies specified for anti-inhibinβA. The antibodies did not present any cross reactions with any other substances. The range of detectable concentration in the assays for activin A was 0.156-10 ng/ml. These antibodies did not present any cross reactions with any other substances. The range of detectable concentration in the assays for activin A was 0.156-10 ng/ml. The ELISA kit for evaluating activin A had polyclonal anti-bodies specified for anti-activinα. The antibodies did not present any cross reactions with any other substances. The range of detectable concentration in the assays for activin A was 0.156-10 ng/ml.

Estriol was also assessed by ELISA kit (DRG, USA) according to the manufacturer’s recommendations. This kit evaluated free estriol in saliva and contained antibodies IgG against estriol. The range of detectable concentration of estriol was 2.5-4000 pg/ml. The antibodies used in the measurement displayed slight cross reaction with other substances, derivatives of estriol such as: 16-epi-estriol, 15α-OH-estriol. The results were then read in the Microplate Reader 680 (BioRad, UK).

Statistic analysis was performed with Statistica 9.0 software (StatSoft, USA). Subjects characteristics compared between groups by Student t-test. Subject characteristics measured categorically were compared by Spearman rank test and/or Mann-Whitney’s test. A p-value of ≤ 0.05 was considered statistically significant.

Results

A total of 62 pregnancy were recruited to the study. Twenty nine patients were control group and 33 had pregnancy complicated with preeclampsia. Studied patients received oral antihypertensive therapy in the form of α-methyldopa 750-2000 mg/day for clinical indications. Concentration of inhibin A and activin A was assessed in 33 women. Free saliva estriol was detected in 22 studied cases. A comparison of baseline characteristics was presented in Table 1.

Baseline characteristics between compared group were similar regarding maternal age and number of pregnancies. We found significantly lower gestational age in preeclampsia group.

Mean levels of maternal serum inhibin A, activin A and saliva free estriol are shown in Figures 1, 2 and 3. The mean saliva estriol in control cases was 3072.592 pg/ml and in preeclampsia 2198.933 pg/ml (p = 0.4004). The mean activin A concentration in control cases was 568.558 pg/ml and studied cases 890.002 pg/ml (p = 0.0729). Inhibin A in normal pregnancies group was 259.078 pg/ml and in preeclampsia 283.92 pg/ml (p = 0.581). There were no statistically significant differences between mean levels of analyzed substances between the control and examined cases.

The correlation between concentrations of activin A and inhibin A in patients with preeclampsia was determined. We found statistically significant correlation between levels of two analyzed substances (p = 0.00003).
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Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control ((n = 29))</th>
<th>Preeclampsia ((n = 33))</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 4.27</td>
<td>29.9 ± 5.45</td>
<td>ns</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>39.51 ± 1.27</td>
<td>36.51 ± 4.49</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>19 (65.5%)</td>
<td>21 (63.6%)</td>
<td>ns</td>
</tr>
<tr>
<td>Multiparous</td>
<td>10 (34.5%)</td>
<td>12 (36.4%)</td>
<td>ns</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>114.31 ± 9.42</td>
<td>160.60 ± 15.19</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>74.31 ± 8.63</td>
<td>102.72 ± 10.46</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

\(n\) – number of patients, \(p\) – statistic value

Therefore having the value of concentration of activin A \((x)\) we can estimate the value of inhibin A (Fig. 4).

We summarized the data of maternal age and its influence on the levels of activin A and inhibin A. The results are shown in Figure 5.

Pregnant women with preeclampsia in middle age were characterized by the highest level of activin A with the highest rise of inhibin A.

The data of serum and saliva maternal levels of detected substances are shown in Fig. 6. We discovered correlation between concentration of activin A, inhibin A and estriol. The highest levels of activin A and inhibin A coexist with the lowest levels of estriol in preeclampsia group.
In one study the influence of α-methyldopa therapy made the effect of reducing maternal serum levels of inhibin A in PE group [12]. In the current study, we found that the concentration of activin A and inhibin A was on equal levels in control and preeclampsia group. This management may have an effect on the synthesis and/or release of placental hormones in preeclampsia. This result may be independent of its known antihypertensive action.

In our study we found that the levels of activin A and inhibin A in preeclampsia correlate. The antihypertensive therapy influenced on placental hormones proportionally by decreasing their levels evenly.

The correlation between maternal age and levels of activin A and inhibin A showed a pattern. The lowest concentration of these proteins in patients with preeclampsia was in the youngest and the oldest patients. Those women belong to the high risk group of developing preeclampsia [15]. The antihypertensive therapy made the most significant effect comparing to the women in middle age. The cause of this apparition is still to be determined.

In our study we found that levels of saliva estriol were the same in control and preeclampsia group. Estriol, as one of the weakest estrogens in women organism, plays a crucial role in developing of pregnancy [16]. Results of other studies showed that concentration of estriol in maternal serum can be lower [17], higher [18] or the same level [19] in preeclampsia as in normal pregnancies. The concentration of salivary estriol in pregnancy corresponds with free serum levels of this hormone [20]. However, no studies until now evaluated the salivary estriol in preeclampsia under antihypertensive therapy. In our study we found that the levels of estriol in saliva did not significantly differed in preeclampsia and in normal pregnancy.

The correlation of levels of activin A, inhibin A and estriol indicate their collective participation in development of preeclampsia. In preeclampsia group the lowest levels of estriol were in patients with the highest levels of activin A and inhibin A. Our results suggest that the weakest competence of fetoplacental unit (lowest saliva estriol) is responsible of the highest production and/or release of other placental hormones (highest activin A and inhibin A).

Because of the effect of the gestational age on the levels of activin A, inhibin A [21] and estriol [22] we matched the gestational age between groups. The period of collecting specimens was in third trimester.

The first-choice drug in Poland used in antihypertensive therapy in preeclampsia is α-methyldopa [23].
The $\alpha$-methyl-dopa acts on $\alpha_2$-adrenergic receptors in central nervous system (CNS) and partially in peripheral $\alpha_2$-adrenoceptors. Stimulation of these receptors in CNS leads to reduction of sympathetic outflow and lowering the blood pressure. The $\alpha_2$-adrenoceptors have been identified in myometrium and placenta [24]. Stimulating the $\alpha_2$- receptors leads to reduced production of cAMP. The decrease of cAMP in placenta reduces the secretion of activin A and inhibin A [25]. However the effect is not seen in gestational hypertension, where those proteins remain on the same levels [12].

The pathophysiology of preeclampsia is uncertain. The increase of concentration of activin A and inhibin A may play a role in pathogenesis of hypertension and proteinuria in pregnancy or may be a manifestation of maternal disease. The participation of estriol in development of preeclampsia is still to be conducted. This hormone is a marker for the competence of the fetoplacental unit. The antihypertensive treatment is associated with decreasing of concentration of placental proteins in maternal serum to the normal range. It also maintains the saliva estriol on the level obtained in normal pregnancy. It is unknown if these medicaments act directly on trophoblast or through placental substances.

Conclusion

Levels of activin A and inhibin A in serum and estriol in saliva in preeclampsia with antihypertensive therapy did not significantly vary from normal pregnancy. Further studies on the influence of antihypertensive management on placental production and/or secretion of hormones in preeclampsia is needed.

Declaration of interest:
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


