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The reduction in circulating levels of estrogen and progesterone in women with preeclampsia

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ABSTRACT

Abnormalities in the development of placental vasculature in early pregnancy and the failure of transformation of the spiral arteries are associated with the pathogenesis of preeclampsia. Sex hormones influence neovascularisation during pregnancy. However the profiling of estrogen and progesterone in preeclampsia is controversial. In this study we investigated the serum levels of estrogen and progesterone in women with preeclampsia. Blood samples were collected from 86 preeclamptic and 97 gestation-matched normotensive pregnancies. The levels of 17β-estradiol (E2), progesterone and 2-methoxyestradiol (2-ME) in serum were measured. In addition, the levels of E2 and progesterone in conditioned media from preeclamptic or normotensive term placental explant cultures or placental explants that had been treated with hydrogen peroxide (H_2O_2) were measured. The expression of estrogen receptors (ER) and progesterone receptors (PR) in preeclamptic and control placentae were measured by immunohistochemistry. The serum levels of E2, progesterone and 2-ME were significantly reduced in women with preeclampsia compared to controls. There was no difference in the serum levels of E2 and progesterone between severe and mild or between early-onset and late-onset preeclampsia as well as between preeclampsia with and without fetal growth restriction (FGR). The levels of E2 and progesterone in preeclamptic placental explants cultures were significantly lower than in normotensive term placental explant cultures. Treatment with H_2O_2 was found to be associated with a reduction in E2 production by the placenta. We demonstrated lower levels of estrogen in preeclampsia and speculate that this reduction may be due to the impairment of placental function in preeclampsia.

1. Introduction

Preeclampsia, a pregnancy specific disorder is characterised by the onset of hypertension and either proteinuria or end-organ dysfunction after 20 weeks of gestation [1]. Although the pathogenesis of this disease is unclear, abnormalities in the development of placental vasculature in early pregnancy and the failure of transformation of the spiral arteries by extravillous trophoblast which result in relative placental under perfusion/hypoxia/ischemia have been suggested to be involved in the pathogenesis of this disease [2,3].

During pregnancy, circulating levels of estrogen and progesterone, which are the main pregnancy hormones, are significantly increased throughout the gestation and reach their peak level in the third trimester [4]. Both estrogen and progesterone are produced by placental trophoblast from 6 to 8 weeks of gestation [4]. The increase in estrogen during pregnancy enables the uterus and placenta to improve vascularization, transfer nutrients, and support the developing fetus [5,6] as well as regulating cardiovascular adaptation such as increasing blood flow during pregnancy (review in [7]). Recent studies suggested that estrogen also promotes extravillous trophoblast invasion through its receptor G-protein-coupled receptor-30 (GPR30) [8] and estrogen deficiency affects endothelial cell function which is a key feature of preeclampsia [9]. In animal models, inhibition of estrogen production results in pregnancy loss [10,11]. In addition, during pregnancy progesterone keeps the placenta functioning properly and the uterine lining healthy and thick through helping the endometrium to secrete special proteins during the second half of the menstrual cycle. Progesterone is also important in suppressing the maternal immunologic

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Table 1
Clinical characteristics of the study cohort used for analyzing serum estrogen and progesterone.

		Severe PE	Mild PE	Controls	P value
Early onset	No. of cases	N = 15	N = 20	N = 40	N/A
	Age (year, mean ± SD)	31.9 ± 5.5	30.2 ± 5.7	27.8 ± 4.3	P < .05
	Gestational age at sampling (week, mean ± SD)	29^{+2} ± 2.8	31^{+3} ± 2.5	30^{+3} ± 2.7	p > .05
	Systolic blood pressure (mmHg, mean ± SD)	172 ± 18	145 ± 6	112 ± 8	p < .0001
	Diastolic blood pressure (mmHg, mean ± SD)	109 ± 13	99 ± 8	76 ± 10	p < .0001
Late onset	No. of cases	N = 16	N = 35	N = 57	N/A
	Age (year, mean ± SD)	31.4 ± 6.6	30.2 ± 7.0	27.4 ± 4.2	P < .05
	Gestational age at sampling (week, mean ± SD)	36^{+6} ± 1.6	37^{+3} ± 1.7	37^{+2} ± 1.8	p > .05
	Systolic blood pressure (mmHg, mean ± SD)	171 ± 16	142 ± 8	123 ± 6	p < .0001
	Diastolic blood pressure (mmHg, mean ± SD)	115 ± 12	95 ± 6	68 ± 8	p < .0001

PE: preeclampsia; N/A: not applicable.

response to fetal antigens, thereby preventing maternal rejection of the trophoblast. Furthermore, progesterone stimulates endometrial endothelial cell proliferation and enhances endometrial angiogenesis in early pregnancy in animal models to allow implantation [12].

There is growing evidence that there are changes in circulating levels of estrogen and progesterone in preeclampsia suggesting sex hormones may be associated with the pathogenesis of preeclampsia [13–18]. However, the profiling of estrogen and progesterone in preeclampsia is controversial. Some studies suggested that there was no change in the levels of estrogen in preeclampsia compared to normotensive pregnancy [14,17], while other studies reported reduced levels of estrogen in preeclampsia [13,15,16,18]. One study reported increased levels of progesterone in preeclampsia [14], however other studies reported that there was no difference in the levels of progesterone in preeclamptic compared to normotensive pregnancies [16,17]. The reasons for these discrepancies are probably multifactorial, but small sample size in those previous studies could be one of the main reasons. Indeed, most of the previous studies involve < 30 patients including all the subtypes of preeclampsia. In addition, whether the changes of estrogen and progesterone levels in preeclampsia are associated with the time of onset or the severity of preeclampsia has not been investigated yet.

Given the importance of estrogen and progesterone in maintaining a successful pregnancy, in this study with a relative large sample size, we investigated the serum levels of estrogen and progesterone in women with preeclampsia taking into account the time of onset and severity status. In addition, we also investigated whether the production of estrogen or progesterone differed from preeclamptic and normotensive placenta and the potential cause of the changes in the production of estrogen.

2. Materials and methods

This study was approved by the Ethics Committee of Wuxi Maternity and Children Hospital, Nanjing Medical University, Wuxi, China. All patient-derived blood samples and tissues were obtained with written informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

2.1. Collection of blood samples for estrogen and progesterone analysis and placentae for estrogen and progesterone receptors analysis

Blood samples from 86 women who presented with preeclampsia at diagnosis with a singleton pregnancy and 97 gestation-matched normotensive pregnant women were collected by venepuncture into plain Vaccutainer® tubes prior to any treatment between January 2013 and March 2014 at the Wuxi Maternity and Children Health Hospital of Nanjing Medical University of China. The blood was allowed to clot, centrifuged at 2500*g* and the serum was aspirated and stored in aliquots at −80 °C. The gestation matched blood samples from normotensive pregnant women were collected and stored until the completion of pregnancy to confirm that the pregnancy was free from complications before use. Of the 86 women with preeclampsia, 35 were diagnosed with severe preeclampsia, and 31 were diagnosed with early onset preeclampsia.

In addition, 5 preeclamptic placentae from singleton pregnancies (including two from severe preeclampsia and three from mild preeclampsia) and 5 term placentae from uncomplicated pregnancies were collected after caesarean section.

Preeclampsia was defined as a maternal systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured on two occasions separated by at least 6 h, and proteinuria > 300 mg in a 24 h period or qualitative, > 1 +, or impaired liver function, after 20 weeks of gestation in accordance with the guidelines of the American College of Obstetricians and Gynecologists [19]. A maternal systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg was defined as severe preeclampsia. Early onset of preeclampsia was defined as occurring at < 34 weeks of gestation.

The summary of clinical characteristics of 86 women with preeclampsia for blood collection is shown in Table 1.

2.2. Determination of the levels of 17β-estradiol (E2), progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in serum

The serum levels of E2, progesterone, FSH and LH in women with preeclampsia and gestation-matched normotensive pregnant women were measured using ELISA kit following the manufacturer's instructions (Beckman Coulter, USA).

2.3. Determination of the levels of 2-methoxyestradiol (2-ME) in serum

Maternal serum levels of 2-ME in women with preeclampsia and gestation-matched normotensive pregnant women were measured using a 2-ME EIA kit following the manufacturer's instructions (Cayman Chemical, MI, and USA).

2.4. Levels of estrogen and progesterone in placental explants culture medium

Placental explants (approximately 400 mg) were dissected from preeclamptic (n = 5, including two from severe preeclampsia and three from mild preeclampsia) or normotensive term placentae (n = 5) and then cultured in 12 well culture plates for 24 h at 37 °C in DMEM/F12 containing 10% FBS in an ambient oxygen atmosphere containing 5% CO_2. In some experiments, first trimester placental explants from elective abortion (approximately 400 mg) (n = 5) were cultured in the presence or absence of hydrogen peroxide, H_2O_2 (200 μM or 400 μM) for 24 h. The viability of placental explants after treatment with H_2O_2 was examined with CMFDA, a live cell tracker dye (InVitrogen,