Levels of sex steroid hormones and their receptors in women with preeclampsia

Kuo-Chung Lan (✉ lankuochung@gmail.com)

Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University college of Medicine, Kaohsiung, Taiwan <https://orcid.org/0000-0003-3702-0091>

Yun-Ju Lai

Chang Gung Memorial Hospital Kaohsiung Branch

Hsin-Hsin Cheng

Chang Gung Memorial Hospital Kaohsiung Branch

Ni-Chin Tsai

Chang Gung Memorial Hospital Kaohsiung Branch

Yu-Ting Su

Chang Gung Memorial Hospital Kaohsiung Branch

Ching-Chang Tsai

Chang Gung Memorial Hospital Kaohsiung Branch

Te-Yao Hsu

Chang Gung Memorial Hospital Kaohsiung Branch

Research

Keywords: preeclampsia, placenta, sex steroids, steroid receptor

Posted Date: December 3rd, 2019

DOI: <https://doi.org/10.21203/rs.2.18092/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Reproductive Biology and Endocrinology on February 18th, 2020. See the published version at <https://doi.org/10.1186/s12958-020-0569-5>.

Abstract

Background: Pregnant women have high serum concentrations of sex steroid hormones, which are major regulators of paracrine and autocrine responses for many maternal and placental functions. The main purpose of this study is to compare patients with preeclampsia and patients with uncomplicated pregnancies in terms of serum of steroid hormones (estradiol [E2], progesterone [P4], dehydroepiandrosterone sulfate [DHEAS], and testosterone [T]) throughout pregnancy, and the levels of cord blood and placental steroid receptors during the third trimester.

Methods: Quantitative real-time reverse transcription PCR, western blotting, and immunohistochemistry were used to determine the levels of steroid hormones in the serum and cord blood, and the placental levels of estrogen receptor- α (ER α), ER β , androgen receptor (AR), and progesterone receptor (PR).

Results: There were 45 women in the uncomplicated pregnancy group and 30 women in the preeclampsia group. Serum levels of T were greater and serum levels of E2 were reduced in the preeclampsia group, but the two groups had similar levels of P4 and DHEAS during the third trimester. Cord blood had a decreased level of DHEAS in the preeclampsia group, but the two groups had similar levels of P4, E2, and T. The two groups had similar placental mRNA levels of ER α , ER β , AR, and PR, but the preeclampsia group had a higher level of ER β protein and a lower level of ER α protein. Immunohistochemistry indicated the preeclampsia group had a greater level of ER β in the nucleus and cytoplasm of syncytiotrophoblasts and stromal cells.

Conclusions: Women with preeclampsia had lower levels of steroid hormones, estrogen, and ER α , but higher levels of T and ER β . These molecules may have roles in the pathogenesis of preeclampsia.

Background

Preeclampsia is a pregnancy-specific hypertensive disorder with multisystem involvement that is associated increased risk of future cardiovascular disease [1]. The pathogenesis of preeclampsia has not been fully elucidated, but much progress has been made during recent decades [2]. In particular, it is widely accepted that preeclampsia is associated with abnormal placentation, reduced placental perfusion, and systemic vasospasm. A two-stage model of preeclampsia proposed that incomplete spiral artery remodeling in the uterus contributes to placental ischemia and the release of antiangiogenic factors from the ischemic placenta into the maternal circulation, thus contributing to endothelial damage [2].

During pregnancy, the placenta is the primary endocrine organ for maintaining pregnancy and fetal growth. The placenta releases hormones, including androgens, estrogens, and progestogens, and these occur at extremely high concentrations in the maternal circulation. These hormones are important paracrine and autocrine regulators that affect the growth and differentiation of the placental trophoblast, growth and maturation of the placental vascular tree, and uterine endovascular invasion by the extravillous cytotrophoblast [3, 4].

Progesterone and estrogens also function as modulators of uterine vessels, in that they decrease resistance of the spiral uterine arteries and modulate the synthesis and release of angiogenic factors by placental cells. Androgens have the opposite effect. Previous studies have compared the serum levels of sex steroid hormones and signaling in women who had preeclampsia with women who had uncomplicated pregnancies [4–7]. Unsurprisingly, women with preeclampsia exhibited altered serum concentrations of sex steroid hormones. However, no studies have yet examined the role of placental steroid hormone receptors and cord blood steroid hormone concentrations in the pathogenesis of preeclampsia.

The main purpose of the present study is to compare pregnant women with preeclampsia and women with uncomplicated pregnancies in terms of the serum and cord blood levels of sex steroid hormones (estradiol [E2], progesterone [P4], dehydroepiandrosterone sulfate [DHEAS], and testosterone [T]) and placental steroid receptors during the third trimester.

Materials And Methods

Study Design

Following approval of the Ethics Committee of the Institutional Review Board of Chang Gung Memorial Hospital (CGMH 201601484A3) and obtaining informed written consent from all subjects, this prospective study was conducted from June 2017 to January 2019. Singleton pregnant women ($n = 78$) were recruited to donate blood samples during the first (gestational age: 8–14 weeks), second (gestational age: 20–24 weeks), and third (gestational age: 32–38 weeks) trimesters of pregnancy, and to provide cord blood and placenta samples during delivery (Figure 1). Among the 50 participants who donated 3 serum samples, 45 women had uncomplicated pregnancies and 5 women (11%) developed preeclampsia. During the study period, 25 women with preeclampsia during the second or third trimesters were referred from other hospitals to the high-risk pregnancy prenatal care center of our institute. Thus, there were 45 women in the uncomplicated pregnancy group and 30 women in the preeclampsia group.

Women in the uncomplicated pregnancy group had no diagnoses of pre-eclampsia or hypertension during pregnancy and no hospitalization due to premature delivery or bleeding. Women in the preeclampsia group were diagnosed based on the presence of new-onset hypertension (systolic blood pressures of 140 mmHg or more, diastolic blood pressures of 90 mmHg or more, or both) on two occasions 6 h or more apart after 20 weeks of gestation, and the presence of significant proteinuria (≥ 300 mg/24 h). None of the patients had a previous history of any known endocrinopathy. Women were excluded if they were smokers, alcoholics, had a chronic maternal disease (essential hypertension, connective tissue diseases, hyperthyroidism, hypothyroidism, chronic glomerulonephritis, renal failure, and diabetes mellitus), or gestational diabetes.

Tissue and blood collection

Human placental tissues and blood samples of the healthy group (n = 45) and the preeclampsia group (n = 30) were collected and immediately stored at -80°C . Blood was collected in plastic tubes under aseptic conditions, with EDTA as an anti-coagulant, and centrifuged at $18,472 \times g$ for 10 min at 4°C to separate the serum. Serum concentrations of E2 and P4 were analyzed using commercially available immunoassay systems (ADVIA Centaur XP; Siemens USA). The intra-assay and interassay coefficients of variation were 5.0% and 4.1%, respectively, for E2; and 5.2% and 3.5%, respectively, for P4.

Immunohistochemistry

Tissues were paraffin-embedded and subjected to immunohistochemical staining. For this procedure, 4 μm -sections were deparaffinized and rehydrated, rinsed in purified water, and treated with 3% H_2O_2 for 15 min at room temperature. After rinsing three times with purified water, they were heated in an autoclave for 1 h with 10 mM citrate buffer, and then incubated with primary antibodies against the estrogen receptor- α (ER α ; EP1 diluted 1:50, Bio SB, CA, USA), ER β (14C8 diluted 1:100, Abcam, Cambridge, UK), progesterone receptor (PR; NCL-L-PGR-312 diluted 1:50, Leica Biosystems, Benton Lane, UK), and androgen receptor (AR; Clone SP107 diluted 1:50, ZECA, CA, USA). After addition of the appropriate secondary IgG antibody, sections were incubated with DAB (K5007, Dako, Denmark) and counterstained with hematoxylin and eosin (H&E; 1.05174, Merck, MA, USA). Finally, sections were dehydrated in a graded series of ethanol, cleared with xylene, mounted using Histomount (008030, Life technologies, MD, USA), and coverslips were applied for evaluation by light microscopy.

Western blot analysis

Placental tissues were washed in PBS and lysed in RIPA Lysis Buffer (20–188, Merck, MA, USA). Proteins were separated by electrophoresis using 8% SDS/PAGE, and then transferred to PVDF Blotting Membranes (10600022, GE Healthcare, Germany). Blots were probed with a primary antibody, and then developed using ImmobilonTM Western (WBKLS0500, Millipore, MA, USA). The primary antibodies were against ER α (MA1–39540, Thermo Fisher, IL, USA), ER β (PA1–310B, Thermo Fisher, IL, USA), PR (MA1–411, Thermo Fisher, IL, USA), AR (06–680, Millipore, CA, USA), and GAPDH (MAB374, Millipore, CA, USA).

RNA extraction and quantitative real-time reverse transcription PCR

Total RNA was isolated from placental tissues using the RNA Clean & Concentrator–5 kit (R1014, Zymo Research, CA, USA) and reverse transcribed. Quantitative real-time reverse transcription-PCR (qRT-PCR) was performed using the Fast SYBR[®] Green Master Mix (Applied Biosystems, CA, USA) and the ABI 7500 Fast Real-Time PCR System (Applied Biosystems), with the primers listed in Table 1.

Statistical analysis

SPSS version 10.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Proportions were compared using the chi-square test or Fisher's exact test, as appropriate, and a *P* value below 0.05 was considered statistically significant.

Results

The preeclampsia group (n = 30) and the uncomplicated pregnancy group (n = 45) had similar age, hemoglobin level, parity, gravidity, and maternal body mass index (Table 2). However, the preeclampsia group had higher blood pressure and markedly decreased infant birth weight.

Comparison of serum data during the third trimester indicated that the uncomplicated pregnancy group had a greater level of serum T and a decreased level of E2, but the two groups had similar levels of P4 and DHEAS (Figure 2A). Comparison of cord blood indicated the preeclampsia group had a decreased level of DHEAS, but the two groups had similar levels of P4, E2, and T (Figure 2B).

We determined the expression of placental hormone receptors in the two groups using western blotting and qRT-PCR. The two groups had similar mRNA levels of ER α , ER β , AR, and PR (Figure 3). However, the preeclampsia group had a greater protein level of ER β and decreased protein level of ER α . The two groups had similar protein levels of AR and PR (Figure 4B).

We further characterized the expression patterns of these hormone receptors in placental tissues by H&E staining and immunohistochemical analysis of formalin-fixed tissues. The H&E staining results indicated the cytotrophoblast was surrounded by a layer of syncytiotrophoblasts, a general morphological feature of the placenta. The immunohistochemical staining indicated that ER β was present in cyto- and syncytiotrophoblast cells of women in the uncomplicated pregnancy group, but was mostly present in the nuclei of syncytiotrophoblast cells of women in the preeclampsia group (Figure 4A). The two groups had no evident differences in the placental distributions of other hormone receptors.

Measurements of the serum levels of hormones throughout pregnancy indicated that the preeclampsia group had increased T levels but decreased E2 levels (Figure 5). However, the two groups had similar P4 levels throughout pregnancy.

Discussion

Female sex steroid hormones, including E2 and P4, have significant physiological roles during menstruation and pregnancy. The ovaries, adrenal glands, and placenta produce these hormones, and the placenta is the major endocrine organ during pregnancy. During pregnancy, the complex process of steroidogenesis occurs in multiple organs, including the maternal uterus, placenta, fetal membrane, and the maternal and fetal hypothalamic-pituitary-adrenal axis, although most steroidogenesis occurs in the placenta.

During pregnancy, the placenta primarily produces estrogen by conversion of androgen precursors that originate in maternal and fetal adrenal glands. Many studies have reported that serum estrogen levels increase progressively from 22 week of gestation, and are important regulators of placental and embryo growth [8]. This is consistent with our findings (Figure 5)

Furthermore, we also found that women in the preeclampsia group had lower serum estrogen levels in the third trimester, and lower ER α and higher ER β protein expression in their placentas. A recent study reported that altered serum concentrations of steroid hormones, including E2, may be associated with preeclampsia [9]. Estrogen has specific metabolic effects, in that it stimulates the expression of vascular endothelial growth factor (VEGF) and angiogenesis. However, aberrant production of estrogens could promote preeclampsia because they are exclusively produced by the placenta and they promote angiogenesis and vasodilation. More recent studies suggested a marked decrease in E2 levels in women with preeclampsia [7]. This is consistent with our findings (Figure 2A and Figure 5).

Estrogen acts by binding to ER α or ER β , which are both located in the nucleus but have distinct tissue-specific expression patterns, or by binding to other receptors located in the plasma membrane and cytoplasmic organelles. ER α and ER β are similar in terms of ligand-binding and nuclear DNA-binding regions, and there are numerous mRNA splice-variants for both receptors in diseased and normal tissues. In a ligand-dependent fashion, each ER pool contributes to the overall effects of estrogens on biological outcomes. Kim et al. suggested that placental ER α and ER β had higher expression at term period compared with early preterm, and that they were located in cyto- and syncytiotrophoblast cells [8]. The placentas of our preeclampsia group had a decreased protein level of ER α , and an increased protein level of ER β , but no alterations in the levels of the corresponding mRNAs. Many studies have measured the concentration of estrogen during pregnancy, but only Yin et al. examined ER α expression in the placenta of preeclampsia patients [10]. Their findings are consistent our findings, although they did not measure the level of ER β .

P4 reduces vascular resistance by decreasing the sensitivity to angiotensin and increasing the production of endothelial vasodilator, which directly affects muscles [11]. A previous study reported that the serum P4 level was markedly increased in women with preeclampsia compared with healthy pregnant women [6]. These results are inconsistent with our data.

The relationship between androgens and maternal cardiovascular and placental function deserves particular consideration, because the serum T level in preeclamptic women is elevated and correlates with vascular dysfunction [5]. Our finding of elevated serum T in women with preeclampsia supports previous studies, thus suggesting that increased androgens and androgen signaling may contribute to preeclampsia [5, 12, 13].

The adrenal cortex produces large amounts of DHEAS and androstenedione during the fetal period, but this production decreases rapidly after birth. However, the physiological role of these changes is still unclear. DHEA is a precursor to testosterone. Interestingly, our results demonstrated that women in the uncomplicated pregnancy and preeclampsia groups had similar serum levels of DHEAS, but the preeclampsia group had a lower cord blood level of DHEAS. The physiological role of DHEAS requires further study.

Our research provided novel insights into the relationship of sex hormone status and preeclampsia. However, our study was limited by the small number of patients. Thus, confirmation requires examination

of a larger cohort, with longitudinal data, and using highly reliable technology, such as gas chromatography/mass spectrometry [7].

In summary, our findings suggest that preeclampsia is associated with distinct hormonal milieus. In particular, we found that E2 and ER α are down-regulated, and T and ER β are upregulated in preeclamptic pregnancies. This suggests the involvement of these molecules in the pathogenesis of preeclampsia.

List Of Abbreviations

E2: estradiol; ER α : estrogen receptor- α ; ER β : estrogen receptor- β ; DHEA: dehydroepiandrosterone; DHEAS: dehydroepiandrosterone sulfate; VEGF: vascular endothelial growth factor; P4: progesterone; qRT-PCR: Quantitative real-time time reverse transcription-PCR; T: testosterone; VEGF: vascular endothelial growth factor

Declarations

Ethics approval

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (CGMH 201601484A3).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by CMRPG8G0101 from Chang Gung Memorial Hospital.

Authors' contributions

Conception and design of study: CCT, TYH, YJL; enrolled the subjects: CCT, TYH, YJL; analysis of data and writing of the manuscript: KCL,NCT; critical revision of the article for intellectual content: KCL NCT,YJL, HHC,YTS. All authors read and approved the final manuscript.

References

1. Shah S, Gupta A: *Hypertensive Disorders of Pregnancy. Cardiol Clin* 2019, 37:345–354.
2. Phipps E, Prasanna D, Brima W, Jim B: *Preeclampsia: Updates in Pathogenesis, Definitions, and Guidelines. Clin J Am Soc Nephrol* 2016, 11:1102–1113.
3. Armaly Z, Jadaon JE, Jabbour A, Abassi ZA: *Preeclampsia: Novel Mechanisms and Potential Therapeutic Approaches. Front Physiol* 2018, 9:973.
4. Maliqueo M, Echiburru B, Crisosto N: *Sex Steroids Modulate Uterine-Placental Vasculature: Implications for Obstetrics and Neonatal Outcomes. Front Physiol* 2016, 7:152.
5. Kumar S, Gordon GH, Abbott DH, Mishra JS: *Androgens in maternal vascular and placental function: implications for preeclampsia pathogenesis. Reproduction* 2018, 156:R155-R167.
6. Hertig A, Liere P, Chabbert-Buffet N, Fort J, Pianos A, Eychenne B, Cambourg A, Schumacher M, Berkane N, Lefevre G, et al: *Steroid profiling in preeclamptic women: evidence for aromatase deficiency. Am J Obstet Gynecol* 2010, 203:477 e471–479.
7. Berkane N, Liere P, Oudinet JP, Hertig A, Lefevre G, Pluchino N, Schumacher M, Chabbert-Buffet N: *From Pregnancy to Preeclampsia: A Key Role for Estrogens. Endocr Rev* 2017, 38:123–144.
8. Kim SC, Park MN, Lee YJ, Joo JK, An BS: *Interaction of steroid receptor coactivators and estrogen receptors in the human placenta. J Mol Endocrinol* 2016, 56:239–247.
9. Zheng JJ, Wang HO, Huang M, Zheng FY: *Assessment of ADMA, estradiol, and progesterone in severe preeclampsia. Clin Exp Hypertens* 2016, 38:347–351.
10. Yin G, Zhu X, Guo C, Yang Y, Han T, Chen L, Yin W, Gao P, Zhang H, Geng J, et al: *Differential expression of estradiol and estrogen receptor alpha in severe preeclamptic pregnancies compared with normal pregnancies. Mol Med Rep* 2013, 7:981–985.
11. Albrecht ED, Pepe GJ: *Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. Int J Dev Biol* 2010, 54:397–408.
12. Hsu TY, Lan KC, Tsai CC, Ou CY, Cheng BH, Tsai MY, Kang HY, Tung YH, Wong YH, Huang KE: *Expression of androgen receptor in human placentas from normal and preeclamptic pregnancies. Taiwan J Obstet Gynecol* 2009, 48:262–267.
13. Shin YY, Jeong JS, Park MN, Lee JE, An SM, Cho WS, Kim SC, An BS, Lee KS: *Regulation of steroid hormones in the placenta and serum of women with preeclampsia. Mol Med Rep* 2018, 17:2681–2688.

Tables

Table 1. Primer sequences		
	Forward	Reverse
18S	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGTG
ER- α	CAGGAACCAGGGGAAAATGTG	AACCGAGATGATGTAGCCAGC
ER- β	ACTTGCTGAACGCCGTGACC	CAGATGTTCCATGCCCTTGTT
PR	TGAATCCGGCCTCAGGTAGTT	CGCGCTCTACCCTGCACTC
AR	TCACCGCACCTGATGTGTG	ACATGGTCCCTGGCAGTCTC

Table 2. Patient characteristics

	Normal pregnant women (N=45)	Preeclampsia women (N=30)	p
Age (yr)	33.44 $\pm$ 4.38	35.45 $\pm$ 5.10	NS
Hemoglobin (g/dL)	11.6 $\pm$ 1.53	12.17 $\pm$ 1.48	NS
Gestational week (wk)	38.99 $\pm$ 2.31	36.38 $\pm$ 1.85	< 0.001
Parity nulliparity	15 (33%)	18 (60%)	< 0.01
Parity nulliparity	30 (67%)	12 (40%)	< 0.01
Body Mass Index (BMI)	27.83 $\pm$ 5.06	32.33 $\pm$ 6.28	< 0.01
Birth weight (g)	3045.98 $\pm$ 717.33	2273.55 $\pm$ 886.27	< 0.001
Systolic blood pressure (mmHg)	118.89 $\pm$ 23.68	163.04 $\pm$ 18.71	< 0.001
Diastolic blood pressure (mmHg)	74.23 $\pm$ 11.98	101.79 $\pm$ 12.37	< 0.001

* Data are expressed as mean $\pm$ standard deviation. NS = not significant.

Figures

Fig 1

Figure 1

Flow chart of study population

Fig 2

Figure 2

Levels of estradiol, testosterone, DHEAS, progesterone in women with preeclampsia compared to women with uncomplicated pregnancies (A) serum in third trimester (B) cord blood * $P < 0.05$

Fig 3.

Figure 3

Quantitative evaluation of ER α , ER β , AR and PR mRNA in placenta by real time reverse transcriptase polymerase chain reaction.

Fig 4

Figure 4

The localization and expression of ER α ,ER β ,AR and PR proteins in uncomplicated pregnancies and preeclampsia placenta (bottom panel) were analyzed by immunohistochemical analysis. Specific and robust ER β immunostaining was detected exclusively in the nucleus and cytoplasm of syncytiotrophoblasts and stromal cells. Magnification $\times 400$. ER α and ER β protein expression was significantly lower and higher in placenta from preeclampsia compared to controls by Western blot.

Fig 5

Figure 5

Trajectories of hormone concentrations in women with preeclampsia compared to women with uncomplicated pregnancies