Archives of Gynecology and Obstetrics (2019) 299:1551–1556
https://doi.org/10.1007/s00404-019-05126-8

MATERNAL-FETAL MEDICINE

Wnt signaling pathway in early- and late-onset preeclampsia: evaluation with Dickkopf-1 and R-Spondin-3 glycoproteins

Ahter Tanay Tayyar[1] · Resul Karakus[2] · Mefkure Eraslan Sahin[3] · Nura Fitnat Topbas[4] · Erdem Sahin[3] · Seren Karakus[2] · Emel Tugce Yalcın[2] · Ahmet Tayyar[5]

Received: 20 November 2018 / Accepted: 16 March 2019 / Published online: 23 March 2019

Abstract

Objective Wnt signaling has been identified as an essential pathway that can direct cell proliferation, migration, and tissue homeostasis. This study aimed to evaluate the role of Wnt signaling pathway in early-onset and late-onset preeclampsia (PE) using serum Dickkopf-1 and R-Spondin-3 glycoproteins.

Study design A total of 80 pregnant women were included in this study. The patients were divided into three groups: (1) control (2) early-onset PE, and (3) late-onset PE. The serum levels of Dickkopf-1 and R-Spondin-3 were measured using an enzyme-linked immunosorbent assay.

Results Of the 80 pregnant women enrolled in the study, 27 were control, 27 had early-onset PE, and 26 had late-onset PE. No differences were found in the maternal age, gravida, parity, and body mass index among the groups ($P=0.536$, 0.230, 0.202, and 0.642, respectively). The serum level of Dickkopf-1 was significantly higher in the early-onset PE group compared with the control group ($P=0.006$). The serum level of Dickkopf-1 was statistically similar in control group compared to late-onset PE group ($P=0.064$). However, no significant difference was found in the serum levels of Dickkopf-1 and R-Spondin-3 between the early- and late-onset PE groups ($P>0.05$). Additionally, the Spearman's correlation analysis revealed a significant negative correlation between maternal serum level of Dickkopf-1 and maternal age ($r=-0.522$, $P=0.005$).

Conclusion The increased serum level of Dickkopf-1 might be associated with the process of pathogenesis of early-onset PE. Further studies would elucidate their exact roles in the pathogenesis of PE.

Keywords Dickkopf-1 · Early-onset preeclampsia · Late-onset preeclampsia · R-Spondin-3 · Wnt signaling pathway

Introduction

Preeclampsia (PE) is a pregnancy condition characterized by proteinuria and hypertension that is one of the main causes of mortality and morbidity in pregnancy, affects 5–7% of all pregnancies [1]. It can develop at any time after the 20th week of pregnancy. The first stage of the disease is far more serious than the later stages, and the results are very risky for both the mother and the child [2, 3]. As the only solution for this syndrome is the removal of the placenta out of the body during the birth, the pathogenesis of PE is thought to be caused by the placenta itself [4].

Multiple signaling pathways mediate the function of trophoblasts during the placentation process. The exact mechanism of this process is not yet fully understood. Indeed, the pathological mechanisms in these processes are quite complex. Several recent studies have investigated the relationship between the Wnt pathway and complications in

✉ Mefkure Eraslan Sahin
mefkureee@hotmail.com

1 Department of Obstetrics and Gynecology, Acıbadem University Medical Faculty Maslak Acıbadem Hospital, Istanbul, Turkey

2 Department of Obstetrics and Gynecology, Zeynep Kamil Maternity and Children's Training and Research Hospital, Health Sciences University, Istanbul, Turkey

3 Department of Obstetrics and Gynecology, Sivas Sarkisla Government Hospital, Sivas, Turkey

4 Department of Obstetrics and Gynecology, Kanuni Sultan Süleyman Education and Research Hospital, Health Sciences University, Istanbul, Turkey

5 Department of Obstetrics and Gynecology, Medipol University Medical Faculty, Istanbul, Turkey

human pregnancies. Wnt signaling is defined as an important pathway that mediates cell proliferation, cell migration, and tissue homeostasis [5]. When a Wnt ligand protein binds to Frizzled receptors and their co-receptors, the canonical Wnt signal is activated. By transmitting the signal to the cytoplasm by a number of cellular factors, β-catenin stabilization is achieved in the cytoplasm. This allows β-catenin to accumulate and localize to the nucleus and afterwards stimulate a cellular response via a targeted gene transduction by forming complexes with the T-cell factor/lymphoid-enhancing factor [5]. In the literature it is well documented that Wnt signaling pathway especially serum Dickkopf-1 levels was associated with breast, pancreas, thyroid and gynecologic cancer and serum Dickkopf-1 can be a novel serological biomarker for the diagnosis and prognosis [6–10].

Dickkopf-1 is a glycoprotein that is secreted to antagonize the Wnt signaling pathway. It influences a large number of biological processes in this pathway [11, 12]. In contrast, the R-Spondin (RSpo1–4) protein family plays a role in the activation and regulation of the Wnt signaling pathway [13, 14]. A relationship might exist between the Wnt signaling pathway and the abnormal existence of PE. This study aimed to comprehend the role of Wnt signaling pathway in the early-onset PE (eo-PE) and late-onset PE (lo-PE) using serum Dickkopf-1 and R-Spondin-3 glycoproteins.

Materials and methods

Study population and inclusion criteria

The present study was conducted between March and December 2017 at the Health Sciences University Zeynep Kamil Women and Children's Health Education and Research Hospital and approved by the Ethics Committee (Decision number: 162/2017). It was carried out in accordance with the Declaration of Helsinki. All participants gave their informed consent to participate in the study.

A total of 80 pregnant women were included in the study. The patients were divided into three groups: (1) control (2) eo-PE, and (3) lo-PE. The exclusion criteria included multiple pregnancies, early membrane rupture, chromosomal or fetal anomaly, type 2 diabetes mellitus or gestational diabetes mellitus, chronic hypertension, collagen vascular disease, and chronic systemic diseases. We included health volunteers (control group) in their routine clinical visits and preeclamptic pregnants were included in the study when hospitalized.

The patients, who had no history of high blood pressure formerly, were diagnosed with PE according to the following criteria: a systolic blood pressure (SBP) 140 mmHg and a diastolic blood pressure (DBP) 90 mmHg, measured at least two times with a 6-h interval after 20 weeks of gestation, and a proteinuria (0.3 g/24 h) or spot urine protein creatinine ratio (PCR) 30 mg/mmol [15].

The patients with a gestational age lower than 34 weeks were classified into the eo-PE group and those with a gestational age of 34 weeks or more into the lo- PE group [16]. A diagnosis of severe PE was made if the patients had a blood pressure 160/110 mmHg, thrombocytopenia (< 100,000/mL), elevated liver enzymes, severe right-upper-quadrant pain that was resistant to medical treatment, pulmonary edema, cerebral and visual symptoms, and progressive renal failure (serum creatinine > 1.1 mg/dL) [15]. The gestational age was determined according to the first day after the last menstrual period and was confirmed by first-trimester crown rump length measurement. The patients were examined using the Toshiba Xario (Tokyo, Japan) with a 3.5-MHz color pulsed Doppler according to the methods described in the previous studies [17]. The diagnosis of fetal growth restriction (FGR) was defined as predictive fetal weight < 10 percentile [1].

Data collection

Venous blood samples were collected from normotensive women having eo-PE or lo-PE who were admitted to the hospital before any drugs were administered. None of the patients were at the delivery stage when the blood samples were taken. A total of 5 mL venous bloods were taken in biochemistry tubes. The samples were centrifuged at 1000*g* for 20 min at 4 °C. The supernatant serum samples were then transferred to clean 1.5-mL eppendorf tubes and stored at 80 °C for enzyme-linked immunosorbentassay (ELISA).The serum level of Dickkopf-1 was measured using a sandwich enzyme-linked immunosorbent assay kit (Human Dickkopf-1 PicoKine ELISA Kit, catalog no. EK0867; Boster Biological Technology, CA, USA). The assay sensitivity was < 10 pg/mL. The serum level of R-Spondin-3 was also measured using a sandwich ELISA kit (Human R-Spondin-3 PicoKine ELISA Kit, catalog no. EK1512; Boster Biological Technology). The assay sensitivity was < 10 pg/mL.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Science software version 24 (IL, USA). Conformity of the data to a normal distribution was evaluated using the Kolmogorov–Smirnov test, and the homogeneity of variance was assessed using the Levene test. The variables with normal distributions were compared between groups using the analysis of variance (ANOVA)/Bonferroni test and were expressed as mean ± standard deviation. The Kruskal–Wallis test was used to analyze nonnormally distributed variables, and the results were expressed as median and interquartile range. The Chi-square test was used for

comparison of categorical variables, and the data were presented as proportions. Correlations were assessed using the Spearman's correlation coefficient, along with related *P* values. All analyses were two tailed, and a *P* value less than 0.05 was considered statistically significant.

Results

Of the 80 pregnant women enrolled in the study, 27 were control, 27 had eo-PE, and 26 had lo-PE. The clinical and demographic characteristics of the study groups are illustrated in Table 1. No differences were found in the maternal age, gravida, parity, and BMI among the groups ($P = 0.536$, 0.230, 0.202, and 0.642, respectively). Maximum SBP and DBP were significantly higher in the eo-PE and lo-PE groups compared with the control group ($P < 0.0001$ and < 0.0001, respectively). The gestational age at blood sampling was statistically different among groups due to disease severity ($P < 0.0001$).

The mean gestational age at delivery was 39.09 ± 0.96, 31.17 ± 3.44, and 36.58 ± 1.54 weeks in the control group, eo-PE group, and lo-PE group, respectively. The mean gestational age at delivery was significantly different among groups ($P < 0.0001$). The birth weight was 3284.44 ± 255 g, 1392.296 ± 542.04 g, and 2565.58 ± 626.08 g in the control group, eo-PE group, and lo-PE group, respectively. The birth weight was significantly different among the groups ($P < 0.0001$). The FGR, neonatal intensive care unit, and 5-min Apgar < 7 were significantly increased in the eo-PE group compared with the lo-PE group ($P < 0.0001$, 0.006, and 0.005, respectively).

Table 2 shows the serum levels of Dickkopf-1 and R-Spondin-3. The serum level of Dickkopf-1 was significantly higher in the eo-PE group compared with the control group ($P = 0.006$). The serum level of Dickkopf-1 was

Table 1 Clinical characteristics of patients in the early-onset PE, late-onset PE, and control groups

	Control ($n = 27$)	Early-onset PE ($n = 27$)	Late-onset PE ($n = 26$)	*P* value
Age (years)	29.44 ± 3.66	28.56 ± 4.85	28.08 ± 4.92	0.536
Gravida (*n*)	2 (1)	1 (2)	2 (2)	0.230
Para (*n*)	1 (1)	0 (1)	1(2)	0.202
BMI at blood sampling (kg/m^2)	29.67 ± 2.40	29.35 ± 4.79	30.3 ± 3.83	0.642
Maximum SBP (mmHg)	104.96 ± 9.71	153.52 ± 12.93*	148.04 ± 13.24*	**< 0.0001**
Maximum DBP (mmHg)	66.70 ± 8.29	96.59 ± 9.16*	93.19 ± 7.10*	**< 0.0001**
GA at blood sampling (weeks)	32.93 ± 1.83	29.35 ± 4.09*	35.98 ± 1.69*,†	**< 0.0001**
GA at delivery (weeks)	39.09 ± 0.96	31.17 ± 3.44*	36.58 ± 1.54*,†	**< 0.0001**
Birth weight (g)	3284.44 ± 255	1392.296 ± 542.04*	2565.58 ± 626.08*,†	**< 0.0001**
FGR *n* (%)[a]	–	22 (%81.5)	7 (%26.9)	**< 0.0001**
NICU *n* (%)[a]	–	18 (66.7)	7 (%26.9)	**0.006**
5-min Apgar < 7*n* (%)[a]	–	10 (%37)	1 (%3.8)	**0.005**

Data are expressed as median (interquartile), mean (± standard deviation), or numbers (%). ANOVA followed by Bonferroni post hoc tests and the Kruskal–Wallis test was used for two-group pairwise comparisons

Bold values are statistically significant

BMI body mass index, *DBP* diastolic blood pressure, *FGR* fetal growth restriction, *GA* gestational age, *NICU* neonatal intensive care unit, *SBP* systolic blood pressure

*$P < 0.05$ versus controls

†$P < 0.05$ versus early PE

[a]Chi-squared test was used to compare the proportions between early-and late-onset PE groups

Table 2 Levels of Dickkopf-1and R-Spondin-3 in the early-onset PE, late-onset PE, and control groups

	Control ($n = 27$)	Early-onset PE ($n = 27$)	Late-onset PE ($n = 26$)	*P* value
Dickkopf-1	1667.05 (573.1)	2159.47 (763.9)*	1990.02 (490.4)	**0.006**
R-Spondin-3	34.48 (90.38)	40.38 (59.53)	32.62 (57.96)	0.578

The Kruskal–Wallis test was used for two-group pairwise comparisons. DKK, C/early: 0.006, C/late: 0.064, E/late: 1.000

Bold values are statistically significant

*$P < 0.05$ versus controls

statistically similar in control group compared to lo-PE group $P = 0.064$). However, no significant differences in the serum levels of Dickkopf-1 and R-Spondin-3 were found between the eo-PE and lo-PE groups ($P > 0.05$). Comparison of Dickkopf-1 and R-Spondin-3 levels is illustrated in Figs. 1 and 2.

Correlation analyses between the maternal serum levels of R-Spondin-3, Dickkopf-1 and other parameters are illustrated in Table 3. The Spearman correlation analysis revealed a significant negative correlation between the maternal serum level of Dickkopf-1 and maternal age ($r = 0.522$, $P = 0.005$).

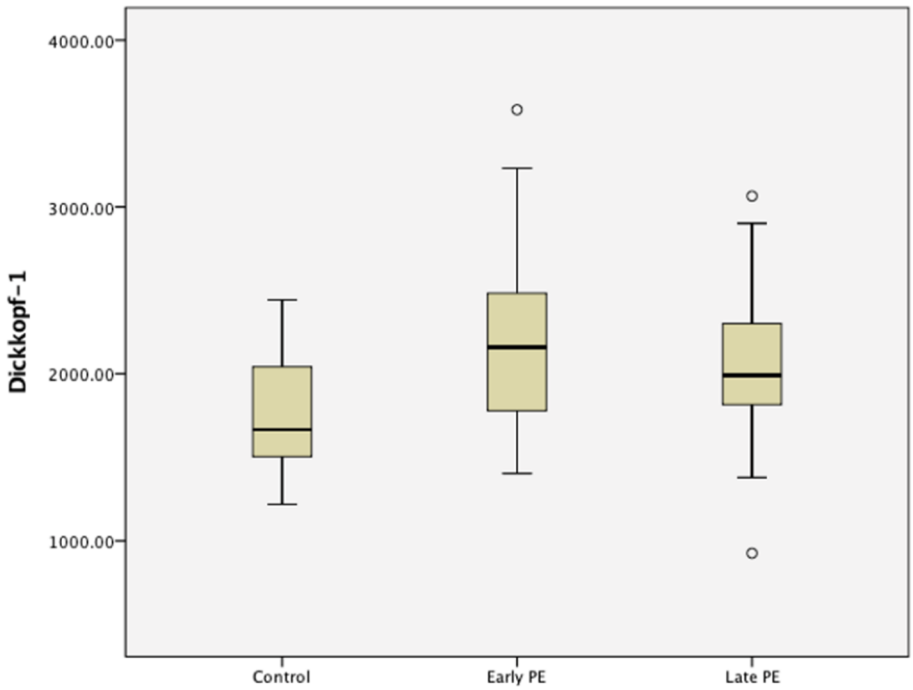

Fig. 1 Comparison of Dickkopf-1 levels among groups

*$P<0.05$ versus controls. DKK, C/early: 0.006, C/late: 0.064, E/late:1.000.

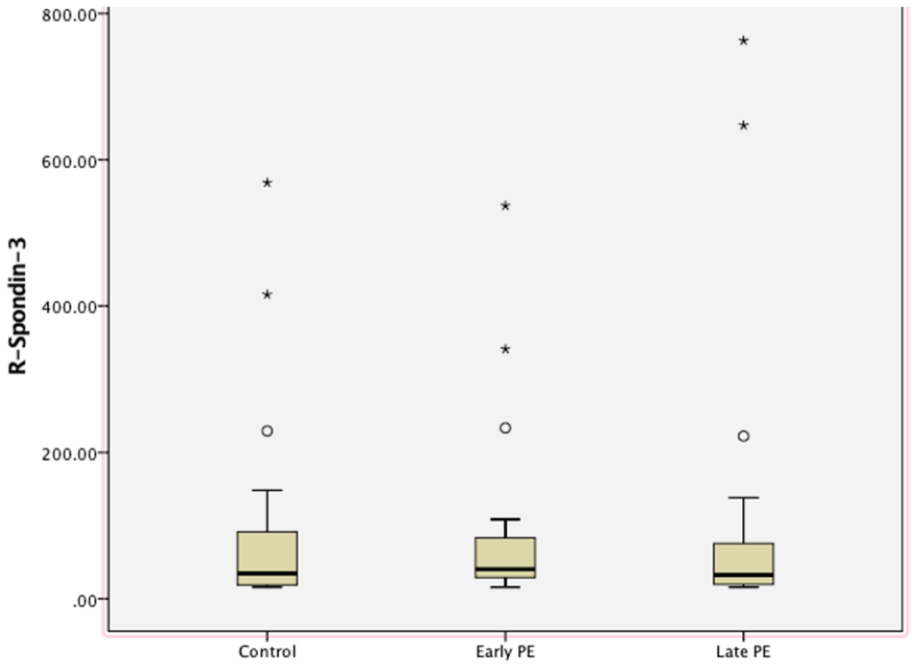

Fig. 2 Comparison of R-Spondin-3 levels among groups

*$P<0.05$ versus controls

Table 3 Correlation analyses between Dickkopf-1 and R-Spondin-3 levels in the maternal serum and clinical parameters

	Dickkopf-1		R-Spondin-3	
	r_s	*P*	r_s	*P*
Age (years)	0.522	0.005	0.073	0.716
BMI at blood sampling (kg/m^2)	0.030	0.883	0.021	0.916
GA at blood sampling (weeks)	0.039	0.848	0.032	0.875

BMI body mass index, *GA* gestational age, r_s Spearman's correlation coefficient

Discussion

PE is a leading cause of morbidity and mortality in maternal and perinatal cases in developing countries. It is a disease of placental origin, and the key factor in its pathogenesis is abnormal trophoblast invasion [3]. The present study aimed to evaluate the role of Wnt signaling pathway in patients with early-and late-onset PE using R-Spondin-3 and Dickkopf-1 glycoproteins. The results indicated that the maternal serum level of Dickkopf-1 was significantly higher in the eo-PE group compared with the control group. However, no significant differences in the serum levels of Dickkopf-1 and R-Spondin-3 were found between the eo-PE and lo-PE groups.

A few previous studies have examined the Wnt pathway in healthy and preeclamptic patients.

Zhan Zhang et al. in their study analyzed 31 patients with severe PE and 30 healthy pregnant women. They reported that β-catenin and Dickkopf-1 were expressed substantially in extravillous trophoblast and syncytiotrophoblast. In preeclamptic placental tissues, mRNA and protein levels of β-catenin decreased while the expression of Dickkopf-1 increased significantly compared with the normal placental controls [5]. Xiaofang Wang et al. in their study evaluated the expression levels of Dickkopf-1, β-catenin, Wnt-1, and glycogen synthase kinase 3β protein using Western blot and immunohistochemical techniques in preeclamptic placental tissues. The results of this study reported that the expression level of Dickkopf-1 was significantly increased in the severe PE group. Also, the staining density of Dickkopf-1 in the preeclamptic placenta was stronger [18].

The present study found that the maternal serum level of Dickkopf-1 was significantly higher in the eo-PE group compared with the control group. However, no significant differences in the serum levels of Dickkopf-1 and R-Spondin-3 were found between the eo-PE and lo-PE groups. The results may be due to the abnormal state of Wnt signaling pathway. It is well documented that Dickkopf-1 antagonizes the Wnt signaling pathway and increases the Dickkopf-1 level, resulting in the abnormal function of this pathway [5, 18].

Both angiogenesis and vasculogenesis processes affect human placenta during pregnancy. The formation and maturation of placenta vasculature were very important for normal pregnancy. However, in the PE etiopathogenesis, the major pathological abnormality in the placenta was an insufficiency in the maternal spiral artery remodeling. Cytotrophoblast cells cannot form endothelial phenotype and cannot reach myometrial spiral arteries. This results in permanent outcomes such as placental hypoxia and dysfunction [19]. The Wnt signaling pathway was found to be related in the differentiation, proliferation, and invasion of trophoblast cells.

However, the classification of PE as early and late onset is a well-accepted fact by all researchers [20]. Studies have shown that the pathogenesis of eo-PE and lo-PE is different. Eo-PE can be due to the placental impairment of the inadequate perfusion of the spiral artery, whereas lo-PE appears to be a symptom of metabolic disorders [21, 22]. Recent studies have reported that lo-PE may be related with trophoblastic dysfunction, leading to decreased intervillous perfusion and increased hypoxia [23]. In this study, our results indicated that abnormal Wnt signaling pathway was found to be associated with eo-PE etiopathogenesis. This argument was the most important finding of the present study. However, this study had some limitations: (1) the small sample size and cross-sectional feature of the study can be considered as the leading constraints. (2) In result section the absence of baby's sex, percentile at birth and delivery mode are another limitation of study. (3) Additionally, the differences gestational age at sampling between control group and PE groups seem to be another limitation.

Conclusions

In conclusion, the increased serum level of Dickkopf-1 might be associated with the process of pathogenesis of early-onset PE. Further studies would elucidate their exact roles in the pathogenesis of PE.

Author contributions ATT, AT, RK: project development. NFT, SK, ETY: data collection management and data analysis. MES, ES: manuscript writing and editing.

Funding This study did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in regard to this study.

References

1. Ozdemir F, Tayyar AT, Acmaz G et al (2016) Comparison of blood and urine nephrin levels in preeclampsia and intrauterine growth retardation. Pak J Med Sci 32(1):40–43
2. Tayyar A, Tayyar AT, Abdülrezzak Ü et al (2012) The role of midtrimester amniotic fluid leptin and endothelin-1 levels in prediction of preeclampsia. J Turk Soc Obstet Gynecol 9(1):37–41
3. Chaiworapongsa T, Chaemsaithong P, Yeo L et al (2014) Preeclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol 10(8):466–480
4. Steegers EA, von Dadelszen P, Duvekot JJ et al (2010) Preeclampsia. Lancet 376(9741):631–644
5. Zhang Z, Li H, Zhang L et al (2013) Differential expression of beta-catenin and Dickkopf-1 in the third trimester placentas from normal and preeclamptic pregnancies: a comparative study. Reprod Biol Endocrinol 4(11):17
6. Liu JT, Guo WB, Sun JY (2017) Serum Dickkopf-1 acts as a new biomarker in human breast cancer. Minerva Med 108(4):334–340
7. Zhao YP, Wang W, Wang XH, Xu Y, Wang Y, Dong ZF, Zhang JJ (2015) Downregulation of serum DKK-1 predicts poor prognosis in patients with papillary thyroid cancer. Genet Mol Res 14(4):18886–18894
8. Han SX, Zhou X, Sui X, He CC, Cai MJ, Ma JL, Zhu Q (2015) Serum dickkopf-1 is a novel serological biomarker for the diagnosis and prognosis of pancreatic cancer. Oncotarget 6(23):19907
9. Jiang T, Wang S, Huang L, Zhang S (2009) Clinical significance of serum DKK-1 in patients with gynecological cancer. Int J Gynecol Cancer 19(7):1177–1181
10. Jiang T, Huang L, Zhang S (2013) DKK-1 in serum as a clinical and prognostic factor in patients with cervical cancer. Int J Biol Markers 28(2):221–225
11. Mao B, Wu W, Davidson G et al (2002) Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signalling. Nature 417(6889):664
12. Niehrs C (2006) Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25(57):7469–7481
13. Wei Q, Yokota C, Semenov MV et al (2007) R-Spondin1 is a high affinity ligand for LRP6 and induces LRP6 phosphorylation and β-catenin signaling. J Biol Chem 282(21):15903–15911
14. Kim KA, Wagle M, Tran K et al (2008) R-Spondin family members regulate the Wnt pathway by a common mechanism. Mol Biol Cell 19(6):2588–2596
15. American College of O, Gynecologists, Task Force on Hypertension in P. Hypertension in pregnancy (2013) Report of the American College of Obstetricians and Gynecologists' Task Force on hypertension in pregnancy. Obstet Gynecol 122(5):1122–1131
16. Aksornphusitaphong A, Phupong V (2013) Risk factors of early and late onset pre-eclampsia. J Obstet Gynaecol Res 39(3):627–631
17. Redman CW, Staff AC (2015) Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. Am J Obstet Gynecol 213(4 Suppl):S9e1–S9e11. https://doi.org/10.1016/j.ajog.2015.08.003 **(PubMed PMID: 26428507)**
18. Wang X, Zhang Z, Zeng X et al (2018) Wnt/β-catenin signaling pathway in severe preeclampsia. J Mol Histol 49(3):317–327
19. Cerdeira AS, Karumanchi SA (2012) Angiogenic factors in preeclampsia and related disorders. Cold Spring Harb Perspect Med 2(11):a006585
20. Valensise H, Vasapollo B, Gagliardi G et al (2018) Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 52(5):873–880
21. von Dadelszen P, Magee LA, Roberts JM (2013) Subclassification of preeclampsia. Hypertens Pregnancy 22(2):143–148
22. Herzog EM, Eggink AJ, Reijnierse A et al (2017) Impact of early- and late-onset preeclampsia on features of placental and newborn vascular health. Placenta 49:72–79
23. Redman CW, Sargent IL, Staff AC (2014) IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? Placenta 35(Suppl):S20–S25