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Association of maternal serum Netrin-1 and Netrin-4 levels with placenta accreta spectrum

S. KAYA[1], U. TURHAN[2], İ. DAĞ[3], İ. POLAT[4]

[1]Department of Maternal-Fetal Medicine, Istanbul Medipol University, İstanbul, Turkey
[2]Department of Maternal-Fetal Medicine, Private Perinatology Clinic, Samsun, Turkey
[3]Department of Clinical Biochemistry, Eyüp State Hospital, Istanbul, Turkey
[4]Department of Maternal-Fetal Medicine, Istanbul Basaksehir Cam and Sakura City Hospital, Istanbul, Turkey

Abstract. – OBJECTIVE: This study aimed to evaluate maternal serum Netrin-1 and Netrin-4 levels in pregnancies complicated with placenta accreta spectrum.

PATIENTS AND METHODS: This cross-sectional study enrolled 49 pregnant women with the diagnosis of placenta accreta spectrum as the study group. Gestational age-matched 30 uncomplicated pregnant women with prior cesarean delivery and normal placentation were randomly selected as the control group. Maternal serum Netrin-1 and Netrin-4 levels were measured between weeks 34 and 36 of gestation.

RESULTS: There was no significant difference between the groups in terms of demographic characteristics. Maternal serum Netrin-1 levels were significantly lower in placenta accreta spectrum cases compared with those in the control group (p=0.038). There was no significant difference between the groups in terms of maternal serum Netrin-4 levels (p>0.05). There was a significant negative correlation between maternal serum Netrin-1 levels and the number of prior cesarean deliveries (r=-0.313, p=0.005).

CONCLUSIONS: The observed decrease in maternal serum Netrin-1 levels in placenta accreta spectrum cases associated with increased angiogenesis might be one of the factors involved in the pathophysiology of this disease.

Key Words:
Angiogenesis, Netrin-1, Netrin-4, Placenta accreta spectrum.

Introduction

Placenta accreta spectrum (PAS), characterized by an abnormal trophoblastic invasion into the myometrium including placenta accreta, increta and percreta, is a crucial cause of maternal morbidity and mortality due to the associated increased risk of massive hemorrhage[1]. The incidence of PAS has been gradually increasing as well as the increasing cesarean delivery rates in the last few decades[2,3]. In addition to the cesarean delivery, other uterine interventions (e.g., operative hysteroscopy, uterine curettage, and endometrial resection) can also cause PAS. It is also reported[4,5] that PAS might develop due to uterine abnormalities such as adenomyosis and Mullerian anomalies in cases without a history of surgery. IVF has been suggested as an independent risk factor for PAS and has been associated with slightly adverse newborn neurological outcomes[6,7]. There are mainly two theories asserted in PAS pathophysiology: the first theory is the abnormal invasion of the abnormal trophoblastic cells, and the second one is the deep infiltration of the trophoblastic cells into the abnormal and insufficient decidualization areas[5,8].

Netrins, which are members of the laminin-related protein family, are secreted extracellular matrix proteins[9-11]. Netrins have been reported to take a role in axonal guidance and neuronal migration[9,12]. In addition to their role in central nervous system development, netrins also take part in several biologic processes, such as angiogenesis, morphogenesis, proliferation, apoptosis, and adhesion of the endothelial cells. Netrin-1 and Netrin-4 examined in this study are the most well-known members of the netrin protein family[9,10,13,14].

Angiogenesis is a crucial process in placental development. Moreover, the placental vascular development is regulated probably by angiogenic factors secreted by the placenta itself[15]. Netrin-1 and Netrin-4 have been shown to take a role in placental angiogenesis[10,16,17]. PAS, characterized by abnormal and excessive vascularization, is associated with an increase in angiogenic factors, such as vascular endothelial growth factor (VEGF) and a decrease in anti-6angiogenic factors, such as VEGF receptor-2, tyrosine kinase receptor-2 in trophoblastic tissue[18,19]. It has been

Corresponding Author: Serdar Kaya, MD; e-mail: kayaserdar75@hotmail.com

asserted that pregnancy-associated plasma protein-A (PAPP-A), human chorionic gonadotrophin (hCG), and alpha-fetoprotein (AFP) could be used as biomarkers in the antenatal diagnosis of PAS[20].

Since the angiogenic factors play a crucial role in PAS pathophysiology, this study hypothesized that the levels of Netrin-1 and Netrin-4, as the regulators of angiogenesis, might be altered in PAS cases. Thus, this study aimed to investigate maternal serum Netrin-1 and Netrin-4 levels in PAS cases and compare them with the control group.

Patients and Methods

In this cross-sectional study, 49 pregnancies diagnosed with placenta accreta spectrum were included in the study group. Thirty uncomplicated pregnancies with prior cesarean delivery and normal placentation were included in the control group. The study protocol received approval from the Istanbul Medipol University Clinical Research Ethics Committee. The study complies with the provisions of the Declaration of Helsinki and its latest amendments. An informed consent form was obtained from all participants before enrollment. PAS was diagnosed with grayscale and color Doppler sonography according to American College of Obstetricians and Gynecologists and the Society of Maternal-Fetal Medicine clinical guideline in PAS cases and planned cesarean delivery was performed between weeks 34 and 36 of gestation in line with the recommendation of this guideline1. Before the planned cesarean delivery, venous blood samples were taken from the study group before any medication was given. Maternal venous blood samples were taken from the control group during their routine outpatient clinic examinations between weeks 34 and 36 of gestation. The collected venous blood samples were immediately centrifugated at 2500 rpm for 10 minutes and were stored at -80°C. The prenatal diagnosis of PAS was confirmed with a histopathological examination performed postoperatively.

The presence of multiple pregnancies, fetal structural or chromosomal anomaly, smoking, and maternal chronic disease were the exclusion criteria.

Serum Netrin-1 and Netrin-4 levels were measured with Enzyme-Linked Immunosorbent Assay (ELISA) kit (standardized with an intra-assay CV <10% and inter-assay CV <12%) (Netrin-1 Catalog Number: SEB827Hu, Netrin-4 Catalog Number: SEB835Hu; Cloud- Clone Corp. 23603 W. Fernhurst Dr. Unit 2201, Katy, TX, USA) according to the manufacturer's protocol.

Statistical Analysis

The Statistical Package for the Social Science software, version 24 (IBM Corp., Armonk, NY, USA) was used to analyze data. The Shapiro-Wilk test was performed to evaluate the conformity of data to normal distribution. Levene's test was used to assess the homogeneity of variance. Normally distributed variables were compared between the groups using the independent samples t-test and were expressed as mean ± SD. Non-normally distributed variables were compared among the two groups with Mann-Whitney U test and the results were expressed as median and minimum-maximum. The correlations between variables were evaluated with Spearman's correlation analysis. p-value <0.05 was considered statistically significant.

Results

The baseline characteristics, maternal serum Netrin-1, and Netrin-4 levels are shown in Table I. No significant differences were noted between the groups in terms of maternal age, gravida, parity, gestational age, and body mass index at blood sampling (p>0.05). The number of prior cesarean deliveries was also found to be similar between the groups (p>0.05). The gestational age at delivery and birth weight were found to be significantly lower in PAS cases compared with those in the control group (p<0.001). Maternal serum Netrin-1 levels were significantly lower in PAS cases compared with those in the control group (p=0.038). There was no significant difference between the groups in terms of maternal serum Netrin-4 levels (p>0.05). In the study group, cesarean hysterectomy was performed in 36 (73.5%) patients, and partial resection was performed in 13 (26.5%) patients.

The correlation analyses between maternal serum Netrin-1 levels and other parameters are shown in Table II. A significant negative correlation was detected between maternal serum Netrin-1 levels and the number of prior cesarean deliveries (r=-0.313, p=0.005). Also, there was a significantly positive correlation between maternal serum Netrin-1 levels and the gestational age at delivery (r=0.349, p=0.002) and birth weight (r=0.281, p=0.012).

Table I. The baseline characteristics of the groups.

	PAS (n=49)	Control (n=30)	*p*-values
Age (years)	29.6±3.9	29.8±4.3	0.833
Gravida (n)	3 (2-7)	3 (2-6)	0.797
Parity (n)	2 (1-6)	2 (1-5)	0.913
The number of prior cesarean deliveries (n)	2 (1-6)	2 (1-5)	0.978
GA at blood sampling (weeks)	34.4±0.5	34.6±0.5	0.182
BMI at blood sampling (kg/m^2)	26.1±3.1	27.0±2.2	0.175
GA at delivery (weeks)	34.7±0.5	39.3±0.8	**<0.001**
Birth weight (grams)	2581.0±203.5	3156.6±221.7	**<0.001**
Netrin-1 (pg/ml)	355.6 (128.7-936.4)	474.9 (209.5-1814.9)	**0.038**
Netrin-4 (pg/ml)	1227.7 (477.9-1500.1)	1223.6 (861.5-1366.7)	0.533

PAS: placenta accreta spectrum; GA: gestational age; BMI: body mass index. Data are expressed as means (±SDs) or medians (minumum-maximum).

Table II. Correlation analyses between maternal serum Netrin-1 levels and other parameters in (**A**) both PAS and control groups (**B**) control group.

	(A) Netrin-1 (n=79)		(B) Netrin-1 (n=30)	
Age	r=-0.100	*p*=.382	r=0.100	*p*=.601
Gravida	r=-0.273*	***p*=.015**	r=-0.077	*p*=.684
Parity	r=-0.335**	***p*=.003**	r=-0.154	*p*=.416
The number of prior cesarean delivery	r=-0.313**	***p*=.005**	r=-0.103	*p*=.587
BMI at blood sampling	r=0.233	*p*=.063	r=0.330	*p*=.075
GA at blood sampling	r=0.297**	***p*=.008**	r=-0.089	*p*=.639
GA at delivery	r=0.349**	***p*=.002**	r=-0.120	*p*=.528
Birth weight	r=0.281*	***p*=.012**	r=-0.222	*p*=.239

PAS: placenta accreta spectrum; BMI: body mass index; GA: gestational age. r= Spearman's correlation.

Discussion

This study, which investigated maternal serum Netrin-1 and Netrin-4 levels in PAS cases, hypothesized that maternal serum levels of Netrin-1 and Netrin-4, shown to be involved in the regulation of angiogenesis, would be altered in PAS cases and these proteins would be used as biomarkers for PAS. This study detected that maternal serum Netrin-1 levels were significantly lower in PAS cases compared with those in the control group. In addition, no significant difference was noted between the groups in terms of maternal serum Netrin-4 levels.

Several vitamins and nutraceutical supplementation may have an important role in women's health from the reproductive age to the postmenopausal period. Adverse outcomes may occur in their imbalance and deficiencies[21-23]. Increasing cesarean delivery rates cause an increase in the incidence of PAS and thus adversely affect maternal health. Recently, PAS has become one of the leading causes of maternal morbidity and mortality[1]. Angiogenesis refers to the development of new vessels from a previously existing vessel. It is a physiologic process that exists during fetal and placental development. Many factors affecting angiogenesis such as angiopoietins, cytokines, and endogenous inhibitors have been identified and these factors carry out the process in balance[24,25]. An in-vitro study demonstrated that mesenchymal stem cells facilitate the angiogenic process through a paracrine effect by inducing the expression of angiogenic factors[26]. Blood vessel development and neuronal development are complex processes and have some similarities. In addition to their roles in axonal guidance and neuronal migration, netrins have been reported to involve in vessel pathfinding and network formation[10,27]. Prieto et al[28] observed that the pharmacological blockage of Netrin-1 leads to decreased angiogenesis, and the exogenous Netrin-1 stimulation causes endothelial vascular migration in human umbilical vein endothelial cell cultures. Additionally, Netrin-1 is shown to induce angiogenesis in vivo and to ensure the survival of endothelial cells by inhibiting apoptosis, thus supporting angiogenesis[29].

Netrin-4 is observed to reduce pathological angiogenesis and inhibit placental endothelial cells' proliferation, migration, and tube formation. It is stated that Netrin-4 has a potential regulator role in placental angiogenesis, and its antiangiogenic effect is prominent in the placenta[30,31]. Contrarily, it is asserted that Netrin-4 has a pro-angiogenic effect in vivo and in vitro, and Netrin-4 is essential for endothelial cell survival, proliferation, and angiogenesis[32]. The angiogenic effects of Netrin-1 and the anti-angiogenic effects of Netrin-4 are prominent according to the current literature. However, it is suggested that the biological effects of these proteins may vary through different receptors in different tissues[33].

The trophoblastic invasion and angiogenesis are controlled by several regulator molecules, such as VEGF, placental growth factor (PlGF), and soluble fms-like tyrosine kinase 1 (sFlt-1) during normal pregnancy[15,34]. The imbalance between the angiogenic and anti-angiogenic stimulus is suggested as the underlying cause of many diseases. Endoglin which has an angiogenic effect is upregulated in preeclamptic patients like many other factors[35,36]. Excessive angiogenesis has been associated with tumoral, ocular, and autoimmune diseases[24]. The optimal placental vascular bed development has a considerable effect on fetal development, and the impaired placental vascular bed development has been associated with preeclampsia and fetal growth restriction[24,37]. Hypertensive disorders in pregnancy and PAS are disorders in which abnormal trophoblast invasion and angiogenic and antiangiogenic factors are accused in their etiopathogenesis. Scholars[38] observed an association between hypertensive disorders in pregnancy and PAS. It is suggested that PAS might cause hypertension through abnormal trophoblast invasion. Currently, the use of anti-angiogenic biological therapies in the prevention and treatment of these diseases is being investigated[35].

Significantly lower maternal serum VEGF levels were detected in pregnancies with abnormally invasive placenta compared with the control group, then it is stated that maternal serum VEGF levels could predict the abnormally invasive placenta and the invasion degree[39]. Similarly, significantly lower preoperative maternal serum VEGF, PlGF, and sFlt-1 levels and higher postoperative maternal serum VEGF and sFlt-1 levels were observed in the placenta percreta cases[40]. In addition, Wehrum et al[41] detected lower circulating VEGF levels in the placenta previa cases with excessive myometrial invasion. Unlike these observations, Biberoglu et al[42] observed similar maternal serum sFlt-1, PlGF, VEGF levels, and sFlt-1/PlGF ratio in pregnancies with abnormal placentation compared to the control group regardless of the invasion degree and localization. Duzyj et al[43] observed that the angiogenic and growth factor levels altered locally at the placental invasion site in cases with placenta accreta. Then, the authors suggested that these local alterations induce the invasion degree by activating the hyperinvasive trophoblasts and dysregulating the placental vascular remodeling.

As mentioned above, the levels of angiogenic factors were evaluated in pregnancies with PAS in several studies. However, there is no information about the maternal serum netrin levels or the expression of netrin in trophoblastic tissue in PAS cases. To the best of our knowledge, this study is the first to offer information about maternal serum netrin levels in PAS cases. The lack of evaluation of placental Netrin-1 expression, the study's cross-sectional design, and the limited number of patients are the limitations of this study.

This study detected significantly lower maternal serum Netrin-1 levels in PAS cases. The observed decrease in maternal serum Netrin-1 levels could be associated with the accumulation of Netrin-1 in the placental tissue, however, this should be evaluated in studies examining Netrin-1 expression in the trophoblastic tissues. Furthermore, this observed alteration in maternal serum Netrin-1 levels suggested that Netrin-1 has a crucial role in PAS etiopathogenesis with other angiogenic factors. Maternal serum Netrin-1 levels might be used in the prediction of PAS and the invasion degree. No significant difference was observed in maternal serum Netrin-4 levels between the groups. Since the anti-angiogenic effect of Netrin-4 is prominent, it can be concluded that Netrin-4 did not have a considerable impact on the pathogenesis of PAS cases with excessive angiogenesis. Maternal serum Netrin-4 levels could be evaluated in abnormal pregnancies with impaired vascularization, such as preeclampsia and fetal growth restriction in further studies.

Conclusions

Imaging methods, such as ultrasonography and magnetic resonance imaging (MRI), are widely used in the diagnosis of PAS and the evaluation of

the invasion degree. In addition to these methods, many biochemical markers are being investigated in the evaluation of PAS cases. This study, which investigated maternal serum Netrin levels in PAS cases, detected a significant decrease in maternal serum Netrin-1 levels in PAS cases in accordance with its hypothesis. Considering the role of netrins in angiogenesis, it can be asserted that Netrin-1 might play a role in the pathogenesis of PAS, and it might be used as a biomarker in the evaluation of PAS.

Conflict of Interest
The authors declare no conflict of interests for this article.

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Ethics Approval
The study conforms to the provisions of the Declaration of Helsinki and was approved by the Istanbul Medipol University Clinical Research Ethics Committee (No. 607 on 08/06/2020).

Informed Consent
Informed consent was obtained from all participants prior to enrollment.

Authors' Contributions
Serdar Kaya: design of the study, acquisition of data, analysis and interpretation of data, drafting the article; Uğur Turhan: acquisition of data, analysis and interpretation of data; İsmail Dağ: analysis and interpretation of data, administering technical support; İbrahim Polat: making critical revision, supervision. All authors have read and agreed to the version of the article to be published.

ORCID ID
Serdar KAYA: https://orcid.org/0000-0002-4092-7316
Uğur Turhan: https://orcid.org/0000-0002-0653-0630
İsmail Dağ: https://orcid.org/0000-0002-9432-7965
İbrahim Polat: https://orcid.org/0000-0001-9434-3976

Data Availability Statement
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Cahill AG, Beigi R, Heine RP, Silver RM, Wax JR. Placenta Accreta Spectrum. Society of Gynecologic Oncology; American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine. Am J Obstet Gynecol 2018; 219: B2-B16.
2) Bowman ZS, Eller AG, Bardsley TR, Greene T, Varner MW, Silver RM. Risk factors for placenta accreta: a large prospective cohort. Am J Perinatol 2014; 31: 799-804.
3) Fitzpatrick KE, Sellers S, Spark P, Kurinczuk JJ, Brocklehurst P, Knight M. Incidence and risk factors for placenta accreta/increta/percreta in the UK: a national case-control study. PLoS One 2012; 7: e52893.
4) Jauniaux E, Collins SL, Jurkovic D, Burton GJ. Accreta placentation: a systematic review of prenatal ultrasound imaging and grading of villous invasiveness. Am J Obstet Gynecol 2016; 215: 712-721.
5) Carusi DA. The Placenta Accreta Spectrum: Epidemiology and Risk Factors. Clin Obstet Gynecol 2018; 61: 733-742.
6) Salmanian B, Fox KA, Arian SE, Erfani H, Clark SL, Aagaard KM, Detlefs SE, Aalipour S, Espinoza J, Nassr AA, Gibbons WE, Shamshirsaz AA, Belfort MA, Shamshirsaz AA. In vitro fertilization as an independent risk factor for placenta accreta spectrum. Am J Obstet Gynecol 2020; 223: 568.e1-568.e5.
7) Gullo G, Scaglione M, Cucinella G, Perino A, Chiantera V, D'Anna R, Laganà AS, Buzzaccarini G. Impact of assisted reproduction techniques on the neuro-psycho-motor outcome of newborns: a critical appraisal. J Obstet Gynaecol 2022; 42: 2583-2587.
8) Jauniaux E, Collins S, Burton GJ. Placenta accreta spectrum: pathophysiology and evidence-based anatomy for prenatal ultrasound imaging. Am J Obstet Gynecol 2018; 218: 75-87.
9) Barallobre MJ, Pascual M, Del Río JA, Soriano E. The Netrin family of guidance factors: emphasis on Netrin-1 signalling. Brain Res Rev 2005; 49: 22-47.
10) Dakouane-Giudicelli M, Alfaidy N, de Mazancourt P. Netrins and their roles in placental angiogenesis. Biomed Res Int 2014; 2014: 901941.
11) Ylivinkka I, Keski-Oja J, Hyytiäinen M. Netrin-1: A regulator of cancer cell motility? Eur J Cell Biol 2016; 95: 513-520.
12) Serafini T, Kennedy TE, Galko MJ, Mirzayan C, Jessell TM, Tessier-Lavigne M. The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. Cell 1994; 78: 409-424.
13) Bradford D, Cole SJ, Cooper HM. Netrin-1: diversity in development. Int J Biochem Cell Biol 2009; 41: 487-493.
14) Yang Y, Zou L, Wang Y, Xu KS, Zhang JX, Zhang JH. Axon guidance cue Netrin-1 has dual function in angiogenesis. Cancer Biol Ther 2007; 6: 743-748.

15) Reynolds LP, Redmer DA. Utero-placental vascular development and placental function. J Anim Sci 1995; 73: 1839-1851.

16) Xie H, Zou L, Zhu J, Yang Y. Effects of netrin-1 and netrin-1 knockdown on human umbilical vein endothelial cells and angiogenesis of rat placenta. Placenta 2011; 32: 546-553.

17) Castets M, Mehlen P. Netrin-1 role in angiogenesis: to be or not to be a pro-angiogenic factor? Cell Cycle 2010; 9: 1466-1471.

18) Tseng JJ, Chou MM, Hsieh YT, Wen MC, Ho ES, Hsu SL. Differential expression of vascular endothelial growth factor, placenta growth factor and their receptors in placentae from pregnancies complicated by placenta accreta. Placenta 2006; 27: 70-78.

19) Tseng JJ, Chou MM. Differential expression of growth-, angiogenesis- and invasion-related factors in the development of placenta accreta. Taiwan J Obstet Gynecol 2006; 45: 100-106.

20) Bartels HC, Postle JD, Downey P, Brennan DJ. Placenta Accreta Spectrum: A Review of Pathology, Molecular Biology, and Biomarkers. Dis Markers 2018; 2018: 1507674.

21) Gullo G, Carlomagno G, Unfer V, D'Anna R. Myo-inositol: from induction of ovulation to menopausal disorder management. Minerva Ginecol 2015; 67: 485-486.

22) Bezerra Espinola MS, Laganà AS, Bilotta G, Gullo G, Aragona C, Unfer V. D-chiro-inositol Induces Ovulation in Non-Polycystic Ovary Syndrome (PCOS), Non-Insulin-Resistant Young Women, Likely by Modulating Aromatase Expression: A Report of 2 Cases. Am J Case Rep 2021; 22: e932722.

23) Menichini D, Imbrogno MG, Basile L, Monari F, Ferrari F, Neri I. Oral supplementation of α-lipoic acid (ALA), magnesium, vitamin B6 and vitamin D stabilizes cervical changes in women presenting risk factors for preterm birth. Eur Rev Med Pharmacol Sci 2022; 26: 8879-8886.

24) Carmeliet P. Angiogenesis in life, disease and medicine. Nature 2005; 438: 932-936.

25) Asahara T, Bauters C, Zheng LP, Takeshita S, Bunting S, Ferrara N, Symes JF, Isner JM. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. Circulation 1995; 92: II365-371.

26) Edwards SS, Zavala G, Prieto CP, Elliott M, Martínez S, Egaña JT, Bono MR, Palma V. Functional analysis reveals angiogenic potential of human mesenchymal stem cells from Wharton's jelly in dermal regeneration. Angiogenesis 2014; 17: 851-866.

27) Eichmann A, Le Noble F, Autiero M, Carmeliet P. Guidance of vascular and neural network formation. Curr Opin Neurobiol 2005; 15: 108-115.

28) Prieto CP, Ortiz MC, Villanueva A, Villarroel C, Edwards SS, Elliott M, Lattus J, Aedo S, Meza D, Lois P, Palma V. Netrin-1 acts as a non-canonical angiogenic factor produced by human Wharton's jelly mesenchymal stem cells (WJ-MSC). Stem Cell Res Ther 2017; 8: 43.

29) Castets M, Coissieux MM, Delloye-Bourgeois C, Bernard L, Delcros JG, Bernet A, Laudet V, Mehlen P. Inhibition of endothelial cell apoptosis by netrin-1 during angiogenesis. Dev Cell 2009; 16: 614-620.

30) Lejmi E, Leconte L, Pédron-Mazoyer S, Ropert S, Raoul W, Lavalette S, Bouras I, Feron JG, Maitre-Boube M, Assayag F, Feumi C, Alemany M, Jie TX, Merkulova T, Poupon MF, Ruchoux MM, Tobelem G, Sennlaub F, Plouët J. Netrin-4 inhibits angiogenesis via binding to neogenin and recruitment of Unc5B. Proc Natl Acad Sci U S A 2008; 105: 12491-12496.

31) Dakouane-Giudicelli M, Brouillet S, Traboulsi W, Torre A, Vallat G, Si Nacer S, Vallée M, Feige JJ, Alfaidy N, de Mazancourt P. Inhibition of human placental endothelial cell proliferation and angiogenesis by netrin-4. Placenta 2015; 36: 1260-1265.

32) Lambert E, Coissieux MM, Laudet V, Mehlen P. Netrin-4 acts as a pro-angiogenic factor during zebrafish development. J Biol Chem 2012; 287: 3987-3999.

33) Lai Wing Sun K, Correia JP, Kennedy TE. Netrins: versatile extracellular cues with diverse functions. Development 2011; 138: 2153-2169.

34) Dimitriadis E, Nie G, Hannan NJ, Paiva P, Salamonsen LA. Local regulation of implantation at the human fetal-maternal interface. Int J Dev Biol 2010; 54: 313-322.

35- Margioula-Siarkou G, Margioula-Siarkou C, Petousis S, Margaritis K, Vavoulidis E, Gullo G, Alexandratou M, Dinas K, Sotiriadis A, Mavromatidis G. The role of endoglin and its soluble form in pathogenesis of preeclampsia. Mol Cell Biochem 2022; 477: 479-491.

35) Sun XW, Li XH, Zhang C, Meng FQ, Xing YG, Ding Y. Correlation analysis of serum placental growth factor, pregnancy-related plasma protein-A and disease severity in patients with hypertensive disorder in pregnancy. Eur Rev Med Pharmacol Sci 2021; 25: 1788-1795.

36) Kusinski LC, Baker PN, Sibley CP, Wareing M. In vitro assessment of mouse uterine and feto-placental vascular function. Reprod Sci 2009; 16: 740-748.

37) Usta IM, Hobeika EM, Musa AA, Gabriel GE, Nassar AH. Placenta previa-accreta: risk factors and complications. Am J Obstet Gynecol 2005; 193: 1045-1049.

38) Schwickert A, Chantraine F, Ehrlich L, Henrich W, Muallem MZ, Nonnenmacher A, Petit P, Weizsäcker K, Braun T. Maternal Serum VEGF Predicts Abnormally Invasive Placenta Better than NT-proBNP: a Multicenter Case-Control Study. Reprod Sci 2021; 28: 361-370.

39) Uyanıkoğlu H, İncebıyık A, Turp AB, Çakmak G, Sak S, Hilali NG. Serum Angiogenic and Anti-angiogenic Markers in Pregnant Women with Placenta Percreta. Balkan Med J 2018; 35: 55-60.

40) Wehrum MJ, Buhimschi IA, Salafia C, Thung S, Bahtiyar MO, Werner EF, Campbell KH, Laky C, Sfakianaki AK, Zhao G, Funai EF, Buhimschi CS. Accreta complicating complete placenta previa is characterized by reduced systemic levels of vascular endothelial growth factor and by epithelial-to-mesenchymal transition of the invasive trophoblast. Am J Obstet Gynecol 2011; 204: 411.e1-411.e11.

41) Biberoglu E, Kirbas A, Daglar K, Timur H, Demirtas C, Karabulut E, Danisman N. Serum angiogenic profile in abnormal placentation. J Matern Fetal Neonatal Med 2016; 29: 3193-3197.

42) Duzyj CM, Buhimschi IA, Laky CA, Cozzini G, Zhao G, Wehrum M, Buhimschi CS. Extravillous trophoblast invasion in placenta accreta is associated with differential local expression of angiogenic and growth factors: a cross-sectional study. BJOG 2018; 125: 1441-1448.