Research Article / Araştırma Makalesi

Investigation of PLGF, sFlt-1 Expression in Placenta Previa, Placenta Accreta and Normotensive Placentas in the 3rd Trimester of Pregnancy

Gebeliğin 3. Trimestrinde Plasenta Previa, Plasenta Akreata ve Normotensif Plasentalarda PLGF, sFlt-1 ekspresyonunun incelenmesi

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Abstract

Background: We aimed to immunohistochemically examine the expression level of Placental growth factor (PLGF) and Soluble FMS-like tyrosine kinase-1 (sFlt-1) proteins in normotensive, Placenta accreta (PA) and Placenta previa (PP).
Materials and Methods: Three groups were created for the study: 20 Nomotensive placentas, 20 PP and 20 PA from women diagnosed with placenta samples. 4-6 µm thick sections were taken from the placentas, PLGF and sFlt-1 immunostaining were applied to the obtained sections, and their expression intensities and localizations in the tissues were determined.
Results: As a result of our observations, normotensive placenta group; sFlt-1 expression was observed in hofbauer cells and syncytial nodes and PLGF positive expression was observed in nuclei of muscle cells in some tunica media region. PP and PA group; sFlt-1 expression was positive in decidua cells, hofbauer cells and dilated vascular endothelium. PP and PA group; negative PLGF expression was observed in syncytial nodes and positive PLGF expression was observed in hofbauer cells and endothelial cells. There was a significant difference between normotensive, PP and PA groups ($p<0.05$).
Conclusions: It can be thought that sFlt-1 and PLGF may be important signal stimulators and markers in the trophoblastic degeneration, apoptotic cells, increase in angiogenesis and imbalance in implantation seen in cases of PP and PA.

Keywords: Normotensive, Placenta accreta, Placenta previa, PLGF, sFlt-1, immunohistochemistry

Öz

Amaç: Normotensif, Plasenta akreata (PA) ve Plasenta previa (PP); Plasenta büyüme faktör (PLGF) ve Soluble Fms-Benzeri Tirozin Kinaz-1 (sFlt-1) proteinlerin ekspresyon düzeylerinin immünohistokimyasal olarak incelenmesini amaçladık.
Materyal ve Metod: Çalışmaya 20 Nomotensif plasenta, 20 PP ve 20 PA tanısı konmuş kadına ait plasenta örnekleri olmak üzere 3 grup oluşturulmuştur. Plasentalardan 4-6 µm kalınlığında kesitler alındı, elde edilen kesitlere PLGF ve sFlt-1 immün boyama uygulanarak dokulardaki ekspresyon şiddetleri ve lokalizasyonları belirlendi.
Bulgular: Gözlemlerimiz sonucu normotensif plasenta grubu; hofbauer hücreler ve sinsityal nodlar sFlt-1 ve bazı tunika media bölgesindeki kas hücrelerin nukleuslarında PLGF pozitif ekspresyonu izlendi. PP ve PA grubu; desidua hücreler, hofbauer hücrelerinde ve dilate damar endotelinde sFlt-1 ekspresyonu pozitif izlendi. PP ve PA grubu; sinsityal düğümlerde negatif, hofbauer hücrelerinde ve endotel hücrelerinde pozitif PLGF ekspresyonu izlendi. Normotensif, PP ve PA grupları arasında anlamlı bir fark vardı ($p<0.05$).
Sonuç: PP ve PA olgularında görülen trofoblastik dejenerasyon ve apoptotik hücreler, anjiyogenezisin artmasına ve implantasyonda dengesizliğin gerçekleşmesinde sFlt-1 ve PLGF etkili olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Normotensif, Plasenta akreata, Plasenta previa, PLGF, sFlt-1, immünohistokimya

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Introduction

The formation of the placenta, the unique organ of exchange between mother and fetus, is essential for successful human pregnancy and fetal health. Throughout pregnancy, the placenta performs many tasks, from maternal physiological adaptation to immunologic acceptance, nutrition and support of the developing embryo (1). In addition to meeting the needs of the developing fetus, the placenta profoundly alters maternal metabolism by secreting numerous hormones into the maternal bloodstream (2). Placenta previa (PP) is the complete or partial covering of the internal os of the cervix. It is a major risk factor for postpartum hemorrhage and may lead to morbidity and mortality both in the mother and the newborn (3). This condition prevents a safe vaginal delivery and necessitates cesarean delivery of the newborn. Most cases are diagnosed early in pregnancy by sonography and others may present to the emergency department with painless vaginal bleeding in the second or third trimester of pregnancy. The presence of PP may also increase a woman's risk of PAS (4). The underlying cause of PP is unknown. However, there is an association between endometrial damage and uterine scarring. PP affects 0.3% to 2% of pregnancies in the third trimester and has become more prominent secondary to increasing cesarean section rates (5). Placenta accreta spectrum (PAS) disorder, also called abnormally invasive placenta, describes a clinical condition in which the placenta does not detach spontaneously after delivery and cannot be forcibly removed without causing massive and potentially life-threatening bleeding (6). The incidence of placenta accreta (PA) is increasing worldwide (7). This is most likely due to increasing rates of cesarean delivery, which is the most important risk factor for PAS in subsequent pregnancies (8). It is thought that an iatrogenic defect of the endometrial-myometrial interface may lead to a failure of normal decidualization at the site of a uterine scar that allows abnormally deep trophoblast infiltration. The degree of penetration of villus tissue into the myometrium is probably related to the degree of deciduo-myometrial damage (9). PLGF and sFlt-1 are circulating angiogenic factors. During pregnancy, these factors are released into the maternal circulation by the placenta (10). sFlt-1 plays an important role in the regulation of angiogenesis and lymphangiogenesis. sFlt-1 is a soluble VEGF antagonist with a fundamental effect in maintaining vascular growth balance. (11). PLGF is a member of the VEGF family and is usually expressed in the placenta, although it is also expressed at low levels in heart, lung, thyroid, liver, skeletal muscle and tissue (12). In addition, it exerts anti-angiogenic activity that induces endothelial cell dysfunction and impairs vascular wall permeability and integrity. It is also speculated that sFlt-1 may play an important role in promoting excessive invasion of trophoblasts and vascular remodeling, this series of changes may be secondary to the endometrial-myometrial injury microenvironment of PA patients (13). PLGF and sFlt-1 are pro-angiogenic and anti-angiogenic factors that may play a role in placental vascular growth and maturation during neovascularization of the PAS (14).

The underlying causes related to the pathogenesis of PP and PA are not fully known. Complications of PA and PP are important causes of maternal and fetal mortality and morbidity globally. We wanted to observe the changes in sFlt-1 and PLGF in patients diagnosed with PP and PA. In these cases, we think that trophoblastic degeneration, apoptotic cells and endothelial dysfunction in the placenta together with differences in the sFlt1/PLGF exchange ratio may cause important complications by increasing the likelihood of preterm delivery and sFlt1/PLGF expression levels may be an important factor in determining this process.

In this study, we aimed to immunohistochemically assess the expression levels of sFlt-1 and PLGF proteins in order to identify potential biomarkers for patients with PP and PA.

Materials and Methods

Collecting the plasental samples

The research was commenced with the approval of the Ethics Committee of the Faculty of Medicine at Dicle University, identified as number 67 and dated 17.03.2022. Patients who underwent transvaginal ultrasonography were diagnosed as PP when the placenta completely covered the cervix and PA was diagnosed when myometrial invasion and myometrial irregularity were observed in patients who underwent colour doppler examination. If the placenta was located normally in healthy pregnant women who did not meet these diagnostic criteria, they were accepted as normotensive patients. Placental tissues of 20 pregnant women with PA, 20 pregnant women with PP diagnosis and 25 Normotensive (healthy) pregnant women from Dicle University Medical Faculty Hospital Gynecology and Obstetrics Clinic were included. The tissues fixed in 10% formalin was applied a paraffin wax embedding procedure (15). 2x2x2 cm pieces were taken from the placentas. Fixation was completed in 16 hours. The fixed tissues were washed in tap water for 1 night. For dehydration, tissue pieces were kept in the following alcohol series (%50, %70, %80, %90, %96), xylol for 2x15 minutes for clearing. For infiltration, they were kept in liquid paraffin for 2 hours in an oven set at 58°C and then blocked. The tissues will then be embedded in paraffin blocks and sections of 5µm thickness will be taken for immunohistochemical staining using a microtome (catalog no: Leica RM2265, Wetzlar, Germany) (16).

Immunohistochemical analysis

Sections from paraffin blocks were deparaffinized. For dehydration, they were passed through decreasing alcohol series and brought to distilled water. Sections were then microwaved in EDTA buffer solution (pH: 8.0, catalog no: ab93680, Abcam, Cambridge, USA) for antigen retrieval. Sections cooled to room temperature were preserved in PBS solution. Sections were kept in 3% H_2O_2 (catalog No:

TA-015-HP, Thermo Fischer, USA) to block endogenous peroxidase activity. Ultra V Blocking solution (catalog No: TA-015-UB, Thermo Fischer, USA) was applied to the sections that were taken back to PBS to prevent non-specific binding. Blocking solution was removed from the sections and incubated overnight at +4 °C with PLGF (Santa Cruz, cat no: sc-518003 CA, ABD) and sFlt-1 (Abcam cat no: ab9540 Cambridge, UK) primary antibody diluted 1/250 with antibody diluent without washing. The sections were then kept at room temperature, the sections were washed in PBS solution and biotinylated secondary antibody (catalog no: TP-015-BN, ThermoFischer, Fremont, CA, USA) was applied. Sections washed with PBS solution were then kept in streptavidin peroxidase solution (catalog no: TS-015-HR, Thermo Fisher, USA). Sections washed in PBS were treated with diaminobenzidine (DAB) (catalog no: TA001 HCX, Thermo Fischer, USA). After counterstaining with Gill III hematoxylin, they were covered with entellan and evaluated and imaged under light microscopy using Zeiss Imager A2 Zen 3.0 software (Germany).

Morphological measurement and quantification

For the diameters of blood vessels, syncytial node, fibronoid area and chorionic villus, 5 random bases were taken in each group and 20 diameters for each group from 4 different areas for diameters and 60 diameters in total were measured and evaluated with Zeiss Imager A2 Zen 3.0 software (Germany). In the immunohistochemistry scoring, 5 random areas were taken from each group and examined in 4 different areas. PLGF and sFlt-1 expression intensity was analyzed in these areas. Semi-quantitative analyses were performed using the H-score method. It was scored as 0, 1, 2, 3 and 4 according to absent, weak, moderate, strong and very strong staining intensity, respectively (17). PLGF and sFlt-1 positive expressions were H-scored by detecting the expression areas with contrast staining based on chromogen DAB and hematoxylin counterstaining. According to these analyses, PLGF positive expression increased in PP and PA groups.

Statistical analysis

SPSS 25.0 IBM, version 25.0., US software and Analyze-it for Microsoft Excel Method Comparison Edition (v30.2, Analyze-it Software Ltd., Leeds, UK) were used for statistical evaluation of our research data. The Shapiro-Wilk test was used for the normality test to determine if the samples were distributed among the groups. All findings were presented in the form of mean ± standard deviation(SD), with a significance level of $p<0.05$ being selected for statistical analysis.

Results

Demographic and hematologic findings

Based on the medical records, the data of demographic characteristics such as age, gravida, parity, gestational age (week) and birth weight of the Normotensive, PA and PP groups are given as mean±SD (Standard deviation). There was a difference in age, gestational week and birth weight in the Normotensive, PA and PP groups (Table 1). In the hematologic evaluation of the groups; "white blood cells (WBC)", "platelet (Plt)", "neutrophil (NEU)", "lymphocyte (LYM)", "hemoglobin (Hgb)" and "haematocrit (Hct)" values were evaluated and the data were given as mean±sd. It was determined that hemoglobin and hematocrit values decreased in the PP and PA groups as a result of excessive bleeding (Table 2).

Table 1. Demographic characteristics of Normotensive, PP and PA groups

	Normotensive (N=20) Mean±SD	PP (N=20) Mean±SD	PA (N=20) Mean±SD
Age (year)	28 ±5	34 ±5	31±5
Gestational age	38 ±2	36 ±3	32 ±3
Gravida	4±2	6 ±3	6±3
Parity	2±2	4 ±3	4±3
Birth Week	37 ±2	36 ±2	34 ±4
Birth Weight(gr)	2851 ±750	2855 ±435	2920±547

Morphometric findings

According to the results of statistical analysis, a significant difference was found between the blood vessel, syncytial node, fibronoid area and chorionic villus diameters of the Normotensive, PP and PA groups ($p<0.0001$) (Table 3).

Immunohistochemical Findings

There was a significant difference between the normotensive and patient groups ($p<0.0001$). sFlt-1 positive expression increased in PA and PP groups. There was a significant difference between the normotensive, PP and PA groups ($p< 0.0001$) (Table 4). As a result of our observations, normotensive placenta group; endothelium negative, hofbauer cells and syncytial node positive, in PA group sections; decidua cells, vascular endothelium and hofbauer cells positive, in PP group sections; dilated vascular endothelium, hofbauer cells positive and syncytial node negative sFlt-1 expression was observed. As a result of our observations, normotensive placenta group; vascular endothelium, syncytial node and muscle cell nuclei were positive, in PA group sections; syncytial node negative and hofbauer cells positive, in PP group sections; hofbauer cells, syncytial node and endothelial cells positive PLGF expression was observed (Figure 1). Graphical demonstration of the statistical analysis of Morphometric measurements and Immunohistochemistry of PLGF and sFlt-1 (Figure 2).

Table 2. Haematological values of Normotensive, PP and PA groups

	Normotensive (N=20) Mean± SD	PP (N=20) Mean± SD	PA (N=20) Mean± SD
WBC			
Prepartum	12,23±2,89	10,27±4,29	11,50± 4,63
Postpartum	15,09±3,92	14,05 ±4,67	15,48± 6,77
Plt x10³/mm³			
Prepartum	226,65±82,67	196,85 ±65,30	203,58± 78,98
Postpartum	210,40±65,58	171,00± 57,54	186,35± 53,64
NEU x10³/mm³			
Prepartum	10,03 ±3,75	7,79 ±3,99	7,67 ±4,35
Postpartum	12,65±3,68	11,78 ±4,25	12,11± 6,08
LYM x10³/mm³			
Prepartum	1,77 ±,58	1,13±,50	1,70± ,62
Postpartum	1,91 ±1,93	1,54± ,49	1,56± ,59
HGB(gr/dl)			
Prepartum	11,01± 4,32	11,38± 1,60	16,93 ±2,12
Postpartum	15,92± 1,61	9,39±1,36	10,01± ,83
HCT(%)			
Prepartum	33,28 ±4,14	34,02±3,79	34,78 ±3,04
Postpartum	32,53± 4,18	29,06± 3,69	31,09 ±2,52

Table 3. Morphometric measurement analysis of Normotensive, PP and PA groups

	Normotensive (20) Mean± SD	PP (20) Mean± SD	PA(20) Mean± SD	P value
Blood vessel diameter (µm)	26,95 ±2,76	25,05±2,43	61,92 ± 46,27	<0,0001
Syncytial node diameter (µm)	6,07 ±2,01	13,14±3,22	18,78±2,12	<0,0001
Fibronoid area diameter (µm)	26,31 ±2,31	38,28±9,90	50,35±20,82	<0,0001
Chorionic villus diameter (µm)	23,43± 4,12	32,81±2,47	139,28±14,45	<0,0001

p<0.05 is accepted as significant. Existence of different superscripts on the results indicate statistically significance between the related groups. PP: Placenta previa, PA: Placenta accreta

Table 4. Plgf and sFlt-1 immunohistochemical statistical analysis values

	Normotensive (20) Mean± SD	PP (20) Mean± SD	PA(20) Mean± SD	P value
PLGF	0,8 ±0,6[a]	3,4±0,9[b]	3,6 ± 0,7[b]	[a-b] p <0,0001
sFlt-1	0,8±0,7[a]	3,5±0,6[b]	3,4±0,6[b]	[a-b] p <0,0001

p<0.05 is accepted as significant. Existence of different superscripts on the results indicate statistically significance between the related groups. PP: Placenta previa, PA: Placenta accreta

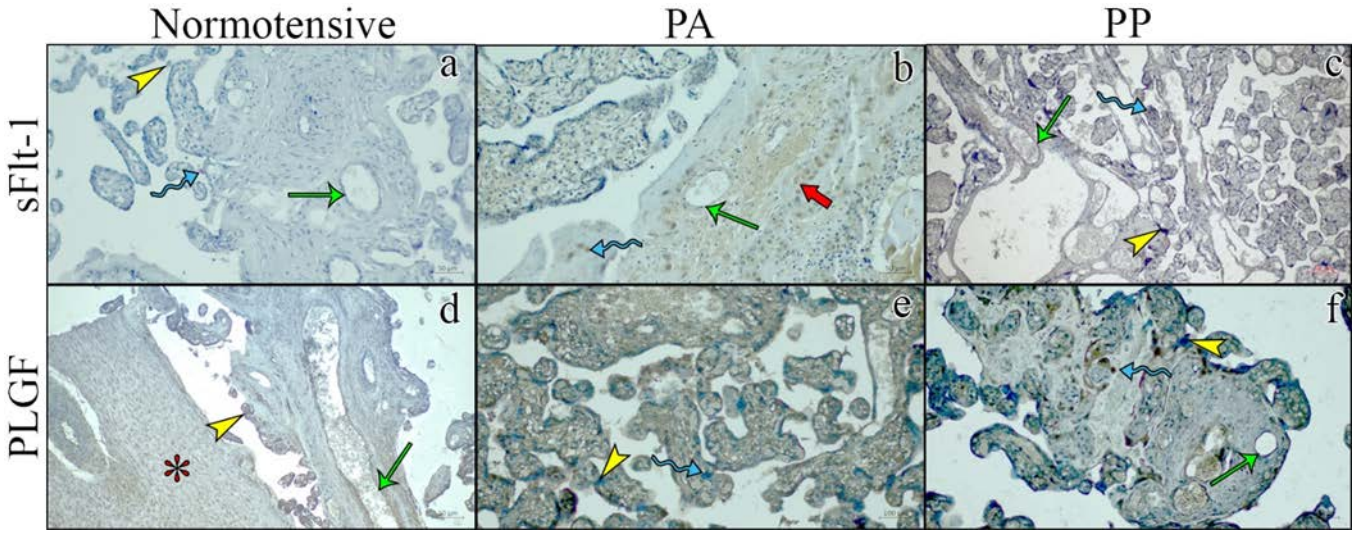

Figure. 1. Representative immunoexpression micrographs of sFlt-1 and PLGF in Normotensive, PA and PP groups. Normotensive (a&d), PA(b&e) and PP(c&f) groups. Normotensive group endothelium negative (green arrow), hofbauer cells (blue curved arrow) and syncytial node (yellow arrowhead) positive expression sFlt-1 Bar: 50 µm **(a)**. PA group decidua cells (red thick arrow), vascular endothelium (green arrow) and hofbauer cells (blue curved arrow) positive expression sFlt-1 Bar: 50 µm **(b)**. PP group dilated vascular endothelium (green arrow), hofbauer cells (blue curved arrow) positive and syncytial node negative (yellow arrowhead) expression sFlt-1 Bar: 50 µm **(c)**. Normotensive group vascular endothelium (green arrow), syncytial node (yellow arrowhead) and nuclei of muscle cells (red asterix) positive expression PLGF Bar: 50 µm **(d)**. PA grubu syncytial node negative (yellow arrowhead) and hofbauer cells positive (blue curved arrow) expression PLGF Bar: 100 µm **(e)**. PP group hofbauer cells (blue curved arrow),syncytial node (yellow arrowhead) and endothelium cells (green arrow) positive expression PLGF Bar: 50 µm (f)..

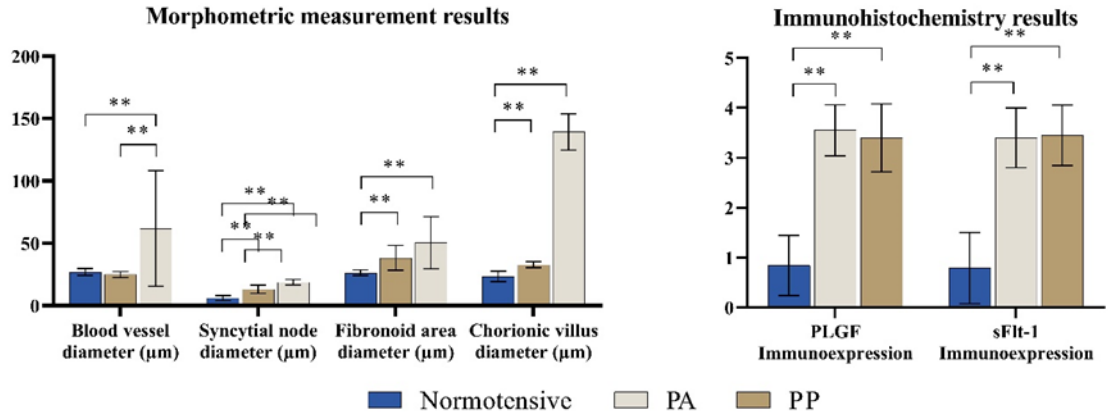

Figure 2. Graphical demonstration of the statistical analysis of Morphometric measurements and Immunohistochemistry of PLGF and sFlt-1. Different symbols between the bar graphs indicate statistically significance. **p<0.0001
PA: Placenta accreta, PP: Placenta previa

Discussion

Recombinant sFlt-1 can block the development of endothelial tubes and inhibit the vasodilator effects of VEGF and PLGF in the vasculature (18). In normal pregnancy, sFlt-1 concentration increases in the third trimester. sFlt-1 binds to PLGF and a recent study suggests that decreased circulating PLGF levels are largely mediated by excess circulating sFlt-1 (19). In normal pregnancies, sFlt-1 levels start to rise after 30-32 weeks of gestation and PLGF levels start to fall after 30 weeks of gestation. In fact, cellular stress in the syncytiotrophoblast that occurs in the last 8-10 weeks of pregnancy leads to biochemical changes in sFlt-1 and PLGF levels in normal pregnancies. (20). In a study, it was shown that plasma sFlt-1 levels and sFlt-1/PLGF ratio of the PAS group were significantly lower than those of the control and PP groups, whereas PLGF levels were the opposite. sFlt-1 concentration and sFlt-1/PLGF ratio were correlated with the volume of blood loss, and it was reported that blood loss may be related to excessive trophoblast invasion of the large vessels of the deep myometrium in PAS patients (21). It has been reported that sFlt-1 and PLGF concentrations

and sFlt-1/PLGF ratio in maternal plasma can be used as biomarkers to predict PA and as a more effective tool to assist ultrasound in the preoperative diagnosis of PA. PA has been reported to have decreased oxidative stress, increased invasion in the placenta and increased oxidative stress in preeclampsia. However, PAS showed a decrease in the level of oxidative stress, possibly related to a decrease in the concentration of the anti-angiogenic factor sFlt-1 (22).

In our study, when sFlt-1 PA was analysed together with the fetal field junction, sFlt-1 reaction was observed in decidual cells with increased intensity, while sFlt-1 reaction was positive in endothelial cells in some degenerated and dilated vessels. sFlt-1 is a signalling molecule formed by pre-signalling when evaluated in terms of VEGF, we can say that this will be exacerbated at a common level in VEGF. When we looked at the free circulating structures of chorionic villi, negative cells were found especially where syncytial nodes were located. However, we observed a positive increase in sFlt-1 reaction in some small hofbauer and connective tissue cells showing inflammatory cell reaction. When the chorionic structure descending from the root villi in PP was analysed, positive sFlt-1 expression was observed especially in conjugated and thrombosed dilated vessels, while negative sFlt-1 expression was observed in syncytial bridges and nodes. Increased sFlt-1 expression was observed in some Hofbauer cells. Although the thrombosed state of the vessels was important, sFlt-1 expression was found to be positive in general, especially in the dilated endothelium.

Yamashita reported that sFlt-1 expression in PP patients was locally high in the caudal part of the internal uterine orifice and placental sFlt-1 levels were also elevated in mouse experiments. Accordingly, it was concluded that the increase in sFlt-1 in the caudal part of the PP causes placental degeneration in the internal uterine os (23). Lower immunostaining for soluble fm's such as tyrosine kinase (sFlt-1), a potent antiangiogenic growth factor, showed low expression in extravillous trophoblast cells (EVT) of women with PA, and has been reported to play a role in the pathological programming of EVT towards increased motility and invasion in PAS (6).

An imbalance of sFlt-1 and PLGF is thought to cause a PAS that results in excessive trophoblast invasion of the endometrium. This excessive invasion can increase maternal and fetal morbidity and is a life-threatening condition, especially during labor (24). Therefore, research has been conducted on whether the PLGF/sFlt-1 ratio can be used as a marker for PAS staging. In this study, it was reported that sFlt-1 was low in cases of PA, PLGF was moderate and high in cases of sFlt-1. Increased PLGF levels would increase the process of placental angiogenesis and increase the depth of placental implantation in the myometrium, and PLGF plays a more important role in the PA process as a pro-angiogenesis factor (25).

In our study, we observed a significant increase in PLGF expression in hofbauer cells and PLGF positive reaction in syncytial nodes in PP. Again, there was a prolonged epiplasmic state with endothelial vessel dilatation and an increase in PLGF positive reaction. We observed PLGF positive expression in some decidual cells. In the examination of PLGF expression in the free floating and stem villi of chorionic villi in PA, negative expressions in the syncytial areas and syncytial bridges were found to be prominent. PLGF positive reaction was detected in the endothelial cells of small capillary vessels in the villi and in some hofbauer cells. This may be related to the increased inflammatory changes.

The stroma of placental villi contains a large number of macrophages, termed hofbauer cells, which are of mesenchymal origin and are thought to function in many processes. Although there are many studies on placental vasculogenesis and angiogenesis, there is a lack of evidence about the possible role of hofbauer cells in these processes. In the study by Seval et al. it was suggested that the location and number of hofbauer cells may be related to the vascular structures in the placental villus nucleus and therefore may play a role in placental vasculogenesis and angiogenesis (26). In our study, PLGF positive reaction in endothelial cells and some hofbauer cells of small capillary vessels in the villus may suggest that these cells may play a role in vasculogenesis and angiogenesis. The increase in sFlt-1 positive reaction in some small hofbauer cells and connective tissue cells showing inflammatory cell reaction may be a sign that induces angiogenesis. The syncytial nod is primarily identified under light microscopy and is defined as a distinct clustering of syncytial nuclei. The increase in syncytial nodes and villus clustering are defined as markers of preeclampsia. They are then thought to indicate placental ischaemia. Instead of being trophoblastic proliferations, most of the nodes appear as slice artefacts of deformed villi due to villous angiogenesis, especially in the last trimester. However, regardless of their aetiology, syncytial nodes are known to show alterations in trophoblastic transformation. An increase in the number of syncytial nodes is not the only sign of hypoxia and may not coexist with other morphological changes of hypoxia (27). VEGF, which increases in response to placental hypoxia, has been reported to be nonfunctional during the pathogenesis of pre-eclampsia. Furthermore, as ligand-receptor binding is impaired, the amount of free VEGF increases in the preeclamptic placenta and impairs vascular function as a result of endothelial cell damage. A significant increase in VEGF expression was observed in syncytial nodes and bridges due to hypoxia effect after placenta previa (28). In our study, when we looked at the free circulating structures of chorionic villi in PP and PA, we observed negative sFlt-1 expression, especially in syncytial nodes. We also observed positive PLGF expression in syncytial nodes in PP. PLGF expression may be an important factor in determining syncytial nodes and both angiogenic and inflammatory aspects.

The limitation of our study is that although the changes in PLGF and sFlt-1 expressions in PA and PP cases are directly involved in both placental pathogenesis, different methods

need to be used to validate the data we obtained. The limitation of these methods is one of the limitations of our study. In addition, there is a need for comprehensive, large and new studies examining and interpreting the interaction of growth factors and kinases, which are directly involved in angiogenesis and many processes in the tissue, with the microframe.

Conclusion

It has been observed that in cases of PP caused by different causes and associated with oxidative stress formation, trophoblastic degeneration, apopitotic cells and endothelial dysfunction seen in the placenta together with the rate of differences in sFlt1/PLGF exchange increases the likelihood of preterm delivery and may cause significant complications. sFlt1/PLGF expression levels have been suggested to be an important marker in determining this process. Since the variation in the expression distribution of sFlt-1 and PLGF induces a spectrum of PA in the endometrium with trophoblastic invasion and trophoblastic increase in the endometrium leading to PAS causing excessive trophoblast invasion and increased angiogenesis leading to an imbalance in implantation, it is thought that sFlt-1 and PLGF duo may be an important signaling stimulus in this process.

Ethical Approval: *This study was carried out in accordance with the rules of research and publication ethics. The study was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee, numbered 2022/67.*

Author Contributions:
Concept: G.E:, E.D.
Literature Review: G.E., S.K.
Design : G.E., S.K., E.D.
Data acquisition: G.E., N.P., I.I.
Analysis and interpretation: G.E., E.D.
Writing manuscript: G.E.
Critical revision of manuscript: N.K., S.P., I.I.

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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