



Placental Growth Factor-soluble FMS-like Tyrosine Kinase-1 Ratio in Placenta Accreta Spectrum Disorder: Case Control Study

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Abstract

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AIM: The aim of the study was to assess the ratio of placental growth factor (PLGF)/soluble FMSlike tyrosine kinase (sFLT-1) as a marker for in placenta accreta spectrum disorder stage.

METHODS: We enrolled 50 participants in this study, 25 participants diagnosed with placenta accreta spectrum disorder and 25 participants with normal pregnancy as controls. Diagnosis is based on ultrasonographic criteria from the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) for spectrum disorders of placenta accreta. Up to 3 cc peripheral venous blood was taken before cesarean section to measure PLGF and sFlt-1 level by ELISA. All data then analyze using SPSS version 26.

RESULTS: In this study, we found that the levels of sFlt-1 in the placenta accreta group (Placenta accreta spectrum stage 0/1/2) were 1711 (136.87) pg/ml, 1474 (122.88) pg/ml, and 1417 (125.45) pg/ml each. This level was higher than the control group of 1246 (98) pg/ml ($p = 0.004$). In measuring PLGF levels, we found that PLGF levels in the control group were lower than those in the placenta accreta group (PAS 0/1/2) with levels of 404 (33.12) pg/ml, 612 (48.96) pg/ml, 805 (53.48) pg/ml, and 785 (53.64) pg/ml, respectively. We found a correlation between placenta accreta spectrum staging with sFlt-1 levels ($r = 0.27$ and $p = 0.015$) and PLGF levels ($r = 0.6646$ and $p = 0.001$). Ratio of sFlt-1/PLGF with cutoff point 1.8 has sensitivity 97% and specificity 67% (Area under the curve (AUC) 0.784).

CONCLUSIONS: There is a correlation between sFlt-1 and PLGF levels with placenta accreta staging based on PAS score. sFlt-1/PLGF ratio can be considered as a predictor of placenta accreta to help establish the diagnosis of placenta accreta.

Introduction

Placenta accreta spectrum (PAS) [1] is a general term used to describe abnormal trophoblast invasion into the myometrium of the uterine wall. It is the result of implantation of the placenta in the damaged dedualization area usually caused by pre-existing damage to the endometrial-myometrial surface. Clinically, the placenta does not spontaneously separate at the time of delivery and attempts at manual removal can cause bleeding, which can be life-threatening [2]. In a 2019 systematic review that included 7001 cases of PAS among nearly 5.8 million births in general population, the aggregated overall prevalence was 0.17% (range 0.01–1.1%) [2]. This is much higher than the 0.003% prevalence in the United States in the 1950s [3]. A very significant increase in PAS, starting in the 1980s and 1990s, has been observed worldwide, and is associated with the increasing prevalence of cesarean delivery in recent decades [4].

Role of sFlt-1 and PLGF in the placenta

Placental “migration” is a phenomenon previously examined using serial sonography

documenting the presence of a low-lying placenta or placenta previa in the second trimester of pregnancy, with subsequent conversion to the upper uterine segment at the end of the third trimester. Approximately 10%–20% of placenta previa at 20 weeks’ gestation remains in the late third trimester. One of the widely proposed mechanisms is placental “trophotropism” or “dynamic placentation”, which is associated with atrophy of thin placental margins due to poor vascular supply, compared to other areas of the placenta that continues to grow and therefore the placenta migrates in a more abundant direction of blood vessel [5]. Despite this hypothesis, the mechanism of placental migration has not been fully elucidated. Some pregnant women recover from placenta previa due to “migration” of the placenta. In contrast, another woman had persistent placenta previa [1]. The human placenta is formed by trophoblast invasion of the uterine endometrium; thus, it is difficult to imagine that the placenta “migrates” from its original position. Assuming that the “migration” is due to placental degeneration due to ischemia, these two patterns of placenta previa during pregnancy may have different angiogenic status [1]. Yamashita [5] showed that the level of sFLT-1 expression is locally high in the caudal

part of the internal uterine orificium in patients with placenta previa and that placental weight decreases with obstruction of the blood supply. Placental sFlt-1 levels were also elevated in mouse experiments. From these results, it was concluded that an increase in sFLT-1 in the caudal portion of placenta previa causes placental degeneration of the internal uterine os [5]. Lower immunostaining for soluble fms such as tyrosine kinase (sFLT-1), which is a potent antiangiogenic growth factor, has been observed in (extravillous trophoblast) EVT cells of women with placenta accreta. These findings suggest that (vascular endothelial growth factor) VEGF and sFLT-1 play an important role in the pathological programming of EVT toward increased motility and invasion in PAS [1]. EVT cells lose their invasive phenotype through syncytial-type fusion into multinucleated giant cells (MNGCs), and VEGF secretion by MNGCs is likely one of the signals initiating and coordinating vascularization in the decidua and placenta during implantation. PIGF has the same structure as VEGF-A and is also a potent angiogenic growth factor, which is thought to amplify VEGF signaling by displacing VEGF from the Flt-1 receptor as well as facilitating PIGF to bind to the receptor domain kinase (RDK) or VEGFR-2. PIGF has four isoforms that have different sizes. PIGF-1 and PIGF-2 are believed to be the main isoforms, consisting of PIGF-1 and PIGF-2 having 131 and 152 amino acid residues, respectively. PIGF is a dimeric and monomeric protein bound by disulfide bonds. Each PIGF monomer has eight cysteine residues and two of them are linked by disulfide bonds between the monomers. PIGF can also form heterodimers with VEGF, especially when both proteins are expressed on the same cell [6], [7]. PIGF is expressed by endothelial cells, trophoblasts, monocytes, and erythroid cells. Glial cell missing transcription factor 1 (GCM1) is a transcription factor for PIGF in trophoblasts. Hypoxia triggers GCM1 degradation, resulting in decreased PIGF expression. In addition, the transcription factor MTF-1 has been reported to regulate PIGF expression in trophoblast cells. Like GCM1, MTF-1 expression is decreased due to hypoxia caused by decreased PLGF expression [6]. PIGF stimulates angiogenesis under conditions of ischemia, inflammation, and wound healing and can lead to atherosclerosis. An imbalance of sFlt-1 and PLGF is suspected to cause a spectrum of placenta accreta which, in turn, causes excessive trophoblast invasion of the endometrium. This excessive invasion can cause increased morbidity to the mother and fetus and is a life-threatening condition, especially during childbirth [6]. Therefore, we were interested in investigating whether the PLGF/sFLT-1 ratio could be used as a marker for staging the placenta accreta spectrum, so we conduct this pilot study in our center.

Methods

Ethics statement

This study obtained ethical approval from the Research Ethics Committee of Universitas Sumatera Utara and Haji Adam Malik General Hospital, Medan no: 273/KEP/USU/2020. Written informed consent to participate (through signature) was obtained from all the participants. Written informed consent for publication of the patients' details was also obtained from the patients/a guardian of the patient.

Population and sampling

In this study, we enrolled 50 participants (according to the prior study by jauniaux, we are using that PAS prevalence in general population is 0.17) [2] consisting of 25 case groups and 25 control groups (normal pregnancy). Eligible patients were outpatients at the polyclinic for fetomaternal at Haji Adam Malik General Hospital from February to May 2020. Patients included in this study were patients who would have their pregnancy terminated in the next 2 days. The PAS' score was assessed by two obstetricians who had ultrasound certification (the obstetricians blind about this study). Patients who participated in this study provided informed consent and signed a letter of consent to participate in this study.

Prenatal ultrasound staging system for placenta accreta spectrum disorders

The diagnosis of PAS was obtained through ultrasound examination (Voluson P6) [8]. In the semi-recumbent position, the ultrasound transducer is positioned either in the right or left abdominal iliac fossa, toward the lateral wall of the uterus and down into the pelvis, to obtain a sagittal view of the uterus and cervical canal. Then, a PAS assessment is carried out which includes: (1) Placental lacunae, (2) loss of clear zone, and (3) interruptions to the bladder wall. The result of the ultrasound examination was classified as follow, PAS0: Placenta previa without ultrasound signs of invasion or placenta previa with placental lacunae but no evidence of an abnormal uterine-bladder surface (loss of clear zone and/or bladder wall disruption); PAS1: Presence of at least two ultrasound signs: (1) Placental lacuna, (2) loss of clear zone, and (3) interruptions to the bladder wall; PAS2: PAS1 plus uterovesical hypervascularity; PAS3: PAS1 or PAS2 plus evidence of increased vascularity in the inferior portion of the lower uterine segment extending to the parametrial region [9].

sFlt-1 and PLGF examination procedures

We took 3 ml of blood from the median cubital vein and placed it in a vacuum tube containing ethylenediaminetetraacetic acid (EDTA) to be sent to the laboratory. Blood samples were taken during cesarean section. Increased or decreased levels of sFlt-1 were checked in the laboratory. We used the sFlt-1 Elisa kit and the PLGF Elisa kit (MyBioSource) [10], [11]. We added 100 ul of assay diluent RD1W into the tube, added 100 ul of standard, control, and sample into one tube, then closed with a sealer for 2 h at a speed of 500–50 rpm and orbital 0.12. We then discarded the contents of each tube and washed them by adding 400 ul of wash buffer to the tube. We repeated the washing process 3 times. In the last wash, we removed the contents of the tube and wash buffer. We then added 200 ul of conjugate to the tube. Then, tube was then covered with sealer, incubated for 2 h, and the whole process repeated. Then, 200 ul of substrate solution was added to the tube, which was then covered with a sealer and incubated for 30 min. A total of 50 l of stop solution was added to the tube, read at a wavelength of 450 nm and a wavelength of 540 nm or 570 nm. Both groups were followed until termination of pregnancy then, the diagnosis of accreta was confirmed through the results of anatomical pathology examination. After all the data are obtained, the data are processed and analyzed statistically.

Statistical analysis

Analysis in this study was done with the use of SPSS version 26 [12]. All data are presented in mean and standard deviation (SD). Independent Student t-test and Mann–Whitney U test were used for the analysis of two variables, while for more than two variables ANOVA and Kruskal–Wallis tests were performed. *Post hoc* test was used if the ANOVA and Mann–Whitney analysis indicated a significant difference ($p < 0.05$). The results of the study were considered significant with $p < 0.05$ (95%).

Results

Participant characteristics

From the analysis of patient characteristics (Table 1), it was found that there were differences in the number of history of cesarean section, history of curettage, and duration of previous surgery between the PASD group and the control group ($p < 0.001$; $p = 0.03$; and $p < 0.001$).

sFlt-1 and PLGF level

There were differences in sFlt-1 levels in the case group and the control group. The levels of sFlt-1

Table 1: Respondent characteristic

Characteristics	PASD (n = 25)	Control (n = 25)	p*
Age (years) (means ± SD)	33 (9)	30 (6.5)	0.08
Weight (kg) (means ± SD)	54 (5)	54 (4.5)	0.72
Height (cm) (means ± SD)	145 (10)	150 (10)	0.08
UAC (cm) (means ± SD)	24.8 (2.1)	25.9 (4.1)	0.36
Gravida (means ± SD)	3 (2)	3 (3)	0.09
Paritas (means ± SD)	2 (2)	2 (2)	0.50
Gestational age (weeks) (means ± SD)	36 (3)	37 (4)	0.68
History of cesarean section (means ± SD)	2 (1.5)	1 (1.5)	< 0.001
History of curettage (means ± SD)	0 (1)	0	0.03
Prior surgery (years) (means ± SD)	4 (3)	1 (1.5)	< 0.001

*Wilcoxon. UAC: Upper arm circumference, PASD: Placental accreta spectrum disorder, SD: Standard deviation.

in the control group were lower than those in the PAS group (Figure 1), but there was a downward trend in sFlt-1 levels when compared between PAS 0, PAS 1, and PAS 2. In this study, there was no PAS 3 group.

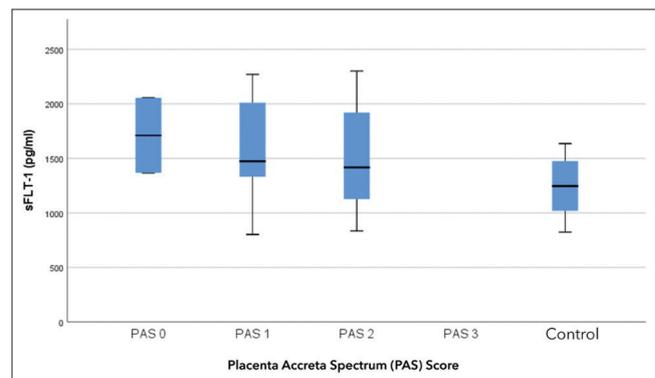


Figure 1: Comparison of sFlt-1 levels in placenta accreta spectrum disorder (PAS 0/1/2/3) and the control group

There were differences in PLGF levels in the case group and the control group. The levels of PLGF in the control group were lower than in the PAS group (Figure 2), but there was an increasing trend of PLGF levels when compared between PAS 0, PAS 1, and PAS 2. In this study, there was no PAS 3 group.

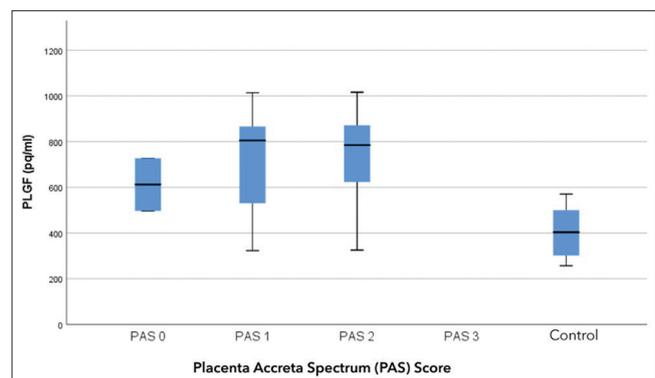


Figure 2: Comparison of PLGF levels in placenta accreta spectrum disorder (PAS 0/1/2/3) and the control group

sFlt-1 and PLGF correlation to placental accreta spectrum disorder

In Table 2, we can see that there is a significant correlation between sFlt-1 levels and PAS staging ($r = 0.270$ and $p = 0.015$), as well as in PLGF, found a significant correlation with the staging of PAS ($r = 0.646$ and $p = 0.001$). Based on the analysis of the

Table 2: Correlation of soluble FMS like tyrosine kinase-1 and placental growth factor level and placenta accreta spectrum score

Parameters	Means \pm SD			r^s	P
	PAS 0	PAS 1	PAS 2		
sFlt-1 (pg/ml)	1711 (136.87)	14.474 (122.88)	1417 (125.45)	0.270	0.015
PLGF (pg/ml)	404 (33.12)	612 (48.96)	805 (53.48)	0.646	0.001

*Spearman correlation. PAS: Placenta accreta spectrum, SD: Standard deviation, PLGF: Placental growth factor, sFLT: Soluble FMS like tyrosine kinase.

receiver operating characteristic (ROC) curve, it was found that the sFlt-1/PLGF ratio of 1.8 had a specificity of 97% and a sensitivity of 67% in diagnosing the incidence of placenta accreta. In this analysis, it was found that the sensitivity value is quite high, but the specificity value of this examination is very low so that it can cause a high incidence of false positives in this examination when used in diagnosing placenta accreta.

Discussion

In this study, the researchers compared two groups (the PAS group and the normal pregnant group as controls) by first matching the characteristics of the two groups to avoid bias in the analysis of sFlt-1 and PLGF levels in both groups. In Table 1, we can see the data on the characteristics of respondents found in the study. Differences in the characteristics of the case group and the control group were only found in the history of cesarean section, previous history of curettage, and the duration of the last surgery before the patient was diagnosed with placenta accreta. This is in line with the study of Uyanikoglu *et al.* [13], who tried to compare serum levels of sFlt-1 in pregnant patients with placenta percreta. In this study, they used a sample size of 50 people, consisting of 25 people in the case group with a diagnosis of placenta accreta and 25 people with a normal pregnancy. The mean age in this study was 33.73–4.36 and 30.09–5.63, gravida 6.18–2.10 and 4.50–1.05, parity 4.64–2.08 and 3, 23–0.97, history of abortion 0.5–0.74 and 0.32–0.47, gestational age at sampling 34.59–2.70 and 35.36–1.86, and history of previous CS 3, 32–0.89 and 2.77–1.06 in the placenta percreta group and the control group, respectively. The characteristics of the sample that they used were the same as our study, but in some variables, no matching was found between the placenta accreta group and the control group, namely, age, gravida, and parity variables ($p = 0.022$; $p = 0.002$; and $p = 0.024$) while in our study, almost all variables were matched except for the number of previous cesarean sections, history of curettage and previous surgery distance ($p < 0.001$; $p = 0.03$; and $p < 0.001$) [16]. Another study by Shainker *et al.* [14] also compared the expression of sFlt-1 in placenta accreta with a control group, where the characteristics of respondents based on age and gestational age were 40 (35–44) and 34.6 (32.1–35.1); 35 (34–36) and 32.9 (32.9–35.1) in each group. The age ranges in the placenta accreta group and

the control group used in the Shainker *et al.* [14] study was slightly higher than in our study. In the study of Shainker *et al.* [14], no matching was performed on each variable between groups. Tseng *et al.* [15] also conducted the same study with the same characteristics of respondents between the placenta accreta group and the control group with gestational ages 34 (31–35.5) and 36 (32–37) in each group. In the research of Tseng *et al.* [15], no matching was also performed. In Figure 1, we can see the difference in serum sFlt-1 levels based on PAS stage in respondents compared to the control group. In this case, the levels of sFlt-1 in the PAS group were higher than in the control group, but there was a decreasing trend along with the higher staging of PAS. In Figure 2, we can see that the mean PLGF levels in the control group were lower than those in the PAS 0, 1, and 2 groups, while the PAS 3 group was not found due to the few cases found. This indicates that PLGF increased in the PAS group. The authors have not found any literature comparing levels of PLGF and sFlt-1 based on PAS staging. Based on the graph of sFlt-1 and PLGF levels based on PAS staging, we conclude that sFlt-1 levels show a decreasing trend with increasing PAS scores, in contrast to PLGF which shows an increasing trend with higher PAS staging. We tried to find a correlation between PAS staging and sFlt-1 and PLGF levels and found a weak relationship between sFlt-1 levels in the blood and PAS staging, while in PLGF, we found a moderate relationship between PLGF levels in the blood and PAS staging. Hence, we think that PLGF plays a more important role in the process of placenta accreta. PLGF is a pro-angiogenesis factor. Increased levels of PLGF will increase the process of placental angiogenesis and will increase the depth of placental implantation in the myometrium. Based on the analysis of the ROC curve, it was found that the sFlt-1/PLGF ratio of 1.8 had a specificity of 97% and a sensitivity of 67% in diagnosing the incidence of placenta accreta. In this analysis, it was found that the sensitivity value is quite high but the specificity value of this examination is very low so that it can cause a high incidence of false positives in this examination when used in diagnosing placenta accreta. In this study, we are aware of some shortcomings, where the number of samples that we use is small and this study is only a pilot study, so that a multicenter study with a larger number of samples is needed. However, these findings open new horizons for us regarding the pathogenesis of placenta accreta and may be used as markers to aid in the diagnosis of placenta accreta.

Conclusions

This study showed that sFlt-1 was decreased and PLGF was increased in placenta accreta. These findings can be taken into consideration to see the potential of these two biomarkers as markers to help diagnose placenta accreta. This research is basic

research, so further research is needed to see this potential in the future.

Data Availability

Underlying data

Mendeley data: Raw Data, sFlt-1, and PLGF. <https://data.mendeley.com/datasets/97zt9fzky8/3> [12]. This project contains the following underlying data:

- Raw Data accreta publish.xlsx (sFlt-1 Result, PLGF, result and patient raw data).

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

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