

Predictive value of vascular endothelial growth factor and placenta growth factor for morbidly adherent placenta among women with previous history of cesarean section

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Abstract

Background The present study aims to determine the predictive value of vascular endothelial growth factor (VEGF) and placenta growth factor (PLGF) for placenta accreta and for the comparison of the serum level of VEGF and PLGF in normal pregnant women and women with placenta accreta spectrum (PAS).

Methods This prospective case-control study was conducted during the 2 years in two hospital in Shiraz, Iran from 2017 to 2019. The inclusion of the study was 90 singleton pregnant women: 45 of them in gestational ages of 28-34 weeks with pathological confirmation of placenta accreta spectrum and 45 healthy pregnant women. In the PAS group, according to pathology reports on the placenta, we divided all cases to placenta accreta, percreta, and increta. Maternal serum level of VEGF and PLGF were measured before the termination of pregnancy and diagnostic accuracy of each factor was evaluated.

Results In PAS group, 75 percent of patients had placenta previa and unlike the control group, there was a significant difference in the gravidity, number of cesarean section, maternal age, and serum level of PLGF ($p < /001$); while in VEGF, the difference was not statistically significant.

Conclusions The results of the present study revealed that the maternal serum level of PLGF could be considered as an accurate predictive test in patients with PAS.

Background

With the rising rate of cesarean section (C/S) worldwide, placenta accreta is considered as a new pathological matter[1]. According to the depth of invasion of placenta to myometrium, the placenta accreta spectrum (PAS), formerly known as morbidly adherent placenta, is divided into three groups: placenta accreta with superficial myometrial invasion, placenta increta with deep myometrial invasion, and placenta percreta with deep invasion of placenta to serosa of uterus and sometime to adjacent organs [1, 2].

In normal placentation, extravillous trophoblasts containing interstitial and endovascular cells invade decidua and superficial myometrium and cause remodeling of the basilar and spiral artery[3, 4].

Thus, excessive trophoblastic invasion, abnormal decidualization, abnormal angiogenesis, and decrease of apoptosis of trophoblasts can result in abnormal adherence of placenta[5, 6].

In uterine scars such as site of C/S, total or partial absence of decidua, thinning, irregularity or absence of Nitabuch's layer, and subsequently, placenta accreta can happen [4, 7-9].

Also, deficient trophoblastic invasion can be harmful and is related to abortion, preeclampsia, intrauterine growth restriction (IUGR), and preterm delivery[3].

The most important risk factors of PAS are previous C/S and intrauterine operation [10-12]. Other risk factors included placenta previa, multiparity, maternal age, any previous uterine surgery, surgical abortion, radiation, endometrial ablation, in vitro fertilization (IVF), chemotherapy, and adenomyosis [7, 10-12].

The rate of C/S in the United States is 32-33% and placentas accreta is equal to 1/540-1/2500 of total deliveries[1]. It has increased since the early 1900s and according to one meta-analysis , overall prevalence of PAS is 0.17% (range from 0.01 -1.1%) [12, 13]. The rate of PAS in women with placenta previa without previous C/S is 3% and with 1, 2, 3 times C/S reaches 11%, 40%, and 60%, respectively[1].

Placenta accreta causes serious complications in pregnancy and increases the maternal mortality rate up to 0.7%[14, 15].

Due to the high maternal mortality (0.7%) and morbidity rate (37.1-56/7%), antenatal diagnosis is important and can improve maternal and perinatal outcome [2, 16].

The gold standard for diagnosis is the microscopic and pathologic report after removing the uterus[1] but in pregnancy, grayscale ultrasonography is the first modality for the detection of placenta accreta.

Color and power Doppler ultrasonography have improved the diagnosis of placenta accreta and Magnetic Resonance Imaging (MRI) is a good tool for evaluation of the depth of the invasion particularly in post placenta[1, 17].

Sonographic criteria for diagnosis of PAS are as follow:

- Loss or irregularity of retroplacental echolucent area
- Thinning of myometrium in site of the implantation of placenta
- Bulging of placenta on the bladder wall
- Increased vascularity of the uterine serosa on the bladder wall with presence of bridging vessels
- Multiple lacuna in placenta[7]

There are not high sensitive serum markers for antenatal diagnosis of accreta[7]. Many hormones and regulatory molecules have important roles in decidualization and trophoblastic adhesion and invasion[1].

Normal placental implantation requires fine balance between the levels of angiogenic and anti-angiogenic factors such as placenta growth factor (PLGF), vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase 1 (sFlt-1), and oxidative status [18].

In cases of placenta accreta, placental and serum level of the receptor of TNF-related apoptosis-inducing ligand (TRAIC) as an apoptotic factor and placental level of sFlt-1 as an anti-angiogenic factor decrease [19, 20][19, 20]; and placental level of intercellular adhesion molecule 1 increases [21].

In PAS, the VEGF messenger RNA expression, the receptor of VEGF, and Epidermal growth factor (EGF) in syncytiotrophoblast increase, but serum level of VEGF can be low [6, 22, 23].

Although several studies have investigated the role of VEGF and its receptor in predicting the placenta accreta, the results are conflicting. Thus, further studies are required to evaluate the predictive value of VEGF and PLGF. In this regard, the present study aims to determine the predictive value of VEGF and PLGF in patients with PAS.

Methods

Study Population

This prospective case-control study was conducted during 2 years period from March 2017 to March 2019 on 90 patients in Shahid Faghihi and Hazrat-e- Zeinab hospital, both tertiary health care and obstetric referral center in southern Iran affiliated with Shiraz University of Medical Sciences (SUMS). The study protocol was approved by Institutional Review Board (IRB) and the Medical Ethics Committee of Shiraz University of Medical Sciences (Registration code: IR.SUMS.MED.REC.1397.495 and IR.SUMS.MED.REC.1398.023). Before inclusion in the study, all the patients produced their written informed consents.

The inclusion of the present study was 45 singleton pregnant women who were registered to the centers for prenatal care and were diagnosed PAS by ultrasonography or MRI with the histological confirmation after delivery and with the gestational age of 28-34 weeks (approved according to the last menstrual period and first-trimester sonography).

Also, 45 healthy singleton pregnant women with previous history of C/S and without PAS were randomly selected as a control group. Exclusion criteria were the pregnancy with gestational age before 28 weeks and after 34 weeks, multiple pregnancies, and any maternal or fetal morbidities including IUGR, diabetes, hypertension, inflammatory or connective tissue disorders, liver or renal failure, pulmonary and cardiovascular disorders, fetal malformations, and placenta separation. The maternal blood serum of all patients enrolled in the study were analyzed after overnight fasting.

Sample size calculation

Considering the results of the study by Wehrum et al[6], the mean difference of 71 and standard deviation of 100 for PLGF variable, and type one and two error of 0.05 and 0.10 respectively, the minimum sample size was estimated as 43 in each group.

Study protocol and intervention

All the patients were initially examined by the obstetrics-gynecology resident and their baseline characteristics, demographic information, and obstetrics history were recorded. There were 2 groups of patients: with PAS and without PAS. Diagnosis of PAS in pregnancy was based on the transabdominal

grayscale, transvaginal grayscale, and Doppler sonography which was performed by a perinatologist. Besides, in the patients that sonography was not conclusive, the diagnosis was based on the report of MRI by radiologist.

All the patients with PAS and gestational age ≥ 32 weeks admitted in the hospital. Earlier hospitalization was done for patients with vaginal bleeding or any obstetric complications, and non-cooperative patients for regular visit. In non-complicated patients, the termination was done at the end of the 34th week according to my country protocols. All patients received betamethasone for fetal lung maturity before the termination. Patients without confirmation of placenta accreta excluded from the study after the operation. Finally, after hysterectomy, the uterus and placenta were sent to the pathology for the detection of the depth of invasion.

Laboratory analysis

To measure the PLGF and VEGF 3 mm of venous blood was collected from each participant in the PAS group at the time of admission for delivery, and before the administration of betamethasone or blood transfusion. The blood sample was centrifuged and serum stored at -80°C until analysis. Also, in control group, we match the sampling time to PAS group and blood sampling was done in gestational age of 28-34 weeks but the termination of pregnancy was at term. Plasma level of VEGF and PLGF were measured by enzyme-linked immune sorbent assay (ELISA) in all patients. VEGF was measured with ELISA kit (Bioassay Technology Laboratory, Shanghai, China, product no. E0080Hu) with 20 to 6000pg/ml and PLGF with ELISA kit (Bioassay Technology Laboratory, Shanghai, China, product no. E0138Hu,) with 8 to 1800pg/ml detection rate based on the manufacturer's instructions. Cut off point of VEGF and PLGF were 135/1(pg/ml) and 59/7(pg/ml), respectively.

Immediately, after hysterectomy, uterus and placenta were stored in 10% neutral-buffered formalin contains and sent to the pathology department. Serial bread-loaf sections of the uterus were examined for areas of increta/percreta. Multiple sections were taken from the suspicious area to accreta and myometrial invasion. Also, two sections of the cervix or lower uterine segment (in supra-cervical hysterectomy) were taken to represent the placenta previa. The absence of decidua between the placental villi and myometrium was considered as the placenta accreta. Deeper invasion into the myometrium was considered as the increta and complete invasion through the uterine wall as the percreta. [24] In addition, the patients with PAS were divided into two groups: the group with PAS and placenta previa and the group with PAS, without placenta previa.

Statistical methods

Descriptive statistics were reported using number (%) and median (IQR) for qualitative and quantitative variables, respectively. For statistical analysis, Mann-Whitney, Kruskal-Wallis, and Fisher's exact tests were used in SPSS 19.0 and $P < 0.05$ considered as statistically significant.

Results

In the present study, the participants were 45 accreta cases and 45 women with normal pregnancy. The mean± SD of the age of the participants was 30.96± 5.22 years: 32.82 ± 0.74 in accreta group and 29.08 ± 0.72 in the control group. The majority (53.3%) of them had previous history of one C/S and numbers of C/S: 1.76 ± 0.127 in accreta group and 1.06 ± 0.097 in the control group. Based on the severity of the invasion, 45 cases of accreta were divided into 3 subgroups: accreta (64.4%), percreta (17.8%), and increta (17.8%). As shown in Table 1 and Graph 1, the mean age of accreta group was significantly higher than control group (33 versus 29, P=0.001).

All the data on the comparison of the three subgroups of accreta and normal pregnancy cases were summarized in Table 2. The results showed that there was a significant increase in the gravidity of the placenta accreta, placenta increta, and placenta percreta groups compared to the normal pregnancy group (p<0.001).

Also, there was a significant increase in the maternal age and number of C/S in the abnormal adhesion of placenta group compared to the normal pregnancy group (p<0.001). (Graph 1). Percreta cases with the mean age of 38 years were significantly older than the other groups (P=0.001). Although the level of the VEGF was lower in the accreta group, the difference was not statistically significant (P=0.17). Moreover, the average level of the PLGF was 88.4(pg/ml) in accreta group which was significantly higher than the control group (35.9, P<0.001) and also had the highest diagnostic accuracy based on the AUC. The AUC for the PLGF was found to be 0.84 (95%CI: 0.75-0.93) indicating a moderate-high accuracy. The AUC for the VEGF was reported to be 0.58(95%CI: 0.46-0.70) indicating a low accuracy. The ROC curves for all the tested variables are shown in Fig. 1.

The level of the PLGF among all of the accreta subgroups were significantly higher than the control group (P<0.001). However, there was no significant difference between 3 groups of placenta accreta (p>0.05) (Figure 2). No significant difference was detected among the groups in term of the level of the VEGF. Also, in comparison of PLGF and VEGF, the results did not indicate a statistically significant difference between the placenta previa and no previa group (p>0.05)

Although the proportion of previa among the increta group was higher than the percreta (100% versus 87.5%) and in percreta higher than the accreta (87.5% versus 65.5%), Fisher's exact test showed that the differences were not statistically significant (P=0.10) which may be due to low sample size (Table 3)

Discussion

The placenta is a vital specialized organ connecting the mother and the fetus and plays an important role in the healthy normal pregnancy. The key role of villous trophoblasts in the development of PAS is well-known. The molecular mechanism of PAS is not clearly described.

For the placental development, there should be coordination between the angiogenic and anti-angiogenic growth factors and their receptors. The Antenatal diagnosis of placenta accreta spectrum provides an opportunity to coordinate a multi-disciplinary team for managing the placenta accreta in a tertiary center.

Pre-operative diagnosis and planned managements decrease the maternal and neonatal morbidity and mortality.

In Iran, the rate of C/S is much higher than the world health standards. It includes 50–60% of total deliveries and 90% of which occurs in the cities and private hospitals [25].

Most of the studies have investigated the placental level of the angiogenic and antiangiogenic factors while the studies on the serum level of these factors are considerably low. According to the results of this study, the maternal serum levels of angiogenic factors could be have predictive value for the prenatal diagnosis of PAS.

In the present study, the value of VEGF and PLGF in predicting PAS was investigated. The maternal serum level of the PLGF in pregnancy was found to be accurate in diagnosing the PAS, whereas maternal serum level of the VEGF had no diagnostic accuracy. Also, though the maternal serum level of the VEGF was lower in the PAS group, the difference was not statistically significant and the level of the PLGF was not affected by the depth of invasion.

In the present study, the gravidity, number of C/S, and age in the accreta group were significantly higher than the normal group that is similar to previous studies [18].

Overall, the present findings indicated that the maternal serum of the VEGF is not an accurate predictor of PAS, whereas the PLGF has more accuracy and might be suitable for the diagnosis of the PAS in pregnancy.

The PAS is a multifactorial complex: abnormal decidualization, abnormal maternal vascular remodeling and increased trophoblastic invasion are the important cause of the PAS[2]. Decidua is the source of the VEGF and PLGF and with the paracrine mechanism regulate the trophoblastic invasion[18].

Both VEGF and PLGF are glycoproteins. These factors with 4 isoforms have an important 'role in the angiogenesis, decidual vascularization, growth, differentiation and trophoblastic invasion. The VEGF has 3 receptors and is bound to both receptor 1 and 2 with high affinity. The VEGFR-1 is a kinase insert domain-containing receptor (KDR) and the VEGFR-2 is Fms-like tyrosine kinase receptor (Flt-1). The PLGF only exhibit the high affinity binding to the Flt-1 receptor [26].

In vitro and in normoxic conditions, in term of the placenta, the level of the PLGF was higher than the VEGF and in hypoxic condition, the VEGF expression was upregulated, while the PLGF expression was decreased. Thus, according to the oxygen tension, the level of these factors could be changed [26].

The Soluble Flt-1 is the Antagonist of the VEGF. The hypoxia leads to a decrease in the sFlt-1 and hyperperfusion can increase the expression of the sFlt-1 in the villous trophoblasts [20].

The PAS has tumoral behavior with epithelial mesenchymal transition in trophoblasts [27]. With the excessive neovascularization, it upregulates the angiogenic factors such as VEGF and angiopoietin 2

(Ang-2) and downregulates the antiangiogenic factors such as sFlt-1 and VEGFR-2 in syncytiotrophoblast [22].

Compromised oxygenation in the scars of C/S is the cause of excessive trophoblastic invasion to the myometrium. Thus, in the PAS, maternal serum level of the total oxidative stress decreases and the total antioxidative stress increases [28]. In this study, the placenta previa was relatively associated with the placenta percreta indicating the implantation in the area with the lesser blood supply results in hypoxia and further more placental invasion.

Several studies revealed that the expression of angiogenic factors such as VEGF, PLGF, ICAM-1, EGF, and TGF- β increase in the placenta and myometrium of the patients with the placenta accrete and expression of VEGF-R decreases [21–23, 28].

These studies did not evaluate the maternal serum level of the angiogenic factors. In a study by Hacer Uyanıkoğlu et al, the maternal serum level of the VEGF, PLGF, and sFlt-1, compared to the control group, was lower in the patients with the placenta percreta [29].

Wehrum et al analyzed the maternal serum level of the VEGF, PLGF, and sFlt-1 between patients with and without complete placenta previa. He found that the serum level of the VEGF decreases in the patients with the placenta previa and accrete, while only placenta previa did not affect the serum level of these 3 factors and also, serum level of sFlt-1 and PLGF in both groups were similar.

Also, placenta level of the VEGF was high in the patients with the PAS [6]. In another study by Biberglu et al, it was found that maternal serum level of VEGF, PLGF and sFlt-1 have no statistical difference between patients with and without PAS and these angiogenic factors have no predictive value for the diagnosis of the PAS [30]. In the present study, the low serum levels of the VEGF were detected which were not statistically significant.

The serum level of the VEGF in the PAS may decrease due to various causes: consumption of the VEGF in the placenta or the increase in the serum level of the sFlt-1 which is binding to VEGF; however, in this study the serum level of sFlt-1 has not been checked.

Lack of decidualization and consequently a decrease in the production of the VEGF in decidua could be another cause of the low serum level of the VEGF.

In hypoxic conditions such as preeclampsia, VEGFR-1 increases and causes a decrease in the serum level of the PLGF in the patients.

In concordance with the present study, in the hypervascularity conditions such as placenta accreta, the antiangiogenic factor including VEGFR-1 decreases leading to decreased attachment of PLGF to its receptor. The final consequence of this phenomenon is increased serum level of PLGF [22, 31, 32].

There were some limitations in the present study, including inability to check the maternal serum levels of the angiogenic factors in the first trimester, in implantation time, and serial evaluation in each trimester. The study also did not check the placenta levels of the angiogenic factors and their receptors in placenta

Though the age, gravidity, and the number of C/S in both groups were different, there was no correlation between these factors and the level of the VEGF and PLGF. However, the study had some strength points including the evaluation of the correlation between the age, gravidity, and the number of C/S with the rate of the placenta accreta. Also, the blood sampling in the control group was performed before 34th weeks. Another strength point was the large sample size of the PAS group, while the previous studies had a more limited sample size. Besides, the present study excluded the patients with the comorbidities that could affect the angiogenic factors.

In conclusion, regardless of the depth of the myometrial invasion, the maternal serum level of the PLGF was found to be the most accurate accreta predictor of the PAS and the VEGF showed a low diagnostic accuracy. It seems that there need further studies for evaluating these angiogenic factors as new targets in the diagnosis and management of the PAS.

Abbreviations

VEGF

vascular endothelial growth factor

PLGF

placenta growth factor

C/S

cesarean section

PAS

placenta accreta spectrum

IUGR

intrauterine growth restriction

IVF

In vitro fertilization

MRI

Magnetic Resonance Imaging

sFlt-1

soluble fms-like tyrosine kinase 1

TRAIC

TNF-related apoptosis-inducing ligand

EGF

Epidermal growth factor

SUMS

Shiraz University of Medical Sciences

IRB

Institutional Review Board

ELISA

enzyme-linked immune sorbent assay

KDR

kinase insert domain-containing receptor

Ang-2

Angiopoietin 2

Declarations

Declaration of Conflicting Interests

All the authors are active participants of the work and are in agreement with the content of manuscript. The manuscript is not under consideration for publication by other journals currently. Medical ethics committee of Shiraz university of Medical sciences has approved the work. All the patients produced their written informed consents. The study does not violate the policies and/or procedures established by journal and no financial support or complicit of interest is declared by the authors.

It is our pleasure if you could give us your valuable comments about our manuscript.

Authors' contribution

Nasrin Asadi: Project development, Data Collection, Data analysis

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Mojgan Akbarzadeh-Jahromi, Shima Bahrami, Sayeh Gharamani: Data Collection, Data analysis

Hadi Raeisi Shahraki, Khadije Bazrafshan: Data analysis

Azam Faraji: Project development, Data collection, Manuscript writing/editing

All authors read the manuscript and approved it.

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Tables

Table 1: comparison of the baseline characteristics and the serum level of PLGF and VEGF between those with and without placenta accreta

Characteristic	Normal group (n=45) median (IQR)	Accreta group (n=45) median (IQR)	P-value*
Age	29 (26.0, 32.5)	33 (29.0, 37.0)	0.001
Number of gravidity	2 (1.0, 2.0)	3 (3.0, 4.0)	<0.001
Number of C/S	1 (1.0, 1.0)	2 (1.0, 2.0)	<0.001
PLGF(pg/ml)	35.9 (8.3, 59.3)	88.4 (69.4, 278.9)	<0.001
VEGF(pg/ml)	285.9 (49.3, 344.4)	229.8 (193.7, 819.7)	0.17

Results of the t-test, considered significant at values <0.05, IQR: Interquartile range

Table 2: comparison of the baseline characteristics and the serum level of PLGF and VEGF between the normal pregnancy and all subgroups of placenta accreta.

Variable	Normal group (n=45) median (IQR)	Accreta group median (IQR)			P-value*
		Accreta (n=29)	Percreta (n=8)	Increta (n=8)	
Placental thickness (cm)	29 (26.0, 32.5)	30 (28.5,35.0)	38 (34,39.8)	29.0 (28.5,37.0)	0.001
Placental vascularity	2 (1.0, 2.0)	3 (3,4)	4 (3,6.5)	3 (3,4.7)	<0.001
Placental depth (cm)	1 (1.0, 1.0)	1 (1,2)	2 (1.3,3)	2 (1.25,2.0)	<0.001
Placental weight (kg)	35.9 (8.3, 59.3)	89.0 (68,807.7)	87.4 (74.4,124)	84.5 (62.5,130)	<0.001
Placental volume (L)	285.9(49.3,344.4)	260 (2628)	205.9 (132.8)	226.6 (177,324.4)	0.06

*Results of the t-test, considered significant at values <0.05, IQR: Interquartile range

Table 3: the comparison of the rate of the placenta accreta, placenta increta, and placenta percreta in patients with and without the placenta previa

	Accreta (n=29)	Percreta (n=8)	Increta (n=8)	P-value*
Previa	19 (65.5)	7 (87.5)	8 (100)	0.10
No previa	10 (34.5)	1 (12.5)	0 (0)	

*The results of the chi square test, considered significant at values <0.05

Figures

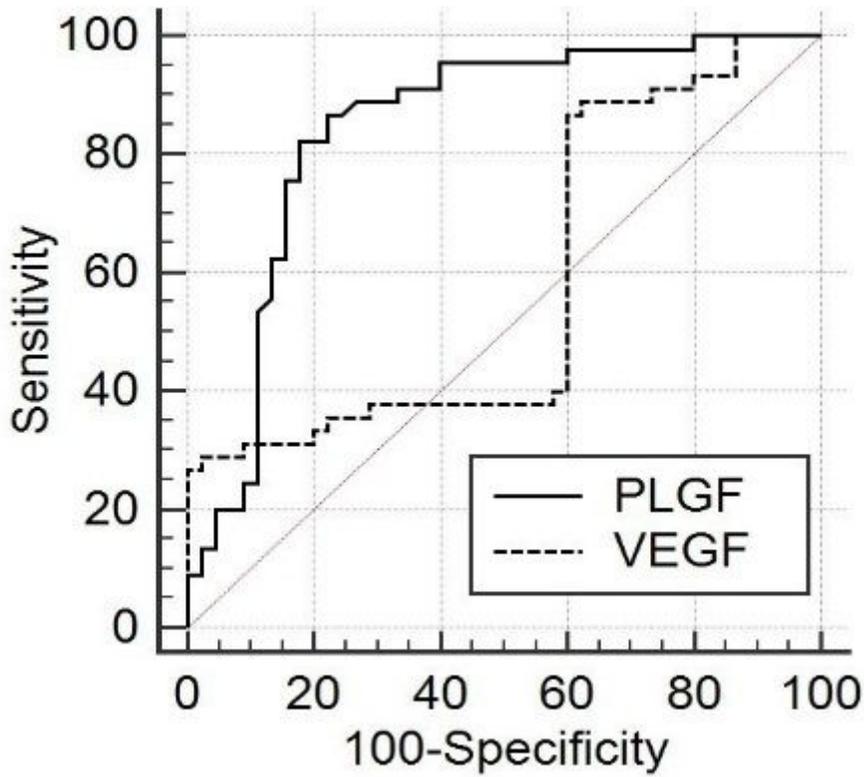


Figure 1

The Receiver-Operating Characteristic (ROC) curves, placenta growth factor (PLGF), and vascular endothelial growth factor (VEGF) to predict the placenta accreta spectrum in 90 singleton pregnant women with the previous cesarean section. The Area under Curve (AUC) indicates the diagnostic accuracy of each test.

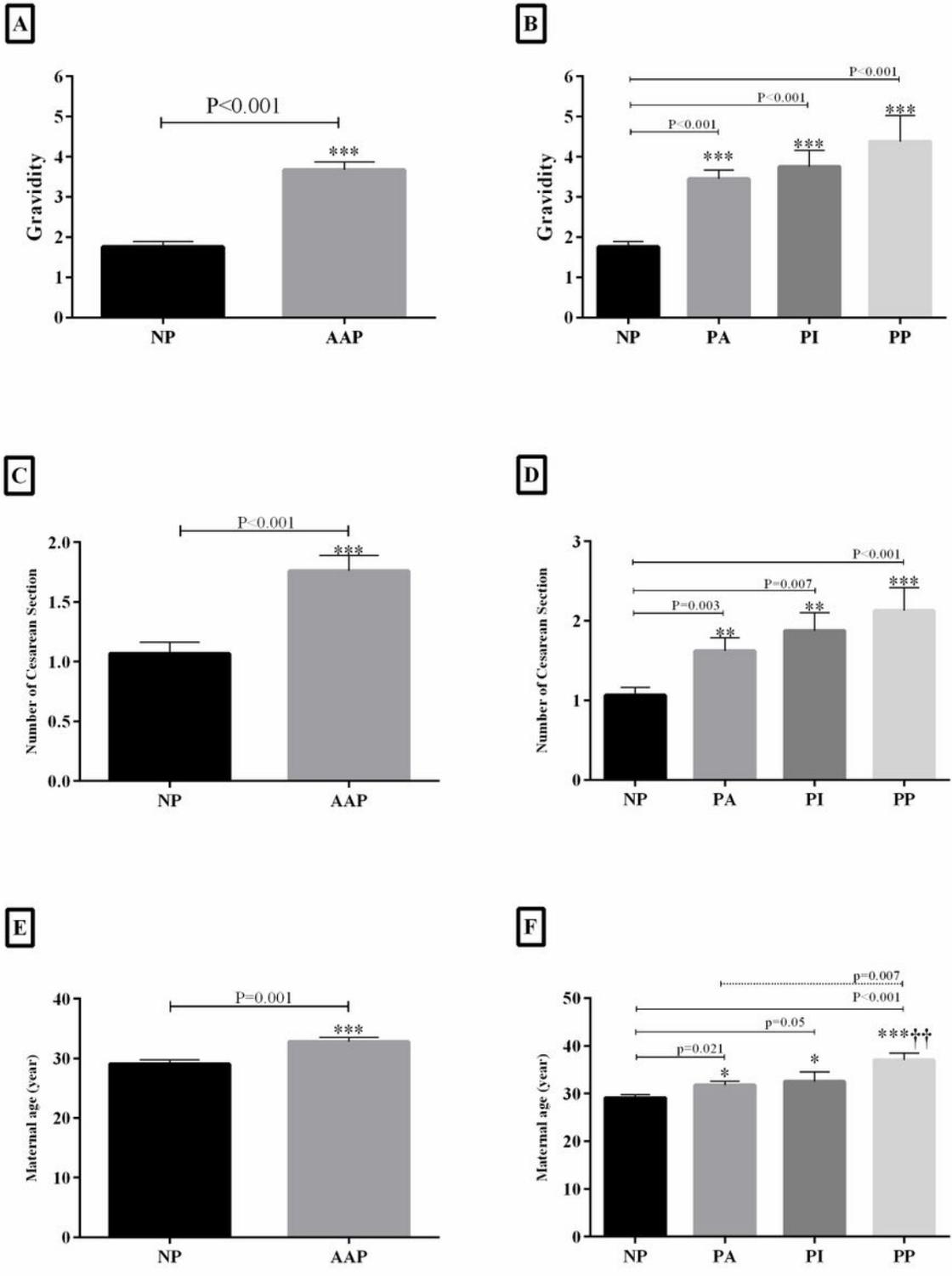


Figure 2

The comparison of the patients' gravidity, number of C/S and age in normal pregnancy, placenta accreta, placenta increta, and placenta percreta. *NP: Normal Pregnancy; PA: Placenta Accreta; PI: Placenta Increta; PP: Placenta Percreta; AAP: Abnormal Adhesion of Placenta