Vascular endothelial growth factor (VEGF): A Noninvasive Biomarker for the Prediction of Invasive Placenta

Abstract: Background: Abnormally invasive placentation (AIP) is a life-threatening obstetric condition which is responsible for massive obstetric hemorrhage that is a leading cause of pregnancy related death. The optimal management of this complication requires a valid antenatal diagnostic tool other than Doppler ultrasound (US). Of these, a handful of laboratory markers have been so far used for the antenatal detection of invasive placenta. Objective: This study sought to establish whether low maternal serum VEGF is related to AIP. Materials and Methods: Over a period of one year, a case control study was conducted in the department of Obstetrics and Gynecology at AL-Yarmouk Teaching Hospital, including 90 pregnant ladies with gestational age 28 week and beyond, of whom 43 cases were diagnosed with placenta accrete using Doppler ultrasound and 47 healthy pregnant ladies with normal placenta. The serum vascular endothelial growth factor was measured by Enzyme-linked immunosorbent assays in all participants, and each individual was followed up regarding intraoperative and postoperative outcomes. Results: a significantly lower serum vascular endothelial growth factor level was found in women having placenta accrete when compared with control group with (P<0.001). At a level of 95.85 pg/ml, serum vascular endothelial growth factor was 90% sensitive, 64% specific in the identification of cases with placenta accrete spectrum. Conclusion: Vascular endothelial growth factor levels may be a potential noninvasive biomarker to aid in the antepartum diagnosis of invasive placenta.

Keywords: Vascular endothelial growth factor, VEGF, Placenta accrete, invasive placenta.

INTRODUCTION

The management of abnormally invasive placentation (AIP) is one of the most challenging conditions in obstetrics. This condition is defined as abnormal trophoblast invasion of part or all of the placenta into the myometrium that leads to the failure of placental separation partially or totally from the uterine wall after delivery (Cunningham, F. G. et al., 2014). This condition is a group of disorders which has more recently been relabeled as placenta accrete spectrum (PAS), that covers all varieties including placenta acrrete, placenta increta, and placenta percreta (Cahill, A. G. et al., 2018). This update was made so as to include both the normal adhesion and invasion of trophoblasts into the uterine wall given that the two conditions may coexist alongside one another (Jauniaux, E. et al., 2018).

Considering the global increase of the cesarean section (CS) rate to 30% of labors over the last 40 years, the incidence of PAS has increased by more than 10-fold (Diag, F. P. A. et al., 2018). In turn, this has led to the emergence of multiple maternal related morbidities, including massive intrapartum or postpartum hemorrhage and its sequel, the need for multiple transfusions, hysterectomy, damage to surrounding organs, and the need for vascular, bowel, or bladder surgery. In addition, the maternal mortality rate attributed to PAS might be as high as 7% (Bauer, S. T., & Bonanno, C. 2009, April; & RCOG. 2011).

The PAS management at level III or IV centers by a multidisciplinary team is known to reduce maternal morbidity and mortality outcomes (Cahill, A. G. et al., 2018). To achieve a successful result, the antenatal diagnosis of the condition is of utmost importance to ensure adequate time for referrals to the multidisciplinary team at the time of delivery (Bauer, S. T., & Bonanno, C. 2009, April). Other than the clinical anticipation, which is based on the previous history of CS or uterine surgery, maternal age, and the presence of placenta previa, ultrasound (US) is still preferable to other radiological modalities such as magnetic resonance imaging (Bauer, S. T., & Bonanno, C. 2009, April; Thompson, O. et al., 2015; & Lyell, D. J. et al., 2015).
In spite of being ultrasound is still recommended as the mainstay for the antenatal diagnosis of PAS, the US neither identifies all cases nor relates to the depth of invasion or histopathological findings (Diag, F. P. A. et al., 2018; Bauer, S. T., & Bonanno, C. 2009, April; & Lyell, D. J. et al., 2018). In addition, its successful use depends on the expertise of the operator, with an interobserver variation in the diagnosis which is entirely possible (Cahill, A. G. et al., 2018). Recent studies have shown that AIP remains undiagnosed before delivery in between half to two thirds of cases resulting in poorer maternal outcomes. Due to limited availability of specialized ultrasound scans that are able to detect the frequently subtle ultrasound features of AIP entities such as invasion of the lower posterior bladder wall or the parametria. Therefore, suitable maternal serum biomarkers might aid doctors additionally to ultrasound to improve the antenatal diagnosis of PAS, particularly in the first trimester and for planning the appropriate management for pregnant women with AIP including pregnancy-associated plasma protein-A (PAPP-A), beta human gonadotropin (β-hCG), alpha-fetoprotein, and most recently, cell-free fetal deoxyribonucleic acid and micro ribonucleic acid (Bartels, H. C. et al., 2018; & Adami, R. R. et al., 2019).

Data derived from animal and human studies demonstrate that the signaling components of the vascular endothelial growth factor (VEGF) family are present in the decidua and play crucial roles in the normal development of the fetoplacental vascular network and oxygen tension has a key role in regulating their expression thus, the alteration of the VEGF system could lead to placental malfunction (Wehrum, M. J. et al., 2011). Placenta accreta develops as a result of abnormal expressions of growth-, angiogenesis- and invasion-related factors in trophoblast (Bartels, H. C. et al., 2018).

**PATIENTS AND METHODS**

**Study Design**

A case control study was carried out in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital in Baghdad, Iraq from February 2019 to March 2021 after gaining the Ethical approval from the scientific council of Obstetrics & Gynecology Specialization /Arab Board of Health Specializations.

**Study Population**

Ninety patients were enrolled in the current study. included Forty three pregnant women their age between (22-44) years were diagnosed with placenta accrete by Gray Scale ultrasound and color Doppler ultrasound and confirmed by intraoperative findings and histological confirmation after delivery and forty seven pregnant women with uncomplicated pregnancy with the same maternal age and body mass index (BMI) of the case group, they are diagnosed to have normal placenta by gray scale ultrasound and confirmed by the delivery outcome whether cesarean section or vaginal delivery.

**Women who met the following inclusion criteria were chosen as study participants:**

- Singleton uncomplicated pregnancy beyond 28 weeks of gestation at time of delivery.
- Diagnosed in the current pregnancy as having placenta accreta by history, ultrasound, and Doppler ultrasound and subsequently confirmed by intraoperative findings and histopathological reports.

On the other hand, women who met the following exclusion criteria were removed from the study

- multiple pregnancies,
- Fetal morbidities including IUGR, , fetal malformations, and placenta separation.
- Medical illness (e.g., hypertensive disorders including preeclampsia, diabetes mellitus, and liver and renal disease, inflammatory or connective tissue disorders, and cardiovascular disorders, fetal malformations, and placenta separation.

Participants’ medical history was collected , regarding, age, gravity, last menstrual period (LMP), gestational age (GA) , medical history ,past obstetrical & Gynecological history with attention regarding uterine surgery (numbers of previous cesarean sections, myomectomy), endometritis and previous dilatation and curettage. Then a general examination, vital signs and BMI measurement, and an obstetric examination were taken. A peripheral blood sample (5 mL) was also taken from each women before delivery, and then placed in a sterile container and labeled. Following centrifugation at 3000 rpm for 20 minutes, the supernatant serum was collected in a separate sterile polyethylene container and stored at -20° C until further analysis for measuring the serum VEGF. All patients were sent for confirmatory Doppler US study performed by the same operator, and all surgical operations were done by senior obstetricians in the same department.

**Serum VEGF measurement:**

Quantitative determination of VEGF were measured using specific immunoassays, Enzyme-linked immunosorbent assays based on biotin double antibody sandwich technology to assay Human Vascular Endothelial cell Growth Factor(VEGF).

The management of AIP was discussed with the patients. Focal resection was performed in women who wished to conserve their uterus provided that sufficient myometrium for an optimum closure of the uterus was available after the resection. All Patients were followed up till the time of delivery to observe the outcome of placenta delivery whether it is normally located non adherent placenta, or morbidly adherent placenta as shown in figure (1), which is further classified into
focal or completely adherent placentas, and in the latter group when hysterectomy was performed histopathological examination was sent to assess the degree of placental invasion whether it is (accrete, increta, or percreta).

Grading of PAS was done intraoperatively according to the classification adopted by the FIGO from grades 1 to 3C. where placenta accrete was considered as grade 1, placenta increta as grade 2, placenta precreta as grade 3A if it was limited to uterine serosa, grade 3B if it invaded urinary bladder and grade 3C if it invaded other pelvic organ or tissue.

Figure 1: Intraoperative findings of placenta accreta (thin and vascular lower uterine segment noted during caesarean section) figure from AL-Yarmouk Teaching Hospital

Statistical Analysis:
Analysis of data was carried out by using the statistical package of Statistical Package for Social Science- Version 24. The data presented in simple measures of frequency, percentage, mean, standard deviation.

The significance of different means (quantitative data) was tested by using student t-test for difference between two independent data, or by using ANOVA test used for difference between more than two independent means. The quantitative data was tested by using Pearson Chi-square test for the significance of difference of different means. Statistical significance was considered when the P-value was equal or less than 0.05 and highly significant when the p value <0.001. In order to determine the cut-off value for the test with its optimum sensitivity and specificity, we applied Receiver Characteristic Operating (ROC).

RESULTS
A total of 90 pregnant women fulfilled the inclusion and exclusion criteria, were included in this study. Among the participants, 43 women were confirmed by ultrasound, color Doppler ultrasound, and histopathological results to have placenta accrete considered as the case group. The remaining 47 healthy pregnant women showed normal placentation considered as the control.

The maternal age, BMI, gravidity, parity, and gestational age presented no significant differences between the groups P value 0.11, 0.40, 0.09, 0.39, and 0.068 respectively (table 1). However, Women with PAS had a higher number of prior cesarean deliveries when compared with controls. 69.76% of pregnant patient with placenta accrete had 3& more cesarean deliveries which is significantly higher than patient with normal placentation (17.03%), p value=0.001. The prevalence of curettage among the pregnant women with placenta accrete (37.21%) was found to be significantly higher than the patient with normal placentation (8.51%) with a p value=0.023.

Table (1): Socio-demographic & clinical characteristics of the study groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placenta accrete (No.=43)</th>
<th>Control (No.=47)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
<td>33.73±4.13</td>
<td>32.37 ± 4.04</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.40 ± 4.14</td>
<td>29.11± 3.95</td>
<td>0.406</td>
</tr>
<tr>
<td>Gestational age at delivery in weeks a</td>
<td>35.87 ± 1.26</td>
<td>35.99 ± 1.58</td>
<td>0.068</td>
</tr>
<tr>
<td>Gravidity</td>
<td>4.04 ± 0.99</td>
<td>3.11 ± 0.88</td>
<td>0.09</td>
</tr>
<tr>
<td>Parity a</td>
<td>4.93 ± 0.86</td>
<td>3.78± 0.85</td>
<td>0.39</td>
</tr>
<tr>
<td>Number of prior cesarean deliveries a No. (%)</td>
<td>None</td>
<td>0 (0%)</td>
<td>N.C*</td>
</tr>
<tr>
<td></td>
<td>1-2 prior cesareans</td>
<td>13 (30.23%)</td>
<td>0.342</td>
</tr>
<tr>
<td></td>
<td>≥2 prior cesarean</td>
<td>30 (69.76%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Number of prior curettage a

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>&gt;1 prior curettage</th>
<th>0 (no prior curettage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (37.21%)</td>
<td>4 (8.51%)</td>
<td></td>
</tr>
</tbody>
</table>

Note. aSignificance at 0.05, No.: Number, N.C : Not computed as cases had zero value in this category,
b Data presented as mean (95% CI) and analyzed by t test
b Data present as n (%) and analyzed by chi-squared test

The maternal serum levels of VEGF were significantly lower in the pregnant women with placenta accrete group than in the control group (AIP mean 75.53 ±56.73 vs. control 182±160.91, with a p value = 0.001) as presented in (table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases Mean± S.D.</th>
<th>Control Mean± S.D.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>75.53± 56.73</td>
<td>182± 160.91</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Further, a serum VEGF level of 95.85 (pg./ml) as a cut off value, or below was specifically associated with placenta accrete with a sensitivity rate of f (90%) and specificity rate of 64%) (Figure 2 and Table 3).

![RCC curve](image)

**Figure (2):** Receiver Operating Characteristic Curve for Estimating the Predictive Value of Serum VEGF for the Stratification of the Cases of Placenta Accreta from those of normal placentation

| Table (3): Diagnostic characteristics of the VEGF to predict placenta accrete |
|---------------------------------|-----------------|--------|--------|--------|--------|--------|--------|
| Cut-off point                   | AUC     | Sensitivity % | Specificity % | PPV %  | NPV %  | P-value |
| 95.85                           | 88%     | 90%        | 64%      | 66%    | 88%    | 0.0001 |

Statistically significant difference was found in the level of VEGF with the delivery finding, operative intervention and histopathological findings as presented in table (5). A lower VEGF level in cases of placenta accrete patients who undergo hysterectomy, the mean VEGF was (66.62±23) than the mean of VEGF level in those who underwent local adherent area resection with over sewing of the defect (72.64±35.6) and those underwent over sewing (74.63±35.6) with p value =0.001

Maternal serum VEGF levels exhibited a significantly negative correlation with the AIP degree of invasion. Serum VEG level was lower in the cases that revealed percreta by histopathological reports, the mean VEGF level in percreta was (69.92±24.42), the median VEGF level for increta was (70.85±31) and the mean VEGF level in accreta was(78.89±70.07), (P value 0.001).

| Table (4): Distribution of VEGF level means according to operative interventions & histopathological findings. |
|-------------------------------------------------|--------|--------|
| Operative interventions                        | Accrete | Not accrete |
|                                               | VEGF Mean± SD | No. (%) | VEGF Mean± SD |
| Caesarean hysterectomy                         | 34 (79.06%) | 66.62±23.1 | 0 (0%) | 0 |
| Caesarean section with focal resection of adherent area | 7 (16.2%) | 72.64±35.6 | 0 (0%) | 0 |
| Cesarean section with over swing& packing      | 2(4.65%) | 74.63±35.6 | 0 (0%) | 0 |
| Cesarean section                               | 0 (0%) | 26(55.31%) | 158±143.2 |
**DISCUSSION:**

As patients with PAS are at the risk of a number of morbidities and even maternal mortality, antenatal diagnosis remains crucial to ensure adequate time for referrals to specialized centers for planned delivery (Büke, B. et al., 2018). Besides Doppler US, variations in levels of a handful of serum biomarkers were added to enhance the antenatal identification of PAS. Among these, the first and second trimester markers of aneuploidy such as PAPP-A, β-hCG, and maternal serum alpha-fetoprotein (MSAF) and other markers including micro ribonucleic acid (miRNA) were suggested (Schwickert, A. et al., 2021). In this work, maternal serum VEGF was hypothesized as a feasible biomarker for the detection of PAS based on the understanding of the pathophysiology of PAS: the role of oxidative stress. Placental hypoxia—and therefore reduced oxidative stress—stimulates increased placental invasiveness by enhancing the expression of VEGF (Wu, F. et al., 2015). It is known that placental invasiveness is promoted by neoangiogenesis (Bartels, H. C. et al., 2018). Extensive neovascularization in PAS involves the enhancement of angiogenesis and the instigation of the vascular endothelial growth factor. AIP mainly develops when nidation has taken place in the uterine scar—a region of localized hypoxia due to abnormal vascularization that has resulted from the scarring process after surgery (Wehrum, M. J. et al., 2011). We presume that VEGF levels might be elevated during the first and second trimester, which would explain increased neoangiogenesis in AIP. When normoxia—or even hyperoxia—has been achieved through increased neoangiogenesis in AIP, augmented oxidative stress might lead to a down regulation of VEGF. As blood was sampled during the third trimester (median GA 35 weeks), this hypothesis could explain the paradoxically low serum VEGF levels in cases of AIP or the molecular mechanisms responsible for the excessive invasion of the myometrium may facilitate local accrual of VEGF and a significant decrease in the maternal circulatory levels of this angiogenic factor (Wehrum, M. J. et al., 2011). Lack of the 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.

The findings of this study revealed that among women with AIP, Serum VEGF level was significantly reduced compared with controls, (AIP mean 75.53±56.73pg/ml, vs. control 182±60.91 pg/ml, p < 0.001), these results were also reported by Alexander Schwickert et al., (2021) and Wehrum et al., (2011). In a study by 11 Hacer Uyanıkoglu et al., (2018), 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.

A serum VEGF level at 95.85 pg/ml and below can be used to detect the presence of placenta accrete with sensitivity rate >90% and specificity rate=64.7%, with p value 0.001. Implying that serum VEGF may offer support as a screening tool in the exclusion of placenta accrete in high-risk population. A study of Alexander Schwickert et al., (2021) found VEGF level cut-off value at 328.0 pg/ml had a negative likelihood ratio of 0.4 and a positive likelihood ratio of 2.5 for the same outcome (AUC = 0.729, 95% CI 0.622–0.836, p < 0.001).

The present study attempted to shed light on the risk factors and management options of PAS. Women with placenta accrete had a higher number of prior cesarean deliveries when compared with controls and it was significantly higher in patient with ≥3 cesarean deliveries with a p value =0.001, as agree with Kathryn et al., (2012), and Silver et al., (2011) They found that the risk of placenta accrete appears to be significantly increased in women who have a previous cesarean delivery decreased maternal serum VEGF levels significantly predicted the higher probability and need to perform a peripartum hysterectomy, due to deep trophoblastic invasion compared to those undergo conservative treatment, P value =0.002 Similarly reported by Alexander (2021).

Maternal serum VEGF levels exhibited a significantly negative correlation with the FIGO AIP degree of invasion as serum VEG level was lower in the cases that revealed perceta by histopathological reports than serum VEG level of cases with increta and acccreta p < 0.001), which is in line with study by Na Wang (2021) who found that the levels of VEGF was the lowest in placenta previa combined with placenta increta, and gradually increased in placenta previa combined placenta accreta, placenta previa alone, and healthy control. Recently reported by Alexander Schwickert et al., (2021) that no linear negative correlation from normal placentation to AIP degrees of invasion 3B + 3C exists, as the median of VEGF in AIP degree of invasion 2 (placenta increta) and 3 (placenta percreta) is higher than for AIP grade 1 (placenta accrete).
CONCLUSION:

Maternal serum VEGF might be a new biomarker for enhancing the use of Doppler US in the antenatal diagnosis of PAS among those with placenta previa or could be used as an inexpensive screening test in situations where color Doppler is unavailable, especially in areas with poor resources. Further evaluations are needed to support the validity of this new hypothesis.

REFERENCES:


