Vascular Endothelial Growth Factor (VEGF) Expression on Placenta Accreta Spectrum (PAS) FIGO Grading

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Abstract

This study aims to analyze the role of placenta VEGF level using immunocytochemical techniques and comparing the obtained results with PAS FIGO grading. This retrospective cross sectional study on total sampling of formalin fixed paraffin-embedded tissue from placenta sampling of elective surgery which were diagnosed as PAS. Sample were immunostained using VEGF and semi-quantitatively Immuno Reactive Score (IRS) measured based on modified Remmele scale index. The result obtained 32 samples that meet inclusion criteria which are found into grade I (n=5), grade II (n=11) and grade III (n=16). This study showed average VEGF at grade I (4.68±1.03), grade II (3.81±1.41), grade III (4.46±1.82) and no significant difference of VEGF expression between grade I, grade II and grade III (p = 0.264). Intraplacental VEGF do not describe the severity of PAS FIGO grading.

Keywords: *Grading, placenta accreta, VEGF*

Introduction

Major cause of maternal mortality in Indonesia are postpartum hemorrhage, hypertension during pregnancy and infection. One of the causes of heavy bleeding and difficult to control during surgery were placenta accreta spectrum (PAS). The incidence of PAS in Indonesia has risen from 2013 to 2016 with the highest rate being in Dr. Soetomo Surabaya, amount to 0% to 4% of total deliveries[1].

One of the placental angiogenic factors is Vascular Endothelial Growth Factor (VEGF). VEGF is secreted in the placenta, causing vasodilation, vasculogenesis and angiogenesis[2,3]. Marked increase in VEGF has been postulated as the cause of placental hypervascularity in PAS[4,5,6]. PAS grading describes the severity of villi invasion into the myometrium. FIGO (The International Federation of Gynecology and Obstetrics) in 2018 divides into grade 1-3C based on clinical and histological[7]. The molecular mechanism of this grading remains unknown. This study aims to analyze the role of placenta VEGF level using immunocytochemical techniques and comparing the obtained results with PAS FIGO grading.

Method

Retrospective analytic study using total sampling of pregnant women with PAS who underwent elective surgery at Dr. Soetomo Hospital Surabaya in 2020. Subjects compiled using inclusion criteria of paraffin block derived from placenta accreta tissue of patients with third trimester gestational age with PAS which was performed elective surgery that had examined by transabdominal sonography prior to surgery with complete data. Meanwhile, the exclusion criteria were incomplete data using previous criteria. From 149 PAS subjects, only 32 subjects have met the inclusion criteria. The confirmation of PAS was

done during surgery performed by a Maternal-Fetal Medicine staff, Dr. Soetomo hospital. Tissue collected during surgery, namely the placenta samples were microscopically examined using VEGF immunohistochemical staining were obtain pathology anatomy Department, Dr. Soetomo Hospital. The paraffin blocks of samples were cut into 4 µm sections with Leica microtome into slide. The slides then were staining with monoclonal antibodies for VEGF (C-1–sc7269 dilution 1:200; Santa Cruz Biotechnology, Dallas, TX, USA). For the evaluation of VEGF expression, a modified semiquantitative IRS scale of Rammele was applied. The method takes into account both percentage of positive immunoreactive cells and intensity of reaction colour (Table 2)[8,9]. Data were analyzed using SPSS version 24. Kruskal Wallis test was used to measure the comparison between variables. A p-value of less than 0.05 was considered statistically significant.

Results

Subject Characteristics

In this study the mean age of the mother, gestational age at ultrasound, age at termination, BMI, parity, in grade I, grade II and grade III groups did not differ significantly or homogeneous. Subjects' characteristics of the study were shown in the table below:

Table 1. PAS Subjects Characteristics.

Variables	PAS(Grades 1-3C) (n=32)			
	Grade I (n=5)	Grade II (n=11)	Grade III (n=16)	P-value
Details:	**(mean±SD)**	**(mean±SD)**	**(mean±SD)**	
Maternal age (years)	34.80±3.76	32.27±6.38	33.80±3.36	0.544 A
Gestational age at US	33.0±2.44	34.08±4.20	33.40±3.04	0.807 A
BMI (mean±SD)	28.43±4.65	27.21±4.56	25.88±3.68	0.585 A
Normal	1 (20%)	3 (25%)	6 (40%)	0.388 C
Overweight	2 (40%)	6 (50%)	8 (53.3%)	
Obesity I	2 (40%)	2 (16.7%)	0 (0%)	
Obesity II	0 (0%)	1 (8.3%)	1 (6.7%)	
Parity				0.059 C
GII	1 (20%)	4 (33.3%)	2 (13.3%)	
GIII	2 (40%)	1 (8.3%)	8 (53.3%)	
GIV	0 (0%)	5 (41.7%)	3 (25%)	
GV	0 (0%)	2 (16.7%)	0 (0%)	
GVI	2 (40%)	0 (0%)	1 (6.7%)	
GVII	0 (0%)	0 (0%)	1 (6.7%)	
Gestational age during termination	33.60±2.07	34.58±3.91	34.67±2.05	0.768 A

Cont... **Table 1. PAS Subjects Characteristics.**

Risk Factors				
History of curettage				0.704 C
No	5 (100%)	11 (91.7%)	13 (86.7%)	
Yes	0 (0%)	1 (8.3%)	2 (13.3%)	
C-section number				
Prev CS 1x	4 (80%)	8 (72.7%)	5 (31.3%)	0.045 C
Prev CS 2x	1 (20%)	2 (18.2%)	10 (62.5%)	0.042 C
Prev CS 3x	0 (0%)	1 (9.1%)	1 (6.3%)	0.785 C
Placenta previa	5 (100%)	11 (100%)	16 (100%)	-

Result were analyzed by C : chi-squared test; A : one way-anova test

Table 2. Semiquantitative IRS scale taking into account both percentage of positive cells (A) and intensity of the reaction colour (B), with the final score representing product of two variables (A×B).

A	B
0 pts - no cells with positivie reaction	0 pts no colour reaction
1 pt - to 10% cells with positivereaction	1 pt - low intensity of colour reaction
2 pts - 11% - 50% cells with positve reaction	2 pt - moderate intensity of colour reaction
3 pts - 51% - 80% cells with positive reaction	3 pts - intense colour reaction
4 pts- > 80% cells with positive reaction	

Comparison Analysis of VEGF levels and PAS Grading

Table 3. Comparison Results of VEGF in Grade I, Grade II, dan Grade III Groups.

Variables	PAS Grading (n=32)			P values
	Grade I (n=5)	Grade II (n=11)	Grade III (n=16)	
VEGF	4.68±1.03	3.81±1.41	4.46±1.82	0.264*

Notes: * = Significant at alpha 0.05. Result were analyzed by Kruskal Wallis test.

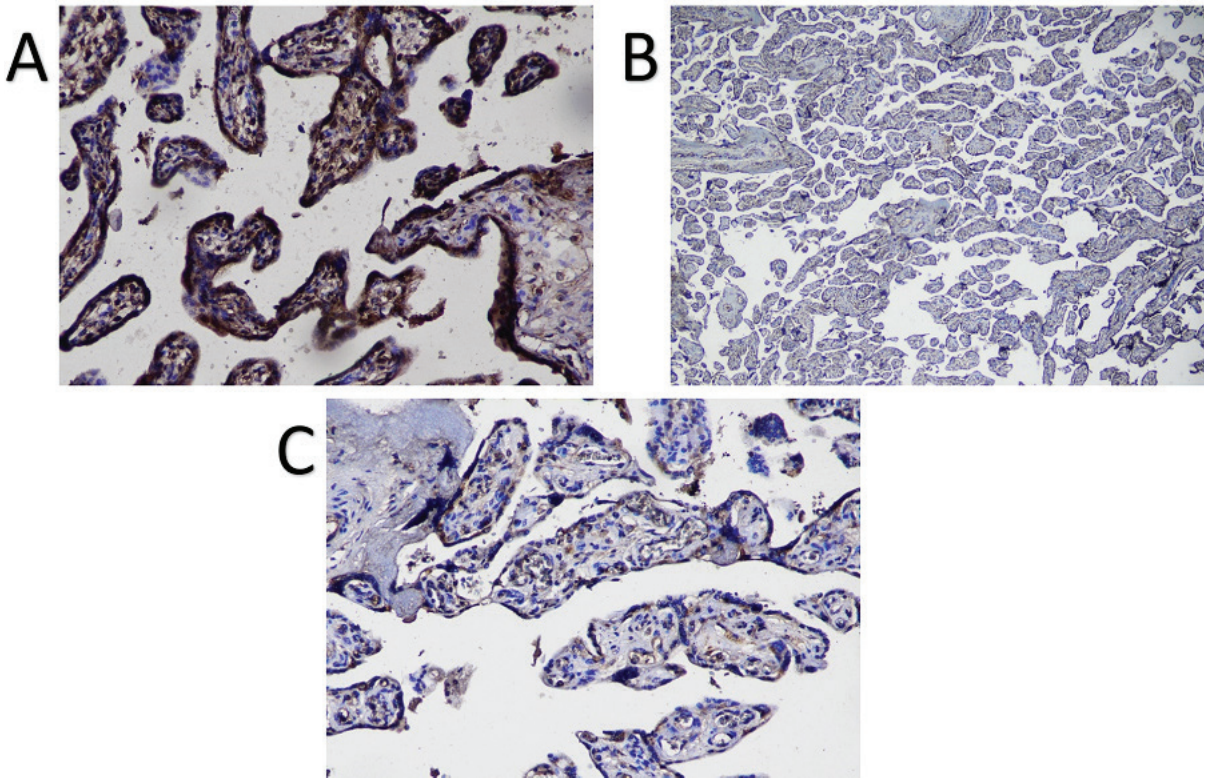

Figure 1. Immunohistochemical expression of VEGF in various grade of PAS. A: 80% placenta cell were stained with strong intensity, 200× magnification; B: 51 % placenta cell were stained with moderate intensity, 40× magnification; C: 25% placenta cell were stained weak intensity, 200× magnification.

Discussion

In cases of PAS, trophoblast invasion occurs through the decidua basalis to the uterine myometrium, uterine serosa or even to the bladder serosa, extending to the parametrium and its surroundings. Based on the histopathological classification, it is divided into three, namely the placenta accreta when trophoblast cells attach to the surface of the myometrium and no decidual layer is found, placenta increta when trophoblast cells invade into the myometrium, and placenta percreta when trophoblast cells invade to the uterine serosa or to the bladder[10]. Meanwhile, the grading of the PAS from FIGO is clinical and histopathological[7]. In PAS, there is an imbalance in angiogenesis, namely an increase in angiogenic factors and a decrease in antiangiogenic factors[11,12,13]. One of the angiogenic factors was vascular endothelial growth factor (VEGF), which is produced by syncytiotrophoblast in greater numbers than in normal placentations, thereby increasing the degree of placental invasion of the uterine wall[14,15]. In our study, based on the results of the Kruskal Wallis test, it showed a significance value of 0.264 ($p > 0.05$), which means that there was no significant difference in VEGF between grade I, grade II, and grade III groups. It may be not only intraplacental VEGF that affect the increase in angiogenesis in PAS, but it is also affected by other factors, such as decreased expression of mRNA 34a, E-cadherin (E-CAD), epidermal growth factor EGF c-(erbB-2), transforming growth factor beta (TGF B), vascular endothelial growth factor receptors-2 (VEGFR-2), the endothelial cell receptor tyrosine kinase (RTK), Tie-2 or increased for the epidermal growth factor receptor (EGFR) and the TIMP-1 tissue inhibitor of matrix metalloproteinase[16].

Conclusion

In summary, there is no difference in placental VEGF between grade I, grade II, and grade III, because Intraplacental VEGF do not describe the severity of PAS FIGO grading.

Conflict of Interest: The author declare that they have no conflict of interest.

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