JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Comparison of Placental Growth Factor Serum Levels in Normal Pregnancy and Pregnancy with Invasive Placenta.

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Abstract
Significantly increase in PAS cases have been observed worldwide associated with increasing cesarean delivery incidences in recent decades. This research purposed to evaluated comparison of PLGF serum levels in normal and placental invasive pregnancies. This is an observational study with case control study design then research data will be analyzed using t test and p value <0.05 was considered statistically significant. Our analysis results showed moderate relationship between FIGO stage and respondents PLGF serum levels which is also found based on PAS degree ($r = 0.627$, $p < 0.001$; $r = 0.646$, $p < 0.001$). The sensitivity value is quite high but specificity value is very low therefore can cause high false positives incidence when used in diagnosing placenta accreta

Keywords: Placenta Accreta Spectrum, FIGO staging, PAS degree, PLGF Serum, Normal Pregnancy

I. INTRODUCTION

In 2019 systematic review that included 7001 Placenta Accreta Spectrum (PAS) cases among nearly 5.8 million births, overall prevalence was found 0.17 percent (range 0.01 to 1.1 percent).[1] This is much higher than 0.003 percent prevalence in United States.[2,3] Significantly increase in PAS cases, beginning in 1980s and 1990s and have been observed worldwide associated with increasing cesarean delivery incidences in recent decades.[4]

The most common theory for pathogenesis PAS is defective decidualization (thin, non-formed, partial, absent or dysfunctional decidua) in scar tissue areas caused by previous uterine surgery involving endometrial-myometrial interface allows retaining villi of placenta to adhere directly or invade myometrium.[5,6] Other theories, attribute PAS to excessive trophoblastic invasion or defective maternal vascular remodeling in scar tissue areas.[7]

PLGF is 25-kd pleiotropic cytokine, originally found in human placenta, that belongs to vascular endothelial growth factor (VEGF) family which also includes VEGF-A (hereinafter also referred to as VEGF), VEGF-B, VEGF-C, VEGF- D and VEGF-E. Unlike VEGF, PLGF homodimer binds to VEGFR1, but not VEGFR2. Several stimuli ranging from hypoxia, inflammatory cytokines, growth factors, hormones, and oncogenes, are able to regulate PLGF expression under pathological conditions. Hypoxia upregulates PLGF VEGFR1 receptors and NRP1 receptors under pathological conditions.[8]

II. RESEARCH METHODOLOGY

This is an observational study with case control study design at Haji Adam Malik General Hospital Medan and USU Medical Faculty network hospital from February 2020 until number of samples is met.

Seventy research samples needed will be selected using consecutive sampling technique that meets inclusion criteria, namely normal pregnant women and women with PAS 1,2 and 3 diagnosis based on USG Stage of PAS disorder without hypertension history (chronic hypertension, preeclampsia and eclampsia).) and endocrine diseases such as diabetes mellitus, singleton pregnancy, third trimester pregnancy and exclusion criteria were having a disease that required premature pregnancy termination, fetal anomalies and history of smoking.

PAS diagnosis was obtained through ultrasound examination. Then PAS assessment was carried out which included placental lacunae, loss of clear zone, bladder wall interruption. Both groups were taken from median cubital vein and put into a vacuum tube containing EDTA for PLGF examination. Add 100 µl of assay diuent RD1W into tube, add 100 µl of standard, control, and arrive into tube, then closed with a sealer for 2 hours at speed 500 ± 50 rpm and orbital 0.12". Then wash by adding 400 µl of Wash Buffer. Add 200 µl of PLGF conjugate. Then cover with sealer, incubated for 2 hours, process is repeated. Add 200 µl of substrate solution, then cover it with a sealer and incubate for 30 minutes. Add 50 µl of stop solution to the tube. Read at a wavelength 450 nm and a wavelength 540 nm or 570 nm.

For research permission, approval was obtained from research subject and Ethics Committee of Medicine Faculty, Universitas Sumatera Utara. The research subjects characteristics data will be arranged in frequency distribution table. For numerical data, normality test will be carried out using Kolomogorov-Smirnov test. If normality test results obtained p <0.05, it means that data is not normally distributed, then will be analyzed using Mann-Whitney test. If data is normally distributed, then will be analyzed using t test. Data analysis used SPSS version 25. This research used 95% confidence level, and p value <0.05 was considered statistically significant.

III. RESULTS AND DISCUSSIONS

This research involved 50 patients who were divided into 2 groups, case group which is pregnancy with PAS and normal pregnancy as a control.

Table 1. Respondents Characteristics

	PAS	Control	*p**
	Median (Min- Max)	Median (Min- Max)	
Age (years)	33 (26-44)	30 (25-38)	0.08
Body weight (kg)	54 (50-60)	54 (50-59)	0.72
Body height (cm)	145 (140-160)	150 (140-160)	0.08
Upper Arm Circumferences (cm)	24,8 (23,5-27,7)	25,9 (23,5-27,6)	0.36
Systole (mmHg)	112 (100-120)	109 (102-120)	0.83
Diastole (mmHg)	63 (60-68)	65 (60-69)	0.26
MAP	80 (74-84)	80 (75-86)	0.49
Gravida	3 (2-6)	3 (2-5)	0.09
Parity	2 (1-4)	2 (1-4)	0.50
Abortus	0 (0-3)	0 (0-2)	0.13
Gestational Age (weeks)	36 (32-37)	37 (30-38)	0.68
History of C-section	2 (0-3)	1 (0-3)	<0.001
History of Curretage	0 (0-2)	0 (0-0)	0.03
Distance to last surgery (years)	4 (1-7)	1 (0-3)	<0.001

* *Wilcoxon test*

PLGF levels differences in normal pregnancy and pregnancy with invasive placenta

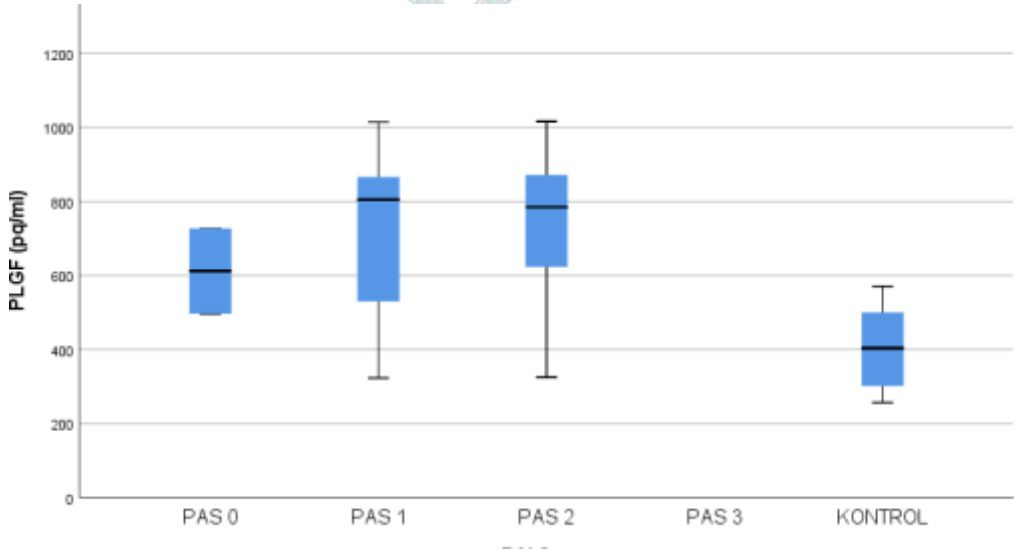

Figure 1. PLGF levels based on PAS stage compared to controls

Mean PLGF level in control group was lower than PAS 0, 1 and 2 groups, while PAS 3 group was not found due to few cases found. This indicates that PLGF increased in PAS group and higher PAS degree indicates higher mean PLGF level.

Tabel 2. Post-Hoc Analysis of placental invasive PLGF based on PAS

	PLGF(pg/ml) Median (min- max)	p*	Post Hoc **			
			Control	PAS 0	PAS 1	PAS 2
Control	404 (257-571)	0,001	-	0.542	0,001	0.001
PAS 0	612 (497-727)		-	-	1.00	1.00
PAS 1	805 (323-1014)		-	-	-	1.00
PAS 2	785 (325 -1016)		-	-	-	

PLGF values in control, PAS 0, PAS 1 and PAS 2 group were 404 (257-571); 612 (497-727); 805 (323-1014) and 785 (325-1016). Based on statistical analysis, there were significant PLGF values differences in control group and PAS group (p = 0.001). Significant differences were only found between control group and PAS 1 and PAS 2 groups (p = 0.001; p = 0.001) .

Table 3. Post-Hoc Analysis of placental invasive PLGF based on FIGO

	PLGF (pg/ml) Median (Min-Max)	Post Hoc**						
		Control	FIGO 1	FIGO 2	FIGO 3	FIGO 4	FIGO 5	FIGO 6
Control	404 (257-571)		NA	0,001	NA	NA	<0,001	<0,001
FIGO 1				NA	NA	NA	NA	
FIGO 2	806 (342-1011)	<0,001		NA	NA	1,00	1,00	
FIGO 3	-				NA	NA	NA	NA
FIGO 4	-						NA	NA
FIGO 5	1732 (323-1016)							0,386
FIGO 6	799 (459-1014)							

Table 4. Correlation of PAS and FIGO on PLGF Serum levels of patients with placental invasiveness.

Variable		PLGF serum levels (pg/ml) Median (min-max)	r*	p*
PAS		404 (257-571)	0,646	0,001
	PAS 0	621 (497-727)		
	PAS 1	805 (323-1014)		
FIGO				
	FIGO 1	-		
	FIGO 2	806 (342-1011)		
	FIGO 3	-	0,627	0,001
	FIGO 4	-		
	FIGO 5	1732 (323 -1016)		
	FIGO 6	799 (459-1014)		

**Spearman Correlation Test*

Spearmen correlation test was done to evaluate correlation between PLGF serum levels with PAS and FIGO stages. Our analysis results showed moderate relationship between FIGO stage and respondents PLGF serum levels which is also found based on PAS degree (r = 0.627, p < 0.001; r = 0.646, p < 0.001).

This research results are supported by Azam et al. research conducted on 90 single pregnant women of which 45 in 28-34 weeks gestational age which is had been diagnosed with Placenta Accreta Spectrum (PAS) as a comparison with 45 healthy pregnant women and it was found that PLGF serum levels were significantly higher in Placenta Accreta Spectrum subgroup (PAS) and Placenta Accreta Spectrum (PAS) were compared with Normal Placenta group.[9] This research is also supported by f Fengge et al. research from January 2017 to September 30, 2019 with total of 177 pregnant women which is: 35 cases with placenta previa-acreta, 30 cases of non-adherent placenta previa, and 112 cases as controls i.e. healthy pregnant women found that serum PIGF increase in first trimester was significantly positively related to placenta accreta suggests a potential role

for PIGF in identifying high-risk placenta accreta pregnancies. Serum PIGF was found to be significantly positively associated with placenta accreta after gestational week adjusted for blood sampling, BMI, and age (OR: 4.83; 95% CI: 1.91-12.24；p = 0.0009 < 0.01).[10]

However, this research is contradict with Ebru et al. research which is evaluate circulating tyrosine kinase 1 (sFlt1), placental growth factor (PLGF) and vascular endothelial growth factor (VEGF) levels in women with abnormal placentation and compare results of women with normal pregnancies, which is 68 pregnant women in third trimester pregnancy diagnosed with vaginal bleeding due to complete placenta previa with and without placenta accreta, increta and percreta as a study group and 30 pregnant women without placental abnormalities who gave birth at 37 weeks gestational age as a control group then this research didn't found statistical differences maternal serum values of sFlt1, PLGF, sFlt1/PLGF and VEGF in group with placental abnormalities compared with group without placental abnormalities.[11]

According to Jenn-Jhy et al. research which is also contradicting the authors' results, his study of Western blot analysis and Reverse Transcription Polymerase Chain Reaction (RT-PCR) showed that VEGFR-2 expression correlated with trends in immunohistochemical data ($p<0.05$). Furthermore, an enzyme-linked immunosor-bent assay (ELISA) on placental lysates showed that women with placenta accreta had significantly higher VEGF concentrations (p 0.001) and lower sVEGFR-2 concentrations (p 0.015) than those with normal pregnancies. . However, PLGF and sVEGFR-1 concentrations did not show a dramatic difference between cases and controls (p 0.149 and 0.354, respectively).[12]

This research results were also contradicted by Wehrum et al which is conducted on 90 pregnant women whereas 45 women with complete placenta previa and 45 pregnant women without complications. As a result, authors limited the analysis to patients with histologically confirmed accreta, increta, or percreta. They don't found difference in maternal serum levels of sFlt-1 (CPP with accreta, increta or percreta: 1.343 [792-2.325] vs controls: 1.301 [821-1.661] pg/mL, P=0.758, Figure 2A) or PLGF (CPP). with accreta, increta or percreta: 823 [292–1,029] vs. control: 370 [249–572] pg/mL, P=0.137). However, women with aberrant myometrium invasion had significantly lower VEGF serum levels compared with matched control group (CPP with accreta, increta or percreta: 0.8 [0.02–3.4] vs. controls. : 6.5 [2.7-10.5] pg/mL, P=0.02, Figure 2C). The ratio of 3 angiogenic factors was not affected in above clinical scenario ($P > 0.05$ for all).[13]

The research were supported by Saffer et al. It was found that 1366 evaluable samples were collected from 247 subjects (242, 238, 226, 223, 222, and 215 samples in each GA interval, 20-24, 24-29, 29-32, 32-35, 35-37, and 37-40 weeks, respectively). The 5th percentile PLGF was 76.4, 141.1, 139.3, 65.5, 31.7, and 23.4 pg/mL based on GA interval, respectively. The distribution of PLGF is close to log normal with parameters continuously varying as a function of GA. The distribution of PLGF is highly dependent on maternal age, race/ethnicity, parity, and maximum systolic blood pressure (taken between weeks 20 and 24). Although statistically significant, these factors did not change PLGF levels by more than ±15%. This study supports that PLGF can provide a reference range for normal placental pregnancies. This proves that there is a relationship between PLGF levels interpretation with normal placenta or abnormalities placenta.[14]

IV. CONCLUSION

PLGF serum levels correlated with PAS and FIGO stages. The sensitivity value is quite high but specificity value is very low therefore can cause high false positives incidence when used in diagnosing placenta accreta.

ACKNOWLEDGEMENT

The researcher would like to thank all parties involved in this research. However, researcher admits there are shortcomings in this research, so that further research on same topic is needed considering number of supporting journals is still limited

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