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Maternal urinary vascular endothelial growth factor (VEGF) versus N.T. pro brain natriuretic peptide (NT-pro BNP) as novel biomarkers for placental accreta spectrum (PAS): A case-control study

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Abstract

This study planned to compare the predictive ability of maternal urinary vascular endothelial growth factor (VEGF) versus N-terminal pro B-type natriuretic peptide (NT-pro BNP) for prediction of placenta accreta spectrum (PAS). This was a prospective case-control study carried out in a tertiary university hospital. It included pregnant women between 37-39 weeks. The study included 50 pregnant women classified in two groups. Group (I, n=25) were pregnant women with PAS, and group (II, n=25) women with uncomplicated pregnancies, as controls. Urine samples were collected, and quantitative analyses of VEGF and NT-pro BNP were performed by ELISA. VEGF was assessed with a cut point of 215.6 pg/ml and NT-pro BNP with a cut point of 182.2 pg/ml to predict the condition of PAS. Both biomarkers were good predictors of PAS with the area under the ROC curve (AUC) equal to (0.871 and 0.904), respectively. However, maternal urinary VEGF levels could predict PAS better than NT-pro BNP (OR=9.967, 95%CI 2.032–48.879, *p*=0.005) versus (OR=8.066, 95% CI 1.520 – 42.811, *p*=0.014) in NT-pro BNP. In conclusion, third trimester urinary levels of both VEGF and NT-pro BNP appear to be s crucially good predictors for PAS. However, VEGF is superior to NT-pro BNP in predicting women with PAS. These biomarkers present promising candidates as they can help to detect patients at high probability of PAS. They can be assessed by non-invasive, simple, and low-cost procedures.

Keywords: VEGF, NT pro Brain Natriuretic Peptide, Placenta Accreta Spectrum, Abnormal invasive placenta

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Introduction

Placenta accreta spectrum (PAS) disorders, previously defined as abnormally adherent placenta, define a variety of conditions characterized by abnormal adhesion and/or invasion of the placental trophoblast to the uterine myometrium.[1]

The pathophysiologic mechanisms of PAS remain incompletely clarified. Still, its clinical sequel are clear: placental invasion and the associated hypervascularity involvingthe uterus and placenta which put women at a great risk for exposure to caesarean hysterectomy, massive hemorrhage, disseminated intravascular coagulopathy, and in the most catastrophic cases, death.[2]

Ultrasound is the gold standard for diagnosing PAS. Its sensitivity and specificity can reach over 90%. However, other population-based studies showed that PAS remains undetected before delivery in between half to two-thirds of cases. This is due to the limited access to specialized ultrasound scans (US) that can detect PAS entities' frequently subtle ultrasound criteria, such as invasion of the lower posterior wall of the urinary bladder or the parametrium.[3] So, appropriate maternal urine and serum biomarkers might help obstetricians in the early detection of PAS and for the accurate management for these cases.[4]

Normal placentation needs a balance between the levels of angiogenic and antiangiogenic factors such as placenta growth factor (PLGF), vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase 1 (sFlt-1), and oxidative status.[5]

VEGF and N-terminal pro B-type natriuretic peptide (NT-pro BNP) are known to have a contribution in the action of placentation. VEGF exhibited by the amniotic epithelium and the cytotrophoblast, promotes angiogenesis, chemotaxis, and vasodilation. NT-pro BNP has recently been shown to augment vasculogenesis.[4]

So, The study aim was to compare the predictive ability of maternal urinary VEGF versus NT-pro BNP-levels for better prediction of women with PAS.

Subjects and Methods

This was a prospective case-control study conducted at Assiut University hospital between December 2020 and August 2021. The maternal urine samples were taken at delivery from 50 women with single pregnancies.

The participants were divided into Group 1 (PAS, n=25), who had been suspected of PAS by prenatal ultrasound and confirmed after surgery by histopathology. Group 2 (controls, n=25) were apparently healthy pregnant women. Both groups were matched for maternal age and gestational age (37–39 weeks). The exclusion criteria included women with a history of conditions that may affect the serum and/or the urinary level of VEGF and NT-pro BNP, such as preeclampsia, hypertension and heart failure, or cancer. Women who declined to participate in the study were also excluded.

Body Mass Index (BMI)

Weights and heights of all participants were measured to calculate BMI using this formula “weight in kilograms divided by height in meters squared”.

Ultrasonographic assessment and Diagnosis of PAS

The prenatal diagnosis of PAS was approached by an expert sonographer (level 2) through gray scale ultrasound and color Doppler ultrasound imaging according to the PAS's published standardized ultrasound parameters.[6] Confirmation of the diagnosis of PAS was done by histopathological examination of the hysterectomy specimens.

Urine Collection, Storage, and Immunoassay Procedures

Fresh urine samples without preservatives were collected in clean containers centrifuged at 1,600 g for 10 min at room temperature, then the supernatant was collected and stored at -80°C until used.

Determination of human N-terminal pro-brain natriuretic peptide (NT-pro BNP) and human Vascular Endothelial cell Growth Factor (VEGF)

Quantitative assay of Human NT-pro BNP, and Human VEGF were performed using ELISA kits

(Sinogene catalog no. SG-10015 and SG-10402, respectively, China), according to the manufacturer's instructions. The optical density of the final product was measured at a wavelength of 450 nm using a microtiter plate reader (Bio-Rad, PR 4100, Singapore). The concentrations of NT- pro BNP and VEGF in the samples were then determined by comparing the optical density of the samples to the standard curve.

Statistical Analysis

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 21. Data were statistically described in terms of mean ± standard deviation (±SD), or median and range when not normally distributed, frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was done using the student t test for normally distributed data and the Mann Whitney U test for non-normally distributed data. The Chi square (χ2) test was used to comparing categorical data. Exact test was used alternative to Chi square (χ2) when the expected frequency was less than 5. Odds ratio (OR) with 95% confidence interval (CI) and logistic regression was calculated to differentiate between different diagnostic prognostic factors of PAS. The p-value was always 2 tailed, set significant at 0.05 levels. The receiver operating characteristic (ROC) curve was used for determination of the sensitivity and specificity for the different cut-off values of the markers.

Results

The demographic, clinical, and pregnancy outcomes features of the study participants are summarized in Table 1. Both study groups were comparable regarding maternal age, residence, parity, the Body Mass Index (BMI, kg/m^2), gestational age, and the incidence of prior abortions with no significant difference between them (p>0.05). Meanwhile, the PAS group had a higher number of previous caesarean section than the control group (64% of women in the PAS group have ≥ 3 prior caesarean section versus 28% in the control group, p=0.003). The urine level of both VEGF (pg/ml) and NT-pro BNP (pg/ml) was significantly higher in the PAS group as compared to controls (p<0.000) Table (1).

Table 1. Demographic, clinical, and outcome characteristics of the 50 studied participants.

Characteristics	PAS group (n=25)		Control group (n=25)		*p-value*
Age (years)	30 (20 – 35)		26 (21 – 34)		NS
Parity	4 (1 – 8)		2 (1 – 6)		NS
Living	4 (1 – 8)		2 (1 – 6)		0.003
BMI (kg/m^2)	28 (24 – 35)		26 (21 – 44)		NS
Gestational age	38 (37 – 39)		38 (37 – 39)		NS
Number of prior cesarean deliveries					0.003
1 prior CS	4	(16.0)	6	(24.0)	
2 prior CS	5	(20.0)	12	(48.0)	
≥ 3 prior CS	16	(64.0)	7	(28.0)	
Prior abortions					NS
No	21	(84.0)	20	(80.0)	
Yes	4	(16.0)	5	(20.0)	
Residence					NS
Rural	23	(92.0)	24	(96.0)	
Urban	2	(8.0)	1	(4.0)	
VEGF (pg/ml)	235.43±34.96		191.64±22.08		0.000
BNP (pg/ml)	226.29±39.01		174.27±21.79		0.000

PAS: Placental Accreta Spectrum; BMI: Body mass index; VEGF: Vascular Endothelial Growth; BNP: Brain Natriuretic Peptide. CS: caesarean section. Variables are presented as mean ± standard deviation or median (range) and frequency (%) as appropriate. * P > 0.05 is not significant (NS).

The ROC curve shows that both VEGF (pg/ml) and NT-pro BNP (pg/ml) were significantly good predictors of PAS with high the area under the curve (AUC), which was 87.1% for VEGF (pg/ml) and 90.4% for NT-pro BNP (pg/ml) (p=0.000, for both) (Table 2 and Figure 1).

Table 2. Sensitivity, specificity, and area under the curve (AUC) values of determined VEGF and NT-pro BNP cut-off levels for PAS detection.

	Cut off	95%CI	Sensitivity	Specificity	AUC	*p*-value
VEGF (pg/ml)	215.6	0.769 - 0.973	80.0%	84.0%	0.871	0.000*
NT-pro BNP (pg/ml)	182.2	0.824 – 0.984	88.0%	72.0%	0.904	0.000*

VEGF: Vascular Endothelial Growth; BNP: Brain Natriuretic Peptide; AUC: Area under the curve; CI: confidence interval.
*$P \leq 0.05$ is significant.

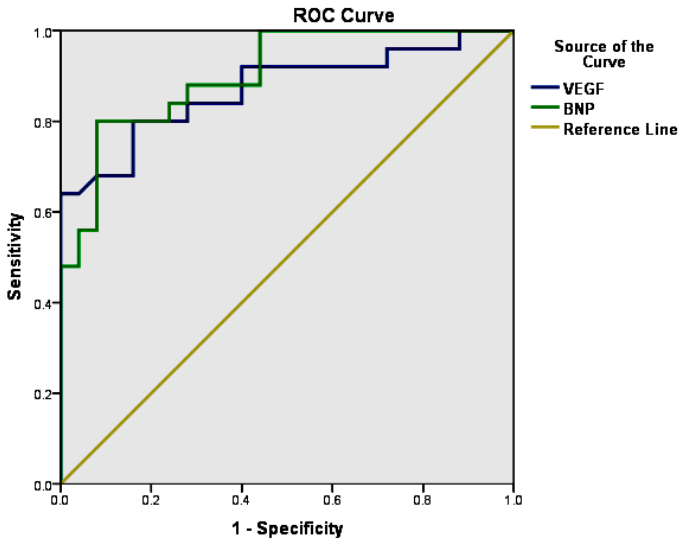

Figure 1. Receiver operating characteristic (ROC) curves showing the predictive value of serum VEGF and NT-pro BNP in PAS. VEGF (blue), NT-pro BNP (green) and Reference line (brown). Area under the curve = 0.871 (0.769 to 0.973), $p < 0.0001$ and 0.904 (0.824 to 0.984), $p< 0.0001$, respectively.

The predictive ability of both biomarker levels and the number of the previous caesarean section for detection of PAS were examined by univariate and multivariate logistic regression model. Patients with urinary VEGF level ≥215.6 pg/ml or NT-pro BNP ≥182.2 pg/ml were found to be significant risk factors for the development of PAS. Interestingly, maternal urinary VEGF levels was superior to NT-pro BNP (OR=9.967, 95% CI 2.032–48.879, *p*=0.005) versus (OR=8.066, 95% CI 1.520 – 42.811, *p*=0.014) for detection of PAS (Table 3).

Meanwhile, the number of previous caesarean section was not found to be an obvious risk factor for the occurrence of PAS Table 3.

Table 3. Univariate and multivariate regression analyses showing the value of variables (number of prior cesarean deliveries, maternal serum VEGF and NT-pro BNP levels) to predict PAS.

Variables	n	Univariate analysis			Multivariate analysis		
		OR	p value	95% CI	OR	p value	95% CI
VEGF (pg/ml)							
< 215.6	26	ref			ref		
≥ 215.6	24	21.00	<0.0001	4.924 – 89.561	9.967	0.005	2.032 – 48.879
NT-pro BNP (pg/ml)							
< 182.2	21	ref			ref		
≥ 182.2	29	18.857	<0.0001	4.254 – 83.592	8.066	0.014	1.520 – 42.811
Number of prior CS							
1 prior CS	10	ref			Not included in the model		
2 prior CS	17	0.625	0.574	0.121 – 3.221			
≥ 3 prior CS	23	3.429	0.118	0.731 – 16.086			

VEGF: Vascular Endothelial Growth; BNP: Brain Natriuretic Peptide; CS: cesarean section; OR: odds ratio; CI: Confidence Interval. *$P \leq 0.05$ is significant.

There were no correlations between markers levels (VEGF and NT-pro BNP) and age, parity, BMI, or number of prior caesarean section. However, NT-pro BNP level was significantly positively correlated with gestational age in contrast to VEGF (Table 4).

Table 4. Correlations between maternal urine VEGF and NT-pro BNP levels and other clinical characteristics among the 25 PAS patients.

Variable name		VEGF (pg/ml)	NT-pro BNP (pg/ml)
Age (years)	r	0.129	0.202
	p-value	NS	0.159
Parity	r	-0.077	0.039
	p-value	NS	0.790
BMI	r	0.073	-0.035
	p-value	NS	0.808
Gestational age	r	0.147	0.308
	p-value	NS	0.030*
Number of prior CS	r	-0.094	-0.002
	p-value	NS	0.989
NT-pro BNP (pg/ml)	r	0.483	1
	p-value	0.000*	

BMI: Body Mass Index, $P > 0.05$ is not significant (NS). r=correlation coefficient; CS: caesarean section.

Discussion

This study planned intended to compare the ability of maternal urinary VEGF and NT-pro BNP levels to predict PAS in pregnant women. The study confirmed that the maternal urine levels of both investigated angiogenic biomarkers (VEGF and NT-pro BNP) are increased in pregnant women with PAS as compared to controls. Furthermore, the ROC curve analysis demonstrated that both VEGF and NT-pro BNP have high predictive values for occurrence of PAS.

Interestingly, maternal urinary VEGF levels were found to be superior to NT-pro BNP. These results indicated that urine VEGF and NT-pro BNP levels should be used as a part of screening for high-risk patients for developing PAS based on combined predictive values, clinical suspicion, and ultrasound findings.

Our study findings agreed with the only published study by Munoz et al., 2021. They studied 24 subjects (12 PAS, 6 placenta previa and, 6 controls), which aimed to identify a minimally invasive biomarker for PAS diagnosis. The authors reported that the average urine VEGF quantities were 8.7, 10.2, and 24.1 pg/mL for control, Previa, and PAS subjects, respectively (p=0.01). In addition, the VEGF expression was 7.9, 18.3 and 29.6 pg/mL for accreta, increta and percreta respectively (p<0.016).[7] Meanwhile, the current study is the first one to introduce the maternal urine level of NT-pro BNP as a novel biomarker for PAS prediction. Both studies suggested that angiogenic biomarkers (VEGF and NT-pro BNP) can be used as sound, reproducible, and minimally invasive accurate methods for screening and early detection of women with PAS and patient referral to more specialized centers for optimal pregnancy outcomes. However, these findings must be further studied in larger well-designated studies to verify our results.

The pathophysiological mechanism by which PAS leads to alteration in maternal levels of VEGF and NT-pro BNP is through extensive neovascularization, which is evident in most PAS cases.[4] Tseng and Chou, 2006 showed increasing in several angiogenic growth factors including VEGF and angiopoietin-2 (Ang-2), in PAS lysates.[8] Decreased exhibition of antiangiogenic proteins such as VEGF receptor-2 (VEGFR-2), endothelial cell tyrosine kinase receptor Tie-2, and soluble fms-like tyrosine kinase 1 (sFlt-1) in syncytiotrophoblastic cells from PAS cases in comparison to normal placenta specimens proposes a proangiogenic phenotype.[8]

PAS-related angiogenesis may not be confined to the trophoblast. Placental relaxin and its receptor (RXFPI) carry on a crucial contribution in angiogenesis in the endometrium by reviving the expression of VEGF.[9] Raised expression of placental relaxin gene and protein has been showed in the PAS basal plate. At the same time, the receptor RFXP1 is manifested in both the basal plate and villous trophoblast in PAS specimens in comparison to controls suggesting that PAS may make some autocrine and paracrine components that enhance the increase of angiogenic- stimulating factors merged with suppression in antiangiogenic factors leading to excess neovascularization.[9] Nevertheless Tseng et al., 2006 and Ersoy et al., 2016 hypothesized that serum NT-pro BNP levels might be related to the abnormal invasion of the placenta.[10,11]

The VEGF angiogenic biomarker was widely studied as a serum biomarker for diagnosing PAS. The case-control study of Wehrum et al., 2011, aimed to identify serum angiogenic factor figure of patients with complete placenta previa and to detect if, invasive trophoblast differentiation characteristic of accreta, increta, or percreta dispenses features of epithelial to mesenchymal transition. It included 90 pregnant women (complete placenta previa, n=45) and (uncomplicated pregnancies, n=45). The authors reported that serum VEGF level was not different between both studied groups.[12]

The study of Biberoglu et al., 2016 evaluated to evaluate the circulating soluble fms-like tyrosine kinase 1 (sFlt1), PLGF, and VEGF levels in patients with abnormal placental invasion and to compare the data with the results of women with normal placentation. The study included 68 third trimester pregnant women, diagnosed to have complete placenta previa with and without associated placenta accreta, increta and percreta as the study group and 30

uncomplicated pregnant as the control group. The author reported that the serum VEGF level in both groups showed no difference.[13.]

Asadi et al., 2020 in a prospective case-control study, aimed to determine the predictive power of VEGF and PLGF for diagnosis of placenta accreta and to compare their serum levels among women with normal pregnancy and pregnant women with PAS. The study included 90 singleton pregnant women: (45 per group) with gestational ages of 28-34, measured the maternal serum level of both at the time of delivery. The author reported that the serum level of VEGF showed no difference between both groups.[14]

The case-control study of Faraji et al., 2021, aimed to assess the maternal serum level of VEGF and PLGF in the diagnosis of the PAS. It included 90 pregnant women, divided in two groups (PAS, n=45) and (Normal Placenta, n=45). The author reported that the serum level of VEGF showed no difference between both studied groups (p>0.05).[15]

Schwickert et al., 2021 in a case-control study, aimed to test if maternal serum VEGF or NT-pro BNP better predicts abnormally invasive placenta (AIP). The study included 99 pregnant women (AIP=44 and uncomplicated pregnancy= 55), the authors reported conflict in results about the maternal serum VEGF level as he found that AIP women had remarkably low maternal serum VEGF levels (AIP mean 285 pg/ml, 95% CI 248–322, vs. control: 391 pg/ml, 95% CI 356–426, p <0.01) and higher NT-pro BNP levels (AIP median 329 pg/ml, IQR 287–385, vs. control 295 pg/ml, IQR 273–356, p = 0.03) as compared to controls. Also, the authors concluded that the maternal serum VEGF could help in predicting AIP and its depth of invasion more than NT-pro BNP.[4]

These conflicting results were explained by the authors as they presumed that VEGF levels might be increased in the first and second trimesters, which would explain increased neo-angiogenesis in AIP. However, during the third trimester as in the cases of our study when normoxia, or, hyperoxia, has been reached through increased neo-angiogenesis in AIP, augmented oxidative stress might lead to a down-regulation of VEGF.

Ersoy et al., 2016 in a case-control study aimed to determine whether venous NT-pro BNP), creatine kinase, a cardiac form of creatine kinase (CK-MB), and Troponin I biomarkers are related to placenta previa and placenta accreta. They included 100 pregnant women (54-cases and 46-matched controls), and concluded that NT-pro BNP levels in placenta previa cases were higher than controls and that NT-pro BNP could predict placenta accrete.[11]

The NT-pro BNP levels can be affected by different physiological conditions. It can be raised by physiological stress and/or cardiac tensity.[16-18] To control these confounding factors, we collected the urine samples at the time of delivery. Also, as it is known that the VEGF level can be affected by the BMI of the studied participants, we have matched this item between studied cases and controls. Hunter et al., 2000,[19] Bosio et al., 2001[20] and Palm et al., 2011[21] report a minimal increase in serum VEGF level during pregnancy course. For this reason, we also matched our studied groups for gestational age.

The strength of our study appears in that it is the first study that introduced NT-pro BNP as a novel maternal urine biomarker for the prediction of PAS and confirmed that maternal urine VEGF expression could be used for early prediction of PAS. In addition, this is the first study that compared the predictive ability of both angiogenic biomarkers for detection of PAS for early interventions as a subsequent better outcome. Our study limitation is the small sample size, therefor, more extensive multicenter studies are needed to confirm our results, detect accurate cut off values for both studied biomarkers and to relate their levels to the degree of placental invasions and with different pathology of PAS. Also, it may be helpful to integrate ultrasound data with biomarkers data to offer a more reliable assessment of PAS risk and prognosis.

In conclusion, we identified two novels, minimally invasive urine angiogenic biomarkers that may be useful for predicting and early diagnosing PAS disorder.

Author Contributions

RE, AS and NA shared in this study by designing and writing the initial draft. MI, HA, AY and AHY contributed equally to this work by data obtaining, analysis and ultrasound diagnosis of placenta accreta. RE, EB and ER carried out the laboratory work MI, EB and ER contributed in the study formulation, design and supervision. All authors revised and agreed for the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Institutional Ethical Review Board of the Faculty of Medicine, Assuit University (approval date July 2020).

Informed consent

A signed consent form was obtained from each study participant.

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