ECRONICON OPEN ACCESS

EC GYNAECOLOGY
Research Article

Placental Proteins in Predicting Preeclampsia

Jayasri Basu*, Ernesto Arostegui, Leonardo Gregorio Ramos, Stephanie Rodriguez, Alireza Mehdizadeh and Magdy Mikhail

Department of OB/GYN, BronxCare Health System, Bronx, NY, USA

***Corresponding Author:** Jayasri Basu, Director-Graduate Medical Education, Department of OB/GYN, BronxCare Health System, Clinical Assistant Professor of Obstetrics and Gynecology and Reproductive Science, Icahn School of Medicine of Mount Sinai, Bronx, NY, USA.

Received: May 01, 2025; **Published:** June 04 , 2025

Abstract

Objective: Preeclampsia is a hypertensive disorder of pregnancy that affects an estimated 2 - 10% of pregnant women worldwide. In this study, the expression profiles of four placental proteins: vascular endothelial growth factor ($VEGF_{165}$), $VEGF_{165}b$, matrix metalloproteinase-9 (MMP-9) and tumor necrosis factor-a (TNF-a) were compared between placentas obtained from either normal pregnancy or preeclampsia. By applying binary logistic regression analysis we determined if the protein levels could be predictive of preeclampsia.

Methods: Placentas from normotensive women and from women with preeclampsia, diagnosed by the American College of Obstetricians and Gynecologists' (ACOG) criteria, were collected after term delivery. Chorionic villi were isolated. Proteins were analyzed independently by Enzyme-linked immunosorbent assay (ELISA) using kits from R&D Systems. Independent t-test, Spearman's correlation, linear regression and binary logistic regression analysis, using preeclampsia as the dependent variable, were performed. A receiver operating characteristic (ROC) analysis was additionally performed to evaluate the performance of the four proteins in detecting preeclampsia.

Results: Placental protein expressions of 113 normotensive pregnancies were significantly different from 36 pregnancies with preeclampsia. Linear regression analysis revealed that the variables $VEGF_{165}$, $VEGF_{165}b$, MMP-9, TNF-a, maternal age, gestational age (GA), and placental weight were not collinear. Binary logistic regression analysis further revealed a Chi^2 statistics of 70.8 with a p value <0.001, when all variables were simultaneously included in the model. Hosemer and Lemeshow test showed that the model provided a good fit of the data (>0.05). Cox and Snell R^2 and Nagelkerke R^2 revealed that the model explained between 38% and 57% of the variance in the dependent variables. Binary logistic regression analysis showed that the model's overall percentage of correct predictions improved from 75.8% at baseline to 84.6%. The "Variables in the Equation" table of binary regression analysis revealed that all four studied proteins were significant predictors of preeclampsia. The Area Under the Curve (AUC) scores of the ROC analyses revealed that both $VEGF_{165}b$ and MMP-9 proteins can moderately distinguish preeclampsia from normal.

Conclusion: The findings of 9% improvement in the percentage of correct prediction when $VEGF_{165}$, $VEGF_{165}b$, MMP-9 and TNF-a placental proteins were included in the binary logistic regression model indicates, that the model's ability to classify preeclampsia accurately has been enhanced by including these proteins. The AUC scores for $VEGF_{165}b$ and MMP-9 demonstrate modest discriminatory ability to distinguish between positive and negative cases; suggesting that the protein-based tests are potentially useful tools, and further research or refinement might be needed.

Keywords: *Placental Protein Expressions; Normal Human Pregnancy; Preeclampsia; $VEGF_{165}$ Protein; $VEGF_{165}b$ Protein; TNF-a Protein; MMP-9 Proteins; Linear Regression Analysis; Binary Logistic Regression Analysis; ROC Curve Analysis; AUC*

Abbreviations

$VEGF_{165}$: Vascular Endothelial Growth $Factor_{165}$; $VEGF_{165}b$: Vascular Endothelial Growth $Factor_{165}b$; MMP-9: Matrix MetalloProteinase-9; TNF-a: Tumor Necrosis Factor-Alpha; GA: Gestational Age in Days; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; NBWt: NewBorn Weight; PlWt: Placental Weight; ROC: Receiver Operating Characteristic; AUC: Area Under the Curve; ACOG: American College of Obstetricians and Gynecologists

Introduction

The placenta is the first fetal multifunctional organ to develop during pregnancy. Its intimate involvement with both the mother and the developing fetus throughout gestation makes it a desirable organ to study to better understand the intrauterine environment. For the past few years we have investigated several placental proteins during normal pregnancy to understand the changes in their expression levels at different gestational age. Cytotrophoblasts during human pregnancy secrete several VEGF proteins, of which $VEGF_{165}$ is considered as the key contributor of the angiogenic process [1]. $VEGF_{165}b$, the alternate spliced inhibitory form of $VEGF_{165}$, is generated from the same transcript and is present in appreciable amounts in human placenta during pregnancy [2]. Placental angiogenesis is accompanied by extensive breakdown and reorganization of the decidual extracellular matrix (ECM) that facilitates cytotrophoblasts migration and proliferation, and is regulated by specific proteolytic enzymes called matrix metalloproteinases (MMPs) [3]. Remodeling of ECM further facilitates maternal and placental tissue morphogenesis and fetal development [4]. The invasion of the uterine spiral arteries by cytotrophoblasts is yet another physiological event that is prominent during pregnancy that results in vascular remodeling of the uterine spiral arteries. During the event the endothelial and smooth muscle cells of the spiral arteries are replaced by cytotrophoblasts that transforms the arteries from the high resistance low capacity blood vessels to low resistance high capacity ones. This transformation is crucial in allowing significant increase in blood flow to the placenta, so that adequate nutrients can be provided to the developing fetus. Defect in remodeling of the uterine spiral arteries, specifically the failure of physiological transformation of these blood vessels is postulated to be a key factor in the development of preeclampsia, leading to impaired uteroplacental perfusion and other complications [5-7]. Placental TNF-a is a cell signaling protein that during pregnancy induces the synthesis of MMP-9 [8]. It allows the cytotrophoblasts to reach the spiral arteries deeper within the uterine decidua and augments the apoptosis of vascular smooth muscle cells surrounding the spiral arteries [9]. Hence, for a number of years we have focused our attention on these four placental proteins: $VEGF_{165,}$ $VEGF_{165}b$, MMP-9 and TNF-a individually in normal pregnancies and preeclampsia. In the present study we have analyzed all four proteins from the same placenta, that were obtained either from normal pregnancy or preeclampsia, to first determine if quantitation of the proteins simultaneously from the same placenta would yield results similar to our previous reports, showing marked differences in expression profiles between normal pregnancy and preeclampsia. Our second goal for the study was to determine by using binary logistic regression analysis if we could evaluate whether the placental proteins $VEGF_{165}$, $VEGF_{165}b$, MMP-9 and TNF-a have the capability in predicting preeclampsia. Additionally, our third goal for the study was to determine if ROC analysis could ascertain whether the placental proteins could be used as diagnostic tools in discriminating normal cases from preeclampsia.

Materials and Methods

Women were not enrolled for the study. Placental tissues after clinical care following term deliveries from normotensive pregnancies that would otherwise have been discarded were collected under a protocol approved by the Institutional Human Subject Committee of the BronxCare Health System, Bronx, New York. The protocol also allowed collection of placentas from women with preeclampsia. The diagnosis of preeclampsia was made by the criteria of the American College of Obstetricians and Gynecologists (ACOG) [10]. These women had no history of hypertension before pregnancy, their systolic and diastolic blood pressure was ≥ 140/90 mm Hg on at least two occasions 6 hours apart, and urinary protein ≥ 300 mg/24 hr. The IRB approved protocol allowed certain clinical information to be collected at the time of tissue collection without the identification of the patients' names and their medical record numbers. These

included: maternal age, race, gestational age as determined by ultrasound and/or by the initial date of the last menstrual period, and method of delivery. Importantly, the excluding criteria for both the study groups included pregnancies complicated with any symptom of infection, diabetes, hypertension, chronic renal disease, chronic peripheral vascular disease, multifetal gestation or with major fetal anomalies. To keep the preeclampsia and the third trimester normotensive group comparable, the gestational age of both groups was limited from 37 to 42 weeks. Placental tissues were collected within 30 minutes of vaginal or cesarean deliveries. Placentas delivered overnight or when placenta sample collection could not be achieved within the 30 minutes window, the placentas were not collected to keep the sample collection protocol consistent. The protocol for processing the placenta, isolating the chorionic villi tissue and storing the chorionic villi tissues until assay are mentioned in details elsewhere [11].

Four different commercially available Enzyme Immunoassay (EIA) kits were purchased from R&D Systems, Minneapolis, MN to determine the chorionic villi protein expressions of $VEGF_{165}$, $VEGF_{165}$b, MMP-9 and TNF-a individually. The assays were carried out according to the instructions provided by the manufacturer. Sensitivity of the assay kit used was 31.3 pg/ml for $VEGF_{165}$, 62.5 pg/ml for $VEGF_{165}$b, 31.3 pg/ml for MMP-9, and 15.6 pg/ml for TNF-a protein, respectively. Samples were analyzed in duplicates and the intra assay and inter assay variations for all proteins were <10%. Expression of the four chorionic villi proteins is later referred to throughout the text as placental proteins.

Statistical analyses

Statistical evaluation of the data was carried out using SPSS^R statistical package version 28 (IBM Corporation, Armonk, NY, USA) [12]. Chorionic villi tissue samples were grouped based on the presence or absence of preeclampsia and independent t-test was performed to assess the difference in means. Spearman's Rank correlation coefficient test was applied to estimate the strength and direction of relationships between pairs of continuous variables. In the linear regression analysis, variables in the study which are associated with the risk of preeclampsia, e.g. $VEGF_{165}$, $VEGF_{165}$b, MMP-9, TNF-a, GA, maternal age, maternal systolic blood pressure (SBP), diastolic blood pressure (DBP), placental weights and newborn weights were included; and collinearity between these independent variables were determined. All independent variables that linear regression analysis revealed not to be collinear were then used in the binary logistic regression analysis as independent variables. ROC analysis was additionally performed to evaluate the performance of the four proteins as diagnostic tests in predicting preeclampsia. $P<0.05$ was considered significant.

Results and Discussion

Preeclampsia is a hypertensive disorder of pregnancy that affects an estimated 2-10% of pregnant women worldwide [13]. A significant portion of preeclampsia research involves comparing blood chemistry. Circulating factors, probably originating in the placenta are postulated to be responsible for the manifestations of the disease; and dozens of serum markers of endothelial activation and endothelial dysfunction are reported to be deranged in women with preeclampsia [14,15]. In placental explant studies, pieces of preeclamptic placental tissue (explants) are cultured to investigate placental function. Cytotrophoblasts from both early (first) and late (third trimester) pregnancy are used to investigate the production and release of various factors, cell interactions, and the effects of hypoxia or other conditions relevant to the disease. The cell culture studies required a variety of culture conditions to mimic the in utero environments, particularly when different phases of gestation were examined [16-18].

Our focus in investigating the expression profiles of placental angiogenic proteins in normal pregnancies and preeclampsia; stems from the facts that theories postulated regarding the etiology of preeclampsia involve angiogenesis in some form. Despite the vast knowledge of the pathophysiology of preeclampsia, the only available treatment/cure for preeclampsia still remains to be the delivery of the dysfunctional placenta [18]. In the past, we investigated placental angiogenic proteins in women with uncomplicated pregnancy. The studies were mostly focused on determining the expression profiles of individual placental proteins throughout gestation. Other studies that we carried out compared the placental protein profiles between normotensive pregnancy and preeclampsia [8,20,21].

The findings of our studies did not always agree with those reported in the literature. While our findings showed a significantly higher MMP-9 protein expression in preeclamptic placentas, an immunohistochemical study reported that in the majority of the preeclamptic placentas MMP-9 protein was absent. In the remaining cases of preeclamptic placentas, the expression of MMP-9 protein was weak [22]. In another study, MMP-9 was reported to be lower in plasma of patients with preeclampsia and in preeclamptic placental extracts [23].

In our previous studies, we mainly focused on these four placental proteins but in each study we had investigated one or two proteins at a time. Hence, in the present study, we analyzed the expression profiles of all four proteins from the same placenta and compared the expression profiles between normotensive pregnancy and preeclampsia. A total of 149 placentas from term deliveries were collected for this study. Of them, 113 were collected from women who were normotensive throughout gestation and 36 from women who had preeclampsia. Clinical characteristics of women from whom placentas were collected are shown in Table 1. The maternal age of the normotensive and preeclampsia groups was comparable. The gestational age of the preeclampsia group was significantly lower ($p = 0.002$). Newborn weights in the preeclampsia group was significantly lower ($p < 0.001$) as well. Newborn weight is dependent on the duration of gestation and rate of fetal growth. Hence, the significantly lower newborn weight in the preeclampsia group could be due to decreased uteroplacental perfusion which is the hallmark of preeclampsia along with the significantly lower gestational age as seen in this study in the preeclampsia group.

Variables	Groups	N	Means	Std Dev	P values
Age (years)	Normotensive	113	27.9	6.2	0.146
	Preeclampsia	36	26.1	6.4	
Gestational Age (in days)	Normotensive	113	276	7	0.002
	Preeclampsia	36	262	24	
Systolic Blood Pressure (mm Hg)	Normotensive	113	120	15	<0.001
	Preeclampsia	36	156	19	
Diastolic Blood Pressure (mm Hg)	Normotensive	113	71	11	<0.001
	Preeclampsia	36	93	13	
Placental Weights (grams)	Normotensive	113	458	100	0.278
	Preeclampsia	36	430	141	
Newborn Weights (grams)	Normotensive	113	3402	507	<0.001
	Preeclampsia	36	2686	968	

Table 1: *Characteristics of women from whom placentas were collected.*

Chorionic villi samples isolated from each placenta were analyzed for the four proteins: $VEGF_{165}$, $VEGF_{165}b$, MMP-9 and TNF-a. Hence, a total of 149 x 4 assays were performed in duplicates. Table 2 and Figure 1 show that $VEGF_{165}$ protein is higher in the normotensive placentas. However, the remaining three proteins $VEGF_{165}b$, MMP-9 and TNF-a were all higher in preeclamptic placentas; results that are indistinguishable from the individual placental protein studies that we have conducted earlier [11,21].

	Groups	N	Mean	Std Dev	P value
$VEGF_{165}$ (pg/ 100 mg tissue)	Normotensive	113	153.03	101.16	0.001
	Preeclampsia	36	73.94	40.17	
$VEGF_{165}b$ (pg/ 100 mg tissue)	Normotensive	113	333.20	215.92	0.005
	Preeclampsia	36	486.71	290.44	
MMP-9 (ng/ 100 mg tissue)	Normotensive	113	25.05	14.29	0.013
	Preeclampsia	36	32.51	15.44	
TNF-a (pg/ 100 mg tissue	Normotensive	113	36.77	24.94	>0.05
	Preeclampsia	36	46.57	42.24	

Table 2: *Chorionic villi protein concentrations.*

Legend: Chorionic villi were isolated from each placenta. The proteins were analyzed individually by ELISA using kits from R&D Systems. Independent t-test was used for statistical analysis.

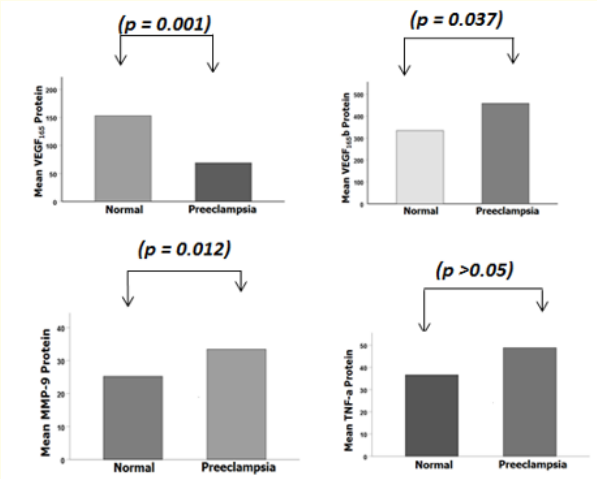


Figure 1: *Legend: Placental VEGF165, VEGF165b, MMP-9 and TNF-a proteins were measured individually by ELISA in 113 placentas collected from normotensive women and in 36 placentas collected from women with preeclampsia. The mean + SD data are shown; pg/100 mg tissue for VEGF165, VEGF165b, and TNF-a proteins and ng/100 mg tissue for MMP-9 protein. The bar graphs depict the comparative values of the placental proteins between the two groups.*

The differences in the expression profiles of the studied protein between normotensive pregnancy and preeclampsia (Table 2) prompted us to carry out a binary logistic regression analysis to determine if the placental proteins have the ability to accurately predict the presence of preeclampsia. Binary logistic regression analysis is a statistical technique that analyzes the relationships between two types of variables, one that is dichotomous (meaning the variable can have only two outputs which in our study "presence of preeclampsia" or "absence of preeclampsia"); and one or more continuous variables (that in this study are the expression values of the four proteins, age, GA, placental weight, etc.). The dichotomous variable in logistic regression analysis is also referred to as the dependent variable, and the continuous variables as the independent variables or predictor variables.

Before proceeding with the binary logistic regression analysis, it is important to ascertain whether the independent variables in the study are collinear. Besides the four placental proteins, variables in this study which are also associated with the risk of preeclampsia include: GA, maternal age, SBP, DBP, placental weights and newborn weights. However, before these variables can be included in the binary logistic regression analysis in this study as independent variables, the collinearity between these variables first needs to be determined. In the linear regression analysis results, the measure of collinearity between independent variables is referred to as tolerance which can range from 0 to 1. Tolerance below 0.4 suggests that the variables are collinear [24]. The results of linear regression analysis are presented in Table 3, which showed that the tolerance values for $VEGF_{165}$, $VEGF_{165}$b, MMP-9, TNF-a, GA, maternal age, and placental weights were .814, .823, .899, .944, .509, .915 and .613, respectively. The values all being >0.4, suggest that the variables are not collinear. However, the tolerance values for SBP, DBP and newborn weights were .332, .314 and .378, respectively. Hence these three variables were not included in the binary logistic regression analysis.

Coefficients								
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std Error	Beta			Tolerance	VIF
1	(Constant)	-.266	.577		-.461	.646		
	VEGF165	-.001	.000	-.182	-3.192	.002	.814	1.229
	VEGF165b	.001	.000	.132	2.322	.022	.823	1.215
	MMP-9	.002	.002	.082	1.499	.136	.899	1.113
	TNF-a	.001	.001	.082	1.553	.123	.944	1.060
	GAD	-.002	.002	-.084	-1.158	.249	.509	1.964
	Age	.000	.004	-.003	-.054	.957	.915	1.093
	Systolic BP	.009	.002	.472	5.268	<.001	.322	3.016
	Diastolic BP	.002	.003	.075	.810	.419	.314	3.182
	Placental weight	.000	.000	.126	1.906	.059	.613	1.631
	Newborn Weight	.000	.000	-.246	-2.928	.004	.378	2.647
a. Dependent Variable: Preeclampsia								

Table 3: *Test for collinearity.*

Legend: The table was generated in linear regression analysis results, using IBM SPSS Statistics, version 28.

Binary logistic regression analysis is normally done in two steps. In the first step, "Step 0" all cases in the data set is classified into one single group. It establishes a starting point for the analysis without any influence from the independent variables. So, for this study the default is set that all cases are normal. We know that of the 149 placentas in the data set, 113 were obtained from the normotensive pregnancy and the remaining 36 were from preeclampsia. However, when the model combined all the placentas into a single group, all 149 placentas were classified as "no preeclampsia" (Table 4). In this scenario, 113 were categorized by the model as "no preeclampsia", and hence were correctly identified. So, the prediction in this case was 100% correct (Table 4, 4^{th} row). The 5^{th} row of Table 4 shows that 36 placentas were also grouped by the model as "No preeclampsia". Hence, the prediction for preeclampsia cases was 0% correct. So, out of 149 placentas 113 were correctly predicted. Therefore, the overall correct prediction score of the model (when no independent variables were included) was 113/149 * 100 = 78.5%. The results of Step 0 are used as a benchmark to assess how much the model's predictive power improves when predictors are added in subsequent steps.

Classification Table					
Step 0			Predicted Preeclampsia		
	Observed Preeclampsia		No Preeclampsia	Preeclampsia	Percentage Correct
		No Preeclampsia	113	0	100.0
		Preeclampsia	36	0	0
	Overall %				75.8

Table 4: *Binary logistic regression analysis result without independent variables in the model.*

Legend: The classification table was generated in the binary logistic regression analysis result when IBM SPSS Statistics, version 28 was used.

In the next step, binary logistic regression analysis determines if there is a statistically significant association between the dependent variable, and the predictor variables, which in the study is the diagnosis of preeclampsia and the four placental proteins, respectively. A Chi Squared test is applied to assess the significance of the relationship between the predictor and the outcome variables. A p-value < 0.05 indicates that the relationship is statistically significant. In SPSS [12] output, the Omnibus Test of Model Coefficients table presents the Chi-square statistics, the degrees of freedom, and the significance (p-value) for the overall model. The result of the Chi square statistics is presented as Figure 2. The figure shows that the Chi square statistics is 70.8, the degree of freedom is 7, because 7 independent variables were included in the analysis, and the total sample size was 149. The p-value of < 0.001 depicted in the result suggests a very strong statistical significance, meaning that the predictor variables together significantly improved the prediction of preeclampsia. The Cox and Snell R^2 and Nagelkerke R^2 values in the binary logistic regression analysis output were 0.378 and 0.565, respectively, which indicated that the model explained 37.8% and 56.5% of the variations in the outcome. Both R-squared values suggested that the model provided a meaningful improvement in predicting preeclampsia compared to a model without the independent variables.

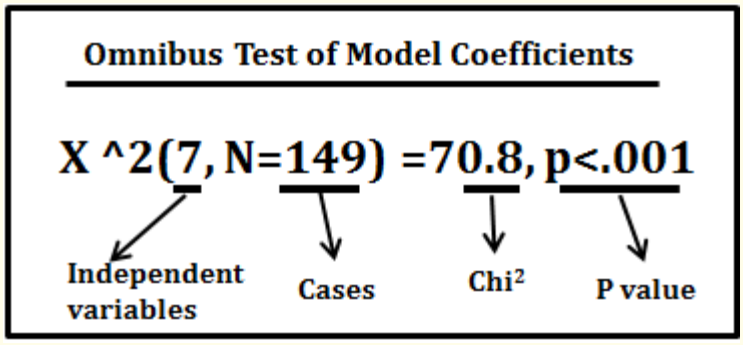


Figure 2: *Legend: The table shown is the Chi square statistics results generated in a binary logistic regression analysis performed by using IBM SPSS Statistics, version 28. The number 7 denotes the number of independent variables that were included in the model, 149 is the total number of cases analyzed, and 70.8 denotes the Chi square statistics.*

The next output in the binary logistic regression analysis result was the "classification" table which is represented as Table 5. This table informs the accuracy of the model's predictions when the independent variables were included in the model, and shows how well the model correctly classifies cases into the two categories, "no preeclampsia and preeclampsia". Table 5 shows that when the independent variables were included in the model, 107 placentas out of 113 collected from the normotensive pregnancy were grouped as "No preeclampsia", and the remaining 6 were classified as preeclampsia (Table 5, line 4). Hence, the percentage of correct prediction by the model is 107/113 * 100 = 94.7% (Table 5, line 4). The 5th row of the same table shows that 19 placentas out of 36 were classified by the model as preeclampsia. However, we know that in our study we had 36 placentas in the preeclampsia group, and 19 were correctly predicted by the model as preeclampsia when all seven independent variables were included in it. Hence, the percentage of correct prediction by the model in predicting preeclampsia was 19/36 * 100 = 52.8% (Table 5, line 5). Hence, on the whole, the model predicted a total of 107 + 19 cases correctly when the independent variables were included in it. Hence, the overall prediction score of the model is 107 + 19 out of 149 cases which is equal to 84.6% (Table 5 line 6). Therefore, binary logistic regression analysis results demonstrated that the model's overall percentage of correct prediction improved by 9% from 75.8% at baseline to 84.6% when all four proteins were included in the model.

Classification Table					
Step 1			Predicted Preeclampsia		
	Observed Preeclampsia		No Preeclampsia	Preeclampsia	Percentage Correct
		No Preeclampsia	107	6	94.7
		Preeclampsia	17	19	52.8
	Overall %				84.6

Table 5: *Binary logistic regression analysis results with independent variables in the model.*

Legend: The classification table was generated in the binary logistic regression analysis results when IBM SPSS Statistics, version 28 was used.

The "variables in the equation" table of the binary logistic regression analysis identifies whether each individual predictor variable has contributed to the model, and how each individual variable is associated with the prediction of the model. It also informs the direction and the strength of the relationship between the predictor variables and the outcome. The "Variables in the Equation" table obtained from the binary logistic regression analysis results using SPSS is presented as Table 6. The p values are all significant and as shown in the table are: <0.001, = 0.005, = 0.038, = 0.027 and .002 for the four proteins: $VEGF_{165}$, $VEGF_{165}$b, MMP-9, TNF-a and GA, respectively. The findings indicate that all four proteins and gestational age each have individually contributed significantly to the model in improving the model's ability in predicting preeclampsia, when the other variables were held constant. The specific relationships of each variable in predicting preeclampsia as shown in the "Variables in the Equation" table can be explained as follows:

- $VEGF_{165}$ (B = -0.022, S.E. = 0.006, Wald = 13.973, Sig. <.001, Exp(B) = 0.98) is a significant predictor of the outcome with a p value of <.001. The data additionally informs that for each one unit increase in $VEGF_{165}$ protein value, the odds of having preeclampsia will decrease (because the B value is negative) by 0.98% (Odds ratio Exp(B) = 0.98).

- $VEGF_{165}b$ protein (B = .004, S.E. = 0.001, Wald = 8.027, Sig. <.005, Exp(B) = 1.004) is also a significant predictor of the preeclampsia (p <.001). In addition, the data informs that for each one unit increase in $VEGF_{165}b$ value, the odds of having preeclampsia will increase (because the B value is positive) by 1% (Odds ratio Ex(B) = 1.004 = 1).
- MMP-9 protein (B = .039, S.E. = 0.019, Wald = 4.320, Sig. = 0.038, Exp(B) = 1.040) is also a significant predictor of the preeclampsia. The data informs that for each one unit increase in MMP-9 protein value, the odds of having preeclampsia will increase (because the B value is positive) by 4% (Odds ratio Ex(B) = 1.04).
- TNF-a protein (B = .026, S.E. = 0.012, Wald = 4.895, Sig. = 0.027, Exp(B) = 1.026) is also a significant predictor of the preeclampsia. The data informs that for each one unit increase in TNF-a protein value, the odds of having preeclampsia will increase (because the B value is positive) by 3% (Odds ratio Ex(B) = 1.03).
- GA (B = -.087. S.E. = .029, Wald = 9.221, df = 1, Sig. = .002, Exp(B) = .944) is also revealed in the "Variables in the Equation" as a significant predictor of preeclampsia with a p value of .002. The data informs that with each unit increase in gestational age in days, which in our study is referred to as GA, the risk associated with preeclampsia will decrease (because the B value is negative) by 0.87% (Odds ratio Exp(B) = .866), meaning the gain in days will provide more time for the fetus to develop and mature.

Table 6 further shows that the p values for the remaining two variables, age and placental weight, were .193 and .665, respectively; indicating that there was no relationship between these two variables and the outcome.

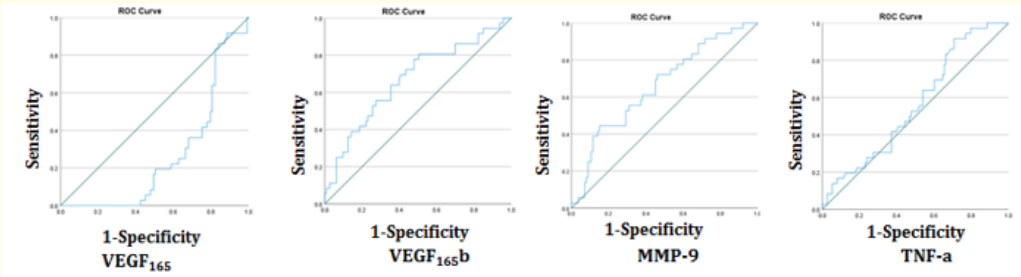
Variables in the Equation									
								95% C.I. for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1 [a]	$VEGF_{165}$	-.022	.006	13.973	1	<.001	.978	.967	.989
	$VEGF_{165}b$	.004	.001	8.027	1	.005	1.004	1.001	1.006
	MMP9	.039	.019	4.320	1	.038	1.040	1.002	1.079
	TNF-a	.026	.012	4.895	1	.027	1.026	1.003	1.049
	GA	-.087	.029	9.221	1	.002	.917	.867	.970
	Age	-.058	.044	1.693	1	.193	.944	.866	1.030
	Placental Weight	.001	.002	.188	1	.665	1.001	.996	1.006
	Constant	22.049	7.736	8.124	1	.004	3764003960.3		
a. Variable(s) entered on Step 1: $VEGF_{165}$, $VEGF_{165}b$, MMP9, TNF-a, GA, Age and Placental weight									

Table 6: *Results of binary logistic regression analysis.*

Legend: The variables in the equation table was generated in the binary logistic regression analysis results when IBM SPSS Statistics, version 28 was used.

The four proteins in the study are continuous variables that were also evaluated as a diagnostic test to differentiate between preeclampsia and normal. To assess the performance of a continuous test, it needs to be converted into a binary (positive/negative) classification, which is done by setting a threshold value. The threshold value is the point at which the continuous results are classified as positive or negative. Choosing a threshold involves a trade-off between sensitivity and specificity. Sensitivity refers to the test's ability to correctly identify individuals with the disease, while specificity refers to its ability to correctly identify individuals without the disease. Receiver Operating Characteristic (ROC) Curve can help visualize the performance of the biochemical index (classifier) across all possible threshold values. The ROC curve plots the True Positive Rates (sensitivity) against the False Positive Rates (1 - specificity) at various threshold settings. It is a graphical representation of the trade-off between sensitivity and specificity across different threshold values. It helps in determining the optimal threshold for a given test. The AUC value is a summary metric of the ROC curve that reflects the test›s ability to distinguish between diseased and non-diseased individuals. The AUC represents the model's overall performance. An AUC = 1.0 indicates a perfect classifier, meaning it can perfectly distinguish between positive and negative classes. AUC > 0.5 indicates that the model has some predictive ability, with higher values than 0.5 indicating better discrimination. However, when AUC score equals to 0.5, it indicates that the model performance is no better than random guessing, meaning it cannot distinguish between the classes.

In the study, the ROC Curve analysis was performed for all four placental proteins using SPSS, and the data is presented in Figure 3. The figure shows that the AUC for $VEGF_{165}$ protein is 0.264. The AUC values for both placental $VEGF_{165}$b and MMP-9 proteins were 0.669 and 0.662, respectively. These two AUC scores demonstrate fair, but not excellent, discriminatory ability, distinguishing between positive and negative cases, suggesting that the tests are potentially useful tools, but are not perfect, and further research or refinement might be needed. The AUC value for TNF-a protein was .563, indicating the model's performance is no better than random guessing.



				Asymptotic 95% Confidence Interval	
Placental Proteins	AUC	Std. Error	Asymptotic Sig.	Lower Bound	Upper Bound
$VEGF_{165}$	.264	..041	.000	.185	.344
$VEGF_{165}$b	.669	.053	.002	.564	.773
MMP-9	.662	.051	.004	.561	.762
TNF-a	.563	.052	.256	.462	.664
a. Under Nonparametric assumption					
b. Null Hypothesis: true area =0.5					

Figure 3: *Legend: Receiver operating characteristic curve (ROC) for predicting preeclampsia based on the placental expression of $VEGF_{165}$, $VEGF_{165}$b, MMP-9 and TNF-a protein as measured by enzyme-linked immunosorbent assay. AUC: Area under the curve.*

The scatter plots of the four proteins against gestational age in days show significant overlap between different data points in all four proteins, and in both studied groups (Figure 4). Similar scatter plot result of yet another placental protein, placental growth factor, has been reported by Roberts, *et al* [19]. The possible explanation of the data could be that at the same gestational age some women in both groups may have similar protein levels. Or the protein levels might be influenced by factors besides preeclampsia. Or it could mean that the studied groups are not homogenous and may include subgroups.

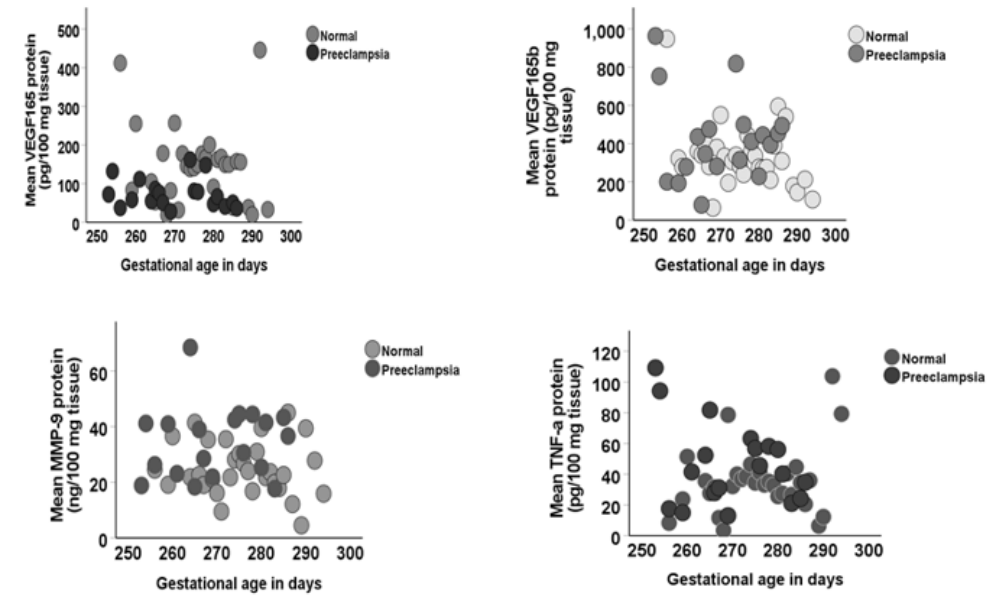


Figure 4: *Legend: Placental VEGF$_{165}$, VEGF$_{165}$b, MMP-9 and TNF-a proteins were analyzed in 149 placental samples, 113 placentas obtained from normotensive pregnancy and 36 placentas from preeclampsia. The scatter plots depict the mean + SD values of the proteins and show heterogeneity of the data between normal and preeclampsia groups for all four proteins.*

To date, supportive management remains the current standard of care for treating preeclampsia as there are no therapeutic interventions other than delivery. The pathophysiological mechanisms underlying preeclampsia highlight an angiogenic imbalance. A 9% improvement in preeclampsia prediction using binary logistic regression with VEGF$_{165}$, VEGF$_{165}$b, MMP-9 and TNF-a placental proteins suggests a modest but potentially clinically relevant improvement in predictive accuracy, which warrants further investigation and validation.

Conclusion

VEGF is a key growth factor involved in angiogenesis and endothelial cell survival. Isoforms of VEGF, VEGF$_{165}$ and VEGF$_{165}$b are secreted by cytotrophoblasts and our studies have demonstrated the coexistence of placental VEGF$_{165}$ and VEGF$_{165}$b proteins throughout gestation in normal pregnancy [2,21]. VEGF$_{165}$b can bind to the same receptor VEGFR2 as VEGF$_{165}$, and can effectively block the effect of VEGF$_{165}$ [25] We have proposed that VEGF$_{165}$b protein in the human placenta is involved in regulating the angiogenic balance in pregnancy, and in restraining the overexpression of VEGF$_{165}$ during placental angiogenesis, which if left unchecked could lead to pregnancy complications. In the third trimester of pregnancy when the placenta requires a robust vascular network to facilitate the exchange of nutrients and oxygen between the mother and fetus; when the fetal demand is at its peak, the two placental VEGF isoforms work synergistically and

show a significant positive correlation. In the current study, similar significant positive correlation between $VEGF_{165}$ and $VEGF_{165}b$ protein were seen (r^2 = .266, p = .004) as well. In normal pregnancy, placental $VEGF_{165}b$ and MMP-9 are both involved in vascular remodeling and show distinct expression patterns. MMP-9 protein increases with gestational age, while $VEGF_{165}b$ is downregulated in the third trimester compared to the second. The interplay between $VEGF_{165}b$ and MMP-9 is crucial for proper placental development and function. The negative association seen between placental $VEGF_{165}$ and MMP-9 protein, in the third trimester shown in the present study (r^2 = -0.201, p = 0.032) may be suggested as a mechanism to restrict the overexpression of $VEGF_{165}$ and placental angiogenesis as pregnancy approaches term.

The present study additionally confirms that $VEGF_{165}b$ and MMP-9 proteins have modest discriminatory ability in predicting preeclampsia. Measuring these proteins in maternal serum or in the cervicovaginal lavage earlier in the pregnancy, could help identify women at high risk of developing the disease. In our previous studies in normal pregnancy, placental TNF-a protein expression showed variations across trimesters. A peak in placental TNF-a protein was seen in the second trimester which we suggested to be essential in regulating vascular smooth muscle cell apoptosis and in the induction of MMP-9 protein synthesis; events that are crucial for successful pregnancy outcome [8]. In the present study the increase in the expression of placenta TNF-a protein seen in women with preeclampsia could suggests a role for inflammation in the development of the disease.

Preeclampsia is a serious pregnancy complication that can lead to severe maternal and fetal complications. An improved predictive value of biochemical indices in predicting preeclampsia, even by a small margin like 9%, can have significant clinical implications, potentially leading to earlier intervention and better outcomes for both mother and baby. Improved predictive accuracy empowers healthcare providers to provide better informed and proactive care to pregnant women. Early intervention can reduce the risk of maternal organ damage, premature birth, and fetal growth restriction. Future research can investigate the proposed role of angiogenic imbalance and the complex interplay between these placental proteins and preeclampsia. Studies can improve the clinical applicability of these biomarkers, identify the best biomarkers for early detection and prevention, and develop guidelines for their use in clinical practice.

Acknowledgements

The study was funded by the Residency Program Grant of the BronxCare Health System. The authors would like to thank Dr. Liaisan Uzianbaeva and Dr. Ashley Thakur, and the staff members of the Department of Obstetrics and Gynecology at BronxCare Health System for their support in carrying out the research. The three authors: EA, LGR and SR were former Residents of BronxCare Hospital System, in the Department of OB/GYN, when the study was carried out. The present address for EA is MomDoc, LLC, Phoenix, Az. The present address for LGR is Lincoln Medical and Mental Health Center, Department of OB/GYN, Bronx, NY, USA; and for SR is Loyola University Medical Center, Department of OB/GYN, Division of Maternal Fetal Medicine, Maywood, IL, USA.

Conflict of Interest

The authors declare no conflicts of interest with respect to the research, authorship and/or publication of this article.

Bibliography

1. Ferrara N. "Role of vascular endothelial growth factor in regulation of physiological angiogenesis". *American Journal of Physiology-Cell Physiology* 280.6 (2001): C1358-C1366.

2. Basu J., *et al.* "Chorionic villi expression of two vascular endothelial growth factor proteins in normal human pregnancy". *Integrative Gynecology and Obstetrics Journal* 1.3 (2018): 1-6.

3. Rossi F., *et al.* "Extracellular matrix and pregnancy: functions and opportunities caught in the net". *Reproductive Biology and Endocrinology* 23.1 (2025): 24.

4. Wang X and Khalil RA. "Matrix metalloproteinases, vascular remodeling, and vascular disease". *Advances in Pharmacology* 81 (2018): 241-330.

5. Raghupathy R. "Cytokines as key players in the pathophysiology of preeclampsia". *Medical Principles and Practice* 22.1 (2013): 8-19.

6. Brosens IA., *et al.* "The role of the spiral arteries in the pathogenesis of preeclampsia". *Obstetrics and Gynecology Annual* 1.1 (1972): 177-191.

7. Hong K., *et al.* "Defective uteroplacental vascular remodeling in preeclampsia: key molecular factors leading to long term cardiovascular disease*". International Journal of Molecular Sciences* 22.20 (2021): 11202.

8. Basu J., et al. "Placental tumor necrosis factor-α protein expression during normal human gestation". *Journal of Maternal-Fetal and Neonatal Medicine* 29.24 (2016): 3934-3938.

9. Liu H., *et al.* "Contribution of vascular smooth muscle cell apoptosis to spiral artery remodeling in early human pregnancy". *Placenta* 120 (2022): 10-17.

10. American College of Obstetricians and Gynecologists: Diagnosis and management of preeclampsia and eclampsia - ACOG practice bulletin No 22. *Obstetrics and Gynecology* 99 (2002): 159-167.

11. Basu J., *et al.* "Can chorionic villi angiogenic protein expression clarify the vascular abnormality seen in preeclampsia?" *EC Gynaecology* 11.9 (2022): 27-38.

12. IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, N.Y., USA).

13. Fox R., *et al.* "Preeclampsia: Risk factors, diagnosis, management, and the cardiovascular impact on the offspring". *Journal of Clinical Medicine* 8.10 (2019): 1625.

14. Sunjaya AF and Sunjaya AP. "Evaluation of serum biomarkers and other diagnostic modalities for early diagnosis of preeclampsia". *Journal of Family and Reproductive Health* 13.2 (2019): 56-69.

15. Akolekar R., *et al.* "Competing risks model in early screening for preeclampsia by biophysical and biochemical markers". *Fetal Diagnosis and Therapy* 33.1 (2013): 8-15.

16. Miller RK., *et al.* "Human placental explants in culture: Approaches and assessments". *Placenta* 26.6 (2005): 439-448.

17. Roberts JM., *et al.* "Preeclampsia: an endothelial cell disorder". *American Journal of Obstetrics and Gynecology* 161.5 (1989): 1200-1204.

18. Brockelsby JC., *et al.* "The effects of vascular endothelial growth factor on endothelial cells: A potential role in preeclampsia". *American Journal of Obstetrics and Gynecology* 182.1 (2000): 176-183.

19. Roberts JM, *et al.* "Subtypes of preeclampsia recognition and determining clinical usefulness". *Hypertension* 77.5 (2021):1430-1441.

20. Basu J., *et al.* "Regional variations in placental protein expression of matrix metalloproteinase-9 in term uncomplicated pregnancy". *EC Gynaecology* 9.9 (2020): 26-33.

21. Basu J., *et al.* "Comparative study of chorionic villi protein expression between normotensive pregnancy and preeclampsia". *EC Gynaecology* 8.10 (2019): 943-956.

22. Shokry M., *et al.* "Expression of matrix metalloproteinases 2 and 9 in human trophoblasts of normal and preeclamptic placentas: Preliminary findings". *Experimental and Molecular Pathology* 87.3 (2009): 219-225.

23. Kolben M., *et al.* "Proteases and their inhibitors are indicative in gestational disease". *European Journal of Obstetrics, Gynecology and Reproductive Biology* 68.1-2 (1996): 59-65.

24. Allison PD. «Multiple regressions: A primer». Pine Forge Press, Thousand Oaks, CA (1999).

25. Woolard J., *et al.* "$VEGF_{165}$b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, *in vivo* effect on angiogenesis and endogenous protein expression". *Cancer Research* 64.21 (2004): 7822-7835.

Volume 14 Issue 6 June 2025