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Maternal Serum Vascular Endothelial Growth Factor in Patients with Preeclampsia

Mohammed Salah El-Sokkary, Tamer Ahmed El-Refaie, Noha Bassiouny Hassan Mostafa, Mona Mohamed Soliman Elaassar

Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University

ABSTRACT

Background: Vascular endothelial growth factor and placental growth factor levels were significantly lower in patients of sever or early onset (34 weeks or less gestational age at onset of PET) pre-eclampsia than in controls. **Aim of the Work:** Will there be a rise in serum vascular endothelial growth factor in pregnant women suffering from preeclampsia than in normotensive pregnant women.

Patients and Methods: 150 pregnant women from 28 weeks of gestation till term will be enrolled into this comparative clinical trial at the time of their antenatal visit to the outpatient department of obstetrics and gynecology Ain Shams University maternal hospital. **Results:** We recruited 150 pregnant women after 28 weeks of gestation and they were classified into 3 groups: Group1:50 pregnant women with mild preeclampsia from 28 weeks of gestation till term, Group2: 50 pregnant women with severe preeclampsia from 28 weeks of gestation till term and Group 3: 50 normal pregnant women serving as controls. **Conclusion:** We conclude that serum VEGF level measured after 28 weeks of gestation can be used as a predictor for preeclampsia and its degrees (mild and severe) with high sensitivity and specificity.

Keywords: Nulliparous, Gastroesophageal, Hypochondrium, Cerebrovascular.

INTRODUCTION

Pre-eclampsia is a hypertensive disease that is characterized by the onset of high blood pressure and proteinuria after 20 gestational weeks; it complicates 3–8% of all pregnancies [(1)].

Normal placental Implantation shows proliferation of extra villous throphoblasts, forming a cell column beneath the chorionic (anchoring) villi. The extravillous trophoblasts invade the deciduas and extend down the inside of the spiral arterioles. This results in replacement of the endothelium and the muscular wall of the blood vessels [(2)].

In pre-eclampsia, the fact that the pathophysiology of the disease remains unclear, abnormal vascular growth and impaired endothelial function have been thought to be the major cause of the pathology [(3)].

The pathophysiological considerations in the development of pre-eclampsia, include: the existence of initiators e.g. genetic factors, immmunological factors, inflammatory factors, maternal vascular disease, faulty implantation [(4)].

The initiators cause the endovascular trophoblastic injury (acute atherosis) of the decidual blood vessels through certain mediators like: vascular endothelial growth actor (VEGF), prostaglandins, nitric oxide (previously termed, endothelium- derived relaxing factor), endothelins, cytokines, lipid peroxidases [(5)].

Reduced uteroplacental perfusion as a result of abnormal cytotrophoplast invasion of spiral arterioles leads to placental ischemia which is thought to cause a wide spread dysfunction of the maternal vascular endothelium which results in enhanced formation of endothelin, thromboxane, and superoxide, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as nitric oxide and prostacyclin.

Vascular endothelial growth factor and placental growth factor levels were significantly lower in patients of sever or early onset (34 weeks or less gestational age at onset of PET) pre-eclampsia than in controls**.** Soluble vascular endothelial growth factor receptor 1 (sVEGFR-1), which antagonizes VEGF functions, has been found to increase in preeclamptic patients. These observations suggest the participation of VEGF and sVEGFRI in the pathaphysiology of pre-eclampsia [(6)].

VEGF is an endothelial cell-specific mitogen in vitro and an angiogenic inducer in a variety of in vivo models. Hypoxia has been shown to be a major inducer of VEGF gene transcription. The tyrosine kinases'FlT-1 (VEGFR-l) and Flk-l/KDR (VEGFR-2) are high-affinity VEGF receptors [(7)].

VEGF is a well-known promote of angiogenesis; it also induces nitricoxide and vasodilatory prostacyclins in endothelial cells suggesting a role in decreasing vascular tone and blood pressure [(8)].

Other proteins are related in structure and receptor specificity to vegf-a. The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (P1GF) [(7)].

Exogenous VEGF was shown to ameliorate post-cyclosporine- mediated hypertension, endothelial dysfunction, and nephropathy [(9)].

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AIM OF THE WORK

To estimate maternal serum vascular endothelial growth factor (VEGF) level in patients with preeclampsia compared to normotensive pregnant women.

PATIENTS AND METHODS

150 pregnant women from 28 weeks of gestation till term will be enrolled into this comparative clinical trial at the time of their antenatal visit to the outpatient department of obstetrics and gynecology Ain Shams University maternal hospital and will be divided into 3 groups.

- **Group 1:** 50 women diagnosed with mild preeclampsia from 28 weeks of gestation till term.
- **Group 2:** 50 women diagnosed with severe preeclampsia from 28 weeks of gestation till term.
- **Group 3:** 50 normal pregnant women serving as controls.

Sample Size

The required sample size has been calculated using the G*Power Software (Universität Düsseldorf, Germany).

The primary outcome measure is the level of the VEGF in the three study groups.

A previous study reported that the mean ± SEM for the VEGF was 184 ± 18pg/ml, 396 ± 35pg/ml, or 608 ± 111 pg/ml in normotensive pregnant women, women with mild PE, or women with sever PE, respectively. The sample size for the three groups included in that study was 20 patients, 55 patients, and 16 patients, respectively [(10)].

From the data of ***Shaarawy et al.*** [(10)], the SD of the VEGF in normotensive pregnant women, women with mild PE, or women with sever PE is estimated to equal 81pg/ml, 260pg/ml, or 444 pg/ml, respectively. This calculation used the following equation:

$SD = SEM * \sqrt{n}$, where *SD* is the standard deviation, *SEM* is the standard error of the mean, and *n* is the group sample size.

So, it is estimated that a sample of 28 patients in each study group (total 84 patients) would achieve a power of 80% (type II error, 0.2) to detect a statistically significant difference among the three groups as regards the level of VEGF for a medium-to-large effect size (eta squared, η^2) of 0.35. The effect size (eta squared, η^2) is calculated as follows:

η^2 = between-group sum of squares/total sum of squares. This calculation used a two-sided F test with a numerator degree of freedom *(k-1)* of 2, a denominator degree of freedom *(n-k)* of 81, and a confidence level of 95% (type I error, 0.05), where *k* is the number of groups and *n* is the total sample size. An effect size equivalent to an eta squared (η^2) of 0.35 has been chosen as it could be regarded as a clinically relevant difference to seek in this exploratory study.

Inclusion criteria

- Primigravida.
- Of gestational age between 28 till term.
- Singleton, viable pregnancy.
- With no medical or obstetric complications of pregnancy.
- No medications received during pregnancy except tonics and vitamins.

Exclusion criteria

Women with:

1) Chronic hypertension
2) Collagen vascular disorders
3) liver disease
4) Renal disease
5) Thyroid disease
6) Tobacco abuse

Definitions and grades of preeclampsia

Preeclampsia is defined as a systolic blood pressure greater than or equal to 140mmHg and/or diastolic blood pressure greater than or equal to 90mmHg and the presence of + or more of proteinuria after 20 weeks of gestation. The blood pressure will be measured in lateral recumbent position in the left arm with the women's legs resting on a flat surface, by a mercury sphygmomanometer kept at the level of the heart. The first sound (korotkoff 1) heard taken as the systolic blood pressure while the diastolic blood pressure taken at complete disappearance of sounds (korotkoff 5).In the event of absence of korotkoff 5, muffling (korotkoff 4) is accepted.

Preeclampsia is graded as mild if the systolic blood pressure is between 140to159mmHg and the diastolic blood pressure is between 90to109mmHg and as severe if the systolic and diastolic blood pressure is more than 160and 110mmHg, respectively.

Severity criteria

- Blood pressure > 160\110mmHg.
- Severe thrombocytopenia (platelets ≤ 50.000\cmL).
- ALT and/or AST > 70U\L.
- Serum creatinine >1.2mg/dl unless to be known previously elevated.
- Serum LDH ≤ 600IU/L.
- Proteinuria > 5g\24h urinary protein or >2+dipstick.

All women were undergo:

1) 1. Informative consent.
2) Detailed history for symptoms like headache, nausea, vomiting, epigastric pain, blurring of vision and lower limb edema.
3) General physical examination for:

- Hypertension: systolic more than 140mmHg and diastolic more than 90 mmHg.

- Abdominal examination for presence or absence of associated complications (eg: IUGR, hepatic tenderness or hepatomegaly).
- Edema which may be occult detected by rapid weight gain more than 1 kg per 2 weeks, or manifest over the dorsum of foot, shin of tibia or absent in dry preeclampsia.

4) Complete blood picture.
5) Renal function test: urea, creatinine and uric acid.
6) Liver function test: AST and ALT are elevated in HELLP, bilirubin is also elevated.
7) Proteins in urine: proteinuria is measured by dipstick test and is graded as +, ++,+++,++++.

The serum vascular endothelial growth factor (VEGF) will be measured at recruitment by ELISA technique using commercial kits

Sample collection:

1) 5 ml venous blood will be collected from antecubital vein in coded bulbs.
2) Sample is centrifuged within 30 minutes.
3) Clear serum collected in coded bulbs and store in refrigerator at -20 c.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

Statistical analysis

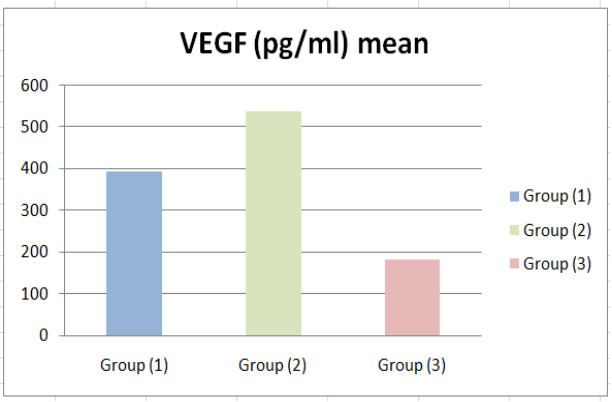
Data will be collected , tabulated, then analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY).

RESULTS

Table (1): VEGF level in the three study groups

Variable	Group 1 (n=50)	Group 2 (n=50)	Group 3 (n=50)	p-value
VEGF (pg/ml)	394.2 ± 25.8†	538.0 ± 89.1†‡	181.4 ± 12.4	**<.001 ¶ HS**

Data are mean ± SD.
¶One-way analysis of variance (ANOVA).
†Statistically significant difference from Group 3 (Tukey-Kramer test).
‡Statistically significant difference from Group 1 (Tukey-Kramer test).



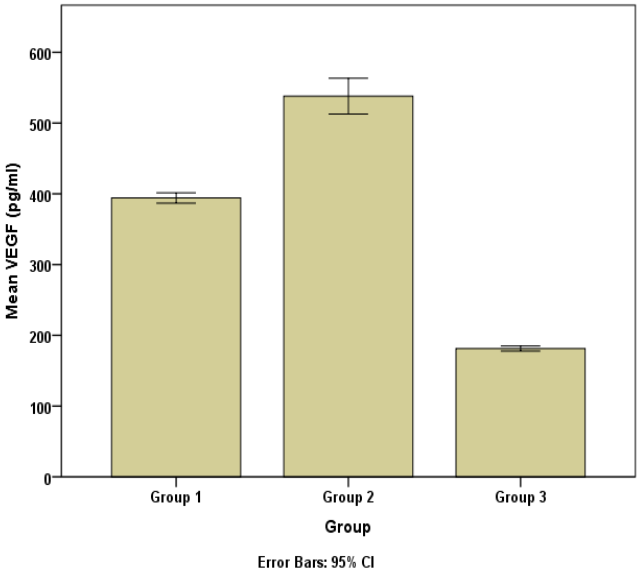


Figure (1): Mean VEGF level in the three study groups.

Table (2): Receiver-operating characteristic (ROC) curve analysis for discrimination between patients with PE and controls using VEGF

Parameter	Value
AUC	1.000
Standard error	.000
Lower bound (95%)	1.000
Upper bound (95%)	1.000
Difference from AUC0	.500
z (Observed value)	∞
z (Critical value)	1.960
alpha	.05
p-value	**<.0001 HS**
Cut-off for VEGF	>201.0 pg/ml
Sensitivity	100.0%
Lower bound (95%)	95.4%
Upper bound (95%)	100.0%
Specificity	100.0%
Lower bound (95%)	91.3%
Upper bound (95%)	100.0%
PPV	100.0%
NPV	100.0%
LR+	∞
LR-	0.00
J index	1.000
Accuracy	100.0%

AUC = area under ROC curve; AUC0 = null AUC of. 5; PPV, positive predictive value; NPV, negative predictive value; LR+ likelihood ratio positive; LR-, likelihood ratio negative.

Table (3): Receiver-operating characteristic (ROC) curve analysis for discrimination between patients with severe PE and those with mild PE using VEGF

Parameter	Value
AUC	.978
Standard error	.006
Lower bound (95%)	.967
Upper bound (95%)	.989
Difference	.478
z (Observed value)	86.164
z (Critical value)	1.960
p-value (Two-tailed)	**<.0001 HS**
Cut-off value for VEGF	> 416.0 pg/ml
Sensitivity	92.0%
Lower bound (95%)	80.5%
Upper bound (95%)	97.3%
Specificity	90.0%
Lower bound (95%)	82.3%
Upper bound (95%)	94.6%
PPV	82.1%
NPV	95.7%
LR+	9.20
LR-	0.09
J index	1.820
Accuracy	90.7%

AUC = area under ROC curve; AUC0 = null AUC of. 5; PPV, positive predictive value; NPV, negative predictive value; LR+ likelihood ratio positive; LR-, likelihood ratio negative.

Table (4): Ordinal logistic regression for the relation between VEGF and severity of PE

	Parameter Estimates	Estimate	Std. Error	Wald	Df	p-value	95% Confidence Interval	
							Lower Bound	Upper Bound
Threshold	PE	19.873	8.292	5.743	1	0.017 S	3.62	36.126
	Severe PE	31.083	9.838	9.982	1	0.002 HS	11.801	50.366
Location	VEGF (pg/ml)	0.074	0.019	15.045	1	**<.001 HS**	0.037	0.112
	BMI (kg/m^2)	0.084	0.129	0.427	1	.513 NS	-0.168	0.336
	GA (weeks)	-0.1	0.153	0.422	1	.516 NS	-0.4	0.201

DISCUSSION

Preeclampsia is a disorder of pregnancy characterized by elevated blood pressure and proteinuria [(11)].

Preeclampsia remains a major cause of maternal and neonatal morbidity and mortality [(12)].

Despite the still unexplained pathogenesis, preeclampsia is thought to be resulted from generalized endothelial dysfunction [(13)].

There is mounting evidence that an imbalance between angiogenic factors, such as vascular endothelial growth factor or placental growth factor (PIGF), and factors that inhibit angiogenesis, such as soluble fms-like tyrosine kinase 1 (sFlt1), are related closely to the pathogenesis of pre eclampsia [(14)].

VEFG is the key factor promoting vasculogenesis and angiogenesis in the embryo and the factor orchestrating most, if not all, processes of adult neovascularization [(15)].

The effect of VEGF performs nonvascular developmental functions and plays a number of homeostatic roles in the adult. The latter includes maintenance of endothelial fenestrations, a role in vasodilatation, neurogenic and neurotrophic activities among others [(16)].

The aim of this study was to estimate maternal serum vascular endothelial growth factor (VEGF) level in patients with preeclampsia compared to normotensive pregnant women.

We recruited 150 pregnant women after 28 weeks of gestation and they were classified into 3 groups: Group1:50 pregnant women with mild

preeclampsia from 28 weeks of gestation till term, Group2: 50 pregnant women with severe preeclampsia from 28 weeks of gestation till term and Group 3: 50 normal pregnant women serving as controls.

Preeclampsia was graded as mild if the systolic blood pressure is between 140 to 159 mmHg and the diastolic pressure is between 90 to 109 mmHg and as severe if the systolic blood pressure is more than 160mmHg and the diastolic pressure is more than 110 mmHg.

In our study, the total VEGF levels were elevated in severe preeclampsia compared with normal pregnancy and there are difference between mild preeclampsia and normal pregnancy. There were statistical differences in the levels of total VEGF between mild and severe preeclampsia.

Maternal serum total VEGF, levels were found to be significantly (p<0, 01) higher in preeclamptic patients. The mean value of the maternal serum levels of VEGF in mild and severe preeclamptic patients was 394.2 pg\ml and 538 pg\ml respectively while in the control group it was 181.4 pg\ml.

These results are in agreement with those previously reported by ***Baker et al.*** [17], who reported elevated levels of VEGF in patients with preeclampsia, which suggests that the growth factor has a role in the endothelial cell activation that occurs in the disease.

The plasma VEGF was significantly elevated (p<0, 01) in preeclamptic group with the normotensive group, VEGF is a potent regulator of endothelial cell function, the increased level found in women with preeclampsia indicated that VEGF may be involved in the maternal endothelial cell dysfunction associated with this condition. An increased VEGF is a potent regulator of microvascular permeability, may also contribute to the extravasation of plasma proteins and the subsequent development of proteinuria, both characteristic features of preeclampsia.

Bosio et al. [18], found that at 10 to 14 weeks of gestation plasma VEGF levels in all subjects were 4 to 5 times greater than the levels measured post partum (p<0.01). Mean VEGF concentration were similar in the control and gestational hypertension; in both group levels remained stable until 34 to 36 weeks gestation, when levels increased further1.3 fold (p<0.01). In comparison, VEGF concentrations in subjects with preeclamptic group were greater at 28 to 32 weeks gestation (p=0.02) and at 34 to 36 weeks gestation (p<0.01).VEGF concentrations were associated with elevated total peripheral resistance observed during the clinical disorder in preeclamptic group, but not in the other groups, so they concluded that raised plasma VEGF levels precede the clinical onset of preeclampsia and subsequently show a positive association with the elevated peripheral resistance observed during the clinical course of the disease.

Trollmann et al. [19] performed a prospective clinical study to compare maternal serum VEGF concentrations of pregnancies complicated by severe preeclampsia with those pregnancies of a well matched normotensive pregnant control group and demonstrated that they found significantly increased VEGF serum levels in preeclamptic pregnancies.

Hunter et al. [20] demonstrated that the predelivery median serum VEGF concentration in preeclamptic group was 51.7ng/ml and in the control group the concentration was 13.9 ng /ml (p<0.01).Serum VEGF concentrations felt within 24 hours of delivery in both groups, which resulted in median values of 3.8 ng/ml and 3.2 ng/ml respectively, (p<0.3).

Wathén et al. [21] ***and Vatten et al.*** [22] demonstrated that sVEGFR-1(sFLt-1) concentrations were elevated at 16-20 weeks gestation prior to clinical manifestation of preeclampsia. They demonstrated that second trimester combination of low or no increase in PIGF and increase in sFt-1 appeared be a very strong predictor of early onset preeclampsia, yielding 35 –fold higher risk compared with the reference group. Taking in consideration that sFt-1 neutralizes free VEGF, these results appeared to be concomitant with our results. In broad terms, the results of the above studies –including ours- are supporting the hypothesis of plancental hypoxia in explaining the pathophysiology of preeclampsia, where it has been proposed that defective placentation results in ischemic placenta which initiated the release of sFt-1 that binds to essential angiogenic factors e, g.VEGF and PIGF blocking their actions of placental neovascularization and depriving the maternal blood vessels from their vasodilator effect in addition to inducing systemic endothelial dysfunction which result in clinical manifestation of preeclampsia.

El-Salahy et al. [23] found that VEGF concentrations were elevated in plasma of women with preeclampsia compared to controls. Also they found that VEGF could differentiate between preeclamptic women and controls at a cut-off value of 70 ng\ml with sensitivity of 95% and specificity of 90%.

On the other hand, results of the present study are contradictory to those reported by ***Lyall et al.*** [24] who reported that serum concentrations of VEGF were significantly lower in normal pregnant women (the median value was 12.89pg/ml) than in non pregnant women (the median value was 166

pg/ml)($p<0.01$). In preeclampsia VEGF concentrations were significantly lower (the median value was 2.34 pg/ml) than in normal pregnancy ($p<0.01$). Postpartum concentrations of VEGF in the group complicated by preeclampsia (the median value was 76.42 pg/ml) were not significantly different from non pregnant values ($p=0.2$).

Livingston et al. [25] found that women with severe preeclampsia demonstrated significantly lower plasma concentrations of VEGF (6.36±3.96 pg/ml vs 18.25± 5.98 pg/ml; $p <0.01$) than did women with normotensive pregnancy. Logistic regression analysis showed an independent association between plasma VEGF concentration and preeclampsia.

Reuvekamp et al. [26] reported that decreased serum levels of VEGF characterize the development of preeclampsia. They concluded that this selective deficit of angiogenic growth factors might in part explain the shallow placentation found in this pregnancy complication. It is important to consider that platelets and leukocytes release VEGF during blood clotting, thus plasma rather that serum is recommended to be used for analysis[27], while ***Reuvekamp et al.*** [26] used serum samples.

However both investigators reported significant decrease of plasma or serum levels in preeclampsia. In the present investigation serum assay for VEGF were used for both controls and preeclamptic patients.

Hefler et al. [28] demonstrated that median VEGF serum levels in women with preeclampsia and healthy pregnant women were 33 pg\ml (range 11 to 101) and 34 pg\ml (14 to 110) respectively ($p=0.9$). VEGF serum levels did not significantly differ between women with preeclampsia and healthy pregnant women. Furthermore, VEGF serum levels in women with preeclampsia were not useful as a prognostic factor identifying women at elevated risk. No explanations were given by the previous authors about the cause of reduced VEGF levels in preeclampsia.

Finally, we concluded that serum free VEGF level measured after 28 weeks of gestation can be used as a predictor for preeclampsia and its degrees (mild and severe) with high sensitivity and specificity.

CONCLUSION

Serum VEGF level measured after 28 weeks of gestation can be used as a predictor for preeclampsia and its degrees (mild and severe) with high sensitivity and specificity.

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