

A study of vascular endothelial growth factor in the cord blood of pre-eclamptics and healthy pregnant women



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ABSTRACT

Background: Pre-eclampsia (PE) is the most frequently encountered medical complication during pregnancy. In developing countries PE is a principal cause of maternal mortality. A disturbance in the angiogenic/antiangiogenic factors and in the hypoxia/placental re-oxygenation process, seems to activate a maternal endothelial dysfunction. **Aims and Objective:** To estimate Vascular Endothelial Growth Factor (VEGF) level in the cord blood of healthy and Preeclamptic (PEc) pregnant women and to associate this with Preeclamptic pregnancy. **Material and Methods:** A case-control study of Umbilical cord serum VEGF levels from women with uncomplicated pregnancies (control group, $n = 60$) and pregnancies complicated by Pre-eclampsia ($n = 40$). VEGF in the cord serum was estimated by SANDWICH Enzyme Linked Immunosorbent Assay method by using ELISA Kit and then compared between the two groups. **Results:** The mean VEGF concentrations in the women who had pre-eclampsia (578.62 ± 468.3) were lower than in the control group (625.75 ± 533.1), but the difference was not statistically significant ($p = 0.8548$). **Conclusion:** VEGF plays a key role in the instability between endothelial dysfunction and angiogenesis that occurs during Preeclampsia. VEGF levels might be a useful tool for the early diagnosis of Pre-eclampsia.

Key words: Vascular endothelial growth factor, Pre-Eclampsia, Cord blood

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INTRODUCTION

Pre-eclampsia (PE), a pregnancy-specific disorder characterized clinically by new onset hypertension and proteinuria after 20 weeks of gestation, is the most frequently encountered medical complication during pregnancy, affecting ~3–5% of pregnant women worldwide. In developing countries where contact to health care is inadequate, PE is a principal cause of maternal mortality, with estimates of >60,000 maternal deaths/yr.¹ In the developed world, the load of this disease falls on the neonate because of premature deliveries performed to save the health of the mother. The condition resolves after the delivery of placenta implicating the placenta as a main cause in the pathogenesis

of PE. The placental dysfunction, characterized by a disturbance in the angiogenic/antiangiogenic factors and in the hypoxia/placental re-oxygenation process, seems to activate a maternal endothelial dysfunction. It is seen that in PE there are changes in placental development which can compromise the feto-maternal exchange, limiting fetal development, and start a maternal and fetal response to adapt to these changes.^{2,3}

In PE, extra-villous cyto-trophoblasts of fetal origin fail to enter the maternal uterine arteries resulting in a defective utero-placental circulation and subsequent placental ischemia.⁴ Placental factors like VEGF, PlGF and sVEGFR-1/ sFlt-1 are then released by the ischemic placenta into the maternal circulation ultimately causing

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generalized endothelial cell dysfunction and multi-organ failure.⁵

VEGF, a potent angiogenic factor enhances proliferation, migration⁶ and survival of endothelial cells.⁷ It also promotes neovascularization, reduces blood pressure and is vital for the formation and maintenance of the glomerular filtration barrier.⁸⁻¹⁰ Therefore, its deficiency could explain the main clinical manifestations of PE such as hypertension, proteinuria and oedema.

VEGF acts through 2 high affinity tyrosine kinase receptors: VEGFR-1 or Flt-1 and VEGFR-2 or KDR.¹¹⁻¹³ An imbalance in the pro(VEGF)- and anti(VEGFR-1)-angiogenic factors in serum is implicated in the pathophysiology of PE.¹⁴

This study was designed to see the association of umbilical cord blood (UCB) VEGF level with PE pregnancies.

MATERIAL AND METHODS

This case control study was conducted in campus of King George's Medical University, Lucknow, Uttar Pradesh, India. All women gave their informed consent to participate in the study. Ethical clearance was taken from the ethics committee of this university for pursuing the research work. The study group comprised of 80 subjects of 18 to 35yrs age group. Out of 100 subjects, 60 controls (healthy pregnant women) and 40 cases (diagnosed PE pregnant women as per ACOG guidelines) admitted in Queen Mary Hospital, KGMU were enrolled in our study.

Inclusion criteria were (1) healthy pregnant women (2) Pre-eclamptic women as per ACOG guidelines-

- Blood pressure: 140 mm Hg or higher systolic or 90 mm Hg or higher diastolic after 20 weeks of gestation in a woman with previously normal blood pressure. Systolic increased > 30 mm Hg or diastolic increased > 15 mm Hg in a patient with pre-existing chronic hypertension.
- Proteinuria: 0.3 g or more of protein in a 24-hour urine collection (usually corresponds with 1+ or greater on a urine dipstick test).

Severe preeclampsia

- Blood pressure: 160 mm Hg or higher systolic or 110 mm Hg or higher diastolic on two occasions at least six hours apart in a woman on bed rest.
- Proteinuria: 5 g or more of protein in a 24-hour urine collection or 3+ or greater on urine dipstick testing of two random urine samples collected at least four hours apart.

Exclusion criteria were pregnant women having history of (1) Hypertension (2) Multifetal gestation (3) Diabetes (4) Chronic renal disease (5) Miscarriage (6) Antepartum haemorrhage (7) Platelet disorders (8) Maternal or fetal infection (9) Epilepsy (10) Autoimmune disorders (11) Smoking.

Evaluation

Complete information about medical history, obstetric history and complications associated with pregnancy were taken in the proforma. All measurements were made by one investigator using standard techniques. Apgar scores at 1 and 5 min and weight of the newborn were evaluated by the Pediatrician.

Blood pressure

Hypertension was diagnosed when 2 BP readings of 140/90 mm Hg or greater were noted 6 hours apart within a 1-week period. Blood pressure was measured by using mercury sphygmomanometer with stand. Measuring BP with an appropriate-sized cuff (cuff bladder encircling at least 80% of arm) placed on the right arm at the same level as the heart is important. The patient must be sitting and ideally, have had a chance to rest for at least 10 minutes before the BP measurement. Korotkoffs sounds (first and fifth phase) were the criteria for systolic and diastolic B.P respectively.

Urine albumin

Urine albumin was detected by Urine DIPSTICK test in a random midstream sample collected in a clean sterile container.

Blood sample collection and storage

Five-ml of Umbilical cord blood was collected in a syringe immediately after the delivery of baby from the maternal end of cord. Collected blood in the syringe was allowed to clot at room temperature before being centrifuged at 3000 rpm for 20 minutes. The serum was then stored at -80 degree centigrade until assayed. Cord serum VEGF was measured by SANDWICH Enzyme Linked Immunosorbent Assay method by using AviBion Human VEGF Elisa Kit. This assay employs an antibody specific for human VEGF coated onto a 96- well plate. Standards, samples and biotinylated anti-human VEGF were pipetted into the wells and VEGF present in a sample was captured by the antibody immobilized to the wells and by the biotinylated VEGF- specific detection antibody. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted into the wells. The wells were again washed. Following this second wash step, TMB substrate solution was added to the wells, resulting in blue colour development proportional to the amount of VEGF bound. The stop

solution changed the colour from blue to yellow, and the intensity of the colour is measured at 450 nm. The standard curve was used to determine the amount of VEGF in an unknown sample. The standard curve was generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding VEGF concentration (pg/ml) on the horizontal (X) axis.

Data analysis was performed using Stata 11.2 software (college station, Tx, US). Data were presented as mean \pm standard deviation. Statistical differences between the studied groups were calculated by using the two sample t-test with equal variances, Mann–Whitney test, chi–squared with ties and Kruskal–Wallis test. Correlations between studied parameters were performed using the Spearman's coefficient of correlation. $P < 0.05$ was considered as statistically significant.

RESULTS

There were no significant differences ($p > 0.05$) in maternal age and gestational age at delivery between the PEc and control groups. Compared with normal pregnancy, PEc pregnancy presented significantly higher systolic and diastolic blood pressure ($p < 0.001$). LSCS was significantly increased in PEc group ($p = 0.003$) as compared to control group (Table 1).

Both the groups were almost similar in their neonatal characteristics like infant weight, sex and apgar score at 1 min and 5 mins, showing no significant difference ($p > 0.05$) (Table 2).

The mean VEGF concentrations in the women who had pre-eclampsia (578.62 ± 468.3) were lower than in the control group (625.75 ± 533.1), but the difference was not statistically significant ($p = 0.8548$) (Table 3).

DISCUSSION

In present study the mean cord serum VEGF levels were lower in pregnant women having Pre-eclampsia, but the difference was statistically insignificant. Our results corroborate with findings of a study which also observed that the difference in the Levels of VEGF in UCB samples between the two groups was not significant.¹⁵ Some studies observed that umbilical cord plasma free VEGF were significantly decreased in PE compared to the control group.^{16,17} On the other side there are studies which are not in accordance with the present study. These studies found that the cord serum VEGF levels were significantly higher in PEc group as compared to control group.¹⁸⁻²⁰

In the pathophysiology of PE an imbalance in the pro- and anti-angiogenic factors in serum is involved. Therefore, a combined analysis of the circulating pro- and anti-angiogenic factors would be more useful in patients with PE. In the present study we only measured the level of cord serum VEGF and the estimation of level of sVEGFR-1 in the cord serum was not done. The duration of study and the sample size were inadequate to achieve confirmatory results.

VEGF is regulated by alterations in oxygen pressure and by VEGFR-1, as its antagonist.²¹ VEGF production is up-regulated by low oxygen tension.²²

There are several studies carried out in maternal serum associating VEGF with PE which demonstrated that serum sVEGFR-1 levels were significantly elevated in PE women compared with controls. The placental sFlt1, an antagonist of VEGF is up-regulated in PE, leading to increased systemic levels of sFlt1. Increased circulating sFlt1 in patients with PE is associated with decreased circulating levels of free VEGF. The balance

Table 1: Maternal data for control and PEc groups

Variables	Control (n=60)	PEc (n=40)	p-value
Age (years)	25.65 \pm 3.4	24.73 \pm 4.0	0.2212*
Gestational Age (weeks)	38.33 \pm 2.1	37.51 \pm 3.0	0.5056**
Systolic BP (mmHg)	122.10 \pm 6.8	147.80 \pm 11.0	0.000**
Diastolic BP (mmHg)	78.07 \pm 4.2	95.40 \pm 7.5	0.000**
Normal delivery	88.33%	62.50%	0.003#
LSCS	11.67%	37.50%	0.003#

LSCS=Lower segment cesarean section, *: Two sample test, **: Mann - Whitney test, #: Chi 2 test

Table 2: Neonatal data for control and PEc groups

Variables	Normal (n=60)	PEc (n=40)	P-value
Weight (kg)	2.80 \pm 0.5	2.73 \pm 0.5	0.4564*
Male	56.67% (34)	55.00% (22)	1.000**
Female	43.33% (26)	45.00% (18)	1.000**
Apgar Score			
1 minute	6.16 \pm 0.6	6.16 \pm 0.4	0.8585***
5 minute	7.09 \pm 0.4	7.16 \pm 0.4	0.4221*

*: Two sample test, **: 2 sided Fisher's exact, ***: Mann-Whitney test

Table 3: Comparison of means of VEGF (pg/ml) between groups

Variable	Study Group (n=60)	Control Group (n=40)	P value
VEGF (pg/ml)	578.62 \pm 468.3	625.75 \pm 533.1	0.8548

Test used: Mann - Whitney

between VEGF, PlGF, and their receptors is important for effective placental development during pregnancy and hypoxia mediated increase in levels of sVEGFR-1 may lead to dys-regulated angiogenesis associated with PE.²³⁻³⁰ The probable explanation for our finding is that in PE trophoblasts under hypoxic conditions seem to increase sVEGFR-1 production, an antagonist of VEGF.

Although we have not measured the level of sVEGFR-1 in the cord serum in our study but previous studies have demonstrated and confirmed this finding.¹⁷ VEGF presented reduced values in new-borns from PE pregnancies, as compared with normal cases as the main target for sVEGFR-1 (significantly higher in UCB circulation in PE cases) is probably VEGF, explaining the observed reduction in UCB when compared to the normal value. Our data suggest that in PE, in UCB circulation, a close interaction seems to exist between endothelial dysfunction and angiogenesis disturbance, and that VEGF seems to play a central role in these disturbances. To conclude our data suggest that in PE, in UCB circulation, a close interaction seems to exist between endothelial dysfunction and angiogenesis disturbance, and that VEGF seems to play a central role in these disturbances. Further studies are needed to find out whether VEGF levels might be a useful tool for the early diagnosis of Pre-eclampsia. The present study is one of ongoing step in the direction of establishing role of VEGF in pathophysiology of Preeclampsia. However to achieve more confirmatory results further studies on larger sample size are needed.

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Authors Contribution:

AR - Concept and design of the study, reviewed the literature, manuscript preparation and critical revision of the manuscript; **AKG** - Collected data and review of literature and helped in preparing first draft of manuscript; **AG** - Concept and design of the study, reviewed the literature; **NS** - Concept and design of the study, manuscript preparation and critical revision of the manuscript; **ST** - Conceptualized study, prepared first draft of manuscript and critical of the manuscript; **US** - Concept, collected data and review of literature and helped in preparing first draft of manuscript.

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