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Original Research Paper

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CORD SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS ASSOCIATION WITH PREGNANCY OUTCOMES

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ABSTRACT

Pre-eclampsia(PE), a pregnancy-specific disorder is the most frequently encountered medical complication during pregnancy.The load of this disease falls on the neonate because of premature deliveries performed to save the health of the mother.The placental dysfunction, characterized by a disturbance in the angiogenic/antiangiogenic factors and in the hypoxia/placental reoxygenation process, seems to activate a maternal endothelial dysfunction .This study was designed to see the association of umbilical cord blood (UCB) VEGF level with pregnancy outcome.This case control study was conducted in campus of King George's Medical University, India. Out of 100 subjects , 60 controls (healthy pregnant women) and 40 cases (diagnosed PEc pregnant women) were enrolled in our study.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. The mean VEGF concentrations in the women who had pre-eclampsia (357.59±215.76) were lower than in the control group(926.17±353.49) and the difference was statistically significant p=<0.01).Lower VEGF values in PE were associated with more ceaserian section delivery and lower birth weight as compared to control group. 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.

KEYWORDS : Vascular Endothelial Growth Factor , Pre-eclampsia , cord blood

Introduction

During pregnancy, mother's well -being affects directly the newborn development. Some maternal and placental complications, such as preeclampsia (PE) may contribute to fetal growth deviations or fetal development modifications. PE has been associated with an enhancement in physiological blood changes and with placental abnormalities, that may condition its perfusion and, therefore, feto-maternal transfer. Worldwide PE is associated with a perinatal and neonatal mortality rate of 10%.(*Altman D* et al.2002).

A normal placental development is essential for adequate feto-maternal nutrients and gas exchanges. In addition, placenta is an important endocrine organ that synthesizes various hormones, cytokines and angiogenic growth factors. These factors are released into the maternal circulation, and may contribute to changes in endothelial function. It is recognized that in PE there are changes in placental development which can compromise the feto-maternal exchange, limiting fetal development, and trigger a maternal and fetal response to adapt to these changes. (Pijnenborg Ret al 2006 , Young B et al 2010) . In PE, endovascular invasion by the Cytotrophoblast remains shallow, leading to a defective uteroplacental circulation and subsequent placental ischaemia (Brosens IA et al 1972). A hypoxic/ischaemic placenta may then release Placental factors VEGF, PlGF and sVEGFR-1/ sFlt-1 into the maternal circulation eventually causing systemic endothelial cell dysfunction and thereby contributing to renal, cardiovascular and neurological symptoms (Lee ES et al 2007) . A balance between VEGF, PlGF (pro-angiogenic factors) and sVEGFR-1/sFlt-1 (antiangiogenic factor) is essential for effective angiogenesis, vasculogenesis and placental development during pregnancy. An imbalance in the pro- and anti-angiogenic factors in serum is involved in the pathophysiology of PE (Romero R et al 2008). This study was designed to see the association of umbilical cord blood (UCB) VEGF level with pregnancy

Methods

This case control study was conducted in campus of King George's Medical University,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 100 subjects of 18 to 35yrs age group. Out of 100 subjects , 60 controls (healthy pregnant women) and 40 cases (diagnosed PEc pregnant women as per ACOG guidelines) KGMU were enrolled in our study. According to American College Of Obstetrician and Gynaecologists (ACOG) Guidelines, for the diagnosis of PE, both hypertension and proteinuria must be present.

- **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 preexisting 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.

Inclusion Criteria

Healthy pregnant women and Pre-eclamptic women as per ACOG guidelines

.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 (12)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

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-millilitre 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.

Results

Table I summarizes the maternal clinical data for control and PEc groups.

Table 1: Maternal Data for control and PEc group

Variables	control (n=60)	Pec (n=40)	p-value
Age (Years)	27.30±2.73	26.60±2.92	0.225
Gestational Age (Weeks)	38.80±1.82	37.28±2.95	0.002**
Blood Pressure (mmHg)			
Systolic BP	121.67±14.47	148.95±10.18	<0.001**
Diastolic BP	78.13±3.45	96.80±6.81	<0.001**
Mode of delivery			
Normal	48 (80.0%)	12 (20.0%)	<0.001**
LSCS	9 (22.5%)	32 (77.5%)	

Data are represented as mean, ±SD, n (%) and ratio. SD=Standard deviation, Independent T- Test, **=Significant (p=<0.01) LSCS= Lower segment cesarean section

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<0.001) as compared to control group.

Neonatal clinical data are given in table 2

Table 2: Neonatal data for control and PEc group

Variables	Normal (n=60)	PEc (n=40)	P –value
Weight (kg)	2.91±0.39	2.63±0.44	0.001**
Sex			
Male	35 (58.33%)	25 (41.77%)	0.902
Female	22 (55.0%)	18 (45.0%)	
Apgar Score			
1minute	6.15±0.55	6.18±0.45	0.810
5 minute	7.05±0.43	7.18±0.45	0.163

Data are represented as mean, ±SD, n (%) and ratio. SD=Standard deviation, Independent T-Test, **=Significant (p=<0.01)

Both the groups were almost similar in their neonatal characteristics like infant sex and apgar score at 1 min and 5 mins except the infant weight which was significantly lower in d study group(p<0.01).

Detectable levels for VEGF were obtained from all samples. Umbilical cord serum VEGF (pg/ml) data for controls and women with pre-eclampsia is shown in Table 3.

Table 3: Comparison of means of VEGF (PG/ML) between groups

Variables	Study Group (n=40)	Control Group (n=60)	P- value
VEGF (pg/ml)	357.59±215.76	926.17±353.49	<0.001**

Data are represented as mean, ±SD, Mann – Whitney test, **=Significant (p=<0.01)

The mean VEGF concentrations in the women who had pre-eclampsia were significantly lower than in the control group (p<0.01).

Discussion

Pregnancies complicated by PE are associated with poor placental growth and inadequate physiologic changes in the vasculature of the placental bed **(**Roberts JM et al 1989 **).**Some authors, Levine et al. (2004**)** and McKeeman et al. (2004) proposed that circulating maternal sVEGFR-1 could be involved in the pathogenesis of uteroplacental vascular disease associated with PE, as trophoblasts under hypoxic conditions seem to increase sVEGFR-1 production. In our study we found that the mean VEGF concentrations in the women who had pre-eclampsia were significantly lower than in the control group. Our results corroborate with findings of a study which also observed that umbilical cord plasma free VEGF were significantly decreased in PE compared to the control group**.(** Kwon JY *et al* and CRISTINA CATARINO *et al)* 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**[**G. Galazios *et al* , Laskowska M *et al and* Kulkarni AV *et al*].

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 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[CRISTINA CATARINO *et al*]. VEGF presented reduced values in newborns from PEc 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 disturbance. 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 steps 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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