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# ROLE OF FREE SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR-A (VEGF-A) IN PRE-ECLAMPSIA.

**DR. MUNMUN DAS(SARKAR)-[1*] AND DR. TAPAN KUMAR GHOSH-[2]**

*[1.] Assistant secretary, Medical education, Government of West Bengal & Assistant Professor, Department of Microbiology, School of Tropical Medicine, Kolkata.*
*[2.] PhD fellow, Department of Gynecology & Obstetrics, Burdwan University.*

## ABSTRACT

Hypertension is one of the common medical disorders of pregnancy and is reported to complicate up to 1 in 10 gestations. Altered utero-placental blood flow has long been the focus of the pathophysiology of preeclampsia. Vascular endothelial growth factor (VEGF) may be important in mediating the endothelial response that occurs in preeclampsia. The pathophysiology of preeclampsia remains largely undiscovered. In this back ground the present study was conducted with the following objects. To determine whether altered levels of active vascular endothelial growth factor may be implicated in the pathogenesis of preeclampsia, and whether VEGF mediates the endothelial cell activation that is involved in the pathogenesis of the clinical syndrome

**KEYWORDS-** ***Pre-eclampsia, VEGF, Endothelial Cells, Pathogenesis.***

**DR. MUNMUN DAS**
**Assistant secretary, Medical education, Government of West Bengal & Assistant Professor, Department of Microbiology, School of Tropical Medicine, Kolkata.**

***Corresponding author***

# INTRODUCTION

The term “Pre eclampsia” was coined in the early 20th century when it was recognized as hypertension and proteinuria could be responsible for pregnancy specific seizure disorder – eclampsia, which had been recognized since 2000 years (1). Preeclampsia is restricted to hypertension in pregnant women who have no preexisting overt chronic hypertension or renal disease and no high blood pressure or proteinuria before 20 weeks ‘gestation, and in whom hypertension and proteinuria occur for the first time during the second half of pregnancy (after 20 weeks), during labour, and subside after delivery . VEGF play role in the growth and differentiation of cytotrophoblast during implantation. Native VEGF is a basic, heparin-binding, homodimeric glycoprotein of 4500 Daltons (2). The human VEGF Gene is organized in 8 exons, separated by 7 introns and assigned to chromosome 6p 21.3. VEGF family includes VEGF –A, B, C, D subtypes. VEGF- A is one of the key molecules for angiogenesis & survival of vascular endothelium and acts as endothelial cell mitogen. VEGF-A exist as four different molecular species depending on numbers of amino acid. As VEGF 121, VEGF 165, VEGF 189, VEGF 206. VEGF – C & D – mainly regulate the lymph angiogenesis. VEGF – B not detected in free form. VEGF promotes angiogenesis and also known as vascular permeability factor (VPF) based on its ability to induce vascular leakage. It induces vasodilatation and induces colony formation in bone marrow. Pre eclampsia is one of the most common clinical conditions among all other pregnancy induced hypertension and sometimes coined as “Disease of theories” mentioned in various literature. In periphery rural hospitals where most of the attending antenatal mothers are poor and ignorant about regular antenatal checkup very often develop preeclampsia that may progress to feared complication like eclampsia and killing both mother and fetus. VEGF promotes angiogenesis but due to alteration of VEGF in circulation in PIH causes impaired angiogenesis leading to hypoxia and hyperplasia of trophoblastic cells which causes hyper secretion of placental hormone ultimately leading to high level of circulating ß hCG.

# MATERIALS AND METHODS

The present study was conducted in the Department of Obstetrics& Gynecology, Department of Pathology, Department of Microbiology and Department of Biochemistry of Burdwan Medical College, Burdwan, West Bengal, India after taking approval from the Institutional Ethical Committee from 2005 June to December 2010.

The study conducted on total 100 pregnant mothers divided into two groups.

i) Group A - 50 healthy pregnant mothers between 20 to36 weeks of gestation (Control) (Study –I).
ii) Group B- 50 pregnant mothers between 20 to 36 weeks of gestation who were diagnosed as PIH. Out of these 50 PIH mothers, 20 were diagnosed as severe PIH including 3 Eclampsia and 30 were mild PIH (Study-II).

Before initiation of the study, informed consent was taken from each pregnant mother individually, after explaining about the study in regional languages for their participation.

### *Study design*

Comparative study involving antenatal mothers attending to Gynaecology & Obstetrics department, Burdwan Medical College & Hospital.

### *Inclusion criteria*

The mothers were diagnosed as PIH when the systolic blood pressure were persistently ≥ 140 mm of Hg and diastolic blood pressure ≥ 90mmof Hg ,on two occasions each 6 hours apart ,accompanied by proteinuria at least 1+ on dip stick testing at third trimester of gestation having singleton foetus.

***Exclusion criteria***

(i) Pregnant women with Blood Pressure ≥ 140 mm of Hg and diastolic blood pressure ≥ 90mmof Hg before 20 weeks of gestation (Pre-existing chronic hypertension)
(ii) Pregnant Mothers with known Diabetes Mellitus , Multiple pregnancy, foetal abnormalities
(iii) Pregnant mothers with renal disease, UTI.

The following investigations were done: Systolic Blood pressure in mm of Hg, Diastolic Blood pressure in mm of Hg, Serum VEGF in pg/ml Serum VEGF just before delivery, Serum VEGF after delivery, I.H.C of placental tissue for localization of VEGF. Blood pressure was measured in semi fowler position before medication or clinical perturbation to avoid alteration of pressure. All data including the control mothers and preeclamptic mothers were tabulated in (Table-I).Preeclamptic mothers were classified into mild and severe PIH (Table-II) Urine protein was measured by dip stick method. Mid stream urine samples were collected on separate occasions in both groups. Advantage of Spot urine sample for detecting protein is 1) reliable method of giving instant report and 2) it correlates with 24hour urinary protein excretion. Urinary protein was reported as 0(nil) to 4+. (0=nil, 1+=30mg/dl ,2+=100mg/dl , 3+=300-1999mg/dl and 4+=2000mg/dl.

# RESULTS

The data were collected and tabulated after analysis for significance of difference between the mean values.

***Group A Results***

Group A- 50 healthy pregnant mothers between 20 to 36 weeks of gestation (Control).

***Group B Result***

Group B- 50 pregnant mothers between 20 to 36 weeks of gestation who were diagnosed as PIH. (Study Group). Out of these 50 PIH mothers 20 were diagnosed as severe PIH including 3 Eclampsia and 30 were mild PIH.

***Group C Result***

The clinical results of mild PIH and severe PIH mothers are compared.
(Subdivision of study group)

***Result of Group A, Group B & Group C***

The age of enrolled mothers and their gestational age, Parity, Blood pressure (Systolic & Diastolic), Serum free VEGF (between 20 weeks to before mother going to labour, just before delivery and after delivery)

**TABLE- I**

***Clinical / Biochemical's / Hematological parameters of control (Group-A)and preeclampsia mothers (Group-B )***

| Parameter | Normal Pregnancy(control) (Mean ±SD) n=50 | Pregnancy induced hypertension(PIH) (Mean ±SD) n=50 | Significance |
|---|---|---|---|
| Age In Years | **22.36±3.31(18-32)** | **21.62±2.44(18-30)** | Not significant (P= 0.172) |
| Systolic Blood Pressure in mm of Hg | **115.60±7.93(100-130)** | **161.68 ±18.25(140-200)** | Significant (P=<0.0001) |
| Diastolic Blood Pressure In mm of Hg | **73.08±6.77(60-90)** | **103.20±9.57(90-120)** | Significant (P=<0.0001) |
| Serum VEGF in pg/ml | **66.25±11.94(40.11-102.32)** | **25.19±3.09(20-30.78)** | Significant (P=<0.0001) |
| Serum VEGF just before delivery | **57.46±8.16(39.09-77.98)** | **17.44±2.02(15-22.98)** | Significant (P=<0.0001) |
| Serum VEGF after delivery | **75±12.99(50.23-105.23)** | **74.47±5.20(62.29-86.42)** | Not significant (P=<0.779) |

***The clinical data of PIH mothers (n=50) were further evaluated in two groups Mild PIH (n=30) and Severe PIH (n=20).***

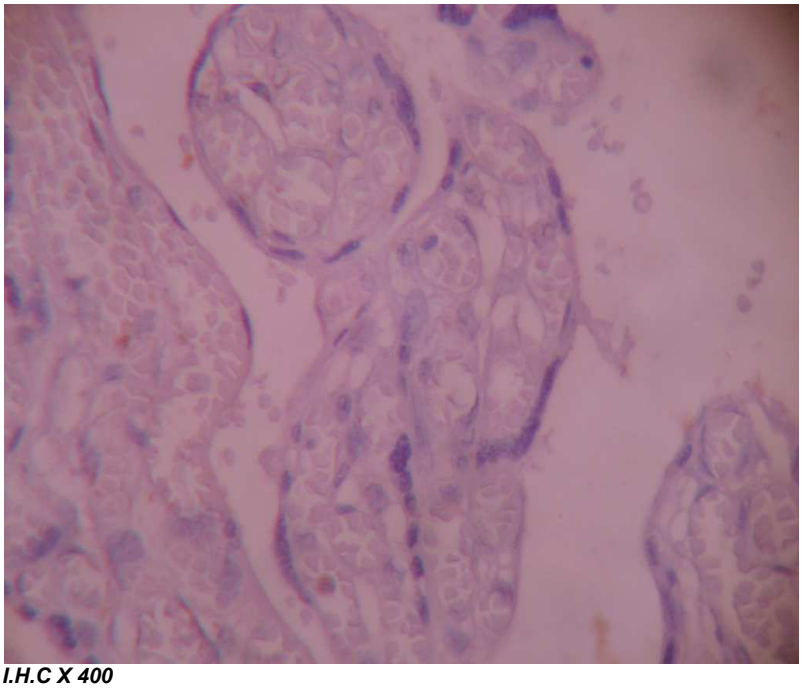
**TABLE-II**
***Clinical / Biochemical's / Hematological parameters of both mild PIH and severe PIH mothers***

| *Parameter* | *Mild PIH* | *Severe PIH* | *Significance* |
|---|---|---|---|
| *Age In Years* | **21.10±1.97(18-25)** | **22.40±2.90(18-30)** | Non sufficient (p=0.174) |
| *Systolic Blood Pressure in mm of Hg* | **149.20±8.60(140-170)** | **180.40 ±11.47(160-200)** | Significant (P=<0.0001) |
| *Diastolic Blood Pressure In mm of Hg* | **97.46±5.48(90-110)** | **111.80±7.78(100-120)** | Significant (P=<0.0001) |
| *Serum VEGF just before delivery* | **18.13±2.15(15.08-22.98)** | **16.40±1.25(15-18.25)** | Significant (p=<0.0001) |
| Serum VEGF after delivery | **74.58±5.21(62.29-84.06)** | **74.32±5.32(62.29-86.42)** | Non significant(p=0.858) |

***The clinical results of mild pre eclampsia and severe eclampsia mothers are compared. (Table-II) group C***

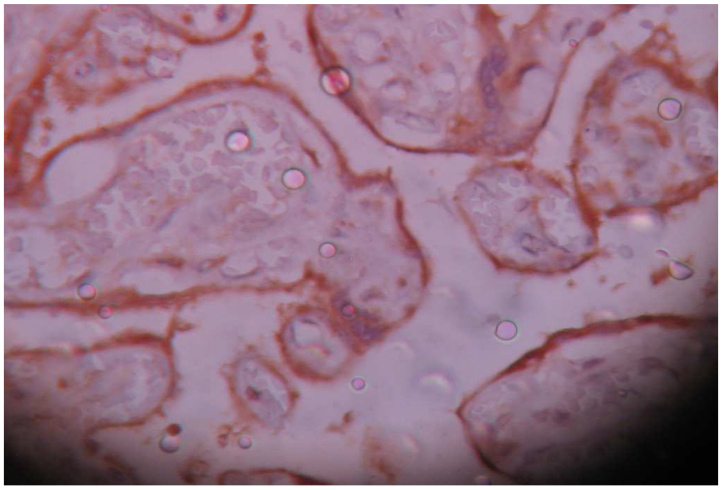
The expression of VEGF in placenta by IHC in preeclampsia and control are shown in figure- 1and figure- 2 respectively. The expression of VEGF in placenta by IHC (qualitative) is reduced in PIH placenta than normal.

**Figure 1**
***SHOWING REDUCED EXPRESSION OF VEGF IN PLACENTA BY I.H.C (IN PRE ECLAMPSIA)***



***I.H.C X 400***

**Figure 2**
***SHOWING EXPRESSION OF VEGF IN PLACENTA BY I.H.C***
***(NORMOTENSIVE NON PROTEINURIC – Control)***



## DISCUSSION

Pregnancy Induced Hypertension is the most common disease in pregnancy and represents a distinct risk for both the mother and fetus. No single risk factor has been found responsible for pathogenesis preeclampsia. Vascular Endothelial Growth Factor (VEGF), a disulphide –linked homodimeric glycoprotein is a selectively mitogenic endothelial cells, plays an important role in vasculogenesis and angiogenesis. Angiogenesis is rare in adults with exceptions of female reproductive tract and in some pathological condition (3,4,5).Mammalian placenta requires extensive angiogenesis to establish a suitable vascular network for supply of oxygen and nutrient to the fetus. Placental growth factor (PLGF) is a member of the VEGF family. VEGF expressions has been found in activated macrophages(6),keratinocytes(7),renalglomerul arvisceralepithelium(8),hepatocytes(9),aortic fibroblast(10). In contrast to the wide spread distribution of VEGF, the expression of PLGF is limited. PLGF is distributed to placental tissue, choriocarcinoma cells and cultured endothelial cells, (11,12). In comparison to VEGF, PLGF is a weak endothelial cell mitogen chemo attractant (13,14) .The biological effect of the VEGF family members are mediated by members of the class III subfamily of receptor tyrosine kinases (RTKs). These contain seven immunoglobulin-like repeats in their extracellular domains (15). At least three RTKs that bind various VEGF family members have been cloned, VEGF-R1 (Flt-1), VEGF-R2 (KDR/Flt-1), and VEGF-R3 (Flt-4) (16, 17, 18, 19). The human VEGF-R1 was originally discovered through the screening of a human placental cDNA library (16). P*L*GF has been shown to bindVEGF-R1 with high affinity but not withVEGF-R2 and VEGF-R3 (19). VEGF has been shown to bind both VEGF-R1 and VEGF-R2 with high affinity but not with VEGF-R3. A soluble form of VEGF-R1 (sVEGF-R1) has also been identified in culture medium conditioned by the growth of human umbilical vein endothelial cells(HUVECs); that is accomplished through an alternate splicing at the pre-mRNA level (Kendall and Thomas1993). The mean plasma free VEGF-A level in male 72.11 ± 17.70 pg/ml and in non pregnant female 92.44±24.20 pg/ml .There is no significant difference in mean plasma free VEGF-A levels between males and females (p=0.49). No correlation is found between free VEGF level and age(r=0.034, p=0.86) (20).

Several studies have demonstrated that circulating total VEGF concentrations significantly elevated in women with PIH (21, 22, 23, 24, 25). Studies showed that serum concentrations of free VEGF-A were significantly lower in normal pregnant women than in non pregnant healthy women ( P < 0.0001).In pre-eclampsia VEGF concentrations were further significantly lower than normal pregnancies (P < 0.0001). Postpartum concentrations of VEGF in the group complicated by pre-eclampsia (median value 76.42 pg/ml) were not significantly different from non pregnant values (P = 0.2) (26). Previous study observed increased VEGF by using radioimmuno assay method for measuring VEGF. Many workers have demonstrated that biologically active free VEGF concentrations were decreased in women with preeclampsia and sFlt-1 (soluble fms-like tyrosine kinase-1) concentrations were elevated in women with PIH (27, 28, 29, 30, 31, and 32). In preeclampsia, one can find in maternal circulation an increased quantity of factors which hinder the action of angiogenic factors that negatively influence the development of the placenta. sFlt-1 is the main antiangiogenic factor & Flt-1 is an endothelial receptor for VEGF and PLGF. Splicing of Flt-1 results in sFlt-1 which is defective in transmembrane and intracellular signaling domain. sFlt-1 is secreted in circulation. Here it gets connected to the angiogenic factors, VEGF and PLGF, the former having higher affinity.

The variations of serum VEGF level finding were due to use of different methods and different commercial kits (33). The competitive type of immunoassay detects total amount of VEGF means both free VEGF and bound form with sflt-1 whereas Sandwich type estimates only free circulating VEGF. The value of serum level of VEGF also depends on the sample used serum or plasma. As platelet and leukocytes secrets VEGF, estimation of VEGF from serum showed higher values than those estimated from plasma (34,35). Some investigators suggested that the causes of endothelial dysfunction in PIH are not due to the increased serum level of total VEGF level, but due to the decreased level of free VEGF. Sharon E. Maynard et al demonstrated that increased circulating sFlt1 in patients with preeclampsia is associated with decreased circulating levels of free VEGF and PLGF, resulting in endothelial dysfunction in vitro that can be rescued by exogenous VEGF and PLGF (30).

In this present study serum VEGF was measured by sandwiched ELISA which measured the Free VEGF only and showed that the free VEGF level in preeclampsia mothers were significantly lower than control mothers, 25.19±3.09(20-30.78), 66.25±11.94 (40.11-102.32 ) (Significant (P=<0.0001) & significantly lowered severe preeclampsia mother (22.37±1.78 range 20-25.69pg/ml)than mild preeclampsia mothers (27.08±2.22 range 22.78-30.78 pg/ml)(p<0.0001)indicating that more lowering of the circulatory VEGF due to more neutralization of circulatory VEGF by sFlt-1 . As more amount of sFlt-1 is produced by increased hypoxic placenta, hypoxia is due to vasoconstriction. Normally mothers were normotensive before pregnancy and the control mothers having normal blood pressure throughout gestation where as PIH mothers had normal blood pressure till $20^{th}$ week of gestation. These findings proved that Placenta is responsible for rising blood pressure in PIH which is further justified by the fact that within seven days after delivery the circulatory free serum VEGF of both control and PIH mothers and mild PIH mothers & Severe PIH mothers have no significant variations.

In the present study the mean serum VEGF level after delivery in control, PIH, Mild PIH, Severe PIH respectively as follows 75±12.99, 74.47±5.20, 74.58±5.21, 74.32±5.32 pg/ml respectively. The level of serum VEGF varies according to duration of gestation and gradually decreases with the advancement of gestation that remarkably reduce in PIH mothers than control mothers .The value of serum Free VEGF just before delivery significantly reduces in severe PIH (16.40±1.25 range 15-18.25pg/ml) than mild PIH (18.13±2.15 range 15.08-22.98pg/ml)(p=<0.0001)in comparison to control

value (57.67±8.16 ,range 39.09-77.98 pg/ml) . It is well documented that less VEGF expressed in the cytotrophoblast and due to less expression of VEGF in preeclampsia the invasion of trophoblastic cells are incomplete and placenta suffered from hypoxia leading to liberations of more sflt1 which reduces the circulating free VEGF. Low VEGF level causes failure of vasodilatation and results vasoconstriction leading to further hypoxia and endothelial dysfunction which are responsible for clinical features of PIH.

A positive correlation was observed between the serum concentrations of total VEGF (bound + free) and sFlt-1 with systolic and diastolic blood pressure in preeclampsia and a negative correlation between the serum concentrations of free VEGF and systolic &diastolic blood pressure in PIH. Strong negative Correlation between free VEGF and sFlt-1 concentrations was observed in PIH.

During embryonic development VEGF expression is first detected within the giant cells of the trophoblast (36, 37) and its altered expression of modulators of angiogenesis causes hypertension, proteinuria, endothelial cell activation and increased platelet aggregation (38) and disappears with resolution of the disease (39). Apart from regulation of blood pressure VEGF also maintains the integrity of the glomerular filtration barrier (40) and has been implicated in glomerular healing. Administration of Anti-VEGF compounds has been found to increase apoptosis, impair glomerular capillary repair, and increase proteinuria in a rat model produce mesangio proliferative nephritis (41). Exogenous VEGF was found to accelerate renal recovery in rat models of glomerulo-nephritis and experimental thrombotic micro angiopathy (42,43) More recently it is found that exogenous VEGF shown to ameliorate post-cyclosporine mediated hypertension, endothelial dysfunction, and nephropathy (44).Finally, in recent antiangiogenic clinical trials, VEGF signaling inhibitors have resulted in hypertension and proteinuria (45).

Collectively, these data suggest that VEGF is important not only in blood pressure regulation but also in maintaining the integrity of the glomerular filtration barrier. Excess circulating sFlt1 secreted by the placenta in preeclampsia leads to endothelial dysfunction, hypertension, and proteinuria by antagonizing circulating VEGF and PLGF. There is strong evidence to suggest that the capillary endothelium of the kidney is extremely sensitive to VEGF which is produced locally by the visceral epithelium (podocytes) and may explain why renal dysfunction is an important and early marker of the disease. VEGF is necessary to maintain the normal fenestration of glomerular endothelial cells. In non-pregnant mice a 50% reduction of VEGF leads to glomerular endotheliosis and proteinuria similar to what is seen in preeclampsia In addition, it has been reported that patient receiving VEGF antagonists for cancer treatment may develop hypertension, proteinuria and endothelial activation .In PIH it is likely that VEGF signaling is decreased in the kidney due to excess sFlt-1 leading to the glomerular changes of endotheliosis and proteinuria.

The placenta is thought to be the main source of the sFlt-1.Approximately 20-fold increase in circulating sFlt-1 levels by the third trimester of normal pregnancy, compared with non pregnant values. The concentration of sVEGFR-1 (sFlt-1) in normotensive pregnant women decreased from 8-12 weeks to16-20 weeks, gradually increased at 26-30 weeks and rapidly increased at 35-40 weeks of gestation (29).Indeed, sFlt-1 mRNA is strongly expressed in the placenta, and maternal serum concentrations of sFlt-1 decline rapidly after delivery. The role of VEGF in PIH has received substantial attention. However, in pregnancy, circulating sFlt1 is present at very high levels as compared with the non pregnant state; in which sFlt1 levels are relatively low (the mean sFlt1 level in healthy female volunteers was 0.15 ± 0.04 ng/ml (46). In the present study it was also noted that the serum VEGF is negatively correlated with systolic and diastolic blood pressure and varied with the severity of the PIH. The bound form of VEGF is biologically inactive. Only the free from of VEGF is biologically active. The total VEGF includes

both free and bound form and does not indicate the total activity of VEGF. This study also explains the pathogenicity of development of clinical features in PIH in relation to active serum VEGF.

## CONCLUSION

Vascular endothelial growth factor (VEGF) is essential for human implantation and placentation, and is critical for trophoblastic invasion into spiral arteries, a key process for successful development and one of the important regulators in the human placenta. The VEGF played a crucial role in the pathogenesis of PIH. All the biochemical changes took place in PIH have directly or indirectly related to VEGF. Due to Low availability of biologically active circulatory free VEGF in PIH there will be vasoconstriction and defective pseudovasculogenesis and generalized endothelial dysfunction& inflammation.

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