A Dissertation on

"Study on Soluble fms like Tyrosine kinase-1/Placental Growth factor ratio(sflt-1/PlGF) as a predictor of Preeclampsia"

Submitted to

**THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600032**

In partial fulfillment of the Regulations
for the Award of the Degree of

M.D. BRANCH - I

GENERAL MEDICINE

**DEPARTMENT OF GENERAL MEDICINE
STANLEY MEDICAL COLLEGE
CHENNAI – 600 001
APRIL 2016**

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CERTIFICATE BY INSTITUTION

This is to certify that **DR. MONICA.K** Post - Graduate Student (JULY 2013 TO APRIL 2016) in the Department of General Medicine, STANLEY MEDICAL COLLEGE, Chennai- 600 001, has done this dissertation on **"STUDY ON SOLUBLE FMS LIKE TYROSINE KINASE-1/ PLACENTAL GROWTH FACTOR RATIO (sFlt-1/PlGF) AS A PREDICTOR OF PREECLAMPSIA"** under my guidance and supervision in partial fulfillment of the regulations laid down by the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for M.D. (General Medicine), Degree Examination to be held in April 2016.

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CERTIFICATE BY GUIDE:

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Dr. EDWIN FERNANDO M.D,D.M,FRCP   Dr.R.JAYANTHI M.D

Prof & HOD,Dept of Nephrology   Prof & HOD,Dept of Gen.Medicine

Stanley Medical College   Stanley Medical College

DECLARATION

I **DR. MONICA.K** declare that I carried out this work on **"STUDY ON SOLUBLE FMS LIKE TYROSINE KINASE-1 / PLACENTAL GROWTH FACTOR RATIO (sFlt-1/PIGF) AS A PREDICTOR OF PREECLAMPSIA"** at the obstetrics OPD and wards of Government Stanley Hospital during the period December 2014 to September 2015. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, or diploma to any other university, board either in India or abroad.

This is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the M. D. Degree examination in General Medicine.

Dr.MONICA.K

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I sincerely thank the members of Institutional Ethical Committee, Stanley Medical College for approving my dissertation topic.

Last but not the least, I sincerely thank all those **patients** who participated in this study, for their co-operation.

STUDY ON SOLUBLE FMS LIKE TYROSINE KINASE(sFlt-1) / PLACENTAL GROWTH FACTOR(PIGF) RATIO AS A PREDICTOR OF PREECLAMPSIA

TABLE OF CONTENTS

ABBREVIATIONS:

BP	Blood Pressure
VEGF	Vascular Endothelial Growth Factor
PIGF	Placental Growth Factor
TGF	Transforming Growth Factor
sFlt-1	Soluble Fms Like Tyrosine Kinase
sEng	Soluble Endoglin
IUGR	Intrauterine Growth Retardation
HELLP	Haemolysis elevated liver enzymes low platelet count
PAPP-A	Pregnancy Associated Plasma Protein -A
PP-13	Placental Preotein - 13
ACEI	Angiotensin Converting Enzyme Inhibitor
ARB	Angiotensin Receptor Blocker
ALK	Activin like kinase

INTRODUCTION:

Significant alterations occur in systemic hemodynamics in normal pregnancy. Plasma volume expands leading to hemodilution.Cardiac output increases by 40-50% and both systolic and diastolic blood pressure decreases especially in second trimester due to decrease in systemic vascular resistance and also unresponsiveness to vasoconstrictor agents. There are also significant changes in renal hemodynamics with increase in GFR and consequent decrease in the serum creatinine.

Preeclampsia is a common disease affecting pregnant patients. Clinically characterised by new onset of hypertension and proteinuria after twenty weeks of pregnancy. It has the potential to lead to unforseen circumstances in the mother and fetus.

Delivery of fetus and placenta is the only effective treatment for preeclampsia which results in premature induction or surgery hence increased risk of preterm birth .Therefore there is a need for identification of highly sensitive and specific diagnostic tests/markers that would help in early detection of patients who are bound to develop preeclampsia even before patient clinically presents with hypertension and proteinuria and hence help in reducing maternal and neonatal morbidity and mortality by improving prenatal care. Further these markers could

aid in differentiating diseases which also would cause hypertension and proteinuria due to other conditions that are misdiagnosed as preeclampsia thereby altering the treatment protocol.

REVIEW OF LITERATURE:

Hypertension is the most prevalent medical disorder occurring in pregnancy. The following classification of hypertension has been described in pregnancy.

1. Chronic Hypertension: Increased BP before 20th week of gestation or hypertension persistent for more than 12 weeks after delivery.

2. Preeclampsia   : Blood pressure more than 140/90 after 20 weeks of gestation and new onset proteinuria (>300 mg protein)

3. Preeclampsia super   : New onset proteinuria in a women with chronic -imposed on existing HT : hypertension.

4. Gestational HT   : Increased BP>140/90 during pregnancy with no proteinuria.

5.Eclampsia   : Seizures in a women with preeclampsia.

PREECLAMPSIA:

Preeclampsia occurs in nearly 5 percent of pregnancies and is a most important cause of maternal and neonatal morbidity and also mortality. Preeclampsia is defined by

(1) Blood pressure of more than 140/90 mmHg occuring after 20 weeks of gestation in a women with previously normal blood pressure

(2)newly developed proteinuria (>300 mg of protein in a 24 hours or a random/spot urine protein/creatinine ratio of >0.3).

Preeclampsia can occur early in pregnancy at <34 weeks of gestation termed "early onset preeclampsia" and if it occurs >34 weeks of gestation termed "late onset preeclampsia"

The pathophysiology of preeclampsia still remains unknown. It is a systemic vascular disorder that affects other organs such as liver, kidney, brain etc. Endothelium has been proposed as the main target. Utero-placental ischemia has been proposed as the main pathophysiological process.[1]The hypertension in pre-eclampsia is associated with vasoconstriction and decreased arterial integrity. In pre-eclampsia the pathognomic glomerular endotheliosis occurs which is the hallmark of the disease process and proteinuria occurs as a result of it. Recently podocyturia has been proposed as possible pathogenic mechanism.There are varied risk factors associated with pre-eclampsia. These include

- primigravida,

- multiple pregnancy,

- gestational diabetes,

- very young and elderly pregnancy,

- obesity,

- pre-eclampsia in previous pregnancy,

- family history

- Chronic kidney disease

- Autoimmune disease

- Gestational trophoblastic disease

- Ethnicity

- Prolonged interval between pregnancies

- Genetic factors and

- other environmental factors. Etc..

Some changes may occur in the systemic circulation of pregnant women who are bound to develop preeclampsia which may occur long before the disease manifests clinically with hypertension and proteinuria. Ambulant blood pressure recordings

showed that the usual dip in blood pressure which occurs at night is abolished in these patients who ultimately develop preeclampsia.[2] These changes usually manifest during second trimester at about 18 - 26 weeks of pregnancy. There is also alteration in resistance to vasopressor substances which also occurs even before the patient clinically presents with hypertension and proteinuria.

Clinically the preeclamptic patients present with increased BP, pedal edema, blurring of vision etc.

Pre-eclampsia is complicated by

- Eclampsia

- HELLP syndrome

- Abruption

- Renal failure

- Hepatic failure

- Pulmonary edema

- Cardiovascular disease

- Recurrent preeclampsia

Neonatal complications include

- Preterm birth

- Intra-uterine growth retardation

- Low birth weight

- Increased neonatal mortality

SEVERE PREECLAMPSIA:

Criteria for diagnosing severe pre-eclampsia include

A) sustained systolic blood pressure more than 160mmHg

B) Sustained diastolic blood pressure more than 110mmHg

C) Pulmonary edema

D) Oliguria less than 500ml in 2 hours

E) Persistent headaches/scotomas

F) Thrombocytopenia

G) Pain in epigastrium or right upper part of abdomen

H) Intrauterine growth retardation

The only management of severe pre-eclampsia that would be most effective is the delivery of fetus and the placenta which could lead to premature induction of labour and hence increased neonatal morbidity and mortality. Hence it is highly

prudent to identify more sensitive and specific biomarkers that would aid in identifying patients who are bound to develop pre-eclampsia thereby improving prenatal care of high risk candidates hence decreased unforseen circumstances.

PREECLAMPTIC PLACENTA:

The development of normal placenta occurs by the following three processes

- Angiogenesis

- Vasculogenesis &

- Pseudovasculogenesis

These processes are governed by proangiogenic and anti angiogenic factors. Abnormality in this regulation could lead to preeclampsia.

During normal placental development the maternal spiral arterioles are invaded by the cytotrophoblasts which transforms the arterioles from high resistance vessels to low resistance conduit vessels. This process usually starts by end of first trimester and ends by twentieth week of gestation. During the process of invasion of vasculature, cytotrophoblasts gets transformed from the epithelial to endothelial phenotype , this process is called as pseudovasculogenesis. A direct contact with maternal circulation is established during this process. The process involves a ennumerable mediators like cytokines, growth factors etc.

In pre-eclampsia, the cytotrophoblasts switching does not occur that is they fail to transform from epithelial cell type to endothelial cell type. Therefore the spiral arterioles continue to remain as high resistance vessels which results in impaired utero-placental circulation subsequently resulting in placental ischemia.
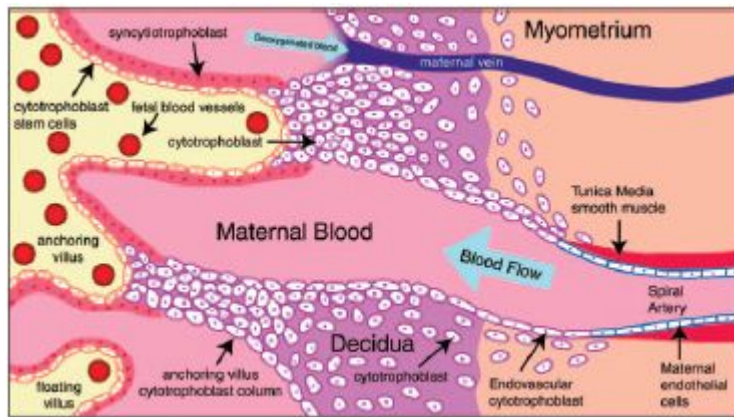
I) Cytotrophobalsts invasion of spiral arteries and process of pseudovasculogenesis

NORMAL
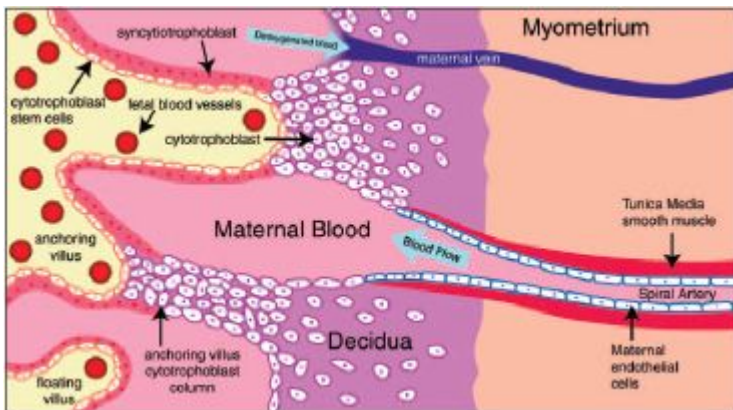
II) Failure of invasion of spiral arteries by cytotrophoblasts

PREECLAMPSIA

The process of pseudovasculogenesis is nothing but transformation of cytotrophobasts from epithelial to endothelial cell type which decreases the resistance in blood vessels hence resulting in increased blood supply to placenta.[2] The adequately perfused placenta which could supply the essential nutrients and also oxygen to the fetus is able to support and sustain the fetus. But in pre-eclampsia there is failure of this process of pseudovasculogenesis which impairs uteroplacental circulation and the placenta remains hypoxic which increases tissue oxidative stress and leads to placental cell death which in turn results in endothelial dysfunction and also leads to abnormal inflammatory response.

Recently, the pathway of preeclampsia that is receiving most attention is the imbalance between proangiogenic and antiangiogenic factors. The most important proangiogenic factors includes Vascular Endothelial Growth factor and Placental Growth Factor and the most important antiangiogenic factors include Soluble Fms like tyrosine kinase and soluble endoglin. Vascular endothelial growth factor plays an important role in stabilizing the endothelial cells in blood vessels. It also maintains and regulates the endothelium in liver,kidney and brain.Placental ischemia plays an important role in preeclampsia.There occurs changes in Hypoxia inducible factors.

Angiogenic proteins like like Flt-1 and VEGF-2 have an important role in regulating these hypoxia inducible factors.Infact placental hypoxia itself leads to

increased production of sFlt-1. Alteration in these pathway could lead to the

failed of cytotrophoblast invading the spiral arterioles - most important factor

leading to the development of preeclampsia.

Genetic factors   Immunological factors   Others

Failed maternal remodelling >   Defective placental implantation

Impaired trophoblastic invasion   > Placental underperfusion Of

                                                        spiral arterioles

Placental Hypoxia

Pro-angiogenic factors   Imbalance   Anti-angiogenic factors   PP-13

↓ VEGF & PlGF   sFlt-1, sEng, NGAL   PAPP-A

Maternal endothelial dysfunction

Clinical symptoms of preeclampsia

Hypertension   Proteinuria   Liver dysfunction   Cerebral edema

CURRENT TOOLS IN MANAGEMENT OF PRE-ECLAMPSIA:

The hallmark features of preeclampsia mainly include

CLINICAL:

- Systolic BP>140mmHg and Diastolic BP>90mmHg occurring after twenty weeks of pregnancy in a women with previously normal blood pressure.

LABORATORY:

- Proteinuria >0.3gm in 24 hours

- Serum uric acid gets elevated in patients with pre-eclampsia

- Provocative tests: Include roll over test, Angiotensin II sensitivity test, isometric exercise test but these tests have disadvantages of being extensive, invasive and time consuming.

- Uterine artery doppler which estimates the rate of flow of blood in the uterine artery has been established as useful tool in prediction of pre-eclampsia and has been used as a useful screening tool between 20-24 weeks. Persisting early diastolic notch even after twenty four weeks of pregnancy or an inappropriate ratio of flow velocity has been associated with impairment of spiral arteries invasion by the cytotrophoblasts.This doppler ultrasound has a good negative predictive value.Also in second half of pregnancy there exists a relation

between between high resistance waveforms in the uterine arteries and preeclampsia.

- Kidney function : the kidneys are the most important organs that are affected in preeclampsia. GFR declines by about 30-40% in preeclampsia although this is not reflected properly by increase in serum creatinine which can be normal.

PROPOSED TOOLS FOR PREDICTING PRE-ECLAMPSIA:

An ideal diagnostic marker of preeclampsia is one that would accurately predict the development of preeclampsia during first few months of pregnancy as it provides a greater chance for the effective management of preeclampsia that may help in better care and survival . Recently, imbalance of proangiogenic and antiangiogenic factors has been postulated as the possible pathogenesis of pre-eclampsia and is arising as a pledging tool as predictor of development of pre-eclampsia.

ANGIOGENIC AND ANTI-ANGIOGENIC FACTORS:

Since the the proangiogenic factors have been proposed to maintain the maternal vascular endothelium, the placental release of antiangiogenic factors into the circulation has been postulated as cause of the endothelial impairment seen in

preeclampsia. The proangiogenic factors namely vascular endothelial growth factor and Placental growth factor - govern placental vascular development. TGF-beta is another angiogenic protein involved in placental vasculogenesis.

The antiangiogenic factor namely soluble Fms like tyrosine kinase which is the soluble form of VEGF receptor produced by alternate splicing. It is an effective inhibitor of VEGF and PIGF which inhibits vasodilatory effect of these factors.

Soluble endoglin is another potent anti-angiogenic protein that has been involved in pathogenesis of pre-eclampsia. Soluble endoglin may combine with sflt-1 to induce the various pathological processes in pre-eclampsia. Soluble endoglin is a coreceptor for TGF-Beta which reduces its binding to receptor thereby inhibiting the vasodilatory effect of TGF-beta.

Recent studies have shown that plasma levels of VEGF and PIGF decrease and plasma levels of sflt-1 and soluble endoglin levels rise have been reported in patients with pre-eclampsia.[7] These biomarkers rise in blood even before clinical hypertension and proteinuria manifests. Hence can be used as a valuable tool in predicting pre-eclampsia in early months of pregnancy and therefore provide a chance for better prenatal care for those patients who are at risk and improved perinatal and neonatal morbidity and mortality.These tests are more sensitive and specific when tested in second trimester.

These factors should be done in patients who satisfy following criteria

- Patients with signs and symptoms of preeclampsia

- Asymptomatic women who are at risk of preeclampsia.
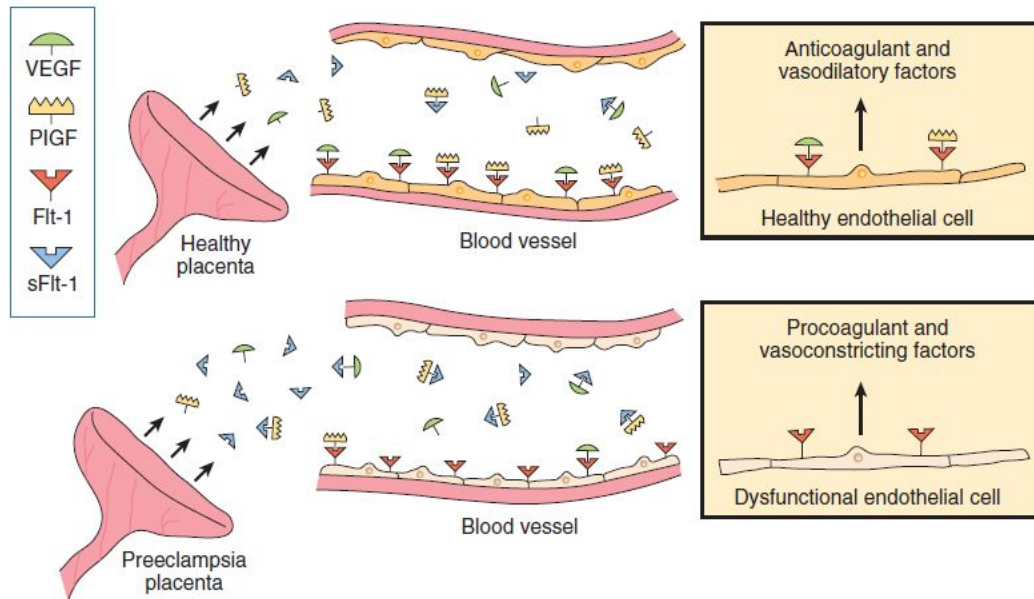
INSIGHTS FROM RISK FACTORS OF PREECLAMPSIA:

Altered expression of angiogenic factors has been seen with risk factors of preeclampsia. High sFlt-1 levels are seen in patients in first versus second pregnancies, in twin versus singleton pregnancy, hydatiform mole and fetus with trisomies. Conversely sFlt-1 levels were decreased in smoking pregnant women thus explaining the protective effect of smoking in preeclampsia. The risk factors

for preeclampsia which include chronic hypertension, obesity, diabetes mellitus, and chronic kidney disease are also characterised by maternal endothelial impairment which might increase the susceptibility of maternal vasculature to underlying effects of the anti-angiogenic factors.

The following lists the important biomarkers in diagnosis of preeclampsia.

1. Vascular Endothelial Growth Factor

2. Placental Growth Factor

3. Soluble Endoglin

4. Soluble Fms like tyrosine kinase-1

5. Pregnancy associated plasma protein- A

6. Placental protein -13

7. Transforming Growth Factor -Beta

8. P-selectin

9. Neutrophil Gelatinase associated Lipocalin (NGAL)

10. Adrenomedullin

11. Activin-A and Inhibin-A

12. Pentraxin

13. Fibronectin

14. Cell Free DNA

15. mRNA

The salient features of each biomarker would be discussed.

VASCULAR ENDOTHELIAL GROWTH FACTOR(VEGF) AND PLACENTAL GROWTH FACTOR(PIGF):

Among the angiogenic factors expressed by the placenta, Vascular Endothelial Growth Factor (VEGF) plays an important role. It is a dimeric glycoprotein involved in vasculogenesis.

The family of VEGF includes VEGF-A,B,C,D and PIGF. VEGF plays an important role in maintaining endothelial integrity. It promotes migration, switching of the endothelial cells and it also maintains permeability of vasculature. Studies have reported that decreased serum levels of VEGF of pre-eclamptic women which takes part in the development of endothelial impairment characteristic of this disease.

The receptors for the VEGF family residing on the vascular endothelial cells mainly include Flt-1 and KDR. VEGF can act on both Flt-1 and KDR receptors,

PIGF acts only on Flt-1. The main action of VEGF on the endothelial cells is primarily mediated by KDR.PIGF plays an important role in angiogenesis. PIGF misplaces VEGF from the Flt-1 receptor, hence the VEGF is able to act on largely thus active KDR receptor.PIGF starts to increase from the second trimester, peak levels are achieved from 29 to 32 weeks and then declines. PIGF levels decrease 9-11 week before the onset of pre-eclampsia, with significant decline during 5 weeks before onset of preeclampsia.

VEGF Signaling

VEGF-A, PIGF   VEGF-A

sFlt1

VEGFR1 (Flt1)   VEGFR2 (KDR/Flk1)

Angiogenesis, Vascular Homeostasis
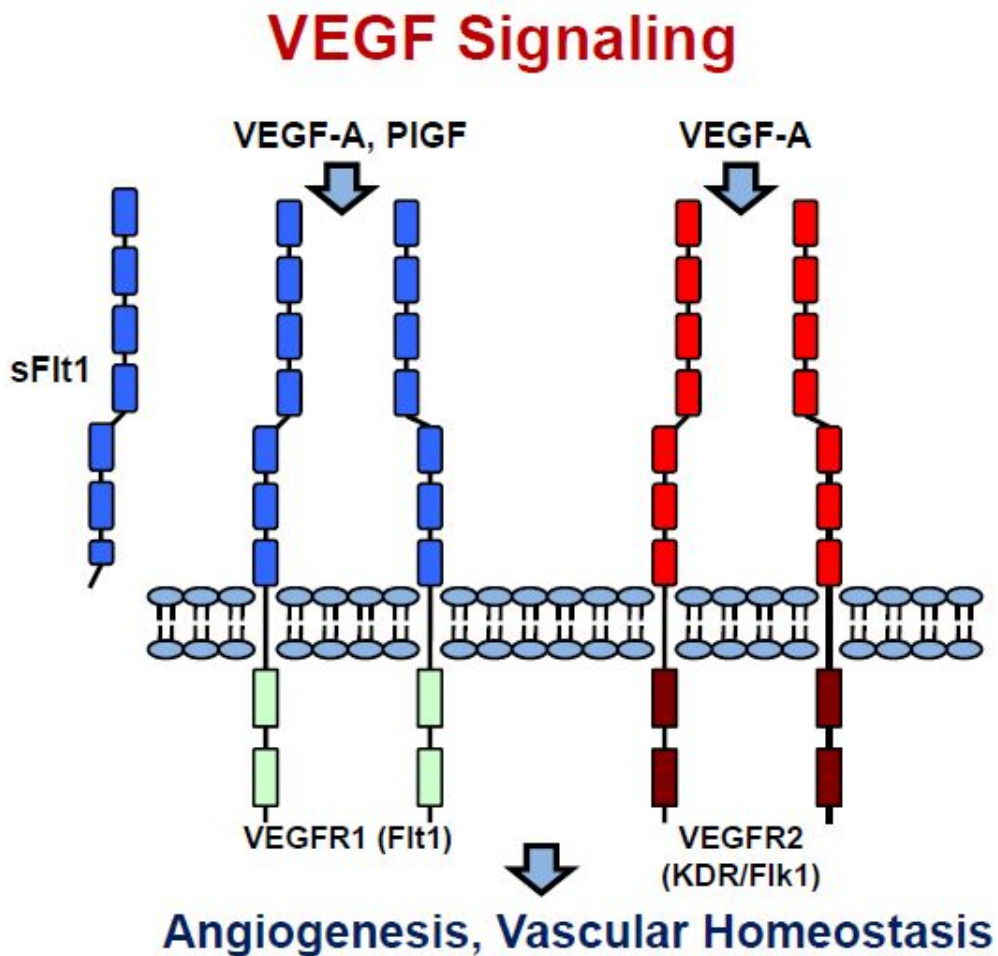
TRANSFORMING GROWTH FACTOR - Beta: (TGF-Beta):

Transforming growth factor group of proteins are known to be involved in the process of angiogenesis. It binds to two receptors namely Type I and Type II receptor.Most of the cells, also the endothelial cells, express TGF-Beta receptor ALK5, but endothelial cells alone express ALK1 receptor.As with TGF-Beta, the ALK1 plays an important role in of the new blood vessel formation.Proliferation and trans-migration of endothelial cells may occur due to ALK1 at small doses of TGF-Beta whereas ALK1 may inhibit these processes at relatively higher doses.

Coreceptors are also involved in signaling of TGF-Beta in the vascular endothelium. These coreceptors also modulate the action of TGF-Beta. TGF-Beta coreceptor which may include Endoglin which is present in the endothelial cells and syncytiotrophoblasts . TGF-Beta apart from its action on the process of angiogenesis in earlier phase it also maintains the vascular homeostasis.

SOLUBLE FMS-LIKE TYROSINE KINASE (sFlt-1):

sFlt-1 is nothing but soluble form of VEGF type I receptor . The alternate splicing of Flt-1 receptor which is the VEGF and PIGF endothelial cell receptor results in this product namely sFlt-1.It results from the alternative splicing of sFlt-1. There is free circulation of the secretory form of this receptor in the serum

where it acts and nullifies the action of VEGF and PIGF.[4] The mechanism of action of this sFlt-1 is by sticking on to the receptor binding areas of PIGF and VEGF thus inhibiting the action of VEGF and PIGF on the endothelial receptors which are present on their cell surface hence resulting in endothelial impairment.Serum levels of sFlt-1 rise in women with pre-eclampsia. The levels rise 5 weeks before the onset of hypertension and proteinuria. It has been proven that sFlt-1/PIGF ratio is a better predictor than either parameters used alone. sFlt-1 concentration rises slowly during the course of pregnancy in woman who have pre-eclampsia and the levels are very high between twenty five and twenty eight week of gestation.The upregulation of sFlt-1 is probably due to hypoxic environment. High levels of sFlt-1 leads to endothelial dysfunction and thus mediate pre-eclampsia development.It has been reported that exogenous sFlt-1 inhibits in vitro process of placental cytotrophoblast invasion. Hence antagonizing sFlt-1 ameliorate symptoms. It has been documented nicotine is proangiogenic which mediates its action by induction of VEGF.

Cigarette smoking is associated with low incidents of pre-eclampsia. Smoking reduces sFlt-1 levels in human beings. Therefore use of nicotine in pre-eclampsia would be an effective treatment. Thus excessive production of sFlt-1 by the placenta contributes to hyper tension,endotheliosis and proteinuria in pre-eclampsia patients . A better understanding of regulation, alternate splicing and the action of sFlt-1 in the placental and vascular functions would lead to better

idea about pathogenesis, prevention & treatment of pre-eclampsia.

There is not much knowledge about the transcription regulation and also the alternate splicing of Flt-1.Alternate splicing had been proposed as the most important rate limiting governing step in the formation of sFlt-1. However it appeared that both the Flt-1 and sFlt-1 were proportional increased in preeclampsia. Future works research into the governing step of sFlt-1 production may clear our doubts regarding the mechanisms.

SOLUBLE ENDOGLIN (sEng):

It is a transmembrane glycoprotein which is present on the membrane of syncitiotrophoblasts and it is also present on the endothelial cells. Soluble endoglin functions as co-receptor for TGF Beta1,TGF Beta3 and also modulates TGF Beta signaling in angiogenesis regulating vascular tone. It is basically truncated form of endoglin. It is a very potent anti-angiogenic protein and its main mechanism of action is by inhibiting binding of TGF Beta 1 to receptor and thus interfering with nitric oxide (NO) formation, it also inhibits the process of vasodilation and formation of capillaries by the endothelium. Recently association of increased soluble endoglin levels and more severe manifestations of pre-eclampsia confirmed. The serum levels of soluble endoglin remain the same throughout the course of pregnancy while the concentration rises during second trimester in

woman who are bound to develop pre-eclampsia.The levels begin to increase about 9 to 11 weeks before the onset of pre-eclampsia and woman with higher soluble endoglin levels are also at an increased risk of small-for-gestational age(SGA) infants.

Normal

Preeclampsia

Preeclampsia

Extracellular space

TGF-β1 — sENG

VEGF — sFLT1

Alk5   TβRII   ENG   FLT1

**Endothelial Dysfunction
Impaired Relaxation**

Cytosol
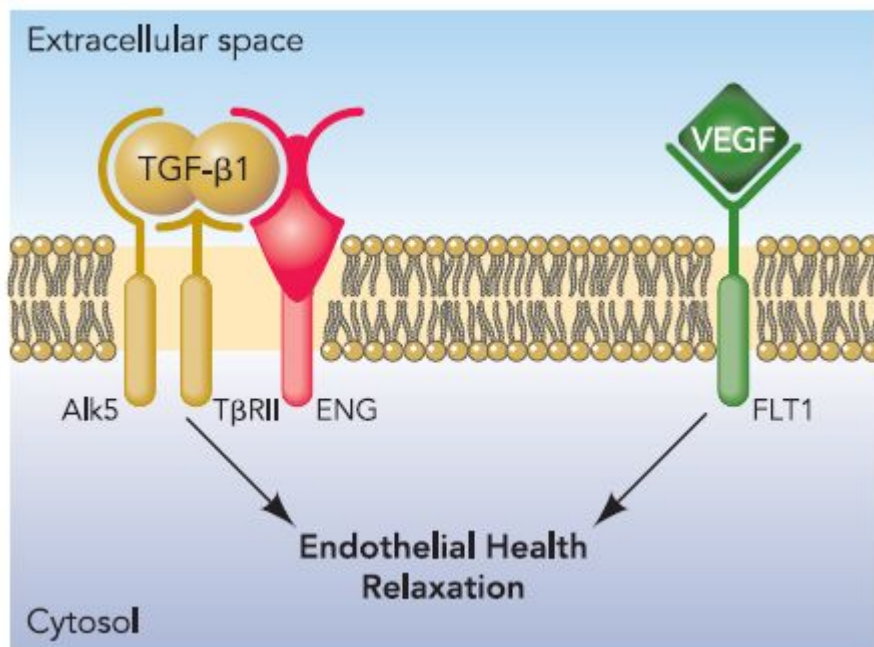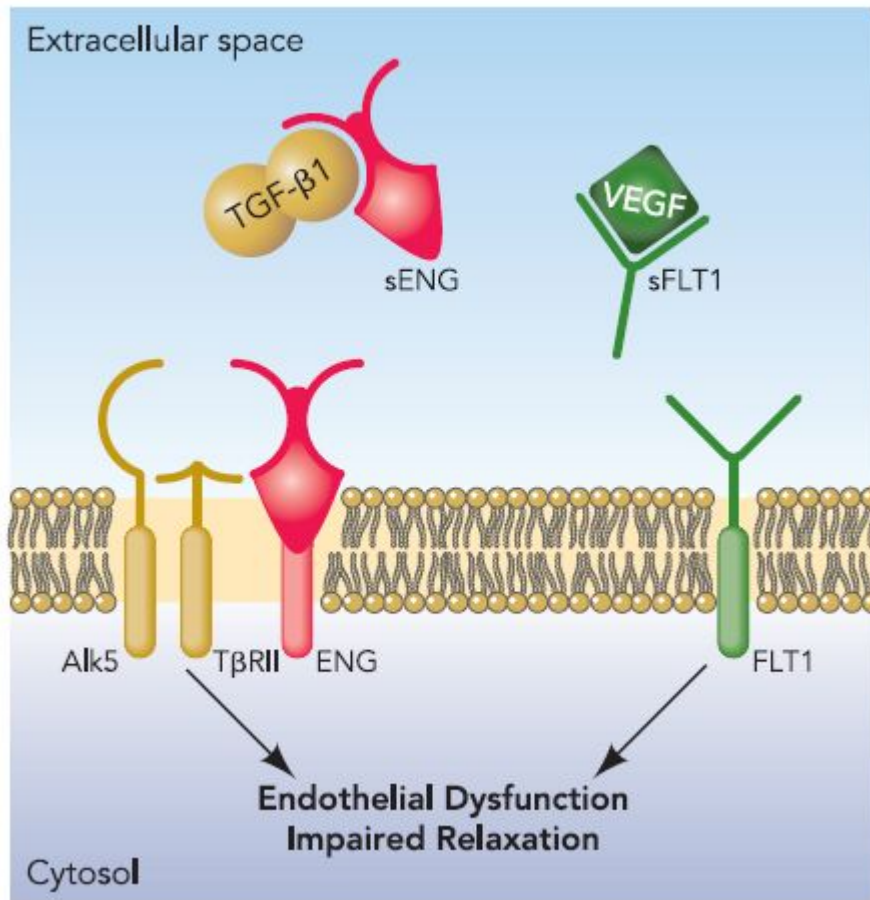
PLACENTAL PROTEIN - 13:

It belongs to galectin superfamily important for placental implantation and remodeling of spiral arteries. It is exclusively produced by placental tissue. The PP13 levels rise in normal pregnancy slowly. In women who are bound to develop preeclampsia the levels of this protein are extremely low. Also, the levels were

found to be high in other problems like IUGR and pre-term birth. It was reported that both the PP13 levels combined with the Doppler of uterine artery were better predictors of preeclampsia especially even in the first trimester.

PREGNANCY ASSOCIATED PLASMA PROTEIN A: (PAPP-A)

It is a protease. The main site of production of this protein is from placental trophoblasts . It cleaves insulin like growth factor binding proteins (IGFBP-4). This IGFBP-4 is very important in governing fetal growth.It had been reported that in preeclampsia the levels of PAPP-A were highly reduced especially early onset pre-eclampsia whereas the levels did not change in case of late onset pre-eclampsia. Hence it can be inferred that the PAPP-A levels were not so useful as a predictor of late onset pre-eclampsia but still larger clinical and cross sectional trials are needed to confirm this prediction.

NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL):

NGAL also known as lipocalin-2 ,uterocalin, citrocalin belongs to family of lipocalins .Its levels are significantly raised in damaged epithelial cells, neoplastic conditions inflammatory disorders , cardio-vascular disease infections and renal diseases. NGAL is considered the best and earliest markers of acute kidney injury. It can be found in urine even wihin 2 hours of kidney injury.It had been reported

that serum levels of the NGAL is high by the last of second trimester in women who are bound to develop pre-eclampsia. A positive correlation of NGAL with blood pressure and and also its association with proteinuria makes it a good biomarker as an early predictor of pre-eclampsia. However large controlled trials are required to establish the accuracy of this biomarker for diagnostic purposes and management of pre-eclampsia.

ADRENOMEDULLIN:

The levels of adrenomedullin are increased in pregnancy in maternal, fetal circulation and also in amniotic fluid. It has a very effective long lasting blood pressure lowering effect in animal models. It has been reported that decreased circulating adrenomedullin concentrations are seen in pre-eclampsia.

ACTIVIN-A AND INHIBIN-A:

Activin-A increases the synthesis of Follicle stimulating hormone and it regulates the differentiation of the trophoblastic cells in the first trimester. Inhibin A downregulates Follicle stimulating hormone formation and secretion. Activin A is a very useful marker of prediction of preeclampsia especially when used at about 21-25weeks of gestation.Inhibin A has higher sensitivity than activin A as a predictor of early onset pre-eclapmsia and can be detected even at about 15-19

weeks of pregnancy.

P-SELECTIN:

It is basically a cell adhesion molecule and is present in high concentrations in platelet granules and it is also present in endothelial cells especially within the Weibel-Palade bodies. It is concerned with interactions between the endothelium and the leukocyte. It has a very high negative predictive value of nearly ninety nine percent as a predictor of preeclampsia. The levels of P-selectin in plasma is highly increased at about 10-14 weeks of pregnancy in women who are bound to develop preeclampsia.

PENTRAXIN-3:

Pentraxin-3 is an inflammatory substance involved in preeclampsia. It is released by cells like the endothelial cells, macrophages-monocytes in response to inflammatory stimuli. The levels are increased in women with preeclampsia which indicate endothelial impairment.The levels rise in pfrom eleventh to thirteenth week of gestation in women who are bound to develop preeclampsia.

FIBRONECTIN:

Levels of fibronectin are significantly higher in pre-eclamptic patients. Levels increase in first trimester of pregnancy. Fibronectin is involved in processes like cell adhesion, differentiation and also in trans-migration and growth of cells.

HEAT SHOCK PROTEINS:

The proteins may be cytoprotective. They prolong the lifetime and safeguards the arterial smooth muscle cells from cell death. Serum levels are increased in women who are bound to develop preeclampsia. It is used not only in the diagnosis but it has also been postulated that it plays a very significant role in pathogenesis of preeclampsia.

CELLFREE FETAL DNA:(cffDNA)

Cellfree fetal DNA rises in pregnancy from eleventh to thirteenth week of pregnancy in women who are bound to develop preeclampsia. The levels of cffDNA are increased when there is decreased oxygen supply within the intervillous space which inturn leads to increased oxidative damage and increases the rate of placental cell death.Hypoxia within intervillous space of placenta leads to oxidative stress and increased cell death. But the disadvantage is that this ccfDNA level is increased not only in preeclampsia but also in other placental disorders. Therefore it is not a very specific marker of preeclampsia.

URIC ACID:

Uric acid is produced in the liver as an end result of purine metabolism The uric acid levels usually fall by about twenty five to thirty five percent in normal pregnancy. This fall is mainly due to the effect of estrogen, increased blood volume and also increase in GFR. By the end of third trimester the levels usually rise to pre-pregnant levels. In preeclampsia, the uric acid begins to rise from 10th week of pregnancy and it continues to increase until about 48hours after delivery. The increase precedes decline in plasma volume. Uric acid is a protectant also has anti-oxidant properties but also it is proinflammatory and it attributes to endothelial impairment. A study has been conducted which has proven that levels of serum uric acid in women with preeclampsia was associated with increased severity of the disease. But still decreasing uric acid with probenecid did not have an influence on hypertension in preeclamptic patients. Similarly another study showed that allopurinol too had no effect on pregnancy in patients who developed preeclampsia.

mRNA:

Recently it has been proven that quantitative PCR assay for podocyte markers is a very fast method to diagnose preeclampsia. Very high levels of mRNA of

nephrin, podocin etc were seen in patients who develop preeclampsia.

AUTOANTIBODIES:

Angiotensin II antibodies that act on angiotensin receptor has been found to be elevated in preeclampic women. But levels are not specific. It is also elevated in antibody mediated renal transplant rejection.

PODOCYTURIA:

VEGF is produced in glomerulus mainly from podocytes.This VEGF produced from podocytes acts on the endothelial cells in a paracrine fashion and acts on the podocytes in autocrine fashion.Recently it has been proved that podocyturia serves as an early marker for prediction of preeclampsia even before the patient clinically presents with hypertension and proteinuria.[13] It has a high sensitivity and specificity. A strong correlation is also established between amount of podocytes in urine and blood pressure but there was no association with proteinuria. The urinary molecules that are most useful for estimating disease activity of disorders of glomerulus are PDX and CD 68 positive cells.

The other suggested biomarkers for prediction of preeclampsia include

- ADAM-12

- Beta-HCG

- 2-Methoxyestradiol

The time frame when these biomarkers get elevated in pregnancy is as follows
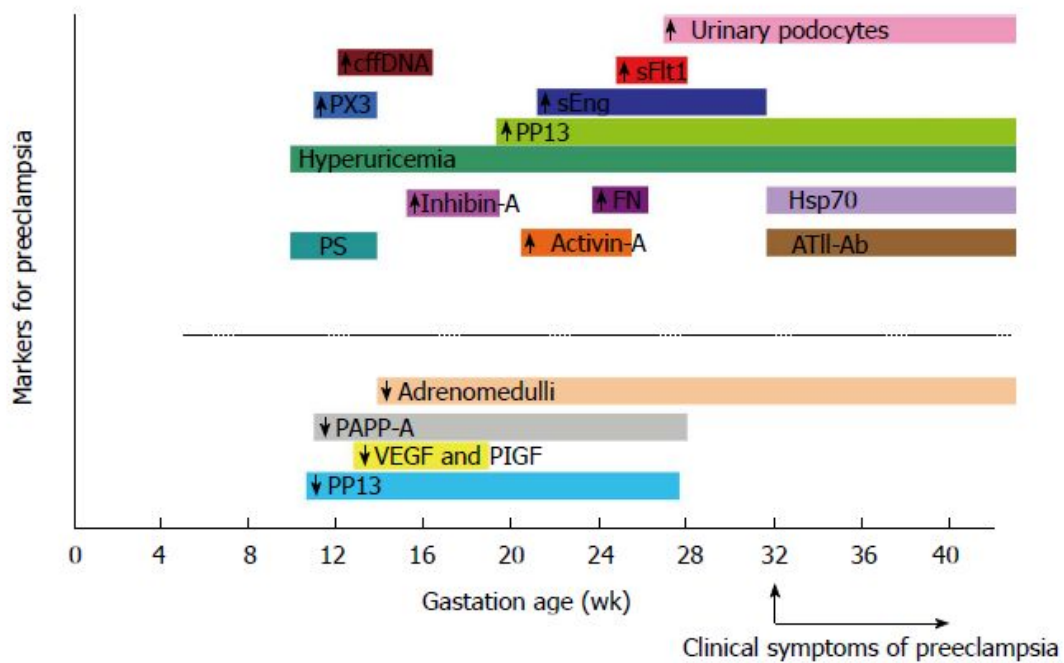
PATHWAYS OF ANGIOGENIC FACTORS DYSREGULATION IN PREECLAMPSIA:

The factors that regulate sFlt-1 and soluble endoglin regulation is being described. Heme oxygenase (HO-1) which is an anti-inflammatory with anti-oxidant property seems to attenuate VEGF induced and proteinase activated

receptor induced sFlt-1 expression. Decreased activity of this enzyme Heme oxygenase has been reported in women with preeclampsia. Therefore it was suggested that downregulation of Heme oxygenase with statins might be helpful. But unfortunately statins are contraindicated in pregnancy.

CLINICAL TRIALS:

Several studies and clinical trials have been done evaluating the reliability of the angiogenic factors in the diagnosis and treatment of preeclampsia.

One such study involving injection of recombinant sFlt-1 into pregnant rats. Control group included the rats that were not injected with rsFlt-1. The pregnant rats injected with rsFlt-1 developed hypertension and proteinuria whereas the control rats did not.

The kidneys of both the control and recombinant sFlt-1 injected rats were biopsied.

Renal pathological changes:

The renal lesion observed in pregnant rats treated with sFlt-1 include

- Glomerular enlargement with occlusion of capillary lumen and glomerular endotheliosis.

- Podocytes showed focal effacement of foot processes.

- Immunofluorescence study of rat kidneys showed deposits of fibrin in the glomerulus.

These findings corresponded to the renal pathological changes observed in preeclampsia in humans whereas the control group did not show any renal pathological changes.

And further it was observed that low dose of injection of sFlt-1 produces a milder phenotype.

Histopathological analysis of sflt-1 treated rats and control group:

B

A
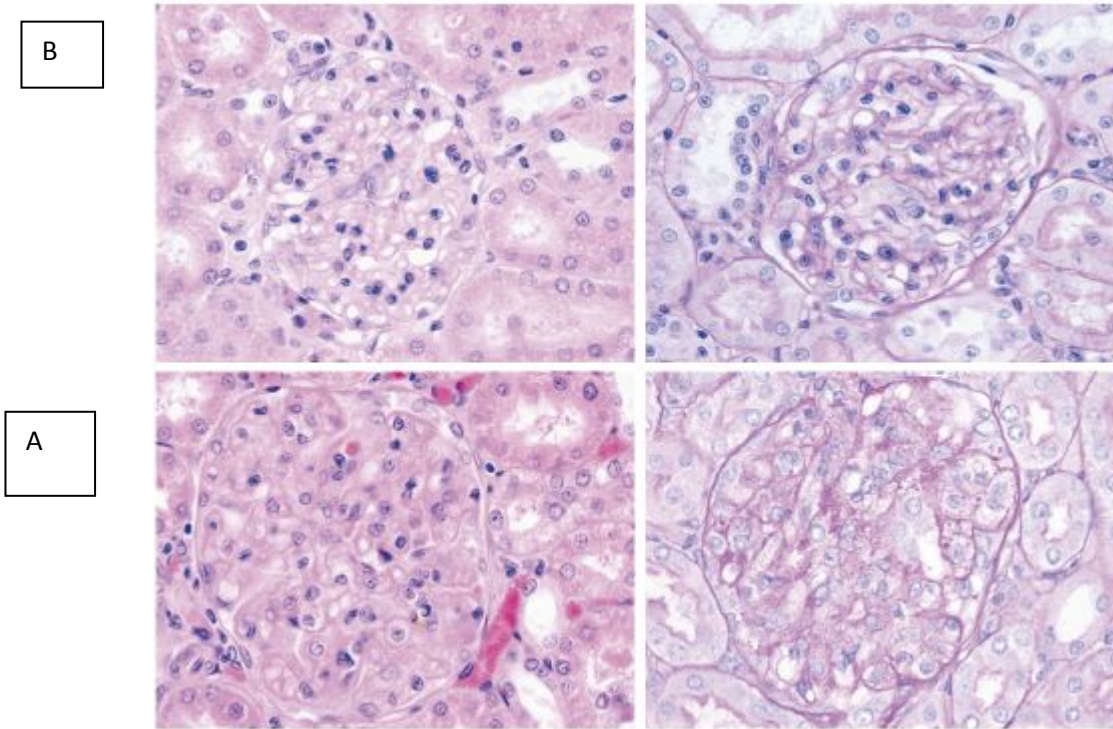
A) **Capillary occlusion with enlarged glomeruli and enlarged endothelial cells in sflt-1 treated rats.**

B) **These pathological changes are absent in control rats**

A study conducted by Rana et al correlated the clinical profile and also outcomes in preeclamptic patients with normal angiogenic profile (non angiogenic preeclampsia) and preeclamptic women with abnormal angiogenic profile (angiogenic preeclampsia). They reported that non angiogenic preeclamptic women were obese, with preexisting diabetes and they all presented during later weeks of gestation on comparing patients with angiogenic preeclampsia. And the women with non angiogenic preeclampsia had better outcomes on comparing patients with angiogenic preeclampsia. Further they had better utilisation of resources. This suggests that these angiogenic biomarkers could be used in identifying severe disease and hence guiding toward better prenatal care and management.

The PROGNOSIS trial which is nothing but Prediction of outcome in suspected women with preeclampsia which studied the usefulness of sFlt-1/PlGF ratio in predicting the outcome in pregnant patients.

USEFULNESS OF THE ANGIOGENIC BIOMARKERS AS AN AID IN DIFFERENTIAL DIAGNOSIS:

Chronic kidney disease and preeclampsia both could present with hypertension and proteinuria. Hence these angiogenic biomarkers were studied in both the population. It was concluded that the sFlt-1/PlGF ratio was elevated in patients

with preeclampsia whereas it was normal in the chronic kidney disease population.

Similarly lupus nephritis can also present with hypertension and proteinuria. And further lupus nephritis itself may predispose to preeclampsia. However the management of both the entities varies. Lupus nephritis involves treatment with immunosuppresives whereas treatment of preeclampsia differs. Hence these angiogenic biomarkers were studied in these two population and it was concluded that both sFlt-1 and sFlt-1/PIGF ratio was elevated in preeclampsia patients whereas it was normal in patients with lupus nephritis. The data further concluded that angiogenic biomarkers could be used in diagnosis of preeclampsia in patients with underlying kidney disease rather than renal biopsy.

Another major challenge in obstetrics is the differentiation between patients with chronic hypertension and patients who develop preeclampsia   with superimposed chronic hypertension because treatment of these two again varies as management of chronic hypertension involves antihypertensives whereas that of preeclampsia is ideally prompt delivery of fetus. Hence study was conducted evaluating the use of angiogenic biomarkers in differentiating the two. The study concluded that sFlt-1 and sFlt-1/PIGF ratio was elevated in preeclampsia superimposed on chronic hypertension whereas it was normal in patients with chronic hypertension.

Further preeclampsia can also present in a variable manner and there are many other conditions mimicking preeclampsia. These angiogenic biomarkers could be

used to differentiate preeclampsia from other disorders.

ANGIOGENIC PROTEINS AND INTRAUTERINE GROWTH RETARDATION:

The normal growth of the fetus is an interplay between fetus, maternal and placenta. A breach of any of the 3 systems could result in Small for Gestational age infants (SGA). SGA infants due to placental insufficiency are the IUGR babies.Although IUGR itself is a consequence of preeclampsia, it may happen even in absence of preeclampsia. As with preeclampsia it has been shown that there is impairment in the transmigration of the trophoblasts and invasion of spiral arteries. These placentas also had defective capillarization. It has been suggested that low oxygen derivation & low oxygen consumption by the growth retarded fetus and increased pO2 inhibits fetoplacental angiogenesis. Abberations in angiogenic proteins is being reported in IUGR babies in few studies. Levels of sFlt-1 in serum are also being shown to be elevated in few studies but strong relation of the pathogenic roles of these angiogenic proteins in IUGR has not yet been established.

CLINICAL UTILITY OF BIOMARKERS IN PREECLAMPSIA:

● The maternal consequences and complications could not be avoided but

however the patients at risk can be identified and hospitalized for safe confinement.

- There is no available data regarding the use of sFlt-1/PIGF ratio to avaid maternal consequences and complications.

- There is also no data that is available to prove that outcome in mother is better now than before the advent of these biomarkers.

- The test should be used in high risk population and also in women with preeclampsia in order to minimize cost utility and resources.

sFlt-1/PIGF RATIO:

It has been proposed that sFlt-1/PIGF ratio is a better predictor of preeclampsia than when either marker is used alone in diagnosis of preeclampsia. This has been highlighted in many studies and it is more sensitive when used in second trimester.

sFlt-1/PlGF ratio

Normal pregnancy

sFlt/PlGF: low

Preeclampsia

sFlt/PlGF: >high
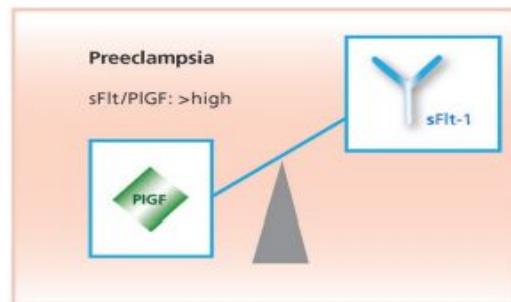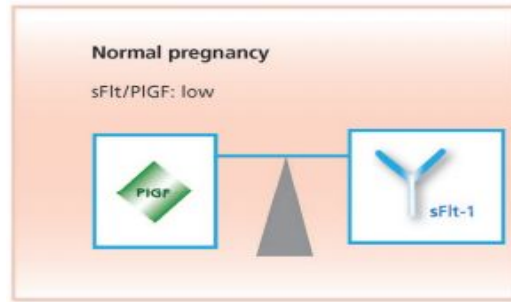
MANAGEMENT OF PREECLAMPSIA:

The optimal management of preeclamptic women would depend on the gestational week and severity of the dsorder. The severity of preeclampsia must be assessed against the risk of premature baby.

- Mild preeclampsia at or beyond 34 weeks of gestation must be delivered.

- In case of severe preeclampsia delivery must be considered at or beyond 34 weeks of pregnancy prior to which corticosteroids are given parenteral to the mother to allow for fetal lung maturity.

- Prevention of seizures and blood pressure control should be the highest priorities.Seizure prevention is by use of parenteral Magnesium sulphate. Magnesium sulphate must be given to all patients with severe preeclampsia.

Blood Pressure Control:

Blood pressure target recommended is Systolic BP of 140-150mmHg and Diastolic BP of 80-90mmHg.

The choice of antihypertensives preferred in pregnancy include

I) Calcium channel blockers mainly Nifedipine

II) Labetalol

III)Centrally acting sympatholytics mainly Methyldopa

IV)Vasodilators mainly Hydralazine.

ACE inhibitors and ARBs are contraindicated in pregnancy.

Low dose Aspirin is being investigated for management of preeclampsia. Since there is alteration in balance between prostacyclin and thromboxane formation in preeclampsia the prescription of low dose aspirin would be reasonable. Further it has been suggested that aspirin reduces the circulating levels of sFlt-1. Other management options which have been tried include albumin infusions, uterine currettage etc..

FUTURE TREATMENT MODALITIES:

Since the recent cross sectional and other studies confirmed the value of use of angiogenic markers as a specific marker of preeclampsia and that these angiogenic markers are inturn involved in the pathogenesis of preeclampsia newer treatment modalities are being evaluated.

The current evidence also suggested that patients with relatively lower sFlt-1 and sFlt-1/PIGF ratio had better outcomes when comparing those with greater elevation of sFlt-1/PIGF. Though the awareness of complications of preeclampsia is increasing, clinicians are hesitant about the management options once it is diagnosed.

The antihypertensive drugs are relatively not so effective in most of the patients and disease management mainly concentrates on prolonging pregnancy as far as possible with good rest, anticonvulsants, and delivering baby and placenta only fully cures this disorder. Therefore the end result is delivery of a premature baby by either inducing delivery or surgery. Therefore newer treatment options would pave way for better management. Recently better understanding of the pathogenesis of preeclampsia have paved way for number of advances in management options and newer approaches have been postulated.

Evidence suggests that recombinant VEGF and PIGF and sFlt-1 antagonists

may serve as effective tool to decrease sFlt-1 induced endothelial dysfunction.[12] Similarly removing sFlt-1 through an extracorporeal device would also serve as an effective management tool.

DEXTRAN SULFATE APHERESIS:

In view of the pathogenic role of the angiogenic proteins in pathogenesis of preeclampsia rather than antagonising sFlt-1 removing the sFlt-1 from the circulation would be an ideal option. An ideal intervention should stabilize or improve maternal and fetal well being without any adverse effects. And it should be titrated given according to the deterioration of the fetus, mother and both. Extracorporeal adsoption of the circulating sFlt-1 would fulfill this criteria.

An adsorption column using dextran sulfate would augment the removal of sFlt-1 from the maternal circulation.[18] The adsorption column creates a concentration gradient that augments removal of sFlt-1.It basically removed the lipoproteins which are also proposed in the pathogenesis of preeclampsia.Maternal blood pressure stabilized but was not lowered significantly after the apheresis therapy. Protein creatinine ratio also fell in conjunction with sFlt-1 levels. Further this treatment reduced the blood pressure and proteinuria without any adverse effects. There are reports of patients with Postpartum HELLP syndrome and thrombocytopenia which recovered following apheresis. Therefore apheresis could

be promising option in treatment of preeclampsia in future. Larger clinical trials are however needed to prove the same.

The following picture depicts the process of dextran sulfate apharesis.

Dextran Sulfate Apheresis

RAAPID TRIAL evaluated the use of this apheresis in preeclampsia. Its main objectives were

- To determine if short term apheresis reduced the circulating levels of sFlt-1 levels in the maternal circulation.

- To determine if short term apheresis stabilized the blood pressure

- If apheresis reduced the proteinuria

- Lead to prolongation of pregnancy

- Increase in fetal birth weight

- To determine the safety of reducing maternal sFlt-1 levels.

The study results are yet to published.

There was one another pilot study evaluating the use of extracorporeal apheresis in preeclampsia.It was concluded that sFlt-1 levels were significantly reduced in apheresis treated patients. And patients had a better outcome. Stabilisation of maternal blood pressure and proteinuria was reported. However data on prolongation on pregnancy and increased fetal weight were not confirmatory.

Other novel therapies include monoclonal antibodies to sFlt-1, sEng, the inhibitors of sFt-1 and sEng or use of substances that increase VEGF, PIGF or TGF-Beta expression. Larger clinical trials are however needed to prove the to confirm the effectiveness of these therapeutic strategies.

Similarly reducing proteinuria has shown to decrease the blood pressure. Reducing proteinuria with ACE inhibitors and ARBs would be helpful but unfortunately ACE inhibitors and ARBs are contraindicated in pregnancy.

SUMMARY:

Preeclampsia is a challenging disease faced by both the obstetrician and physician. The past decade has brought us exhilarating advances in understanding of the pathophysiology of preeclampsia. Eventhough the process that initiates preeclampsia is still not known recently advances in work have suggested the role of angiogenic factors in the circulation which was the key factor in linking the placenta with clinical manifestations of the disease.The options for diagnosis and treatment of preeclampsia may be elusive. However more work need to be performed to study the regulatory factors in placental vascular development and about the mechanisms that lead to variability in maternal response and also to compare these factors in normal pregnancy as well as preeclampsia. And it is still not very clear about the novel strategies of treatment and also about their safety and effectiveness.But still, it is exhilarating to experience the advances that has been made in understanding of the various mechanisms and the promising treatment options available for this disease which is more challenging.

OBJECTIVES:

- To test sFlt-1 levels in patients with preeclampsia.

- To test PIGF levels in patients with preeclampsia.

- To check correlation between development of preeclampsia with sFlt-1 and PIGF levels

- To assess sflt-1/PIGF ratio and its correlation with development of preeclampsia.

- To check if sflt/PIGF ratio correlates with pregnancy outcomes in patients with pre-eclampsia.

PLACE OF STUDY:

Department of Obstetrics & Gynaecology, RSRM Lying in Hospital - Obstetrics and Gynaecology extension of Govt.Stanley Hospital.

STUDY DESIGN:

Case-cohort study

STUDY PERIOD:

December 2014 to September 2015.

OPERATIONAL DEFINITION:

Case Definition: Preeclampsia patients defined by (1) Blood pressure of more than 140/90 mmHg after twenty weeks of pregnancy in a women with previously normal BP(2), newly detected proteinuria (>300 mg of protein in twenty four hours or a random/spot urine protein/creatinine ratio of >0.30)

INCLUSION CRITERIA:

High risk Normotensive normoglycemic Antenatal women >18 years of age between 15-20 weeks of gestation.

- Very young (<20years) and elderly (>30years) Primi
- Family history of Hypertension.
- History of Pre-eclampsia in previous pregnancy
- History of IUD in previous pregnancy
- BMI>30

EXCLUSION CRITERIA:

- Patients with Chronic Hypertension

- Patients with Renal insufficiency

- Patients with Diabetes Mellitus/GDM

- Patients with Autoimmune disorders.

- Patients with multiple pregnancy.

SAMPLE SIZE:

100 Antenatal women.

CRITERIA FOR CONTROL:

Patients who remain normotensive throughout pregnancy.

METHODOLOGY:

This study is a case-cohort study wherein 100 antenatal women between 15-20 weeks of gestation who are at high risk of developing Pre-eclampsia satisfying inclusion and exclusion criteria were selected and informed consent obtained for enrollment in this study.

BP, Urine routine and Random blood sugar was checked for all patients and relevant investigations done.Blood was drawn for sFlt-1 and PIGF. sFlt-1 assay done by Delfia Method.PIGF assay done by ELISA. Patients were followed up throughout pregnancy and blood pressure and urine protein was checked in every antenatal visit. The patients who subsequently developed preeclampsia were assigned as the cases and the patients who remained normotensive throughout pregnancy were assigned as the control. sFlt-1 and PIGF levels and their ratio is then correlated with the cases and controls.

Patients at risk of Developing Preeclampsia selected

↓

sFlt-1 & PlGF levels measured in serum

↓

Patients followed up

Preeclampsia

Normotensive

↓

CASES
n - 20

↓

CONTROL
n - 80
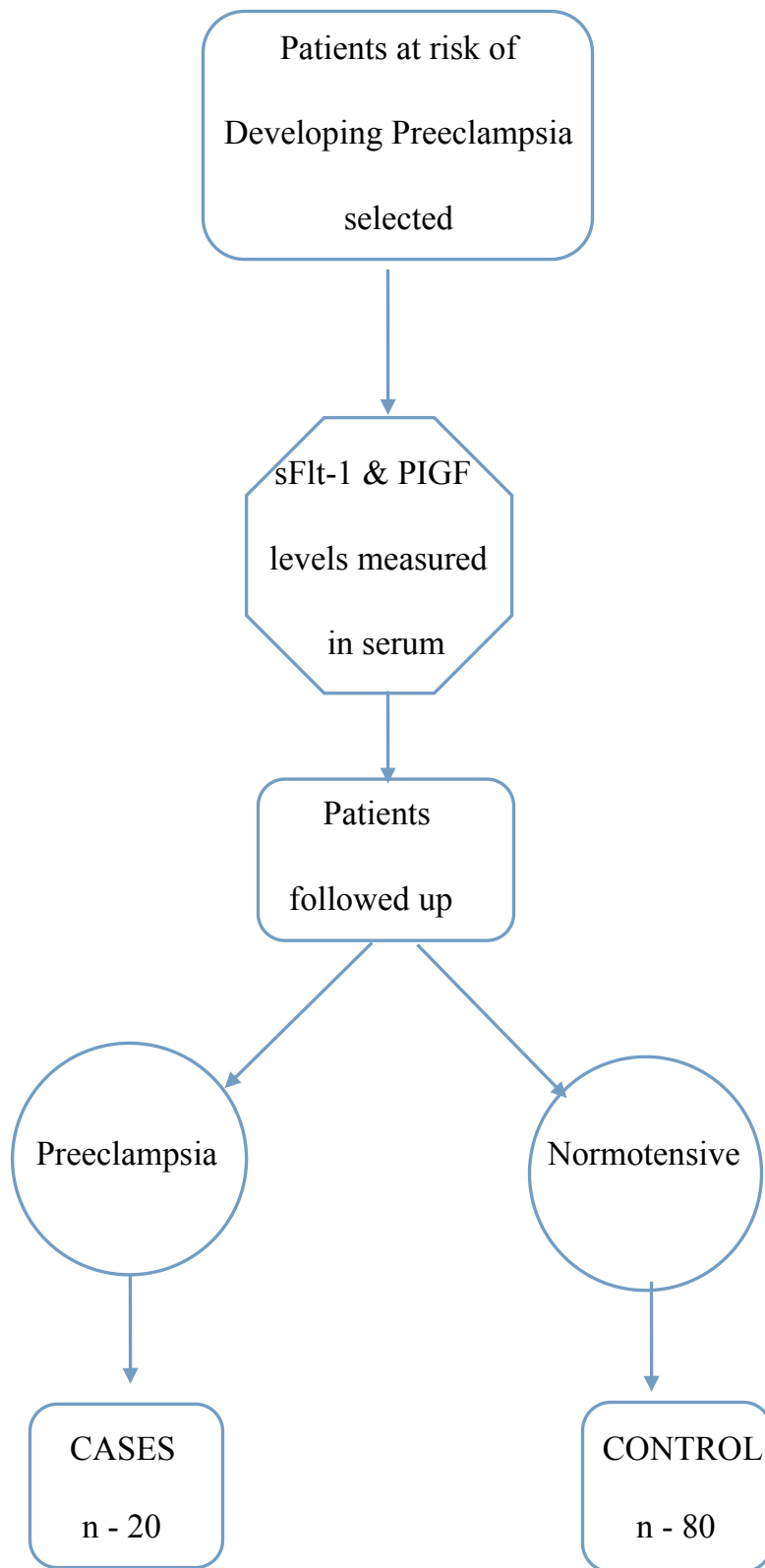
EXPECTED BENEFITS FROM STUDY:

If study confirms association between Sflt-1/PIGF ratio & Pre-eclampsia,

- Sflt and PIGF levels and their ratio may be used as reliable markers and as a predictor of Pre-eclampsia since it antedates symptoms of Pre-eclampsia.

- Extensive monitoring of the positive patients can reduce maternal and neonatal morbidity.

Sflt removal, VEGF analogues and targeted therapies may aid in better management options.

Statistics

All the data will be entered in a master excel sheet and will be analysed using SPSS software. Categorical variables will be expressed as absolute (n) and relative (%) frequencies and continuous variables will be expressed as means and standard deviation.

RESULTS AND DISCUSSION

HIGH RISK PROFILE AND PREECLAMPSIA:

HIGH RISK	NO.	PREECLAMPSIA
PREVIOUS PREECLAMPSIA	30	5
YOUNG PRIMI	48	12
ELDERLY PRIMI	8	2
FAMILY H/O HYPERTENSION	4	0
OBESITY	4	1
BAD OBSTETRIC HISTORY	6	0
TOTAL	100	20

AGE DISTRIBUTION:
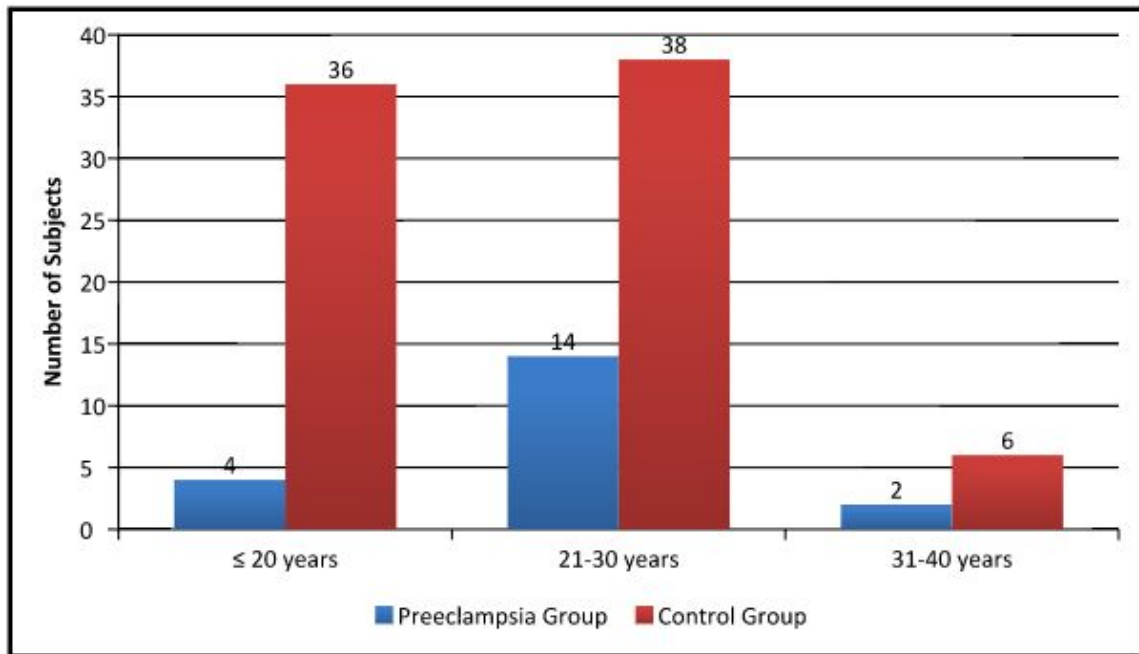
AGE	Preeclampsia Group	%	Control group	%
≤ 20 years	4	20	36	45
21-30 years	14	70	38	47.5
31-40 years	2	10	6	7.5
Total	20	100	80	100

Age Distribution	Preeclampsia Group	Control group
N	20	80
Mean	24.20	23.08
SD	4.54	4.99
P value Unpaired t Test		0.3386

Majority of the Preeclampsia Group patients belonged to the 21-30 years age class interval (n=14, 70%) with a mean age of 24.20 years. In the Control group patients, majority belonged to the same age class interval (n=38, 47.5%) with a mean age of 23.08 years. The association between the intervention groups and age distribution is considered to be not statistically significant since $p > 0.05$ as per unpaired t test.

GRAVIDA STATUS:
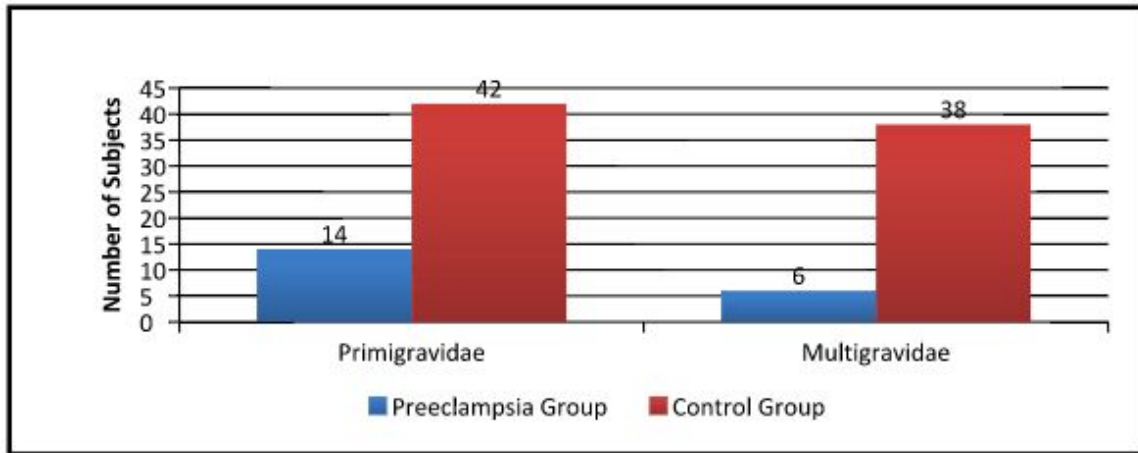
Gravida Status	Preeclampsia Group	%	Control Group	%
Primigravidae	14	70	42	52.5
Multigravidae	6	30	38	47.5
Total	20	100	80	100
P value **Fishers Exact Test**			0.2865	

Majority of the Preeclampsia Group patients belonged to the primigravidae class interval (n=14, 70%). In the Control group patients, majority belonged to the same class interval (n=42, 52.5%). The association between the intervention groups and gravida status is considered to be not statistically significant since p > 0.05 as per fishers exact test.

TIMING OF DEVELOPMENT OF PREECLAMPSIA:
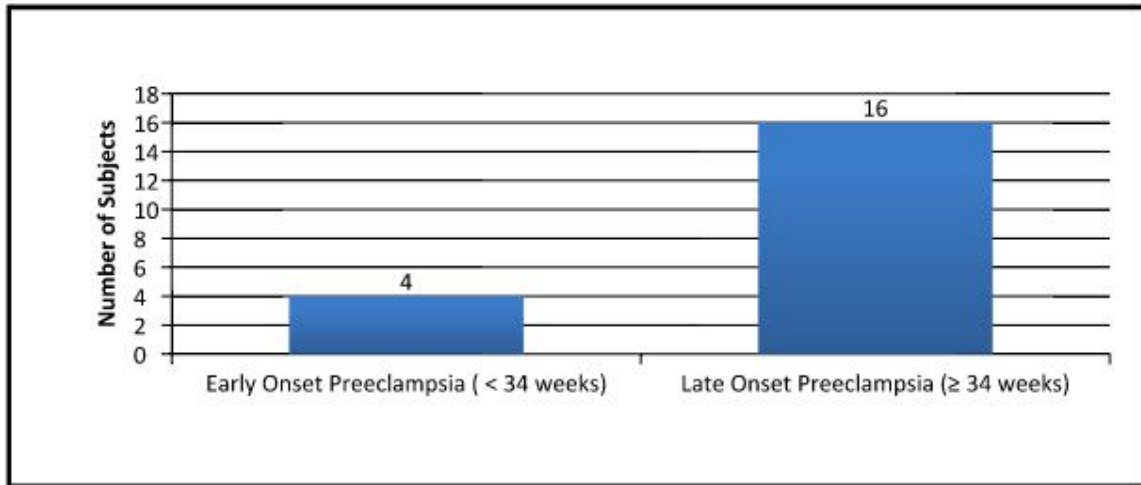
TIME	Preeclampsia Group	%
Early Onset Preeclampsia (< 34 weeks)	4	20
Late Onset Preeclampsia (≥ 34 weeks)	16	80
Total	20	100

GESTATIONAL AGE AT DELIVERY:
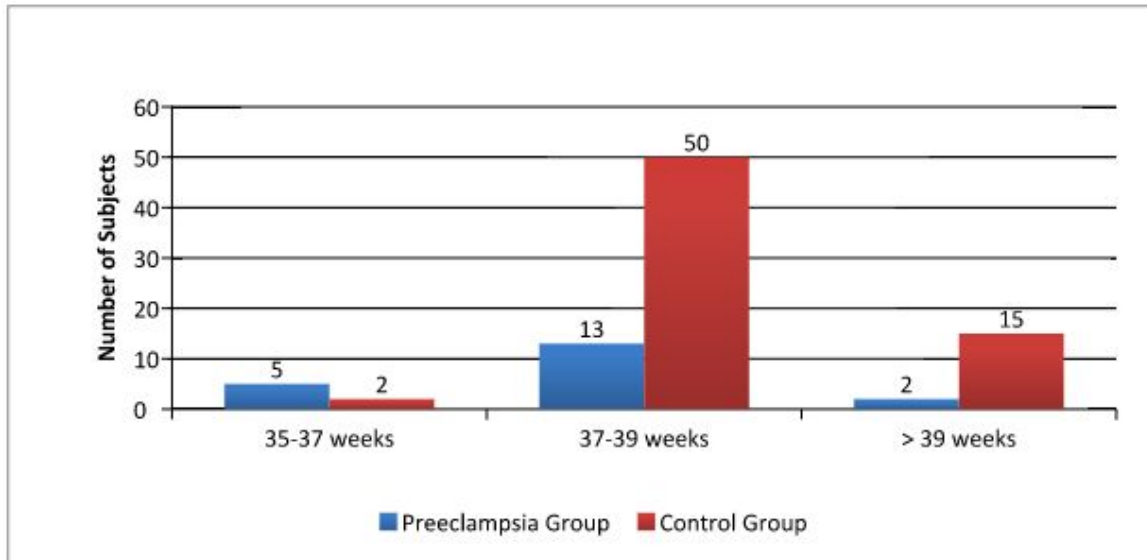
Gestational Age at Delivery	Preeclampsia Group	%	Control Group	%
≤ 33 weeks	0	0	0	0
33-35 weeks	0	0	0	0
35-37 weeks	5	25	2	2.5
37-39 weeks	13	65	50	62.5
> 39 weeks	2	10	15	18.75
Total	0	100	80	100

Gestational Age at Delivery	Preeclampsia Group	Control Group
N	20	67
Mean	38.05	38.81
SD	1.19	0.82
P value Unpaired t Test		0.0136

Results

In patients belonging to Preeclampsia Group, the mean gestational age at delivery was 38.05 weeks. In Control Group, the mean gestational age at delivery was 38.81 weeks. The increased mean gestational age at delivery measurements in Preeclampsia Group compared to the Control Group is statistically significant as the p value is 0.0136 as per unpaired t- test indicating a true difference among study groups.

Discussion

The mean gestational age at delivery was meaningfully less in Preeclampsia Group compared to the Control Group by 0.76 weeks.

This significant difference of 2% decrease in gestational age at delivery in Preeclampsia Group compared to the Control Group is true and has not occurred by chance.

Conclusion

In this study we can safely conclude that mean gestational age at delivery levels were significantly and consistently lower in Preeclampsia Group compared to the Control Group.

COMPLICATIONS IN PREECLAMPSIA Vs CONTROL GROUP:
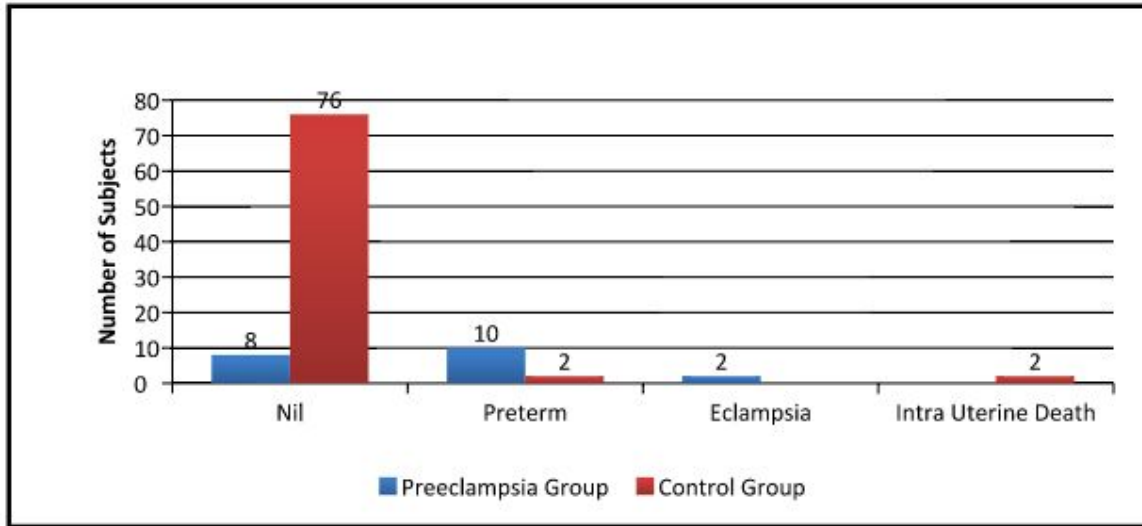
Complications	Preeclampsia Group	%	Control Group	%
Nil	8	40	76	95
Preterm	10	50	2	2.5
Eclampsia	2	10	0	0
Intra Uterine Death	0	0	2	2.5
Total	20	100	80	100
P value Fishers Exact Test			<0.0001	

Results

In patients belonging to Preeclampsia Group, the major complication observed was preterm delivery (n=10, 50%). In Control Group, the major complication observed is again preterm delivery (n=2, 2.5%). The increased incidence of preterm delivery as complication in Preeclampsia Group compared to the Control Group is statistically significant as the p value is 0.0001 as per unpaired t- test indicating a true difference among study groups.

Discussion

The incidence of preterm delivery as complication was meaningfully more in Preeclampsia Group compared to the Control Group by 47.5 percentage points.This significant difference of 20 times increase in incidence of preterm delivery as complication in Preeclampsia Group compared to the Control Group is true and has not occurred by chance.

Conclusion:

In this study we can safely conclude that incidence of preterm delivery as complication was significantly and consistently higher in Preeclampsia Group compared to the Control Group.

SOLUBLE FMS LIKE TYROSINE KINASE-1:(sFlt-1)
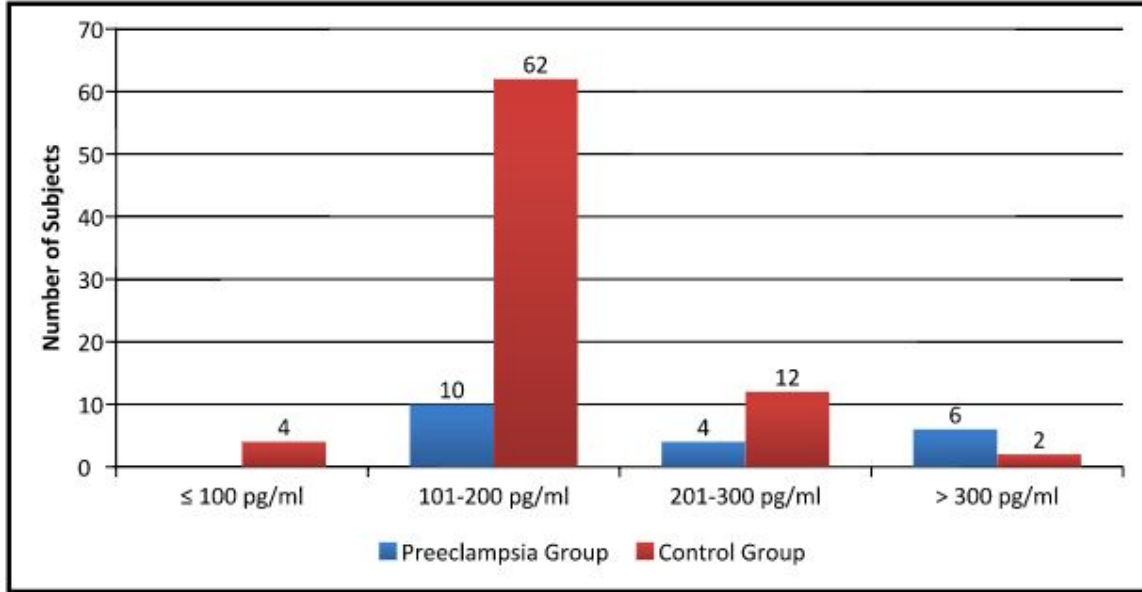
sFlt-1 – 1 Levels	Preeclampsia Group	%	Control Group	%
≤ 100 pg/ml	0	0	4	5
101-200 pg/ml	10	50	62	77.5
201-300 pg/ml	4	20	12	15
> 300 pg/ml	6	30	2	2.5
Total	20	100	80	100

sFlt-1 – 1 Levels	Preeclampsia Group	Control Group
N	20	80
Mean	560.74	202.78
SD	595.86	153.89
P value Unpaired t Test		0.0150

Results

In patients belonging to Preeclampsia Group, the mean sFlt-1 measurements is

560.74 pg/ml. In Control Group, the mean sFlt-1 measurement is 202.78 pg/dl.

The increased mean sFlt-1 measurements in Preeclampsia Group compared to the

Control Group is statistically significant as the p value is 0.0150 as per unpaired t-

test indicating a true difference among study groups.

Discussion

The mean sFlt-1 measurements were meaningfully more in

Preeclampsia Group compared to the Control Group by 357.96 pg/dl. This s

ignificant difference of 177% increase in mean sFlt-1 measurement in Preeclampsia Group compared to the Control Group is true and has not occurred by chance.

Conclusion

In this study we can safely conclude that mean Soluble Fms like Tyrosine kinase – 1 Levels were significantly and consistently higher in Preeclampsia Group compared to the Control Group.

PLACENTAL GROWTH FACTOR:(PIGF)
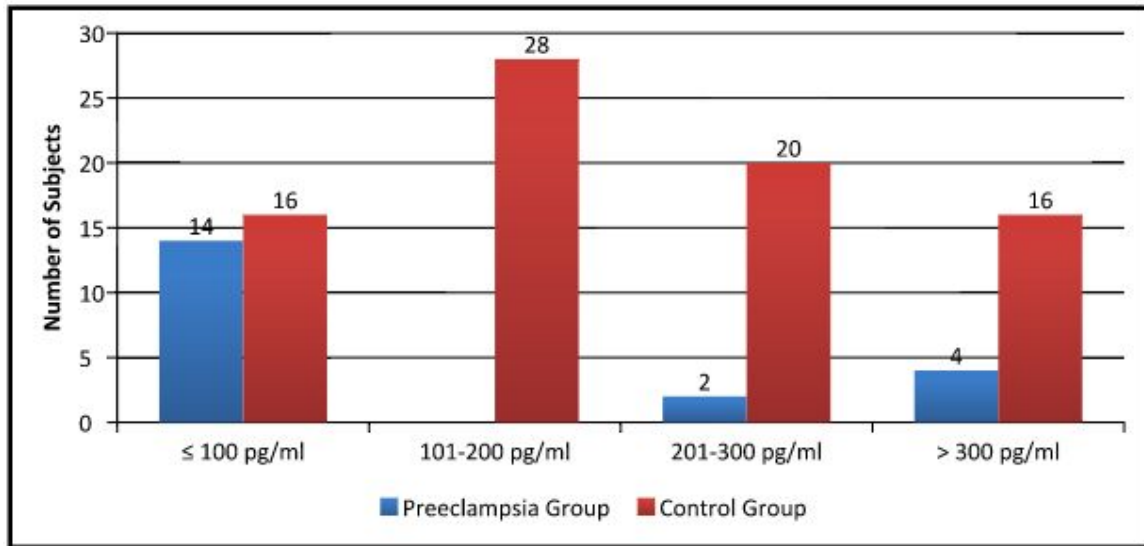
Placental Growth Factor Levels	Preeclampsia Group	%	Control Group	%
≤ 100 pg/ml	14	70	16	20
101-200 pg/ml	0	0	28	35
201-300 pg/ml	2	10	20	25
> 300 pg/ml	4	20	16	20
Total	20	100	80	100

Placental Growth Factor Levels	Preeclampsia Group	Control Group
N	20	80
Mean	162.61	228.20
SD	214.78	170.14
P value Unpaired t Test		0.0216

Results

In patients belonging to Preeclampsia Group, the mean PIGF measurements is

162.61 pg/ml. In Control Group, the mean PIGF measurement is 228.20 pg/dl. The

decreased mean PIGF measurements in Preeclampsia Group compared to the

Control Group is statistically significant as the p value is 0.0216 as per unpaired t-

test indicating a true difference among study groups.

Discussion

The mean PIGF measurements were meaningfully less in PreeclampsiaGroup

compared to the Control Group by 65.59 pg/dl.This significant difference o

f 29% decrease in mean PIGF measurement in

Preeclampsia Group compared to the Control Group is true and has not occurred by chance.

Conclusion

In this study we can safely conclude that mean Placental Growth Factor Levels were significantly and consistently lowered in Preeclampsia Group compared to the Control Group.

sFlt-1/PlGF RATIO:
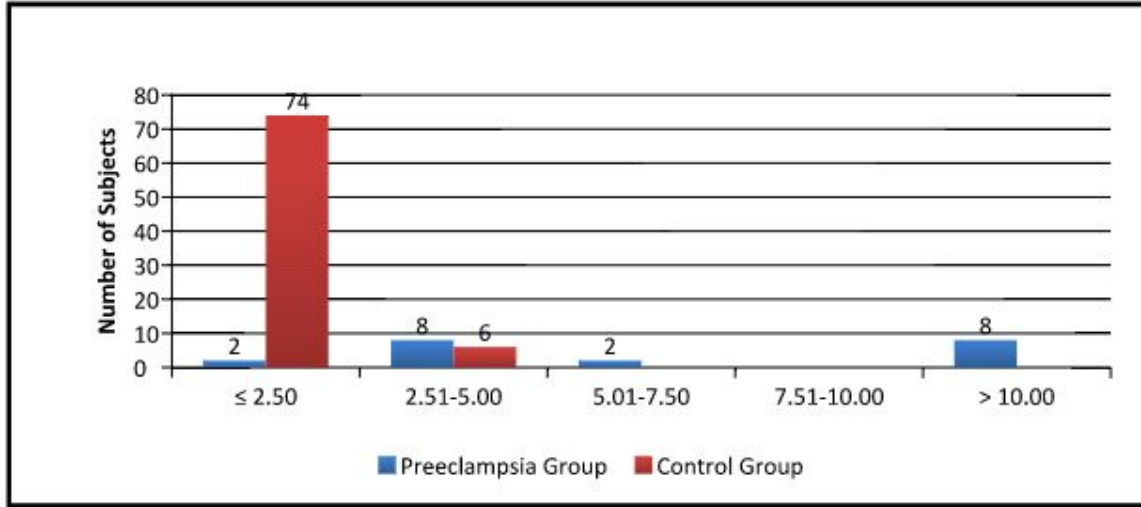
sFlt-1/PlGF Ratio	Preeclampsia Group	%	Control Group	%
≤ 2.50	2	10	74	92.5
2.51-5.00	8	40	6	7.5
5.01-7.50	2	10	0	0
7.51-10.00	0	0	0	0
> 10.00	8	40	0	0
Total	20	100	80	100

sFlt-1/PlGF Ratio	Preeclampsia Group	Control Group
N	20	80
Mean	20.12	5.66
SD	41.20	21.88
P value Unpaired t Test		0.0436

Results

In patients belonging to Preeclampsia Group, the mean sFlt-1/PlGF ratio

measurement is 20.12. In Control Group, the mean sFlt-1/PlGF ratio measurement

is 5.66. The increased mean sFlt-1/PlGF ratio measurements in Preeclampsia

Group compared to the Control Group is statistically significant as the p value is

0.0436 as per unpaired t- test indicating a true difference among study groups.

Discussion

The mean sFlt-1/PlGF ratio measurements were meaningfully more in Preecl

ampsia Group compared to the Control Group by 18.89 points. This signific

ant difference of 16.37 fold increase in mean sFlt-1/PlGF ratio measurement

 in Preeclampsia Group compared to the Control

Group is true and has not occurred by chance.

Conclusion

In this study we can safely conclude that mean sFlt-1/PlGF ratio Levels were

significantly and consistently higher in Preeclampsia Group compared to the

Control Group.

sFlt-1/PIGF ratio Vs SAMPLING WEEK:

sFlt-1/PIGF Ratio Vs Sampling weeks	SFLT/PIGF Ratio ≤ 2.5	SFLT/PIGF Ratio > 2.5
15-16 weeks	6 Preeclampsia - 0	14 Preeclampsia - 8
17-18 weeks	46 Preeclampsia - 0	6 Preeclampsia - 6
19-20 weeks	24 Preeclampsia - 2	4 Preeclampsia - 4
Total	76	24

Sampling at 17-18weeks gave high probability of positive result hence good diagnostic accuracy and sensitivity during this sampling period.

COMPLICATIONS Vs sFlt-1/PIGF RATIO:
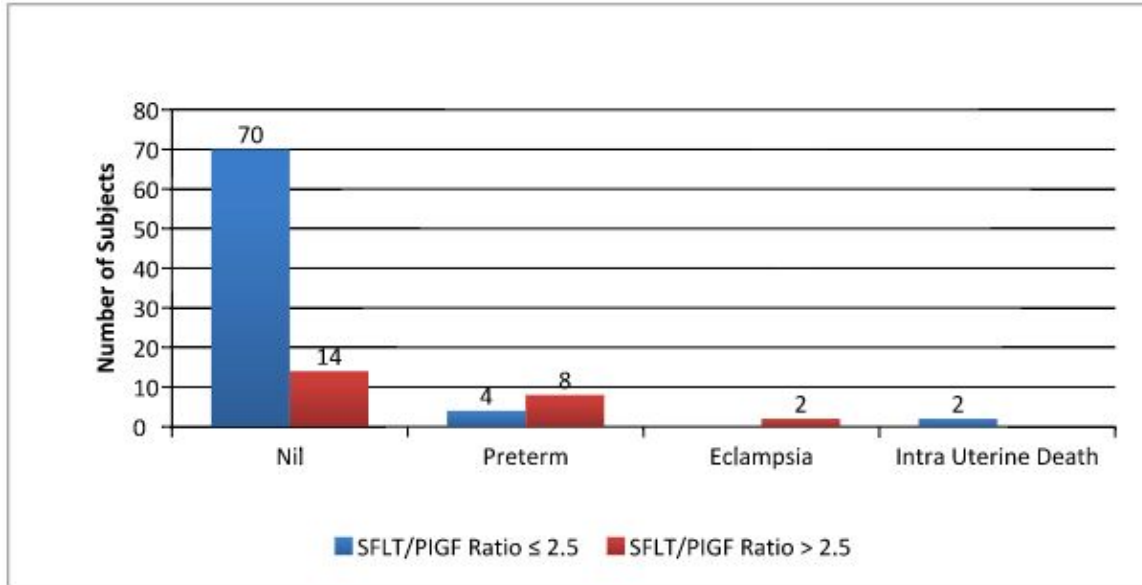
Complications Vs sFlt-1/PIGF Ratio	sFlt-1/PIGF Ratio ≤ 2.5	%	sFlt-1/PIGF Ratio > 2.5	%
Nil	70	92.11	14	58.33
Preterm	4	5.26	8	33.33
Eclampsia	0	0.00	2	8.33
Intra Uterine Death	2	2.63	0	0.00
Total	76	100	24	100
P value Fishers Exact test		<0.0001		

Results

In patients belonging to sFlt-1/PIGF Ratio ≤ 2.5 Group, the major complication observed was preterm delivery (n=4, 5.26%). In sFlt-1/PIGF Ratio > 2.5 Group, the major complication observed is again preterm delivery (n=8, 33.33%). The decreased incidence of preterm delivery as complication in sFlt-1/PIGF Ratio ≤ 2.5 Group compared to the sFlt-1/PIGF Ratio > 2.5 Group is statistically significant as the p value is 0.0001 as per unpaired t- test indicating a true difference among study groups.

Discussion

The incidence of preterm delivery as complication was meaningfully less in sFlt-1/PIGF Ratio ≤ 2.5Group compared to the sFlt-1/PIGF ratio > 2.5 Group by 28.1 percentage points.This significant differenceof 84 % decrease in incidence of preterm delivery as complication in sFlt-1/PIGF Ratio ≤ 2.5 Group compared to the sFlt-1/PIGF ratio>2.5lGroup is true an d has not occurred by chance.

Conclusion

In this study we can safely conclude that incidence of preterm delivery as complication was significantly and consistently lower in sFlt-1/PIGF Ratio ≤ 2.5 Group compared to the sFlt-1/PIGF Ratio > 2.5 Group.

ACCURACY STATISTICS:

Test	Result	Lower Confidence Interval	Lower Confidence Interval
Sensitivity	90%	76.9%	100%
Specificity	92.5%	86.7%	98.3%
PPV	75%	57.7%	92.3%
NPV	97.4%	93.8%	100%

- The sensitivity of sFlt-1/PlGF Ratio is very high (90%). This means that high positive levels of sFlt-1/PlGF Ratio test often occurs in those with preeclampsia. Given a sensitivity of 90% or .90, we can safely conclude that 90% of the patients with the preeclampsia will have a positive sFlt-1/PlGF Ratio test.

- The specificity of sFlt-1/PlGF Ratio is very high (92.5%). This means that negative levels of sFlt-1/PlGF Ratio test often occurs in those without

preeclampsia. Given a specificity of 92.5% or .925, we can safely conclude that 92.5% of the patients without preeclampsia will have a negative sFlt-1/PlGF Ratio test.

- A positive predictive value of 75% means that only 75% of the patients with a positive sFlt-1/PlGF Ratio test actually have preeclampsia. In other words,patients with a positive sFlt-1/PlGF Ratio test have a 75% chance of having preeclampsia.

- A negative predictive value of 97.4% means that 97.4% of patients with a negative sFlt-1/PlGF Ratio test are in fact, preeclampsia free. In other words, patients with a negative sFlt-1/PlGF Ratio test have a 97.4% chance of being preeclampsia free.

The diagnostic effectiveness or diagnostic accuracy of sFlt-1/PlGF Ratio test to predict preeclampsia is very high. It means that the overall value of sFlt-1/PlGF Ratio test in detecting preeclampsia as a combined screening and case finding test is good

CONCLUSION:

- **sFlt-1 levels were elevated in patients who later developed preeclampsia even before the patients developed hypertension and proteinuria and hence can be used as an effective diagnostic marker and an early predictor of preeclampsia.**

- **PIGF levels were significantly low in patients who later developed preeclampsia even before the patients manifested clinically and hence can be used as an effective diagnostic marker and an early predictor of preeclampsia.**

- **sFlt-1/PIGF ratio was elevated in patients who developed preeclampsia even before the patients manifested clinically and hence can be used as a valuable diagnostic marker and an early predictor of preeclampsia.**

- **There was high probability of diagnostic accuracy when sFlt-1 and PIGF were measured at 17-18 weeks of gestation.**

- **sFlt-1/PIGF ratio was higher in those patients who developed complications of preeclampsia and hence higher ratio indicates bad prognosis.**

- **The sensitivity and specificity of sFlt-1/PIGf ratio is high, hence can be used as a reliable screening and diagnostic test of Preeclampsia.**

REFERENCES

1. ACOG Practice Bulletin Committee. Diagnosis and management of preeclampsia and eclampsia. *J Obstet Gynecol.* 2002;99:159–167.

2.*Circulation AHA*;Preeclampsia,A disease of maternal endothelium: The role of antiangiogenic factors and implications for later Cardiovascular disease.Camille E. Powe, Richard J. Levine and S. Ananth Karumanchi. 2011 ;123: 2856-2869

3.American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy.Report of the American College of Obstetricians andGynecologists' Task Force on Hypertension in Pregnancy.*Obstet Gynecol* 2013; **122**: 1122–1131.

4. S. E. Maynard, J.-Y. Min, J. Merchan et al., "Excess placen- tal soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction hypertension, and proteinuria in preeclampsia,"*Journal of Clinical Investigation*,vol.111,no.5,pp. 649–658,2003.

5. R.J.Levine,C.Qian,S.E.Maynard,K.F.Yu,F.H.Epstein,and S. A. Karumanchi, "Serum sFlt1 concentration during pree- clampsia and mid trimester blood pressure in healthy nulli- parous women," *The American Journal of Obstetrics and Gynecology* ,vol.194,no.4,pp.1034–1041,2006

6. Polliotti BM, Fry AG, Saller DN, Mooney RA, Cox C, Miller RK. Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset preeclampsia. *Obstet Gynecol.* 2003;101:1266–1274.

7. C.Lam,K.-H.Lim,andS.A.Karumanchi,"Circulating angio-genic factors in the pathogenesis and prediction of preeclampsia,"*Hypertension*,vol.46,no.5,pp.1077–1085,2005.

8. E. R. Unal, C. J. Robinson, D. D. Johnson, and E. Y. Chang, "Second-trimester angiogenic factors as biomarkers for future- onset preeclampsia," *The American Journal of Obstetrics and Gynecology*,vol.197,no.2,pp.211.e1–211.e4,2007.

9. O. Lapaire, A. Shennan, and H. Stepan, "The preeclampsia biomarkers soluble fms-like tyrosine kinase-1 and placental growth factor: current knowledge, clinical implications and future application," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol.151,no.2,pp.122–129,2010.

10.Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, Ecker J, Karumanchi SA. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab.* 2004;89:770–775

11. T.Chaiworapongsa,R.Romero,Y.M.Kimetal.,"Plasma solu- ble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia," *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 17, no. 1, pp.3–18,2005.

12.Wallner W, Sengenberger R, Strick R, Strissel PL, Meurer B,Beckmann MW, Schlembach D. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (London)* 2007; **112**:51–57.

13. Wikstro¨m AK, Larsson A, Eriksson UJ, Nash P, Norde´n-Lindeberg S, Olovsson M. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. *Obstet Gynecol* 2007; **109**: 1368–1374.

14.Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P,Vaisbuch E,Mazaki-Tovi S, Gotsch F, Edwin SS, Gomez R, Yeo L, Conde-Agudelo A, Hassan SS. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. *J Matern Fetal Neonatal Med* 2009; **22**: 1021–1038.

15.Noori M, Donald AE, Angelakopoulou A, et al. Prospective study of placental angiogenic factors and maternal vascular function before and after pre-eclampsia and gestational hypertension. *Circulation*. 2010; 122:478–487. [PubMed: 20644016]

16.Janina Müller-Deile, Mario Schiffer, Division of Nephrology and Hypertension, Department of Medicine, Hannover Medical School, 30625 Hannover, Germany. Preeclampsia from a renal point of view: Insides into disease models, biomarkers and therapy. *World J Nephrol* 2014 November 6; 3(4): 169-181

17.Sharon E. Maynard, MD* and S. Ananth Karumanchi, MD†*George[DVC1] Washington University School of Medicine and Health Sciences, Washington, DC†Howard Hughes Medical Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA "Angiogenic Factors and Preeclampsia Semin Nephrol" 2011 January ; 31(1): 33–46.

18.Richard J. Levine, M.D., M.P.H., Sharon E. Maynard, M.D., Cong Qian, M.S.,Kee-Hak Lim, M.D., Lucinda J. England, M.D., M.S.P.H., Kai F. Yu, Ph.D.,Enrique F. Schisterman, Ph.D., Ravi Thadhani, M.D., M.P.H.,Benjamin P. Sachs, M.B., B.S., D.P.H., Franklin H. Epstein, M.D.,Baha M. Sibai, M.D., Vikas P. Sukhatme, M.D., Ph.D.,and S. Ananth Karumanchi, M.D. "Circulating Angiogenic Factors and the Risk of Preeclampsia"*N Engl J Med* 2004;350:672-83.

19.Lakshmi Tanuja Petla, Rosy Chikkala, K.S. Ratnakar*, Vijayalakshmi

Kodati** &V. Sritharan "Biomarkers for the management of pre-eclampsia in pregnant women" *Indian J Med Res* 138, July 2013, pp 60-67

20.Pradhan P Department of Obstetrics and Gynaecology, Nepal Medical College Teaching Hospital, Kathmandu, Nepal. "Role of Angiogenic Factors in Preeclampsia" *NJOG* 2014 Jan-Jun; 17 (1):71-73

PROFORMA

NAME: AGE:

SEX: 1.M   2.F

ADDRESS:

CONTACT NO:

OCCUPATION   :

LIFESTYLE: 1.SEDENTARY   2.MODERATE

                   3.HEAVY WORKING

GESTATIONAL CODE:

LMP: EDD:

COMPLAINTS:

PAST H/O:

DIABETES   1. Yes   2. No   If yes specify_____

HYPERTENSION   1.Yes   2. No   If yes specify_____

RENAL FAILURE   1. Yes   2. No   If yes specify_____

AUTOIMMUNE DISORDER 1. Yes   2. No   If yes specify_____

CARDIAC ILLNESS   1. Yes   2. No   If yes specify_____

            OTHERS   _____

H/O CHRONIC DRUG INTAKE:

H/O SMOKING:

H/O ALCOHOL:

OBSTETRIC HISTORY:

FAMILY HISTORY:

RELEVENT CLINICAL EXAMINATION

HEIGHT   :   WEIGHT:

BMI:

BP(each antenatal visit):   PR:

RR:

CVS: RS:

PA:

CNS:

FUNDUS:

INVESTIGATIONS:

URINE ROUTINE:

RANDOM BLOOD SUGAR:

RENAL FUNCTION TEST:

LIVER FUNCTION TEST:

URIC ACID:

PLASMA SFLT-1:

PLASMA PIGF:

SFLT/PIGF:

USG ABDOMEN:

COURSE OF PRESENT PREGNANCY:

IF DEVELOPED PRE-ECLAMPSIA   1.Yes   2.No If yes

specify_____

OTHERS   1.Yes   2.No If yes specify_____

MODE OF DELIVERY:

GESTATIONAL AGE AT DELIVERY:

ANY COMPLICATIONS DURING DELIVERY:

BIRTH WEIGHT, SEX & DETAILS OF BABY:

COMMENT:

INFORMED CONSENT

"Study on Soluble Fms like Tyrosine kinase – 1/Placental Growth Factor ratio (sflt-1/PIGF) as a predictor of Pre-eclampsia"

AT GOVERNMENT STANLEY HOSPITAL, CHENNAI.

I ……………………………………………. have been informed about the details of the study in my own language.

I have completely understood the details of the study.

I am aware of the possible risks and benefits, while taking part in the study.

I agree to collect samples of blood/saliva/urine/tissue if study needs.

I understand that I can withdraw from the study at any point of time and even then, I can receive the medical treatment as usual.

I understand that I will not get any money for taking part in the study.

I will not object if the results of this study are getting published in any medical journal, provided my personal identity is not revealed.

I know what I am supposed to do by taking part in this study and I assure that I would extend my full cooperation for this study.

Volunteer: Witness:
Name and address   Name and address:
Signature/thumb impression: Signature/thumb imp:
Date: Date:

Investigator Signature and date

GOVT. STANLEY MEDICAL COLLEGE, CHENNAI – 600001

INFORMED CONSENT

"Study on Soluble Fms like Tyrosine kinase – 1/Placental Growth Factor ratio (sflt-1/PIGF) as a predictor of Pre-eclampsia"

AT GOVERNMENT STANLEY HOSPITAL, CHENNAI.

நான் இந்த ஆராய்ச்சியில் விவரங்களை முற்றிலும் புரிந்து கொண்டேன்.

ஆய்வில் பங்கு எடுத்து போது, சாத்தியமான அபாயங்கள் மற்றும் பயன்களை பற்றி நான் அறிந்துள்ளேன்.

நான் எந்தவொருவேளையிலும் ஆய்வில் இருந்து திரும்ப முடியும், அதன்பின்னர், நான் வழக்கம் போல் மருத்துவ சிகிச்சை பெறமுடியும் என்று புரிந்து கொள்கிறேன்

நான் ஆய்வில் பங்கு எடுத்து பணம் எதையும் பெற முடியாது என்று அறிந்துள்ளேன்.

இந்த ஆய்வின் முடிவுகள் எந்த மெடிக்கல் ஜர்னலில் வெளியிடப்பட இருந்தால் நான் எதிர்க்கவில்லை, என் தனிப்பட்ட அடையாளத்தை வெளிப்படுத்தப்பட்டு இருக்க கூடாது.

நான் இந்த ஆய்வில் பங்கெடுப்பதன் மூலம் நான் என்ன செய்ய போகிறேன் என்று தெரியும்

நான் இந்த ஆய்வில் என் முழு ஒத்துழைப்பையும் கொடுப்பேன் என்று உறுதியளிக்கிறேன்.

தன்னார்வளர்   சாட்சி
பெயர் மற்றும் முகவரி   பெயர் மற்றும் முகவரி

கையொப்பம் / விரல்ரேகை:   கையொப்பம் / விரல்ரேகை

ஆராய்ச்சியாளராக

கையொப்பம் மற்றும் தேதி

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Study on Soluble FMS like Tyrosine Kinase-1/ placental growth factor ratio (Sflt-1/PIGF) as a predictor of Pre-eclampsia.

Principal Investigator : Dr. K Monica

Designation : PG M D (General Medicine)
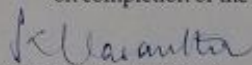
Department : Department of General Medicine Government Stanley Medical College, Chennai-01

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 26.11.2015 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY,
IEC, SMC, CHENNAI

MASTER CHART:

S.NO	AGE	S.WEEK	SFLT	PIGF	SFLT/PIGF	PRE-ECLAMPSIA	G.CODE	COMP	DELIVERED WEEK
1	24	18	159.065	207	0.768	NO	G3P2L1	N	39th wk
2	23	19	1123.05	630	1.783	NO	G3P1L1A1	N	38th wk
3	38	20	51.09	276	0.185	NO	G3P2L1	N	38th wk
4	19	18	286.47	221	1.296	NO	PRIMI	N	38th wk
5	18	18	214.115	200	1.071	NO	PRIMI	N	39th wk
6	20	18	204.655	184	1.112	NO	G2P1L0	N	40th wk
7	31	19	189.86	107	1.774	NO	PRIMI	N	38th wk
8	26	18	177.295	119	1.49	NO	G2P1L1	PRETERM	36th wk
9	25	20	1292.77	563	2.296	YES	G2P1L1	PRETERM	36th wk
10	19	20	180.21	543	0.332	NO	PRIMI	N	39th wk

11	28	17	172.43	127	1.358	NO	G2A1	N	39th wk
12	18	17	286.47	221	1.296	NO	G2P1	N	38th wk
13	19	17	91.745	85.5	1.073	NO	PRIMI	N	38th wk
14	20	17	174.015	178	0.979	NO	PRIMI	N	37th wk
15	27	18	266.105	374	0.712	NO	PRIMI	IUD	37th wk
16	21	18	178.51	220	0.811	NO	G3P2L1	N	39th wk
17	19	19	193.785	111	1.746	NO	PRIMI	N	38th wk
18	20	18	1353.21	214	6.323	YES	PRIMI	PRETERM	36th wk
19	17	17	199.615	406	0.492	NO	PRIMI	N	38th wk
20	21	19	171.565	108	1.589	NO	PRIMI	N	40th wk
21	26	18	156.355	40.2	3.889	YES	PRIMI	N	39th wk
22	20	16	156.08	150	1.041	NO	PRIMI	N	38th wk
23	29	17	167.015	105	1.591	NO	PRIMI	N	37th wk
24	25	18	200.52	224	0.895	NO	G4P3L2	N	39th wk
25	22	15	170.83	15.3	11.165	YES	PRIMI	PRETERM	36th wk

26	28	17	165.96	365	0.455	NO	G3P1L0A1	N	39th wk
27	23	16	179.02	84.6	2.116	NO	PRIMI	N	38th wk
28	24	16	196.79	65.3	3.014	NO	G2A1	N	40th wk
29	19	15	170.3	76.5	2.226	NO	PRIMI	N	39th wk
30	23	16	240.08	81.4	2.949	YES	PRIMI	N	38th wk
31	20	20	132.72	843	0.157	NO	G3A2	N	38th wk
32	22	15	171.835	56.6	3.036	NO	G2P1L1	N	37th wk
33	21	15	209.995	20.5	10.244	YES	PRIMI	ECL	38th wk
34	27	18	167.975	190	0.884	NO	G4P1L1A1	N	39th wk
35	29	20	1663.14	644	2.583	YES	G3P1L1A1	N	38th wk
36	18	16	169.31	37.3	4.539	YES	PRIMI	N	39th wk
37	19	20	175.19	554	0.316	NO	PRIMI	N	40th wk
38	21	18	171.25	72.5	2.362	NO	G2A1	N	39th wk
39	24	20	181.47	1.3	139.592	YES	PRIMI	PRETERM	34th wk
40	22	19	165.57	194	0.853	NO	PRIMI	N	38th wk

41	20	15	198.285	77.3	2.565	NO	G2P1L0A0	N	37th wk
42	19	17	153.69	242	0.635	NO	PRIMI	N	40th wk
43	25	18	163.54	217	0.754	NO	PRIMI	N	39th wk
44	20	17	136.435	289	0.472	NO	G2P1L1	N	37th wk
45	27	18	192.66	96.7	1.992	NO	PRIMI	N	39th wk
46	28	17	189.61	126	1.505	NO	G2A1	N	40th wk
47	20	19	169.1	261	0.648	NO	PRIMI	N	38th wk
48	38	17	176.485	383	0.461	NO	G2P1L0A0	N	38th wk
49	34	17	164.825	9.4	17.535	YES	G2A1	PRETERM	36th wk
50	19	20	184.665	145	1.274	NO	PRIMI	N	39th wk
51	20	15	199.285	77.4	2.574	NO	G2P1L0A0	N	40th wk
52	19	17	154.5	240	0.643	NO	PRIMI	N	38th wk
53	25	18	165.54	215	0.769	NO	PRIMI	N	40th wk
54	20	17	134.56	290	0.464	NO	G2P1L1	N	38th wk
55	27	18	193.67	98	1.976	NO	PRIMI	N	39th wk

56	28	17	190.01	124	1.532	NO	G2A1	N	38th wk
57	20	19	169	260	0.65	NO	PRIMI	N	40th wk
58	38	17	175.56	380	0.462	NO	G2P1L0A0	N	39th wk
59	34	17	175.456	10.2	17.2	YES	G2A1	PRETERM	36th wk
60	19	20	185.66	144	1.289	NO	PRIMI	N	39th wk
61	20	20	133.42	842	0.158	NO	G3A2	N	38th wk
62	22	15	170.83	55.5	3.078	NO	G2P1L1	N	37th wk
63	21	15	208.9	20.2	10.34	YES	PRIMI	ECL	38th wk
64	27	18	166.98	189	0.883	NO	G4P1L1A1	N	40th wk
65	29	20	1664.2	645	2.58	YES	G3P1L1A1	N	38th wk
66	18	16	168.9	37.2	4.54	YES	PRIMI	N	39th wk
67	19	20	175.45	555	0.316	NO	PRIMI	N	39th wk
68	21	18	170.25	72.5	2.348	NO	G2A1	N	40th wk
69	24	20	182	1.3	140	YES	PRIMI	PRETERM	34th wk
70	22	19	166.6	195	0.854	NO	PRIMI	N	39th wk

71	26	18	155.56	40.3	3.86	YES	PRIMI	N	39th wk
72	20	16	155.9	150	1.039	NO	PRIMI	N	40th wk
73	29	17	168.05	108	1.556	NO	PRIMI	N	38th wk
74	25	18	202	222	0.909	NO	G4P3L2	N	37th wk
75	22	15	170.56	15.2	11.221	YES	PRIMI	PRETERM	36th wk
76	28	17	165.08	366	0.451	NO	G3P1L0A1	N	38th wk
77	23	16	178.2	84.2	2.116	NO	PRIMI	N	39th wk
78	24	16	197.02	65.9	2.98	NO	G2A1	N	40th wk
79	19	15	172.3	76.8	2.243	NO	PRIMI	N	39th wk
80	23	16	240.2	80.1	2.998	YES	PRIMI	N	38th wk
81	28	17	173.1	128	1.352	NO	G2A1	N	39th wk
82	18	17	286.5	220	1.302	NO	G2P1	PRETERM	35th wk
83	19	17	91.82	86	1.067	NO	PRIMI	N	40th wk
84	20	17	174.2	178.3	0.978	NO	PRIMI	N	37th wk
85	27	18	266.2	375	0.709	NO	PRIMI	N	39th wk

86	21	18	179	220	0.813	NO	G3P2L1	N	38th wk
87	19	19	194.2	110	1.765	NO	PRIMI	N	39th wk
88	20	18	1354	213.8	6.333	YES	PRIMI	PRETERM	36th wk
89	17	17	199.9	405	0.493	NO	PRIMI	N	40th wk
90	21	19	172.5	106	1.627	NO	PRIMI	N	38th wk
91	24	18	158.99	206	0.771	NO	G3P2L1	N	40th wk
92	23	19	1124.2	629.8	1.785	NO	G3P1L1A1	IUD	35th wk
93	38	20	51.2	275	0.186	NO	G3P2L1	N	39th wk
94	19	18	286.65	220	1.302	NO	PRIMI	N	40th wk
95	18	18	215.2	200	1.076	NO	PRIMI	N	40th wk
96	20	18	205.65	183	1.123	NO	G2P1L0	N	37th wk
97	31	19	189.9	106	1.791	NO	PRIMI	N	36th wk
98	26	18	178.15	120	1.484	NO	G2P1L1	N	38th wk
99	25	20	1293	562.5	2.298	YES	G2P1L1	PRETERM	36th wk
100	19	20	180.5	542.8	0.332	NO	PRIMI	N	39th wk

S week - Sampling week

Comp - Complications

G Code - Gestational Code

N - Normal