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Evaluation Of Soluble Fms-Like Tyrosine Kinase-1 (Sflt-1) In Preeclampsia Patients Attending A Tertiary Care Hospital In North-East India.

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Abstract:
Background and aim*: Preeclampsia is a pregnancy-specific multi-organ syndrome that affects 2% to 8% of pregnancy. It is a unique condition of placental pathogenesis with acute onset of predominantly cardiovascular manifestations attributable to generalized vascular endothelial activation and vasospasm resulting in hypertension and multi-organ hypoperfusion. Elevation of serum sFlt-1 level that is associated with inhibition of nitric oxide signalling can contribute to the pathogenesis of preeclampsia. Therefore the aim of this study is to evaluate the soluble fms-like tyrosine kinase-1 in patients with preeclampsia.*
Materials and Methods*: A case control study was conducted in the Department of Physiology, RIMS, Imphal, Manipur among 40 preeclampsia patients and 40 controls (healthy pregnant women). Soluble fms-like tyrosine kinase-1 (sFlt-1) was analyzed with ErbaLisaScan EM, Automated Microplate ELISA reader- (Model no SR No.120710 Scan EM, Transasia Bio-Medical Ltd, Mumbai) and Human Soluble Vascular Endothelial Growth Factor Receptor 1 ELISA kit. Data collected was analyzed using SPSS version 21(IBM).A $p<0.05$ was taken as significant.*
Results*: In our study, the sFlt-1 concentration was significantly higher in patients with preeclampsia than healthy pregnant women (p value < 0.001).*
Conclusion: *The sFlt-1 concentration differentiated women destined to develop pre-eclampsia from those with chronic hypertension and those who had presented with a diagnostic rise in maternal blood pressure with no other feature of pre-eclampsia. This test has important clinical utility as it targets patients at high risk of developing pre-eclampsia, that is, those women with uncertainty in their diagnosis. Thus, estimation of serum sFlt-1 can be used for early detection and as a prognostic marker of preeclampsia.*
Key Word*: Preeclampsia, Soluble fms-like tyrosine kinase-1, Anti- angiogenic factor, Pregnancy*

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I. INTRODUCTION

Preeclampsia is a pregnancy-specific multi-organ syndrome that affects 2% to 8% of pregnancy.[1]It is a unique condition of placental pathogenesis with acute onset of predominantly cardiovascular manifestations attributable to generalized vascular endothelial activation and vasospasm resulting in hypertension and multi-organ hypoperfusion.[2]

Overproduction of soluble fms-like tyrosine kinase-1 (sFLT1) in the placenta is a major cause of vascular dysfunction in preeclampsia through sFLT1dependent antagonism of VEGF. However, the cause of placental sFLT1 upregulation is not known. Soluble fms-like tyrosine kinase-1 sFLT1 is upregulated in placental trophoblasts, while VEGF is upregulated in adjacent maternal decidual cells. It has been found that endometrial-specific VEGF overexpression induced placental sFLT1 production and elevated sFLT1 levels in maternal serum. This led to pregnancy losses, placental vascular defects, and preeclampsia-like symptoms, including hypertension, proteinuria, and glomerular endotheliosis in the mother. Knockdown of placental sFlt1 with a trophoblast-specific transgene caused placental vascular changes that were consistent with excess VEGF activity. Moreover, sFlt1 knockdown in VEGF-overexpressing animals enhanced symptoms produced by VEGF overexpression alone. These findings indicate that sFLT1 plays an essential role in maintaining vascular integrity in the placenta by sequestering excess maternal VEGF and suggest that a local increase in VEGF can trigger placental overexpression of sFLT1, potentially contributing to the development of preeclampsia and other pregnancy complications.[3]

High blood pressure and proteinuria have low predictive value for preeclampsia and its associated adverse outcomes. Angiogenic and antiangiogenic factors have been implicated in the pathophysiology of preeclampsia.[4]

In preeclampsia there is placental overproduction of anti-angiogenic proteins namely soluble Fms – like tyrosine kinase 1(sFlt-1) & soluble endoglin(sEng).Increased sFlts inactivate & decrease circulating free placental growth factor (PlGF) & vascular endothelial growth factor(VEGF). Increased sEng decreases endothelial nitric oxide dependent vasodilatation.[5]

The abnormal invasion of placenta and the release of placenta-derived adverse factors during the first trimester are thought to be the main cause of the extensive damage to the maternal endothelium and systemic inflammatory response involving many systems and organs in late pregnancy. To date, there is no effective treatment for PE in addition to the termination of pregnancy. Therefore, a reliable predictor for PE would play an important role in early prevention and intervention. PE can be classified into two degrees, mild PE (mPE) and severe PE (sPE), and there are different treatments and clinical outcomes for each degree. It is necessary to predict the severity of PE for rational gestational management.[6]

II. MATERIAL & METHODS

Study design: The study design is a case control study.

Study Location: This study was carried out in the Department of Physiology in collaboration with Department of Obstetrics & Gynaecology, Regional Institute of Medical Sciences (RIMS), Imphal.

Study duration: Two (2) years: September 2018-August 2020

Sample size: 80 participants.

Sample size calculation: A purposive sampling was done. It is a matched case control study, so gestational age matched normal pregnant women were enrolled for each case of pre-eclamptic pregnant women and case to control ratio is (1:1). Sample size based on the previous data from the study of Chauhan P et al[7] was undertaken. The sample size was calculated by using the formula:

$$\frac{(u+v)^2(S_1^2+S_2^2)}{(m_1-m_2)^2}$$

Where: m1 (mean of 1st study population): 222.93
m2 (mean of 2nd study population): 157.18
u=1.28 (power=90)
v= 1.96 (5% level)
S1= (standard deviation of 1st study population): 97.94
S2= (standard deviation of 2ndstudy population): 56.66

Study population: The study population will be divided into two groups as follows:

• Case group consisting of known cases of pre-eclampsia> 20 weeks gestation belonging to age group of 18-45 years attending Obstetrics out-patient department and in-patient antenatal ward, RIMS, Imphal.

• Control group consisting of normal pregnant women > 20 weeks gestation belonging to age group of 18-45 years attending, Obstetrics OPD.

Inclusion Criteria:

- **Controls:** Normal healthy pregnant women in the range of 18 – 45 years of age and >20 weeks gestation for control group.
- **Cases:** Diagnosed case of preeclampsia having fulfilled the following diagnostic criteria:
- Maternal systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90 mmHg measured at resting for two times at 4- hour intervals after 20 weeks of gestation in a previously normotensive woman
- Proteinuria based on either measurement of ≥300mg per 24-hour urine collection or at least one positive dipstick reading.

Exclusion criteria:

- History of thyroid dysfunction, cardiovascular disease, diabetes mellitus, chronic hypertension, chronic liver disease, and chronic renal disease
- Pregnant women of age <18 years &>45years.
- History of multiple gestations.
- History of intake of any antihypertensive drug.

Study variables:
- SBP, DBP
- Weight, height
- sFlt-1

Study tools:
1. ErbaLisaScan EM, Automated Microplate ELISA reader- (Model no SR No.120710 Scan EM, Transasia Bio-Medical Ltd, Mumbai)
2. Mercury Sphygmomanometer-Diamond, Industrial Electronic & Allied Products, Pune, India.
3. Stethoscope-Littmann quality, 113H39682, Made in USA.
4. Weighing machine (Victoria DX, Ramon surgical co.ltd. Delhi)

Procedure: The study was carried out after obtaining clearance from the Research Ethics Board (REB), RIMS, Imphal. All the subjects for the study were explained about the nature and purpose of the study. Those subjects willing to participate in the study were included after obtaining informed consent.

The subjects had undergone detailed general physical and systemic examination. Physical examination of all the subjects included measurement of height in centimetres, weight in kilograms, recording of pulse rate by palpating the radial artery and blood pressure recording with mercury sphygmomanometer using appropriate sized cuff. Clinical examination of the cardiovascular system and respiratory system was done. The findings of the examinations were recorded in proforma.

Blood sample collection:

a. Blood samples of 2ml were drawn from the antecubital vein with aseptic precaution from each subject after taking prior consent.

b. Then blood was collected in a plain sterile vial for ELISA. After blood centrifugation, the serum will be stored at freezer. Prior to use, all the blood samples will be brought to room temperature and estimation of serum sFlt-1 by (ELISA) method. The titres of the ELISA test of each subject will be recorded

Statistical analysis: Data were entered and analyzed using IBM SPSS statistics version 21 for windows. Data were summarized using descriptive statistics like percentages for categorical data, means (standard deviation) and median for continuous data. Student's t- test was used for data analysis with normal distribution. A p value of < 0.05 was taken as significant.

III. RESULTS:

The study included 80 pariticipants, 40 preeclampsia patients were included as cases and 40 normal healthy pregnancy of same gestational age were included as controls. Figure 1 shows the age distribution of the participants. The minimum age of the participants was 18 years and maximum age was 45 years with a mean age of 23.5±5.49 years in the case group and 27.89±8.55 in the control group. Majority of the participants belong to the age group of 18-25 years (51.7%) in case group and in control group belong to the age group of 26 -35years (54.4%.) Difference observed was found to be statistically significant ($p<0.05$).

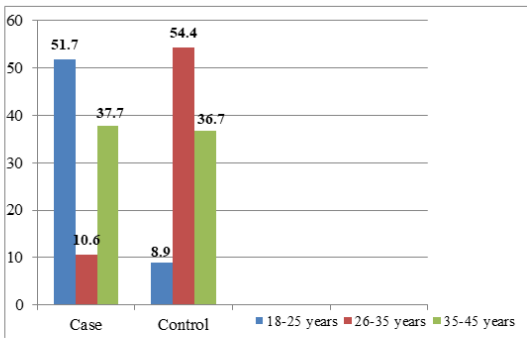

Fig 1: Age distribution of the participants (N=80)

Figure 2 shows Case group having higher SBP (156 ± 10) mmHg and higher DBP (96.6±5.6) mmHg compared to the control group SBP (110 ± 12) mmHg, DBP (76±10) mmHg. Difference observed was found to be statistically significant (p<0.05)

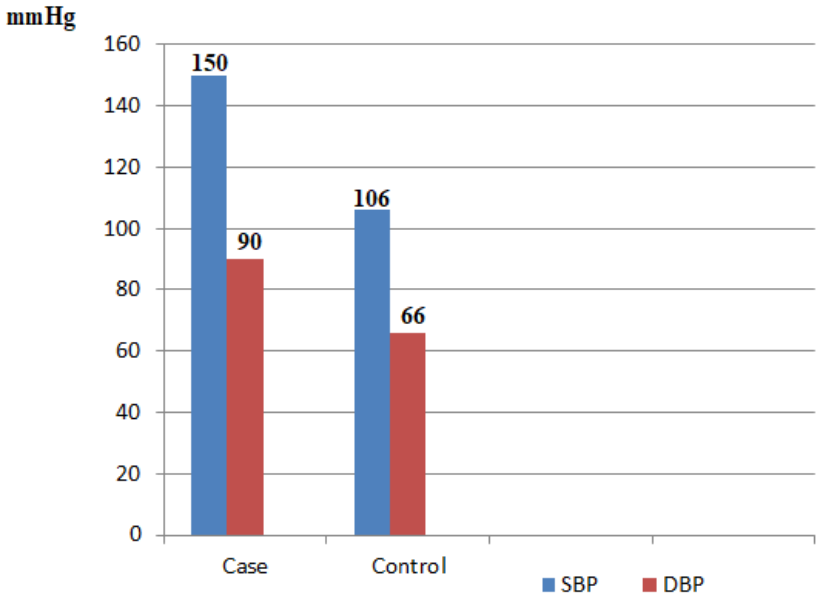

Figure 2. Blood pressure of the participants (N=80)

Figure 3 shows case group having higher BMI (31.6 ± 7.21) kg/m2 compared to the control group (28.14 ± 8.4)kg/m2.Difference observed was found to be statistically significant (p<0.05).

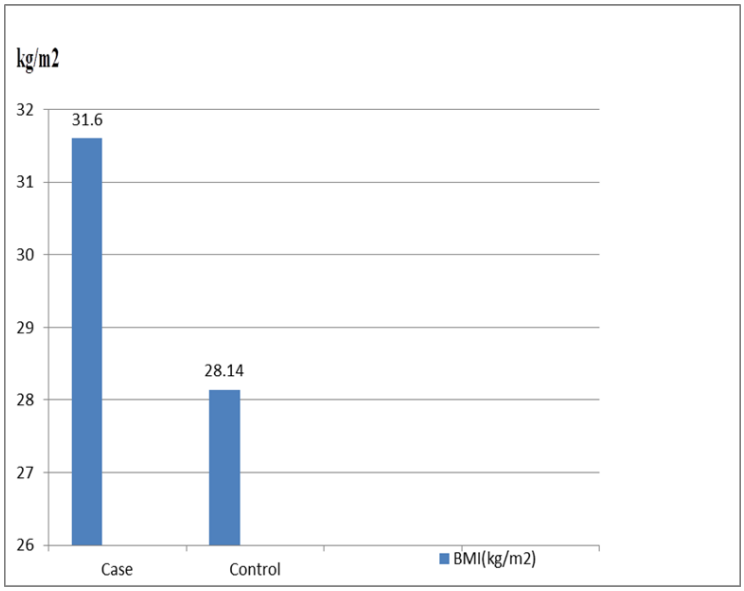

Figure 3. BMI of the participants (N=80

Figure 4.shows that in control group sFlt-1 mean value is (1279±65) pg/ml. In study group sFlt-1 mean value is (6265± 146) pg/ml. Difference observed was found to be statistically significant (p<0.05).

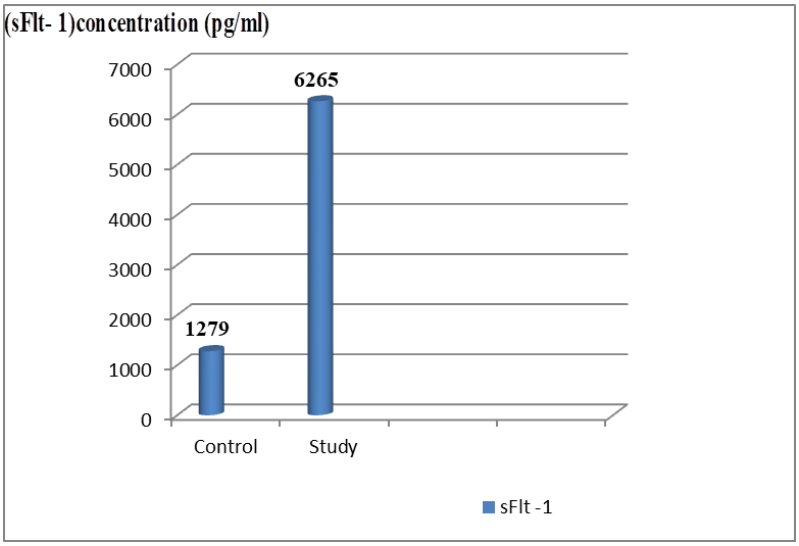

Figure 4: Anti – angiogenic factor (sFlt- 1) concentration (pg/ml) of the participants (N=80)

Table1 shows the baseline study variables and in table2 comparison of outcome variables between the study groups has shown.

Parameters	Study group(n=40) Mean ± SD	Control group(n=40) Mean ± SD	P value
Age,yrs	23.5±5.49	27.89±8.55	0.033
BMI(kg/m2)	31.6±7.21	28.14±8.4	0.06
Mean pregnancy duration, wk	31.2± 6.2	28.6±7.5	0.01
SBP mm Hg	156±10	110±12	0.028
DBP mm Hg	96.6±5.6	76±10	0.017

Table 1. Baseline variables of the study & control group

Variable	Study group (n=40) Mean ± SD	Control group (n=40) Mean ± SD	P value
Hemoglobin, g/dL	11.1 ± 2.3	12.2 ± 3.1	0.642
Hematocrit, %	35.4 ± 2.32	34.3 ± 2.4	0.3471
Red cell distribution width , %	17.14 ± 2.7	16.19 ± 2.0	0.025
sFlt- 1,pg/ml	6265 ± 146	1279 ± 65	0.001

Table 2. Comparison of outcome variables of the study & control group

IV. Discussion:

In the present study, sFlt-1 concentration was higher in case group than in control (6265 vs. 1279 pg/mL,p < 0.001).These results are similar to Maynard SE et al who also concluded that preeclampsia is associated with elevated circulating sFlt1 protein. It is likely that the excess sFlt1 production originates in the placenta, they also have shown that placental sFlt1 mRNA is up regulated in preeclampsia and that levels fall within 48 hours after delivery.[8]

Fan X et al conducted a study among 25 preeclamptic subjects and 34 gestational age-matched normal subjects (without preeclampsia or any other pregnancy complication), which showed strong evidence that overproduction of soluble fms-like tyrosine kinase-1 (sFLT1) in the placenta was a major cause of vascular dysfunction in preeclampsia through sFLT1dependent antagonism of VEGF. In that study they demonstrated that in women with preeclampsia, sFLT1 is upregulated in placental trophoblasts, while VEGF is upregulated in adjacent maternal decidual cells. In response to VEGF, expression of sFlt1 mRNA, but not full-length Flt1 mRNA, increased in cultured murine trophoblast stem cells. They developed a method for transgene expression

specifically in mouse endometrium and found that endometrial-specific VEGF overexpression induced placental sFLT1 production and elevated sFLT1 levels in maternal serum. This led to pregnancy losses, placental vascular defects, and preeclampsia-like symptoms, including hypertension, proteinuria, and glomerular endotheliosis in the mother. Knockdown of placental sFlt1 with a trophoblast-specific transgene caused placental vascular changes that were consistent with excess VEGF activity. Moreover, sFlt1 knockdown in VEGF-overexpressing animals enhanced symptoms produced by VEGF overexpression alone. These findings indicated that sFLT1 plays an essential role in maintaining vascular integrity in the placenta by sequestering excess maternal VEGF and suggest that a local increase in VEGF can trigger placental overexpression of sFLT1, potentially contributing to the development of preeclampsia and other pregnancy complications.[3]

Hagmann H et al discussed about the role of circulating angiogenic proteins in the diagnosis and prediction of preeclampsia. Extensive work clearly identifies sFlt-1, PlGF, and sEng alterations during the early second trimester as powerful tools in predicting preeclampsia, allowing in particular the identification of early forms of the disease.IncreasedsFlt-1,lowPlGF, and/or increased sFlt-1/PlGF ratio were particularly useful for the diagnosis and prognosis of preeclampsia in the triage setting and may be useful in the management of established preeclampsia. They also suggested that clinical utility of these angiogenic markers could directly affect obstetrician's management decisions, improve health outcomes, and/or reduce costs to the health care system.[9]

Verlohren S et al conducted a study in which they have investigated 388 singleton pregnancies with normal pregnancy outcome, 164 with PE, 36 with gestational hypertension, and 42 with chronic hypertension.sFlt-1andPlGFwere measured in serum samples.Patients with preeclampsia had a significantly increased sFlt-1/PlGF ratio as compared with controls and with patients with chronic and gestational hypertension in <34 weeks and ≥34 weeks (P<.001). Time to delivery was significantly reduced in women with preeclampsia in the highest quartile of the sFlt-1/PlGFratio (P<.001). They concluded that sFlt-1/PlGF ratio allows the identification of women at risk for imminent delivery and was a reliable tool to discriminate between different types of pregnancy-related hypertensive disorders.[10]

Kandi S et al studied the effect of pre-eclampsia on renal cardiovascular, hepatic and the thyroid functions. Pre-eclampsia was associated with an increased release of soluble growth factor like sFlt 1 from placenta, which decreases the availability of Vascular Endothelial Growth Factor (VEGF) receptors and decreases the availability of endothelial nitric oxide synthase. This in-turn decreasesd the diastolic relaxation and endothelial dysfunction, podocytes injury of glomerular cells, increased excretion of podocytes specific proteins in urine leads to proteinuria. Cardiac hypertrophy complicates the heart function. There is a raise in liver function, soluble factor like thyroxine kinase 1 (sFlt 1) may cause a disturbance in thyroid hormone function, thus prone to thyroid problem during or after pregnancy.[11]

Omran AA et al conducted a study among 50 pregnant females at second trimester of pregnancy divided into 25 normotensive pregnant females who remained normotensive till delivery (group I) and 25 high risk pregnant females who subsequently developed PE (group II). Twenty five healthy non-pregnant females served as control (group III). Maternal blood samples were collected at 14-18 gestational weeks. EDTA samples were investigated for both neutrophil- and monocyte-flt-1 by flowcytometer. Stored serum samples were analyzed for sFlt-1, PTX3, NO and AFP by ELISA. Alpha fetoprotein, sflt-1 and pentraxin 3 were found to be statistically significantly increased in group II when compared with group I (P- value=0.024, <0.001 & 0.006) and group III (P-value=<0.001). However, there was statistically significant decrease in neutrophil-flt-1 and nitric oxide in group II when compared with group I (P-value=<0.001 & 0.016). Group II had significant negative correlation between soluble flt-1 and both neutrophil- & monocyte-flt-1 (P-value=<0.001 & 0.009) and between neutrophil-flt-1 and PTX3 (P-value=0.007). Soluble flt-1 was found to have the highest predictive value for predicting preeclampsia (AUC=0.941& P- value=<0.001).So from that study it was concluded that soluble flt-1 was the best single biomarker to predict preeclampsia at second trimester of pregnancy.[12]

Any increase in serum sFlt-1 will cause decrease in serum-free VEGF and PlGF. During normal pregnancy, sFlt-1 serum levels increase with advancing gestation but are markedly increased in both serum and placental tissue from preeclamptic pregnancies. Significant rise in the serum sflt-1 value in pre-eclamptic women was found when compared with gestational age matched control women in the present study.

V. Conclusion:

During normal pregnancy, sFlt-1 serum levels increase with advancing gestation but are markedly increased in both serum and placental tissue from preeclamptic pregnancies. Increased serum levels of sFlt-1 in preeclampsia have been argued as a potential direct cause of several manifestations of the disease and it has been postulated that in preeclampsia, the abnormal placentation following placental hypoxia may result in increased sFlt-1 levels, thus contributing to the pathogenesis of this disease.

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