

## Original Article

# Plasma Soluble Fms-like Tyrosine Kinase-1 Works as Sole Predictor of Pre-Eclampsia at Mid-Trimester of Pregnancy

<sup>1</sup>Khalid Yousuf Memon, <sup>2</sup>Ikramdin Ujjan, <sup>3</sup>Nailla Yousuf Memon, <sup>4</sup>Syed Hasan Ala, <sup>5</sup>Fozia Shaikh,

<sup>6</sup>Abid Hussain Chang

<sup>1,2,6</sup>Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro,

<sup>3</sup>Department of Gynecology/Obstetrics, Unit 1 Peoples University of Medical & Health Sciences, Banzirabad

<sup>4</sup>Department of Gynecology/Obstetrics, Dow University of Medical & Health Sciences, Karachi.

<sup>5</sup>Department of biochemistry, Liaquat University of Medical & Health Sciences, Jamshoro.

**Correspondence:** Dr. Khalid Yousuf Memon

Department of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro

Email: kymemon1@gmail.com

## Abstract

**Objective:** To determine the soluble fms-like tyrosine kinase-1 (sFlt-1) as a predictor of pre-eclampsia at mid trimester of pregnancy.

**Methodology:** A Case-Control study was carried out in the departments of Obstetrics/Gynecology and Pathology, at LUMHS, Hospital Jamshoro/Hyderabad over a period of twelve months from 06-02-2016 to 06-02-2017. All the pregnant ladies of different parity with USG findings of >13- weeks of gestation were included in this study. They were divided into two groups. Group-A were those with pre-eclampsia and Group-B, who were normotensive as control group. Blood samples were collected from all the participants at 15-20 weeks of gestation during antenatal visits. The serum was separated by 2,500-g centrifugation for 10-min. Aliquots of serum were stored at -80°C.

**Results:** In this study 384 women with pre-eclampsia group A and 50 normotensives as control Group B were studied. Mean age of group A women was 27.46+3.91 years and mean age of group B was 28.0+4.0 years, (P= 0.362). There was no significant difference between mean gestational age of group A and group B (P=0.346). Mean of systolic BP was 147.43+10.83 mmHg and diastolic 103.15+6.39 mmHg in group A ladies. Mean of soluble fms-like tyrosine kinase-1 was significantly higher 5995.5+12838.87 pg/mL, in group A ladies, when compared to group B (P= 0.0001). There was positive correlation between systolic BP and sFlt-1, (r= 0.095), as well as between diastolic BP and sFlt-1, (r= 0.039).

**Conclusion:** It is concluded that sFlt-1 is an important marker for the diagnosis of pre-eclampsia, as it shows significant association and positive correlation with pre-eclampsia in this study.

**Keywords:** Soluble fms-like tyrosine kinase-1, pre-eclampsia.

Cite this article as: Memon KY, Ujjan I, Memon NY, Ala SH, Shaikh F, Chang AH. Plasma Soluble Fms-like Tyrosine Kinase-1 Works as Sole Predictor of Pre-Eclampsia at Mid-Trimester of Pregnancy J Soc Obstet Gynaecol Pak. 2021; 11(2):117-120.

## Introduction

Pre-eclampsia has been an important cause of fetal and maternal morbidity & mortality.<sup>1</sup> It is described as hypertension with BP ~140/90 mm Hg on 2 events 4–6 hours apart or one reading of diastolic BP of >110 mm Hg and proteinuria development following 20 weeks of pregnancy, up to 6 weeks post-partum in earlier normotensive, non-proteinuric females.<sup>2</sup> Maternal mortality is a concern of public wellbeing globally and >99 % of maternal deaths take place in less developed nations. The frequent factors of direct maternal mortality are hemorrhage, sepsis and pre-eclampsia/eclampsia.<sup>3</sup>

In Pakistan, deaths due to pre-eclampsia/eclampsia represent one-third of maternal deaths reported at tertiary care hospital settings.<sup>3</sup>

Pakistan is the sixth most populous country in the world. A recent systematic analysis of global mortality ranked Pakistan as the country with the third highest burden of maternal, fetal, and child mortality.<sup>3,4</sup> According to the Pakistan Demographic and Health Survey 2006–07, the maternal mortality ratio (MMR) was 279 per 100,000 live births.<sup>5</sup>

Authorship Contribution: <sup>1,2</sup>Final approval of the version to be published, revising it critically for important intellectual content <sup>3,4</sup>Substantial contributions to the conception or design of the work drafting, <sup>5</sup>Literature review, statistical analysis

Funding Source: none

Conflict of Interest: none

Received: Jan 27, 2021

Accepted: June 07, 2021

There are wide variations between rural and urban populations; the MMR for Sindh province (which is predominantly rural) was as high as 345–350 per 100,000 live births.<sup>6</sup> Eclampsia is accountable for 34 % of maternal mortality among females admitted to a tertiary hospital within Pakistan.<sup>7</sup> The pathogenesis of pre-eclampsia remains incompletely explained, but increased attention has been directed toward the role of angiogenic and antiangiogenic factors including elevated sFlt1 and soluble endoglin (sEng, a receptor for members of the TGF $\beta$  superfamily), and lower PlGF.<sup>8-10</sup> In recent years sFlt-1 has been shown to be elevated among pregnant females with pre-eclampsia.<sup>11-12</sup> sFlt-1 is a splice variant of the vascular endothelial growth factor receptor Flt-1 and a potent inhibitor of vascular endothelial growth factor, with increased plasma concentrations detectable approximately 5 weeks before the onset of the disorder.<sup>13-14</sup> However, the studies conducted in this regard are not adequate, and despite explicit search, no local study has been found. Therefore, the purpose of this study is to determine the role of soluble fms-like tyrosine kinase-1 in early detection of pre-eclampsia in our setup, which will help not only in prevention of fetomaternal morbidity and mortality but also save cost and resources spent for the administration & treatment of this dreadful abnormality."

## Methodology

This case-Control study, was conducted at the departments of Obstetrics and Gynecology and Pathology, Liaquat University of Medical and Health Sciences, Jamshoro/Hyderabad, during a period of twelve months from 06-02-2016 to 06-02-2017. Women of all parity with more than 13-weeks of gestation as per USG findings were included in this study. All the women correlated with medical disorders like renal disease, diabetes mellitus, women with multiple gestation, antiphospholipid antibody syndrome, systemic lupus erythematosus, or any other autoimmune disease, and chronic corticosteroid drug use were excluded from the study. Informed consent was obtained from women who fulfilled the inclusion criteria. They were divided into two groups. Group-A were those with pre-eclampsia and Group-B, who were normotensive as control group. All information regarding demographic data, medical history, obstetrical history, general physical examination, and ultrasonography was recorded on a predesigned proforma. Pre-eclampsia was determined by systolic blood pressure (SBP)  $\geq 140$  mmHg or a diastolic BP (DBP)  $\geq 90$  mmHg on 2 successive measurements 4-6 hours apart, along with proteinuria.

Proteinuria was determined by collecting a spot mid-stream urine sample. Total protein concentration was measured by the biuret colorimeter assay. Blood samples were obtained from all participants of group A and Group B at 15-20 weeks of gestation during their antenatal visit. Maternal plasma samples will be isolated by 2,500-g centrifugation for 10-min. Aliquots of maternal plasma were stored at -80°C. Levels for sFlt-1 were measured by a fully automated Elecsys system. This assay was sandwich immunoassay based on the electrochemiluminescence technology. The cutoff value for sFlt-1 was set at 31.2-pg/mL.

The test sFlt-1 was performed as per the manufacturer's recommendations. This test was performed on sandwich principal which takes 18 minutes to complete the test. The kit is composed of three reagents, Reagent M, R1, R2 respectively. Reagent M contains Streptavidin-coated microparticles. Reagent R<sub>1</sub> contains Biotinylated monoclonal anti-sFlt-1 antibody (mouse) and R2 contains Monoclonal anti-sFlt-1 antibody (mouse) labeled with ruthenium. This test was performed on Cobas e411 analyzer which is closed and fully automated. During the test; patient labeled sample which was frozen at -80 degree Celsius was thawed and kept in sample rack of analyzer which incubates, mix, and make photometric calculation of the value of test. Result was determined by calibration curve.

SPSS version 22.0 was used for data analysis. Relevant descriptive statistics, frequencies and percentages were calculated for quantitative data like presence of hypertension, proteinuria, value and pre-eclampsia. Mean and standard deviation were calculated for continuous data like age, parity, systolic blood pressure, diastolic blood pressure, 24-hr urinary protein excretion, sFlt-1 level. Independent t-test was used to analyze the continuous variables whereas chi-square test was used to analyze categorical variables. P-values < 0.05 was considered significant."

## Results

In this study 384 women with pre-eclampsia group A and 50 normotensives as control Group B were studied. Mean age of group A was 27.46 $\pm$ 3.91 years and mean age of control group B was 28.0 $\pm$ 4.0 years, and there was no significant difference between mean ages of both groups ( $P= 0.362$ ). Similarly no significant difference was found between mean of gestational age of two groups ( $P=0.346$ ), as mean gestational age of pre-eclamptic women was 27.08 $\pm$ 2.34 weeks and mean gestation age of normal was 27.42 $\pm$ 2.61 weeks. Mean

of systolic blood pressure was significantly higher (147.43+10.83 mmHg) in group A women as compare to group B women (111.90+6.91 mmHg) ( $P= 0.0001$ ). Similarly, diastolic blood pressure was also significantly high (103.15+6.39 mmHg) in group A women as compare to group B (72.10+7.36 mmHg) ( $P= 0.0001$ ) as shown in **Table I**.

**Table I: Distribution of patients according to demographic characteristics (n=434)**

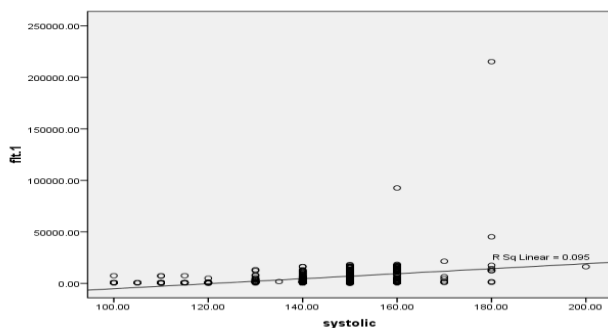
Statistic		Group A (n=384)	Group B (n=50)	p-value
Age	Mean	27.46 years	28.0 years	0.362
	SD	3.91 years	4.0 years	
Gestational age	Mean	27.08 weeks	27.42 weeks	0.346
	SD	2.34 weeks	2.61 weeks	
Systolic BP	Mean	147.43 mmHg	111.90 mmHg	0.001
	SD	10.83 mmHg	6.91 mmHg	
Diastolic BP	Mean	103.15 mmHg	72.10mmHg	0.001
	SD	06.39mmHg	7.36 mmHg	

Mean of soluble fms-like tyrosine kinase-1 was significantly higher 5995.5+12838.87 pg/mL, in group A, while mean of soluble fms-like tyrosine kinase-1 in group B was 130.18.45 pg/mL, ( $P= 0.0001$ ) as shown in **Table II**.

**Table II: Mean and Standard Deviation of sFlt-1 and urinary protein**

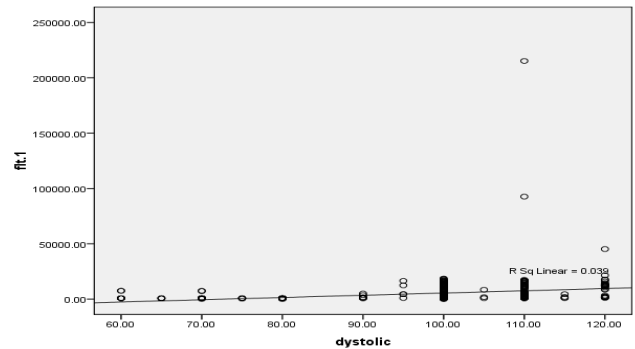
Statistic	Blood pressure		p-value	
	Group A (n=384)	Group B (n=50)		
sFlt-1	Mean	5995.5 pg/mL	130.45 pg/mL	0.0001
	SD	12838.87 pg/mL	18.45 pg/mL	
Urinary protein	Mean	1.54mg/L	1.14mg/L	0.001
	SD	0.72mg/L	0.35mg/L	

In this study there was positive correlation between systolic blood pressure and soluble fms-like tyrosine kinase-1 ( $r= 0.095$ ). **Figure 1**



**Figure 1. Pearson correlation between sFlt-1 and systolic**

In this study there was positive correlation between diastolic blood pressure and soluble fms-like tyrosine kinase-1, ( $r= 0.039$ ). **Figure 2**



**Figure 2. Pearson correlation between sFlt-1 and diastolic blood pressure.**

## Discussion

Soluble fms-like tyrosine kinase-1 (sFlt-1) contributes to pre-eclampsia development and is projected as a prediction marker in healthy pregnant females, but limited data is accessible in females with major pre-eclampsia risk factors.<sup>14</sup> In this study, there were no significant differences in the basic characteristics among women who developed pre-eclampsia compared to those with uncomplicated pregnancy. In this study, the mean age of patients with pre-eclampsia was 27.46+3.91 years, which was almost equal to control group i.e. 28.0+4.0 years. Similarly, in the study of Shibata E et al<sup>15</sup> the mean age of women with pre-eclampsia was 26.5 ± 6.0 years and the mean age of normal women was 26.2 ± 7.1 years which was statistically insignificant. In another study by Elsheikh WA et al<sup>16</sup> there was no difference in the maternal age between the women who developed pre-eclampsia compared to the normotensive controls; 29 ± 2.2 vs. 27 ± 3.8 years respectively, these findings are similar to our study.

In this study, no significant difference was found between the mean of gestational age of patients and normal pregnant women p-value 0.346.

In present study, we observed that elevated soluble fms-like tyrosine kinase-1 is an important marker for pre-eclampsia with 90% sensitivity and 100% specificity. We found mean of soluble fms-like tyrosine kinase-1 was significantly higher at 5995.5+12838.87 pg/mL, in women with pre-eclampsia,  $P= 0.0001$ . Similar results were observed by Gurnadi JI et al<sup>17</sup> that the concentration of sFlt-1 in severe pre-eclampsia is higher (20,524.75 pg/mL) compared with normal pregnancy (6820.4 pg/mL). This was also reported by Hassan MF et al<sup>18</sup> that in women with pre-eclampsia, the median sFlt-1 ratio was significantly higher compared to the control group (44.8 (16.2) versus 14.4 (6.1) with  $P=$

0.0000. This led to many studies<sup>18-25</sup> conducted to explore the role of circulating anti- and pro-angiogenic factors in predicting the development of pre-eclampsia. Although studies<sup>24,25</sup> have been carried out to determine the role of these markers.

## Conclusion

It is concluded that sFlt-1 is an important marker for predicting pre-eclampsia, it showed significant association and positive correlation with pre-eclampsia. However large prospective multicenter studies are required to confirm these results and to employ these in clinical practice besides the assessment of the cost-effectiveness.

## References

- Duckitt K, Harrington D. Risk factors for pre-eclampsia antenatal booking: systemic review of controlled studies *BMJ*. 2005;330:565-7.
- Ayaz A, Muhammad T, Hussain SA, Habib S. Neonatal outcome in pre-eclamptic patients. *J Ayub Med Coll Abbottabad*. 2009;21(2):53-5.
- Khowaja AR, Qureshi RN, Sheikh S, Zaidi S, Salam R, Sawchuck D, Vidler M, von Dadelszen P, Bhutta Z. Community's perceptions of pre-eclampsia and eclampsia in Sindh Pakistan: a qualitative study. *Reproductive Health*. 2016;13(1):36.
- Wikimedia. Islamic Republic of Pakistan. 2014. <http://en.wikipedia.org/wiki/Pakistan>. Accessed 18 Marh 2015.
- Bhutta ZA, Hafeez A, Rizvi A, Ali N, Khan A, Ahmad F, Bhutta S, Hazir T, Zaidi A, Jafarey SN. Reproductive, maternal, newborn, and child health in Pakistan: challenges and opportunities. *The Lancet*. 2013;381(9884):2207-18.
- Pakistan Demographic and Health Survey 2006 - 07. <http://dhsprogram.com/pubs/pdf/FR200/FR200.pdf>. Accessed 18 September 2015.
- United Nations Development Program Pakistan & Government of Sindh. Report on the status of millennium development goals sindh 2012. <http://www.pg.undp.org/content/dam/pakistan/docs/MDGs/UNDP-PK-MDG-sindh-report-2012.pdf>. Accessed 18 March 2015.
- Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, et al. Circulating angiogenic factors and the risk of pre-eclampsia. *New England Journal of Medicine*. 2004;350:672-683.
- Powers RW, Roberts JM, Cooper KM, Gallaher MJ, Frank MP, et al. Maternal serum soluble fms-like tyrosine kinase 1 concentrations are not raised in early pregnancy and decrease more slowly postpartum in women who develop pre-eclampsia. *American Journal of Obstetrics & Gynecology*. 2005;193:185-191
- Levine RJ, Qian C, Maynard SE, Yu KF, Epstein FH, et al. Serum sFlt1 concentration during pre-eclampsia and mid trimester blood pressure in healthy nulliparous women. *American Journal of Obstetrics & Gynecology*. 2006;194:1034-1041.
- Miranda AL, Galicia IC, Salas II, Leanos RR, Arauz JFR, Mendez JAA, Aguirre AU. Changes in circulating concentrations of soluble fms-like tyrosine kinase-1 and placental growth factor measured by automated electrochemiluminescence immunoassays methods are predictors of pre-eclampsia. *J Hypertens*. 2012;30:2173-81.
- Hassan MF, Rund NMA, Salama AH. An elevated maternal plasma soluble fms-like tyrosine kinase-1 to placental growth factor ratio at midtrimester is a useful predictor for pre-eclampsia. [online] *ObstetGynecol Int*. 2013. <http://dx.doi.org/10.1155/2013/202346>.
- Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, et al. An automated method for the determination of the sFlt-1/PIGF ratio in assessment of pre-eclampsia. *Am J Obstet Gynecol*. 2010;202:161.e1-11.
- Cohen A, Lim KH, Lee Y, Rana S, Karumanchi SA, Brown F. Circulating levels of the antiangiogenic marker soluble fms-Like Tyrosine Kinase-1 are elevated in women with pregestational diabetes and pre-eclampsia. *Diabetes Care*. 2007;2:375-7.
- Shibata E, Rajakumar A, Powers RW, Larkin RW, Gilmour C, Bodnar LM, Crombleholme WR, Ness RB, Roberts JM, Hubel CA. Soluble fms-like tyrosine kinase 1 is raised in pre-eclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. *The Journal of Clinical Endocrinology & Metabolism*. 2005 Aug 1;90(8):4895-903.
- Elsheikh WA, Behery MA, Farag EA, Hassan FI, Abdelaziz FA, Alkholi MM. The Role Of Second Trimester Uterine Artery Doppler Ultrasound, Inhibin-A And Placental Growth Factor In Prediction Of Pre-Eclampsia. *Zagazig University Medical Journal*. 2016;22(4):1-1
- Gurnadi JI, Mose J, Handono B, Satari MH, Anwar AD, Fauziah PN, Pramartirta AY, Rihibiha DD. Difference of concentration of placental soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PIGF), and sFlt-1/PIGF ratio in severe pre-eclampsia and normal pregnancy. *BMC research notes*. 2015;8(1):534.
- Hassan MF, Rund NM, Salama AH. An elevated maternal plasma soluble fms-like tyrosine kinase-1 to placental growth factor ratio at midtrimester is a useful predictor for pre-eclampsia. *Obstetrics and gynecology international*. 2013.
- Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension and proteinuria in pre-eclampsia. *J Clin Invest*. 2003; 111(5): 649-58.
- Maynard, SE, Venkatesha S, Thadhani, R, Karumanchi SA. Soluble fms-like tyrosine kinase 1 and endothelial dysfunction in the pathogenesis of pre-eclampsia. *Pediatr Res*. 2005; 57(5 Pt 2): 1R-7R.
- Makris A, Thornton C, Thompson J, et al. Uteroplacental ischemia results in proteinuric hypertension and elevated sFlt-1. *Kidney Int*. 2007; 71(10): 977-84.
- Powers RW, Jeyabalan A, Clifton RG, Van Dorsten P, Hauth JC, Klebanoff MA, Lindheimer MD, et al. Soluble fms-like tyrosine kinase 1 (sFlt1), endoglin and placental growth factor (PIGF) in pre-eclampsia among high risk pregnancies. *PLoS One*. 2010; 5(10): e13263.
- Verlohren S, Galindo A, Schlembach, D, et al. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of pre-eclampsia. *Am J Obstet Gynecol*. 2010; 202(2): 161.e1-161.e11.
- Sibai BM, Koch MA, Freire S, et al. Serum inhibin A and angiogenic factor levels in pregnancies with previous pre-eclampsia and/or chronic hypertension: are they useful markers for prediction of subsequent pre-eclampsia. *Am J Obstet Gynecol*. 2008; 199(3): 268.e1-9.
- Kim SY, Ryu HM, Yang JH, et al. Raised sFlt-1 to PIGF ratio in women who subsequently develop pre-eclampsia. *J Korean Med Sci*. 2007; 22(5): 873-7.