Serum levels of soluble Endoglin, soluble FMS-like Tyrosine kinase-1, and Uterine Artery Doppler in Pre-eclamptic patients

Elattar N.[1], Swelam E[1]., El Anwar A.[2]

Clinical Pathology Department[1], Gynecology &Obstetric Department[2], Faculty of Medicine, Zagazig University
enasswelam19_8@yahoo.com

Abstract: Background: Pre-eclampsia, a pregnancy-specific disorder, contributes substantially morbidity and mortality of both mother and newborn. An increasing number of biochemical agents were evaluated as markers for predicting pre-eclampsia, much effort has been put into assessing noval potential markers and their combination with other screening methods such as Doppler sonography. The aim of the study was to assess the serum levels of soluble Endoglin (sEng) , soluble FMS like tyrosine kinase-1 (sFlt-1), and Uterine Artery Doppler in pre-eclampsia to evaluate their clinical utility in diagnosis, and assessment of severity of the disease. **Patients and Methods:** The study was conducted on 35 pre-eclamptic patients and 20 healthy pregnant control subjects. All individuals were subjected to clinical examination and estimation of sEng and sFlt-1 by enzyme linked immunosorbent assay and estimation of ALT, Platelet count and urinary proteins, Uterine Artery Doppler measured as pulsatility Index (PI). **Results:** revealed highly significant increase in sEng and sflt-1,PI and systolic and diastolic blood pressure (SBP and DBP), proteins in urine, and ALT in patients than control(p<0.001), and in severe preeclampsia than in mild preeclampsia (p<0.001),. sEng and sFlt-1 were significantly increased in pre-term pre-eclampsia than in term per-eclampsia and in patients with abnormal Doppler than in normal Doppler(p<0.001). PI was not significantly increased in pre-term than term pre-eclampsia (p>0.05). sEng is positively correlated to sFlt-1(r =0.94) and both of them are positively correlated to SBP, DBP, PI, urinary proteins, and negatively correlated with gestational age and platelet count. Receiver operating characteristic (ROC) curve analysis was applied to evaluate the diagnostic utility of sEng, sFlt-1,and PI for discriminating the early onset from late onset pre-eclampsia , the best diagnostic cut off levels for sEng and sFlt-1 were >15 ng/ml, >900 pg/ml respectively. Both had a diagnostic sensitivity of 100%, specificity 78.9%,accuracy 88.6%, positive predictive value(PPV) 80% and negative predictive value(NPV) 100%, the area under the curve 0.97, 0.99 respectively . While the cut off levels for P1>1.42 , had a diagnostic sensitivity of 68% specificity, 58%, accuracy 62%, PPV 58% and NPV 58%. Also (ROC) curve analysis was applied to evaluate the diagnostic utility of sEng, sFlt-1,and PI for discriminating the mild from severe pre-eclampsia they had a diagnostic sensitivity 89%, 83% specificity 65%, 70% ,accuracy for both 77%, PPV, 72%, 75% and NPV 84%, 80% respectively .As regard PI had a diagnostic sensitivity of 100%, specificity 94%, accuracy 97%, PPV 95% and NPV 100%. **Conclusion:** The results of this study indicate that s Eng, and sFlt-1 are efficient in prediction of early onset pre-eclampsia and can discriminate between severe and mild pre-eclampsia, and both with pulsatility index of Doppler give better prediction of pre-eclampsia. This results will help in finding a new strategy for early management and so reduction of associated complication of pre-eclampsia.

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1-Introduction

Pre-eclampsia is a multi-system pregnancy specific hypertensive syndrome which is characterized by hypertension and proteinuria after 20 weeks of gestation that causes substantial maternal and fetal morbidity and mortality. The lack of an effective test for identification for women at risk of developing pre-eclampsia remains a contributing factor for the high morbidity of the disease. In most developing countries, where the incidence of the disease is high, women present late with complications.[1]

Although pre-eclampsia is called the disease of theories, the overwhelming evidence points to endothelial dysfunction as the central mechanism in the pathogenesis of the maternal syndrome in pre-eclampsia. The causes of this endothelial dysfunction remain elusive. However, poor placentation has been proposed as a major factor.[2]

Ischemic placenta secretes soluble factors into the maternal vasculature which have been implicated in inducing the endothelial dysfunction and the clinical features of pre-eclampsia. Excess secretion of naturally occurring anti-angiogenic molecule of placental origin referred to as soluble endoglin (sEng) and soluble Fms-like tyrosine kinase 1(sFlt-1) may contribute to the pathogensis of pre-eclampsia.[3]

Soluble endoglin acts by antagonizing an angiogenic and vasodilator molecule know as

transforming growth factor beta-1 (TGF-β1*)* which is important not only in angiogenesis but also in keeping the lining of the blood vessels healthy. As a result the cells lining of the blood vessels begin to sicken and die, the blood pressure increases and the blood vessels leak protein into the tissues and urine[3].Soluble endoglin is elevated not only during clinical pre-eclampsia but also 2-3 months before onset of clinical symptoms. It was also suggested that sEng correlates with disease severity and falls after delivery. Therefore, this anti-angiongenic protein in the maternal blood is a subject research as potential diagnostic and screening test for pre-eclampsia.[4]

Soluble fms-like tyrosine kinase 1 (sFlt1) is truncated form of the Flt-1 receptor [vascular endothelial growth factor receptor-1(VEGFR1)] including the extracellular ligand-binding domain, but not the trans membrane and intracellular domains; it is secreted (hence named "soluble") and antagonizes VEGF and placental growth factor (PIGF) in the circulation by binding and presenting their interaction with their endothelial receptors.[5] Concentrations of sFlt-1 is increased in women with established pre-eclampsia and begin to increase steeply about five weeks before the onset of clinical disease.[6]

The antiangiogenic state which occur in pre-eclmpsia may be reflected by changes in the impedance to flow in the maternal circulation.[7] Uterine artery Doppler is a non-invasive assessment of utero-placental circulation, and its clinical value in high risk pregnant women has been encouraging.[8]

The aim of the study was to assess the serum levels of soluble Endoglin (sEng) ,soluble FMS like tyrosine kinase-1 (sflt-1), and role of Uterine Artery Doppler in pre-eclampsia to evaluate their clinical utility in diagnosis, and assessment of severity of the disease.

2. Subjects and Methods:

SUBJECTS :

This study has been carried out in the outpatients clinics and inpatients departments of Obstetrics & Gynecology and Clinical Pathology departments, Zagazig University Hospitals. It included 35 pre-eclamptic patients in addition to 20 healthy pregnant control subjects. All participants gave their consent to participate in this study.

Patient Group (group I):

Thirty five pre-eclamptic patients. Their ages ranged between 18 to 40 years. Patients were diagnosed according to the diagnostic criteria outlined by **The American College of Obstetrics and Gynecology (AGOG) practice bulletin (2002)[9]:**Blood pressure: systolic blood pressure (SBP) of 140 mmHg or greater or diastolic blood pressure (DBP) of 90 mmHg or greater on 2 occasions at least 6 hours apart, Proteinuria: at least 300 mg in 24-hours urine collection.

Patients were classified according to onset of Pre-eclampsia: Early- onset or preterm pre-eclamptic group: This group included 16 pregnant females who developed pre-eclampsia before 32 weeks of gestation, and late- onset or term pre-eclamptic group: This group included 19 pregnant females who developed pre-eclampsia after 32 weeks of gestation.

The pre-eclamptic patients were re-classified according to severity **(AGOG, 2002)**[9] into: Mild pre-eclamptic group: This group included 17 pre-eclamptic females. They had SBP <160 mmHg , DBP<110 mmHg. 24 hrs urinary protein <3gm/day, ALT<40 IU/L and platelet count >100 x 10^3 /uL ,and severe pre-eclamptic group: This group included 18 pre-eclamptic females. They had SBP $\geq$ 160 mmHg or DBP $\geq$ 110 mmHg or 24 hrs urinary protein $\geq$ 3 gm/day or ALT $\geq$ 40 IU/L or platelet count $\leq$ 100 x 10^3 /uL

Pre-eclamptic patients were re-classified with Doppler velocimetry according to absence and presence of persistent diastolic notch **(Cnossen *et al.*, 2008)**[10] into: Normal uterine artery Doppler velocimetry group: It includes 17 pre-eclamptic patients, among them there are 5 preterm and 12 term pre-eclamptic patients, and abnormal uterine artery Doppler velocimetry group: It includes 18 pre-eclamptic patients having persistent diastolic notch, among them there are 11 preterm and 7 term pre-eclamptic patients.

Control group (group II): It included Twenty apparently healthy pregnant females (neither have hypertension nor diabetes or renal diseases) matched in age with patients group, their ages ranged between 20-35 years.

All individuals included in this study were subjected to the following: Full history taking and clinical examination, assessment of blood pressure, Laboratory estimation of fasting serum glucose , urea and creatinine, ALT, platelet count,24 hours urinary protein, serum soluble endoglin (sEng), serum soluble Fms-like tyrosine kinase-1 (sFlt-1),and uterine artery Doppler assessment. . Individuals with repeated upper urinary tract infections, chronic hypertension, diabetes mellitus or pre-existing renal disease were excluded.

SAMPLES: Five milliliters of venous blood were collected and divided into an EDTA tube for platelet count and a plain test tube for serum separation. , 24-hours urine sample was collected from each subject in a clean container, examined immediately (**National Committee for Clinical Laboratory Standards, 2001).**[11]

Analytical Methods*:*

-Serum glucose, Serum urea and creatinine ,and Serum Alanine Aminotransferase (ALT), were carried out on

ADVIA 1650 auto-analyzer (Siemens Medical Solutions Diagnostic, USA).

-Platelet count was done using cell coulter Sysmex KX21 N (Japan).

-Urine Analysis for 24hrs Protein: by turbidimetric assay using trichloracetic acid (TCA) according to (**Shahanigan *et al.*, 1984).** [12]

-Serum sEng and sFlt-1**:** were assayed by a sandwich enzyme-linked immunosorbent assay (ELISA) using reagents provided by Quantikine R&D International (R&D International, Inc., 614 NC Kinely Place N. E Minneapolis, MN 55413. USA). This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for antigen has been pre-coated on a micro plate. Standards & samples were pipetted into the wells and any antigen present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for antigen was added to the wells. Following a wash to remove an unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of antigen bound in the initial step. The color development was stopped and the intensity of the color was measured by ELISA reader.

B-Uterine Doppler artery:

Pulsed wave and color Doppler ultrasound examination of both uterine arteries was performed on both pre-eclamptic patients and controls using (volusion 730 PRO V),by sitting in semi-recumbent position , recording uterine artery waveforms which was made at the point where the uterine and external iliac arteries appeared to have crossed each others as detected by color flow Doppler then three consecutive pulsed waveforms were recorded and pulsatility index (PI) was measured and diastolic notch if present. Uterine artery Doppler velocimetry was defined as abnormal if either the mean PI was above the 90th percentile for gestational age and/or early diastolic notch was found **(Cnossen *et al.*, 2008).**

C- Statistical methods:

This was done on a personal computer using software SPSS (version 15) (SPSS Inc. Chicago, IL, USA). Descriptive statistics**:** Data were summarized using the arithmetic mean, the standard deviation, median and range for numerical variables. Mann–Whitney *U* test (MW) as non-parametric test for assessing whether two independent samples of observations have equally large values , and finally correlation was done to evaluate correlation between variables using Spearman rank correlation coefficient "r". *P* value of <0.05 indicates a significant results. Receiver Operating Characteristic (ROC) curves analysis was carried out to demonstrate the diagnostic performance of sEng, sFlt-1 and PI as indicators of early versus late onset pre-eclampsia. In addition ROC curve was constructed to evaluate the efficiency of sEng, sFlt-1 and PI for discriminating severe pre-eclampsia patients from mild patients.

3. Results:

Statistical comparison of mean ±SD of some studied parameters among cases and controls revealed a highly significant increase was found regarding SBP, DBP, PI and soluble Flt-1 (t=10.78, 9.04,3.47, 13.44 ;p<0.001 respectively). and non-significant difference regarding gestational age, Statistical comparison of median using Mann Whitney U test(MW) of some studied parameters among cases and controls revealed a highly significant increase was found regarding protein in urine, ALT, and sEng in cases (M.W.= 22.9, 12.4, 22.8 ;p<0.001 respectively) and non-significant difference regarding platelet count (Table1).

Statistical comparison between preterm, term pre-eclampsia regarding soluble endoglin ,and soluble Flt-1, revealed a highly statistically significant difference(t=8.29,7.89,p<0.001)respectively, while PI had no significant different between them. (Table 2).

The descriptive data of the different parameters among mild and severe pre-eclamptic groups. showed a significant increase regarding SBP,DBP, urinary protein, ALT, sEng and sFlt-1 ,except platelet count significant decrease in severe cases (t=0.46, 7.01, 7.23, 2.3, 5.14, 5.79, 3.16; *P*<0.001 respectively). While GA was non-significant. Also PI revealed a significant increase in pulsatility index in severe cases (MW 4.5;*P*<0.001) (Table 3).

Statistical comparison between normal and abnormal Doppler regarding sEng and sFlt-1 revealed a highly statistically significance increase in both s.Eng,sFlt-1 in abnormal Doppler group (t=5.74, 5.79 ;p < 0.001) respectively (Table 4).

Correlation study between sEng , and sFlt-1 level in pre-eclamptic patients ,and correlation between them and other parameters .Regarding sEng it revealed a highly significant positive correlation with SBP, DBP, urinary protein, ALT and PI ,sFlt-1.(r = 0.67,0.66,0.69, 0.3,0.49, 0.94 ;p<0.001) respectively, and high significant negative correlation with gestational age ,and platelet count(t=-0.63,-0.4; *P*<0.001), also as regard sFlt-1 it revealed a highly significant positive correlation with SBP, DBP, urinary protein, PI, and sEng (t=0.65,0.64,0.73, 0.67,0.94 respectively). and high significant negative correlation with gestational age ,and platelet count (t=-0.70,-0.45 *P*<0.001) while ALT non-significant correlate with sFlt-1 (Table5).

Receiver operating characteristic (ROC) curve analysis (Fig 1) was applied to evaluate the diagnostic utility of serum s.Eng ,sFlt-1,and PI for discriminating the early from late pre-eclampsia ,the best diagnostic cut off levels for sEng and sFlt-1 were >15 ng/ml,>900 pg/ml respectively. Both had a diagnostic sensitivity of

100%. Specificity 78.9%, accuracy 88.6%, positive predictive value 80%, and negative predictive value 100%,the area under the curve were 0.97,0.99 respectively.

While the cut off levels for PI >1.42 and had a diagnostic sensitivity68%, specificity 58%,accuracy 62%,PPV 58% and NPV58%,the area under the curve was 0.72.

Also, Receiver operating characteristic (ROC) curve analysis (Fig 2) was applied to evaluate the diagnostic utility of serum sEng, sFlt-1,and PI for discriminating the mild from severe pre-eclampsia. sEng, and sFlt-1 had a diagnostic sensitivity 89%, 83%, specificity 65%, 70%, accuracy for both 77%, PPV 72%, 75%, and NPV 84%,80%, the area under the curve 0.90,0.89, respectively.

As regard PI had a diagnostic sensitivity of 100%, specificity 94%, accuracy 97%, PPV 95%, and NPV 100%, the area under the curve was 0.94 .

Table (1): Clinical and laboratory data among the studied groups.

	Cases (group I) N = 35	**Control (group II) N = 20**	**Test of significance**	***P***
- GA (weeks) $\bar{X}$±SD Range	32.4±3.4 23-36	32.7±2.9 28-36	0.22	>0.05 N.S
-SBP (mmHg) X±SD Range	153.1±13.4 140-190	106.0±5.1 100-110	10.78	<0.001 H.S
- DBP (mmHg) X±SD Range	102.6±10.0 90-120	73±4.2 70-80	9.04	<0.001 .H.S
-Soluble Flt-1 (pg/ml) X±SD Range	917.5±127.98 700-1169	322.8±103.8 137-450	13.44	<0.001 H.S
- Pulsatility index (PI) X±SD Range	1.62±1.0 (0.4-5)	0.52±0.14 (0.4-0.8)	3.47	<0.001 H.S
- Urinary TP(gm/24h) Median range	3.1 0.46-10.9	0.05 0.05-0.06	MW 22.9	<0.001 H.S
- ALT (IU/L)Median range	19 10-68	11 10-15	MW 12.4	<0.001 H.S
- Plt (x 10^3/cmm) Median range	198 99-350	221 197-260	MW 1.86	>0.05 N.S
-Soluble endoglin (ng/ml) Median range	15.5 7.5-34	4.2 1.3-6.5	MW 22.8	<0.001 H.S

P >0.05 Non-significan; P<0.001Highly-significant; M.W:Mann-WhitneyUtest GA: Gestational age; SBP: systolic blood pressure; DBP: Diastolic blood pressure; ALT: Alanine aminotransferase; Urinary TP: urinary protein Plt: Platelet count

Table (2): Soluble endoglin ng/ml and, soluble Flt-1 pg/ml,and pulsatility index according to gestational weeks in pre-eclampic patients.

	Preterm group N=16	Term group N=19	T	*P*
Soluble endoglin (ng/ml) $\bar{X}$±SD (Range)	26.3±6.56 (15.4-34)	12.15±3.2 (7.5-17)	8.29	<0.001 HS
Soluble Flt-1 (pg/ml) $\bar{X}$±SD (Range)	1028.6±66.8 (940-1169)	823.8±83.6 (700-950)	7.89	<0.001 HS
Pulsatility index(PI) $\bar{X}$±SD (Range)	1.87±0.82 (0.8-3)	1.41±1.09 (0.4-5)	1.39	>0.05 NS

P<0.001 Highly significant; *P* >0.05 Non-significant

Table (3): Studied parameters in Mild and severe pre-eclampsia.

	Mild group N = 17	Severe group N = 18	Test of significance	*P*
GA(weeks)	33.2±2.86	31.7±3.7	1.39	>0.05
Blood pressure - Systolic (mmHg) - Diastolic (mmHg)	 142.9±5.8 94.7±4.8	 162.8±11.3 110±7.7	 0.46 7.01	 <0.001 <0.001
TP (gm/24h)	1.46±0.9	5.2±2.1	7.23	<0.001
ALT (IU/L)	19.2±9.2	29.7±16.2	2.3	<0.001
Platelet (x10^3/cmm)	227.9±55.9	166.1±59.1	3.16	<0.01
S. endoglin (ng/ml)	12.4±4.1	24.5±7.7	5.14	<0.001
S.flt-1 (pg/ml)	825.3±96.9	1004.5±86	5.79	<0.001
PI*	0.8(0.4-5)	2.1(1.8-3)	4.5 MW	<0.001

GA: Gestational age; MW :Mann -Whitney-U test; * PI: Uterine artery pulsatility index median & range;
P >0.05 Non-significant *P* <0.01 significant; *P*<0.001 highly significant

Table (4): Statistical comparison between normal, abnormal uterine Doppler artery regarding soluble endoglin ng/ml and soluble Flt-1pg/ml in Pre-eclamptic patients.

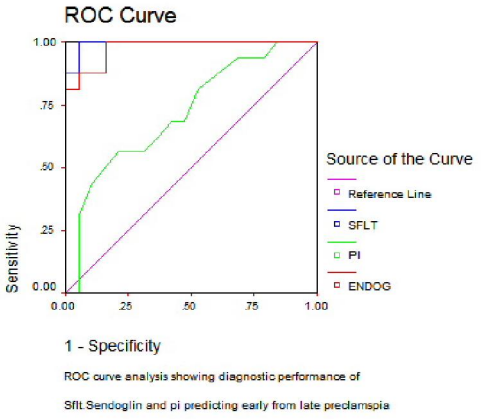
	Normal doppler N=17	Abnormal doppler N=18	t	*P*
Soluble endoglin (ng/ml) $\bar{X}$±SD (Range)	12.4 ±4.1 (7.5-21)	25.2±8.8 (16.4-34)	5.74	<0.001 HS
Soluble Flt-1 (pg/ml) $\bar{X}$±SD (Range)	825.29±96.91(700-965)	1004.5±86.05(870-1169)	5.79	<0.001 HS

P<0.001 Highly significant

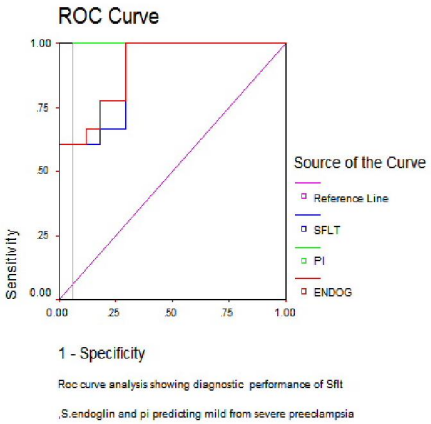
Table (5): Correlation between s- endoglin ng/ml, sflt-1pg/ml in pre-eclamptic patients and correlation between them and other parameters.

	S.Eng	S Flt-1
Gestational age(weeks)	r = -0.63**	r = -0.70**
Systolic Blood pressure(mmHg)	r = 0.67**	r = 0.65**
Diastolic Blood pressure(mmHg)	r = 0.66**	r = 0.64**
TP (urinary total protein)(g/24 h)	r = 0.69**	r = 0.73**
ALT IU/L	r = 0.3*	r = 0.25
Platelet (Plt)(x10^3/cm)	r = -0.4**	r = -0.45**
Pulsatility index (PI)	r = 0.49**	r = 0.67**
S-flt-1 Pg/ml	r = 0.94**	
S Eng ng/ml		r = 0.94**

P>0.05 non significant * *P* <0.05 significant ** *P* <0.001highly significant



AUC of: sEng=0.97 , sFlt-1=0.99 , PI=0.72



AUC of: sEng=0.90, sFlt-1=0.89, PI=0.94

4. Discussion

Pre-eclampsia is a pregnancy specific syndrome and a leading cause of maternal and fetal morbidity and mortality worldwide. It affects about 2-5% of western countries and complicates up to 10% of pregnancies in the developing countries. [13]

Levine *et al.*[1] reported that blood levels of placental derived anti angiogenic proteins might eventually be involved in the endothelial dysfunction which is the hall mark pathological finding in Pre-eclampsia.

The aim of this study was to assess levels of s.Eng and s.flt-1, a circulating placental-derived anti-angiogenic proteins, combined with uterine artery Doppler velocimetry in a group of pre-eclamptic pregnant females to evaluates their clinical utility in diagnosis and assessment of severity of the diseases.

The results revealed highly significantly increased in s.Eng,s.flt-1,PI, SBP, DBP ,proteins in urine, , and ALT in patients than control, and in severe pre-eclampsia than in mild pre-eclampsia. These results are similar to **Eremina *et al.***[14],and **Levin *et al.***[3] who believed that excess levels of sflt-1 lead to endothelial dysfunctions, Hypertension and proteinuria this effect explained also by **Luttun** and **Carmeliet**[5] that it was due to inhibition of VEGF (vascular endothelial growth factor) a factor important not only in blood pressure regulation, but also in maintaining the integrity of glomerular filtration barrier.

These results was also similar to **Masuyama *et al.***[15]. They found that the more severe the condition is, the higher the levels of sEng and s Flt-1 in sera of pre-eclamptic females. They concluded that the relationship of sEng and sFlt-1 to the severity of pre-

eclampsia is possible a casual one in which these markers might be playing a role in the pathogenesis of pre-eclampsia. **Venkatesha *et al.*[16]** reported that exogenous sEng and sFlt-1 administration in pregnant rats lead to severe pre-eclampsia including HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) and restriction of fetal growth.

In the present study pre-term pre-eclampsia has highly significant increase sEng and sflt-1 than term pre-eclampsia while PI was not significantly different. Similar results are reported by **Hirashima *et al.*[17], Levine *et al.*[3]** measured serum levels of sEng and sFlt-1 in pre- eclampsia in the second trimester and found that levels of sEng rose earlier and more steeply in women in whom pre-eclampia developed and were highest in preterm (early onset pre-eclampsia). **Lesili *et al.*[18]** reported that the prediction of term pre-eclampsia is poor with Doppler. **Whitley *et al.*[19]** reported significant association between abnormal uterine artery Doppler with pre-term but not with term pre-eclampsia.

The results showed that not all cases of pre-eclampsia had abnormal Doppler, this agree with **Zahumensky[20].** Also the results revealed that pre-eclamptic patients with abnormal uterine Doppler have higher sEng, and sFlt-1 concentrations than patients with normal Doppler. This is consistent with finding of **Stepan *et al.*[21]** who reported that in patients with pre-eclampsia subgroups with abnormal Doppler findings have higher sEng and sFlt-1 levels in comparison with the group with normal uterine perfusion.

In studying the correlation of sEng and sFlt-1 and other laboratory findings: sEng is positively correlated to sFlt-1 and both of them are positively correlated to SBP,DBP, PI and urinary proteins but AlT with sEng only and negatively correlated to gestational age and platelet count.

As regards positive correlation of s Eng and s Flt-1 with urinary protein go hand in hand with **Venkatesha *et al.*[16]** who demonstrated that sEng act by antagonizing the angiogenic molecule transforming growth factor beta-1 (TGF-B1) which is important both in mediating NO-dependent vasodilation and in keeping lining of blood vessels healthy. So excess secretion of s. Eng lead to intense vasoconstriction, with resultant hypertension, and leakage of proteins into tissues and urine. These data clearly indicate that s.Eng is a major cause of the maternal manifestation of pre -eclampsia.

Also **Buhimschi *et al.*[22]** reported that podocyte and mesengial cell destruction as well as loss of glomerular basal membrane integrity (glomerular endotheliosis) in women with pre-eclampsia resulted from increased exposure to sFlt-1. **Chaiworapongsa *et al.*[7]** who found a significant relationship between sEng and sFlt-1 and Doppler abnormalities in the uterine circulation these findings also consistent with **Gilbert *et al.*[23]**, who demonstrated that reducing uterine perfusion pressure by clamping the aorta above the iliac bi-furcation in pregnant rats led to increase the serum concentration and placental expression of anti angiogenic proteins.

Assessment of the diagnostic performance of sEng and sFlt-1 in early pre-eclampsia patients versus late pre-eclampsia patients using Receiver operating characteristic (ROC) curve analysis revealed that the best diagnostic cut off levels for sEng and sFlt-1 was >15 ng/ml,and >900 pg/ml respectively ,both had a diagnostic sensitivity of 100%. Specificity 78.9%, accuracy 88.6%, positive predictive value 80%, and negative predictive value 100% respectively. Similar results were obtained by **Abdel Fattah *et al.*[24]**this results in agreement with **Chen *et al.*[25]** who reported that serum sFlt-1 levels >350 pg/ml in pateints of early pre-eclampsia. , **Savvidow *et al.*[26]** found that women who developed early onset pre -eclampsia often had sEng levels more than 10.2 ng/ml. **Woolcock *et al.*[27]** explained these findings by the fact that the early onset pre-eclampsia is more associated with placental ischemia than late onset pre-eclampsia, leading to more pronounced alternation in sFlt-1.While **Stepan *et al.*[21]** reported that the increase of circulatory sEng is detectable approximately 2-3 months before the clinical manifestation of preeclampsia, it is known that sFlt-1 increase is described as detectable 5 weeks before onset of disease, so sEng could be an earlier marker for pre eclampsia.

As regard pulstality index cut off levels >1.42 and had a diagnostic sensitivity68%, specificity 58%,accuracy 62%,PPV 58% and NPV58%. That go hand in hand with **Yu *et al.*[28]** who reported that Doppler screening for predicting pre-eclampsia yield sensitivity up to 60% and PPV up to 40%,also **Thangaratinan *et al.*[29]** found that PI have a sensitivity of 60%,and so on **Lapaire *et al.*[30]** demonstrated that abnormal uterine artery Doppler velocimetry was independent risk factors for the occurrence of pre-eclampsia. Assessment of the diagnostic performance of sEng and sFlt-1 in severe pre-eclampsia patients versus mild pre-eclampsia patients using Receiver operating characteristic (ROC) curve analysis revealed that sEng, and sFlt-1 had a diagnostic sensitivity 89%, 83%, specificity 65%, 70%, respectively, while the PI had a diagnostic sensitivity of 100%, specificity 94%,that is mean that all the three items were efficient to discriminate severe pre-eclampsia from mild cases. In consistent with our finding **Buhiomshi *et al.*[22] and Venkalesha *et al.*[16]** who established this strong association between sEng and sFlt-1 and severity of pre-eclampsia .

Also Masuyama *et al.*[15] reported that the relationship of sFlt-1 and sEng to the severity of pre-

eclampsia is possibly a causal one of the severity,and also reported that sEng level >22.0 ng/ml in this cases, and **Alexandre *et al.*[31]** recorded that serum s Flt levels were often > 800 pg/ml in severe pre –eclampsia.

As regard the results of PI its sensitivity 100%,specificity 94% is better than sFlt-1,sEng in severe cases .In agreement with this results **Plasenica *et al.*[32]** who reported a sensitivity 82% of PI in pre – eclampsia at 11-13 weeks. While **Leslie *et al.*[18]** reported that single screening modality by uterine Doppler may not adequately predict all presentation. So that **Stepan *et al.*[21]** said that concurrent measurement of uterine perfusion and anti angiogenic factors sEng, sFlt-1 allows a highly efficient prediction of early onset pre-eclampsia ,Although anti angiogenic factors don't improve the good sensitivity of Doppler sonography they substantially improve the specificity.

Conclusion: The results of this study indicate that s Eng, and sFlt-1 are efficient in prediction of early onset pre-eclampsia and can discriminate between severe and mild pre-eclampsia, and both with pulsatility index of Doppler give better prediction of pre-eclampsia. This results will help in finding a new strategy for early management and so reduction of associated complication of pre-eclampsia.

5.References:

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