Maternal Serum soluble Endoglin Level as Early Predictor for Pre-eclampsia

Mohamed A. Al-Nory[1]; Adel F. Al-Kholy[2]; Mamdouh Z. Abadier [2] and Ibrahim Rageh [3]

Departments of [1]Obstetrics & Gynecology, [2]Medical Biochemistry and [3]Clinical Pathology, Faculty of Medicine, Benha University, Benha, Egypt
adeladel59@yahoo.com

Abstract: Objectives: To compare serum soluble endoglin (sEng) in women developed pre-eclampsia (PE group) versus those free of hypertensive manifestations (Control group) till delivery and to evaluate the predictability of single estimation versus sequential estimations for the development of PE. Patients & Methods: The study included all primipara attending the antenatal care unit prior to the 12th week gestational age (GA) so as to select 90 pregnant PE women. Then, all women attended the clinic 4-weekly till delivery for follow-up and to donate blood sample for serum sEng estimation. Women developed PE were categorized according to time of development of PE into Early- and Late-onset PE and stopped blood donation once diagnosed. Results: Thirty-eight women developed early and 52 women developed late-onset PE. Mean serum sEng estimated at 16th week GA were significantly higher in early compared to late-onset PE, despite the non-significant difference at 12th week GA and so the difference between 16th and 12th week estimations was significantly higher with early versus late-onset PE. Serum sEng estimated at time of PE diagnosis was significantly higher in early compared to late-onset PE. Serum sEng levels estimated at 12th week GA showed positive significant correlation with the possibility of development of PE and systolic blood pressure and at cutoff point of 14 ng/ml could identify women liable to develop PE with high sensitivity, but was a weak point for differentiation between early and late PE, while on 16th week GA at cutoff point of 6 ng/ml could specifically identify women liable to develop early-onset PE Conclusion: Serum sEng at 12th week GA could be used as a sensitive screening test for women liable to development of PE and 4-weeks sequential estimation of serum sEng in susceptible women could specifically identify women liable to develop early-onset PE prior to clinical diagnosis.

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1. Introduction:

Pre-eclampsia is the major cause of maternal and fetal morbidity and mortality, involving 15% to 20% of pregnancies in developed countries and even more in less developed parts of the world. Superficial placentation driven by immune maladaptation, with subsequently reduced concentrations of angiogenic growth factors and increased placental debris in the maternal circulation, are likely responsible (**Dechend and Luft, 2008**).

Central to the pathogenesis of PE is shallow placentation with abnormal maternal-placental vascular development. Shallow placentation causes release of endothelial deranging factors to the maternal circulation. Among these placenta-derived factors are the antiangiogenic proteins such as soluble fms-like tyrosine kinase receptor (sFlt1) which binds and reduces the free circulating levels of the proangiogenic factors such as vascular endothelial growth factor and placental growth factor. Thereby sFlt blunts the beneficial effects of these proangiogenic factors on maternal endothelium, with consequent maternal hypertension and proteinuria (**Maynard *et al.*, 2003; Redman and Sargent, 2005**).

Endoglin (Eng), also called CD105, is a 180-kDa homodimeric transmembrane glycoprotein expressed mainly in endothelial cells, but also in many other cell types. Endoglin is an intriguing molecule that functions as an auxiliary receptor (type III receptor) for several of the transforming growth factor-ß (TGF-ß) superfamily members and modulates TGF-ß signaling by interacting with TGF-ß receptors types I and II (**Guerrero-Esteo *et al.*, 2002; Fonsatti and Maio, 2004**).

The soluble form of Eng (sEng) is a proteolytic derivative of processing of the membrane bound Eng, a partial peptide sequence of purified circulating sEng suggests that it is an N-terminal cleavage product of full-length Eng and membrane-type metalloprotease-1 (interstitial collagenase) present in trophoblasts may induce shedding of sEng from membrane bound Eng. *In vitro* studies suggested that sEng inhibits TGF-ß1 signaling and blocks TGF-ß1-mediated nitric oxide synthase activation in endothelial cells. Furthermore, sEng interfere with

endothelial proliferation and capillary formation (**Venkatesha *et al.*, 2006**).

Multiple studies tried to explore the origin of anti-angiogenic factors in women developed PE. **Guller *et al.* (2010)** documented that the reported increase in the ratio of plasminogen activator inhibitor-1/-2 and elevated levels of sFlt-1 and sEng in maternal serum, are linked to placental damage and maternal endothelial cell dysfunction in PE and attributed this to the shedding of syncytiotrophoblast micro-particles containing these anti-angiogenic factors from the plasma to maternal blood which occurs in normal pregnancy and is enhanced during PE.

A balance between angiogenic and anti-angiogenic factors is essential for feto-placental development; angiogenic factors play crucial roles in the pathophysiology of pre-eclampsia (**Chaiworapongsa *et al.*, 2010**). Thus, the present selective two-arm study was designed to collect 90 pre-eclamptic women among primipara attending the antenatal outpatient clinic and aimed to compare serum sEng in women developed PE versus those completed their pregnancy without hypertensive manifestations and to evaluate and compare the predictability of single estimation level versus sequential estimation level of serum sEng in these pre-eclamptic women for the development of PE on the other arm.

2. Patients and Methods

The present study was conducted at Departments of Obstetrics & Gynecology, Medical Biochemistry and Clinical Pathology, Faculty of Medicine, Benha University and designed to include all primipara attending to antenatal care unit prior to the 12th week GA so as to select 90 pregnant pre-eclamptic women. All women signed a fully informed written consent to participate in the study so as to attend the clinic 4-weekly since 12th week GA till delivery for follow-up and to donate blood sample each visit for serum sEng estimation. Throughout their antenatal visits, women developed the diagnostic criteria for pre-eclampsia were grouped as PE group and were categorized according to time of development of PE into Early-onset PE if diagnosed around the 20th week GA and Late PE if diagnosed later to the 20th week GA. Women developed PE stopped blood donation once diagnosed. Ninety women completed their pregnancy free of PE manifestations were grouped as Control group. Exclusion criteria included multiple gestation and preexisting medical conditions such as diabetes, chronic hypertension, and renal diseases.

Pre-eclampsia was diagnosed by the presence of gestational hypertension beginning after the 12th week of pregnancy with an absolute blood pressure ≥140 mmHg systolic and/or 90 mmHg diastolic on at least two occasions, 4 hours apart, and proteinuria (one dipstick measurement ≥2+ on a voided random urine sample) (**Gifford *et al.*, 2000**).

At time of enrollment in the study, all women underwent full history taking, general and abdominal examination to determine a baseline arterial blood pressure and body mass index (BMI). Ultrasonographic examination was conducted to confirm the gestational age, and to exclude the presence of fetal congenital abnormalities. Body mass index was calculated according to the equation: BMI = [(Weight)/ (Height) ^{2}], a person with a BMI of ≥30 is considered obese (**Vella and Galloway, 2003**).

Throughout the period since baseline data collection, all women were examined during each visit for the progress of pregnancy and fetal wellbeing, the extent of hypertension and occurrence of other complications. Urine analysis was performed for the presence of urinary tract infection and the degree of proteinuria.

Collected maternal blood samples were allowed to clot then serum was separated by centrifugation at 2000 rpm for 10 min. Serum was removed, placed in pyrogen-free Eppendorf tubes and stored at -80°C until ELISA assayed for estimation of serum sEng (AdipoGen Inc., Seoul, Korea) (**Staff *et al.*, 2007**).

Statistical analysis

The obtained results were compared using Wilcoxon Rank test (Z-test) for unrelated data. The Receiver Operating Characteristic (ROC) curve was used to evaluate the predictability of serum sEng levels for the development of PE and to verify the validity of multiple cutoff points to identify the valid cutoff point for differentiation between early-onset and late-onset PE. Statistical analysis was conducted using SPSS statistical program, (Version 10, 2002). P value <0.05 was considered statistically significant.

3. Results

Throughout the study period 38 women developed early-onset PE and 52 women developed late-onset PE. At time of study enrollment, there was non-significant difference between studied and control women as regards age, body weight, height and BMI. At time of development of PE, studied women developed significantly higher blood pressure measures and significantly higher levels of proteinuria compared to control women, (Table 1).

Table (1): Clinical data of the entire study population at time of enrollment

	Control group (n=90)	PE group (n=90)
Age (years)	25±2.5 (20.5-29)	25.3±2 (21-28)
Weight (Kg)	87±4.4 (75-95)	88±3.7 (80-94)
Height (cm)	165.4±3.7 (156-170)	164.7±3.5 (155-169)
BMI (kg/m^2)	31.8±1.9 (26.6-37.5)	32.5±2.3 (28.4-38.5)
Systolic blood pressure (mmHg)	120.2±6 (105-125)	139.1±13.6 (130-160)*
Diastolic blood pressure (mmHg)	78±1.8 (65-75)	91±5.2 (83-98)*
‡Level of protein in urine	0.7±0.5 (0 to +1)	1.8±0.7 (+1 to +4)*

Data are presented as mean±SD, ranges are in parenthesis BMI: Body mass index
*: significant difference versus control group
‡: Level of protein in urine as judged by dipstick measurement and expressed as number of + marks

Mean serum sEng level estimated at 12th week GA, was significantly higher in women developed PE compared to control women, but was non-significantly higher in women developed early-onset PE compared to those developed late-onset PE. However, serum sEng levels estimated at 16th week GA were significantly higher compared to control group with significantly higher levels in women developed early-onset PE compared to those developed late-onset PE and consequently the difference between levels estimated at 16th and 12th week GA was significantly higher in early-onset versus late-onset pre-eclamptics. Moreover, serum sEng estimated at time of diagnosis of PE was significantly higher in women developed early-onset compared to those had late-onset PE (Table 2).

Table (2): Mean serum levels of sEng estimated in study group till development of PE compared to control levels

		Control group	PE group		
			Early-onset	Late-onset	Total
12th week GA		12±1.22 (10-15)	19.3±1.9* (13-20)	18.9±2.3* (13-22)	19±2.1* (13-22)
16th week GA	Level		24.8±1.8*† (19-28)	19.8±2.08* (14-22)	21.8±3.05* (14-28)
	Difference (16th w-12th w level)		5.55±2.45*† (2-14)	0.94±0.87* (0-3)	
Time of clinical diagnosis			40.5±5.32*† (32-52)	38.1±3.41* (32-44)	

Data are presented as mean±SD, ranges are in parenthesis GA: gestational age
*: significant versus control levels †: significant versus late-onset PE

Serum sEng levels estimated at 12th week GA showed negative significant correlation with body weight (r=-0.329, p=0.002) and BMI (r=-0.313, p=0.003), while showed positive significant correlation with systolic (r=0.361, p<0.001) but non-significant (r=0.201, p>0.05) with diastolic blood pressure. Moreover, elevated serum sEng estimated at 12th week GA showed positive significant correlation (r=0.898, p<0.001) with the possibility of development of PE.

ROC curve analysis for the predictability of estimated sEng at the 12th week GA at cutoff point of 14 ng/ml (the median value for all the studied 180 women) could identify women liable to develop PE with high sensitivity judged by AUC=0.313, but was a weak point for differentiation between those liable to develop early or late PE, (AUC=0.386 and 0.427), respectively (Fig. 1).

Using sequential estimation, the extent of increased serum sEng level on the 16th versus the 12th week GA at cutoff point of 6 ng/ml could specifically identify women liable to develop early-onset PE with AUC=0.825 versus AUC=0.175 for late-onset PE. Furthermore, for identification of women liable to develop early-onset PE, AUC at 6 ng/ml was wider than that determined at cutoff point of 5 and 7 ng/ml, (AUC= 0.813 and 0.799, respectively).

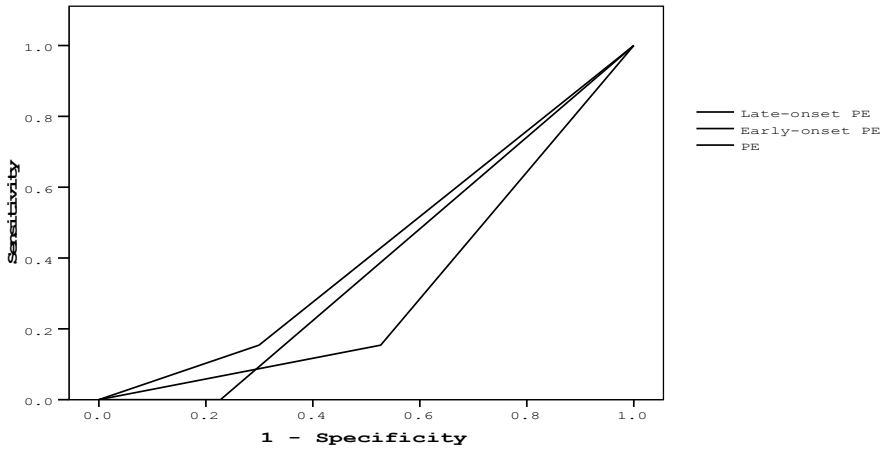

Fig. (1): ROC curve analysis of predictability of serum sEng level estimated at the 12th week GA for development of PE and its both types of PE at cutoff point of 14 ng/ml

4. Discussion

The present study relied on ELISA estimation of only one anti-angiogenic factor; soluble endoglin (sEng) as a representative of angiogenic/anti-angiogenic parameters and this decision depended on the outcome of previous study of **Staff *et al.* (2007)** who found median maternal serum and amniotic fluid sEng concentrations were elevated in pre-eclampsia, compared with control pregnancies, while low concentrations of sEng were found in fetal circulation, which did not differ between pre-eclampsia and control pregnancies; a finding indicating that the fetus appears not to contribute to elevated circulating maternal sEng concentrations in pre-eclampsia, also **Staff *et al.* (2007)** indicated that elevated sEng in maternal circulation in pre-eclampsia correlated with soluble fms-like tyrosine kinase-1 concentrations and soluble fms-like tyrosine kinase-1/placental growth factor ratio, so sEng could represent the changes in angiogenic/anti-angiogenic milieu.

Also, the choice of ELISA procedure go in hand with **Guller *et al.* (2010)** who found ELISA examination of placental microparticles perfusion supernatant fluid revealed the presence of anti-angiogenic factors at the following relative levels: Eng>PAI-2»PAI-1>sFlt-1 and indicated that micro-particles shed from the syncytial surface express factors which may alter the fibrinolytic and angiogenic balance at the maternal-fetal interface and play a role in the pathophysiology of PE and that Eng and PAI-2 were almost exclusively localized to the surface of placental micro-particles, a site with biological potential.

Estimated mean serum s-Eng level at the 12th week GA was significantly higher in PE versus control group with non-significant difference among women had early or late PE, but the difference became significant at the 16th week GA reaching a peak coincident with appearance of clinical manifestations around the 20th week GA in those had early PE and mostly around the 30th week GA in those had late PE. These data indicated early elevation of serum sEng prior to evident clinical manifestations of PE, a finding coincided with **Signore *et al.* (2008)** who found elevated serum levels of the anti-angiogenic factor sEng prior to the development of hypertension and these elevations persist from this time in gestation onward and may be useful for identifying pregnant women at risk for abruption and hypertension and with **Baumann *et al.* (2008)** who reported that serum sEng and sFlt1 are increased in the first trimester in women with subsequent PE and might therefore prove useful to predict PE.

Moreover, at time of development of clinical manifestations of PE, women developed early PE had significantly higher serum sEng levels compared to those had late-onset PE, a finding that was in line with **Zhao *et al,* (2010)** who found serum level of sEng was significantly higher in early onset compared to late onset PE at time of admission for PE and with **Fang *et al.* (2010)** found pregnant women in the second trimester who eventually developed PE had significantly higher serum sEng than age- and gestational age-matched controls and these findings suggest that sEng plays an important role in PE pathogenesis.

Serum sEng levels estimated at 12th week GA showed negative significant correlation with body weight and height, while showed positive significant correlation with the possibility for PE development

and with systolic but non-significant with diastolic blood pressure. These data indicated a relation between serum sEng and constitutional data and with severity of PE and go in hand with **Lim *et al.* (2009)** who found early midtrimester plasma levels of sEng are predictive of subsequence occurrence and severity of PE, in terms of severity of hypertension and proteinuria and with **Staff *et al.* (2009)** who reported that in PE, the antiangiogenic state was more pronounced with clinical characteristics indicative of greater disease severity.

ROC curve analysis for the predictability of estimated sEng at the 12th week GA at cutoff point of 14 ng/ml for PE development could identify women liable to develop PE with high sensitivity, but was a weak point for differentiation between those liable to develop early or late PE. However, using sequential estimations at cutoff point of 6 ng/ml could specifically identify women liable to develop early-onset PE with AUC=0.825 that was wider than that determined at cutoff point of 5 and 7 ng/ml.

These data point to the failure of the 12th week GA level for prediction of early-onset PE, while elevated difference of the 16th week level in relation to the 12th week level showed high specificity for identification of women liable to develop early-onset PE. The data go in hand with **Salahuddin *et al.* (2007)** who reported sensitivity and specificity of sFlt1 in differentiating pre-eclampsia from normal pregnancy of 90% and 90%, respectively, and 90% and 95% for sEng and with **Baumann *et al.* (2008)** who found sEng and sFlt1 serum concentrations were higher in women with subsequent PE than in controls and sensitivities and specificities for predicting PE were 63% and 57% for sEng and 64% and 56% for sFlt1, respectively. Also, **Stepan & Jank (2007)** investigated the predictive value of anti-angiogenic markers in high-risk second trimester pregnancies characterized by abnormal uterine perfusion and found using both sFlt1 and placental growth factor, early-onset PE can be predicted with 83% sensitivity and 95% specificity, while combined analysis of sEng and sFlt1 is able to predict early-onset PE even with a sensitivity of 100% and a specificity of 93.3% and concluded that the concurrent measurement of uterine perfusion and angiogenic factors allows an efficient prediction of early-onset pregnancy complications, particularly PE.

In conclusion, estimation of serum sEng at the 12th week GA could be used as a sensitive screening test for women liable to development of PE and 4-weeks sequential estimations of serum sEng in susceptible women could specifically identify women liable to develop early-onset PE prior to commencement of clinical manifestations. However, larger scale study was warranted for establishment of the validity of proposed cutoff points.

Corresponding author
Adel F. Al-Kholy
Medical Biochemistry Department, Faculty of Medicine, Benha University, Benha, Egypt
adeladel59@yahoo.com

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