An angiogenic marker for early prediction of preeclampsia

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ABSTRACT

Background: Preeclampsia is a pregnancy-specific disease associated with a high incidence of maternal and fetal morbidity and mortality with classical features of hypertension, proteinuria, and edema. **Aims and Objectives:** The aims of the study were to study the angiogenic marker for the early predicting of preeclampsia. **Materials and Methods:** Blood samples were collected from 75 healthy singleton pregnant women from 19 to 29 years between 12 and 16 weeks of gestation with no underlying medical illness. The serum soluble endoglin (sEng) level and serum uric acid levels were estimated. Patients who developed preeclampsia were classified as mild and severe. They were classified as severe preeclampsia if their serum sEng levels are above 8.5 ng/ml and mild preeclampsia was grouped when the serum sEng level varies from 7.1–8.4 ng/ml. The serum sEng and serum uric acid levels were compared. Serum sEng was processed by ELISA technique and serum uric acid was measured by Uricase method in autoanalyzer. **Results:** The mean uric acid level was 4.0 ± 0.5 mg/dl in severe preeclampsia and 3.6 ± 0.2 mg/dl in mild preeclampsia patients. The levels were within normal limits. The serum sEng levels were 8.7 ± 1.4 ng/ml in severe preeclampsia and 7.7 ± 0.3 ng/ml in mild preeclampsia. The levels are elevated in preeclampsia. Women with serum sEng levels between 7.1 and 8.2 ng/ml developed symptoms of mild preeclampsia between 28 and 30 weeks of gestation, whereas women with serum sEng levels between 8.5 and 13.4 ng/ml developed symptoms of severe preeclampsia between 26 and 28 weeks of gestation. **Conclusion:** Serum uric acid levels were within normal limits in preeclampsia patients, whereas serum sEng levels were elevated in these patients. Thus, this study concludes that serum sEng will be a good predictor of severity of preeclampsia compared to serum uric acid level.

Keywords: Serum soluble endoglin; Preeclampsia; Serum uric acid

INTRODUCTION

Preeclampsia is a pregnancy-specific disease associated with a high incidence of maternal and fetal morbidity and mortality[1] with classical features of hypertension, proteinuria, and edema.

The placenta plays a central role in preeclampsia and failure of normal trophoblastic invasion and remodeling of the spiral arteries lead to a high resistance uteroplacental circulation. The delivery of the baby or termination of pregnancy is the only modality of treatment. The clinical symptoms of preeclampsia are resolved after the delivery of the placenta, which indicates that placental factors play a major role in the pathogenesis of the disease.[2]

Although the cause remains unclear, the syndrome may be initiated by placental factors that enter the maternal circulation and cause endothelial dysfunction, that is, vasoconstriction, end-organ ischemia, and increased vascular permeability resulting in hypertension and proteinuria. Moreover, preeclampsia is the primary reason for indicated preterm deliveries before 37 weeks of gestation. Although there is advancement in the clinical management of preeclampsia to prolong the delivery time to improve the outcome of neonates, delivery is still the

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only definitive treatment. Unfortunately, premature delivery of the fetus can be associated with adverse sequelae for the infant. Consequently, balancing maternal and fetal risks in the management of the early-onset preeclampsia is a challenge for care providers.

There is a link between placental disease and the maternal endothelial and inflammatory responses which are seen in preeclampsia are due to number of potential mediators.[3] It is postulated that the existence of one or more vasoactive factors is produced by the placenta and is secreted into the maternal bloodstream.[4]

Soluble fms-like tyrosine kinase 1 (sFlt1) is an anti-angiogenic circulating receptor for placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) thought to be derived from the placenta. Another placentally-derived angiogenic factor implicated in the development of preeclampsia is soluble endoglin (sEng). It is a coreceptor for transforming growth factor (TGF)-β1 and β3.[5] Administration of sEng to pregnant rats also induces hypertension, and when combined with sFlt1 produces HELLP like symptoms.[6] Circulating levels of sEng are also elevated before the onset of preeclampsia.

The women who are at risk of developing preeclampsia cannot be identified before the development of disease due to lack of an effective test and this is the main reason high morbidity of the disease.

There is not only a need to diagnose preeclampsia, but to predict it in early pregnancy to identify women at risk who could be offered intervention to prevent the disorder. A number of biochemical changes in maternal blood are not only present once the disease is overt, but are also present before the onset of disease. Many of these changes are only present in the weeks leading up to diagnosis, but some are significantly different even before 20 weeks of gestation. These setting have commonly assessed placental perfusion or vascular resistance, and serum or urine markers of placental distress, renal and endothelial dysfunction, oxidative stress, and insulin resistance. Although the diagnosis of preeclampsia is usually made after 30 weeks of gestation, the underlying pathophysiology begins much earlier. The disease development is described as occurring in several stages: The early stages involve primarily the placenta, and only the final stages, characterized by hypertension and proteinuria, are clinically detectable. The search for predictive biomarkers has focused largely on placental factors, in particular anti-angiogenic peptides, which are released into the maternal circulation. Elevated levels of sEng have been reported in women with preeclampsia, both at diagnosis and, crucially, before the onset of

clinically detectable disease. Neither these nor other biomarkers, however, have crossed the boundary to routine clinical practice. Endoglin is also highly expressed by the syncytiotrophoblast layer, which is the outer-most layer of the placenta, bathed in maternal blood.[7]

There are few review articles about different angiogenic biomarkers and its importance in early prediction of preeclampsia. Leona et al., have published a review article on early prediction of preeclampsia and stated that effective screening of angiogenic biomarkers during first trimester between 11 and 13 weeks of gestation is useful to assess the risk of preeclampsia.[8] Hagman et al., in 2012, have stated that usage of circulating angiogenic proteins such as soluble fms like tyrosine kinase-1 and serum sEng for the diagnosis and prediction of preeclampsia is still at an evolving stage.[9] Saleh et al., have done studies on soluble fms like tyrosine kinase-1 and PlGF in pregnant women between 20 and 41 weeks and have proved that these two markers are strong predictors of preeclampsia. However, this study was not conducted during first trimester of pregnancy.[10] Kar et al., have written a detailed review article on role of various biomarkers in the early detection of preeclampsia.[11]

In all the above studies, only review has been done on various angiogenic biomarkers. Very few case-based studies were done on angiogenic markers such as soluble fms like tyrosine kinase-1 and PlGF. Hence, the main objective of this study is to measure an angiogenic marker named serum sEng during first trimester to predict preeclampsia at an early stage.

Hyperuricemia was first reported to be elevated in preeclampsia in 1917,[12] and it remains one of the most common blood tests used in assessment of the condition in clinical practice in the UK. From that time on, uric acid measurement was considered as an important parameter in pregnant women with preeclampsia to monitor the severity of the disease and helped to manage it. During early pregnancy, serum uric acid levels fall, often to <3 mg/dl, related to the uricosuric effects from estrogen and from the increase in renal blood flow. Uric acid levels then increase during the third trimester, reaching levels of 4–5 mg/dl by term.[13] However, increased uric acid precedes the reduction in plasma volume. Mothers who develop preeclampsia present with significantly elevated serum uric acid levels. Numerous studies have suggested that the predictive value of serum uric acid is relatively poor for both diagnosis and prognosis, especially for distinguishing preeclampsia from gestational hypertension[14]. It is therefore appearing that while uric acid may be of value in the detection of preeclampsia, it is not useful in the early prediction of disease.

Aims and objectives

The aim is to study the angiogenic marker for the early predicting of preeclampsia.

MATERIALS AND METHODS

Materials

After getting Institutional Ethical Clearance (IEC) on January 19, 2012 from Meenakshi Medical College, Hospital and Research Institute, the clinical study was conducted on pregnant mothers between 12 and 16 weeks of gestation in the Department of Clinical Biochemistry, Meenakshi Medical College, Hospital and Research Institute, Kanchipuram, Tamil Nadu. The sample size was calculated based on the prevalence of hyperuricemia among pregnant women. The prevalence in normal pregnancy is 15%–36% in case of preeclampsia. The formula used for calculation is

$$n = \frac{\left[Z_{1-\alpha/2} \sqrt{\left\{ (1+1/m)\, p * (1-p) \right\}} + Z_{1-\beta} \sqrt{\left\{ p_0 * (1-p_0/m)\, p_1(1-p_1) \right\}} \right]^2}{(p_0 - p_1)^2}$$

Values were incorporated in the above formula and the sample size was calculated as 34.8 which was approximated to 40. After obtaining informed consent, blood samples were collected from 75 healthy singleton pregnant women from 12 to 16 weeks of gestation between 19 and 29 years with no underlying medical illness. The patients were classified as control, mild, and severe preeclampsia, as shown in Table 1.

Methods

The serum sEng level was estimated by ELISA method. The case group was further classified into mild and severe based on their sEng levels. They are classified as mild preeclampsia if their serum sEng levels varies from 7.1 to 8.4 ng/ml and severe preeclampsia when the serum sEng level are above 8.5 ng/ml. The serum uric acid was measured by uricase method in autoanalyzer.

Table 2 shows the demographic and clinical characteristics of the pregnant women. The inclusion criteria consist of primigravida, singleton pregnancy, non-diabetic, non-hypertensive, and no renal disorder. Exclusion criteria are multiparity, multiple pregnancy, diabetes mellitus, chronic

Table 1: Classification of patients

Classification	Number of persons
Control	40
Mild preeclampsia	19
Severe preeclampsia	16

hypertension, collagen vascular disease, renal disease, and thyroid disease.

RESULTS

Serum uric acid levels in controls and cases are represented in Figure 1. The mean uric acid was 2.4±0.2 mg/dl in control. The mean uric acid was 3.6±0.2 mg/dl in mild preeclampsia patients and 4.0±0.5 mg/dl in severe preeclampsia. The normal uric acid level is 2–6 mg/dl. The levels were within normal limits. Hence, it cannot be used for predicting preeclampsia.

The mean serum sEng was 5.9±0.6 ng/ml in control. The mean serum sEng was 7.7±0.3 ng/ml in mild preeclampsia patients and 8.7±1.4 ng/ml in severe preeclampsia which is represented in Figure 2. The normal sEng is 2.54–7.06 ng/ml. The levels were elevated in mild and markedly elevated in severe preeclampsia which suggests that serum sEng would be a better marker for preeclampsia.

The patients grouped under severe preeclampsia developed symptoms of preeclampsia, that is, elevated blood pressure, urine proteinuria between 26 and 28 weeks (mean 27.9±1.0), and patients with mild preeclampsia developed symptoms between 28 and 30 weeks (mean 28.6±0.6), as shown in Figure 3. Control patients did not develop any symptoms of preeclampsia. The mean systolic blood pressure of severe preeclampsia was 134.5±4.0 and diastolic blood pressure was 89.8±2.9. The mean systolic blood pressure of mild preeclampsia was 131.4±2.6 and diastolic blood pressure was 88.5±3.9.

Patients with preeclampsia delivered pre-term when compared to control. Most of the women in control group delivered at term. The average time of delivery was
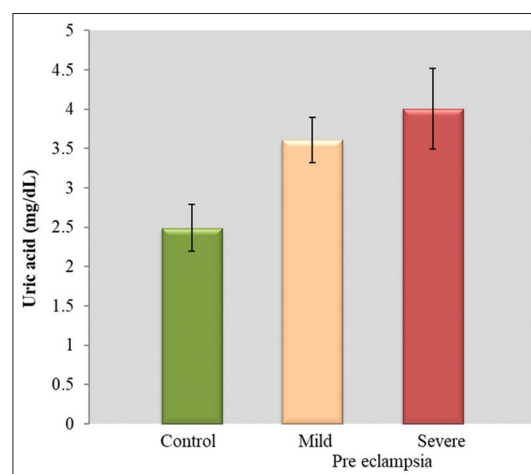

Figure 1: Serum uric acid levels between control, mild, and severe preeclampsia

Table 2: Demographic and clinical characteristics

Parameters	Control group	Mild preeclampsia	Severe preeclampsia
Age (years)	23.5±2.5	24.4±2.5	23.6±2.0
Serum urea (mg/dl)	16.6±4.6	21.6±3.3	24.3±5.1
Serum creatinine (mg/dl)	0.7±0.2	0.7±0.1	0.8±0.2
Serum uric acid (mg/dl)	2.4±0.2	3.6±0.2	4.0±0.5
Serum soluble endoglin (ng/ml)	5.9±0.6	7.7±0.3	8.7±1.4
Onset of symptoms (weeks)	--	28.6±0.6	27.9±1.0
Time of delivery (weeks)	38.9±0.5	37.7±0.7	37.1±1.1
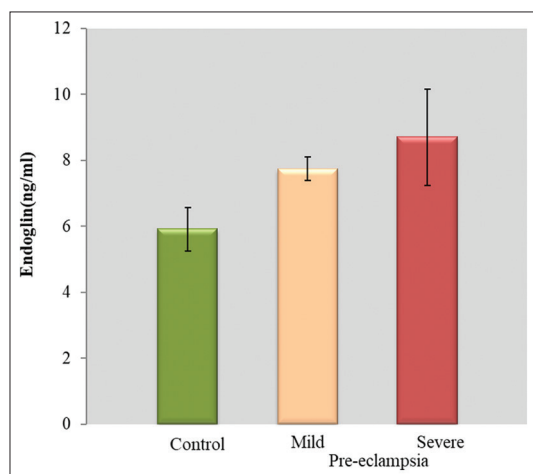
Figure 2: Serum soluble endoglin levels between control, mild, and severe preeclampsia
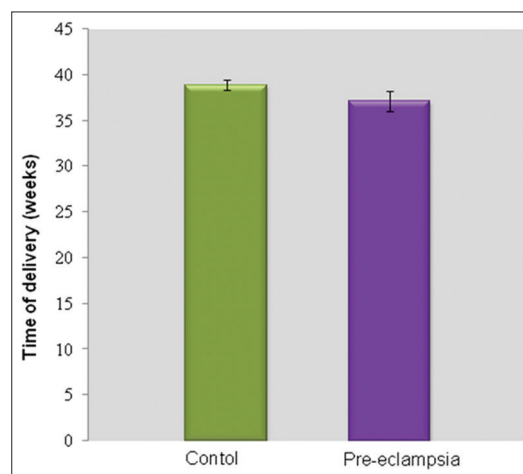
Figure 4: Time of delivery in cases and controls
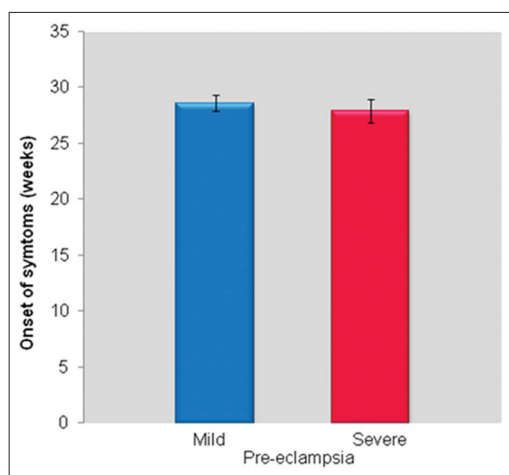
Figure 3: Onset of symptoms between mild and severe preeclampsia
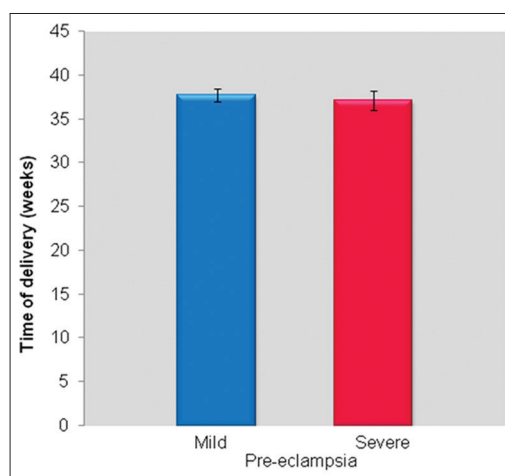
Figure 5: Time of delivery between mild and severe preeclampsia

DISCUSSION

37.1±1.1 in cases and 38.9±0.5 in control, as shown in Figure 4. The correlation between time of delivery among control and cases were significant as $p=0.01$.

Patients with severe preeclampsia delivered even early when compared to mild preeclampsia. The mean weeks of delivery were 37.1±1.1 in severe preeclampsia and 37.7±0.7 in mild preeclampsia, as represented in Figure 5. The $p=0.01$ which is significant.

Serum sEng and serum uric acid

There are few studies which suggest that concentration of angiogenic proteins in blood is good predictors of preeclampsia. In this study, sEng level, one of the angiogenic protein levels which increase between 1st and 2nd trimester proves to be an early predictor when compared to uric acid.

Shallow implementation is one of the classical features seen in placenta of severe preeclampsia. In addition

to this, abnormal vascular remodeling impaired pseudovasculogenesis is seen. These placental changes occur between 12 and 18 weeks of pregnancy which is a major pathogenesis of severe preeclampsia. It is assumed that placentation abnormalities may lead to the elaboration of systemic factors that induce the maternal syndrome of preeclampsia.[15] There are other factors such as genetic factors and hypoxia which are involved in the pathogenesis of preeclampsia.

During normal pregnancy, placenta is relatively hypoxic in the early pregnancies. Hypoxia disappears during the second trimester with increased blood flow to the placenta. However, in preeclampsia, hypoxia plays a major role Expression of both sFlt1 and sEng is elevated in response to hypoxia, where increased expression is mediated by hypoxia inducible factor-1.[16,17]

In normal individuals and in person with chronic hypertension, hyperuricemia acts as an independent predictor of renal and cardiovascular disease. Hyperuricemia has been contributed to reduce renal clearance. Kidney plays a role in filtration, reabsorbtion, and secretion of uric acid. In case of preeclampsia, increase in the concentration of serum uric acid is due to hypovolemia which is an early change in preeclampsia. This is due to increased uric acid reabsorption. However, increased uric acid precedes the reduction in plasma volume.[13] Abnormal renal clearance, increased tissue breakdown, acidosis, and a rise in the activity of the xanthine oxidase/dehydrogenase enzyme are some of the mechanisms for elevation of uric acid level in preeclampsia.[18] Reduction in the clearance of uric acid due to the reduction in the glomerular filtration rate, increased absorption, and a decrease in the secretion may be the cause for the rise in the level of serum uric acid in preeclamptic women.[19] Uric acid is a marker of oxidative stress, tissue injury, and renal dysfunction, and several studies have reported a positive correlation between elevated maternal serum uric acid levels and adverse pregnancy outcomes.[20] Uric acid is a potent inhibitor of endothelial function, induces systemic and glomerular hypertension in animals, and passes freely into the fetal circulation. Uric acid has been found to block VEGF–induced endothelial proliferation and, thus, may have a direct role in blocking fetal angiogenesis, resulting in small-for gestational-age infants. Uric acid can also block trophoblast invasion *in vitro*. Uric acid has also been found to mediate insulin resistance in animals, and levels correlate with the development of insulin resistance in the pregnant patients. A study by Taefi et al., showed no significant correlation between the plasma uric acid level among preeclamptic women and control group and concluded that diagnosing preeclampsia is linked by

measuring serum uric acid levels. Although uric acid is not useful in identifying preeclampsia, still it can play a key role in identifying preeclamptic women who are at greater risk for maternal and fetal morbidities.[19] Weerasekera et al., in 2003, studied serum uric acid levels at 28 weeks and followed the patients by monitoring uric acid level in each visit and BP. They found that there was no significant role of serum uric acid as a predictor of pre eclampsia.[21]

Serum sEng and onset of symptoms

In the present study, women with sEng levels between 7.1 and 8.2 ng/ml developed mild preeclampsia between 28 and 30 weeks of gestation, whereas women with sEng levels between 8.5–13.4 ng/ml developed severe preeclampsia between 26 and 28 weeks of gestation. sEng remains elevated throughout second trimester in women with preterm preeclampsia when compared to normal pregnancies. In women who develop preterm preeclampsia, this finding indicates that placental ischemia may be part of the cause in the increased production of antiangiogenic proteins. Hirashima et al., in 2008, reported alterations in serum sEng levels taken 3 times at 20–23, 27–30, and 36–38 weeks of gestation. They first found that there were several women with persistently high sEng levels during the second half of pregnancy who did not show clinical manifestation of preeclampsia, that is, hypertension or proteinuria. Second, after the onset of preeclampsia, women with early-onset showed high sEng levels compared with those with late-onset.[22] Duhan et al., found significantly elevated serum sEng in preeclamptic women in later pregnancy than early pregnancy in 2011.[23] Nory et al., in 2011, studied the serum sENG levels in women from 12 to 16 weeks. They found that there was no significant change in the levels between the preeclamptic women and normotensive women, whereas during 16 weeks, there was elevated serum sENG levels seen in women who developed preeclampsia.[24] These results suggest that the increase in serum sEng might be linked with the severity of preeclampsia and that the increase in circulating sEng might play an important role in pathology of the disease. Our findings are also consistent with a role for sEng in the pathogenesis and prediction of preeclampsia.

Serum sEng and time of delivery

Women in control group delivered at term. However, most of the women who developed preeclampsia delivered pre-term. Fetus delivered at pre-term will have low birth weight and prone for more complications in future when compared to term delivery. Hence, time of delivery is more important. As delivery is the only option for preeclampsia, predicting early will prevent the chances of preeclampsia and for better fetal care.

Limitations of the study

However, very limited studies have been conducted in Indian population. More number of studies at various gestational weeks in a larger number at different age group needs to be done in order to establish a biomarker for early detecting of preeclampsia.

CONCLUSION

The present study suggests that serum sEng is a good predictor of preeclampsia when compared to serum uric acid. Not all the preeclamptic mothers showed elevated serum uric acid level. On the other hand, serum sEng level was elevated only in mothers who developed preeclampsia between 12 and 16 weeks. This concludes that serum sEng increases at an early stage when serum uric acid level tends to be within normal limits. This suggests that serum sEng will be a better marker for predicting preeclampsia than serum uric acid.

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AA- Concept and design of the study, data collection and processing of samples, prepared first draft of manuscript, interpreted the results, preparation of manuscript; **SA-** Reviewed the literature and manuscript preparation; **SV-** Statistical analysis, interpretation and revision of the manuscript; and **US-** Interpretation and revision of the manuscript.

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