

# Biochemical Markers In The Prediction Of Pre-Eclampsia, Are We There Yet?

S Kanagasabai

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## Abstract

Over the last two decades various biochemical tests that have been proposed for the prediction of preeclampsia are described and evaluated. These tests are apparently better predictors when preeclampsia supervenes shortly. Thus, explaining why screening in the first trimester is unlikely to produce results as in the second trimester. The current use of multiple markers in the screening reflects that different aspects of the disease processes being evaluated hence are increasing the specificity and sensitivity of the screening tests which works on different contributing factors in its etiology. Ultimately to be practical, the screening tests needs to be simple, fast and cost effective. Urinary soluble endoglin, different profiles of Angiogenic factors ,cell-free DNA of the fetal erythrocytes, proteomics are selectively promising as future biochemical markers Some of these tests are currently available for clinical use, whereas others exist only in the laboratory in view of its cost.

## INTRODUCTION

Preeclampsia is a form of hypertension in pregnancy that complicates approximately 5-8% of all pregnancies and is a major cause of maternal morbidity and mortality worldwide.(1) Maternal mortality in the United Kingdom has risen currently not due to preeclampsia ,but attributable to obesity (2),but in countries where prenatal care does not meet the expected expectations ,preeclampsia/eclampsia accounts for 40% to 80% of maternal deaths. Infants of women with preeclampsia have a fivefold increase in mortality compared with infants without the disorder. Much of the mortality is attributable to iatrogenic prematurity especially premature induction to avoid morbidity, mortality from this dreaded disease. Approximately 10% of preeclampsia occurs before 34 weeks gestation, and induced premature delivery resulting from preeclampsia is responsible for approximately 15% of all preterm births.(3) .The specialized care of these premature babies place considerable strain on health care resources of developing nations. Despite the last two decades of research into this condition, the ability of clinicians to predict preeclampsia prior to the onset of symptoms has not improved remarkably. The onset of preeclampsia at or near term is certainly associated with low maternal and neonatal morbidity. However those patients who suffer early onset preeclampsia have significant maternal and perinatal morbidity and mortality. Historically there is a lack of

proven predictors and neither prophylaxis for preeclampsia, implying an urgent need for predictors to identify those who are at risk of contracting the subclinical disease as it is desirable for them to have more intense observation thus averting subsequent morbidity and mortality. Early detection, prediction is aimed at detecting the early pathophysiological changes preceding the development of clinical disease (4).In order to achieve this objective it seems logical to search for early markers of uteroplacental maladaptation that may be used for the later development of the clinical disease (5).Based on the data from patients with established disease with the involvement of various organ systems, the potential markers would include angiotensin II sensitivity, urine albumin excretion, uric acid and microproteinuria, urinary inhibin A, urinary kallikrein –calcium/kallikrein ratio, maternal serum alpha fetoprotein; coagulation and platelet activation, Uric acid, creatinine and urine microprotein, calcium levels, urinary soluble endoglin, urinary proteomics, human chorionic gonadotrophin, urinary and serum oestriols, plasma tumour necrosis factors, collagen levels, amniotic fluid cytokinase, plasma fibronectin levels, serum CA 125 lipoproteins, placental peptide hormones(CRH, CRHbp, activin, inhibin A, hCG); genetic markers and fetal erythrocytes in maternal blood are appropriately discussed. Currently the association between angiogenic (placental growth factor)and antiangiogenic (sFlt-1) seems to be promising, however endoglin (sEng)

acting in concert with sFlt-1 could improve the predictive performance. Alternations in placental proteins were studied using MALDI-TOF MS/MS proteomics analysis and discovered there was over demonstration of chaperonin 60, VDAC, ERp29 and cathepsin D in pre-eclampsia. In this review, the various the databases from Pubmed, Medline and Proquest were searched for relevant articles pertaining to the current biochemical tests that have been proposed for the prediction of preeclampsia have been analysed and evaluated.

### ANGIOTENSIN 11 SENSITIVITY

Over half a century ago, Deckmann and Micheal reported that preeclamptic women showed an elevated pressor response to vasopressin. Although the performance of the test had a 77% sensitivity and 95% specificity in 1973, The largest most recent study of 494 healthy nulliparous women found a disappointing sensitivity of 22% with a specificity of 85% for prediction of pregnancy induced hypertensive disorders..The disappointing results of recent studies raises doubts about usefulness as a continuing test.(6)

### RENAL LABORATORY TESTS.

Urine albumin excretion rate was the best predictive value for preeclampsia in hypertensive patient when blood for fibronectin, antithrombin 3, alpha 1 microglobulin, U-N acetyl-beta-glucosaminidase, uric acid and albumin excretion rate was studied in 68 non pregnancy induced hypertensive and 40 chronic hypertensive, positive predictive value and specificity being 87.5 and 98.9 respectively.(7)

### URIC ACID, CREATININE AND URINE MICROPROTEIN, CALCIUM LEVELS

Elevated serum uric acid levels were associated with clinical severity of preeclampsia and perinatal outcomes. Despite a good amount of studies, not of all studies suggest that the serum uric acid levels may begin to rise before the onset of hypertension and proteinuria. Hyperuricaemia, an early sign of renal involvement in preeclampsia, is the result of reduced renal clearance due to altered tubular processing of uric acid preceding glomerular affliction, which will cause albuminuria. However results from recent studies show that despite its long history of use, the discriminatory value of serum uric acid as a predictor of preeclampsia remains to be proven.(8,9) Microproteinuria levels above 375mg/l may be significant and thus could be utilized as a screening test for the early detection of a woman at risk of developing preeclampsia. Serum uric acid and creatinine had no

predictive value. Rodriques and Susuki showed that in women with a low levels of calcium/creatinine ratio and high microalbuminuria, 84% developed PE.(10,11) Martinez, however says there is no diagnostic value of microalbuminuria and the calcium/creatinine ratio when used alone (13)

### URINARY SOLUBLE ENDOLIN.

When clinical utility of urinary soluble endoglin was compared with soluble fms-like tyrosine kinase 1 to placental growth factor (PIGF) ratio, soluble endoglin levels were significantly raised in preterm preeclampsia. However urinary endoglin has limitations to determine the severity of preeclampsia, and to differentiate between preeclampsia and chronic hypertension (14)

### URINARY PROTEOMICS

Women with preeclampsia appear to present a unique urine proteomic fingerprint composed of SERPINA1 and albumin fragments that predicts preeclampsia in need of mandated delivery with highest accuracy. This characteristic proteomic profile also has the ability to distinguish preeclampsia from other hypertensive or proteinuric disorders in pregnancy.(15)

### URINARY INHIBIN A.

Urine levels of inhibin A showed the greatest discrimination between severe preeclampsia and pregnant control women, when there was cut off of 45pg/mg. for urine creatinine Women with greater than 90pg/mg urinary creatinine had a 17 fold relative risk of a clinically indicated delivery due to preeclampsia. (16)

### URINARY KALLILREIN - CREATININE RATIO(UK:CR)

Measurement of urinary kallikrein(IUK) to creatinine ratio(IUK:ck) between 16-20 weeks of gestation is a simple and practical test for the determining the risk of developing subsequent preeclampsia, with a sensitivity and specificity comparable to those reports as ascertained by other investigators using the well recognized, but less practical, angiotensin II sensitivity test(17)

### CLINICAL VALUE OF MICROTRANSFERRINURIA AND MICROALBUMINURIA

Urinary microtransferrinuria levels in pregnant women who subsequently developed preeclampsia and eclampsia were significantly higher than those pregnant women who remained normotensive. Microtransferrinuria as a predictor for preeclampsia had a sensitivity 93.5%, specificity

65%, positive predictive value of 83% and a negative predictive value of 98.4%, whereas these values for microalbuminuria were 50%, 58%, 50% and 91% respectively. Hence Microtransferrinuria is potentially a more sensitive predictor of preeclampsia than microalbuminuria. (18)

### URINARY EXCRETION OF N-ACETYL-BETA-GLUCOSAMINIDINE

The urinary excretion of N-acetyl-beta-glucosaminidase, a lysosomal enzyme of the renal tubular cells, was increased in normal pregnant women and in women with transient hypertension when compared to non pregnant healthy controls. In preeclampsia women, the increase was much higher than corresponding to their gestational age. (19)

### MATERNAL SERUM MARKERS

#### SERUM INHIBIN A IN PREGNANCIES SUBSEQUENTLY DEVELOPING PREECLAMPSIA

Inhibin-A was determined as a more sensitive marker for the prediction of preeclampsia than hCG. Addition of hCG data to inhibin did not improve the screening efficiency for preeclampsia suggesting that inhibin-A and hCG are markers of the same underlying pathological process. (20)

#### MATERNAL SERUM ALPHA FETOPROTEIN (MSAFP)

Despite using multivariable second trimester clinical factors and biomarkers like history of preeclampsia, elevated mean arterial pressure, low unconjugated serum oestriol, the addition of human chorionic gonadotrophin and alpha-fetoprotein biochemical markers did not enhance the model's predictive value for severe preeclampsia (21)

#### HUMAN CHORIONIC GONADOTROPHIN (HCG)

Midtrimester beta-hCG levels alone correlated significantly with the severity of preeclampsia. However the combination of MShCG levels, body mass index (BMI), parity and age as a predictive test for preeclampsia was far superior to MShCG alone. This multi-factorial model could identify preeclampsia with a sensitivity of 70% and a specificity of 71%. (22)

#### SERUM OESTRIOL

Even after incorporating the strongest risk factors such as nulliparity, history of preeclampsia, elevated mean arterial pressure, low unconjugated serum oestriol concentration, and in addition of hCG and  $\alpha$ -fetoprotein did not improve the predictive value for severe pre-eclampsia (23)

Plasma tumor necrosis factor was measured in the first, second and third trimester prospectively. Although the plasma TNF-alpha levels were higher in preeclamptics compared with normotensives, the levels cannot be used in the first, second trimester as a specific marker however may be useful in the early third trimester. (24)

### COAGULATION FACTORS AND PLATELETS

Platelet count, mean platelet volume and serum uric acid levels were assessed in the prediction of preeclampsia in women with mild hypertension. No test or cut off level was found to be of any use. (25)

Platelet activation is demonstrated by reduced platelet count, increased mean platelet volume and elevated plasma concentrations of beta thromoglobulin and platelet 4 in preeclamptic patients. During the first and second trimester of pregnancy preeclamptic patients have an increased expression of some antigens on their surface. Currently there is no reliable platelet test as yet to predict the onset of preeclampsia (26)

### PLASMA FIBRONECTIN

Plasma fibronectin was assessed to see if it could predict the onset of preeclampsia. This study showed that with increased fibronectin levels the sensitivity, specificity, positive and negative predictive values were 37.5% (95% CI = 3.3-71.7), 96.6% (95% CI = 93.7-99.6), 37.5% (95% CI = 3.3-71.3) and 96.6% (95% CI = 93.7-99.6). Its sensitivity has to be improved for it to be a specific marker for preeclampsia however its negative predictive value strongly argues against the development of preeclampsia within the next 4 weeks of blood sampling (27)

However platelet angiotensin II receptors measurement is not a clinically useful marker in the prediction of preeclampsia.

### SERUM THROMBOMODULIN

Serum thrombomodulin antigen levels were measured in patients with preeclampsia, gestational hypertension and chronic hypertension. Levels were higher in preeclampsia than those with gestational hypertension or chronic hypertension. Thrombomodulin may serve as a clinical marker to differentiate preeclampsia from other disorders of pregnancy. (28)

### SERUM ANDROGEN MARKERS AND SEX

## **HORMONE BINDING GLOBULIN (SHBG) MARKERS**

In obstetrics the low levels of SHBG in the first half of pregnancy were reported as a promising early risk marker of the later development of preeclampsia (29). When controls were compared with those who developed preeclampsia, serum testosterone, sex hormone binding globulin and dehydroepiandrosterone sulphate were measured and compared. As compared to normotensives controls, preeclamptic women exhibited no statically significant differences in the median levels of total testosterone, free androgen index, sex hormone binding globulin or dehydroepiandrosterone. (30)

## **SERUM CA125 VARIABILITY--NEW ASPECTS OF CLINICAL USEFULNESS**

Levels of CA125, has been historically a valuable parameter as a marker of ovarian carcinoma but also in other fields of obstetrics and gynecology. To date, most of the studies dealing with the CA125 and preeclampsia remain experimental and their clinical usefulness is not widely acknowledged (31)

## **LIPOPROTEIN A-LP(A)**

Lipoprotein a concentration in women with preeclampsia and healthy pregnancies was assessed to determine the usefulness as a prognostic factor in preeclampsia. The results show that the determination of lipoprotein (a) levels in preeclampsia may be a significant predictor of Eclampsia (32)

## **ANGIOGENIC FACTORS IN PREECLAMPSIA**

Hypertension and proteinuria, may be due to an excess of circulating anti-angiogenic growth factors, most notably soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng).(33)

sFlt1 is an endogenous protein that is produced by the placenta. sFlt1 is able to bind to the angiogenic growth factors vascular endothelial growth factor and placental growth factor, thereby neutralizing their functions. Soluble endoglin was found to cooperate with the soluble form of vascular endothelial growth factor receptor 1 in the pathogenesis of preeclampsia by inducing endothelial cell dysfunction.(34). High serum concentrations of sFlt1 and low concentrations of free vascular endothelial growth factor and free placental growth factor were evident clinically prior to clinical manifestation of preeclampsia. The excessive production of these two anti-angiogenic proteins induces

endothelial dysfunction by preventing circulating proangiogenic factors such as the placental growth factor, the vascular endothelial growth factor but also the transforming growth factor-beta. Despite numerous placental factors having been experimented, none has been able to reproduce a typical phenotype of preeclampsia.(35) More recently, serum levels of sEng were also significantly elevated in preeclamptic women and levels of sEng correlated strongly with severity of the disease process. Thus, measurement of sFlt1 and sEng in the maternal circulation may be a useful diagnostic and screening tool for preeclampsia and would have significant impact in current obstetrical care by reducing preeclampsia-induced morbidity and mortality. (36). Changes in the maternal plasma concentration of s-Eng, sVEGFR-1, and PlGF precede the clinical presentation of PE(37)) The combined PlGF/sEng ratio and its delta and slope had an excellent predictive performance for the prediction of early-onset preeclampsia, with very high likelihood ratios for a positive test result and very low likelihood ratios for a negative test result(38)(39).Masuyama and colleagues in 2009 have shown that there is sufficient data to suggest that there are different profiles of angiogenic factors and adipocytokines between women who develop early and late onset preeclampsia.(40,)

This discovery has two potential effects. Firstly, the possibility, in measuring these surrogates markers in the blood or urine to predict the onset of preeclampsia before its clinical manifestations. Secondly the postulated possibility of reversing the angiogenic imbalance state of preeclampsia by correcting the maternal syndrome by transiently adding exogenous proangiogenic factor.(41,42)

## **FETAL ERYTHROCYTES IN MATERNAL BLOOD**

Although fetal cell have been demonstrated in the lungs of patients had preeclampsia more than a century ago by Schamarol.(43)This is thought to result from placental damage and subsequent deportation across the placenta. Several studies have demonstrated the increased fetal cell trafficking and cell free DNA, from twenty weeks onwards in the maternal serum of women who developed preeclampsia later(44).The cell free DNA cells could be apoptic DNA fragments from the breakdown of the placental cytotrophoblasts. These particles could contribute to the cytotoxic effect on endothelial cells characteristic of preeclampsia.(45) Cell free fetal DNA in the maternal circulation does not stem from the the transplacental passage of fetal erythroblasts.(46).Currently results overlap between

preeclampsia and control groups, with low sensitivity and specificity.(47)..At present the only likely application of this marker would be a late second trimester marker for preeclampsia. Circulating placental RNA is a potentially useful tool for noninvasive investigation of the placenta. The development of new placental-specific RNA markers that could be detected in maternal plasma could simultaneously analyze >39,000 RNA transcripts in the placenta. This development has implication for the development of new markers/break for studying disease conditions associated with placental pathology, such as preeclampsia.(48)

### SERUM COLLAGEN LEVELS

Serum levels of collagen synthesis, procollagen I, carboxy-terminal peptide (PICP) and procollagen111 amino-terminal peptide (PIIIP), in patients with preeclampsia and controls. The markers were mildly elevated in preeclampsia, but unlikely to be useful in the prediction of preeclampsia. (49)

### AMNIOTIC FLUID CYTOKINASE.

Midtrimester amniotic fluid cytokinase may reflect the maternal immune system in the maternal fetal interface and thus be predictive of preeclampsia, concentrations of interleukin (IL)-6, IL8, IL10, IL11, IL12, IL15, tumor necrosis factor (TNF)-alpha and transforming growth factor (TNF)-beta in the amniotic fluid at 14-16 weeks gestation from women with normal pregnancies and from those who subsequently developed severe preeclampsia. The concentrations of cytokinase did not significantly differ between patients and controls.(50)

### PROTEOMICS

The current development of powerful mass spectrometry-based proteomic techniques capable of identifying and characterizing multiple proteins simultaneously has added a new insights into the field of biomedical research. Application of these methodologies with more conventional techniques in pregnancy-related research has begun to provide a new perspective on the biochemical blueprint of pregnancy and its related disorders. This enables the identification of proteins specific to a disease process; proteomics is likely to contribute, not only to the comprehension of the underlying pathophysiologies, but also to the clinical diagnosis of multifactorial pregnancy disorders. The technology to pregnancy research is in its infancy, identification of the cellular proteome, and it's of functional networks and disease biomarkers can be expected to significantly improve maternal healthcare outcomes in the near future. (51)Proteomics alterations in pre-eclampsia

suggest that possible cellular battle against mitochondria-originated oxidative stress test resulting in recovery or apoptosis. The over expression of chaperonin 60, GST, VDAC, Erp29 and cathepsin D in pre-eclampsia makes it a ideal marker of predicting preeclampsia (52, 53)

### DISCUSSION

Despite decades of research into this clinical condition, the ability of the clinicians to predict preeclampsia prior to the onset of symptoms has improved marginally but not significantly. Neither are there factors for prediction have gained universal acceptance, nor are there strategies for prevention and therapy for this disease .Urinary concentrations of various tests and some serum tests evaluated earlier has failed to establish itself as an important marker for patients with preeclampsia earlier in the disease and is also unlikely to correlate with the later stages of the disease. .However the potential possibility, in the near future, to measure the changes in the maternal plasma concentration of these surrogate markers in the blood or urine such as s-Flt1 s-Eng, sVEGFR-1, and PlGF to predict the onset of preeclampsia before its clinical manifestations remains a possibility. Hence elucidating the theoretical possibility to reverse the angiogenic imbalance state of preeclampsia by adding exogenous proangiogenic factor and consequently to correct the maternal syndrome transiently In order to justify a proper screening test which is still not currently available, there is a need for a large long term longitudinal prospective studies with rigorous definition of outcomes to determine if they are useful at all in predicting preeclampsia. . The World Health Organization (WHO) has just initiated a large prospective, observational study to evaluate the usefulness of sFlt1, soluble endoglin, and PlGF in the prediction of preeclampsia. This study will take place in developing nations, where maternal and fetal deaths from preeclampsia are greatest and tertiary care facilities are limited. If the tests can be validated to predict preeclampsia before onset could considerably reduce maternal and fetal deaths in these developing nations by providing the limited allocations for tertiary care providers and keeping a close surveillance by the appropriate caregivers (54) In conclusion, as of 2010, the only successful treatment for preeclampsia is delivery. No definitive preventive strategies have been identified. However, several of the recent observations based on these biochemical tests provide stimuli for the development of novel therapies for future application as mentioned above.

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**Author Information**

**Sachchithanantham Kanagasabai, FRANZCOG, FRCOG, FACS, FICS, DIMH.**

Senior Const. Obs & Gynae and Head of Dept, Melaka Manipal Medical College