Role of Soluble Fms-like Tyrosine Kinase 1 / Placental Growth Factor Ratio

In Prediction of Preeclampsia

thesis

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By

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List of Content

List of abbreviations

PE	Pre-eclampsia
PIGF	Placental Growth Factor
sVEGFR-1	Soluble Vascular Endothelial Growth Factor Receptor-1
sFLT-1	Soluble Fragments Like Tyrosine Kinase-1
VEGF	Vascular Endothelial Growth Factor
NHBPEP	*National High Blood Pressure Education Program*
IUGR	Intrauterine Growth Restriction
ACOG	American College of Obstetricians and Gynecologists
UPCR	Urine Protein/Creatinine Ratio
SGOT	Serum Glutamic Oxalo-acetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
LDH	lactate dehydrogenase
PET	Pre-EclampticToxaemia
ELISA	Enzyme Linked Immuno Sorbent Assay
RIA	Radio Immuno Assay
IL	Interleukin
AUC	Area Under The Curve
CBC	Complete Blood Count
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
HRP	Horseradish Peroxidase
ROC	Receiver-Operating Characteristic

List of abbreviations

BMI	Body Mass Index
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
OD	Odds Ratio
CI	Confidence Interval
PPV	Positive Predictive Value
NPV	Negative Predictive Value
PLR	Positive Likelihood Ratio
NLR	Negative Likelihood Ratio

List of Figures

List of Tables

Introduction

Preeclampsia (PE) is an important cause of fetal and maternal morbidity and mortality worldwide. It complicates about 5% of pregnancies ***(Hutcheon et al., 2011).***It occurs during second and third trimester of pregnancy. It is characterized by blood pressure of ≥140/90 mmHg or rise in systolic blood pressure of more than 30 mmHg or diastolic blood pressure of more than 15 mmHg after 20 weeks of gestation, in conjugation with proteinuria ≥300 mg/24 hours or greater or equal to +1 or 30 mg/dl by dipstick response ***(Lindheimer et al., 2009).***

In the absence of proteinuria, preeclampsia is diagnosed as hypertension in association with thrombocytopenia (platelet count less than 100.000/microliter), impaired liver function (elevated concentration of liver transaminases to twice the normal concentrations), renal insufficiency (elevated serum creatinine greater than 1.1 mg/dl or doubling of serum creatinine in the absence of other renal disease), pulmonary edema or new onset of cerebral or visual symptoms ***(Roberts et al., 2013).***

Additional signs and symptoms that can occur include visual disturbance, headache, epigastric pain, thrombocytopenia, and abnormal liver function. These clinical manifestations result from mild to severe microangiopathy of target organs, including the brain, liver, kidney and placenta ***(Lain and Roberts, 2002).***

Despite intense research efforts, the pathogenesis of preeclampsia is still mysterious, but it is mostly multifactorial **(J.M. Roberts et al, 2003)**.

The pathophysiology of preeclampsia likely involves maternal, fetal and placental factors. Abnormalities in the development of placental vasculature early in pregnancy may result in relative placental underperfusion/ hypoxia/ischemia, which then lead to release of antiangiogenic factors into the maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease ***(Brosens et al., 2011).***

Introduction

There is growing evidence supporting the role of angiogenic proteins in the diagnosis and prediction of PE. The markers most frequently studied are the proangiogenic protein, **placental growth factor (PlGF)**, and the antiangiogenic protein, **soluble vascular endothelial growth factor receptor 1 (sVEGF R1),** also referred to as **soluble fms-like tyrosine kinase 1 or (sFlt-1)** ***(Sunderji et al, 2010).***

Because of the high prevalence and seriousness of preeclampsia, evaluation of various angiogenic and antiangiogenic factors in both serum and plasma have been tested as diagnostic markers for preeclampsia along with an assessment for the probability of their use in prediction of preeclampsia development (**R. J. Levine et al, 2004; K. Koga et al, 2003; R. J. Levine et al, 2005**).

Recent studies have shown that sFlt-1, an antiangiogenic protein that is produced by the placenta acts by antagonizing two pro-angiogenic molecules **vascular endothelial growth factor (VEGF)** and PlGF - raising the possibility that this antiangiogenic protein might have a pathogenetic role in PE. ***(Maynard et al, 2003; Maynard et al, 2005).***

Elevated serum levels of the antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) are found involved in preeclampsia (**S. E. Maynard et al, 2003; E. Shibata et al, 2005; T. Chaiworapongsa et al, 2004**). A pathological role for the antiangiogenic factor sFlt-1 has been established in pregnant animals (**S. E. Maynard et al, 2003**; ***S. Venkatesha et al, 2006*****).** A preeclampsia-like syndrome has been reported in pregnant rats that have been given adenovirus expressingsFlt-1 (**S. E. Maynard et al, 2003**).

PlGF is a member of the VEGF family and is released from the placenta and the maternal endothelium. Experimental evidence shows that, although PlGF has only little mitogenic effect alone, it potentiates the effects of VEGF ***(park et al, 1994).***

Contrary to sFlt–1, PlGF is thought to exert a direct proangiogenic function in the maternal circulation, and the levels of PlGF are decreased early in pregnancies later complicated by PE ***(Thadhani et al, 2004).***

An increase in sFlt-1 and a decrease in PlGF have been revealed in maternal serum five to ten weeks before the onset of preeclampsia (**R. J. Levine et al, 2004)**. It has been postulated that these changes in sFlt-1 and PlGF may contribute to the pathogenesis of preeclampsia (**R. J. Levine et al, 2004; R. J. Levine et al, 2006)**.Thus, these two particular factors have been proposed as candidates for an efficient screening in the prediction of preeclampsia (**R. J. Levine et al, 2006**; ***T. Chaiworapongsa et al, 2005***;***R. Romero et al, 2008)***.

Formerly, many studies assessed sFlt-1 and PlGF for the prediction of preeclampsia development. However, few studies reported the sFlt-1/PlGF ratio in the prediction of preeclampsia. In the present study, we investigated the ability of late second and early third trimester serum levels of sFlt-1, PlGF, and sFlt-1/PlGF ratio to identify women at high risk for development of preeclampsia and to determine whether the use of sFlt-1/PlGF ratio was superior to the use of individual biomarkers sFlt-1 and PlGF in the prediction of preeclampsia.

The ratio of sFlt–1/PlGF predicts the development of PE in some, but not all studies ***(Verlohren et al, 2014).***

Aim of the Work

This study aims to measure the accuracy of the **sFlt-1 / PlGF ratio** to predict the occurrence of PE accurately.

Research hypothesis

In normal pregnant women between 24-32 weeks, measurement of **sFlt-1 / PlGF ratio** may predict the occurrence of PE.

Research Question

In normal pregnant women between 24-32 weeks, does measurement of **sFlt-1 / PlGF ratio** predict the occurrence of PE?

Chapter (1): **Pre-eclampsia**

Hypertension is one of the most common medical complications of pregnancy. It remains one of the leading causes of maternal deaths all over the world, about 15 % to 20% of maternal deaths in developing as well as developed nations ***(Wagner et al., 2007).***

►Classification of hypertension in pregnancy :

According to the working group of the ***National High Blood Pressure Education Program, 2008,*** (NHBPEP) there are five types of hypertensive disease that include:

[1] Gestational hypertension (formerly pregnancy-induced hypertension or transient hypertension).

[2] Chronic hypertension.

[3] Pre-eclampsia superimposed on chronic hypertension.

[4] Pre-eclampsia.

[5] Eclampsia.

1-Gestational hypertension :

This describes women who develop de novo hypertension without proteinuria. It was noted that a substantial number of these women later develop proteinuria and are reclassified as preeclamptics. Also, a final diagnosis was to be made postpartum in that women whose blood pressure normalized by the 12th postpartum week should be reclassified as "transient hypertension" (the designation of this group in the 1990 report) but if hypertension persisted the diagnosis would be changed to "chronic hypertension" ***(Brown et al., 2001).***

2-Chronic hypertension :

Defined as hypertension known or detected prior to conception or diagnosed before the 20th gestational week, hypertension in pregnancy is defined as a blood pressure more than 140mm Hg systolic, and or more than 90mm Hg (K5) diastolic. In addition, hypertension initially diagnosed during pregnancy that failed to resolve by 12 weeks postpartum would retrospectively be reclassified as chronic hypertension ***(Lindheimer et al., 2009).***

3-Pre-eclampsia superimposed chronic hypertension :

There is substantial evidence that preeclampsia occurs in women already hypertensive of both the essential and secondary variety. There is further evidence that superimposed disease in these populations has an incidence exceeding 20% ***(Brown et al., 2001).***

4-Pre-eclampsia , 5-Eclampsia :

The diagnosis of pre-eclampsia is determined by the de novo appearance of hypertension and proteinuria after mid pregnancy (but can rarely occur earlier with trophoblastic diseases such as hydatidiform mole). Proteinuria was defined as the urinary excretion of more than 0.3 g in 24 hours collection. The Working Group gave tentative approval to qualitative testing, noting that a 24-hour measurement more than 0.3 g "usually correlated with 30 mg/dl, (1+ dipstick) or greater in a random urine determination with no evidence of urinary tract infection." They stressed, however that because there were discrepancies between the two approaches they strongly recommended "diagnosis be based on a 24-hour urine, if at all possible, or a timed collection corrected for creatinine excretion if this is not feasible. Eclampsia is the occurrence of seizures in women with preeclampsia that cannot be attributed to other causes ***(Lindheimer, 2009).***

■Pre-Eclampsia :

Preeclampsia (PE) is a leading cause of maternal and perinatal morbidity and mortality worldwide, with a global incidence of 3%–5% of all pregnancies annually ***(Sibai et al, 2005).***

It occurs during second and third trimester of pregnancy. It is characterized by blood pressure of ≥140/90 mm Hg or rise in systolic blood pressure of more than 30 mmHg or diastolic blood pressure of more than 15 mmHg after 20 weeks of gestation, in conjugation with proteinuria ≥300 mg/24 hours or greater or equal to +1 or 30 mg/dl by dipstick response ***(Lindheimer et al., 2009).***

In the absence of proteinuria, preeclampsia is diagnosed as hypertension in association with thrombocytopenia (platelet count less than 100.000/microliter), impaired liver function (elevated concentration of liver transaminases to twice the normal concentrations), renal insufficiency (elevated serum creatinine greater than 1.1 mg/dl or doubling of serum creatinine in the absence of other renal disease), pulmonary edema or new onset of cerebral or visual symptoms ***(Roberts et al., 2013).***

Additional signs and symptoms that can occur include visual disturbance, headache, epigastric pain, thrombocytopenia, and abnormal liver function. These clinical manifestations result from mild to severe microangiopathy of target organs, including the brain, liver, kidney and placenta ***(Lain and Roberts, 2002).***

Pre-eclampsia is the second leading cause after embolism, of maternal mortality. Early detection and appropriate management of the pregnancy may improve the outcome for both the mother and the fetus ***(Fitzpatrick et al., 2009).***

Preeclampsia may develop before week 20 in patients with extensive hydatidiform changes in the chorionic villi or in the presence of lupus anticoagulant, so preeclampsia is considered as a pregnancy-specific condition characterized by placental dysfunction and a maternal response featuring systemic inflammation with activation of the endothelium and coagulation. This multifactorial disease presents as a syndrome of symptoms and signs, with associated hematological and biochemical abnormalities ***(Mitsuko et al., 2011).***

►Pathophysiology :

The etiology is complex, and, in spite of decades of research, the exact mechanisms behind the disease remain unclear ***(ACOG 2002).***

There are numerous theories concerning the cause and pathophysiology of PE, including trophoblast cell invasion, oxidative stress, endothelial dysfunction and most recently, antiangiogenic proteins ***(Widmer et al, 2007; Mignini et al, 2005).***

There is growing evidence supporting the role of angiogenic proteins in the diagnosis and prediction of PE. The markers most frequently studied are the proangiogenic protein, **placental growth factor (PlGF)**, and the antiangiogenic protein, **soluble vascular endothelial growth factor receptor 1 (sVEGFR1)**, also referred to as **soluble fms-like tyrosine kinase 1** or **(sFlt-1)** ***(Sunderji et al, 2010).***

This pathophysiology of pre-eclampsia is established in the first trimester of pregnancy, when a range of deficiencies in placentation affect the key process of spiral artery remodeling. As pregnancy progresses to the third trimester, inadequate spiral artery remodeling along with multiple