REVIEW ARTICLE

The Use of Soluble FMS-like Tyrosine Kinase 1/Placental Growth Factor Ratio in the Clinical Management of Pre-eclampsia

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Abstract

Hypertensive disorders of pregnancy in particular the category preeclampsia (PE), remains a major cause of both maternal and fetal morbidity and mortality. Angiogenic growth factors (PlGF and VEGF) and their tyrosine kinase receptors -1 and 2 (Flt-1 and KDR) are involved in both fetal and placental development. Inadequate placentation and the consequent release of antiangiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) is thus instrumental in the etiology of this disease. sFlt-1 binds to both angiogenic growth factors and neutralizes their effect thereby creating an angiogenic imbalance. This imbalance is frequently reported in women diagnosed with preeclampsia occurring before the clinical manifestation of the disease. The recent prognostic value of the sFlt-1/PlGF ratio has received considerable attention as a risk indicator of preeclampsia development. The aim of this review is to highlight the current advances in the diagnostic utility of the sFlt-1/PlGF ratio with regards to preeclampsia development. *(Afr J Reprod Health 2018; 22[4]: 135-143).*

Keywords: Preeclampsia, sFlt-1, PlGF, treatment, prediction

Résumé

Les troubles hypertensifs de la grossesse, en particulier de la catégorie pré-éclampsie (PE), restent une cause majeure de morbidité et de mortalité maternelles et fœtales. Les facteurs de croissance angiogéniques (PlGF et VEGF) et leurs récepteurs tyrosine kinase -1 et 2 (Flt-1 et KDR) sont impliqués dans le développement fœtal et placentaire. La placentation inadéquate et la libération consécutive de la tyrosine kinase 1 analogue à la fms soluble anti-angiogénique (sFlt-1) jouent donc un rôle déterminant dans l'étiologie de cette maladie. Le sFlt-1 se lie aux deux facteurs de croissance angiogéniques et neutralise leur effet, créant ainsi un déséquilibre angiogénique. Ce déséquilibre est fréquemment signalé chez les femmes chez lesquelles une pré-éclampsie a été diagnostiquée avant la manifestation clinique de la maladie. La récente valeur pronostique du rapport sFlt-1 / PlGF a fait l'objet d'une attention considérable en tant qu'indicateur de risque de développement de la pré-éclampsie. Le but de cette étude est de mettre en évidence les progrès actuels dans l'utilité diagnostique de l'indice de sFlt-1 / PlGF en ce qui concerne le développement de la pré-éclampsie. *(Afr J Reprod Health 2018; 22[4]:135-143).*

Mots-clés: Pré-éclampsie, sFlt-1, PlGF, traitement, prédiction

Introduction

Low and middle income countries (LMIC) such as South Africa face challenges in reducing maternal and neonatal deaths. Nonetheless, attempts are underway to meet the United Nation's Sustainable Developmental Goals to reduce the maternal mortality ratio of >70 per 100 000 live births by the year 2030. The Saving Mothers Report states that 14% of all deaths are due to hypertensive disorders of pregnancy (HDP)[1]. Preeclampsia (PE) remains a significant cause of maternal and neonatal mortality and morbidity worldwide[2] particularly in LMIC. It is the commonest direct cause of maternal mortality in South Africa, of which almost 70% are associated with avoidable

factors[3]. With the exception of prevention of PE development through the use of low-dose acetylsalicylic acid for women with a previous history of the disorder[4], early detection, stabilization of high blood pressure and timely delivery remain the cornerstone of treatment[5]. Preeclampsia is characterised by mid-gestational new onset hypertension and proteinuria, affecting 5% to 10% of all pregnant women worldwide[6]. If untreated, it leads to maternal and fetal complications such as convulsions, stroke, liver rupture and/or failure, renal failure, and death[7]. Still births, intrauterine growth restriction, preterm deliveries and low birth weight babies are also complications of PE[7]. Diagnosis of PE is clinically dependent on the measurement of blood pressure levels and the presence of proteinuria, however, these tools show low predictive sensitivity and specificity of both the disease progression, and maternal and perinatal outcome[7]. Thus, the need for a reliable biomarker to predict those at risk of PE development is urgently warranted.

Angiogenic vs anti-angiogenic factors in the pathogenesis of PE

Whilst the exact cause of PE remains unresolved, speculations of its origin are diverse. The pathogenesis includes placental dysfunction which is characterised by defective trophoblastic invasion and incomplete physiological remodelling of myometrial spiral arteries during the first 20 weeks of gestation[8]. The absence of the normal remodelling of the spiral arterioles into wide bore channels leads to reduced placental perfusion in the subsequent weeks. The invasive cytotrophoblasts of the pre-eclamptic placenta exhibit a vascular mimicry due to its inability to express epithelial cell-like adhesion molecules but rather express endothelial cell adhesion markers such as integrins alpha 1/beta 1, alpha V/beta 3 and VE-cadherin[9,10]. Studies have speculated that this leads to placental hypoxia, reduced placental perfusion, and the consequent release of placental soluble factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng)[11,12]. Soluble fms-like tyrosine kinase 1 (sFlt-1) is an anti-angiogenic protein produced by the placental syncytiotrophoblasts and is a truncated splice variant of the membrane-bound vascular endothelial growth factor (VEGF) receptor -1 (Flt-1)[13]. It contains an extracellular ligand binding domain without the transmembrane and intracellular signalling domains and is reported to reduce the invasive ability of cytotrophoblast *in vitro*[14]. In contrast, placental growth factor (PlGF), a member of the VEGF family, is a pro-inflammatory protein produced by placental trophoblasts[15]. It has a limited mitogenic effect yet induces angiogenesis[11]. PlGF strengthens VEGF signalling by shifting VEGF from its Flt-1 receptor and redirecting it to bind to the stronger VEGF receptor-2 (KDR)[11].

Circulating sFlt-1 levels are minimal during early pregnancy but rises steadily towards the 3rd trimester, indicative of the consequent physiological anti-angiogenic shift in the placental environment[11]. This angiogenic imbalance linked with maternal endothelial dysfunction, blood coagulation and renal endotheliosis, manifests as increased blood pressure and proteinuria[2]. The proposed utility of sFlt-1, PlGF and its ratio as a prognostic indicator of PE development is widely investigated[16-21]. Based on these angiogenic trends, our study reviews the predictive accuracy of sFlt-1/PlGF ratio in PE diagnosis and its value as a clinical diagnostic marker.

Methods

An electronic MEDLINE search identified studies on the prediction of PE using the biomarkers sFlt-1 and PlGF. The search strategy was based on the MeSH terms and keywords related to preeclampsia and to each of the biomarkers. The search terms were ‘preeclampsia and angiogenic factors and sFlt-1”. The second search included keywords such as ‘clinical utility’ and ‘sFlt-1/PlGF ratio’. Inclusion criteria included studies published to-date on testing of sFlt-1 and PlGF in plasma or serum of pregnant females.

Discussion

sFlt-1 and PlGF in preeclampsia development

The landmark study by Maynard *et al*.[22] stimulated both experimental and clinical studies that evaluated the clinical utility of sFlt-1 and PlGF in the diagnosis and prediction of PE[3,16,19,20,23-27]. Maynard *et al.*[22] demonstrated elevated maternal serum sFlt-1 levels, reduced PlGF and free VEGF levels and an up-regulation of placental Flt-1 mRNA in pre-eclamptic pregnancies. Circulating PlGF levels increase around 28-32 weeks in normal pregnancies but decrease in PE[28]. This reduction manifests a few weeks prior to clinical presentation and may be useful in early diagnosis of women at high risk and with a clinical suspicion of PE at 20–35 weeks gestation. These data are consistent with other studies that support the role of anti-angiogenic factors in PE development[28-32]. sFlt-1 acts as a scavenger receptor for VEGF-A and PlGF, and interrupts their binding affinity and signalling[22,28,33]. Endothelial cell homoeostasis is maintained by VEGF and PlGF however, circulating sFlt-1 in the maternal circulation leads to a net decrease in PlGF and VEGF in the vasculature, thereby disrupting endothelial cell, causing endothelial dysfunction[22,28,33].

The reliability of angiogenic proteins to discriminate between different types of pregnancy-related hypertensive disorders[34], chronic kidney disease[24,35], severe pregnancy complications[36] and future cardiovascular disorders (CVD) is widely reported[37]. Pre-eclamptic individuals are reported to be at a greater risk of developing CVD and diabetes[37]. However, the predictive usefulness of the pre-eclamptic prediction biomarkers is greatly improved when combined with maternal and clinical characteristics[38].The negative predictive value of these biomarkers is remarkable and may provide added benefit on suspicion of PE onset, by preventing excessive testing/management, hospital admission and iatrogenic preterm delivery[38]. Several studies have confirmed the role of angiogenic factors in the pathophysiological outcomes observed during PE, long before the onset of clinical signs and symptoms[28,39-42]. In addition, cross-sectional angiogenic studies conducted amongst Black South African women at term prior to delivery confirmed higher serum levels of the anti-angiogenic sFlt-1 and soluble endoglin in PE pregnancies[43,44].

sFlt-1/PlGF ratio

There is increasing evidence linking the shift in sFlt-1 and PlGF levels to PE development and its-related adverse outcome[28,32,45]. The anti-angiogenic activity index of the sFlt-1/PlGF ratio represents variation in both sFlt-1 and PlGF levels, and correlate exceptionally well with disease severity[46]. Its detection prior to clinical presentation of the disease may improve clinical management, reduce unnecessary hospitalization and preterm deliveries related to PE. Thus, its utility as an anti-angiogenic indicator of PE development has been widely investigated[16-21], with the sFlt-1/PlGF ratio being a better predictor of PE development than either marker alone. These data have contributed to the development of automated angiogenic biomarker platforms (sFlt-1 and PlGF) in an attempt to assist with the diagnosis and prognosis of PE. Recent guidelines from the National Institute for Health and Clinical Excellence advocate regular screening for PE risk factors[47]. It is recommended that those at high risk be identified before the 13th week of gestation and they should commence low-dose aspirin intake until 36 weeks' gestation[47]. These guidelines endorse the use of an automated test (sFlt-1/PlGF ratio), due to its ability to identify women at risk of PE development, thereby enabling better management.

Moreover, negative PlGF-based tests (such as Triage PlGF test; Elecsys immunoassay sFlt-1/PlGF ratio) may be clinically valuable "for the rule-out of pre-eclampsia" in women presenting with suspected PE between 20 and 34 weeks plus 6 days of gestation[48]. Whilst systematic review and meta-analysis biomarker accuracy studies have demonstrated significantly altered biomarker concentrations prior to 30

weeks' gestation in women who developed PE[49], its precision for accurate clinical prediction is limited. Automated tests to-date, have approximated the predictive power of angiogenic proteins for the 2nd trimester as 89%[50]. However, recent meta-analysis studies argue that despite their moderate accuracy for PE detection and its high accuracy for early-onset PE, its translation into clinical practice still warrants further analyses[51]. Yet its clinical potential if combined with other biomarkers is undoubtedly promising. Verhloern *et al.*[16] reported on the 1st trimester predictive value of 0.95 prediction power in a longitudinal multicentre study and suggests a detection rate of 82% and 89% for all PE and early onset PE, at a false positive rate of 5% and 3%, respectively[16]. On the other hand, several studies have demonstrated an 80–100% sensitivity with 89–100% specificity for classifying early-onset PE and/or intrauterine growth restriction (IUGR)[26,45,52].

Angiogenic factors are therefore believed to be promising clinical tools for the early detection of PE at the "pre-symptomatic stage" thereby influencing disease management and prognosis of affected pregnancies. Early antenatal access will allow for consistent screening and detection prior to disease manifestation and thus allow appropriate clinical management. Whilst this strategy may benefit the diagnosis of early onset PE, it lacks specificity in detection of late-onset PE, the type that correlates with increased adverse outcome[53,54]. Recent studies have also demonstrated the diagnostic accuracy for PE detection in pregnancies with persistent atypical uterine artery Dopplers when combined with angiogenic-related biomarkers around 26 weeks' gestation[8].

The automated Roche Elecsys sFlt-1 and PlGF immunoassays investigated by many, are recognised as convenient, fast, fully automated, and ready to use reagent concept for routine hospital laboratories[16,34,55-57]. More recently, the PROGNOSIS Study[58] emphasised its potential to influence clinical practice[58]. The clinical utility of a sFlt-1/PlGF ratio of ≤38 to predict the absence of PE within 1 week and a ratio ≥38 to rule in PE within 4 weeks was substantiated[58]. Results from this combined cohort show that for women with suspected pre-eclampsia between 24-36 wks ±6 days of gestation, sensitivity and specificity for ruling out PE within 1 week was relatively high[58]. Moreover, the sensitivity for ruling in PE within 4 weeks was lower than for ruling out PE within 1 week, but specificity was relatively high[58]. A 38 cut-off point for sFlt-1: PlGF ratio was confirmed to be beneficial in predicting the short-term absence of PE in women in whom the disorder is clinically suspected[58]. These assays also demonstrate excellent technical performance and compared adequately with the current manual ELISA methods, except they showed a wider measuring range.

Comparative studies on the diagnostic accuracy of BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio with the Elecsys immunoassay PlGF/sFlt-1 ratio was recently conducted amongst 39 patients with confirmed PE and 76 normotensive pregnant patients[59]. These investigators reiterated the diagnostic usefulness, regardless of assay platform for early–onset PE, women with PE and preterm delivery or Haemolysis, Elevated Liver enzymes, Low Platelet count (HELLP), and non–obese women developing PE[59]. In addition, prospective, multicenter, case-control studies compared the diagnostic performance and value of Elecsys® immunoassay sFlt-1/PlGF ratio and the Triage® PlGF assay[59]. They reaffirmed the diagnostic utility of the fully automated sFlt-1/PlGF ratio Elecsys assay in contrast to the Triage PlGF assay for the clinical management of women with suspected preeclampsia[59]. More recently, the Preeclampsia Open Study prospectively evaluated the clinical utility of the sFlt-1/PlGF assay in the diagnosis of preeclampsia in women with clinical signs and symptoms in routine clinical practice[17]. A real-time recording of decisions in the clinic was employed by these investigators, which demonstrated the impact of sFlt-1/PlGF ratio on the physicians' clinical decision-making skills for pregnant women with a suspicion of PE and on

patient management[17]. Risk for preeclampsia-associated maternal and fetal outcomes increased along with increasing sFlt-1/PlGF ratios and was highest in women with a sFlt-1/PlGF ratio of 85 and above[17], concurring with a previous study by Rana *et al*[24].

Based on their ROC analysis, De Oliveira *et al*.[60] confirmed an inverse association between sFlt-1/PlGF ratio and the outstanding gestational period plus the utility and accuracy of sFlt-1: PlGF ratio in detecting those at risk of adverse outcomes. Their use in predicting adverse outcomes in women with suspected PE if presented at <34 weeks was corroborated by Rana *et al*.[24] This prospective multicenter study highlights the accuracy and predictive value of the sFlt-1/PlGF ratio in diagnosing preeclampsia-associated consequences[60]. It also demonstrates their clinical importance in the inception of preeclampsia-associated maternal or fetal consequences within two weeks amongst those who presented with clinical signs and symptoms of PE[60]. Their data demonstrates a high sFlt-1/PlGF ratio with a median of 4 (25^{th}–75^{th} centile 2–14) amongst those without and a median of 226 (50–547) in those with early-onset preeclampsia-associated consequences[60]. They further emphasised that a ratio greater than 85 had a significantly reduced gestational period[60].

A systematic review evaluating the link between 1^{st} trimester sFlt1 levels and pregnancy outcome does not support sFlt-1 as a predictive tool[61], whilst others have confirmed that first trimester maternal PlGF and free b-hCG are potential screening utilities for early-onset PE development when integrated with maternal characteristics[62]. The sensitivity of >75% is demonstrated for a prediction value of sFlt-1 /PIGF ratio especially for early-onset preeclampsia development[63]. However, the diagnostic power of the sFlt-1 /PlGF ratio appears to be greater in patients with early-onset preeclampsia compared with late-onset[63]. Stephan *et al*.[26] nevertheless recommends the use of the ratio in combination with the uterine artery Doppler as both sensitivity (83%) and specificity (95%) are higher[26]. Preeclampsia can result in maternal and fetal death, and thus high sensitivity is absolutely essential in screening for preeclampsia. Also, a low specificity will lead to unnecessary use of health resources and cause needless concern and burden for healthy pregnant women. Measurement of maternal angiogenic factors early in pregnancy or onset of preeclampsia may improve maternal and child health, both in the short and in the long term.

Recent longitudinal studies evaluated the angiogenic and Doppler profiles at admission in a cohort of high risk women developing PE[64]. These investigators confirmed a positive link between admission plasma sFlt-1/PlGF levels with negative maternal and neonatal consequences and pre-delivery uteroplacental Doppler flow deviations[64]. The angiogenic imbalance which increased steadily amongst those who presented with negative consequences, may be linked with the characteristic fluctuations observed in uteroplacental flow in PE women[64]. Thus it is believed that the use of angiogenic profiles in management of such women may have a positive link with pregnancy maintenance. An earlier study conducted on pre-eclamptic patients revealed that those with sFlt-1/PlGF ratios < 85 are more likely to be correlated with those women predisposed to obesity, pre-existing diabetes, and limited serious adverse outcomes, than those with sFlt-1/PlGF ratios >85[65]. Furthermore the integration of angiogenic biomarkers in the evaluation of preeclampsia may enable a more accurate and earlier identification of severe disease with possibly prevention of preterm delivery. These data therefore substantiate that an angiogenic imbalance among women with a suspicion of PE may be correlated with negative maternal and neonatal outcome[65]. The sFlt-1/PlGF ratio is reported to have a better diagnostic accuracy compared with current standard of care; however the cut-off values and reference ranges for the test only apply to singleton pregnancies[65]. Thus, it is important that interpretation of results be cautious in women with multiple pregnancies and the test be offered in combination with other established

diagnostic tools, to those who are considered high risk.

The predictive value of the sFlt-1/PlGF ratio for diagnosis of severe-early onset PE in combination with Doppler sonography and other clinical and biochemical biomarkers is notable, however is limited in their prediction of late onset PE[46]. Despite the possible limitations of its use in the first trimester, the fluctuating levels of both sFlt-1 and PlGF approximately 6 weeks before the clinical onset of the disease correlates significantly with disease severity[59]. These observations support its clinical role as a promising biomarker even in the first trimester particularly if combined with extracellular fetal haemoglobin and □1-microglobulin[59]. Thus, it is imperative that prescribed biomarkers for PE have the potential to be measureable prior to the advancement of the disease, thereby assisting in early referrals of those affected by remote health care professionals.

Conclusion

The sFlt-1/PlGF ratio has the potential to be implemented in clinical practice to guide appropriate patient management with respect to hospitalization and therapeutic decision. However in a clinically relevant proportion of pregnant women with signs and symptoms of preeclampsia, eclampsia or HELLP syndrome, large scale studies are warranted.

Contribution of Authors

All authors have read and approved the manuscript.

Nalini Govender: Drafted and conceptualised the manuscript.

Jagidesa Moodley: Contributed conceptually to the manuscript

Thajasvarie Naicker: Contributed conceptually to the manuscript.

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