THE ROLE OF PLATELET ENDOTHELIAL CELL ADHESION MOLECULE-1 (PECAM-1) AND SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (sVEGFR)-1 AND -2 IN THE PATHOGENESIS OF HIV ASSOCIATED PRE-ECLAMPSIA

By

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PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Optics & Imaging Centre, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professor T Naicker and in collaboration with Professor J Moodley.

Miss Semone Thakoorddeen (210500459)

Prof. T Naicker  (Supervisor)
DECLARATION

I, Semone Thakoordeen declare that:

(i) The research reported in this dissertation, except where otherwise indicated is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other person’s data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:

a) Their words have been rewritten but the general information attributed by them has been referenced.

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Signed: __________________________ Date: 31/10/2016
DEDICATION

To my parents and brother, Sherwin,

Your love is beyond bounds. Without your unyielding support, encouragements and faith in me, I would not have pursued a Master of Medical Science degree, with such confidence.

I love you, Mum, Dad and Sherwin.

To my grandparents,

I have learned perseverance and resilience from you, traits that enabled me to never be railroaded from my destination.

I love you, Nana, Nani, Grandma and Grandad.
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<tr>
<td>Angiotensin II type 1</td>
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<td>Blood pressure</td>
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<td>Early-onset pre-eclampsia</td>
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<td>Enzyme-linked immunosorbent assay</td>
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<td>Extracellular signal-regulated kinases</td>
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<td>Human Immunodeficiency Virus</td>
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ABSTRACT

Background: It is proposed that the pathology behind a pre-eclamptic placenta is impaired placentation resulting in an imbalance between angiogenic and antiangiogenic factors leading to widespread vascular endothelial dysfunction. The angiogenic, anti-angiogenesis imbalance is evident in pre-eclampsia and it has been suggested that it could be used to identify women likely to develop the clinical features of pre-eclampsia in early pregnancy. Additionally, a lack of adhesion molecules leads to inadequate cytotrophoblast invasion, augmenting endothelial damage. Current predictive tests for pre-eclampsia have a low sensitivity and specificity. Therefore, this study determined the concentrations of sVEGFR-1 and -2 and PECAM-1 in HIV associated pre-eclampsia, which show promise as a predictive tool.

Method: Retrospectively collected blood serum samples were analysed from 38 normotensive and 38 pre-eclamptic pregnancies, further stratified by HIV status. Quantification of the angiogenic factors were done by use of a multiplex immunoassay.

Results: A significant up-regulation of sVEGFR-1 concentrations were observed in the pre-eclamptic compared to the normotensive pregnant group (p = 0.001), irrespective of HIV status. Specifically, a higher concentration of sVEGFR-1 was observed in HIV negative pre-eclampsia than HIV positive pre-eclampsia. Additionally, there was a significant difference in sVEGFR-1 levels between the HIV positive normotensive and HIV negative pre-eclamptic groups (p = 0.004). A significant down-regulation of sVEGFR-2 levels were noted in pre-eclampsia in comparison to the normotensive pregnant group (p = 0.002). Furthermore, there is a significant difference in sVEGFR-2 concentrations between HIV negative normotensive and HIV positive pre-eclamptic groups (p = 0.01). Despite no statistical significant difference demonstrated in PECAM-1 levels stratified by pregnancy type and HIV status, a down-regulation trend was observed in the pre-eclamptic versus normotensive pregnant groups.

Conclusion: This study demonstrated an imbalance of angiogenic factors, favouring antiangiogenesis, that is less offset when coupled with HIV infection. The levels of sVEGFR-1 and -2 and PECAM-1 in HIV associated pre-eclampsia, may be used as a risk indicator, predicting pre-eclampsia development prior to the manifestations of clinical signs and symptoms.
CHAPTER 1
INTRODUCTION

1.1 Hypertensive disorders in pregnancy

Hypertensive disorders in pregnancies are one of the major causes of maternal and perinatal morbidity and mortality globally. It is associated with an elevated blood pressure and may be classified into four categories namely chronic hypertension, pre-eclampsia/eclampsia, pre-eclampsia superimposed on chronic hypertension and lastly gestational hypertension. This may be attributed to the fact that mothers with high blood pressure are predisposed to an array of lethal complications particularly disseminated intravascular coagulation, hepatic and acute renal failure as well as pre-eclampsia (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000).

1.1.1 Pre-eclampsia

Pre-eclampsia is a pregnancy specific syndrome, indicated by an elevation in blood pressure, reduced organ perfusion and the activation of the coagulation cascade. It is defined as the sudden on-set of hypertension accompanied with proteinuria following the 20th week of gestation (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). This two phase disease incorporates a pre-clinical and a clinical phase. The pre-clinical phase is asymptomatic, characterised by abnormal placentation i.e. inadequate cytotrophoblast invasion, initiating hypoxia, immune dysregulation and oxidative stress (Govender et al., 2013a). The second clinical phase involves the symptomatic manifestation of the disease. Here placental discharge of soluble factors into the maternal circulation results in the characteristic endothelial dysfunction (Govender et al., 2013a).

1.1.2 Epidemiology of pre-eclampsia

Pre-eclampsia complicates 7 – 10% of pregnancies worldwide (Kalumba et al., 2013). In developing countries, where limited access to health care persists, the leading cause of maternal mortality is pre-eclampsia. Its resolution rests on delivering the baby (Wang et al., 2009). World Health Organisation (WHO) approximates a pre-eclamptic burden that is seven times greater in these developing countries than in developed ones (Osungbade and Ige, 2011). The incidence in African countries such as Egypt, Ethiopia and Tanzania range from 1.8% - 7.1% (Osungbade and Ige, 2011). In South Africa, maternal deaths due to hypertension in pregnancy is 14.8%, of which 83% is attributed to pre-eclampsia (Saving
Mothers report, 2013). Specifically, the prevalence of pre-eclampsia in KwaZulu-Natal, is 12% (Saving Mothers report, 2013).

1.1.3. Risks associated with developing pre-eclampsia

Globally, nulliparous women are at higher risk of developing pre-eclampsia, with an incidence rate of 7.5% (Wang et al., 2009). The risk associated with multiparous women who are pregnant by a different partner is similar to that of nulliparous women (Wang et al., 2009). The aforementioned suggests that a paternity change and/or a longer interval between pregnancies is associated with development of the syndrome.

Family history of pre-eclampsia development is a risk factor. Its presence in a first-degree female relative relays in an approximate fivefold and in a second-degree relative a twofold increase, in the threat of developing a severe form of the disease (Carr et al., 2005). Additionally, the occurrence of pre-eclampsia in a father’s mother also contributes to a higher risk of pre-eclampsia development, implying that men born from pre-eclamptic pregnancies are likely to father such pregnancies (Wang et al., 2009). This implicates the fact that the placenta is a product of the mother and father, and is the organ of pre-eclampsia origin (Haram et al., 2014; Wang et al., 2009).

Medical disorders namely diabetes mellitus, chronic hypertension, renal disease and obesity as well as conditions involving an increased placental mass such as hydatidiform mole and multifetal gestation are also connected with the risk of developing pre-eclampsia (Maynard and Karumanchi, 2011). Interestingly, smoking during pregnancy has been found to reduce the existence of pre-eclampsia (Maynard and Karumanchi, 2011).

1.1.4. Pathogenesis of Pre-eclampsia

The placenta is an exceptional transient organ that attaches the foetus to the uterus during pregnancy and enables foetal development by supplying blood between mother and foetus. In this regard it supplies oxygen and nutrients to the foetus and carries away foetal waste products (Cerdeira and Karumanchi, 2012; Maduray et al., 2016). As pregnancy advances, the surface area of the placenta increases ensuring adequate maternofoetal transfer (Griffiths and Campbell, 2014). The structural unit of the placenta is the chorionic villus, which is essentially vascular projections of foetal tissue surrounded by a chorion membrane. The chorion is made up of an inner cytotrophoblast and an outer syncytiotrophoblast layer that creates a physical barrier between the maternal and foetal circulation (Griffiths and Campbell, 2014).
During normal placental formation, the cytotrophoblast cells of embryonic origin, invade the maternal uterine wall. These cells enter the inner third of the myometrium and decidua, via interstitial and endovascular pathways, physiologically converting the maternal spiral arteries into large bore conduits. The significantly lower oxygen concentration (3%), present during the first trimester of pregnancy, creates a hypoxic milieu that favours cytotrophoblast invasion. The oxygen concentration gradient is regulated between the maternal and placental arteries, shifting it into a normoxic environment, as invasion continues (Valenzuela et al., 2012). Throughout the invasion there is altered expression of molecules such as metalloproteinases, cadherins and integrins, in a process known as pseudovascularogenesis. Here, the cytotrophoblasts lose their epithelial phenotype, gain a more endothelial-like one and replace the endothelium of the maternal spiral arteries, forming a pseudoendothelium. By this arterial remodelling process, a shift from a high resistant vessel to a low resistant one occurs, enabling sufficient blood perfusion thereby sustaining the foetus. It is proposed that the pathology behind pre-eclampsia is poor placentaion, i.e. inadequate cytotrophoblast invasion and hence defective spiral arterial wall remodelling. The myometrium arterial lumen remains of a small calibre and of, high resistance. A state of under-perfusion persists, resulting in placental hypoxia and localised oxidative stress. This leads to a systemic inflammatory response causing endothelial dysfunction, culminating in the clinical manifestations of the syndrome (Valenzuela et al., 2012).

![Figure 1. Normal vs abnormal placentation. (adapted from Winn, 2010)](image)

In pre-eclampsia, uterine invasion by cytotrophoblast cells is shallow hence the physiological transformation of spiral arteries is limited to the decidua (Cerdeira and Karumanachi, 2012; Silasi et al., 2010; Uzan et al., 2011). Additionally, the fusion of villous cytotrophoblasts into the syncytiotrophoblast layer allows for the continuous regeneration of apoptotic syncytial knots into the maternal environment. The occurrences of elevated syncytial knots, increased cytotrophoblast proliferation and necrotic subcellular syncytial particles activating the maternal vasculature are all associated with pre-eclampsia (Groten et al., 2010).
The proper formation, maturation and maintenance of the placental vasculature is critical, as failure results in an array of hostile outcomes such as miscarriage and pre-eclampsia (Cerdeira and Karumanchi, 2012).

1.1.5. **Clinical manifestations of pre-eclampsia**

The cardinal manifestations of pre-eclampsia are *de novo* hypertension, defined as $\geq \frac{140}{90}$ mmHg in two instances and proteinuria, which is defined as $\geq 300\text{mg}/24\text{hr}$ and/or a protein:creatinine ratio of $> 0.30$. These appear after the 20th week of gestation. (Powe *et al.*, 2011). Oedema was initially considered one of the symptoms of pre-eclampsia, however due to its non-specificity, it ceases to be a diagnostic sign. Notably, the sudden onset of facial and peripheral (hand) oedema is cause for concern with regard to this surreptitious syndrome (Wang *et al.*, 2009). Numerous diagnostic laboratory tests including quantification of urinary protein and serum creatinine as well as a liver function examination may determine the degree of end-organ damage, yet none are pre-eclamptic specific. Significantly, approximately 20% of eclampsia incidences occur in the absence of a previous history of hypertension and proteinuria, further highlighting the imperfect diagnostic criteria. Hyperuricemia is also often employed as a predictive tool of adverse events occurring in pre-eclampsia, however its predictive accuracy is modest (Powe *et al.*, 2011). Irrespective of the numerous breakthroughs in understanding the pathogenesis and predictive onset of pre-eclampsia, treatment other than delivery of the placenta to reverse its pathology has yet to be found. It is clear that the placenta has a significant role in pre-eclampsia development. Currently the available treatment is delivery of the baby and the placenta, considering maternal health, so as to rid the maternal environment of the placenta. Clinical signs and symptoms regress following its delivery (Govender *et al.*, 2013b).

1.1.6. **Complications of pre-eclampsia**

Complications of pre-eclampsia are intrauterine growth restriction (IUGR) and HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome (Haram *et al.*, 2014; Crispi *et al.*, 2008). HELLP syndrome encompasses haemolysis, thrombocytopenia and acute liver injury due to a widespread intravascular coagulation system (Maynard and Karumanchi, 2011; Powe *et al.*, 2011). IUGR and early-onset pre-eclampsia (EOPE) are commonly interlinked. It has been found that elevated levels of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and declining concentrations of placental growth factor (PIGF) occur in both pre-eclampsia and IUGR (Crispi *et al.*, 2008). This may be a plausible explanation for the reduced endovascular trophoblast invasion and hence inadequate
physiological spiral artery remodelling that characterises pre-eclampsia (Naicker et al., 2003; Kaufmann et al., 2003). Increased apoptosis of villous trophoblast populations occurs in pregnancies with the simultaneous existence of pre-eclampsia and/or IUGR (Naicker et al., 2013; Longtine et al., 2012). Complications of the central nervous system include eclampsia, which is defined as tonic clonic seizures (Maynard and Karumanchi, 2011). Pre-eclampsia may progress to eclampsia and a third of eclampsia cases may transpire postpartum, even weeks after delivery (Silasi et al., 2010).

1.2. HIV and pre-eclampsia

Human Immunodeficiency Virus (HIV) infection creates a compromised immune system predisposing an infected individual to opportunistic diseases, to which they eventually succumb. The HIV pandemic is largely predominant in Sub-Saharan Africa. Specifically in South Africa, according to reports released by the Human Sciences Research Council (HSRC), there was a rise in HIV infection from 10.6% in 2008 to 12.2% in 2012 (Zuma et al., 2016). KwaZulu-Natal is considered the epicentre of this burden, with the highest prevalence of 16.9% amongst the provinces. Furthermore, the HIV prevalence rate of those at a reproductive age is 18.8% (Zuma et al., 2016).

In contrast to a normal pregnancy where there is an altered immune sensitivity, allowing foetal tolerance and infection resistance, pre-eclamptic pregnancies exhibits a hyper active immune response. HIV infection is associated with a decline in an immune response. It is therefore plausible to assume that pre-eclampsia associated with HIV infection may therefore result in a neutral immune response. However, a conflicting paradigm exists in the literature with regards to the immune response in HIV infected pre-eclamptic women (Kalumba et al., 2013; Boyajian et al., 2012; Frank, 2006). Furthermore, the prevalence of pre-eclampsia is reduced in untreated HIV-infected patients than those on highly active antiretroviral therapy (HAART). HAART functions so as to reconstitute immune cell viability and the immune response (Govender et al., 2013a). This may have dire consequences with regard to pre-eclampsia, as enhancing the maternal immune system, consequently increasing the susceptibility to developing the syndrome (Govender et al., 2013a; Govender et al., 2013b; Kalumba et al., 2013).

1.3. Angiogenesis

Vasculogenesis is evident by approximately 21-22 days’ post conception. Placental vascular formation begins when mesenchymal cells, from the placental secondary villi differentiate into endothelial progenitor cells. The subsequent assembly of these differentiated endothelial progenitor cells allow for the development of primitive blood vessels. Lumen formation occurs through the establishment of intercellular spaces between haemangiogenic cell cords (a precursor of endothelial progenitor cells).
When the intracytoplasmic vacuoles of these cells fuse, small microvascular connecting tubes form. These successively make contact to larger vessels, resulting in a network of primitive connections (Cerdeira and Karumanchi, 2012).

Angiogenesis encompasses the transformation of the endothelial tube segments, formed during vasculogenesis, into an organised vascular network as seen in figure 2 (Cerdeira and Karumanchi, 2012). This is done by means of non-branching angiogenesis, *i.e.* elongation of already present tubes or branching angiogenesis, which involves lateral sprouting of tubes. Branching angiogenesis dominates from day 32 to the end of the 24th week post conception, pending its switch to non-branching angiogenesis which prevails until term. Under normal conditions, branching and non-branching angiogenesis takes place in combination. The mesenchymal villi begin to develop into mature intermediate villi which later initiate terminal villi production. At this point, there is a decrease in trophoblast proliferation and an increase in endothelial proliferation throughout the length of the villi. These peripheral branching tubes grow rapidly, exceeding a length of 4000 μm, resulting in its coiling and forming the terminal villi. The vasculosyncytial membranes constitute the aforementioned tubes enclosed in a thin layer of trophoblast cells. This creates a separation between the maternal and foetal circulation, yet is the principal site for gaseous exchange between the mother and foetus. Recruitment of pericytes, supporting and smooth muscle cells, are critical in angiogenesis. These cells function in stabilizing and maintaining the integrity of developing vessels. The penetrating trophoblasts, besides forming the placental villi, also create open endings in the maternal vasculature. This allows for the release of maternal blood into the intervillous space (Cerdeira and Karumanchi, 2012).

![Figure 2. Schematic representation of vasculogenesis and angiogenesis. (adapted from Llevadot & Asahara, 2002)](image-url)
1.4. Angiogenic and antiangiogenic factors

Angiogenic factors are a group of circulating polypeptides that function in blood vessel formation. Understanding angiogenesis and its contributing factors is central to grasping the pathogenesis of pre-eclampsia. An equilibrium between angiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), and antiangiogenic factors, soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and soluble endoglin (sEng) must be maintained for optimal vasculogenesis, angiogenesis and placental development (Govender et al., 2013b). The release of antiangiogenic factors into the maternal circulation is elevated in pre-eclampsia, resulting in the systemic manifestations of the disease (Govender et al., 2013a).

1.4.1. Soluble vascular endothelial growth factor receptor -1 (sVEGFR-1)

Vascular endothelial growth factors (VEGFs) are important regulators of endothelial cells processes. They mediate both physiological and pathological angiogenesis in addition to vascular permeability. There are numerous isoforms of VEGF that arbitrate their signals through high affinity cell surface tyrosine kinase receptors, namely VEGFR-1, VEGFR-2, VEGFR-3 (Karpanen, 2006). sVEGFR-1, also referred to as soluble fms-like tyrosine kinase (sFlt-1), is an antiangiogenic protein that is a shortened spliced variant of VEGFR-1 (Wang et al., 2009). Plasma concentrations of sVEGFR-1 is elevated in imminent pre-eclamptic pregnancies as depicted in figure 3 (Lorquet et al., 2010). It was initially believed that sVEGFR-1 was produced by placental trophoblast cells, in response to an array of stimuli, specifically hypoxia. However, the amount of sVEGFR-1 released by these cells surpasses that, which can be explained by circulating levels evident in pre-eclampsia. Monocytes are an alternate source of sVEGFR-1, yet its stimuli remains undefined (Major et al., 2014). sVEGFR-1 inhibits VEGF and PIGF activity by hindering their vasodilatory and angiogenic effects (Powe et al., 2011). Thadhani and colleagues (2011) hypothesized that sVEGFR-1 facilitates the manifestation of the signs and symptoms evident in pre-eclampsia. They further noted that circulating sVEGFR-1 are highest in early-onset pre-eclampsia, therefore therapeutic intervention should focus on circulating sVEGFR-1 (Thadhani et al., 2011).
Figure 3. The effect of anti-angiogenic factors inducing vascular protection vs dysfunction. (adapted from Ahmed & Ramma, 2015)

Pre-eclamptic patients have an increased vascular responsiveness to vasoconstrictive agents such as angiotensin II. It is recognised to couple with angiotensin II type 1 (AT1) receptor, inducing arterial vasoconstriction maintaining an elevated blood pressure (Powe et al., 2011). Agonistic AT1 receptor autoantibodies have been identified in pre-eclamptic patients, at heightened levels, providing an explanation for the angiotensin II hypersensitivity (Wang et al., 2008). This leads to endothelial cells producing tissues factors which affect trophoblast invasion as well as increase the concentrations of sVEGFR-1 and sEng which contribute to placental injury that results in the production of antiangiogenic factors (Wang et al., 2008; Powe et al., 2011).

1.4.2. Soluble vascular endothelial growth factor receptor -2 (sVEGFR-2)

sVEGFR-2 is a mediator of wide spread signalling pathways that facilitate endothelial cell functions, ranging from proliferation and differentiation to migration. The binding affinity to VEGF-A is approximately 10-fold higher in VEGFR-1 than in VEGFR-2, yet the kinase activity of VEGFR-2 is much greater. Hence it can be said that VEGFR-2 is a major VEGF-A signalling receptor (Ho et al., 2012). Although its role is less established in the pathogenesis of pre-eclampsia, matrix metalloproteinase-9 (MMP-9) may be involved in the degradation of the VEGFR-2 extracellular domain, with consequential vascular dysfunction and impaired angiogenesis (figure 4) (Luizon et al., 2012). Alternatively known as the KDR receptor, sVEGFR-2 originates from an endothelial cell surface proteolytic cleavage as a consequence of a reduced expression of VEGFR-2 (Munaut et al., 2012). In the presence of vascular endothelial cadherin (VE cadherin) coupled with ligand binding, VEGFR-2 initiates cell stabilization and survival. Cell activation via VEGFR-2 signalling occurs in the absence of VE cadherin ligand binding (Groten et al., 2010).
Figure 4. Binding of VEGF and VEGFR-1 & -2 occurring in a normal vs a pre-eclamptic pregnancy. (adapted from Luizon et al., 2012)

The interaction between VEGFs and VEGFRs are mediated by a number of cell surface molecules of varying classes. An example of this reaction involves VEGFR-2, PECAM-1 and VE cadherin (Figure 5).

Figure 5. VEGFs binding to their specific receptors and the formation of a mechanosensory complex VEGFR-2, PECAM-1, VE cadherin and intergrins. (adapted from Olsson et al., 2006)

1.4.3. Platelet endothelial cell adhesion molecule-1 (PECAM-1)

Vascular development and remodelling is influenced by shear stress and blood flow, through the formation of a mechanosensory complex encompassing VEGFR-2, VE cadherin and PECAM-1 (Olsson et al., 2006). Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a homophilic adhesion
receptor, whose cytoplasmic domain binds to β- and γ-catenins. In its phosphorylated form the immunoreceptor tyrosine-based inhibitory motif (ITIM), of PECAM-1, can induce extracellular signal-regulated kinases (ERKs) activation (Tzima et al., 2005). Being a part of the immunoglobulin superfamily, its expression is evident on the surface of circulating platelets, neutrophils, monocytes and certain T cells. It is also implicated in a variety of functions ranging from being a constituent of the endothelial cell intercellular junction to angiogenesis and transendothelial migration of leukocytes. In pre-eclampsia neutrophil and platelet activation promote vascular damage. This is mediated by cell adhesion molecules like PECAM-1, which are expressed not only on the maternal endothelium but also at the utero-placental bed. (Yasemin et al., 2012).

1.5. Aim and objectives of this investigation

Researchers have explored an assortment of avenues yet the gap in the knowledge of pre-eclampsia still persists. Predictive tests for the syndrome have optimum performance after the first trimester, however, this is also a period proven to be too late to reverse development. Hence, much more research is required to identify clinical predictor risk indicator tools for the early identification of pre-eclampsia (Valenzuela et al., 2012). Therefore, the use of angiogenic biomarkers in the early detection of pre-eclampsia is promising.

This study aims:

- to determine the concentrations of platelet endothelial cell adhesion molecule-1 (PECAM-1) and soluble vascular endothelial growth factor receptor (sVEGFR)-1 and -2, in the pathogenesis of HIV associated pre-eclampsia.

The objectives will be:

- to investigate the concentration of serum PECAM – 1 in HIV associated normotensive and pre-eclamptic pregnant patients with the use of a BioPlex Multiplex immunoassay.
- to investigate the concentration of serum sVEGFR - 1 in IIIV associated normotensive and pre-eclamptic pregnant patients with the use of a BioPlex Multiplex immunoassay.
- to investigate the concentration of serum sVEGFR – 2 in HIV associated normotensive and pre-eclamptic pregnant patients with the use of a BioPlex Multiplex immunoassay.
- to compare and contrast PECAM – 1 and sVEGFR – 1 and – 2 across the study population based on pregnancy type (pre-eclamptic vs normotensive) and HIV status (HIV positive vs HIV negative).
CHAPTER 2
The role of Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) and soluble Vascular Endothelial Growth Factor Receptor (sVEGFR)-1 and -2 in HIV associated pre-eclampsia

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SERUM LEVELS OF PLATELET ENDOTHELIAL CELL ADHESION MOLECULE-1 (PECAM-1) AND SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (sVEGFR)-1 AND -2 IN HIV ASSOCIATED PRE-ECLAMPSIA

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Total word count: 3954

Word count excluding abstract and references: 2701

Number of Figures: 1

Number of Tables: 2
Abstract

Objective: The angiogenic-antiangiogenic imbalance evident in pre-eclampsia may be used as a predictive tool to identify women likely to develop the clinical features in early pregnancy. Method: This retrospective study examined normotensive pregnant (n=38) and pre-eclamptic (n=38) HIV infected and uninfected women to quantify sVEGFR-1 and -2 and PECAM-1 levels. Results: In contrast to PECAM-1, sVEGFR-1 and -2 differed according to pregnancy type (p=0.07; p=0.001; p=0.002) but not by HIV status (p=0.68; p=0.13; p=0.43). Conclusion: Irrespective of the HIV status, we report an up-regulation of sVEGFR-1 with concomitant decline of PECAM-1 and sVEGFR-2 levels in pre-eclampsia compared to normotensive pregnancies.

Keywords: Pre-eclampsia, HIV, sVEGFR-1, sVEGFR-2, PECAM-1

Running title: Angiogenic factors in HIV associated pre-eclampsia
Introduction

Sub-Saharan Africa remains the epicentre of the global HIV pandemic. In South Africa, 35.8% of maternal deaths are attributable to non-pregnancy related infections (mostly HIV-related) (1). The province of KwaZulu-Natal is to be considered the global epicentre of this pandemic, with a prevalence rate of HIV in pregnancy of approximately 37.7% (2). Furthermore, there is a high HIV incidence rate (18.8%) in women of reproductive age (3).

Pre-eclampsia (PE) is associated with the clinical signs of an elevation in blood pressure, reduced organ perfusion and activation of the coagulation cascade (3). In South Africa, maternal deaths due to hypertension in pregnancy is 14.8%, of which 83% is attributed to PE (1). Specifically, the prevalence of PE in KwaZulu-Natal is 12% (1).

In contrast to a normal pregnancy where there is an altered immune sensitivity, allowing foetal tolerance and infection resistance, PE exhibits an exaggerated immune response (4). HIV infection is associated with a decline in immune activity (5). It is therefore plausible to assume that the exaggerated immune response in PE is neutralised by HIV infection. However, this hypothesis is met with conflicting reports in the current literature (3,6-7).

Angiogenesis encompasses the transformation of the endothelial tube segments into an organised vascular network (8). This is done by means of branching angiogenesis, which involves lateral sprouting of tubes that dominates from day 32 to the end of the 24th week post conception, pending its switch to non-branching angiogenesis, i.e. elongation of existing tubes which prevails until term gestation. The proper formation, maturation and maintenance of the placental vasculature is critical, as failure results in an array of hostile outcomes such as miscarriage and PE (8). The invasive penetrating trophoblasts, besides forming the placental villi, also create open endings in the maternal vasculature. This allows for the release of maternal blood into the intervillous space (8). Pre-eclampsia
is associated with poor placentation, i.e. inadequate extravillous trophoblast invasion resulting in the physiological transformation of spiral arteries being limited to the decidua (8-10).

An equilibrium of angiogenic-antiangiogenic factors is a requisite for optimal angiogenesis and placental development (11). In PE, the release of antiangiogenic factors into the maternal circulation is elevated, resulting in the systemic manifestation of the disease (4). Moreover, chronic HIV infection contributes to chronic arterial injury and subsequently endothelial damage as well as atherosclerosis (4).

Soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), also referred to as soluble fms-like tyrosine kinase (sFlt-1), a shortened spliced variant of VEGFR-1 (12) is elevated in PE (13). sVEGFR-1 inhibits vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) activity by hindering their vasodilatory and angiogenic effects (14). Thadani et al., (2011) hypothesized that sVEGFR-1 facilitates the manifestation of the clinical signs and symptoms of PE. These authors further noted circulating sVEGFR-1 levels were highest in preterm PE and has been reported to have a predictive risk indicator value for the diagnosis of early onset PE (15).

In comparison to VEGFR-1, vascular endothelial growth factor receptor-2 (VEGFR-2) is understood to be a major VEGF-A signalling receptor owing to its greater kinase activity (16). Alternatively known as the KDR receptor, soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) originates from an endothelial cell surface proteolytic cleavage as a consequence of a reduced expression of VEGFR-2 (16). Although the role of this angiogenic factor is less established in the pathogenesis of PE, matrix metalloproteainase-9 (MMP-9) may be involved in the degradation of the VEGFR-2 extracellular domain, with consequential vascular dysfunction and impaired angiogenesis (18).

Another angiogenic receptor, platelet endothelial cell adhesion molecule-1 (PECAM-1) is influential in vascular development and it forms a complex with VEGFR-2 and VE cadherin (19). Its function ranges from being a constituent of the endothelial intercellular junction to angiogenesis and transendothelial
migration of leukocytes. In PE, PECAM-1 of maternal endothelium and utero-placental origin induce neutrophil and platelet activation which promotes vascular damage (20).

Predictive tests for PE development have optimum value after the first trimester; however, this is also a period known to be too late to reverse PE development. Hence, much more research is required to identify clinical predictor risk indicator tools for the early detection of PE (21). The use of angiogenic factors, sVEGFR-1 and -2 and PECAM-1, as biomarkers show promise in this regard. Therefore, the aim of this study was to determine the concentrations of platelet endothelial cell adhesion molecule-1 (PECAM-1) and soluble vascular endothelial growth factor receptor (sVEGFR)-1 and -2, in HIV associated PE.

Methods and Materials

Study population

This retrospective study (BE256/12) received institutional ethics approval (BE085/16). The study population (n=76) consisted of normotensive (n=38) and pre-eclamptic (n=38; new onset blood pressure of ≥140/90 mmHg and at least 1+ proteinuria) women recruited from a regional hospital in Durban, South Africa. Both groups were further stratified by HIV status. Exclusion criteria for the PE group included polycystic ovarian syndrome, chorioamnionitis, eclampsia, chronic hypertension, intrauterine death, abruptio placentae, gestational diabetes, chronic diabetes, systemic lupus erythematosus, chronic renal disease, sickle cell disease, thyroid disease, antiphospholipid antibody syndrome, cardiac disease, pre-existing seizure disorders, asthma, unknown HIV status as well as women who did not have antenatal care. The control group consisted of healthy normotensive women (blood pressure levels at or below 120/80 mmHg).

Bio-Plex Multiplex method

Maternal blood samples collected in sterile serum separation tubes were centrifuged at 3000 rpm for 10 minutes at 4°C and the supernatant was used for the quantification of sVEGFR-1 and -2 and PECAM-
1 using the multiplex enzyme-linked immunosorbent assay (ELISA). A 3-plex, Bio-Plex Pro Human Cancer Biomarker kit (Panel 1) was used according to manufacturer’s instructions (Bio-Rad Laboratories, Inc., USA). The standards were prepared in a 1:10 and 1:4 dilution series, whilst samples were prepared in a 1:4 dilution.

This bead-based flow cytometric assay allowed for multiplex analyses. The immunoassay involved the incubation of the antigen samples, i.e. sVEGFR-1 and -2 and PECAM-1, with the capture antibody-coupled beads. Subsequently, biotinylated detection antibodies coupled with a reporter conjugate, streptavidin-phcoerythrin (SA-PE) completed the interaction. The samples were read using the Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad Laboratories Inc., USA). Bio-Plex Manager™ software version 4.1 was used to obtain the data from the Bio-Plex multiplex analysis.

Statistical Analysis

Graphpad Prism 5.00 for Windows (GraphPad Software, San Diego California USA) was used to analyse the data. Non-parametric tests were performed. Descriptive statistics for continuous data is presented by median, interquartile range (IQR) and mean ± standard deviation, whilst non-parametrically distributed data are presented as median and IQR. To determine statistical significance across all groups a Mann-Whitney U or Kruskal-Wallis test in combination with the Dunn’s Multiple comparison post hoc test was carried out. Statistical significance was p < 0.05.

Results

Clinical Characteristics

Table 1 provides a summary of the demographics of the study population. Gestational age, systolic and diastolic blood pressures (BP) were statistically different between the normotensive pregnant and PE groups [p < 0.001 each; Two-sample Wilcoxon rank-sum (Mann-Whitney) test]. There were no significant differences in maternal age (p = 0.5) and maternal weight (p = 0.5) between normotensive pregnant versus PE groups.
The birth weight of babies from the normotensive pregnant group was 3.24±0.40 kg, compared to 2.17±0.65 kg from the PE group. The mean body mass index for pre-eclamptic was 32.85±8.10kg/m² and the normotensive pregnant women was 28.89±6.80kg/m².

**Table 1. Patient demographics in the normotensive pregnant (n = 38) and pre-eclamptic (n = 38) groups**

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<th>Median</th>
<th>IQR</th>
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<tbody>
<tr>
<td><strong>Normotensive Pregnancy</strong></td>
<td></td>
<td></td>
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<tr>
<td>Maternal Age (years)</td>
<td>26.5</td>
<td>9</td>
<td>27.11 ± 2.83</td>
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<tr>
<td>Maternal Weight (kg)</td>
<td>72.6</td>
<td>12.5</td>
<td>74.05 ± 2.83</td>
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<td>Gestational Age (weeks)</td>
<td>39</td>
<td>2</td>
<td>38.89 ± 3.54</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>122</td>
<td>17</td>
<td>120.66 ± 18.38</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.5</td>
<td>15</td>
<td>71.18 ± 23.33</td>
</tr>
<tr>
<td><strong>Pre-eclamptic Pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Age (years)</td>
<td>27.5</td>
<td>12</td>
<td>28.45 ± 3.54</td>
</tr>
<tr>
<td>Maternal Weight (kg)</td>
<td>75</td>
<td>34.8</td>
<td>77.70 ± 1.70</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>33.5</td>
<td>6</td>
<td>32.92 ± 4.95</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.5</td>
<td>19</td>
<td>154.55 ± 7.07</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>98.5</td>
<td>14</td>
<td>99.82 ± 15.56</td>
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Proteinuria was noted in all cases of PE, i.e. 50% of the sample population. Twenty-six percent of the PE group had a urine protein concentration of 30-99mg/dL whilst 16% had concentrations of 100-299 mg/dL. Only a marginal portion of this group exhibited 300-999mg/dL or ≥1000mmg/dL urine protein, being 7% and 1% respectively (p = 0.1; Fisher’s exact test).

*Serum concentrations of sVEGFR-1 and -2 and PECAM-1.*

Concentrations of sVEGFR-1 and -2 and PECAM-1 are outlined in Table 2 and Fig. 1 A-C. There was no correlation between gestational age and sVEGFR-1 (r=0.18), sVEGFR-2 (r=0.12) and PECAM-1 (r=0.26).
Irrespective of HIV status, a significant difference of serum sVEGFR-1 was noted between the normotensive pregnant versus PE groups (Mann-Whitney U = 404.0; p = 0.001). In contrast, regardless of pregnancy type, sVEGFR-1 did not differ between HIV negative and HIV positive pregnancies (Mann-Whitney U = 577.0; p = 0.13). Higher concentrations of sVEGFR-1 were observed in PE (mean = 7749 pg/ml; 95%CI: 10860 – 4640) compared to the normotensive pregnant group (mean = 2423 pg/ml; p = 95%CI: 3079 – 1766). Furthermore, the level of sVEGFR-1 was lower in HIV positive PE group (mean = 7443 pg/ml; 95%CI: 13076 – 1810) than in the HIV negative PE group (mean = 8055 pg/ml; 95%CI: 11363 – 4747), albeit non-significantly (Mann-Whitney U = 143.0; p = 0.28). Additionally, there was a significant difference in sVEGFR-1 concentrations between HIV positive normotensive pregnant and HIV negative PE group (Kruskal-Wallis H = 13.21; p = 0.004).

sVEGFR-2 concentration was statistically different between the normotensive pregnant and PE groups, regardless of HIV status (Mann-Whitney U = 416.5; p = 0.002). There was a reduction in the concentration of sVEGFR-2 in the PE group (mean = 519.5 pg/ml; 95% CI: 598.7 – 440.3) compared to the normotensive group (mean = 815.3 pg/ml; 95% CI: 980.9 – 649.6). However, based on HIV status, there was no significant difference in sVEGFR-2 levels (Mann-Whitney U = 646; p = 0.43). There was a significant difference in sVEGFR-2 concentrations between the HIV negative normotensive and the HIV positive PE group (Kruskal-Wallis H = 11.01; p = 0.01).

No statistical significant effect was demonstrated in PECAM-1 concentration stratified by pregnancy type (normotensive vs pre-eclampsia) (Mann-Whitney U = 547.5; p = 0.07), HIV status (negative vs positive) (Mann-Whitney U = 681.5; p = 0.68) and across all groups (Kruskal-Wallis H = 4.21; p = 0.24). Although there was no significance, a decreasing trend in PECAM-1 concentration was observed in PE (mean = 1030 pg/ml; 95%CI: 1207 – 853.1) compared to normotensive pregnant group (mean = 1190 pg/ml, 95%CI: 1389 – 992).
Figure 1. Serum concentration of angiogenic factors: (A) sVEGFR-1 (pg/ml), (B) sVEGFR-2 (pg/ml), (C) PECAM-1 (pg/ml) in HIV negative normotensive, HIV positive normotensive, HIV negative pre-eclamptic and HIV positive pre-eclamptic pregnant groups. Results are represented as median and interquartile range. ** Serum concentrations of sVEGFR-1 are significantly different between HIV positive normotensive pregnant and HIV negative pre-eclamptic group, p = 0.004. *Serum concentrations of sVEGFR-2 are significantly different between HIV negative normotensive pregnant and HIV positive pre-eclamptic group, p = 0.01

Table 2. Serum concentrations (pg/ml) of angiogenic-antiangiogenic factors in all study groups

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<td>HIV Negative (n = 19)</td>
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<tr>
<td>sVEGFR-1</td>
<td>2415 (2689)</td>
<td>1294 (1663.3)</td>
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<td>sVEGFR-2</td>
<td>715.7 (256)</td>
<td>751.6 (600)</td>
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<tr>
<td>PECAM-1</td>
<td>1011 (470.7)</td>
<td>1153 (729.2)</td>
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Results are represented as median (interquartile range), ns: non-significant.
Discussion

An increasing volume of research attention is now focused on non-invasive predictive tests in early pregnancy to identify women likely to develop PE. Angiogenic biomarker levels show promise as a diagnostic tool, as changes in their circulating levels are detectable several weeks before the onset of signs and symptoms of PE (22-23). Their use during early pregnancy, may prevent, the high maternal and perinatal morbidity and mortality rates due to PE in low and middle income countries.

Our findings demonstrate an up-regulation of sVEGFR-1 in PE compared to normotensive pregnant women. These results are corroborated by other studies (4,8,11,24,25). Although sVEGFR-1 diminishes the bioavailability of both VEGF and PI GF, the binding of sVEGFR-1 and VEGF-A alone cannot provide explanation for the clinical signs of PE (26). It is known, that sVEGFR-1 inhibition of VEGF impairs angiogenesis i.e. there is inadequate placental vascularization culminating in reduced placental perfusion (24). Physiologically, VEGF-A increases vascular permeability inducing oedema, as well as vasodilation and angiogenesis, implying that this coupling should prevent the aetiology of PE, rather than inducing it (26). Moreover, the presence of sVEGFR-1 is also believed to be the stimulus for an increase in soluble endoglin, an antiangiogenic factor (11).

Additionally, due to the antagonistic nature of sVEGFR-1 to PI GF, circulating levels of PI GF are decreased, off-setting the balance in favour of antiangiogenesis (24). It is therefore probable that this coupling leads to the systemic manifestation of PE. It is important to note that despite PI GF and VEGF having the same binding affinity to sVEGFR-1, circulating levels of VEGF, is greater (26), thus theoretically most VEGF should bind to sVEGFR-1. The secreted levels of PI GF are reported to be lower in PE therefore sVEGFR-1 binding would be negligible (26). Nonetheless Crispi et al., (2008) found that the differences in the level of sVEGFR-1 and PI GF varied according to gestational age, being more pronounced in early-onset (<33w 6d gestation) compared to the late-onset (>34 w gestation) PE in comparison to normotensive pregnancies. Since abnormal angiogenic levels are detectable 5-14 weeks prior to clinical onset of the syndrome (27), a possible role of both sVEGFR-1 and PI GF may
serve as a risk indicator for early-onset PE development. A limitation in our study is that we did not stratify the groups by gestational age into early and late gestational presentation of PE.

A study by Koga et al. (2003) demonstrated a marked decline in sVEGFR-1 levels following delivery, in both PE and normotensive pregnancies, confirming that the placenta is a source of this antiangiogenic factor. However, considering the association of increased sVEGFR-1 in essential hypertension, the heightened sVEGFR-1 production may be augmented from other tissues in PE (24) and may provide an explanation for higher sVEGFR-1 level in PE compared to the normotensive pregnant women.

Moreover, in our study, the sVEGFR-2 levels were down-regulated in PE compared to normotensive pregnancies. These findings are similar to those of Munaut et al. (2012), Chaiworapongsa et al. (2010) and Tripathi et al. (2009) (17,23,28). In contrast to sVEGFR-1, not much is known about the role of sVEGFR-2 in the aetiology of PE or its potential use as a biomarker for the early detection of the disorder (28). According to Chaiworapongsa et al. (2010), the median plasma sVEGFR-2 concentrations are significantly lower in those women that subsequently go on to develop PE, compared to normotensive pregnancies. Since this decrease is evident as early as 6-10 weeks before the clinical onset of PE (23), it is conceivable that sVEGFR-2 may be used as a risk indicator diagnostic tool for PE development. It must be noted that our samples were collected just prior to delivery.

A pathognomonic lesion in the placental vasculature of PE women is endotheliosis (20). Notably deficient trophoblast invasion is implicated in the pathogenesis of PE (20). Cytotrophoblasts do not express PECAM-1 (20), hence the lower trend in PE as observed in our study may underwrite the impaired trophoblast invasion of PE. Similar findings are reported by, Lyall et al. (1995) who also found no significant difference in the concentration of PECAM-1 between normotensive and PE groups (29). However, conflicting results of a significantly lower PECAM-1 activity in PE compared to normotensive pregnancies have been reported (20).
Our results clearly demonstrate an imbalance of angiogenic factors, sVEGFR-2 and PECAM-1, versus the antiangiogenic factor, sVEGFR-1 in favour of the latter in PE. This imbalance is less offset in HIV infected PE. Govender et al. (2013a), who also reported similar findings, proposed that the immune insufficiency exhibited by HIV infection, reduced the susceptibility to the classic immune hyperactivity evident in PE (4). The administration of highly active anti-retroviral therapy (HAART) reconstitutes immune cell viability and the immune response, consequently increasing the predisposition to PE (4,30). At present, there are contradictory reports on the administration of HAART during pregnancy and its association with pre-eclampsia. Suy et al. (2006) concluded that HIV infected women are at an increased risk of developing PE due to their exposure to HAART prior to pregnancy (31). Moreover, Powis et al. (2013) found that HIV infected pregnant women receiving HAART exhibited a similar risk of developing PE as HIV uninfected pregnant women (32). A limitation of our study is that all HIV infected women received either HAART or anti-retroviral drugs for the prevention of mother-to-child transmission (PMTCT) as part of the standard of care treatment regimen in South Africa. However, Powis et al. (2013), reports that HAART treatment does not significantly alter levels of PI GF or sVEGFR-1 (32). The limitation of this study is that viral load was not done and that only a limited number of women had CD4 counts, hence the biomarkers in our study were not correlated with severity of HIV infection.

Conclusion

This study demonstrates a significant elevation of sVEGFR-1 (antiangiogenic factor) and a significant decrease in sVEGFR-2, with a down-regulation trend in PECAM-1 in PE. However, in HIV associated PE this imbalance is less severe probably due to the combination of opposing immune responses. The level of the angiogenic factors, sVEGFR-1 and -2 and PECAM-1, in HIV associated pre-eclampsia, has a predictive risk indicator value for the early detection of the disease.

Declaration of Interest

There are no conflicts of interest.
Acknowledgements

The authors wish to thank the institutional biostatistician, Dr. C Connolly. Financial support was obtained from College of Health Sciences, UKZN.

Reference


CHAPTER 3
3.1 College of Health Science’s Research Symposium 2016 (8th – 9th September 2016)

College of Health Sciences

Research Symposium 2016

8-9 September

Book of Abstracts
PROGRAMME

THURSDAY, 8TH SEPTEMBER
8h00-8h30 : Registration
8h30-10h00 : SESSION 1
8h30-9h00 : Welcome and Introduction of Sponsors
            Professor Moses Chimbari
9h00-10h00 : KEY-NOTE ADDRESS
            ‘90-90-90: Learnings From The 21st International
            Conference’
            Professor Salim Abdool Karim
10h00-10h30 : TEA
10h30-12h30 : SESSION 2
12h30-13h30 : LUNCH
13h30-15h30 : SESSION 3

FRIDAY, 9TH SEPTEMBER
8h00-10h00 : SESSION 4
10h00-10h30 : TEA
10h30-12h30 : SESSION 5
12h30-13h30 : LUNCH
13h30-15h30 : SESSION 6
13h30-14h00 : Large Grants recipients
14h00-15h00 : Fractional Professors presentation: Professor Per
              Arvidsson, Professor Vivienne Russell, Professor Bob Hickner, Professor Hans
              Peter Lipp and Professor Taha Mekuria.
15h00-15h30 : PRIZE GIVING and LUCKY DRAWS
15h30 : Vote of Thanks and Closure
# SESSION TWO

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## TRACK THREE: AUSSER AND STEIN

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THE ROLE OF PLATELET ENDOTHELIAL CELL ADHESION MOLECULE-1 (PECAM-1) AND SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (sVEGFR-1) AND -2 IN THE PATHOGENESIS OF HIV ASSOCIATED PRE-ECLAMPSIA

Thakoordeen, S*; Moodley, J; Naicker, T*

*Discipline of Optics and Imaging; #Discipline of Obstetrics and Gynaecology

Introduction
The equilibrium between pro- (PECAM-1 and sVEGFR-2) and anti-angiogenic factors (sVEGFR-1) is altered in favour of sVEGFR-1 elevation in pre-eclampsia. It is plausible to assume that the exaggerated immune response in pre-eclampsia is neutralised by HIV infection. Therefore, this study aims to determine the concentrations of the angiogenic factors PECAM-1, sVEGFR-1 and -2 in HIV associated pre-eclampsia.

Methods
Institutional ethical approval was obtained. The sample population (n=76) included normotensive and preeclamptic patients, sub-stratified by HIV status. The levels of the angiogenic factors were analysed using the Bio-Plex Pro Human Cancer Biomarker kit, on the Bio-Plex MAGPIX Multiplex Reader (Bio-Rad).

Results
No significant difference was found in the concentration of PECAM-1 between pre-eclampsia and normotensive pregnancies (p=0.07), there was however an observed trend of down-regulation in pre-eclampsia. sVEGFR-1 was significantly up-regulated in pre-eclampsia compared to normotensive pregnancies (p=0.001). Although the levels of sVEGFR-1 increased in both HIV negative and positive preeclamptic groups, this elevation was less in HIV positive pre-eclampsia. sVEGFR-2 was significantly down-regulated in pre-eclampsia compared to normotensive pregnancies (p=0.002). A significant difference was found between HIV negative normotensive and HIV positive pre-eclamptic pregnancies (p=0.01) for sVEGFR-2.

Conclusion
This study demonstrates that the imbalance of angiogenic factors is less offset in HIV associated pre-eclampsia. Hence HIV exerts a possible protective effect against pre-eclampsia development. The levels of these angiogenic factors in HIV associated pre-eclampsia, may be used as a predictive risk indicator for the early detection of the disease.
3.2 2nd xMAP CONNECT (LUMINEX) 2016 (16th – 17th November 2016)

- **Event:** xMAP® Connect 2016
- **Attending:** Semone Thakoordeen
- **Date:** 16 November, 2016 - 17 November, 2016
- **Location:** De Nieuwe Liefde
- **Address:** Da Costakade 102, Amsterdam 1053 WP, Netherlands
- **Confirmation Number:** VKNM7ZXX9LG
- **Current Registration:**

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My Agenda

Agenda For: Semone Thakoordeen

• Optional

16 November, 2016

09:30 - 17:15   Day 1
• Biomarker analysis and immunology
• Multiplex screening developments

18:15 - 00:00   Social event
(17 November, 2016) Grow your multiplex network and come to our social event!

17 November, 2016

08:30 - 17:00   Day 2
• Clinical research in infectious diseases
• Public health solutions

Thakoordeen, S., Moodley, J., Naicker, T. The role of platelet endothelial cell adhesion molecule-1 (PECAM-1) and soluble vascular endothelial growth factor receptor (sVEGFR)-1 and -2 in the pathogenesis of HIV associated pre-eclampsia. 2nd xMAP CONNECT (LUMINEX), Die Nieuwe Liefde, Amsterdam, Netherlands, 16th-17th November 2016.

Introduction: In South Africa, maternal deaths due to hypertension in pregnancy is 14.8%, of which 83% is attributed to pre-eclampsia. The release of anti-angiogenic factors (soluble vascular endothelial growth factor receptor-1, sVEGFR-1) into the maternal circulation is elevated in pre-eclampsia, instead of the desired equilibrium that should be maintained between pro- (platelet endothelial cell adhesion molecule-1, PECAM-1 and soluble vascular endothelial growth factor receptor-2, sVEGFR-2) and anti-angiogenic factors. Pre-eclamptic pregnancies also exhibits a hyper active immune response, whilst HIV infection is associated with a decline in immune activity. It is therefore plausible to assume that pre-eclampsia associated with HIV infection may result in a neutral immune response. Therefore, the
aim of this study is to determine the concentrations of the angiogenic factors PECAM-1, sVEGFR-1 and -2 in HIV associated pre-eclampsia.

Materials and Methods: Ethical approval was obtained from the Biomedical Research Ethics Committee, University of KwaZulu-Natal. Patient recruitment was conducted at a large regional hospital in, Umlazi, Durban. The sample population included primigravid and multigravid pregnant patients; further divided into normotensive HIV negative (n=19), normotensive HIV positive (n=19), pre-eclamptic HIV negative (n=19) and pre-eclamptic HIV positive (n=19). The levels of each of the angiogenic factors were then analysed using the Bio-Plex Pro Human Cancer Biomarker Assays, on the Bio-Plex MAGPIX Multiplex Reader (Bio-Rad).

Results: Statistical analysis revealed no significant difference between the levels of PECAM-1 between normotensive and pre-eclamptic pregnancies, irrespective of HIV status (p=0.07), between HIV negative and positive groups, irrespective of pregnancy type (p=0.67) and across all study groups (p=0.24). Yet there was an observed trend of PECAM-1 downregulation in pre-eclampsia compared to normotensive pregnancies. sVEGFR-1 was significantly up-regulated in pre-eclampsia compared to normotensive pregnancies (p=0.001). Regardless of pregnancy type, there was no significant difference based on HIV status (p=0.13). Interestingly, a significant difference was found between the levels of sVEGFR-1 in HIV positive normotensive and HIV negative pre-eclamptic groups (p=0.004). Although the levels of sVEGFR-1 increased in both HIV negative and positive pre-eclamptic groups, this elevation was less in HIV positive, than in HIV negative pre-eclamptic pregnancies. Additionally, sVEGFR-2 was significantly down-regulated in pre-eclampsia compared to normotensive pregnancy types (p=0.002). There was no difference observed between HIV positive and negative groups (p=0.43). In combination, a significant difference was found between HIV negative normotensive and HIV positive pre-eclamptic pregnancies (p=0.01) for sVEGFR-2.

Conclusion: This study demonstrates a dramatic increase in sVEGFR-1 (anti-angiogenic factor), while a decrease in pro-angiogenic factors PECAM-1 and sVEGFR-2. However, coupled with an HIV infection, this imbalance is less off-set. It is therefore plausible to assume that HIV exerts a possible protective effect against pre-eclampsia development. The levels of these angiogenic factors in HIV associated pre-eclampsia, may be used as a predictive risk indicator for the early detection of pre-eclampsia.
CHAPTER 4
SYNTHESIS

Pre-eclampsia complicates 7-10% of all pregnancies worldwide (Kalumba et al., 2013) and it continues to be a significant contributor of maternal and perinatal morbidity and mortality. The province of KwaZulu-Natal, in South Africa, has a pre-eclamptic incidence of 12% (Saving Mothers report, 2013). Furthermore, the prevalence of HIV in pregnancy is approximately 37.7% (The 2012 National Antenatal Sentinel HIV and Herpes Simplex Type-2 Prevalence Survey in South Africa, 2012).

A family history of pre-eclampsia is considered a risk factor of this pregnancy specific syndrome (Carr et al., 2005). Importantly, its source is not just of maternal origin, as men born from such pregnancies are likely to father them as well (Harem et al., 2014). This reiterates the fact that the placenta is a product of both maternal and paternal origin (Wang et al., 2009).

During normal placentation, embryonic cytotrophoblast cells invade the maternal uterine wall, physiologically converting the maternal spiral arteries into large bore conduits. Additionally, the cytotrophoblasts switch from an epithelial phenotype to an endothelial-like one. This enables sufficient blood perfusion sustaining the foetus (Valenzuela et al., 2012). Angiogenesis is the transformation of endothelial tube segments into an organised vascular network, while angiogenic factors are the circulating polypeptides that function in angiogenesis. In order to fully grasp the aetiology of pre-eclampsia, angiogenesis and its contributing factors must be understood. Optimum angiogenesis and subsequent placental development occurs when there is an equilibrium between angiogenic factors VEGF and PI GF and antiangiogenic factors sVEGFR-1 and sEng (Govender et al., 2013b). It is proposed that in pre-eclampsia, a state of under perfusion persists, as there is limited spiral artery wall remodelling due to inadequate cytotrophoblast invasion (Cerdeira & Karumanchi, 2012). Hence, proper formation, maturation and maintenance of the placental vasculature is vital.

Soluble vascular endothelial growth factor receptor -1, is an antiangiogenic factor, commonly known as soluble fms-like tyrosine kinase (sFlt-1) (Wang et al., 2009). sVEGFR-1 inhibits VEGF and PI GF, thus hindering vessel vasodilation and adequate angiogenesis (Powe et al., 2011). Its circulating levels are reported to be elevated in imminent pre-eclamptic pregnancies (Stepan et al., 2016). Thandani et al. (2011) hypothesized that sVEGFR-1 facilitates the manifestations of the clinical signs and symptoms in pre-eclampsia.

The results of this study demonstrates an up-regulation of sVEGFR-1 in pre-eclampsia compared to normotensive pregnant women, irrespective of their HIV status. These results corroborate the findings of many studies worldwide (Govender et al., 2013a; Govender et al., 2013b; Cerdeira & Karumanchi, 2012; Koga et al., 2003). Off note, despite sVEGFR-1 diminishing the bioavailability of both VEGF and PI GF, the binding of sVEGFR-1 and VEGF-A alone cannot provide explanation for the clinical signs of pre-eclampsia (Bates, 2011). It is known, that the inhibition of VEGF by sVEGFR-1 impairs
angiogenesis i.e. there is inadequate placental vascularization culminating in reduced placental perfusion (Koga et al., 2003). Physiologically, VEGF-A increases vascular permeability thereby inducing oedema, as well as vasodilation and angiogenesis. This implies that this coupling should prevent the aetiology of pre-eclampsia, and not induce its development (Bates, 2011). Moreover, the presence of sVEGFR-1 is also believed to be the stimulus for an increase in sEng, an antiangiogenic factor (Govender et al., 2013b).

Additionally, due to the antagonistic nature of sVEGFR-1 to PI GF, circulating levels of PI GF are decreased. This overall imbalance in favour of anti-angiogenesis, is postulated to lead to the systemic manifestation of pre-eclampsia (Koga et al., 2003). It is important to note that despite PI GF and VEGF having the same binding affinity to sVEGFR-1, circulating levels of VEGF165 is greater (Bates, 2011), thus theoretically most VEGF should bind to sVEGFR-1. The secreted levels of PI GF are reported to be lower in pre-eclampsia therefore its binding to sVEGFR-1 would be negligible (Bates, 2011). Nonetheless Crispi et al. (2008) found that the differences in the level of sVEGFR-1 and PI GF varied according to gestational age, being more pronounced in early-onset (<33 weeks 6days gestation) compared to the late-onset (>34 weeks gestation) pre-eclampsia in comparison to normotensive pregnancies. Since abnormal angiogenic levels are detectable 5-14 weeks prior to clinical onset of the syndrome (Crispi et al., 2008), the ratio of sVEGFR-1 and PI GF may possibly serve as a risk indicator for early-onset pre-eclampsia development. However, Stepan and colleges (2016) concluded that the utilisation of the automated Elecsy immunoassay sFlt-1/PI GF ratio, can serve as a diagnostic tool, at different cut-offs for early as well as late gestation. A limitation in our study is that we did not stratify the pre-eclampsia into early and late gestational presentation.

A study by Koga et al. (2003) confirmed that the placenta expressed this antiangiogenic factor, as a result of an array of stimuli, specifically hypoxia. However, they further went on to propose that sVEGFR-1 production may be augmented from other tissue in pre-eclampsia, as circulating levels far surpass that which can be secreted by placental trophoblast cells alone. This may provide explanation for basal levels of sVEGFR-1 still being present following delivery, despite its overall marked decline in the pre-eclamptic group in comparison to their normotensive pregnant counterparts. Although the stimulus eludes us, it is suggested that monocytes are an alternate source of sVEGFR-1 (Major et al., 2014).

Soluble vascular endothelial growth factor receptor -2 originates from an endothelial cell surface proteolytic cleavage, due to reduced VEGFR-2 expression (Munaut et al., 2012). It is known that PI GF displaces VEGF from binding to sVEGFR-1, as a result VEGF then binds to the more potent sVEGFR-2; hence the bioavailability of the angiogenic factor is diminished (Chaiworapongsak et al., 2010; Govender et al., 2013a). Alternatively known as the KDR receptor, it plays a role in mediation of endothelial cell functions such as differentiation, proliferation and migration. Although not much is
knows about its role in the pathogenesis of pre-eclampsia, it is believed that MMP-9 may be involved, resulting in the vascular damage and impaired angiogenesis characteristic of pre-eclampsia (Luizon et al., 2012). A study carried out in 2012 by Munaut and co-workers was the first to discover the association of a low plasma concentration of VEGFR-2 with reduced placental expression of sVEGFR-2. This reduced plasma VEGFR-2 concentrations may have a role in the inadequate regenerative capacity exhibited in the endothelial cells of pre-eclamptic women.

Our study also reports a significant down-regulation in sVEGFR-2 concentrations in pre-eclampsia. Munaut et al. (2012), Chaiworapongsa et al. (2010) and Tripathi et al. (2009) had similar findings. Chaiworapongsa et al. (2010) further went on to say that lower median plasma levels of sVEGFR-2 evident in pre-eclamptic pregnancies are apparent 6-10 weeks prior to its clinical onset, the same timeframe at which sVEGFR-1 levels begin to escalate. Thus, it is conceivable that sVEGFR-2 may be used as a risk indicator diagnostic tool for women at risk of developing pre-eclampsia.

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a homophilic adhesion receptor. In its phosphorylated form, PECAM-1 can induce extracellular signal-regulated kinases (ERKs) activation (Tzima et al., 2005). It is a part of the immunoglobulin superfamily, hence expression is evident on the surface of circulating platelets, neutrophils, monocytes and certain T cells. PECAM-1 is also implicated in a variety of functions ranging from being a constituent of the endothelial cell intercellular junction to angiogenesis and transendothelial migration of leukocytes. In pre-eclampsia neutrophil and platelet activation promote endothelial damage. This is mediated by cell adhesion molecules like PECAM-1, which are expressed by both the maternal endothelium and the utero-placental bed. (Yasemin et al., 2012).

Particularly deficient cytotrophoblast uterine invasion is notable in the pathogenesis of pre-eclampsia (Yasemin et al., 2012). Embryonic cytotrophoblast invasion occurs twice in the gestation period. First, at 8-10 weeks gestation that is limited to the decidua, following its second invasion into the myometrium at 16-18 weeks gestation (Lyall et al., 2001). It has been found that these cytotrophoblast cells do not express PECAM-1, a necessary molecule for proper maternal spiral artery wall remodelling (Yasemin et al., 2012). In our study, despite finding no significance, a down-regulation trend of PECAM-1 levels in the pre-eclamptic women were observed. Similar conclusions were made by Lyall et al. in 1995 and 2001. However, conflicting results of significantly lower PECAM-1 concentrations have been reported in pre-eclampsia in comparison to normotensive pregnant women (Yasemin et al., 2012) substantiating the endotheliosis.

Pre-eclampsia and HIV infection continue to be a major cause of maternal deaths in sub-Saharan Africa. A common observation in HIV infected individuals is chronic arterial injury and endothelial
dysfunction. Moreover, if these individuals are untreated, they may be prone to endothelial damage. Importantly, HIV seems to impact the aetiology associated with pre-eclampsia (Govender et al., 2013a).

Finally, our results clearly established an imbalance of angiogenic factors, sVEGFR-2 and PECAM-1, versus the antiangiogenic factor, sVEGFR-1 in pre-eclampsia. However, coupled with HIV infection this imbalance is less offset in pre-eclampsia. Govender et al. (2013a), who also reported similar findings, proposed that the immune insufficiency exhibited by HIV infection, reduced the susceptibility to the classic immune hyperactivity evident in pre-eclampsia. The administration of highly active anti-retroviral therapy (HAART) reconstitutes immune response, consequently increasing the predisposition to pre-eclampsia. A limitation of our study is that all HIV infected women received either HAART or anti-retroviral drugs for the prevention of mother-to-child transmission (PMTCT) as part of the standard of care treatment regimen in South Africa.

It must be noted that the heterogeneous nature of the clinical manifestations in pre-eclampsia often present as a complication in identification (Stepan et al., 2016). A limitation of our study was that we did not sub-stratify pre-eclampsia into early and late onset groups. Also understanding the aetiology, pathogenesis as well as the early diagnosis of this syndrome is vital. At present, its diagnosis relies on blood pressure and urinary protein measurements. In view of the relative non-specificity of these parameters, a much more reliable and precise diagnostic risk indicator is warranted. Thus, current trends in pre-eclampsia research are now focused on non-invasive predictive tests in early pregnancy to identify women likely to develop pre-eclampsia.

Seeing that abnormal circulating levels of angiogenic biomarkers are detectable several weeks before the onset of signs and symptoms of pre-eclampsia, the use of angiogenic factors show promise as a diagnostic tool (Lapire et al., 2010; Chaiworaponsa et al., 2010). This study concludes that the levels of the angiogenic factors, sVEGFR-1 and -2 and PECAM-1 are altered in HIV associated pre-eclampsia, hence may have a predictive risk indicator test value for the early detection of pre-eclampsia. Our results indicated that the depressed immune response associated with HIV infection is insufficient to totally neutralising the hyper-immune activity displayed in pre-eclampsia.
CHAPTER 5


Frank, K.A. (2006). HIV and pre-eclampsia: is there a connection? Master of Medicine, University of the Witwatersrand.


49


Winn, V.D. (2010). Implantation Lecture. The University of Colorado Health Sciences Center, Anschutz Medical Campus.

06 April 2016

Ms Semone Thakoordeen
Discipline of Optics and Imaging
School of Laboratory Science and Medicine Science
semonesthakoordeen@gmail.com

Protocol: The role of platelet endothelial cell adhesion molecule -1 (PECAM-1) and soluble vascular endothelial growth factor receptor (sVEGFR) -1 and-2 in the pathogenesis of HIV associated pre-eclampsia
Degree: MMedSc
BREC reference number: BE085/16

EXPEDITED APPLICATION

The Biomedical Research Ethics Committee has considered and noted your application received on 01 March 2016.

The study was provisionally approved pending appropriate responses to queries raised. Your responses dated 05 April 2016 to queries raised on 02 April 2016 have been noted and approved by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from 06 April 2016. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.


BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its meeting taking place on 10 May 2016.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely,

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc: supervisor: nsicera@ukzn.ac.za
cc: Postgraduate Office
Dear Miss Thakoordeen:

Ref: The role of Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) and soluble Vascular Endothelial Growth Factor Receptor (sVEGFR)-1 and -2 in HIV associated pre-eclampsia

Our referees have now considered your paper and have recommended publication in Hypertension in Pregnancy. We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. Accepted papers will be transmitted for production. The first and most important task for authors at that point will be to complete an online author agreement form. Please make sure you complete it as soon as you receive the publisher notice about it.

You will receive proofs for checking. The publisher requests that proofs are checked and returned within 48 hours of receipt.

Thank you for your contribution to Hypertension in Pregnancy and we look forward to receiving further submissions from you.

Sincerely,
Dr Karumanchi
Editor, Hypertension in Pregnancy
sananth@bidmc.harvard.edu