THE ROLE OF BASIC FIBROBLAST GROWTH FACTOR IN HIV ASSOCIATED PREECLAMPSIA

By

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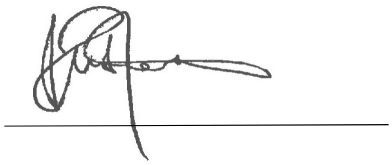
PREFACE

This study contains original work done by the author, it has not been submitted in any form to any other University. Work done by other authors have been used and duly acknowledged in the text.

The research within this dissertation was conducted in the Optics and Imaging Centre, Doris Duke Medical Research Institute, College of Health Science, University of Kwa-Zulu Natal, Durban, South Africa under the supervision of Professor Thajasvarie Naicker.



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DECLARATION

I, Charline Sangany (211559876) declare that:

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DEDICATION

All letters cannot find the words to say...

All the words cannot express my gratitude, love, respect...

I dedicate this thesis...

To my Lord Jesus Christ all powerful, who inspired me and guided me in the right path; I owe you what I became. Praise and thanks to your clemency and mercy.

To my dad Francois Sangany, thank you for your endless love and support that you have shown me through my life. Without you I would not have been granted this wonderful opportunity therefore I dedicate this Dissertation to you as my inspiration.

To my mother Hortense Tshikomba, dear mama, thank you for countless and huge sacrifices that you have made for my education and well-being. Your love, your goodness, your extreme generosity and your support are limitless. Your dedication was for me a constant encouragement. Today, I put in your hands the result of your dedication and your sacrifice as well as the expression of my love and my respect for you. Your prayers have been for me, very supportive. May God give you long life and happiness and may your blessing be with me always. I love you.

To all the Sangany and Bokana, you are my joy, my strength, my positive energy...

To all my friends for unforgettable friendship, countless memories, to the infinite loyalty, to the strong links which have sprayed our cohesion. This work is a testimony and recognition of your noble morals.

To all who are dear to me and I forgot to mention. To anyone who spent a moment to help, advice, encourage or simply smiled at me

Charline

"Life is a matter of decision, what you decide is what you become "- Pastor Jonas Mpoyi

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
AKT	Protein kinase B
ECM	Extracellular matrix
EOP	Early onset preeclampsia
ER	Endoplasmic reticulum
ERS	Endoplasmic recticulum stress
EVT	Extravillous trophoblast
FGF	Fibroblast growth factor
FGF-1	Acidic fibroblast growth factor
FGF-2	Basic fibroblast growth factor
FGFR	Fibroblast growth factor receptor
HAART	Highly active antiretroviral therapy
HELLP	Hemolysis elevated liver enzymes and low platelet count
HIF	Hypoxia-inducible factor
HSPG	Heparan sulphate proteoglycan
HIV	Human immunodeficiency virus
IFN	Interferon
Ig	Immunoglobulin
IL-6	Interleukin 6
Kda	Kilo Dalton

KZN	KwaZulu-Natal
LOPE	Late onset preeclampsia
MAPK	Mitogen-activated protein kinases
N	Normotensive
N+	Normotensive HIV positive
N-	Normotensive HIV negative
NF-kb	Nuclear factor kb
NO	Nitric oxide
NK	Natural killer cell
PlGF	Placental growth factor
PE	Preeclampsia
PI	Protease inhibitor
RAS	Receptor activated serine kinases
VEGF	Vascular growth factor
SA	South Africa
SFlt	soluble fms-like tyrosine kinase (sFlt-1)
TGF	Transforming growth factor
Th	T helper
UNAIDS	United nations programme on HIV and AIDS
UNICEF	United nations international children's emergency fund
UPR	Unfolded protein response

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ABSTRACT

Background: HIV/AIDS and preeclampsia (PE) remain the main causes of maternal death in sub-Saharan Africa. In South Africa, 12% and 38% of maternal deaths are attributed to PE and HIV/AIDS respectively. Both HIV infection and PE are characterised by opposing inflammatory responses that affect cell signalling. Basic fibroblast growth factor FGF-2 is a multifunctional protein that regulate structural reorganization of uterine and placental vascular bed during pregnancy. In light of the high prevalence of HIV infection and PE in the province of KwaZulu-Natal, this study investigates FGF-2 expression in HIV associated PE using the Bio-Plex Multiplex Immunoassay.

Method: Retrospectively collected serum samples from 40 normotensive pregnant and 40 preeclamptic women attending the antenatal clinic in a large regional hospital in eThekwini, South Africa were analysed. Samples were further stratified according to HIV status. FGF-2 was quantified using a Bio-Plex immunoassay.

Results: A downregulation of FGF-2 expression was observed in PE irrespective of HIV status (p=0.0011). Similarly, a downregulation of FGF-2 expression was reported in HIV positive patients irrespective of pregnancy type (p=0.0471). Across all study groups, there was a significant difference in the expression of FGF-2 between normotensive HIV negative *vs* pre-eclamptic HIV negative (p=0.0255). A significant difference was also noted between normotensive HIV negative vs pre-eclamptic HIV positive (p = 0.0002) and between normotensive HIV positive vs pre-eclamptic HIV positive (p = 0.0149).

Conclusion: This study demonstrates a downregulation of FGF-2 in PE implicating a defect in the endothelial loop feedback. The downregulation of FGF-2 in HIV infected subjects may be attributed to HIV *Tat* that interferes with the Src homology, which phosphorylates FGF-2. The use of highly active antiretroviral (HAART) results in endothelial toxicity and vascular dysfunction which affect FGF expression. Future studies will include a genetic appraisal of the variants FGF-1 rs34011 and FGF-2 rs2922979 in African women with PE.

CHAPTER I

BACKGROUND AND LITERATURE REVIEW

1.1 Preeclampsia

Preeclampsia (PE) is an obstetric condition that clinically occurs after the 20th gestational week. It is characterised by hypertension with a diastolic blood pressure ≥ 90 mmHg and a systolic blood pressure ≥140 mmHg, recorded twice in less than four hours apart, with proteinuria > 300 mg/24h, in a previously normotensive woman (Brown *et al*., 2018). The severity of PE lies in the possible occurrence of severe maternal complications (hemolysis elevated liver enzymes and low platelet count (HELLP) syndrome, eclampsia, haemostasis disorder, retro-placental hematoma and death) and perinatal complications (hypotrophy, induced prematurity and death *in utero*) (Ai-ris *et al*., 2018). PE may be classified as early onset (EOPE) or late onset (LOPE) according to the gestational age at diagnosis (Tranquilli *et al*., 2013). EOPE is diagnosed before 34 weeks of gestation and LOPE after 34 weeks of gestation (Brown *et al*., 2018; Tranquilli *et al*., 2013).

The aetiology of PE remains unknown. Studies suggest that PE could be established by inadequate signalling between the placenta and the mother (Brown *et al*., 2018). Nonetheless, abnormal placentation related to immune maladaptation and angiogenic dysregulation are implicated in PE development (Naicker *et al*., 2003). Preeclampsia is biologically associated with an increase of anti-angiogenic factors namely soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) that impair the signal of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) in the maternal circulation (Karumanchi *et al*., 2015). The pathological origin of PE is suggested to be the placenta hence the only curative treatment is the removal of the foetus allowing the delivery of the placenta (Ijomone *et al*., 2018).

1.1.1 Epidemiology and risk factors

Approximately 50 000 women worldwide die annually from PE (WHO, 2018). Preeclampsia affects 5-8% of pregnancies worldwide (Adams *et al*., 2016). In high-income countries, PE has a prevalence of 2-5% (Iyengar, 2001; Villar *et al*., 2006). However, in low-middle income countries, it occurs more frequently and is the primary cause of direct maternal mortality (15-20%) and morbidity, peri-natal death and intra-uterine growth retardation (Sharma *et al*., 2018; Alkema *et al*., 2016).

In South Africa, 14.8% of maternal deaths are attributed to hypertension in pregnancy with PE/eclampsia accounting for the majority of deaths (Moodley, 2018). Nulliparity (Myers, 2017), multiparity (Paré *et al*., 2014), age (below 18 and above 40 years) (English *et al*., 2015), medical

history of pre-existing high blood pressure (English *et al*., 2015), trophoblastic disease (Wright *et al*., 2015), obesity (Duckitt and Harrington, 2005), and prior history of PE are associated with a high probability of PE development (Duley *et al*., 2007). Other risk factors include duration of exposure to paternal sperm (Lie *et al*., 1998) and environmental factors such as climate and poverty (Fisher *et al*., 2015).

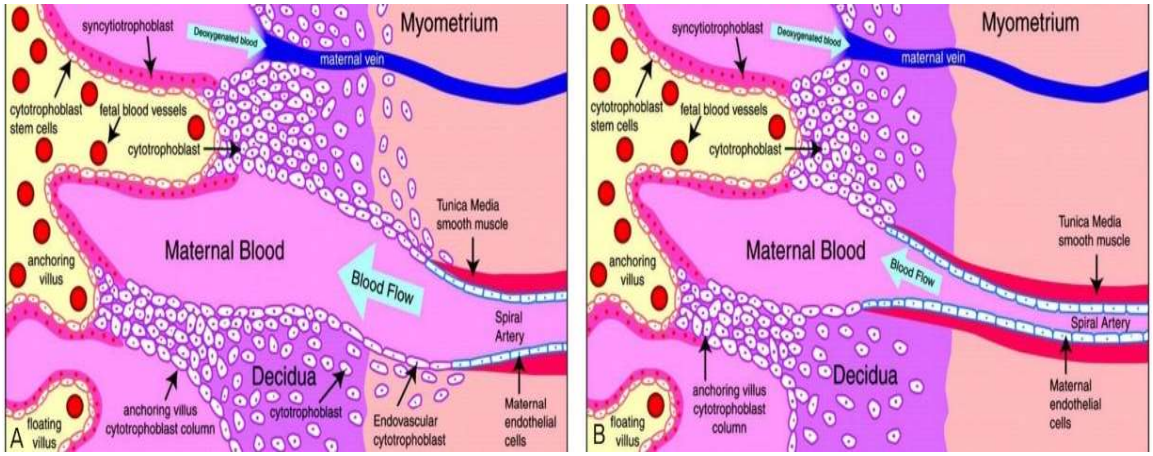
1.1.2 Pathogenesis of Preeclampsia

Several theories explaining the pathogenesis of PE have been put forward that directly or indirectly contribute to placental malfunction.

1.1.2.1 Angiogenesis

Angiogenesis is the formation of new capillary/blood vessels and occurs in a wide range of important biological processes (Heymach *et al*., 2016). In human pregnancy, the formation of the placenta undergoes angiogenesis, vasculogenesis, and pseudovasculogenesis as cytotrophoblasts convert from an epithelial to an endothelial phenotype (Karumanchi *et al*., 2015). Cytotrophoblast invasion generates vascular changes that favour materno-placental blood exchange necessary for fetal development (Karumanchi *et al*., 2015). Placental angiogenesis is characterized by an invasion of spiral arteries in the decidua by extravillous cytotrophoblasts (EVT) (Thakoordeen *et al*., 2017). Vascular changes are mediated by an increase in local and systemic VEGF secretion favouring vascular proliferation (Govender *et al*., 2013).

Failure of angiogenesis during early development has been linked to the pathogenesis of PE (Figure 1.1). The placenta in preeclamptic women produces an excess of anti-angiogenic factors (sFlt-1 and Eng) that enter the maternal circulation and impair vascular endothelial cell signaling of pro-angiogenic factors such as VEGF and PlGF (Karumanchi *et al*., 2015). The study of factors that are responsible for insufficient trophoblastic invasion is limited as PE is diagnosed after the natural process of trophoblastic invasion. Therefore, abnormalities observed in PE do not establish whether they are the cause or the consequence of this syndrome (Lecarpentier *et al*., 2016).



Normal pregnancy

Preeclampsia

Figure 1.1: Physiological modification of the spiral arteries and trophoblast invasion in normal pregnancy and in PE. Preeclampsia is characterized by ineffective trophoblast invasion and defective vascular remodelling leading to reduced maternal blood flow, which in turn may compromise foetal growth. Adapted from (Karumanchi *et al*., 2015).

1.1.2.2 The oxidative stress theory

One of the intrinsic trophoblastic factors involved in uteroplacental artery invasion is the surrounding oxygen tension. Extravillous trophoblasts (EVTs) encounter an increasing oxygen gradient during their migration to the spiral arteries and the more the tension increases, the more they differentiate into an invasive phenotype (Genbacev *et al*., 1997). Abnormal remodelling of the spiral arteries results in an abnormal oxygen supply to the placenta leading to a hypoxic state causing activation of diverse mechanisms that result in a high level of oxidative stress and inflammation (Salsoso *et al*., 2017). This phenomenon results from increased expression and activity of hypoxia inducible factor 1a (HIF-1α) that ends in nuclear factor kB (NF-kB)-dependent inflammation (Salsoso *et al*., 2017). HIF-1 induces transcription of TGF-β3 which inhibits trophoblast invasion (Caniggia *et al*., 1999). Oxidative stress results in endothelial dysfunction and increases endoplasmic reticulum stress (ERS), a condition evidenced by increased unfolded protein response (UPR) and trophoblast apoptosis (Caniggia *et al*., 1999).

1.1.2.3 Adhesion molecules

A study conducted by Zhou *et al* in 1993 revealed an abnormal pattern of molecules on the surface of cytotrophoblasts in placental bed biopsies performed in PE. In PE, EVTs lose their ability to switch from a proliferative phenotype to an invasive phenotype (Zhou *et al*., 1993). Thus, the expression of integrins α6β4, αvβ6 and E-cadherin persists whereas integrin α1β1 does not appear on the EVT surfaces (Karumanchi *et al*., 2015).

1.1.2.4 Immune maladaptation

T-helper (Th) 1 and Th2 cytokines play a role in cell-mediated and humoral immunity. The Th1 immune response inhibits the Th2 response and *vice versa* by cytokine production (Mikovits *et al*., 1994). In healthy non-pregnant women, there is a balance between the Th1 and Th2 response (Laresgoiti-Servitje *et al*., 2010). In a normal pregnancy, with the presence of the placenta, a shift from the Th1 to Th2 immune response occurs (Laresgoiti-Servitje *et al*., 2010).

However, in PE, the regulation of the maternal immune system is further altered as this shift does not occur (Saito *et al*., 2010). Wegmann *et al*. (1993) proposed that the placenta is a Th2 organ that stimulates the production of Th2 cytokines in the maternal immune system. When the placenta is abnormal, the maternal immune system responds by producing Th1 cytokines (Wegmann *et al*., 1993). Additionally, transforming growth factor-β cytokines surge thereby retarding cytotrohophoblast migration (Wegmann *et al*., 1993, Laresgoiti-Servitje *et al*., 2010). Moreover, in PE, syncytiotrophoblast fragments are increased in maternal circulation due to the ischemic placenta which is possibly responsible for triggering the Th1 response (Germain *et al*., 2007). Th2 cytokines respond to extracellular infection and are known to suppress the immune system (Sargent *et al*., 2006). In HIV infected patients there is a decrease in the Th1 and an increase in Th2 cytokines (Clerici and Shearer, 1993). However, the circulating levels of cytokines in PE is still conflicting (Kumar *et al*., 2013).

1.2 Human immunodeficiency virus infection

Human immunodeficiency virus (HIV) is an RNA virus from the retroviridae family, characterized by the presence of an enzyme-reverse transcriptase (Garofalo *et al*., 2016). HIV infection is known to alter the body's immune response by infecting immune cells and making the body more vulnerable to infections (McCormack *et al*., 2016). Its preferred targets are the CD4 + T lymphocyte through their CCR5 and CXCR4 receptors (Bengoa *et al*., 2017). HIV *Tat* plays an important role in the activation of the transcriptional proviral DNA and it is known as a

powerful angiogenic factor (La Venuta *et al*., 2015). Opportunistic diseases that take advantage of the reduced immunity lead to the Acquired immunodeficiency syndrome (AIDS) which is fatal (Bengoa *et al*., 2017).

1.2.1 Epidemiology

Sub-Saharan Africa accounts for 56% of people living with HIV infection globally (UNAIDS, 2018). In 2017, women accounted for a disproportionate 59% of new adult HIV infections (> 15 yrs) (UNAIDS, 2018). South Africa (SA) is home to the largest global HIV epidemic (UNICEF Eastern and Southern Africa, 2018). In SA, 13.1% of the total population is HIV positive, one-fifth been women in their reproductive ages (15–49 years) (Statistics South Africa, 2018). The province of KwaZulu-Natal (KZN) is the global "HIV hotspot" where 40.8% of adults (aged 15 years and older) are living with HIV infection (Cuadros *et al*., 2018). The highest prevalence of HIV infected parturients (37%) occurs in the province of KZN (Statistics South Africa, 2018). Hence, health care professionals providing maternity care are faced with a severe dilemma.

1.2.2 Influence of HIV infection on pregnancy

Premature births and foetal hypotrophy are more frequent in the cohorts of women infected with HIV compared to uninfected women (Adam, 2015). In the absence of antiretroviral therapy (ART), the severity of the immunosuppression is also a risk factor for obstetric complications or foetal malformations (Yudin *et al*., 2016). The benefit of ART in pregnancy, including the reinforcement of immune defences, is counterbalanced by its potential toxic effect (Danforth *et al*., 2017). A European study found that treatment with protease inhibitors (PI) was associated with a significant increase in the rate of premature delivery (Woodsong and Holt, 2015). Other preliminary studies have suggested that the use of triple ART is associated with an increased rate of PE (Maharaj *et al*., 2017).

1.3 HIV associated Preeclampsia

HIV and PE are disorders that affect the immune response by causing an alteration in both the innate and the adaptive immunity (Moodley, 2013). As these disorders generate an inflammatory response, activated neutrophils, macrophages and NK cells that travel to the inflammatory site induce endothelial dysfunction, while activated T cells may support inadequate tolerance during pregnancy (Moodley, 2013). Previous data have indicated that the expression of inflammatory cytokines such as TNF-alpha, IL-1beta, and IFN-gamma in PE are similar to those in HIV infected patients (Udenze *et al*., 2015). The combination of these cytokines as found in conditioned media

from activated T cells induce normal endothelial cells to acquire the features of being responsive to the effects of HIV *Tat* protein (Maharaj *et al*., 2017). Both large and small vessel endothelium chronically exposed to inflammatory cytokines produce and release angiogenic factors in the absence of cell death, a key feature of PE (Maharaj *et al*., 2017). Highly active antiretroviral therapy (HAART) is reported to enhance maternal immune reconstitution by re-establishing the mother's immune response to foetal antigens, however it makes HIV infected women susceptible to the development of PE (Adams *et al*., 2016).

1.4 Fibroblast Growth Factors (FGFs)

Cellular signalling is a complex communication system that governs the fundamental functional processes of cells and coordinates their activity (Wright and Dyson, 2015). The ability of cells to perceive and correctly respond to their microenvironment is at the root of their developmental processes such as in proliferation, differentiation, migration, invasion, and apoptosis (Sun *et al*., 2015). A correct signalling pathway contributes to a well-established immune response as well as to normal tissue homeostasis (Sun *et al*., 2015). Both HIV infection and PE are characterised by an inflammatory response (Maharaj *et al*., 2017) therefore, assessing analytes that participate in cell signalling pathways in both physiological and pathological conditions is important.

The fibroblast growth factors (FGF) are multifunctional proteins that, among other roles, regulate structural reorganization of uterine and placental vascular bed during pregnancy (Chrusciel *et al*., 2010). Fibroblast growth factors are synthesized by endothelial cells and fibroblasts (Blotnick *et al*., 1994). Fibroblast growth factors are only released on physiological and pathological demand hence their role in tissue repair and in angiogenesis is vital (Ornitz and Itoh, 2015).

1.4.1 FGF superfamily and subfamily

According to a phylogenetic analysis, the human FGF superfamily consists of 22 members or ligands (Figure1.2). These may be further stratified into seven subfamilies: FGF 1/2, FGF 4/5/6, FGF 3/7/10/22, FGF 8/17/18, FGF 9/16/20, FGF 11/12/13/14, and FGF 19/21/23, coded by as many different genes varying between 155 and 268 amino acids (Nunes *et al*., 2016).

Members of FGF-1 to 10 bind to the fibroblast growth factor receptors (FGFR) (Nunes *et al*., 2016). Fibroblast growth factor *ie*. FGF-11to 14, also known as FGF-homologue 1-4 (FHF-1-FHF4) are involved in intracellular processes, but they do not bind to FGFRs (Nunes *et al*., 2016). The FGF-18 subfamily is involved in cell development and morphogenesis in various tissues,

including cartilage. The FGF-19 was only described in humans whilst FGF-15 only in mouse. The human FGF-19 is suggested to be the ortholog of mouse FGF-15 (Ornitz and Itoh, 2015).

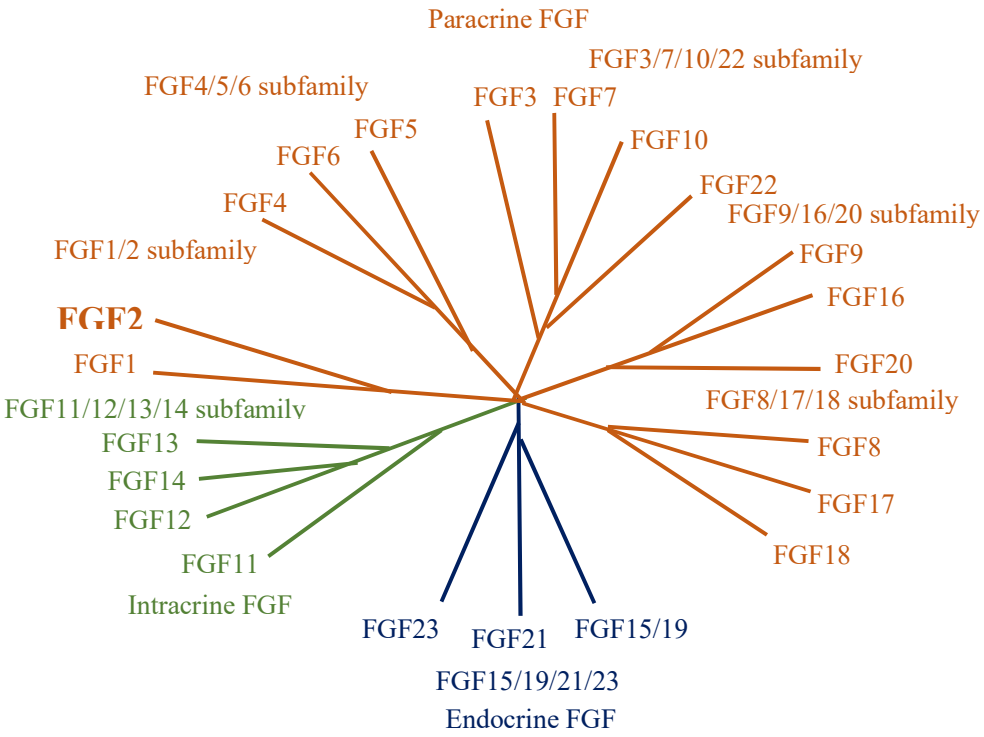


Figure 1. 2: Phylogenetic tree of human FGF family. Adapted from (Ornitz and Itoh, 2015).

1.4.2 Fibroblast growth factor receptor and signaling pathway

In humans, the FGFR consists of four members: FGFR1, FGFR2, FGFR3 and FGFR4 (Sarabipour and Hristova, 2016). Each FGFR comprises three extracellular immunoglobulin-like domains *i.e.*, IgI, IgII, and IgIII that are important in receptor dimerization; a transmembrane domain and a tyrosine kinase intracellular domain (Sarabipour and Hristova, 2016). The FGFR1-3 have a varied IgIII domain and use it alternatively to generate different isoforms that are expressed in different types of tissues with diverse binding specificities (Ornitz and Itoh, 2015). FGFR4 can only use its unique IgIII form.

FGF binds to heparan sulfate proteoglycan (HSPG) that are present in the extracellular matrix (ECM); mediated by the IgIII domain (Sarabipour and Hristova, 2016). This binding triggers a signal causing tyrosine kinase phosphorylation and FGFRs activation (Sarabipour and Hristova, 2016). The activated FGFRs then mediates signalling by recruiting specific molecules that bind

to the phosphorylated tyrosine at the cytosolic part of the receptor (Yashiro and Matsuoka, 2016). Finally, this triggers a number of signalling pathways that mediate specific cellular responses (Ornitz and Itoh, 2015). The best understood pathways are the RAS/MAP kinase pathway, PI3 kinase/AKT pathway, and PLCγ pathway (Yashiro and Matsuoka, 2016). These signalling pathways contribute to cell proliferation, migration, differentiation, and angiogenesis (Yashiro and Matsuoka, 2016).

1.4.3 FGF Mechanism of action

The FGF family may also be divided into three groups according to their mode of action (Figure1.3). These include:

a) *Paracrine* action- mediated by five sub-families namely FGF-1/2, FGF -4/5/6, FGF -3/7/10/22, FGF -8/17/18 and FGF -9/16/20;
b) *Endocrine* action- mediated by FGF-19/21/23 subfamily;
c) *Intracrine* action- mediated by FGF-11/12/13/14 subfamily (Ornitz and Itoh, 2015).

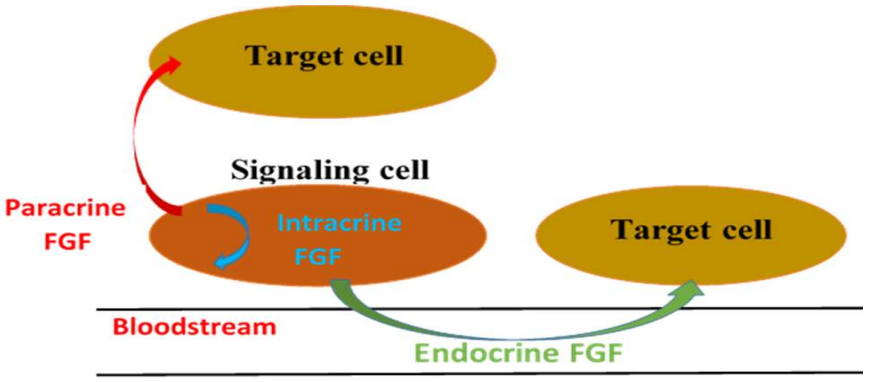


Figure 1.3: Diagram of the mechanism of action of FGF through paracrine, intracrine and endocrine manner. Adapted from (Nunes et al., 2016).

Paracrine FGF contains a cleavable N-terminal secreted signal peptide (Revest *et al*., 2000). The bipartite secreted signal peptide sequence of FGF-9/16/20 is not cleaved in mature proteins (Revest *et al*., 2000). However, the FGF1/2 subfamily lacks the N-terminal signal peptide sequence and is released from damaged cells by an exocytotic mechanism of which the ER/Golgi pathway is not required (Zacherl *et al*., 2015;Mohan *et al*., 2010; Nickel, 2010). Paracrine FGFs

are heparan binding molecules that bind to the tyrosine kinase FGFR (Nickel, 2010). This results in functional dimerization, receptor transphosphorylation, and the activation of downstream signalling pathways (Ornitz and Itoh, 2015). Paracrine FGFs mediate the biological response of neighbouring cells from a distance, and their affinities for heparan-like molecules of the ECM regulate the range of their signalling (Antoine *et al*., 1997).

Like paracrine FGFs, endocrine FGF function by binding to FGFRs. However, their affinity for heparin sulfate is not that strong thus they are restricted to act in an endocrine manner (Zhang *et al*., 2006; Goetz *et al*., 2007). Intracrine FGFs are intracellular proteins that primarily work as regulators of the electrical excitability for neurons (Goldfarb *et al*., 2007; Xiao *et al*., 2007).

1.4.4 Basic Fibroblast Growth Factor (FGF-2)

The first members of the FGF family "FGF 1/2" was described in 1986 *(Jaye et al., 1986; Abraham et al*., 1986). Fibroblast Growth Factor-1 was isolated at an acidic pH, and was named "acidic fibroblast growth factor" whereas FGF-2 was isolated at basic pH, hence termed "basic fibroblast growth factor". Basic fibroblast growth factor is a potent mitogen and powerful angiogenic factor that is involved in repair of damaged tissues (Sonmez and Castelnuovo, 2014). Basic fibroblast growth factor is a heparan-binding protein involved in a variety of pathological conditions including angiogenic dysregulation and solid tumour growth hence it is regarded as a target for cancer chemopreventative and therapeutic strategies.

Basic fibroblast growth factor lacks a signal peptide-mediated sequence and is released by direct translocation across the plasma membrane (Rothman and Wieland, 1996) or through vesicular intermediates such as autophagosomes or exosomes derived from multivesicular bodies (Rabouille *et al*., 2012). An unconventional mechanism involves FGF-2 oligomerization that forms pores in the plasma membrane thus allowing direct protein translocation (La Venuta *et al*., 2015). As illustrated in Figure 1.3, the translocation process is mediated by FGF-2 recruitment by the phosphatidylinositol 4,5-bisphosphate PI(4, 5)P_2 (Zacherl *et al*., 2015). As a result, FGF-2 undergoes oligomerization that, in turn, causes membrane insertion and the formation of membrane pores (Steringer *et al*., 2012)

The main features of the unconventional secretion of FGF-2 are:

1. Serial molecular connections with the inner leaflet along with Tec kinase dependent tyrosine phosphorylation of FGF-2,
2. PI(4, 5)P_2 dependant oligomerization and membrane pore formation,
3. Extracellular trapping of FGF-2 mediated by HSPG on cell surfaces.

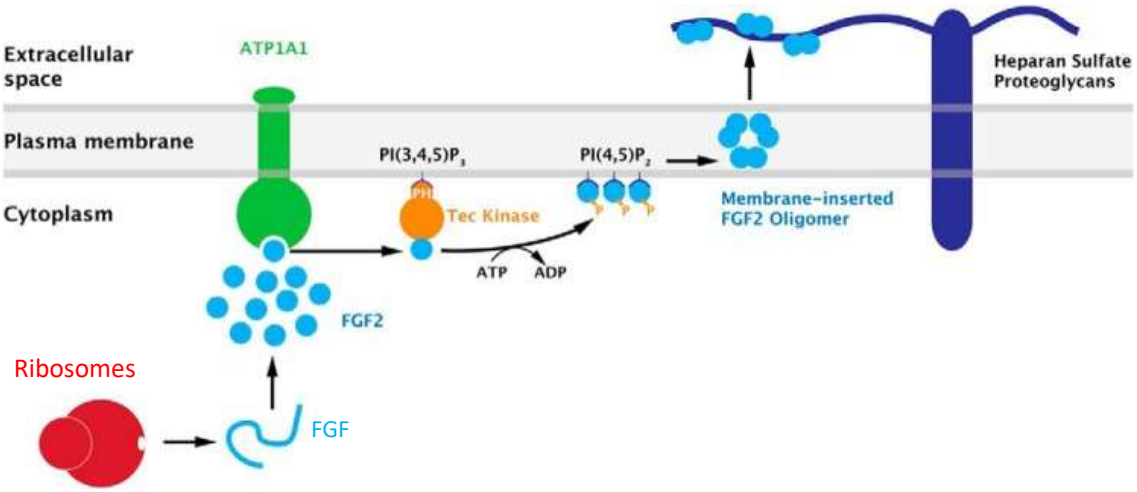


Figure 1.4: Illustration of the molecular mechanism of unconventional secretion of FGF-2. FGF2 secretion is mediated by direct translocation across the plasma membrane. This process involves sequential interactions of FGF-2 with components at the inner leaflet, including ATP1A1 (the α subunit of the Na/K ATPase), the phosphoinositide PI(4,5)P2, and Tec kinase. The ATP1A1 is efficient as its downregulation was shown to cause a substantial drop in FGF-2 secretion. The phosphoinositide PI(4,5)P2 dependant membrane binding contributes to the secretion of FGF-2, a protein that lacks peptide signal. The HIV accessory protein called Tat exits the cell in the same manner as FGF. Tec kinase is a non-tyrosine kinase receptor that regulates FGF-2 secretion. It is also known in the context of immune cell development and activation. Tec kinase phosphorylates FGF-2, a process that favours the formation of membrane pores by FGF-2 oligomers. Tec kinase contains a pleckstrin homology domain that mediates PI(3,4,5)P3 – dependant membrane recruitment. Tec kinase becomes phosphorylated by plasma membrane-resident Src kinases or by autophosphorylation within its activation loop resulting in enzymatic activation. Heparan sulfate proteoglycans are required to trap FGF-2 on cell surfaces, the final step in the overall process of FGF-2 secretion. Adapted from (Zacherl *et al*., 2015).

1.4.4.1 Basic Fibroblast growth factor & HIV infection

The molecular mechanism by which FGF2 is secreted from cells seems to be used by other proteins such as the HIV-1 transactivator of transcription (HIV-*Tat*) (Ruben *et al*., 1989). HIV-*Tat* is a regulatory protein that is synthesised early in HIV infection, involved in viral gene expression by enhancing transcriptional rates and responsible for viral variability (Ruben *et al*., 1989).

Like FGF-2, HIV *Tat* is a protein that lacks a signal peptide. Despite the lack of signal peptide, HIV *Tat* can be secreted from damaged or infected cells, therefore, it may be classified as an unconventional protein (Debaisieux *et al*., 2012). HIV-*Tat* has been previously demonstrated to

be released from infected T cells in a PI(4,5)P_2-dependent manner which is similar to the exit manner of FGF-2 (Rayne *et al*., 2010) . In addition, HIV-*Tat* has been demonstrated to bind to heparan sulfate proteoglycans (Debaisieux *et al*., 2012), which contributes to HIV-*Tat* secretion similarly to those of FGF-2 (Nickel, 2007). Therefore, the activities of FGF-2 and HIV *Tat* are synergistic (Zhou *et al*., 2013).

1.4.4.2 Basic Fibroblast Growth Factor (FGF-2) & Preeclampsia

In PE, there is a deregulation of decidual and placental FGFR2 expression (Marwa *et al*., 2016). Nonetheless, FGF-2 is widely expressed during embryonic development and acts by controlling neovascularization (La Venuta *et al*., 2015). Variation in FGF-2 gene is associated with hypertension, which was attributed to upregulation of FGF-2 expression in glomerular mesangial and endothelial cells (Marwa *et al*., 2016). To our knowledge, there is no available data on the pathological condition of PE comorbid with HIV infection.

1.5 Hypothesis

Basic Fibroblast growth factor would be altered in the opposing immune responses of PE and HIV infection.

1.6 Aim and objectives

This study investigated FGF-2 expression in HIV associated preeclampsia using the Bio-Plex Multiplex Immunoassay.

Objectives will include:

- To determine the effect of pregnancy type (normotensive and preeclampsia) on the expression of FGF-2 irrespective of HIV status,
- To determine the effect of HIV status (HIV positive and HIV negative) on FGF expression irrespective of pregnancy type,
- To compare FGF-2 expression across the study population.

CHAPTER II

Inflammation Research

The role of basic fibroblast growth factor (FGF-2) in HIV associated preeclampsia

--Manuscript Draft--

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Abstract:	Purpose Cell signalling is vital to ensure successful trophoblast invasion, hence this study assessed the level of serum basic fibroblast growth factor (FGF-2) in HIV associated preeclampsia. Method Using a Bio-plex Multiplex Immunoassay, FGF-2 serum concentration (pg/ml) was analysed in blood sera collected from 80 pregnant women attending a large regional hospital in Durban, South Africa. Study groups consisted of normotensive and preeclamptic pregnant women stratified according to their HIV status. All HIV positive groups received highly active antiretroviral therapy (HAART). Results In this study, we report a significant decrease of FGF-2 serum levels ($p=0.0011$) in preeclamptic compared to normotensive pregnant women, irrespective of HIV status. Also, there was a significant decrease in FGF-2 serum levels ($p=0.0471$) in HIV positive compared to HIV negative group, irrespective of pregnancy type. Across all groups, a significant difference ($p=0.0020$) in FGF-2 expression was noted. Conclusion This study demonstrates a down-regulation of FGF-2 expression in preeclamptic compared to normotensive pregnant women and may be attributed to the defective trophoblast invasion and/or to preeclampsia severity. The decline in FGF-2 expression in HIV infection is probably due to the effect of HIV Tat protein on angiogenesis. Further investigations on the effect of HAART on FGF-2 expression are required.
Suggested Reviewers:	

The role of basic fibroblast growth factor (FGF-2) in HIV associated preeclampsia

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Abstract

Purpose: Cell signalling is vital to ensure successful trophoblast invasion, hence this study assessed the level of serum basic fibroblast growth factor (FGF-2) in HIV associated preeclampsia.

Method: Using a Bio-plex Multiplex Immunoassay, FGF-2 serum concentration (pg/ml) was analysed in blood sera collected from 80 pregnant women attending a large regional hospital in Durban, South Africa. Study groups consisted of normotensive and preeclamptic pregnant women stratified according to their HIV status. All HIV positive groups received highly active antiretroviral therapy (HAART).

Results: In this study, we report a significant decrease of FGF-2 serum levels (p=0.0011) in preeclamptic compared to normotensive pregnant women, irrespective of HIV status. Also, there was a significant decrease in FGF-2 serum levels (p=0.0471) in HIV positive compared to HIV negative group, irrespective of pregnancy type. Across all groups, a significant difference (p=0.0020) in FGF-2 expression was noted.

Conclusion: This study demonstrates a down-regulation of FGF-2 expression in preeclamptic compared to normotensive pregnant women and may be attributed to the defective trophoblast invasion and/or to preeclampsia severity. The decline in FGF-2 expression in HIV infection is probably due to the effect of HIV *Tat* protein on angiogenesis. Further investigations on the effect of HAART on FGF-2 expression are required.

Keywords: FGF-2; Preeclampsia; HIV; HAART

Introduction

South Africa (SA) is the 4th largest Human Immunodeficiency Virus (HIV) epidemic in the world [1]. In SA, the HIV prevalence rate is 13.1% with 19.0% of infections occurring within the reproductive age (15–49 years) [2, 3]. In the province of KwaZulu-Natal (KZN) SA, the overall HIV prevalence in women is 39.0% hence is a serious obstetric problem [4]. In SA, 14.8 % of maternal deaths emanate from hypertension in pregnancy with preeclampsia (PE) accounting for 83% of deaths [5]. The prevalence of PE in SA is 12% [6].

Preeclampsia is a pregnancy specific complication unique to humans that affects 3 to 10% of pregnancies worldwide [7]. It is characterised by *de novo* hypertension (blood pressure ≥ 140/90 mm Hg) and proteinuria (> 300g/24 h or ≥ + 1 dipstick) occurring after 20 weeks of pregnancy [8]. The underlying pathogenesis of PE is defective trophoblast invasion that eventuates in an absence of the physiological remodelling of spiral arteries [9]. This creates a hypoxic, oxidative stressed microenvironment, which produces factor(s) that lead to the clinical manifestation of PE [10].

Successful placentation depends on the correct expression of angiogenic factor [11]. However, this is compromised in PE where there is an imbalance between increased expression of the anti-angiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and Endoglin (Eng) with a concurrent decrease in the pro-angiogenic placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) [12, 13]. The systemic release of sFlt-1 pre-empts the discharge of cytokines such as tumour necrosis factor (TNF)-α which contributes to an exaggerated inflammatory response [14].

During embryonic development acidic fibroblast growth factor (aFGF; FGF1; FGF-1) and basic fibroblast growth factor (bFGF; FGF2; FGF-2) are expressed as angiogenic factors [15]. Acidic fibroblast growth factor and FGF-2 are members of the super FGF family which is composed of 22 ligands, with 4 receptors that act in a paracrine, intracrine and/or endocrine manner [16]. The heparan sulfate proteoglycan (HSPG) protects FGF-2 against degradation and confers high affinity for binding to its receptors [17]. The FGF-2 protein lacks signal peptides hence it is directly translocated across the plasma membrane and is secreted through autophagosomes or exosomes and by the non-conventional FGF-2/oligomerization with resultant membrane pore formation and by extracellular trapping mediated by HSPG on cell surfaces [18].

The binding of FGF-2 to HSPG triggers a signal causing tyrosine kinase phosphorylation as well as its receptors activation [19]. The activated FGF-2 receptors then mediate signalling by recruiting specific molecules that bind to the phosphorylated tyrosine at their cytosolic part [20].

Finally, this triggers a number of signalling pathways via the RAS/MAP kinase pathway, PI3 kinase/AKT pathway and PLCγ pathway that mediate specific cellular responses [21].

Fibroblast growth factor-2 promotes cell growth, differentiation and proliferation [22]. It stimulates the migration and proliferation of endothelial cells in existing vessels leading to the formation of capillary tubes and recruits other cell types to generate and stabilise new blood vessels [23]. In hypertension, a vasopressor may be balanced by an up-regulation of FGF-2 secretion that is initiated by the activity of the endothelial loop [24]. In the scenario of endothelial loop dysfunction such as in PE, FGF-2 secretion may be dysregulated [24].

In HIV infection, the HIV-1 (HIV-*Tat*) has similar properties to those of FGF-2 [18]. The transactivator of transcription *Tat* can also act in synergy with VEGF or FGF-2 to modulate angiogenesis [25]. In view of the role of FGF-2 and the high prevalence of PE and HIV infection in SA, this study attempts to examine the effect of FGF-2 in HIV associated PE based on pregnancy type (PE *vs* normotensive pregnancy), HIV status (HIV-ve *vs* HIV+ve) and across all study groups.

Material and Methods

Study population

Following ethical approval (BCA 338/17), regulatory health authority permission and informed consent, pregnant women were recruited from a large regional hospital in KZN, SA. The study population (n=80) was determined by the Fischer's test and consisted of normotensive pregnant (n=40) and pre-eclamptic (n=40) women. These groups were further stratified according to HIV status into HIV+ve normotensive (n=20), HIV+ve PE (n=20), HIV-ve normotensive (n=20) and HIV-ve PE (n=20). Pre-eclampsia was defined as hypertension with systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, measured on two occasions, no less than four hours apart, and with proteinuria (+1 urine dipstick test) after the 20th gestational week. HIV status was determined following a rapid HIV test.

All newly HIV infected women were initiated on HAART. Exclusion criteria included those without informed consent, chorioamnionitis, eclampsia, chronic hypertension, intrauterine death, abruption placentae, diabetes, systemic lupus erythematous, chronic renal disease, polycystic ovarian syndrome, sickle cell disease, cardiac disease, pre-existing seizure disorders, active asthma that requires medication during the gestational period and unknown HIV status.

Samples

This is a retrospective study using archived venous blood. These were collected in K3-EDTA tubes, serum was extracted and stored in cryovials at -80ºC until immunoassay.

Bio-Plex Multiplex immunoassay

The immunoassay was performed according to the manufacturer's instructions (Bio-Rad Laboratories, Inc., USA).

Maternal blood samples were collected in sterile tubes were centrifuged at 3000 rpm for 10 min at 4° C and serum was used for quantifying FGF-2 levels using the Bio-Plex Pro Human Cancer Biomarker Panel 1 (Bio-Rad Laboratory, Inc., USA). Standards were prepared in a 1:10 and 1:4 dilution series, whilst samples were prepared in a 1:4 dilution.

The immunoassay involved the incubation of the antigen sample, *i.e*., FGF-2, with the capture antibody-coupled beads. Subsequently, biotinylated detection antibody with streptavidin-phycoerythrin (SA-PE), a reporter conjugate was added to the reaction. Following washing for removal of any unbound SA-PE, fluorescence of the SA-PE bounded to each bead was measured by a Bio-Plex Multiplex Reader. The analyte concentration was extracted in picograms per millilitre (pg/ml) using the Bio-plex manager software version 4.1.

Statistical analysis

Data analysis was performed using GraphPad Prism statistics software, version 5.00 (California, USA). The Student's unpaired t-test was used to analyse parametric data (mean ± standard deviation) and the Mann-Whitney U test was used to measure non-parametric data (median ± inter-quartile range). An analysis of variance (ANOVA) was performed to compare difference of serum FGF-2 concentration in the sample population based on HIV status (HIV+ve *vs* HIV-ve) and pregnancy type [normotensive *vs* pre-eclamptic]. A probability level of $p< 0.05$ was considered statistically significant.

Results

Clinical characteristics and demographic data

Table 1 provides a summary of the demographics of the study population. Gestational age, diastolic and systolic blood pressures (BP) were statistically different between the normotensive and PE groups ($p<0.0001$: Mann-Whitney test). There were no significant differences in maternal

age (p=0.14) and maternal weight (p=0.5) between normotensive *vs* PE groups. Proteinuria was noted in all cases of PE and HAART were given to all HIV positive women.

The mean ± standard deviation of maternal body mass index was 28.76.86±6.79 kg/m^2 *vs* 32.84±8.12kg/m^2 for normotensive and for PE groups respectively. The mean ± standard deviation of baby weight from the normotensive *versus* PE pregnant group was 3.18±0.37 *vs* 2.14±0.57 kg; Mann-Whitney test.

Table 1: Clinical profile and demographic data of participant across all study groups.

Variable	**Normotensive HIV-ve** n=20	**Normotensive HIV+ve** n=20	**Preeclamptic HIV-ve** n=20	**Preeclamptic HIV+ve** n=20	**P value**
Maternal age (years)	25.3±1.322	28.84 ±1.447	26.21±1.761	29.11±1.459	0.1446
Gestational age (weeks)	39.37±0.4133	38.53±0.4733	32.63±1.140	31.05±0.927	***<0.0001
Diastolic BP (mmHg)	67.10±2.035	67.42±2.016	104.4±1.813	107.3±2.254	***<0.0001
Systolic BP (mmHg)	120.3±2.285	114.6±2.696	159.3±3.500	163.7±3.25	***<0.0001
Maternal weight (kg)	77.36±2.971	76.17±3.654	76.63±4.762	77.04±5.586	0.9096
Parity	1.053±0.3008	1.47±0.2662	0.894±0.2405	1.316±0.2301	0.2350

Data are presented as Mean± standard deviation, a one-way ANOVA test was used for statistical analysis. n=80 ***p<0.001.

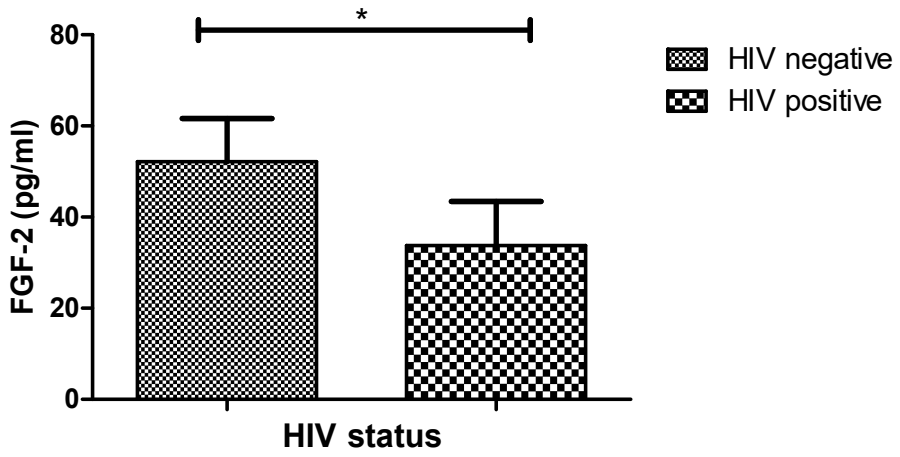

Figure 1: FGF-2 expression based on HIV status. *p=0.0471. Results are represented as Mean±Standard deviation.

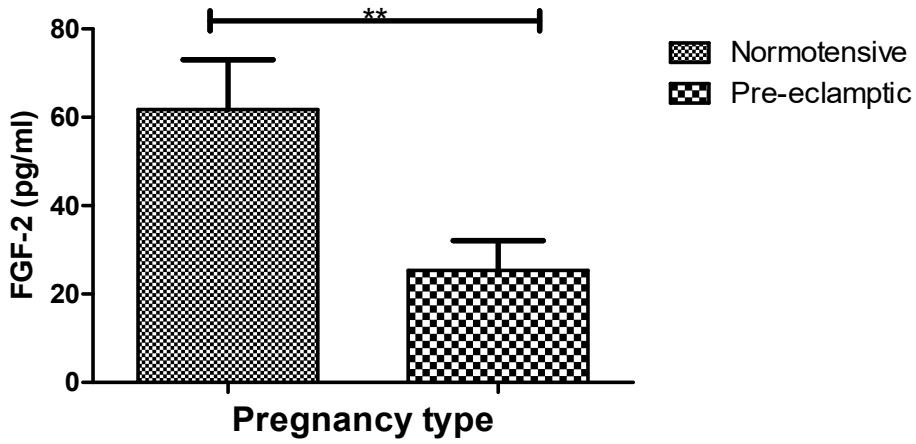

Figure 2: FGF-2 expression based on pregnancy type. ** p=0.0011.Results are represented as Mean±Standard deviation.

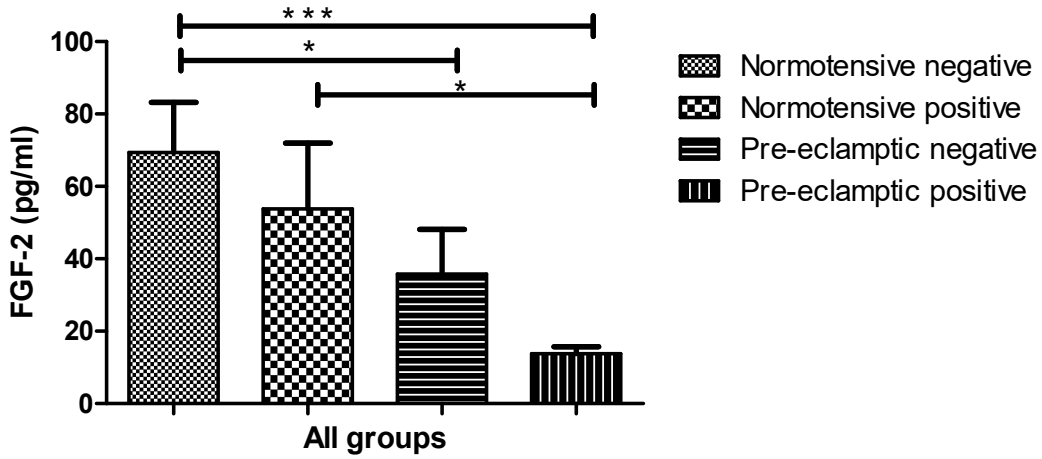

Figure 3: FGF-2 expression across all study groups. ***p*=0.0020 Results are represented as Mean±Standard deviation;

Table 2: Serum concentration of FGF-2 and P value across all study group.

	Normotensive HIV-ve n=20	**Normotensive HIV+ve** n=20	**Pre-eclamptic HIV-ve** n=20	**Pre-eclamptic HIV+ve** n=20	**P value**
FGF-2 (pg/ml)	64.63 (77.03)	11.81(58.87)	11.81(26.58)	11.81(0)	** 0.0020

Results are represented as: median (interquartile range)

FGF-2 serum concentration

Concentrations of FGF-2 across study group are outlined in table 2 and figures 1, 2 and 3.

HIV status: Based on HIV status (figure 1), there was a significant difference of serum FGF-2 between the HIV-ve versus the HIV+ve groups (52.15±9.491 pg/ml *vs* 33.80±9.622 pg/ml; Mann Whitney U=544.5; $p = 0.0471$) respectively.

Pregnancy type: Based on pregnancy type (figure 2), there was a significant difference of serum FGF-2 between normotensive and pre-eclamptic groups (61.79±11.25 pg/ml *vs* 25.38±6.690 pg/ml; Mann Whitney test U=445.0; $p = 0.0011$) respectively.

Across study groups: A significant difference of serum FGF-2 expression was noted across all groups (figure 3) *i.e*., normotensive HIV-ve *vs* pre-eclamptic HIV-ve [69.34±13.83 pg/ml *vs* 35.83±12.26 pg/ml; p=0.0255]; normotensive HIV-ve *vs* pre-eclamptic HIV+ve [69.34±13.83 pg/ml *vs* 13.78±1.969 pg/ml; p = 0.0002] and normotensive HIV+ve *vs* pre-eclamptic HIV+ve [53.81±18.17 pg/ml *vs* 13.78±1.969 pg/ml; p = 0.0149].

Discussion

Elevated levels of FGF-2 have been implicated in the pathogenesis of several diseases characterized by a dysregulated angiogenic/inflammatory response, including cancer [26]. In contrast, our study reports that FGF-2 expression was significantly down-regulated in PE compared to normotensive pregnancies. The disparity in FGF-2 levels may be attributed to the lowered angiogenesis in PE. Since FGF-2 is a mitogen for endothelial cells, our results are congruent with the physiological absence of myometrial spiral artery remodelling that occur in PE. Moreover, vasopressors such as endothelin and angiotensin II correlate with an increase in serum FGF-2 content in hypertension [27]. Similarly, the work of Ozkan *et al*. (2008) reports a significant increase of FGF-2 immunostaining in chorionic villi and in amniochorionic membranes of PE. The latter author suggests that the low serum FGF-2 levels observed in severe compared to mild PE may be explained by the severity of endothelial damage [28]. A limitation of our study was the heterogeneity of the study population, early onset and late onset PE may reflect varying degrees of endothelial damage [29].

Notably, both FGF and VEGF are potent angiogenic factors [30]. Preeclampsia is associated with hypoxia and an oxidative stressed microenvironment [31]. Oxidative stress controls the steroid receptor coactivator 3 (Src-3) expression, influencing angiogenesis through the PI3K/Akt/mTor signaling pathways [32]. VEGF-induced angiogenesis requires integrin αvβ5 unlike, FGF-induced angiogenesis hinges on ligation of integrin αvβ3, rather than Src kinase activity [33]. Also, FGF-2 mediated angiogenesis is blocked by mitogen-activated protein kinase inhibitor, PD98059 [34].

Preeclampsia characterised by an ischemic placenta and hypoxia that induces the release of stored FGF-2 from damaged extracellular matrix. The enhanced FGF-2 promote proliferation and differentiation of endothelial cells and tissue repair [35]. Umbilical vein endothelial cell line (HUVE-12 cells) cultured under hypoxic conditions produced higher FGF-2 levels when compared to cells that are maintained under normal condition [35]. The increased FGF-2 is

associated with duration of hypoxia [36]. The FGF-2 down-regulation reported in our study may be attributed to the dysregulated endothelial loop feedback mechanism.

Furthermore, matrix metalloproteases (MMPs) mediate trophoblast cell invasion [37]. Matrix metalloproteinase 9 (MMP9) is an endopeptidase responsible for ECM degradation specifically [37]. Tissue inhibitor of metalloproteinase 1 (TIMP1) is an inhibitor of MMP9. The balance between MMP9 and TIMP1 influences cell permeation [38, 39]. This homeostasis correlates negatively with FGF-2 levels in HUVE-12 cells; suggesting that endothelial FGF-2 release may contribute to MM9/TIMP1 regulation [40]. In breast cells, FGF-2 promotes MMP9 secretion through the Ras/ERK signalling pathway [41]. Since MMP-9 mediates the discharge of FGF-2 [42], the FGF-2 decline in our study probably indicates the inhibition of ECM proteolysis with the subsequent inadequate trophoblast invasion of PE.

Our study reports a down-regulation of FGF-2 in HIV infected women compared to uninfected women, irrespective of pregnancy type. These result are corroborated by [43] who demonstrated a high serum FGF-2 in untreated HIV infected patients compared to HIV infected patients on HAART.

The HIV-1 *Tat* protein is a potent angiogenic factor since the *Tat* arginine- and lysine-rich sequence is similar to that of VEGF-A [44]. During HIV infection, VEGF, FGF-2 and *Tat* work in synergy to modulate endothelial cells phenotype [25]. Fibroblast growth factor-2 was shown to synergize with HIV-1 *Tat* protein and promotes Bcl2 that prevents endothelial cell apoptosis via p53 inhibition [23]. HIV-1 *via* gp120 to the negative charge of HSPG on endothelial cells amplifying viral infectivity and facilitating the release of *Tat* [45]. The *Tat* protein competes with FGF-2 for binding to HSPG on cell surfaces and within the ECM [46]. The combined *Tat* FGF-2 is likely dependant on FGF-2 capability of inducing the expression of the $\alpha_v\beta_{3\text{ and }}\alpha_5\beta_1$ integrins which, in turn, function as *Tat* receptors [46]. The *Tat* also binds and activates the Flk-1/kinase insert domain receptor (Flk-1/KDR), a VEGF-A tyrosine kinase receptor independent of FLT-1 [47]. It is therefore plausible that *Tat* interaction with HSPG/FGF-2 on endothelial cells contributes to the characteristic pathognomic lesion of endotheliosis that occurs in PE. In our study, the FGF-2 downregulation in HIV positive group could be due to antiretroviral administration that may have confounded the action of *Tat* [44].

Notably, antiretroviral therapy (ART) is implicated in FGF dysregulation and may account for the downregulation of FGF-2 observed in our study. Antiretroviral drugs affect FGF21–obligatory co-receptor β-Klotho expression in an *in vitro* model using human cells [48]. Intracellular ER stress/oxidative stress triggered by ART causes induction of FGF. In addition, several studies

have reported dysregulation in FGF21 [49-51]. In addition, HAART treatment increases TNF-α which has anti-apoptotic and anti-viral replication effects.

Gestational age correlates with the severity of PE [52]. In our study, the gestational age varied amongst study groups. The PE group irrespective of HIV status delivered at an earlier gestational period compared to the normotensive group. PE is also associated with intrauterine growth restriction [53].

Limitations

Limitations of this study include a small sample size as well as non-stratification of PE into early and late sub-type. PE is a heterogeneous disease, where EOPE is associated with defective trophoblast invasion [54]. Another limitation of this study is that all HIV infected individuals were on dual anti-retroviral (HAART and nevirapine) management.

Conclusion

In conclusion, this study demonstrates a significant down-regulation of FGF-2 in preeclamptic compared to normotensive pregnancies. These results echo a hypoxic oxidative stressed microenvironment that reflects deregulated angiogenesis with a dysfunctional endothelial loop that affects the FGF secretory mechanism leading to the deficient trophoblast invasion in PE.

In contrast, we report a significant downregulation of FGF-2 in HIV infection where VEGF, FGF-2 and *Tat* work in synergy to modulate endothelial cell phenotype and angiogenesis. The expression of FGF-2, has potential as a predictive risk indicator value for the early detection in HIV associated PE.

However, the management of comorbidities (PE) resulting from the side effect of HAART on FGF in HIV infected pregnant women warrants investigation. Future studies will include a genetic appraisal of the variants FGF-1 rs34011 and FGF-2 rs2922979 in African women with PE.

Declaration of interest

The authors declare no conflict of interest.

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CHAPTER III

SYNTHESIS

Sub Saharan Africa has many challenges in maternal health management (Sebitloane *et al*., 2017). In South Africa, 13.1% of the total population is HIV positive, the 4th highest prevalence rate globally (Statistics South Africa, 2018, UNAIDS, 2016). Human Immunodeficiency Virus infection/AIDS and PE are the leading causes of maternal and perinatal morbidity and mortality in SA (Sebitloane *et al*., 2017). In SA, 40% of pregnant women are found to be HIV infected (Saving Mothers Report 2014-2016, 2017). The province of KZN is the global "HIV hotspot" where 40.8% of adults (aged 15 years and older) are living with HIV infection (Cuadros *et al*., 2018). This province also has a PE prevalence of 12% (Moodley *et al*., 2018).

As PE affects both the mother and foetus, predictive tests early in pregnancy would have a greater impact in reducing the morbidity and mortality (Magee *et al*., 2014). A number of angiogenic factors such as sFlt-1 and PlGF have been proposed in the prediction of PE (Vatten *et al*., 2007; Rana *et al*., 2013). The sFlt-1:PlGF ratio has been approved as a diagnostic aid for preeclampsia in conjunction with other clinical findings (Zeisler *et al*., 2016).

Basic fibroblast growth factor is a potent mitogen and angiogenic factor that contributes to cell growth, differentiation, and angiogenesis, hence would be implicated in PE aetiology (Ornitz and Itoh, 2015). *In vitro* studies report that FGF-2 induces endothelium-derived *de novo* synthesis of vasodilators such as endothelial nitric oxide (NO) (Hohlagschwandtner *et al*., 2002). Circulating FGF-2 levels are reported to be elevated in PE (Ozkan *et al*., 2008, Hohlagschwandtner *et al*., 2002). In view of the absence of data on PE comorbid with HIV infection, our study evaluates FGF-2 expression in HIV associated PE.

In our study, a downregulation of FGF-2 expression was noted in PE compared to normotensive pregnant women irrespective of their HIV status. FGF-2 is produced by endothelial cells and fibroblast (Pintucci *et al*., 2002). PE is a state of endotheliosis and has a heightened inflammatory milieu, hence FGF-2 is released albeit lower (Moscatelli *et al*., 1986). The circulating free FGF-2 acts in repairing endothelial cell damage and promoting angiogenesis (Tepper *et al*., 2004). FGF binds with HSPG present within the ECM where it triggers a signal that initiates tyrosine kinase phosphorylation and FGFR activation (Ornitz and Itoh, 2015). This results in a cascade of phosphorylation events via the RAS/MAPK pathway, PI3 kinase/AKT signalling pathways, and the PLCγ pathway that control migration (Murakami *et al*., 2007). Notably, the consequences of the inhibition of FGFR phosphorylation promotes aberrant protein activation that explains the abnormal invasion of the trophoblast population associated with the PE (Yang *et al*., 2011) and

explains the FGF downregulation expressed in our study. Nonetheless, in contrast to our findings, elevated levels of FGF-2 have been implicated in the pathogenesis of several diseases characterized with a deregulated angiogenic/inflammatory response such as cancer (Andrés *et al*., 2009). Similarly, FGF-2 expression was reported to be upregulated in PE compared to normotensive pregnancies (Hohlagschwandtner *et al*., 2002).

Nonetheless Ozkan *et al* (2008) suggested an upregulation of FGF-2 in mild PE concurrent with a downregulation of FGF-2 in severe PE due to the severity of endothelial damage. In severe PE, the endothelial loop is subject to major damage compared to mild PE, implicating less FGF-2 release, hence the inadequate trophoblast invasion that occurs in PE. A limitation of our study was the non-stratification of the study population by disease severity into mild or severe type or by gestational age into EOPE and LOPE.

PE is associated with a hypoxic microenvironment associated with high reactive oxidative stress (Verma *et al*., 2018). Both FGF and VEGF are powerful angiogenic factors (Cao *et al*., 2004). Hypoxia induces the release of stored FGF-2 from a damaged extracellular matrix (Cao *et al*., 2004). The enhanced FGF-2 promotes proliferation and differentiation of endothelial cells and tissue repair (Lindner *et al*., 1990). Oxidative stress regulates the steroid receptor coactivator 3 (Src-3) expression, manipulating angiogenesis via the PI3K/Akt signaling pathways (Reuter *et al*., 2010).

Since FGF is a mitogen for endothelial cells, our results are congruent with the physiological absence of myometrial spiral artery remodelling that occurs in PE. Moreover, vasopressors such as endothelin and angiotensin II correlate with low serum levels of FGF content in hypertension (Hohlagschwandtner *et al*., 2002).

Endothelial FGF-2 release may contribute to MM9/TIMP1 regulation (Luo *et al*., 2011). FGF-2 promotes MMP9 secretion through the Ras/ERK signaling pathway (Luo *et al*., 2011). The FGF-2 decline in our study probably indicates the inhibition of ECM proteolysis with the subsequent inadequate trophoblast invasion of PE.

According to HIV status, we report a downregulation of FGF-2 in HIV positive compared to HIV negative pregnant women irrespective of pregnancy type. Our results were found to be in contrast to those of (Barillari *et al*., 1999), who reported a high expression of FGF-2 in HIV positive infection. HIV *Tat* protein is a transactivator of viral gene expression and is released extracellularly in HIV acute infection (Romani *et al*., 2010). Biologically, this HIV regulatory protein *Tat* acts in synergy with FGF-2. Both FGF-2 and VEGF, are potent angiogenic factors

that are highly expressed in AIDS, where they synergize in promoting neoangiogenesis and oedema (Zhou *et al*., 2013).

Since *Tat* has a similar arginine and lysine-rich sequence to VEGF it is a powerful angiogenic factor (Zhou *et al*., 2013). *Tat* induces endothelial cells to migrate, adhere and grow as a capillary-like network *in vitro* (Barillari *et al*., 1999). HIV *Tat* has also been shown to bind F1K-1/KDR, one of the receptors for the VEGF, suggesting an additional mechanism for *Tat* to exert its angiogenic effect (Albini *et al*., 1996). Moreover, *Tat* exerts activities that are linked to diseases of vascular origin such as in Kaposi sarcoma, hence would also correlate with PE (Zhou *et al*., 2013).

During HIV infection, VEGF, FGF-2, and *Tat* work in synergy to modulate endothelial cells phenotype (Das *et al*., 2016). FGF-2 works in concert with *Tat* and promotes Bcl2 that impedes endothelial cell apoptosis via p53 blockade (Sgadari *et al*., 2011). The inoculation of *Tat* alone in nude mice has little or no effect, however, when injected with a small amount of FGF-2, it promotes the development of angio-proliferative lesions in the inoculated animal (Sgadari *et al*., 2011). The *Tat* protein contests with FGF-2 for binding to HSPG on the plasmalemma and within the ECM (Alghisi and Rüegg, 2006).

Tat can promote endothelial cell adhesion through the binding of its arginine-glycine-aspartic acid (RDG) region to the $\alpha_v\beta_3$ and $\alpha_5\beta_1$integrins and the VEGFR-2/KDR via the basic domain of *Tat* (Zhou *et al*., 2013). The combined *Tat*/FGF-2 effect is due to FGF-2 inducing the expression of the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, which aids *Tat* binding (Alghisi and Rüegg, 2006). HIV-1 *via* gp120 binds to HSPG on endothelial cells amplifying viral infectivity thereby expediting the release of *Tat* (Crublet *et al*., 2008).

Tat causes aberrant cell signaling and leads to altered endothelial cell morphology, gene expression, and survival. HIV-1 Tat protein stimulates the MAPK pathway in primary T cells which is connected with the movement from G0 to G1 phase in naïve T cells enabling productive HIV infection (Li *et al.*, 1997). HIV-1 also utilizes the *Tat* protein to hijack intracellular functions and evades the immune response of the host, and thus may contribute to the high inflammatory response in HIV associated PE (Abbas and Herbein, 2013)

The FGF-2 downregulation in the HIV positive group observed in our study could also possibly be due to HAART. In our study, all HIV positive women received HAART and nevirapine, as part of the standard of HIV treatment in SA. HAART acts by suppressing HIV-1 viral load by restoring natural killer cells NK ability to secrete chemokines. In pregnancy, this NK activation has negative effects as it may inhibit trophoblast invasion which is associated with PE (Phoswa

et al., 2018). Clinical and *in vitro* studies suggest that the long-term use of HAART results in endothelial toxicity and vascular dysfunction (Francisci *et al*., 2009).

Conclusion

This study demonstrates a downregulation of FGF-2 in PE compared to normotensive pregnant women irrespective of HIV status reflecting endothelial damage congruent with FGF secretion. The downregulation of FGF-2 in HIV positive compared to HIV negative irrespective of pregnancy type may be attributed to the non-phosphorylation of HIV regulatory protein tat or to the MAPK pathway inhibition. Notably, the use of HAART results in endothelial toxicity and vascular dysfunction which affect FGF expression. Future studies will include a genetic appraisal of the variants FGF-1 rs34011 and FGF-2 rs2922979 in African women with PE.

CHAPTER IV

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CHAPTER V

APPENDICES

Appendix 1

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17 April 2018

Prof T Naicker
Discipline of Optics and Imaging
School of Laboratory Medicine and Medical Sciences
naickera@ukzn.ac.za

Dear Prof Naicker

Title of Project: Exploring the pathogenesis HIV associate pre-eclampsia syndrome in a homogenous South African population group.
BREC Ref No.: BCA338/17

We wish to advise you that your response received on 03 April 2018 to BREC letter dated 16 March 2018 has been **noted** by a sub-committee of the Biomedical Research Ethics Committee.

Your request received on 07 March 2018 to append the studies below to the above study has now been **approved** by a sub-committee of the Biomedical Research Ethics Committee

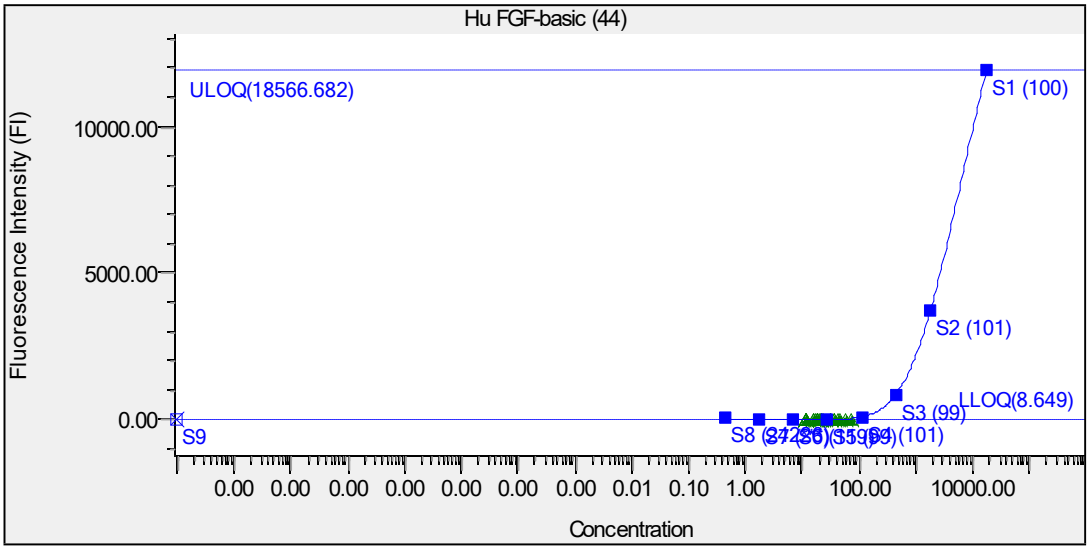
Name	Student number	Title
Deneshree Varaden	211510564	Morphometric image analysis of placental clec2d and HLA-G immunolocalization in HIV-associated pre-eclampsia
Merantha Moodley	214514757	The role of histone 2A in NETosis of HIV-associated pre-eclampsia
Kyle Kupsamy	214504430	EGF and HGF in HIV-associated pre-eclampsia
Mikaila Moodley	214558958	Immunoglobulin isotypes (IgG1, IgG2, IgG3, IgG4, IgM, IgA) in HIV-associated pre-eclampsia
Charlene Mukasa Sangany	211559876	The role of acidic and basic fibroblast growth factor in HIV-associated pre-eclampsia
Mduduzi Mazibuko	214504614	The role of sTIE2 and sHER2 in HIV-associated pre-eclampsia
Siphesihle Mdlalose	217011521	The role of follistatin and G-CSF in HIV-associated pre-eclampsia

This approval will be **ratified** at the next BREC meeting to be held on **08 May 2018**.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix 2



■ **Standard** □ **Partial Outlier** ☒ **Outlier**

▲ **Unknown** ▲ **Control**