THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-3 IN THE PLACENTA IN HIV ASSOCIATED PREECLAMPSIA

By

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in the

Discipline of Optics and Imaging

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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, Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban, South Africa and under the supervision of Professor Thajasvarie Naicker.

_____	_____
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DECLARATION

I, **Miss Saieshni Pillay,** 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.

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DEDICATION

I humbly dedicate this dissertation first and foremost to my Dearest Bhagawan Baba, my Master, my Guru and my Guide. Thank you, my Lord, for your divine strength, love, care and encouragement throughout this beautiful journey.

To my amazing, wise and unconditionally supportive parents, I lovingly dedicate this Master's dissertation to you. I am most appreciative for your presence, guidance, understanding and invaluable advice throughout this journey. Thank you for being my strength in the toughest battles. I have grown, learnt and overcome many obstacles, with your blessings, thank you.

ACKNOWLEDGEMENTS

Professor T. Naicker, thank you for taking me under your guidance for this project and your knowledgeable input that has greatly assisted me throughout this year. I am most grateful to have had the opportunity of being under your supervision.

The National Research Fund, thank you for giving me the opportunity of being a recipient of the DST-NRF Innovation Master's Scholarship for the current year (2019).

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PUBLICATIONS

Pillay, S. and Naicker, T. (2019). Morphometric image analysis of vascular endothelial growth factor receptor-3 in preeclamptic, HIV infected women. **Submitted to** *European Journal of Obstetrics and Gynecology and Reproductive Biology*, Manuscript ID: EJOGRB-19-20890

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LIST OF ABBREVIATIONS

Antiretroviral therapy	ART
Antiretrovirals	ARVs
Early onset preeclampsia	EOPE
Extracellular signal-regulated protein kinases 1 and 2	ERK1/2
Highly active antiretroviral therapy	HAART
Human immunodeficiency virus	HIV
Hypertensive disorders of pregnancy	HDP
Hypoxia inducible factor-2α	HIF-2 α
Intrauterine growth restriction	IUGR
Late onset preeclampsia	LOPE
Nucleoside reverse transcriptase inhibitors	NRTIs
Phosphatidylinositol-3-kinase/Protein kinase B	PI3K/Akt
Placental growth factor	PlGF
Preeclampsia	PE
Reactive oxygen species	ROS
Receptor tyrosine kinase	RTK
Soluble endoglin	sEng
Soluble fms-like tyrosine kinase	sFlt1
Syncytiotrophoblast	STB
Trans-activator of transcription	Tat
Vascular endothelial growth factor	VEGF
Vascular endothelial growth factor receptor-3	VEGFR-3

ABSTRACT - ENGLISH

Summary: PE and HIV dualism in pregnant HAART women are yet to be investigated. Furthermore, the effect on VEGFR-3 and subsequent downstream influence on angiogenesis is poorly understood. Therefore, this investigation evaluates the immuno-expression of VEGFR-3 in placental conducting and exchange villi from normotensive, preeclamptic and HIV+ African women, using morphometric image analysis.

Study design: This is a prospective study utilizing retrospectively collected, paraffin wax-embedded, placental samples (n=90) that were immuno-stained for VEGFR-3. The study population consisted of normotensive (n=30) and pre-eclamptic (n=60) groups which were further stratified on the basis of HIV status (negative - and positive +), and early and late onset preeclampsia (EOPE and LOPE respectively). The final groups were as follows; N- (n=15), N+ (n=15), EOPE- (n=15), EOPE+ (n=15), LOPE- (n=15) and LOPE+ (n=15). Brightfield microscopy and morphometric image analysis of VEGFR-3 immuno-expression within conducting and exchange placental villi was performed.

Results: HIV status analysis did not demonstrate a significant difference in VEGFR-3 immunostaining for both villous types. The N *vs*. PE comparative analysis showed a downregulated immuno-expression of VEGFR-3 in both conducting ($p = 0.0107$) and exchange ($p < 0.0001$) villi. Results from analysis of pregnancy subtypes showed a significant difference in VEGFR-3 expression between N *vs*. EOPE regardless of villous type.

Conclusion: This study demonstrates that HIV infection does not significantly alter VEGFR-3 placental immuno-expression in pregnant women receiving HAART irrespective of pregnancy type. Furthermore, VEGFR-3 immuno-expression was dysregulated in EOPE women, irrespective of HIV status. These novel findings emphasize severe down-regulation of VEGFR-3 in preeclamptic women, and provide compelling evidence for a future investigation into placental angiogenic and lymphangiogenic regulation in the early onset of PE.

ABSTRACT – ISIZULU

Isifinyezo: Inhlanganyela yePE neHIV kwabesifazane abakhulelwe abemukela iHAART kusamele kuphenywe. Ngakho-ke, lolu phenyo luhlola ukubonakaliswa kwe-VEGFR-3 kwiplacental ekuqhubeni nasekushintshaniseni i-villi kusuka ku-normotensive, preeclamptic ne-HIV+ kwabesifazane abansundu, kusetshenziswa ukuhlaziywa kwezithombe.

Umklamo wokutadisha: Lokhu kucwaninga okungenzeka kusetshenziswa amasampula eplacental aqoqiwe, afakwe kwi-paraffin wax (n=90) abegcinelwe i-immuno-stain for VEGFR-3. Inani labantu abacwaningwayo liqukethe amaqembu: i-Normotensive (n=30) kanye ne-pre-eclamptic (n = 60) abephinde ahlukaniswa ngesisekelo sesimo se-HIV (abangenayo i-HIV Kanye nabanayo i-HIV+), kanye nabaqalwa yi-preeclampsia nalabo abakhombisa i-preeclamsia emva kwesikhathi eside (i-EOPE ne-LOPE) ngokulandelana.). Amaqembu esewonke ngokulandelayo; N- (n=15), N+ (n=15), EOPE- (n=15), EOPE+ (n=15), LOPE- (n=15) no-LOPE+ (n=15). IBrightfield microscopy nokuhlaziywa kwezithombe imorphometric yeVEGFR-3 nokuziveza kwayo ngokuqhuba nokushintshaniswa kweplacental villie kwenziwa.

Imiphumela: Ukuhlaziywa kwesimo seHIV akuvezanga umehluko omkhulu kwiVEGFR-3 immuno-staining kuzo zombili izinhlobo zeVillous. Ukuhlaziywa kokuqhathaniswa kwe-N vs. PE kukhombise ukubonakaliswa okuphansi kwe-immuno-expression kwe-VEGFR-3 kokubili kuqhuba ($p = 0.0107$) kanye nokushintshaniswa ($p < 0.0001$) villi. Imiphumela yokuhlaziywa kwezinhlobo ezahlukene zokukhulelwa zikhombise omkhulu umehluko ekubonisweni kweVEGFR-3 phakathi kwe N iqhathaniswa neEOPE kungakhathaleleki uhlobo lwevillous.

Isiphetho: Lomkamo ukhombisa ukuthi ukutheleleka nge HIV akunawo umthelela omkhulu ekuvimbeni iVEGFR-3 immuno-expression kwabesifazane abakhulelwe abemukela iHAART kungakhathalaleki uhlobo lokukhulelwa. Ngaphezu kwalokho, iVEGFR-3 immuno-expression kwabesifazane abaneEOPE ayisebenzanga, kungakhathaleki isimo sabo seHIV. Okutholakale emibhalweni lugcozelela ngokukhulu ukwehla kwezinga kweVEGFR-3 kwabesifazane aba-preeclamptic, futhi kunikeza ubufakazi obuqanda ikhanda obungasetshenziswa kucwaningo oluzayo kweplacental angiogenic nelymphangiogenic regulation kulabo abaqedwa yiPE.

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

Spiritual scriptures emphasize the importance of women and how they should be revered; however, women are no longer treated with the highest respect and honour, and have become plagued with sorrow and disease (Baba, 2005). It is one of many duties of scientists and spiritualists, to unite and piece together a puzzle of varying knowledge for the healing and upliftment of the mothers of this land (Baba, 2005).

1.1 Maternal mortality in South Africa

A staggering 7.97 million South Africans are infected with the human immunodeficiency virus (StatsSA, 2019). Females in their child-bearing years (15-49 years) comprise an estimated 22.47% (StatsSA, 2019). Despite major advances in antiretroviral therapy (ART), a vertical transmission of <10% still exists (UNAIDS, 2018).

Pregnancy is also complicated by hypertensive disorders of pregnancy (HDP). Low and middle income countries, *e.g.* South Africa, have experienced an alarming 14% increase in maternal mortality between 2011-2016, due to HDPs (NCCEMD, 2018; Nathan *et al.*, 2018). The most pronounced contributor is preeclampsia (PE), which accounts for more than 83% of deaths reported (NCCEMD, 2018; Moodley *et al.*, 2019).

1.2 The Human Immunodeficiency Virus (HIV) in pregnancy

In vertical transmission of HIV, the virus infected cells cross the uteroplacental mucosa and permeate the trophoblast epithelia (Figure 1.1) where they may remain undetected by surrounding maternal immune defense cells (Milligan *et al.*, 2018; Ander *et al.*, 2019). The high mutation rate of HIV causes CD4+ and CD8+ T cell and B cell exhaustion (Bussmann *et al.*, 2010; Moir and Fauci, 2009). T cell availability and functioning is also dysregulated during HIV infection (Shive *et al.*, 2016; Saito *et al.*, 2018), leading to the suggestion that this anomaly decreases maternal tolerance of the fetus (Altfeld and Bunders, 2016). Furthermore nullification of natural killer cell function, impedes their ability to contribute towards optimal immune defense and placental artery formation (Wang, 2010). Nonetheless, the physiological maternal-fetal equilibrium is altered (Wang, 2010).

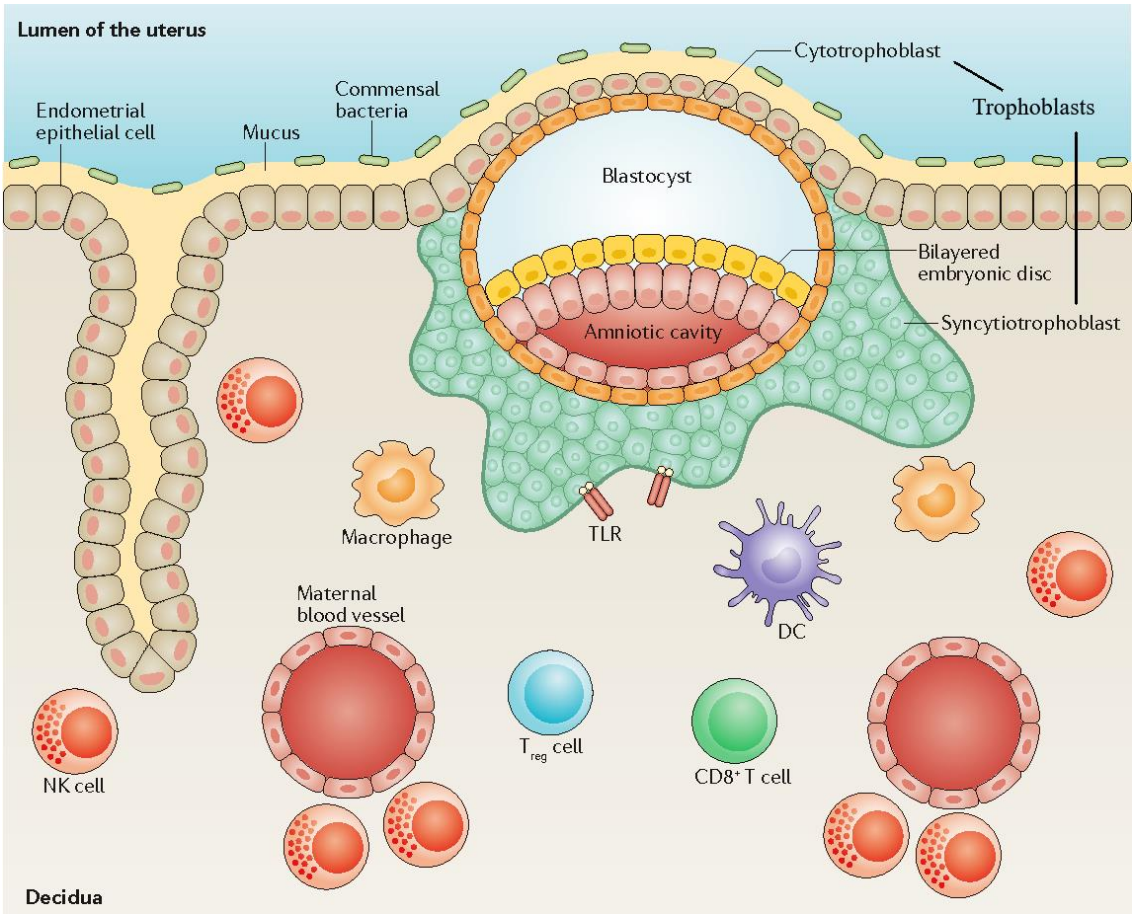

Figure 1.1: Depiction of the site of implantation which also forms the maternal-fetal interface. Immediately surrounding the blastocyst are the trophoblastic layers of cells which are differentiated into a cytotrophoblast and syncytiotrophoblast layers. Beyond the syncytiotrophoblast, in the decidua, are maternal immune defensive cells. Adapted from (Mor *et al.*, 2017).

South Africa is recognized as having the highest rollout of antiretroviral (ARV) therapy in the world (Naicker *et al.*, 2019). The usage of ARVs reduce the rate of vertical transmission and prevent progression of HIV infection to acquired immunodeficiency syndrome (Roberts and Escudero, 2012).

1.3 Preeclampsia

1.3.1 Clinical characteristics

PE is a medical disorder developing at/after 20 weeks gestation (Brown *et al.*, 2018), and is caused by the presence of the placenta (Roberts and Escudero, 2012). PE women present with a new onset of hypertension (≥ 140/90 mmHg) and may have associated proteinuria (Kalumba *et al.*, 2013; Brown *et al.*, 2018). However, not all women present with these clinical manifestations (Douglas and Redman, 1994; Knight, 2007; Morikawa *et al.*, 2012; Ohno, 2018).

PE may be sub-classified as an early onset (EOPE; < 34weeks gestation) or late onset (LOPE > 34 weeks gestation, near term) sub-types (James *et al.*, 2010; Staff and Redman, 2018). Presently, emergency delivery and subsequent expulsion of the placenta is the primary method of restoring maternal and neonatal health (Jeyabalan, 2013; Rana *et al.*, 2019).

1.3.2 Etiopathogenesis

1.3.2.1 The placenta: Anatomical location and function

The placenta is a highly vascular organ that is anatomically located (Figure 1.2) to the anterior or posterior wall of the uterus (Fidan *et al.*, 2017). Centrally arising from the placenta and connecting to the fetus is the umbilical cord. Physiologically, the placenta functions in exchange of gases, waste excretion, hormone secretion, maintenance of homeostasis, *etc.*, between the maternal and fetal circulation (Young *et al.*, 2014).

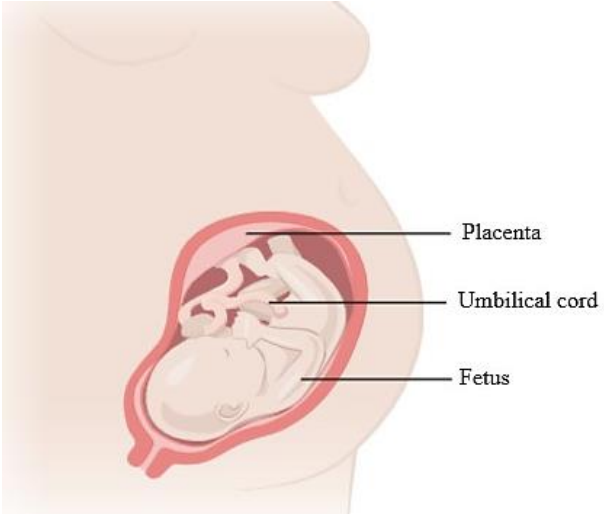

Figure 1.2: Simple illustration of the placenta, fetus and umbilical cord *in utero.* The placenta is seen as a disk or pan-shaped organ located to the posterior uterine wall. Adapted from Biorender.com.

1.3.2.2 Synopsis of the genesis of the placenta

At the time of implantation, trophoblast cells immediately surrounding the blastocyst begin to differentiate and thereafter mediate blastocyst implantation into the decidua of the endometrium (Kliman, 1999). Trophoblasts proliferate to form an inner cytotrophoblast and outer syncytiotrophoblast (STB) (Baergen, 2011) layer of cells. Trophoblast cells actively invade the decidua (James *et al.*, 2010), penetrate the endometrial spiral arteries (Kliman, 1999) and remodel both the decidual and myometrial arteries in a set time sequence (Naicker *et al.*, 2019).

Fetal circulation is established three weeks after fertilization and comprises chorionic villi (Figure 1.3). Conducting villi (stem) branch into exchange villi *viz*., intermediate (mature and immature) and terminal villi (Benirschke and Kaufmann, 1942; Slator *et al.*, 2018). These finely branched structures are important for exchange of gases, nutrients and waste between the maternal and fetal circulations (Kliman, 1999). At four weeks gestation, a mature placenta is established (Kliman, 1999).

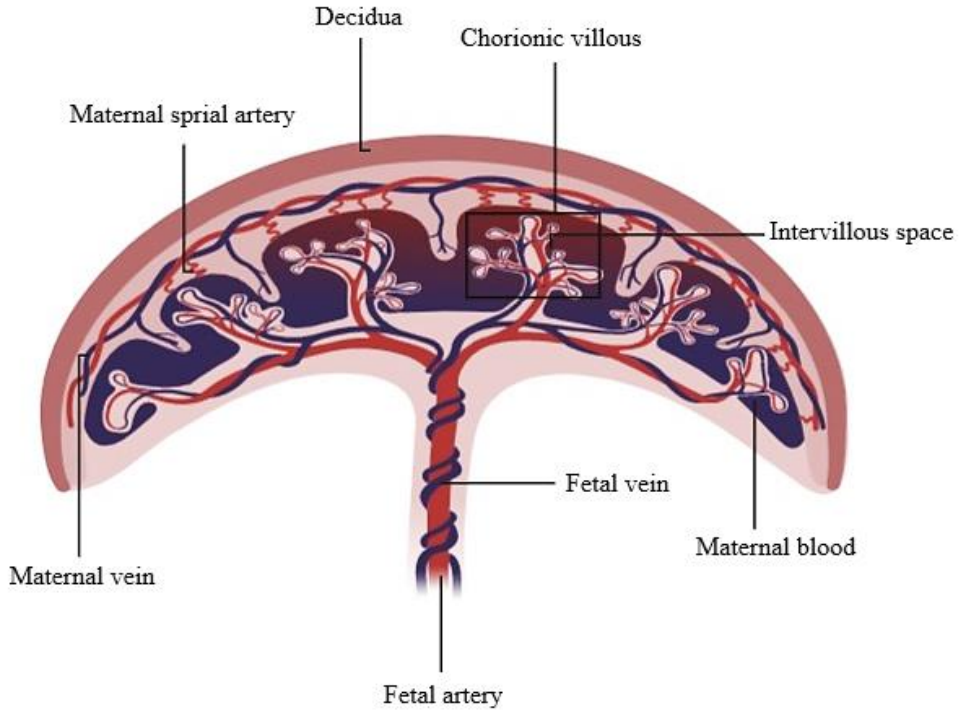

Figure 1.3: Simple, labelled illustration of placental anatomy. Adapted from Biorender.com.

1.3.2.3 Two stage theory of PE

The etiopathogenesis of PE is not completely understood, but it has been well established that it begins in the placenta and consists of two stages (Figure 1.4). Vasculogenesis is the *de novo* synthesis of the fetal vascular system, whilst angiogenesis is pertinent for (i) amplification of the primordial embryonic vascular network as embryogenesis progresses, and in adults (Patan, 2004; Li, 2014), as well as (ii) remodeling of uterine spiral arteries into high capacitance and low resistance vessels in pregnancy (Cerdeira and Karumanchi, 2012).

The development of EOPE begins with the pathological upregulation of anti-angiogenic factors, *e.g.* soluble fms-like tyrosine kinase (sFlt1) and soluble endoglin (sEng), with simultaneous inhibition of pro-angiogenic factors *e.g.* vascular endothelial growth factors (VEGFs) and placental growth factor (PlGF), thereby promoting an angiogenic imbalance (Burke and Karumanchi, 2018). The angiogenic imbalance observed in EOPE results in defective uterine spiral artery remodeling, subsequently limiting expansion of the embryonic vasculature and thereby giving rise to abnormal placentation (Huppertz, 2018; Staff and Redman, 2018). Additionally there is disruption of optimal uteroplacental blood flow and perfusion (Patan, 2004).

In healthy pregnancies, a delicate balance of reactive oxygen species (ROS) production and oxidative stress is maintained to meet the requirements of energy-demanding cells within the placenta and the growing fetus (Wang, 2010). However, the abnormal placentation observed in EOPE and the effects of maternal factors (*e.g.* obesity and smoking) in the development of LOPE, adversely affect the balance of ROS production and oxidative stress (Wang and Walsh, 1998). This ultimately results in the first stage of preeclampsia which is characterized by STB stress (Staff and Redman, 2018). STB stress stimulates endothelial cell activation, thereby prompting the release of pro-inflammatory molecules into the maternal circulation (Staff and Redman, 2018). Resultantly, systemic oxidative stress causes widespread inflammation and endothelial dysfunction, thereby leading to the second stage of the maternal syndrome of PE, which is inclusive of the onset of hypertension and multiple organ failure (Pereira *et al.*, 2015; Rana *et al.*, 2019). Furthermore, ischemia (Huppertz, 2018), poor fetal vasculature and intra-uterine growth restriction occur (Egbor *et al.*, 2006).

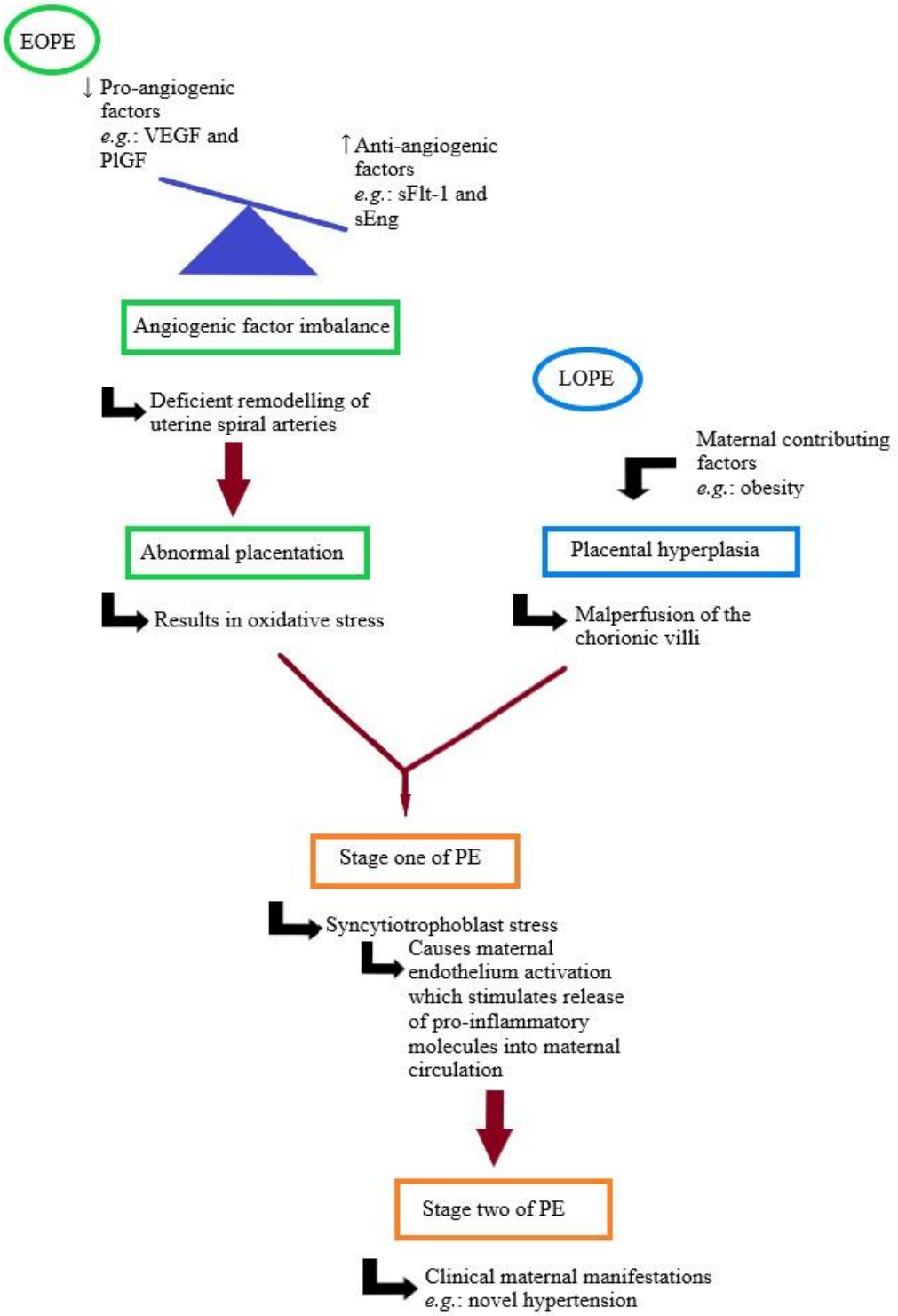

Figure 1.4. Supplementary illustration of the two stage model of PE development. Abbreviations included, (i) early onset of preeclampsia (EOPE); (ii) late onset of preeclampsia (LOPE); (iii) vascular endothelial growth factors (VEGF); (iv) placental growth factor (PlGF); (v) soluble fms-like tyrosine kinase (sFlt1) and (vi) soluble endoglin (sEng).

1.4 Vascular endothelial growth factor (VEGF) family

1.4.1 Molecular structure and function

Vascular endothelial growth factors (Figure 1.5) are 40 kDa dimeric glycoproteins (Olsson *et al.*, 2006) which have a fundamental role in angiogenesis and lymphangiogenesis (Chen and Zheng, 2014). This family comprises (i) 5 ligands, VEGF-A to VEGF-D and placental growth factor (PlGF) (Olofsson *et al.*, 1996; Wang, 2010); (ii) 3 tyrosine kinase receptors, *viz*., VEGFR-1 - VEGFR-3; (iii) and 2 non protein kinase co-receptors; (a) neuropilin-1/2 and (b) heparin sulphate proteoglycans (Wang, 2010). VEGFs are located on both endothelial and non-endothelial cells (Koch *et al.*, 2011).

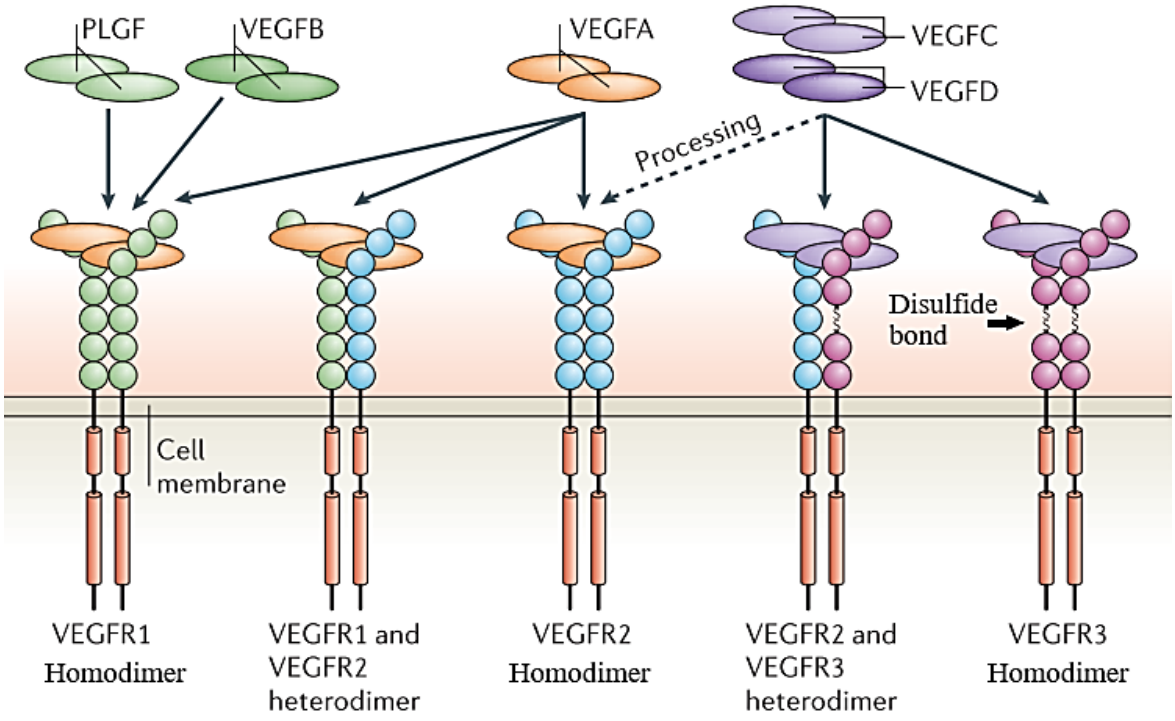

Figure 1.5. Schematic of vascular endothelial growth factor ligands and respective receptor counterparts. Adapted from (Olsson *et al.*, 2006).

1.4.1 VEGFR-3

1.4.2.1 Signaling

VEGFR-3 is a receptor tyrosine kinase (RTK) with an extracellular component for binding of VEGF-C and VEGF-D (Figure 1.5). It comprises 6 immunoglobulin loops with the 5th loop cleaved by a disulphide bond (Olsson *et al.*, 2006). VEGF-C/VEGFR-3 signaling exclusively facilitates angiogenesis and lymphangiogenesis during embryonic development and in adults (Maynard and Karumanchi, 2011). This is achieved in the downstream activation and functioning of the phosphatidyl inositol 3' kinase/protein kinase B (PI3K/Akt) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) pathways (Mäkinen *et al.*, 2001). VEGFR-3 is pertinent for endothelial cell proliferation, migration and remodeling, as well as expansion of existing vasculature in angiogenesis (Leach *et al.*, 2002). Stimulation by hypoxia-inducing factors (HIFs) (Hong *et al.*, 2004) or an increase in ROS, promotes binding of VEGF-C to VEGFR-3, causing VEGFR-3 activation by phosphorylation (Randi *et al.*, 2009). In contrast, VEGFR-3 is down-regulated by de-phosphorylation, induced by phospho-tyrosine, a potent modulator of VEGFR-3 signaling (Kappert *et al.*, 2005). However, regulatory anomalies may have lethal consequences. VEGFR-3 gene polymorphisms in animal models cause intrauterine death (Dumont *et al.*, 1998). Furthermore, pathophysiological concentrations of ROS leads to endothelial cell damage which sequentially reduces VEGFR-3 bio-availability for angiogenesis and lymphangiogenesis (Pereira *et al.*, 2015).

1.4.2.2 VEGFR-3 and HIV infection

The HIV trans-activator of transcription (Tat) protein is an angiogenic protagonist (Zhang *et al.*, 2012; Shisadeh *et al.*, 2019), thereby contributing to heightened maternal systemic inflammation and endothelial dysfunction (Abbas and Herbein, 2013). In contrast, HAART ameliorates HIV-induced inflammation and endothelial dysfunction (Naicker *et al.*, 2019).

However, it has been suggested that HAART may increase oxidative stress (Filardi *et al.*, 2008), leading to further endothelial dysfunction, impairment of RTKs (Fiala *et al.*, 2004; Divi *et al.*, 2010) and exacerbate the pathophysiology of PE (Naicker *et al.*, 2019). This is attributed to HAART association with premature deliveries and end organ disease (*e.g.*: metabolic and cardiovascular diseases), both of which are characteristic of PE pregnancies (Townsend *et al.*, 2010). Therefore it may be concluded that the combined effects of PE, HIV infection and HAART

may exacerbate VEGFR-3 signal dysfunction and subsequently downregulate angiogenesis during pregnancy.

1.5 Aim

To assess and compare the immuno-expression of VEGFR-3 between conducting (stem) and exchange (terminal and intermediate) placental chorionic villi from normotensive, preeclamptic and HIV infected women.

1.6 Objectives

- To determine the effect of pregnancy type (normotensive *vs.* preeclampsia) on the immuno-expression of VEGFR-3 regardless of HIV status (HIV- *vs.* HIV+) in the placenta using immunocytochemistry with allied morphometric analysis.
- To determine the effect of HIV status (HIV- ***vs.*** HIV+) on the immuno-expression of VEGFR-3 regardless of pregnancy type (normotensive *vs.* preeclamptic) in the placenta with allied morphometric analysis.
- To compare and contrast the immuno-expression of VEGFR-3 in the across all study groups with the use of immunohistochemistry and allied morphometric analysis.
- To compare and contrast the immuno-expression of VEGFR-3 between conducting and exchange villi across all study groups.
- To correlate VEGFR-3 immuno-expression with maternal and infant demographic and clinical data.

CHAPTER TWO

MANUSCRIPT

Original research article entitled: "Morphometric image analysis of vascular endothelial growth factor receptor-3 in preeclamptic, HIV infected women"

Chapter two is presented as an original research article which has been submitted to a DoHET accredited international journal - *European Journal of Obstetrics and Gynecology and Reproductive Biology*.

The first author is the author of this Master's Dissertation (Ms. Saieshni Pillay), contributing to the literature and protocol review, experimental procedures and interpretation of the results.

This research article investigates the immuno-expression of VEGFR-3 in placental villi from normotensive, preeclamptic and HIV infected women of African ancestry.

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Abstract: Objective: To ascertain the expression of vascular endothelial growth factor receptor-3 (VEGFR-3) in placental conducting and exchange villi from normotensive, preeclamptic (PE) and antiretroviral treated pregnant women, using morphometric image analysis.
Study design: This study utilizes retrospectively collected, paraffin
wax-embedded, placental samples (n=90) that were immuno-stained for VEGFR-3. The study population consisted of normotensive (n=30) and preeclamptic n=60) groups which were further divided on the basis of HIV status (negative - and positive +), and early and late onset preeclampsia (EOPE and LOPE respectively). The resulting groups were as follows; N-(n=15), N+ (n=15), EOPE- (n=15), EOPE+ (n=15), LOPE- (n=15) and LOPE+ (n=15). Microscopic examination and morphometric image analysis were performed on the immuno-stained placental tissue samples.
Results: Analysis on HIV status did yield a significant difference in conducting ($p = 0.3015$) or exchange ($p = 0.4535$) villi, regardless of pregnancy type. The N vs. PE analysis showed a reduced immuno-expression of VEGFR-3 in both conducting ($p = 0.0107$) and exchange ($p < 0.0001$) villi. Results from a multiple group comparative analysis of N vs. EOPE vs. LOPE, showed a significant difference in the N vs. EOPE analysis regardless of villous type.
Conclusion: The results presented provide compelling evidence that HIV

infection does not significantly alter angiogenesis in placental villi. PE however, has caused angiogenic dysregulation and trophoblast pathology was observed. We report a severe downregulation of VEGFR-3 in placental villi from EOPE woman, regardless of HIV status. Hence we suggest a future investigation into EOPE's aetiology and its downstream effects on pregnancy.

Suggested Reviewers: Nalini Govender
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Basic medical scientist who has expertise in the field of obstetrics and gynecology and has a vast knowledge of preeclampsia.

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Basic medical scientist in the field of preeclampsia.

Opposed Reviewers:

Morphometric image analysis of vascular endothelial growth factor receptor-3 in preeclamptic, HIV infected women

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INTRODUCTION

Globally, 5-7% of pregnant women in their second or third trimester develop the elusive medical disorder known as preeclampsia (PE) [1]. Novel hypertension (≥140/90mmHg) is mandatory for diagnosis and it may or may not be accompanied with proteinuria [2]. Some women however, experience a normal and healthy pregnancy, but succumb to PE or eclampsia, prior to or during labour, respectively [3]. PE may be classified by gestational age into early onset (EOPE < 34 weeks) or late onset of PE (LOPE ≥ 34 weeks) [4].

The placenta, a highly vascular organ, which constitutes the essence of sustaining and developing life *in utero*, is the causative organ in this multi-faceted disorder [5]. EOPE is a direct result of deficient maternal spiral artery remodeling and LOPE is posited to arise from abnormal placental growth and size, thereby not conforming to uterine capacity [6]. The lack of myometrial spiral artery transformation from low to high capacitance vessels, creates a hypoxic micro-environment [7]. This stimulates the release of pro-inflammatory and anti-angiogenic factors into the maternal blood circulation [6], which initiates the maternal syndrome [8, 9] and eventuates in intra-uterine growth restriction [10].

Anti-angiogenic factors (soluble endoglin and soluble fms-like tyrosine kinase) inhibit pro-angiogenic factors (vascular endothelial growth factors / VEGFs) [11, 12]. VEGFs bind to tyrosine kinase receptors (VEGFR1-3) and non-kinase co-receptors, neuropilin 1 and 2, as well as heparin sulphate proteoglycans [13]. VEGFR-3 has an indispensable regulatory role in angiogenesis and lymphangiogenesis [14]. VEGF-C and VEGFR-3 are cardinal proteins for crucial vascular development during early embryonic growth and in adults [15]. The establishment and development of the placental vascular and lymphatic network is crucial for both the mother and fetus [16].

The human immunodeficiency virus (HIV) induces inflammation, thereby altering the existing maternal-fetal equilibrium [17]. Both HIV infection and PE account for the high maternal, fetal and neonatal morbidity and mortality in South Africa [18]. The HIV accessory protein, trans-activator of transcription (Tat), is a strong pro-angiogenic factor [19]. In contrast, treatment for HIV infection in South Africa includes highly active anti-retroviral therapy (HAART), which is anti-angiogenic [20].

There is a paucity of information on VEGFR-3 expression and function in angiogenesis in the duality of preeclamptic and HIV infection. Also, in light of the high prevalence and pervasive nature of PE and HIV infection in South Africa, this research project evaluates the immuno-expression of VEGFR-3 in placental tissue from normotensive, preeclamptic and HIV infected

women. This was achieved by comparing the immuno-expression of VEGFR-3 in conducting (stem) and exchange (terminal and intermediate) chorionic villi between normotensive and HIV associated PE women.

MATERIALS AND METHODS

Ethics

This research investigation utilized retrospectively collected placental samples of which institutional ethics approval was obtained (BCA 338/17). Post permission to utilize these samples (BCA 338/19), immunohistochemistry, brightfield microscopy and morphometric image analysis was performed.

Study population

Normotensive pregnant and preeclamptic women of African ancestry between 15 and 41 years of age were recruited at an antenatal clinic in a large regional hospital in eThekwini, Durban, KwaZulu-Natal. A total of 90 women were included and divided into normotensive (N; n=30) and preeclamptic (PE; n=60) categories. Further stratification was based on HIV status (negative – or positive +) and PE subtypes, such as early or late onset of PE (EOPE; LOPE). The resultant groups were N- (n=15), N+ (n=15), EOPE- (n=15), EOPE+ (n=15), LOPE- (n=15) and LOPE+ (n=15).

Preeclampsia diagnosis, as defined by Brown et al., (2018) was applied [2]. Additionally, women with an unknown HIV status; have chronic disease(s) (diabetes mellitus type I, II or gestational diabetes, chronic hypertension, renal disease, connective tissue disease, systemic erythematosus, sickle cell anemia, anti-phospholipid antibody syndrome or thyroid disease); who were on medicinal treatment (Aspirin, Warfarin or non-steroidal anti-inflammatory drugs or asthma medication); had gestational abnormalities (placental abruption or intra-uterine fetal death) or did not deliver via caesarean section, were filtered out during selection. Lastly, maternal and neonatal demographic and clinical data were recorded and archived together with consent forms.

Immunohistochemistry technique

Following cesarean delivery, a wedge biopsy from the central and peripheral regions of the placentae were performed. After overnight fixation in 10% buffered formaldehyde, the samples were processed using conventional dehydration, clearing and paraffin wax embedding techniques [21]. Thereafter, 3µm sections were cut using a rotary microtome (Leica Biosystems Inc., IL, USA) and collected onto X-tra adhesive slides (Leica Biosystems Inc., IL, USA). The primary antibody was a VEGFR-3 antibody (clone 54703; catalogue MAB 3491; R&D Systems, USA) which was linked to a horseradish peroxidase secondary antibody and finally, visualization was mediated by incubation in the 3, 3'-diaminobenzidine (DAB) chromogen using an EnVision™ Flex immunostaining kit (Dako, Denmark). Sections were washed, dehydrated and embedded with dibutylpthalate polystyrene xylene.

Microscopic examination

Conducting and exchange villi were viewed using the Zeiss Axio Imager 2 light microscope (Carl Zeiss, Germany, Europe) and images were captured at an initial magnification of x20. Two field areas of conducting and exchange villi per slide were captured.

Morphometry

The Axiovision Image Analysis Software (Version 4.8.3, Carl Zeiss, Germany, Europe) was employed for measurement and data analysis of each image.

Conducting villi

All regions of interest (ROI; DAB-stained areas of VEGFR-3) of the conducting villi underwent a one-phase segmentation using an auto measurement mode. This preceded cleaning, whereby any automated labelling of debris, red blood cells *etc*., were excluded from analysis. The perimeter of each villous was framed; thereafter the software generated the percentage of VEGFR-3 within the framed area of the villous.

Exchange villi

Two-phase segmentation was conducted to determine the percentages of ROI and villous area, using an auto measurement mode. Cleaning ensued, followed by framing of the entire field area. The average ROI and average villous area percentages were calculated and substituted into the equation below to establish the percentage of VEGFR-3 expressed in the total field area of exchange villi.

(Average ROI / Total field area of villi) x (100 / 1) = Total field area of VEGFR-3 immunoreactivity (%)

Statistical analysis

The output values were input to GraphPad Prism (version 8.3.0; California, USA). Normality testing showed that the data was non-parametric. For the comparative analysis across all 6 study groups, as well as for the PE subtype analysis (N *vs.* EOPE *vs.* LOPE), the one-way ANOVA non-parametric Kruskal-Wallis test was employed, followed by Dunn's Multiple Comparisons test. The Mann Whitney test was utilized for pregnancy type (N *vs.* PE) and HIV status (HIV- *vs.* HIV+) for both villous subtypes. Descriptive statistics have appropriately been presented as median and interquartile range for all study group analyses. A p value of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

Demographics and neonatal information is outlined in table 1. Based on HIV status, there was significant difference observed in the maternal age ($p = 0.001$) and birth weight ($p < 0.05$). Notably, the EOPE+ group had the lowest birth weight ($p < 0.05$), when compared to the other groups. The mean gestational age in the EOPE group was statistically lower ($p < 0.05$), when compared to N and LOPE groups.

VEGR-3 immunolocalization and histopathology

VEGFR-3 was immuno-expressed within conducting and exchange villi in trophoblast cell types (cytotrophoblast and syncytiotrophoblast), endothelial cells, mesenchymal cells and fibroblasts within the stroma. Trophoblast cell pathology included cytotrophoblast and syncytial knot increase with areas of intra- and extra- villous fibrinoid necrosis.

Morphometric image analysis

Conducting villi

The comparative results of VEGFR-3 immuno-expression across all 6 groups is outlined in table 2 and in Figure 3. A significant difference between N- *vs.* EOPE- ($p = 0.0022$) and N+ *vs.* EOPE- ($p = 0.0212$) was noted. VEGFR-3 immuno-expression was significantly reduced ($p = 0.0107$) in the PE (EOPE and LOPE) group, compared to the normotensive group (figure 1a).

Analysis of the immunostaining pattern of VEGFR-3 differed ($p = 0.0159$) across the study groups (N *vs.* EOPE *vs.* LOPE; figure 1b) with an additional difference between N *vs.* EOPE ($p = 0.0121$), irrespective of HIV status.

Regardless of pregnancy type, HIV status (figure 1c) did not have a significant ($p = 0.3015$) effect on VEGFR-3 immuno-expression.

Exchange villi

The results from comparative analysis of all 6 study groups are shown in table 2 and in Figure 4. Similar to the conducting villi, significance is observed across the groups ($p = 0.0016$) and significant differences also exist between N- *vs.* EOPE+ ($p = 0.0092$) and N+ *vs.* EOPE+ ($p = 0.0071$).

Irrespective of HIV status, VEGFR-3 was expressed significantly lower ($p < 0.0001$) in the PE group, when compared with the normotensive group expression (figure 2a). Sub-stratification of

the PE groups (figure 2b) showed a decline in VEGFR-3 immuno-expression (p = 0.0002) and significant differences between N *vs.* EOPE (p = 0.0003) and N *vs.* LOPE (p = 0.0031).

HIV status analysis (figure 2c) showed that irrespective of pregnancy type, VEGFR-3 immuno-expression is not significantly (p = 0.4535) effected in exchange villi.

COMMENTS

The primary finding of our investigation was that VEGFR-3 immuno-expression in both conducting and exchange chorionic villi was not significantly affected; however it was downregulated by HIV status, regardless of pregnancy type. It is important to note that all HIV+ women in our study received anti-retroviral therapy (Efavirenz, Nevirapine and HAART), hence, the similar VEGFR-3 immuno-expression observed in HIV negative and positive groups. Tat has an array of highly specialized functions [22], inclusive of being pro-angiogenic [23]. In a model of corneas from HIV infected rabbits, binding of Tat to VEGF co-receptor, heparin sulphate glycan, resulted in an up-regulation of angiogenesis [23]. This emanates from the molecular similarities between Tat and VEGF, allowing for VEGF mimicry [24]. Specifically, Tat binds to the VEGFR-2 receptor and up-regulates angiogenesis via the downstream phosphorylation pathways [25]. HIV is also able to elevate VEGF expression via activation of hypoxia inducible factor-2α (HIF-2α) [26]. HIF-2α has an important role in the hypoxic micro-environment of PE [27, 28] by regulating VEGF signaling [29, 30]. Hence, we posit that HAART may ameliorate the effect of HIV on VEGF expression in the hypoxic state of PE.

The receipt of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) impairs angiogenesis and lymphangiogenesis by disrupting endothelial cell proliferation and migration [20, 31, 32]. More specifically, NRTIs affect VEGFR-2 and VEGFR-3 [20]. Moreover, Conroy et al. (2017) reported an increase in anti-angiogenic factors with a concomitant decline in VEGF rather than VEGFR-3 in HIV+ woman receiving antiretroviral therapy [33]. In our study, there was no association between VEGFR-3 and HIV status. Nonetheless, cohorts in Europe [34] and the United States [35], and a meta-analysis review by Conde-Agudelo et al. (2008) [36] have reported that irrespective of whether or not HIV patients are treated with HAART, PE still develops.

In our study VEGFR-3 immuno-expression was significantly reduced in the conducting and exchange chorionic villi (p = 0.0107; p = 0.0016) in PE (EOPE and LOPE groups combined) compared to the normotensive group. It is plausible VEGF/VEGFR-3 signaling is dysregulated in the anti-angiogenic micro-environment of PE compared to normotensive pregnant women. Moreover, in an animal model, VEGFR-3 silencing results in early embryonic loss due to a halt in endothelial development [13, 37]. The VEGF-C/VEGFR-3 signaling is vital in lymph-endothelial migration [38]. VEGF/protein kinase B/endothelial nitric oxide synthase 3 (VEGF/Akt/eNOS) pathway inhibition prevents VEGF driven nitric oxide secretion thereby causing vasoconstriction [39]. Also, VEGFR-3 stimulates the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) and extracellular signal-regulated protein kinases 1 and 2

(Erk1/2) pathways. However, these pathways are dysregulated in PE [40]. Furthermore, polymorphisms in the VEGFR-3 tyrosine kinase domains inactivates the kinase enzyme [41].

In our study the immunostaining pattern of VEGFR-3 differed significantly within conducting ($p = 0.0121$) and exchange villi ($p = 0.0003$) when analyzing N *vs.* EOPE groups. In contrast to the normotensive pregnant group, the EOPE group is associated with defective placentation with an absence of physiological conversion of myometrial spiral arteries [42, 43]. Unexpectedly, we also observed a difference in VEGFR-3 immunoreactivity between N *vs.* LOPE ($p = 0.0031$) in exchange villi. The gestational age of the LOPE group does not reflect the inadequate trophoblast cell migration [44].

In our study, VEGFR-3 was immunolocalized within vascular endothelial cells, trophoblast cells and fibroblasts thereby adding to the complex signaling transduction role of VEGFR-3. Nonetheless, our findings within non-endothelial cells are corroborated by Orlandini (2006) [45]. Recently an absence of lymphatic vessels within the chorionic villi was shown [46]. Pathological observations observed in our study such as vasculo-syncytial membrane deficiency may be indicative of villous mal-perfusion and villous immaturity [47]. The qualitative increase in syncytial knots and cytotrophoblasts in the EOPE group is corroborated by the findings of other researchers [48-51]. The cytotrophoblasts are often characteristic of failed cytotrophoblastic regression which occurs in immature villi [47].

There are many risk factors that govern the onset of preeclampsia, one of which is increased fold risk in women aged ≥ 35 years [52]. Additionally in our study, the EOPE women delivered lower birth weight infants compared to the normotensive and LOPE groups. These results are in accordance with current literature on PE pregnancies [53-55] and are independent of HIV status.

To encapsulate our findings based on the reduced morphometric immuno-expression of VEGFR-3 within conducting and exchange villi, our findings suggests significant defective VEGF-C/VEGR-3 signal transduction in PE compared to normotensive pregnant women. However, VEGFR-3 immunostaining in both conducting and exchange chorionic villi was not significantly affected by HIV status. This similarity may be attributed to the synergistic effect of Tat and antiretroviral therapy in HIV infected women.

These molecular interactions in humans is not well understood, henceforth, research into genetic variants of VEGFR-3 in healthy and PE women is essential to understand deregulation of angiogenesis and lymphangiogenesis in PE. We also suggest further research into EOPE and LOPE to provide affordable early diagnostic testing and therapy in economically deprived countries such as Sub-Saharan Africa.

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Contribution to authorship

Pillay S and Naicker T have equally contributed to the writing and reviewing of this manuscript. Pillay S collated and performed analysis of the data, as well as produced the tables and figures presented.

Disclosure of interests

The authors do not report any conflicts of interest.

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Table 1. Maternal and neonatal demographics and clinical data across all 6 study groups.

Parameters	Normotensive		EOPE		LOPE	
	HIV- (n=15)	HIV+ (n=15)	HIV- (n=15)	HIV+ (n=15)	HIV- (n=15)	HIV+ (n=15)
Maternal age (years)	26.5 ± 6.4	27.7 ± 5.5	25.2 ± 6.5 **	31.8 ± 5.9 **	24.2 ± 6.4	26.7 ± 4.6
Gestational age (weeks)	38.5 ± 1.1	38.7 ± 0.7	32.9 ± 2.6 *	33 ± 3.60 *	37.1 ± 6.1	37.4 ± 1.2
Baby weight (g)	3346 ± 321	3255 ± 396	2187 ± 799 *	2083 ± 852 *	3078 ± 457 *	2962 ± 277 *
Baby sex (M:F)	14:16	14:16	14:16	12:18	15:15	20:10

Data reported as mean ± SD and statistical significance considered *$p < 0.05$ and **$p \leq 0.001$.

Table 2. Immuno-expression of placental VEGFR-3 across all 6 study groups.

Villous type	Normotensive		EOPE		LOPE	
	HIV- (n=15)	HIV+ (n=15)	HIV- (n=15)	HIV+ (n=15)	HIV- (n=15)	HIV+ (n=15)
Conducting villi	9.82 (13.32-5.15)**	9.00 (9.00-6.60)*	6.08 (6.08-5.25)*, **	8.86 (11.03-6.17)	8.71 (9.43-8.71)	8.45 (8.45-8.45)
Exchange villi	36.48 (41.19-27.80)**	35.88 (37.89-32.11)**	30.10 (34.81-26.52)	26.23 (28.98-19.14)**	29.94 (32.67-26.30)	27.48 (34.37-27.28)

Data reported as median and IQR and statistical significance considered *$p < 0.05$ and **$p \leq 0.001$.

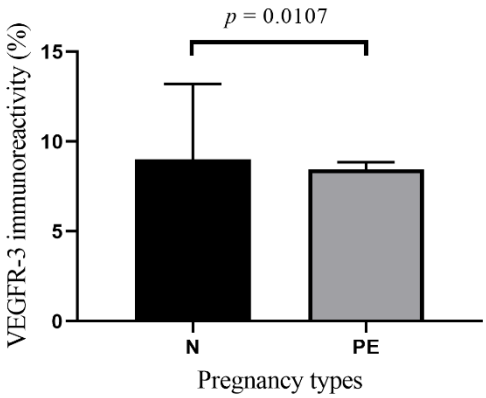

Figure 1a.

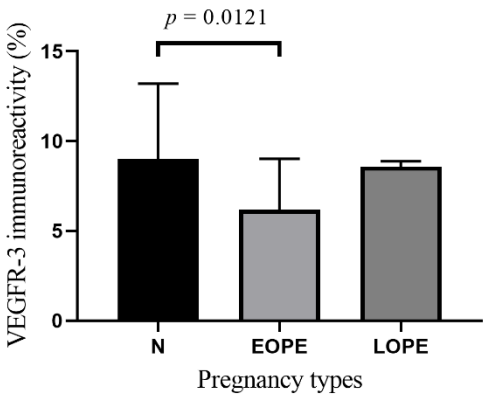

Figure 1b.

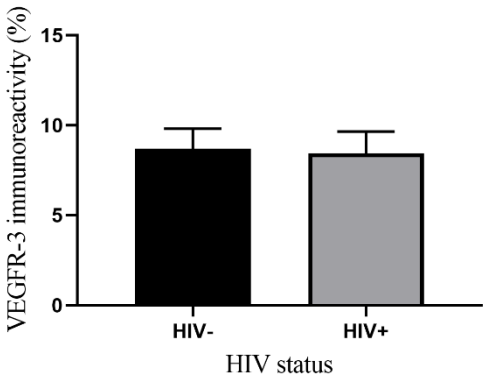

Figure 1c.

Figure 1. Histogram illustrating field area percentage of VEGFR-3 immuno-expression within conducting villi.

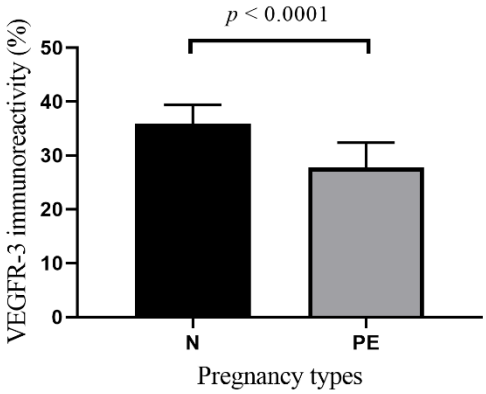

Figure 2a.

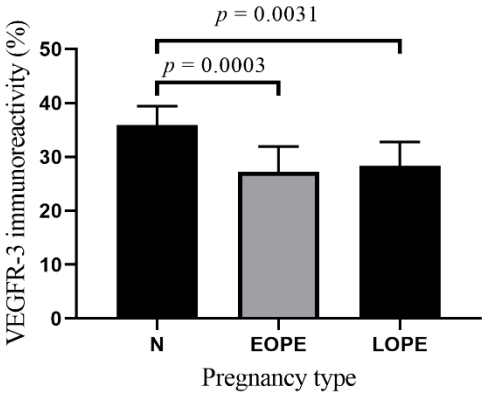

Figure 2b.

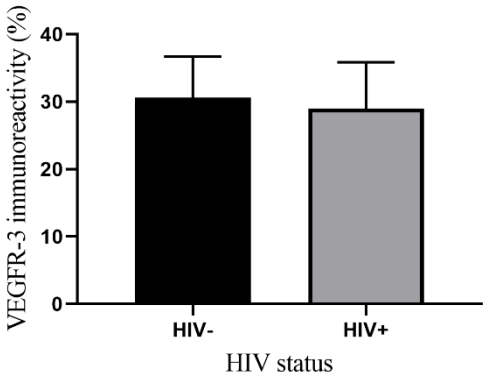

Figure 2c.

Figure 2. Histogram outlining VEGFR-3 immuno-expression (per frame area) of exchange villi.

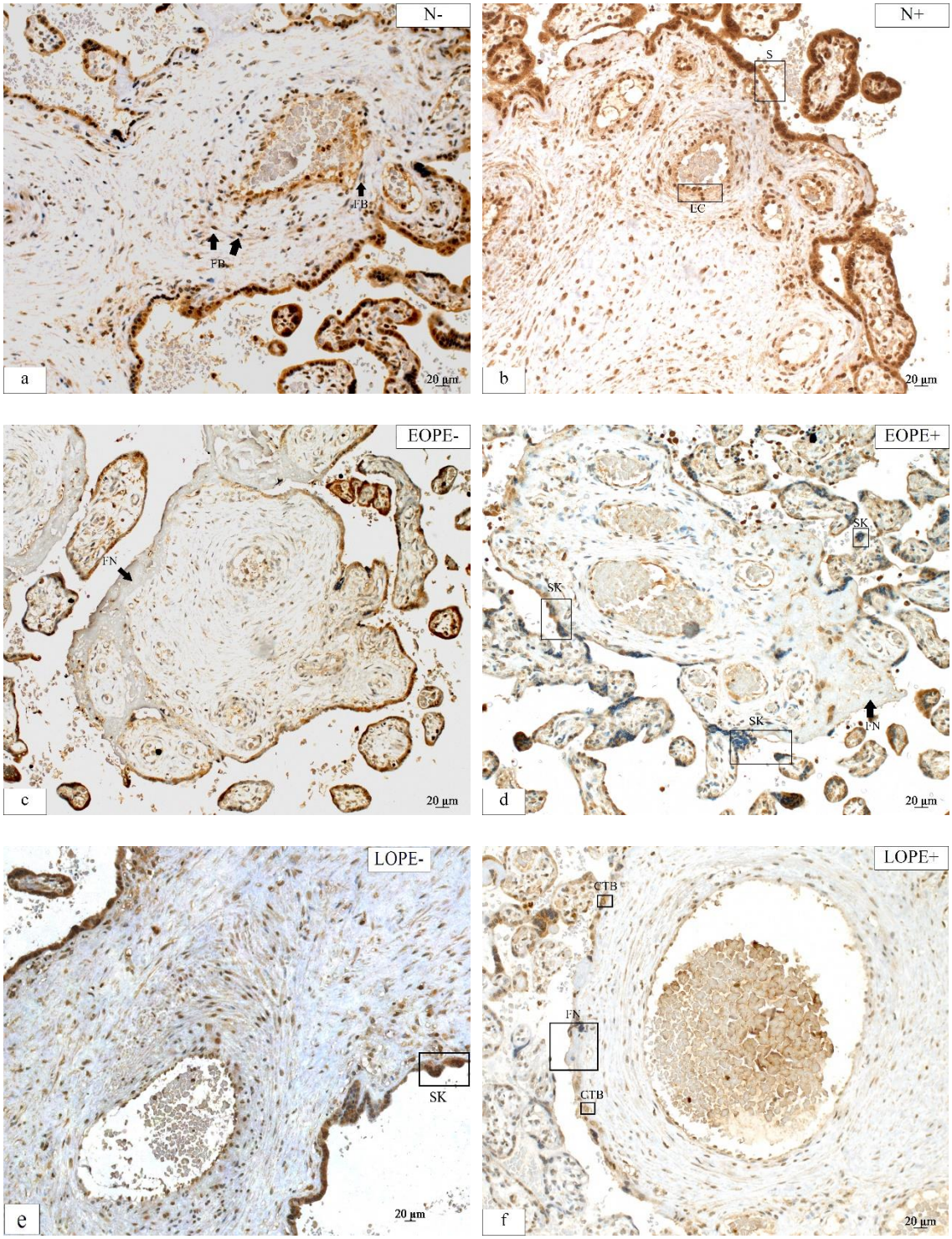

Figure 3. Light micrographs illustrating immuno-expression of VEGFR-3 within conducting villi from a) N-; b) N+; c) EOPE-; d) EOPE+; e) LOPE- and f) LOPE+ placenta. Initial Magnification: x20. Morphological observations denoted: FB = Fibroblast; S = Syncytium; EC = Endothelial cell; FN = Fibrinoid necrosis; SK = Syncytial knot; CTB = Cytotrophoblast.

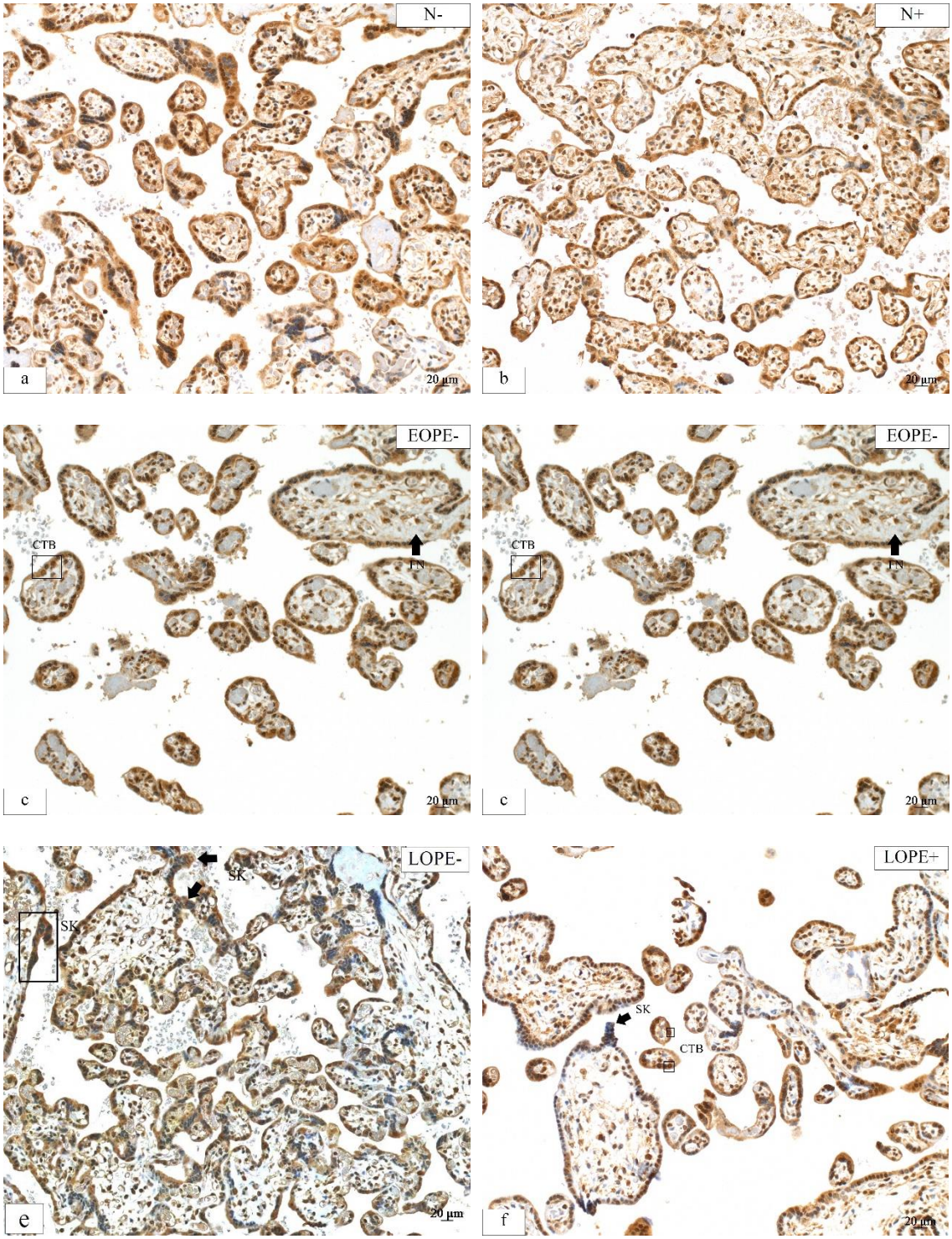

Figure 4. Light micrographs illustrating immuno-expression of VEGFR-3 within exchange villi from a) N-; b) N+; c) EOPE-; d) EOPE+; e) LOPE- and f) LOPE+ placenta. Initial Magnification: x20. Morphological observations denoted: FN = Fibrinoid necrosis; SK = Syncytial knot; CTB = Cytotrophoblast.

CHAPTER THREE

SYNTHESIS, CONCLUSION AND FUTURE PERSPECTIVES

3.1 Synthesis

South Africa is a low-middle income country with 13.5% of its overall population being HIV+; moreover more than 1/5 of women in their reproductive age are infected with the virus (StatsSA, 2019). Since 2016, mother to child transmission with ARV usage has plateaued at 0.71% (AVERT, 2018). Pregnancies are further complicated by HDPs, which are responsible for 14% of maternal mortality (NCCEMD, 2018). Of these occurrences, PE is the most prominent (Moodley *et al.*, 2019).

Due to PE and HIV infection being the leading causes of maternal and fetal morbidity and mortality in South Africa (NCCEMD, 2018), these conditions are currently under intense scrutiny. It has since been established that a disproportion of angiogenic factors is induced in both conditions (Karumanchi and Granger, 2016) and the VEGF family has been observed as being targets of HIV accessory protein, Tat (Levine *et al.*, 2004).

In light of the high incidence of HIV infection and preeclampsia in South Africa, it is urgent that the duality of these co-morbidities on VEGF/VEGFR-3 signaling during angiogenesis and lymphangiogenesis be investigated.

This study demonstrates a reduced placental VEGFR-3 immuno-expression in the HIV+ groups. Although not significant, it is plausible that the HIV Tat protein stimulates endothelial cells to activate angiogenesis (Arasteh and Hannah, 2000). The binding region of the Tat protein is arginine- and lysine-rich, similar to that of VEGFs (Albini *et al.*, 1996). This mimicry allows Tat to bind to the VEGFR co-receptor, heparin sulphate glycoprotein (Albini *et al.*, 1996). Furthermore, endothelial cell transmembrane integrin proteins (potent modulators of VEGFR-3) are targeted by Tat to upregulate downstream signaling within the PI3K/Akt pathway for activation of angiogenesis (D'Oria *et al.*, 2017). Also, HIF-2α upregulates VEGF (i) during embryonic development; (ii) for tissue growth and (iii) in oxygen poor micro-environments induced by pregnancy complications (PE) and viral infections (HIV) (Korgaonkar *et al.*, 2008). Subsequently, as a compensatory mechanism VEGF/VEGFR signaling stimulates angiogenesis (Verma *et al.*, 2018).

However, it is notable that all HIV+ women in our investigation were administered ARVs (combined HAART, Efavirenz and Nevirapine) which inhibit (although not 100% effective) HIV viral replication and upregulation of angiogenesis (Song *et al.*, 2018). Nucleoside reverse

transcriptase inhibitors (NRTIs) and non-NRTIs (Efavirenz and Nevirapine) are HIV viral suppressors and are anti-angiogenic/anti-lymphangiogenic (McNeil *et al.*, 2017). Since VEGFR-3 is a critical regulator of both processes, binding with VEGF-C is blocked (Song *et al.*, 2018). This is substantiated by the results observed in our study where HIV status did not significantly affect VEGFR-3 immunostaining in both conducting and exchange villi. Henceforth, based on the mechanisms of ARV's and the results from our study it may be posited that while ARVs do not completely inhibit viral action, they are able to sufficiently prevent over stimulation of VEGFR-3 activation-induced angiogenesis.

This study has observed low VEGFR-3 immuno-expression in conducting (p = 0.0107) and exchange (p = 0.0016) villi from the preeclamptic pregnancies. In PE, the overproduction of placental ROS creates an ischemic micro-environment (Pereira *et al.*, 2015). Maynard *et al.*, (2003) have reported that consequent to a high ROS concentration, anti-angiogenic factors (*e.g.* sFlt-1) are released into the maternal circulation, which cause systemic endothelial cell damage and dysfunction (Maynard *et al.*, 2003). Furthermore, targeted gene inactivation or inhibition of VEGFR-3 result in blood vessel anomalies (Ferrara, 2001). Karkkainen *et al.*, (2001) have observed RTK inactivity in mouse models carrying VEGFR-3 polymorphisms (Karkkainen *et al.*, 2001). Damages to the molecular structure of VEGFR-3 would reduce its expression within endothelial cells where it would affect placental vascular formation and development (Chen and Zheng, 2014). The results of a significant downregulation of VEGFR-3 in both the conducting and exchange villi from the preeclamptic women in our study possibly emanates from dysregulation of VEGF-C/VEGFR-3 signaling.

It is important to note that some of the women included in this study were preeclamptic and HIV+, thus the dual nature of the opposing inflammatory conditions may further dysregulate VEGF-C/VEGFR-3 signaling. Notably, as aforementioned, PE and ARVs are anti-angiogenic whilst the HIV Tat protein has a pro-angiogenic effect. The combined effects of PE, HIV infection and ARVs may present severe consequences. Bahram and Claesson-Welsh (2010) report that VEGFR-3 degradation renders the RTK inactive (Bahram and Claesson-Welsh, 2010). Loss of activation of PI3K/Akt and ERK1/2 pathways in the duality of PE and HIV (D'Oria *et al.*, 2017; Song *et al.*, 2018) lead to under-developed placental vasculature.

Interestingly, a comparison of the immuno-expression of VEGFR-3 across the normotensive, EOPE and LOPE groups in our study, showed that the most significant multiple group comparison was between normotensive and EOPE (conducting villi: p = 0.0121; exchange villi: p = 0.0003). EOPE is a direct result of defective placentation and superimposed defective angiogenesis (Burke and Karumanchi, 2018). It is plausible that the PI3K/Akt and ERK1/2 pathways are dysregulated

in the duality of PE and HIV infection. Chen and Zheng (2014) confirm that activation of these pathways by VEGFs is pertinent for stimulation of angiogenesis and sequentially, endothelial cell proliferation, migration and vessel formation (Chen and Zheng, 2014). Several studies corroborate reduced angiogenesis by VEGF activation as reported in our study (Zheng *et al.*, 2008; Feng *et al.*, 2012). Furthermore, heightened ROS production and systemic inflammation reduce VEGFR-3 bio-availability (Wang, 2010). This coincides with abnormal placentation as observed in the development of EOPE and substantiates the reduced VEGFR-3 immuno-expression in placental villous samples from women with EOPE.

Additionally, EOPE women included in our study were associated with early delivery and low birth weight ($p < 0.05$), compared to the normotensive and LOPE groups. Furthermore, these findings were independent of HIV status. Due to reduced VEGFR-3 immuno-expression observed in the EOPE groups, it is not surprising that the reduced angiogenesis was associated with defective placental and fetal vasculature. Furthermore, women aged $\geq$ 35 years have a predisposition to PE development (Bartsch *et al.*, 2016). Notably, there was significance difference ($p \leq 0.001$) in maternal age between the normotensive and the EOPE groups.

This research presents a novel finding of significantly reduced VEGFR-3 immunostaining in LOPE exchange villi ($p = 0.0031$) when compared to the normotensive group. This is unexpected as LOPE is not a result of angiogenic imbalance and abnormal placentation, as its onset occurs much later in pregnancy, near term. LOPE is primarily caused by factors that are extrinsic to the placenta (Staff and Redman, 2018). Such factors include maternal smoking and obesity (Wang and Walsh, 1998). However, keeping in mind that 67% of women in our study population were HIV+ and receiving ARVs, it is plausible that the introduction of antiretroviral therapy caused down-regulation of VEGFR-3 immuno-expression. ARV inhibition of angiogenesis and lymphangiogenesis is corroborated by several researchers (Song *et al.*, 2018; Naicker *et al.*, 2019; Monini *et al.*, 2003).

Prominent pathological observations in our study included trophoblast anomalies such as, increased appearance of syncytial knots and cytotrophoblasts, vasculo-syncytial membrane deficiencies, as well as fibrinoid necrosis. These findings are substantiated by several studies on placental pathology in PE complicated pregnancies (Hansen *et al.*, 2000; Sebire *et al.*, 2005; Heazell *et al.*, 2007; Huppertz, 2011).

3.2 Conclusion and future prospectives

This study is the first to observe the immuno-expression of VEGFR-3 in the placenta of preeclamptic and HIV+ women receiving ARV treatment. We find that HIV infection does not have a significant effect on VEGFR-3 immuno-expression and angiogenesis, with or without the presence of additional PE. However, we have posited that combined ARV therapy may shadow the true effect of HIV infection. These novel findings specifically infer serious dysregulation in EOPE women, regardless of HIV infection. Furthermore, our findings draw attention to the need to re-evaluate the effects of ARV therapy on angiogenic dysregulation in PE concomitant HIV infection. Further large scale studies to confirm the biomarker values of VEGFR-3 is important to develop therapeutic interventions and specialized care for affected women. We advocate the establishment of economically viable strategies for the early detection of PE, particularly in financially constrained countries.

CHAPTER FOUR

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APPENDICES

4.1 Appendix 1

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17 December 2019

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 letter received on 13 December 2019 submitting an application for amendments to change the title for the study below has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committe.

Study details:

PI: Saieshni Pillay (215044397)

Title: The role of VEGFR-3 in the placenta and placental bed in HIV associated preeclampsia

NEW TITLE: The role of VEGFR-3 in the placenta and in HIV associated preeclampsia

The committee will be notified of the above at its next meeting to be held on 11 February 2020.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

215044397@stu.ukzn.ac.za