THE REGULATION OF OSTEOPONTIN AND SOLUBLE NEUROPILIN-1 IN HIV-ASSOCIATED PREECLAMPSIA

By

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PREFACE

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

The research described in this dissertation was carried out in the Optics & Imaging Centre, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, under the supervision of Professor Thajasvarie Naicker.

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Professor Thajasvarie Naicker — Date

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DECLARATION

I, **Nitalia Naidoo**, 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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Signed: _____ Date: 03 December 2020

DEDICATION

To God Almighty

For his guidance, protection, and favourable blessings

'...If ye have faith as a grain of mustard seed, ye shall say unto this mountain, Remove hence to yonder place; and it shall remove; and nothing shall be impossible unto you.' - Matthew 17:20

To my mother, late grandparents, and great grandmother

I dedicate this dissertation to my mother, late grandparents, and great grandmother. Thank you for your endless love, support, and encouragement. You are the source of my success.

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PUBLICATIONS

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2. Naidoo N., Moodley J. & Naicker T. (2020). The regulation of osteopontin and soluble neuropilin-1 in HIV-associated preeclampsia. **Submitted** to *Angiogenesis*, Manuscript ID: AGEN-D-20-00293

Table of Contents

LIST OF ABBREVIATIONS

Angiotensin I, II, (1-7), (1-9)	Ang I, II, (1-7), (1-9)
Angiotensin-converting enzyme and/or 2	ACE and ACE 2
Antiretrovirals	ARV
Antiretroviral therapy	ART
Coronavirus disease 2019	COVID-19
Dolutegravir	DTG
Extracellular matrix	ECM
Endothelial cell	EC
Highly active antiretroviral therapy	HAART
Human immunodeficiency virus	HIV
Interleukin	IL
KwaZulu-Natal	KZN
Lopinavir	LPV
Matrix metalloproteinase	MMP
Nuclear factor-kappa B	NF-κB
Osteopontin	OPN
Placental growth factor	PlGF
Preeclampsia	PE
Protease Inhibitor	PI
Remdesivir	RDV
Ritonavir	/r
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2
Soluble and/or endoglin	sEng or Eng
Soluble and/or fms-like tyrosine kinase	sFlt-1 or Flt-1
Soluble and/or neuropilin-1	sNRP-1 or NRP-1
South Africa	SA
Trans-activator of transcription	Tat
Vascular endothelial growth factor	VEGF
Vascular endothelial growth factor receptor	VEGFR

LIST OF FIGURES

THESIS LAYOUT

CHAPTER ONE

*Note: Only abbreviations not expanded in the text are listed in captions in Chapter One.

CHAPTER TWO

CHAPTER THREE

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

ABSTRACT

Background: The prevalence of preeclampsia (PE) and human immunodeficiency virus (HIV) infection in pregnant women remains a concern to the South African healthcare system. The conflicting angiogenic roles of osteopontin (OPN) and soluble neuropilin-1 (sNRP-1) has been highlighted in many diseases. However, there is a dire paucity of data on the influence of OPN and sNRP-1 in PE. Moreover, there is an absence of information of OPN and sNRP-1 in HIV-associated PE. In light of the aforementioned criteria, it was important to study the influence of OPN and sNRP-1 in the synergy of HIV-infection and PE. Therefore, this study evaluated the concentration of angiogenic proteins, OPN and sNRP-1, in the synergy of HIV infection and PE. Additionally, in light of the coronavirus disease 2019 (COVID-19) pandemic, we reviewed maternal endothelial dysfunction in HIV-associated PE comorbid with COVID-19. In this review article, we also explored the potential of antiretroviral therapy (ART) in the therapeutic intervention of COVID-19.

Study design: A Bio-plex multiplex immunoassay was used to quantify serum OPN and sNRP-1 concentrations in preeclamptic *vs* normotensive pregnancy type stratified by HIV status (n=19 per subgroup).

Results: Significant differences across all groups, i.e., HIV-negative normotensive (n = 19), HIV-negative preeclamptic (n = 19), HIV-positive normotensive (n = 19) and HIV-positive preeclamptic (n = 19), were reported in maternal age ($p = 0.0211$), gestational age ($p = 0.0004$), parity ($p = 0.0042$), systolic blood pressure ($p < 0.0001$) and diastolic blood pressure ($p < 0.0001$). The concentration of OPN was significantly downregulated by pregnancy type (preeclamptic *vs* normotensive; $p = 0.0033$) and showed a non-significant elevation when stratified by HIV status (HIV-positive vs HIV-negative; $p = 0.5099$). In addition, there was a significant upregulation in sNRP-1 concentration by pregnancy type (preeclamptic *vs* normotensive; $p = 0.0054$) and by HIV status (HIV-positive *vs* HIV-negative; ($p = 0.0005$). In comparison to the normotensive HIV-negative group, there was a significant difference in sNRP-1 concentrations between the sub-groups normotensive HIV-positive ($p = 0.0049$), preeclamptic HIV-negative ($p = 0.0244$), and preeclamptic HIV-positive ($p < 0.001$), respectively.

Conclusion: This innovative study validates a significant systemic downregulation of OPN in PE compared to normotensive pregnancies in contrast to an upregulation of sNRP-1, conforming to the anti-angiogenic milieu of PE. Moreover, based on HIV-status, both the systemic OPN and sNRP-1 levels were upregulated in HIV-positive pregnancies. This may be attributed to the HIV trans-activator of transcription protein mimicry of VEGF and/or the immune reconstitution following ART as well as

to integrin expression that mediates endothelial cell tube formation during angiogenesis. Additionally, adverse effects associated with HIV infection and ART promote endothelial dysfunction predisposing PE development; however, higher prevalence and mortality rates among PE cases are still associated with ART use. Pregnancies complicated by the COVID-19 exploitation of angiotensin-converting enzyme 2 have a strong correlation with PE-like symptoms such as endothelial injury, implicating COVID-19 in PE onset. Inconsistent data on the potential effectiveness of ART in COVID-19 and their safety in pregnancy warrants further investigations.

OKUNGAQONDAKALI (ISIZULU)

Isendlalelo: Ukudlanga kwegciwane le-preeclampsia (PE) kanye nokutheleleka ngegciwane lesifo sokugonywa kwabantu besifazane abakhulelwe kuhlala kuyinkinga ohlelweni lwezempilo lwaseNingizimu Afrika. Ngakho-ke, lolu cwaningo luhlole ukugcwala kwamaprotheni e-angiogenic, i-osteopontin (OPN) ne-soluble neuropilin-1 (sNRP-1), ekuhlanganyeleni kokutheleleka nge-HIV kanye ne-PE. Ngaphezu kwalokho, ngokubheka isifo se-coronavirus 2019 (COVID-19)) ubhubhane, sibukeze ukungasebenzi kahle kwe-endothelial in comorbid ehambisana ne-COVID-19. Kulesi sihloko sokubuyekezwa, siphinde sahlola amandla we-antiretroviral therapy (ART) ekungeneleleni kwezokwelapha kwe-COVID-19.

Umklamo wokutadisha: Kusetshenziswe i-Bio-plex multiplex immunoassay ukukala ukugxila kwe-serum OPN kanye nokugxila kwe-sNRP-1 kuhlobo lokukhulelwa lwe-preeclamptic vs normatensive oluhlukaniswe yisimo se-HIV (n = 19 iqembu elingaphansi).

Imiphumela: Umehluko obalulekile kuwo wonke amaqembu abikwa eminyakeni yobudala bomama ($p = 0.0211$), iminyaka yokuthinta ($p = 0.0004$), ukulingana ($p = 0.0042$), umfutho wegazi we-systolic ($p < 0.001$) nomfutho wegazi we-diastolic ($p < 0.00$). Ukuqoqwa kwe-OPN kwehliswe kakhulu ngohlobo lokukhulelwa (preeclamptic vs Normadensive; $p = 0.0033$) futhi kwakuvame ukwanda ngesimo se-HIV (i-HIV-positive vs virus-negative; $p = 0.5099$). Ngaphezu kwalokho, kube nokukhushulwa okukhulu ekuhlolweni kwe-sNRP-1 ngohlobo lokukhulelwa (preeclamptic vs Normatensive; $p = 0.0054$) nangesimo se-HIV (HIV-positive vs HIV-negative; ($p = 0.0005$). Ngokuqhathanisa neqembu le-HIV-negative elijwayelekile, kube nomehluko omkhulu ekugxileni kwe-sNRP-1 phakathi kwamaqembu amancane ajwayelekile ane-HIV-positive ($p = 0.0049$), i-preeclamptic HIV-negative ($p = 0.0244$), ne-preeclamptic HIV-positive ($p < 0.01$), ngokulandelana.

Isiphetho: Lolu cwaningo olusha luqinisekisa ukwehliswa okukhulu kwe-OPN ku-PE ngokuqhathaniswa nokukhulelwa okujwayelekile ngokungafani nokuphakanyiswa kwe-sNRP-1, okuhambisana ne-anti-angiogenic milieu ye-PE. Ngaphezu kwalokho, ngokususelwa ku-HIV-status, bobabili i-OPN ne-sNRP-1 baphakanyiswa ekukhulelweni okuhle kwe-HIV. Lokhu kungahle kuvezwe yi-HIV trans-activator ye-protein mimicry ye-VEGF kanye / noma ukubuyiselwa kabusha komzimba okulandela i-ART kanye ne-integratein expression ethi mediates endothelial cell tube ngesikhathi se-angiogeneis. Ngaphezu kwalokho, imiphumela emibi ehambisana nokutheleleka nge-HIV kanye ne-ART ikhuthaza ukungasebenzi kahle kwe-endothelial dysformance deposing PE; kodwa-ke, ukwanda okuphezulu kanye namazinga okufa kwabantu phakathi kwamacala we-PE kusahlotshaniswa nokusetshenziswa kwe-ART. Ukukhulelwa okuyinkimbinkimbi ngokuxhashazwa kwe-COVID-19

kwe-angiotensin-converting enzyme 2 kunokuhlangana okuqinile nezimpawu ezifana ne-Pe njengokulimala kwe-endothelial, okufaka i-19 ekuqaleni kwe-PE. Idatha engahambisani nokusebenza okungenzeka kwe-ART ku-COVID-19 nokuphepha kwabo kuma-warrant okukhulelwa kuyaqhubeka nokuphenya.

DISSERTATION LAYOUT

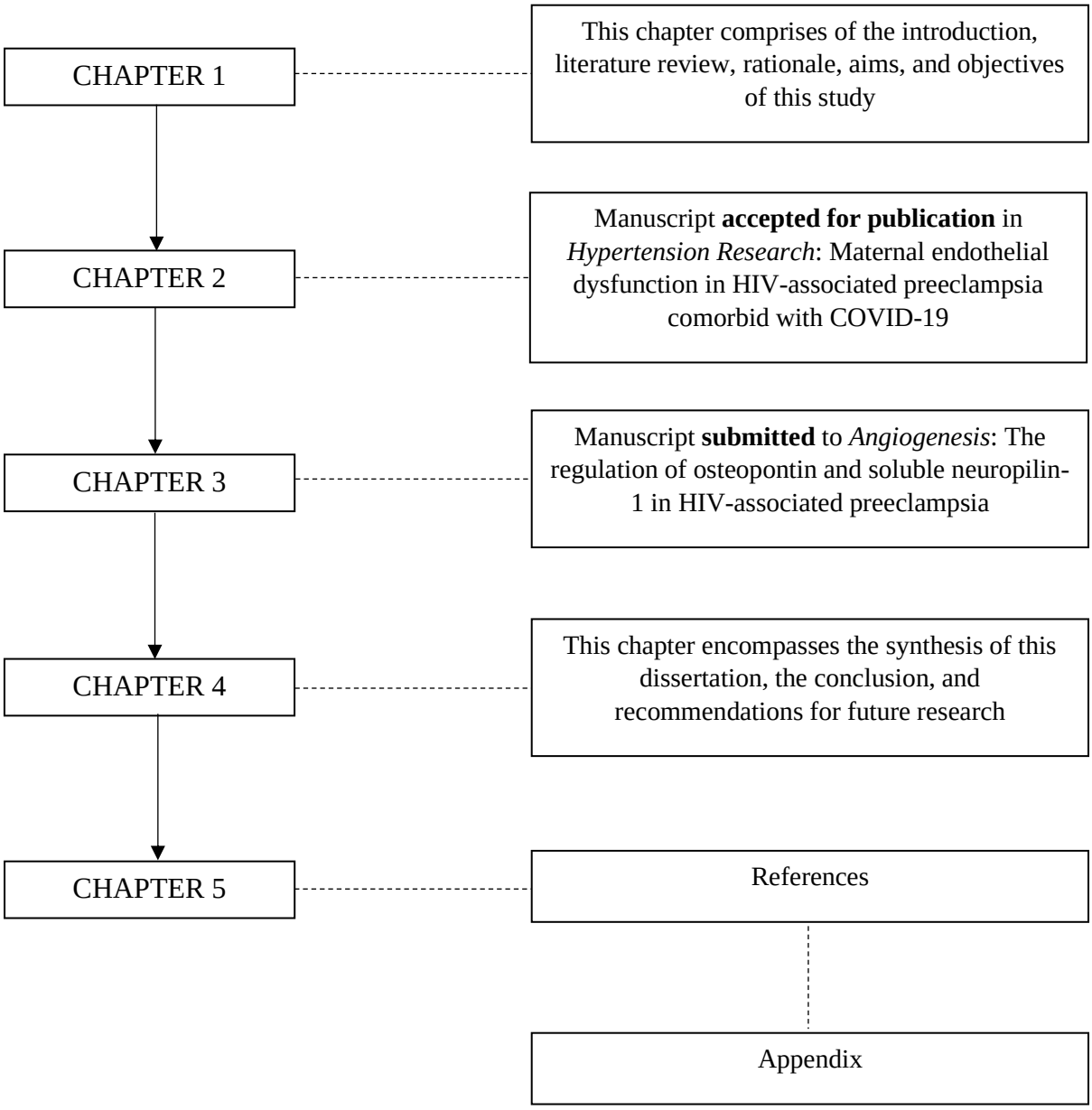

Figure 1: Schematic diagram showing the dissertation layout

CHAPTER ONE

BACKGROUND/LITERATURE

1.1 Maternal mortality

Maternal mortality is defined as deaths during pregnancy or within 42 days of pregnancy termination, regardless of gestational period and site of pregnancy, emanating from any cause associated with pregnancy or its management (World Health Organization, 2020). The prevalence of maternal mortality is highly concentrated in low-middle income countries (LMICs) such as South Africa (SA) (Lewis, 2008; Girum and Wasie, 2017). In SA, non-pregnancy-related infections, predominantly HIV infection, and hypertension account for 32.78% and 23.20% of maternal deaths, respectively (National Committee for Confidential Enquiry into Maternal Deaths, 2018).

1.2 Preeclampsia

Hypertensive disorders of pregnancy (HDP) are responsible for 14.8% of maternal deaths in SA, the majority of which are due to preeclampsia (PE) (National Committee for Confidential Enquiry into Maternal Deaths, 2018). The province of KwaZulu-Natal (KZN) in SA, however, has a 12% prevalence of PE (Pattinson, 2014). Preeclampsia is a disorder characterized by new-onset hypertension (blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic) after 20 weeks of gestation (Brown *et al.*, 2018). PE is usually accompanied by proteinuria (not mandatory for diagnosis) and/or multiple organ dysfunction such as acute kidney injury, neurological features, liver dysfunction, the HELLP syndrome (Haemolysis, Elevated Liver enzymes, Low platelets), and/or uteroplacental dysfunction such as fetal growth restriction (Brown *et al.*, 2018). The etiology of PE is unclear; however, reduction in uterine natural killer (uNK) cells, impaired spiral arterial modification, abnormal placentation, imbalance of angiogenic factors, apoptosis, as well as oxidative stress have been implicated in its development (Rana *et al.*, 2019). The most prominent risk factors for PE development include pre-gestational diabetes mellitus, antiphospholipid syndrome, and obesity (Bartsch *et al.*, 2016). Other factors include advanced maternal age (Lamminpää *et al.*, 2012), nulliparity (Odegård *et al.*, 2000), a history of chronic kidney disorders (English *et al.*, 2015), family history of PE (Bezerra *et al.*, 2010), and assisted reproductive technology (Omani-Samani *et al.*, 2018).

1.3 Decidualization

Decidualization is the process of endometrial stromal cell (ESC) differentiation to prepare for and maintain pregnancy (Okada *et al.*, 2018). Changes to the endometrium consist of functional cyclic

phases, *i.e.*, the proliferative phase and secretory phase (Reed and Carr, 2000). During the secretory phase, the endometrial tissue is most receptive and suitable for blastocyst implantation concomitant with decidualization (Vinketova *et al.*, 2016). The decidual cells within the extracellular matrix (ECM) interact with trophoblast cells during invasion (Sharma *et al.*, 2016). The presence of adherens junctions between the decidual cells and their arrangement of gap junctions aid trophoblast invasion (Okada *et al.*, 2018). The decidualized stromal cells produce proteins such as fibronectin, laminin, decorin, type IV collagen, and heparin sulphate proteoglycans for effective ECM remodelling (Gellersen and Brosens, 2014; Okada *et al.*, 2014). This is vital for preparing the endometrium for implantation and is believed to be under hormonal control (Okada *et al.*, 2018). The decidualized endometrium plays a pivotal role in embryonic development by protecting the embryo from maternal immunological rejection, i.e., enabling decidual stromal cells to acquire specific functions related to recognition, selection, and acceptance of the allogeneic embryo, as well as to development of maternal immune tolerance (Vinketova *et al.*, 2016). It also provides the embryo with adequate oxygen and nutrients prior to the formation of the placenta (Mori *et al.*, 2016). Decidual stromal cells also secrete proteins such as osteopontin (OPN), which induce trophoblast growth, invasion, and promote angiogenesis during placentation (Xia *et al.*, 2009; Wang *et al.*, 2018).

1.4 Spiral artery remodelling, placentation, and the pathophysiology of preeclampsia

The uteroplacental vasculature undergoes a significant morphological and physiological transformation to sustain a healthy pregnancy (Singh *et al.*, 2011). Inadequate cytotrophoblast (CT) cell migration, spiral artery remodelling, and placentation predispose PE development (Moffett-King, 2002; Roberts and Hubel, 2009), seen in **Figure 1.1**. The lack of physiological transformation of spiral arteries within the myometrium leads to a reduced blood flow creating a hypoxic environment. The decreased bioavailability of endothelial nitric oxide synthase (eNOS) results in vasoconstriction. Thereafter, hypoxia inducible factor 1 alpha (HIF-1α) is upregulated promoting hypoxia and reactive oxygen species (ROS) generation (Kvietys and Granger, 2012). These events promote an imbalance in pro-angiogenic and anti-angiogenic homeostasis with an increased sFlt-1/PlGF ratio (Redman and Sargent, 2005; Jardim *et al.*, 2015). This phenomenon is believed to be the initiator of pervasive multi-organ endothelial dysfunction leading to the clinical manifestation of PE (Redman and Sargent, 2005; Rana *et al.*, 2019). These events are highlighted in chapter two.

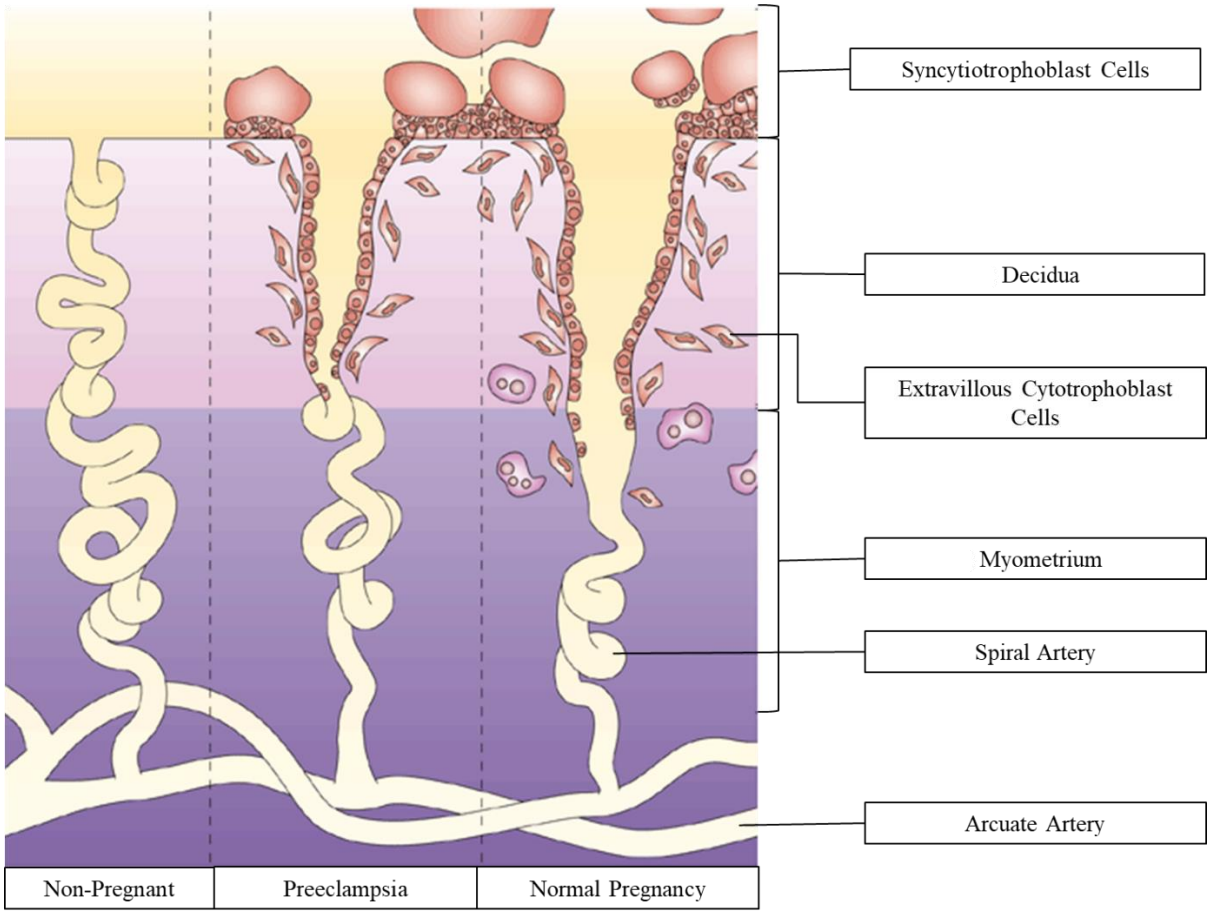

Figure 1.1: Spiral artery remodelling in non-pregnant, preeclamptic pregnancy, and normal pregnancy conditions (Adapted from Moffett-King, 2002).

1.5 Human immunodeficiency virus

Africa accounts for 25.7 million HIV infections; however, sub-Saharan Africa alone constitutes approximately 21.2 million of the global HIV-infected population (World Health Organization, 2018). South Africa has a total population of 57.8 million people, of which 7.79 million are estimated to be burdened with HIV infection (Stats SA, 2019). The HIV-1 accessory protein, trans-activator of transcription (Tat), is implicated in vascular endothelial growth factor (VEGF) mimicry; however, its role in angiogenesis is conflicting. Due to the immune incompetency observed in HIV-infection, antiretroviral therapy (ART) is a global standard of care (World Health Organization, 2010). However, HIV-infected pregnant women may be at risk for severe comorbidity with PE as ART re-establishes immunological responses predisposing PE development (Tooke *et al.*, 2016). Furthermore, various ARTs influence decidualization, placentation and enhance maternal endothelial dysfunction (Autran *et al.*, 1999; Powis and Shapiro, 2015; Sandra Hernández *et al.*, 2017; Song *et al.*, 2018).

1.6 Osteopontin

Osteopontin is an angiogenic factor involved in vascular remodelling and injury repair (Wang *et al.*, 2018). It has three major isoforms (OPN-a, OPN-b, and OPN-c) (Cao *et al.*, 2012). All three isoforms share identical domains, *i.e.*, the aspartate domain, arginine-glycine-aspartate (Arg-Gly-Asp) domain, the serine-valine-valine-tyrosine-leucine-arginine (Ser-Val-Val-Tyr-Gly-Leu-Arg) domain, thrombin cleavage domain, calcium-binding domain as well as the heparin-binding domain, all of which are strung together via various linkers (Cao *et al.*, 2012). Variation in the length of the linkers between the signal peptide and aspartate domain distinguishes the isoforms, with OPN-a being the longest and OPN-c being the shortest, as seen in **Figure 1.2** (Cao *et al.*, 2012). However, knowledge of the exact variant of OPN expressed in pregnancy-related processes is unknown.

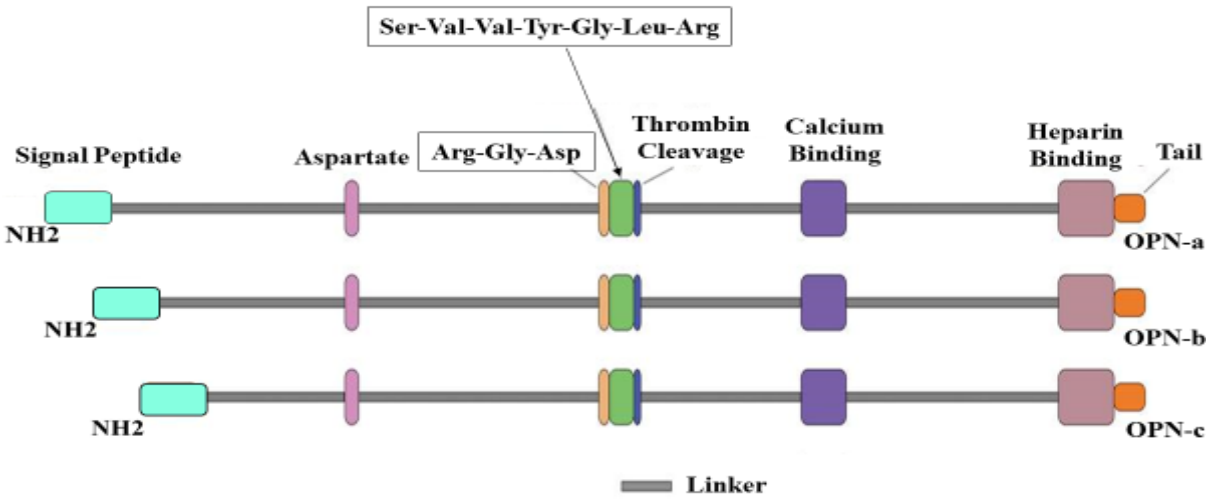

Figure 1.2: Structure features of osteopontin isoforms (Adapted from Cao *et al.*, 2012). Abbreviations: NH2 (Amino acid group)

Osteopontin mediates VEGF expression upon binding to the αvβ3 integrin (Chakraborty *et al.*, 2008). Osteopontin signalling results in the activation of anti-apoptosis and pro-survival pathways via phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and nuclear factor kappa B (NF-κB) signalling molecules, angiogenesis modulation via VEGF induction, and ECM degradation via matrix metalloproteinases (MMPs).

OPN is expressed in the epithelial cells of the secretory phase of the endometrium, invading trophoblast cells, endometrial glands, and the placenta (Wang *et al.*, 2018). After binding to its integrin receptor, αvβ3, OPN facilitates cell adhesion and signalling, promoting implantation, cell migration, invasion, and placentation (Xia *et al.*, 2009). The expression of OPN at the maternal-fetal interface implicates its fundamental role in maintaining the uterine-embryonic micro-environment (Wang *et al.*, 2018).

Similarly, studies in mice show an ovarian oestrogen surge promotes OPN expression in the uterine glandular epithelium that induces blastocyst endometrial adhesion during implantation (Qi *et al.*, 2014). Proceeding decidualization, OPN is greatly expressed in decidual cells and is under progesterone control. OPN is then involved in trophoblast cell invasion through the enzymatic activity of MMPs, which are implicated in various processes such as angiogenesis, as seen in **Figure 1.3** (Qi *et al.*, 2014). Matrix metalloproteinases are enzymes that proteolytically degrade the extracellular matrix (ECM). Angiogenesis requires degradation of the vascular basement membrane and remodeling of the ECM to allow endothelial cells to migrate and invade into the surrounding tissue. MMPs participate in this remodeling of basement membranes and ECM (Rundhaug, 2005). MMPs detach pericytes from vessels undergoing angiogenesis, by releasing ECM-bound angiogenic growth factors, therefore displaying integrin binding sites. This cleaves endothelial cell-cell adhesions (Rundhaug, 2005).

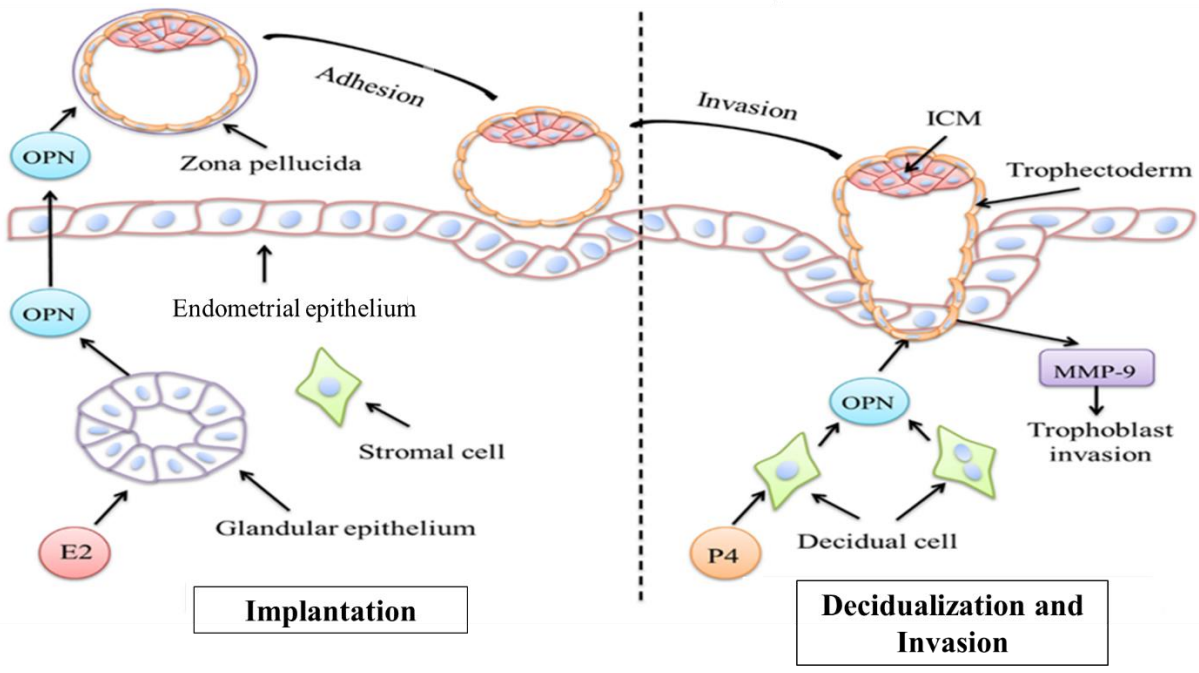

Figure 1.3: Role of osteopontin in implantation, invasion, and decidualization (Adapted from Qi *et al.*, 2014). Abbreviations: Oestrogen (E2), Intracellular matrix (ICM), Progesterone (P4)

1.6.1 Osteopontin in preeclampsia

A study conducted by Xia *et al.* reported a downregulation of both OPN and αvβ3 in the placenta of preeclamptic women compared to normotensive pregnancies implying its involvement in the pathogenesis of PE (Xia *et al.*, 2009). Proper modulation of proliferative CT to an invasive phenotype and a signalling cascade is needed for normal placentation (Gabinskaya *et al.*, 1998). The decreased expression of αvβ3 in preeclampsia hinders these intrinsic signal transduction pathways, with sustained OPN expression in the absence of αvβ3 receptors, accompanied by abnormal invasion (Gabinskaya *et al.*, 1998). The latter study also demonstrated a gestational age-related pattern of OPN expression in decidual extravillous trophoblast (EVT) cells in PE. In contrast, a study conducted in PE showed a notable elevation in plasma OPN with concurrent endothelial damage (Stenczer *et al.*, 2010).

1.6.2 Osteopontin in HIV

In a retrospective observational study, plasma OPN was significantly higher in HIV positive individuals receiving ART in comparison to HIV negative individuals (Bryant *et al.*, 2016). A four-month study conducted on human subjects showed a persistent elevation of OPN levels in the plasma of HIV-infected patients despite clinical improvements following HAART initiation (Chagan-Yasutan *et al.*, 2009). This elevation may emanate from the pro-inflammatory cytokine function of OPN following exposure to ART (Chagan-Yasutan *et al.*, 2009).

Another function of OPN is immune regulation at the maternal-fetal interface (Johnson *et al.*, 2003). The source of OPN during ART emanates from the activation of T-cells and antigen-presenting cells (Chagan-Yasutan *et al.*, 2009) because OPN is involved in CD4+ T helper (Th1) cell linage commitment (Shinohara *et al.*, 2006) and is an early T-lymphocyte-activating factor within the ECM (Ashkar *et al.*, 2000; O'Regan *et al.*, 2000).

1.7 Neuropilin-1 and soluble neuropilin-1

Neuropilin-1 (NRP-1) is a non-signalling transmembrane protein and co-receptor of VEGF receptors (Arad *et al.*, 2017). It has a high affinity to $VEGF_{165}$ and placental growth factor (PlGF) -2 (Felmeden *et al.*, 2003). The specificity of NRP-1 co-receptors for $VEGF_{165}$ and their binding capacity is mediated by amino acids located at the carboxyl-terminal chain of the exon 7-encoded peptide of $VEGF_{165}$ (Neufeld *et al.*, 1999; Wu *et al.*, 2010). VEGF-A can also bind to VEGFR-2, with a lower affinity, in the presence of NRP-1, but this is an essential mediator of angiogenesis, promoting endothelial cell migration and proliferation (Shibuya, 2006; Pandey *et al.*, 2018). NRP-1 binds to VEGF2 in the

presence of $VEGF_{165,}$ which stimulates PI3K to activate Akt, thereby promoting angiogenesis, as seen in **Figure 1.4** (Chen *et al.*, 2007).

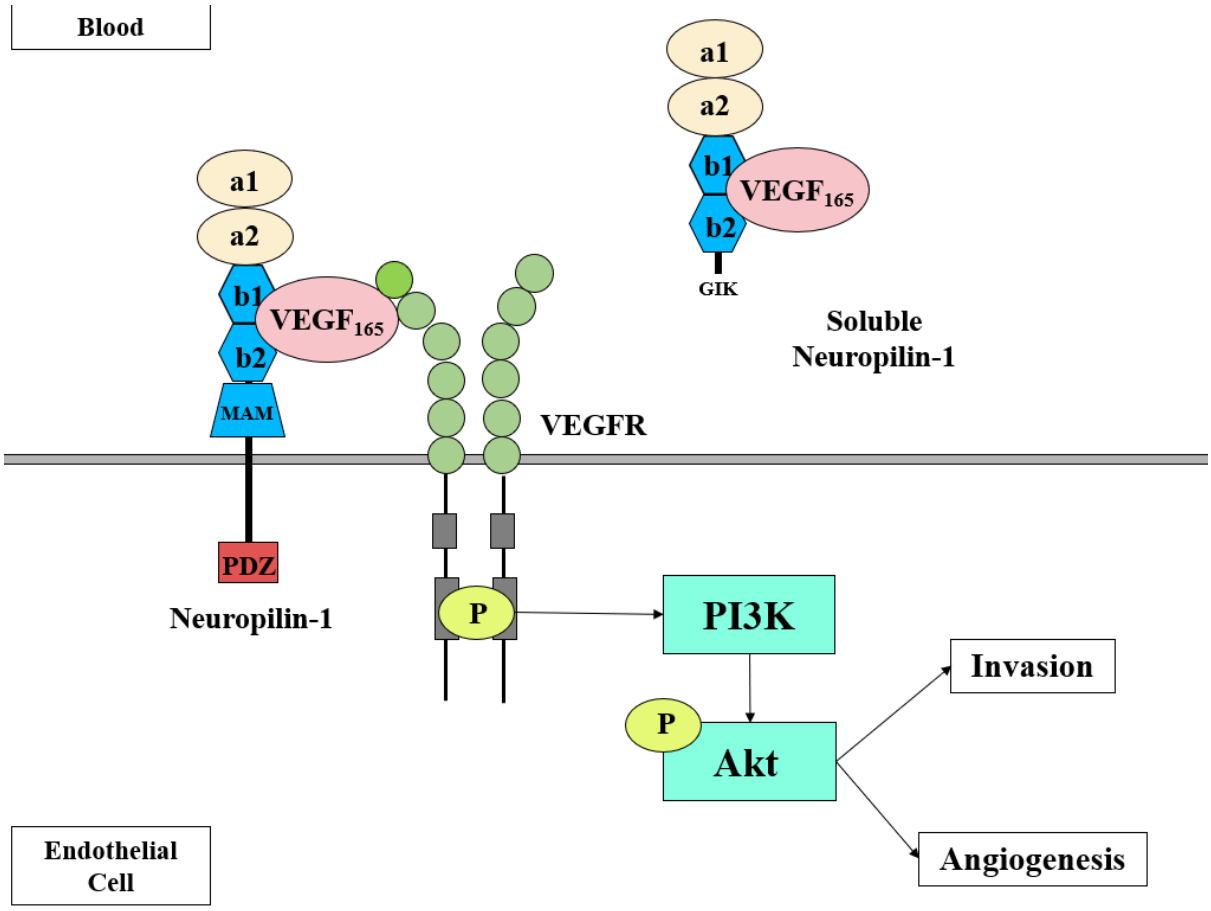

Figure 1.4: Structure and mechanism of NRP-1 in angiogenesis (Adapted from Chen *et al.*, 2007). Binding domains: a1,a2,b1,b2, MAM, PDZ and GIK. *Abbreviations: Phosphate group (P)

Soluble neuropilin-1 (sNRP-1) is an anti-angiogenic molecule (Cackowski *et al.*, 2004) that binds to and sequesters VEGF-A ($VEGF_{165}$) and PlGF (Klagsbrun *et al.*, 2002). An overexpression of sNRP-1 in tumour cells leads to damaged vasculature, subsequently promoting tumour cell apoptosis in prostate cancer (Gagnon *et al.*, 2000). In another study, sNRP-1 counteracted the upregulation in inflammation and oedema induced by VEGF overexpression in cutaneous delayed-type hypersensitivity reactions (Mamluk *et al.*, 2005). A previous study implicated sNRP-1 in the inhibition of human breast carcinoma cell migration, suggesting the antagonistic role of sNRP-1 in angiogenesis and tumorigenesis compared to the full-length NRP-1 (Cackowski *et al.*, 2004). Notably, NRP-1 functions as a receptor for both VEGF-A and semaphorin 3A (SEMA 3A) (Romano *et al.*, 2016). However, SEMA 3A exerts anti-angiogenic effects such as impaired endothelial cell adhesion, migration, and survival *in vitro* (Romano

et al., 2016). Considering that $VEGF_{165}$ and SEMA 3A are competitive inhibitors, their imbalance may affect the degree of tumour angiogenesis and in turn, alter sNRP-1 levels (Miao and Klagsbrun, 2000). Full-length NRP-1 is expressed in the human decidua and trophoblast cells, proposing its involvement in embryonic implantation and placentation (Baston-Buest *et al.*, 2011).

1.7.1 Soluble neuropilin-1 in preeclampsia

The anti-angiogenic and apoptotic potential of sNRP-1 implicates it in PE development as defective vasculature, and elevated apoptosis is a characteristic feature of the disorder (Naicker *et al.*, 2013). However, there is a lack of research on the role and regulation of sNRP-1 in pregnancy. A reduced expression of full-length NRP-1 was observed within the syncytiotrophoblast villous layer in PE compared to normotensive pregnancies, implicating its role in the development of PE (Arad *et al.*, 2017). Another study analysing the expression NRP-1 and VEGF also showed significantly lower NRP-1 and VEGF levels in both preeclamptic women and in homocysteine-induced PE in mice, which are suggested to promote endothelial damage and dysfunction predisposing to PE development (Xu *et al.*, 2016). Moreover, the downregulation of placental NRP-1 expression in fetal growth-restricted pregnancies complicated with absent end-diastolic flow in the umbilical artery correlates with PE development (Maulik *et al.*, 2016). The opposing nature of full-length NRP-1 makes sNRP-1 a possible candidate in PE pathogenesis.

1.7.2 Soluble neuropilin-1 in HIV

Contrary to PE, research on NRP-1 and HIV-infection show an upregulation in VEGFR2 and its co-receptor NRP-1 (Korgaonkar *et al.*, 2008). The lack of literature on sNRP-1 in HIV infection prompts future research on sNRP-1 and NRP-1 regulation in viral infection. Lane *et al.* reported that pre-incubation of β-herpesvirus murine cytomegalovirus with sNRP-1 dramatically inhibits infection by reducing virus attachment (Lane *et al.*, 2020). Moreover, NRP-1 has been implicated in binding the novel severe acute coronavirus 2 to cell surface NRP-1 upon entry (Cantuti-Castelvetri *et al.*, 2020; Daly *et al.*, 2020). So far, studies only shed light on the anti-angiogenic ability of sNRP-1; however, knowledge on its effects in systemic inflammation and HIV-associated conditions is lacking.

1.8 Rationale

Although the implementation of The Millennium Developmental Goals from 1990-2015 globally decreased maternal deaths by 44%, South Africa failed to reach the target set by the United Nations (World Health Organization, 2015). Thereafter, SA adopted the Sustainable Development Goals 2016-2030 to lower its maternal mortality ratio to less than 70 deaths per 100 000 live births. Maternal mortality from HIV and obstetric haemorrhage decreased between 2011-2017; however, deaths emanating from HDP, predominantly PE, remained unchanged and are the most common direct cause of maternal mortality (Naicker *et al.*, 2019).

This study contributes to demystifying the condition of PE and assists in achieving these developmental goals. This study addresses three areas of concern identified by the World Health Organization and United Nations, i.e., maternal health, child mortality, and HIV infection (World Health Organization, 2015; United Nations, 2016).

A greater risk of mortality was observed due to HDP among women who received ART compared to untreated women (HM Sebitloane and J Moodley, 2017). Notably, the opposing immune response of PE and HIV infection would indicate a neutralization (Phoswa *et al.*, 2019). However, the initiation of ART increases the prevalence of PE in HIV-infected women by facilitating immune reconstitution (Kalumba *et al.*, 2013; HM Sebitloane and J Moodley, 2017). In South Africa, more than 95% of pregnant women are reportedly living with HIV infection and receive various forms of ARVs (World Health Organization, 2019). The province of KwaZulu-Natal has an HIV prevalence of more than 40%, which includes a large number of women in their reproductive age (15-49 years) (UNAIDS, 2018). With a significant contribution to the HIV pandemic, KZN has the highest rollout of HIV treatments making it a suitable region for this study. Research on the dual-burden of HIV infection and PE is still lacking.

The conflicting angiogenic roles of OPN and sNRP-1 has been highlighted in many diseases. However, there is a dire paucity of data on the influence of OPN and sNRP-1 in PE. Moreover, there is an absence of information of OPN and sNRP-1 in HIV-associated PE. In light of the aforementioned criteria, it was important to study the influence of OPN and sNRP-1 in the synergy of HIV-infection and PE.

1.9 Aim, objectives, hypothesis, and research question

Main Aim

To evaluate the regulation of OPN and sNRP-1 concentration in HIV-infection comorbid with PE compared to normotensive pregnancies.

Specific Objectives

1. To quantify serum osteopontin and soluble neuropilin-1 levels in normotensive and preeclamptic pregnant women (irrespective of HIV status) and based on HIV status (irrespective of pregnancy type) using a BioPlex Multiplex Immunoassay.

2. To quantify and compare serum osteopontin and soluble neuropilin-1 concentration across all groups (normotensive HIV-positive, normotensive HIV-negative, preeclamptic HIV-positive, and preeclamptic HIV-negative) using a BioPlex Multiplex Immunoassay.

3. To analyse patient demographic and clinical data.

Hypothesis

OPN and sNRP-1 concentration will be dysregulated in PE compared to normotensive pregnancies, complicated by HIV infection.

Research Question

How is OPN and sNRP-1 serum concentrations regulated in PE, HIV infection, and HIV superimposed on PE compared to normotensive pregnancies?

CHAPTER TWO

Review Article: Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19.

This review article discusses the role and regulation of angiogenic, inflammatory, and other factors in preeclampsia, HIV infection. Moreover, in light of the COVID-19 pandemic and also in line with the South African COVID-19 Lockdown, a DOHET approved peer-reviewed review article that scrutinizes the use and safety of antiretroviral therapy in preeclampsia concurrent with the HIV-COVID-19 syndemic was submitted and accepted. The citation for this manuscript is outlined below. Note, the format of the manuscript is congruent with the journal.

Citation: Naidoo N., Moodley J., and Naicker T. (2020). Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19. **Accepted** by *Hypertension Research*. Manuscript ID: HTR-2020-0647.R2. Impact Factor: 3.150

Hypertension Research

Maternal Endothelial Dysfunction in HIV-Associated Preeclampsia Comorbid with COVID-19: A Review

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Manuscript ID	HTR-2020-0647.R2
Manuscript Type:	Review Article
Date Submitted by the Author:	07-Nov-2020
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Keyword:	Antiretrovirals, Endothelial dysfunction, HIV, Preeclampsia, SARS-CoV-2
Category:	Blood Vessels, Pregnancy, Renin-Angiotensin-Aldosterone System, Therapeutics

Hypertension Research

Decision Letter (HTR-2020-0647.R2)

From: eic-htr@jpnsh.jp

To: nitaliatally@gmail.com, 216013288@stu.ac.za

CC: admin-htr@jpnsh.jp

Subject: Hypertension Research - Decision on Manuscript ID HTR-2020-0647.R2

Body: 07-Nov-2020

Dear Dr. Naidoo:

It is a pleasure to accept your manuscript entitled "Maternal Endothelial Dysfunction in HIV-Associated Preeclampsia Comorbid with COVID-19: A Review" in its current form for publication in Hypertension Research. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

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MATERNAL ENDOTHELIAL DYSFUNCTION IN HIV-ASSOCIATED PREECLAMPSIA COMORBID WITH COVID-19: A REVIEW

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ABSTRACT

This review assesses markers of endothelial dysfunction (ED) associated with the maternal syndrome of preeclampsia (PE). We evaluate the role of antiretroviral therapy (ART) in human immunodeficiency virus (HIV)-infected preeclamptic women. Furthermore, we briefly discuss the potential of lopinavir/ritonavir (LPV/r), dolutegravir (DTG), and remdesivir (RDV) in drug repurposing and their safety in pregnancy complicated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In HIV infection, the trans-activator of transcription protein, which has homology with vascular endothelial growth factor, impairs angiogenesis, leading to endothelial injury and possible PE development despite neutralization of their opposing immune states. Markers of ED show strong evidence supporting the adverse role of ART in PE development and mortality compared to treatment-naïve pregnancies. Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 infection, exploits angiotensin-converting enzyme 2 (ACE 2) to induce ED and hypertension, thereby mimicking angiotensin II-mediated PE in severe cases of infection. Upregulated ACE 2 in pregnancy is a possible risk factor for SARS-CoV-2 infection and subsequent PE development. The potential effectiveness of LPV/r against COVID-19 is inconclusive; however, defective decidualization, along with elevated markers of ED, was observed. Therefore, the safety of these drugs in HIV-positive pregnancies complicated by COVID-19 requires attention. Despite the observed endothelial protective properties of DTG, there is a lack of evidence of its effects on pregnancy and COVID-19 therapeutics. Understanding RDV-ART interactions and the inclusion of pregnant women in antiviral drug repurposing trials is essential. This review provides a platform for further research on PE in the HIV-COVID-19 syndemic.

KEYWORDS: Antiretrovirals, Endothelial dysfunction, HIV, Preeclampsia, SARS-CoV-2

INTRODUCTION

Maternal mortality is a major concern worldwide, with its prevalence being particularly high in low- and middle-income countries (LMICs) [1,2]. Sub-Saharan Africa has the highest burden of maternal deaths, namely, 66% of the global estimate [3]. The leading direct cause of maternal mortality in South Africa (SA) is preeclampsia (PE) [3].

Hypertensive disorders of pregnancy (HDP) are classified as follows: chronic hypertension (high blood pressure predating pregnancy or present at/or before 20 weeks of gestation); gestational hypertension, which is persistent *de novo* hypertension that develops at/or after 20 weeks of gestation without evidence of other organ involvement; PE without severe features; and PE with severe features [4]. PE is defined as new-onset hypertension presenting after 20 weeks of gestation in conjunction with one or more characteristic features, such as proteinuria and/or acute kidney injury, persistent headache, visual disturbances, epigastric pain, HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), eclampsia (hypertension-associated seizures in pregnancy), and uteroplacental dysfunction, including fetal growth restriction [4]. Maternal mortality is present in all categories of HDP with eclampsia and PE, with severe features being the most common diagnosis before death [5]. It has been reported that PE accounts for >70,000 maternal deaths and 500,000 fetal deaths worldwide every year [4]. Globally, PE complicates 5-7% of pregnancies, and this incidence often increases to greater than 10% in LMICs [6].

Although the exact etiology of PE remains elusive, endothelial dysfunction (ED) initiates the maternal syndrome of PE as a result of placental hypoxia, a reduction in uterine natural killer (uNK) cells, oxidative stress (OS), angiogenic imbalance and an exaggerated inflammatory response [7]. Human immunodeficiency virus (HIV) infection and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection also impact the inflammatory response and endothelial function. It is unclear whether HIV infection increases or decreases the frequency of PE. Nonetheless, the synergistic effect of these inflammatory conditions occurring concurrently requires investigation.

PATHOPHYSIOLOGY OF PREECLAMPSIA

In a normal pregnancy, the uteroplacental vasculature undergoes a significant morphological and physiological transformation to sustain fetal development [8]**.** Usually, cytotrophoblast (CT) cells derived from the tips of the chorionic villi migrate into the decidua and the inner myometrium in a set-time sequence [9,10]. Thereafter, they fuse to form the multinucleated syncytiotrophoblast (ST) layer, which encloses the floating villi of the placenta and establishes the maternal-fetal interface for efficient gaseous and nutrient exchange [9,11]. Extravillous trophoblast (EVT) cells infiltrate fibrinoid-type material that replaces the musculo-elastic media of the spiral arteries, converting them into low-resistance large flaccid sinusoidal-like arteries [11,12]. In the decidua, uNK cells regulate the depth of placentation and spiral artery remodeling [7]. The lumen of the spiral arteries is dilated five- to tenfold, ensuring an adequate supply of blood to the developing fetus [13]. These changes are typically achieved by 20 weeks of gestation [14].

Aberrant vascular remodeling predisposes the individual to PE development. Preeclampsia is considered a two-stage placental disease where stage 1, often referred to as the fetoplacental or asymptomatic stage, occurs during the first and second trimesters of pregnancy [15]. In this stage, CT cells fail to take on the invasive endothelial phenotype; hence, CT migration is deficient, and there is a lack of physiological transformation of the myometrial spiral arteries [16,17]. The resulting small arterial lumen, surrounded by vasoactive medial cells, is unable to provide adequate blood to meet the oxygen and nutrient demands of the fetus [17,18]. This reduction in blood flow creates a hypoxic-ischemic microenvironment that marks the second stage [7,19]. Stage 2, also referred to as the maternal stage, prompts the release of antiangiogenic factors and other mediators that initiate systemic inflammation, OS, and endothelial cell (EC) dysfunction. These mediators pre-empt the maternal syndrome of PE (presence of hypertension, proteinuria, liver dysfunction, cerebral edema, eclampsia, *etc.*) [4,20]. An imbalance in circulating angiogenic factors persists during the pathogenesis of maternal syndrome [7].

PATHOGENESIS OF THE MATERNAL SYNDROME IN PREECLAMPSIA

Neovascularization (new blood vessel formation) results from either vasculogenesis or angiogenesis [21]. Vasculogenesis is the *de novo* construction of blood vessels from precursor cells, such as angioblasts, which differentiate into ECs that shape lumens to form simple blood vessels. In contrast, angiogenesis is the formation of new capillaries from the pre-existing vasculature [21,22]. Angiogenesis is strongly associated with female reproductive conditions such as decidualization, implantation and embryonic development [23]. Proangiogenic factors such as vascular endothelial growth factors (VEGFs) and placental growth factor (PlGF) are released into circulation, thereby increasing vascular permeability and promoting proteolysis of the extracellular matrix (ECM) via proteases, leading to EC proliferation, migration and infiltration into the lumen and subsequent endothelial maturation [24,25]. An array of VEGF isoforms, namely, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF, are present in the blood circulatory system and are responsible for various vascular processes. VEGF-A binding to VEGFR-1 does not produce significant receptor activation (in this case, the receptor acts as a decoy), whereas VEGF-B binding to VEGFR-1 promotes cell survival [26]. VEGF-A can also bind to VEGFR-2, with a lower affinity, in the presence of NRP-1, a coreceptor of VEGF, thereby promoting EC migration and proliferation (Figure 1) [26,27]. However, VEGFs and their receptors are significantly downregulated in preeclamptic conditions due to the overexpression of their antiangiogenic counterparts [28].

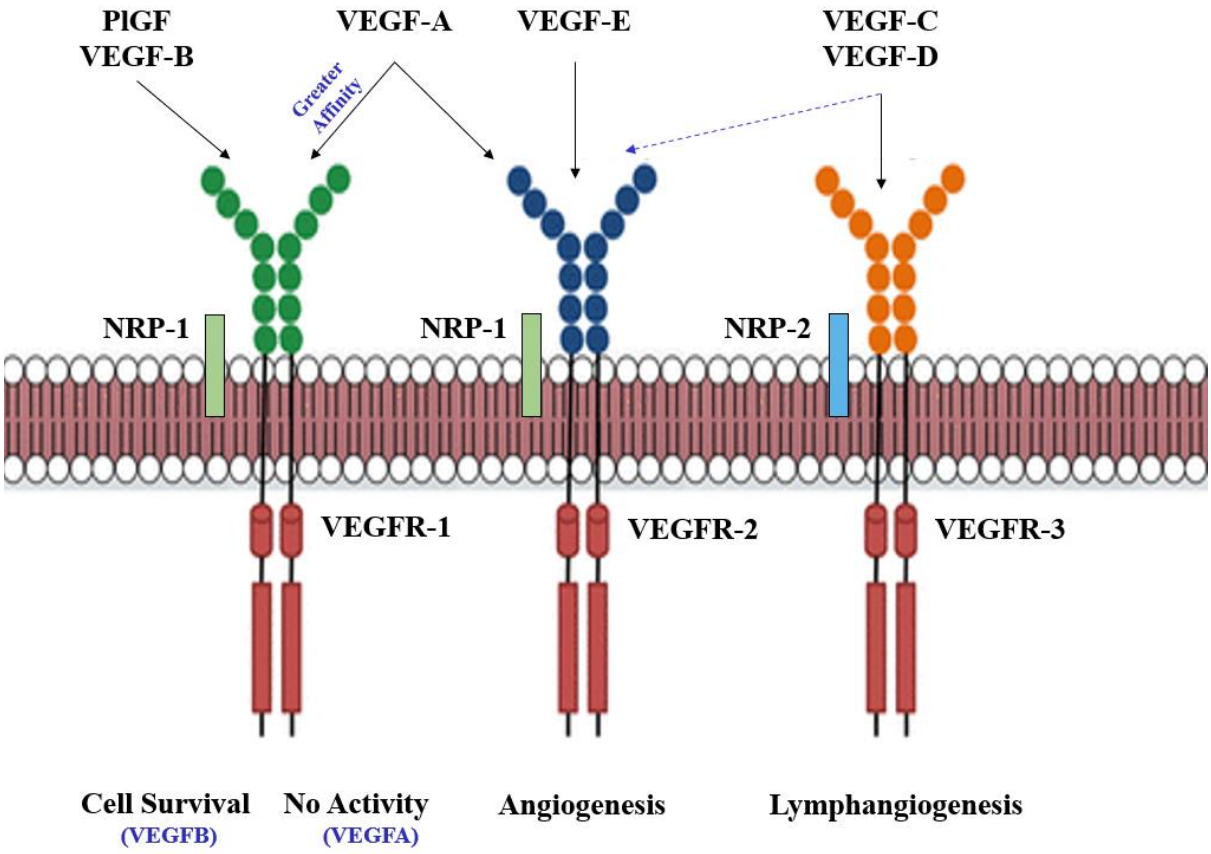

Figure 1: Differential functions of vascular endothelial growth factor receptors. Adapted from Pandey *et al.* [27]. Abbreviations: Vascular endothelial growth factor-A, B, C, D, and E (VEGF-A, B, C, D, and E); VEGF receptor-1, 2, and 3 (VEGFR-1, 2, and 3); Neuropilin-1 and 2 (NRP-1 and 2); Placental growth factor (PlGF)

Soluble fms-like tyrosine kinase (sFlt-1), also known as sVEGFR-1, is the soluble form of endothelial-bound VEGF receptors and functions as a VEGF antagonist to maintain angiogenic homeostasis [29]. Elevated sFlt-1 prevents VEGF and PlGF binding to VEGFR-2 on ECs, thus hindering angiogenic signal transduction leading to EC injury [30]. Concentrations of sFlt-1 are markedly elevated in pregnancy and are even higher in PE [31,32]. Studies have demonstrated that the overexpression of sFlt-1 in rats induces PE-like syndrome early in pregnancy, supporting the role of antiangiogenic factors in PE development [33]. Assessment of the imbalance in the sFlt-1/PlGF ratio is currently used in the diagnosis

and management of PE; however, more accurate and effective modes of early detection are urgently needed [34].

Endoglin (Eng), a coreceptor for the transforming growth factor (TGF) group of factors, is involved in vascular remodeling and hemostatic events via the activation of the endothelial nitric oxide synthase (eNOS) pathway that facilitates angiogenesis. In contrast, soluble endoglin (sEng), an extracellular variant of Eng, is highly expressed by trophoblasts and opposes TGF-β interactions with its receptor, thereby preventing vasodilation [35]. Upregulation of sEng impedes potent production of the vasodilator nitric oxide (NO) in ECs via its binding with TGF-β [36]. Therefore, exaggerated levels of sEng observed in PE may be central to the characteristic hypertension encountered during the maternal syndrome of the disease [35].

Oxidative/nitrosative stress that causes endothelial injury in PE emanates from an imbalance between pro-oxidants and their therapeutic antagonists (antioxidants) [37]. This stress includes an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) production and/or diminished availability of antioxidant mechanisms [38]. The release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6 and IL-8 from the ischemic placenta is intensified by syncytiotrophoblast microparticle (STMB) recruitment of monocytes and neutrophils to damaged EC sites [36]. These inflammatory cytokines not only decrease the bioavailability of NO and prostaglandin I2 (PGI2) but also produce ROS, which stimulates the elevation of endothelin-1 (ET-1), a potent vasoconstrictor. Vascular smooth muscle contraction results from an imbalance of endothelial vasodilators (NO and PGI2) and vasoconstrictors [Angiotensin II (Ang II), ET-1, and thromboxane A2 (TXA2)] during EC damage [39]. Vasoconstrictors decrease calcium ion efflux from smooth muscle cells through protein kinase C and Rho-kinase activation [39]. This leads to sustained vascular resistance and the hypertensive hallmark of endothelial injury observed in PE [33], depicted in Figure 2.

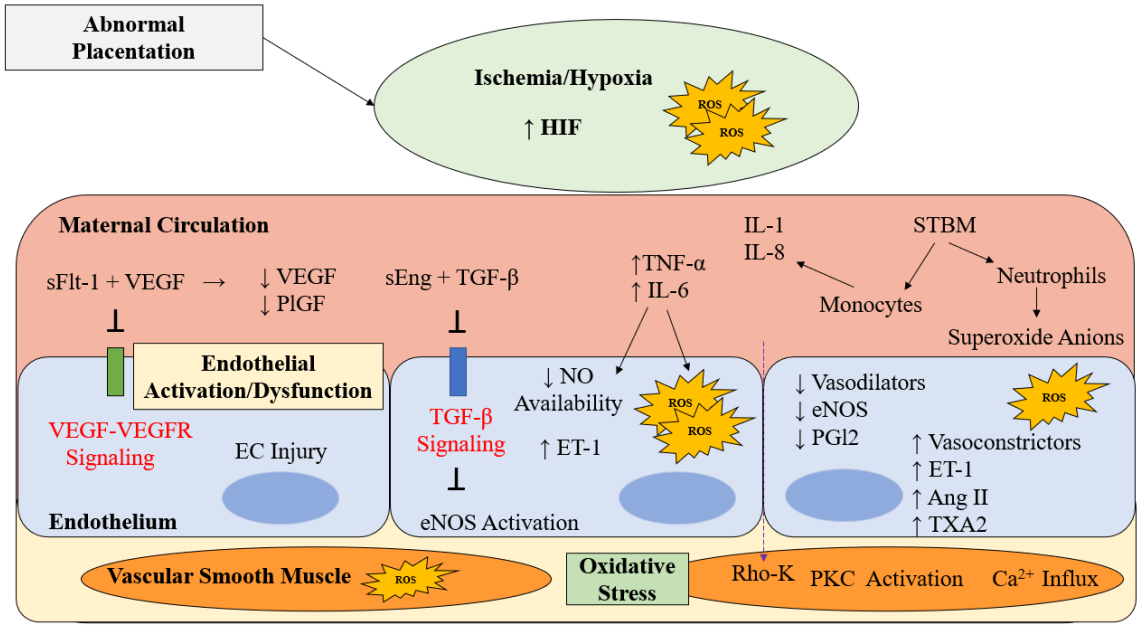

Figure 2: Endothelial dysfunction in Preeclampsia. Adapted from Moghaddas *et al.* [36]. Abbreviations: Angiotensin II (Ang II); Endothelial nitric oxide synthase (eNOS); Endothelin-1 (ET-1); Hypoxia-inducible factor (HIF); Interleukin-1, 6, and 8 (IL-1, IL-6, and IL-8); Nitric oxide (NO); Prostaglandin (PGI2); Protein kinase C (PKC); Placental growth factor (PlGF); Reactive oxygen species (ROS); Soluble endoglin (sEng); Soluble fms-like tyrosine kinase-1 (sFlt-1); Syncytiotrophoblast microparticles (STBMs); Transforming growth factor-β (TGF-β); Tumor necrosis factor-α (TNF-α); Thromboxane A2 (TXA2); Vascular endothelial growth factor (VEGF); VEGF receptor (VEGFR)

MATERNAL ANTIOXIDANT IMBALANCE and OXIDATIVE/NITROSATIVE STRESS IN PREECLAMPSIA

The endothelial cell activation encountered during PE exacerbates systemic inflammation and increases the expression of leukocyte adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin (SELP), and E-selectin (SELE) [40,41]. Leukocytes such as neutrophils and macrophages express nicotinamide adenine dinucleotide-phosphate (NADPH) oxidase, which generates superoxide (O^{2-}) with subsequent production of other free radicals

leading to a respiratory burst [42]. Usually, this process is tightly regulated; however, an increase in this phenomenon greatly overwhelms reducing agents such as glutathione, glutathione peroxidase, superoxide dismutase and catalase, resulting in OS and endothelial damage [43]. Elevated proinflammatory cytokines, such as TNF-α, observed in PE not only promote NO degradation leading to O^{2-} generation but also induce free radical production during oxidative phosphorylation, further contributing to EC injury [44,45]. Moreover, increased levels of proinflammatory cytokines such as IL-1 and TNF-α upregulate lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which consequently elevates the receptor for oxidized low-density lipoprotein (oxLDL), thereby facilitating O_2^- production via NO degradation [46,47]. In maternal circulation, STBMs evoke ED via the activation of LOX-1 with an increase in O_2^- and a subsequent reduction in NO-mediated vasodilation [48]. However, contradictory studies demonstrate a significant upregulation in oxLDL with reduced concentrations of LOX-1 in PE [49]. Elevated agonist autoantibodies against angiotensin receptors (AT1-AA) due to placental ischemia enhance Ang II sensitivity via angiotensin II type I receptor (AT1) in PE [50]. Higher levels of AT1-AA have demonstrated increased placental OS [51] due to superoxide production through NADPH activation [52,] which may result in vascular injury, deficient trophoblast invasion, placental hypoxia, inflammation, angiogenic imbalance and reduced bioavailability of NO [53]. Additionally, free fetal hemoglobin and circulating xanthine oxidase induce ROS production through various mechanisms [38]. These pathways can lead to eNOS uncoupling, generating O_2^- [54], which may prompt NO-O_2^- interactions and the production of the potent oxidant peroxynitrite, which inevitably predisposes cells to damage and DNA fragmentation and alteration [55]. Peroxynitrite can also hinder eNOS activity and disrupt endothelium-dependent vasodilation [56]. Reactive oxygen species have also been shown to downregulate the calcium-activated potassium channels KCa2.3 and KCa3.1, which are vital for electrical stimulation of vascular smooth muscle to ensure effective vasodilation [38].

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 AND PREECLAMPSIA

The outbreak of coronavirus disease 2019 (COVID-19) was declared a pandemic in March 2020 by the World Health Organization (WHO) [57]. At present, over 43.3 million cases of COVID-19 have been confirmed, with approximately 1.15 million deaths in over 218 countries and territories [58]. Genetic

analysis of the novel beta-coronavirus revealed that its entry mechanism exploits the renin-angiotensin system (RAS) [59,60]. The virus thereafter induces an array of symptoms, including vasoconstriction, elevated blood pressure and profibrotic pathway activation via coagulation [61]. An observational study conducted on COVID-19-infected pregnant women revealed that severe to critical cases of COVID-19 present with PE-like symptoms exclusive to placental maladaptation [62]. PE mimicry by COVID-19 was confirmed following the alleviation of preeclamptic symptoms without delivery of the placenta, which is currently the only known method for obtaining resolution of the clinical signs and symptoms of PE [62]. This prompted further insight into COVID-19's role in PE.

During normal RAS activation, renin catalyzes the conversion of angiotensinogen into angiotensin I (Ang I). Angiotensin I is further cleaved by angiotensin-converting enzyme (ACE) to form Ang II [63]. The physiological antagonist of ACE and Ang II, angiotensin-converting enzyme 2 (ACE 2), serves to cleave Ang I and Ang II into angiotensin 1-9 and angiotensin 1-7 [Ang (1-7)], respectively, bringing about vasodilatory, anti-inflammatory, and antifibrotic effects upon binding to its Mas receptor [61,64]. RAS activation is, therefore, dependent on the balance between ACE and ACE 2. Pregnant women are partially unreceptive to circulating Ang II to maintain low vascular resistance. However, this adaptation is reversed in PE, leading to an angiogenic imbalance [65]. In SARS-CoV-2 infection, ACE 2 receptors are increased and exploited for effective viral infectivity, which decreases ACE 2 function, subsequently upregulating Ang II activity [66]. The decrease in ACE 2 function, along with an increase in the Ang II/Ang (1-7) ratio, may result in hypoxia-induced upregulation of sFlt-1 [67,68], which further sensitizes ECs to Ang II [69]. Similar to PE, COVID-19 infection shows an increase in the sFlt-1/PlGF ratio due to the pathologic Ang II/Ang (1-7) imbalance [70]. Angiotensin II acts through its receptors (AT1 and AT2) to induce vascular impairment, which is the initiator of the maternal syndrome of PE, thereby reinforcing Ang II-mediated ED [71], as depicted in Figure 3.

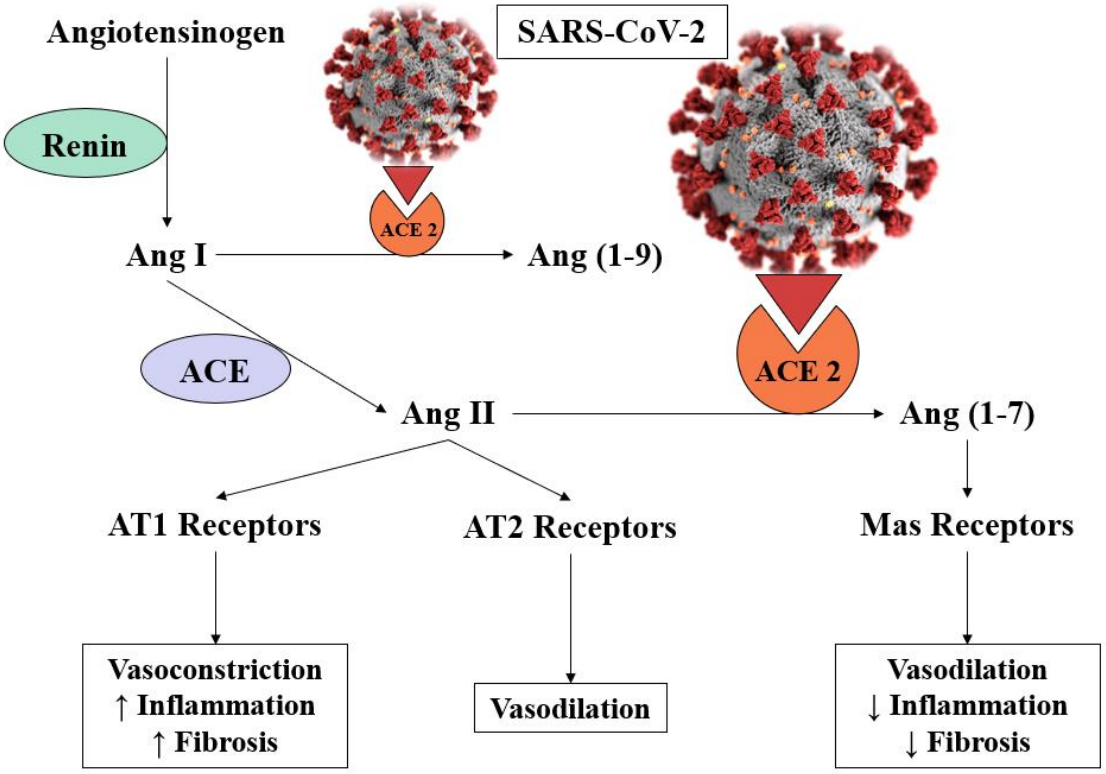

Figure 3: Manipulation of RAS by SARS-CoV-2 in COVID-19. Abbreviations: Angiotensin-converting enzyme (ACE); Angiotensin-converting enzyme 2 (ACE 2); Angiotensin (1-7), (1-9), I and, II [Ang (1-7), (1-9), I, and II]; Angiotensin type 1 (AT1) receptors; Angiotensin type 2 (AT2) receptors; Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

The elevated expression of ACE 2 in STs, CTs and the placental vasculature is imperative for blood pressure mediation for sufficient perfusion of the developing fetus. Therefore, SARS-CoV-2 infection and its alteration of ACE 2 expression may lead to dire adverse outcomes [72,73]. A recent review highlighted that both normal pregnancy and COVID-19 infection show upregulation of ACE 2, IL-8, and IL-10; thus, pregnancy may be a risk factor for COVID-19 morbidity [74]. They also postulated that increased expression of ACE 2 receptors in the placenta might escalate the risk of vertical transmission of SAR-CoV-2 infection [74]. This suggestion is supported by the predominant localization of SARS-CoV-2 in STs at the maternal-fetal interface of the placenta, potentiating severe comorbidity among

COVID-19-complicated pregnancies [75]. Conflicting evidence has revealed no significant differences in ACE 2 expression between normotensive pregnant women and preeclamptic women in the third trimester; however, the data are inconclusive, as PE onset occurs earlier in gestation [76]. Another study showed no significances in the prevalence rates of intrauterine growth restriction (IUGR) and PE between COVID-19-negative and COVID-19-positive pregnant women. The observed ED in this study was attributed to the 'cytokine storm' of COVID-19, similar to the proinflammatory state of PE. This is further supported by Shanes et al., who showed altered maternal vascular perfusion following placental hypoxia, conceivably due to systemic inflammation, in sixteen placentas obtained from COVID-19-infected women [77]. In contrast to PE, acute lung injury, and acute respiratory distress syndrome (ARDS) have upregulated VEGF levels, which increases vascular permeability. Moreover, the same study identified VEGF-D as the most prominent indicator related to the severity of clotting in COVID-19 [78].

SOLUBLE ANGIOTENSIN-CONVERTING ENZYME 2 IN THE THERAPEUTIC INTERVENTION OF COVID-19

Unlike the ACE 2 receptor, soluble angiotensin-converting enzyme 2 (sACE 2) is unable to facilitate SARS-CoV-2 entry into cells due to its lack of cell membrane interactions along with the absence of transmembrane serine protease 2 (TMPRSS2), a corequisite for SARS-CoV-2 endocytosis [79]. Soluble ACE 2 is formed through ACE 2 receptor cleavage/shedding by disintegrin and metalloproteinase 17 (ADAM17) and is suggested to have protective effects against SARS-CoV-2 infection [80]. sACE 2 may serve as a competitive decoy for the coronavirus, thereby reducing the binding of viral particles to membrane-bound ACE 2 and consequently reducing viral infectivity [81]. Research on therapeutic interventions using sACE 2 has revealed its higher affinity for COVID-19, thereby neutralizing the virus without altering endogenous ACE 2 homeostasis [82]. However, research on the effects and safety of sACE, TMPRSS2 and ADAM17 manipulation in pregnancy and HIV-infected individuals comorbid with COVID-19 has yet to be established.

HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND PREECLAMPSIA

The trans-activator of transcription (Tat) protein is a regulatory protein of HIV-1 that improves the efficiency of viral infectivity [83]. The rich arginine and lysine arrangement seen in Tat resembles the VEGF sequence [84]. Therefore, Tat mimics the role of VEGF by promoting EC adhesion and αvβ3 and α5β1 integrin expression [84,85], which also binds osteopontin (an angiogenic factor involved in decidualization) [86]. A study conducted on HIV-1 Tat-induced angiogenesis demonstrated that Tat protein notably reduced endothelium-dependent vasorelaxation and eNOS expression and regulation in ECs of porcine coronary arteries [37]. The latter study also implicated Tat in coronary artery disease, which is associated with the long-term effect of PE [37]. In addition, Tat protein was also shown to induce the expression of ICAM-1 and VCAM-1, suggesting a possible mechanism by which HIV-1 infection contributes to endothelial injury and accelerates atherosclerosis [87,88]. Therefore, it is plausible that Tat's homology with VEGF affects angiogenesis in PE.

In contrast to the exaggerated immune state of PE, there is significant immune suppression after HIV infection [89,90]. Although the infection has been shown to reduce the risk of developing PE, most studies show that pregnant women receiving highly active antiretroviral therapy (HAART) have an increased prevalence of PE development [90,91]. This increase is believed to be due to immune restoration [92]. Recent studies show no difference in the risk of PE development between treated and untreated HIV-infected pregnant women [93], but others have reported findings that do not support the notion that HIV infection has protective qualities against HDP development [94].

ROLE OF HIV THERAPY IN MATERNAL ENDOTHELIAL DYSFUNCTION

The WHO recommends that all individuals living with HIV infection receive HAART, regardless of their $CD4^+$ count and disease stage (including pregnant and breast-feeding women) [95]. HAART or antiretrovirals (ARVs) not only improve life expectancy but also decrease the risk of mother-to-baby (vertical) transmission of the infection *in utero* during birth and breastfeeding [95]. However, ARVs may trigger severe PE development [96]. A study conducted on nucleoside/nucleotide reverse transcriptase

inhibitors (NRTIs), namely, azidothymidine, tenofovir disoproxil fumarate and lamivudine, revealed dysregulation of EC proliferation and migration [97]. The study also suggested that NRTIs induce mitochondrial OS, which hinders the activation and transduction of endothelial receptor tyrosine kinase signals and VEGFR-2 pathways in vascular ECs [97]. Additionally, this adverse effect on angiogenesis may predispose the individual to PE development [98]. Excessive production of ROS is associated with increased trophoblast apoptosis, which may occur in placental-mediated disorders, such as PE and/or IUGR, overpowering antioxidant defenses with deleterious effects [98].

Protease inhibitors (PIs) deter HIV aspartyl protease, causing reconstitution of the immune microenvironment, which may predispose the individual to PE development [99]. *In vivo*, three PIs [atazanavir, lopinavir (LPV), and ritonavir (r)] significantly lower progesterone in trophoblast cells, thus indicating its hindrance of trophoblast proliferation and migration [100]. In a recent study, Kala et al. showed that LPV-based ART dysregulated uterine decidualization and spiral artery remodeling in both human *ex vivo* and mouse *in vivo* models [101]. Lower expression of the chemokines VEGF, PlGF, angiopoietin-2, granulocyte-macrophage colony-stimulating factor, interferon-gamma and matrix metalloproteinase 9 (MMP-9) was observed upon LPV exposure of primary decidual cell cultures [101]. They reported uNK cell depletion and deficient trophoblast invasion as a result of decreased expression of the transcription factor STAT3, which mediates decidualization [101]. These observations highlight the events that precede widespread ED in PE and its associated adverse neonatal outcomes. HAART impairs nuclear factor kappa B (NF-κB) transcription factors that decrease MMP and VEGF expression, which inevitably dysregulate angiogenesis, promoting ED and PE development [102]. The placentae of HIV-infected women receiving zidovudine-containing ART showed evidence of mitochondrial DNA depletion, elevated OS levels, and apoptosis, implicating secondary mitochondrial failure potentiating PE development and adverse perinatal outcomes [103].

Increased immune-expression of Flt-1 and sFlt-1 was observed within trophoblast cells during PE, regardless of HIV status, implying autocrine signaling in trophoblast invasion and differentiation [104]. This is believed to promote abnormal placentation with subsequent EC dysfunction in PE [104]. Pre-

HAART exposure in HIV infection showed lower PlGF levels and increased sFlt-1 in women who developed PE compared to normotensive pregnant women [105]. Multivariate analysis demonstrated that PlGF and viral load were significantly related to PE development, and no significant shifts were observed in angiogenic factors following HAART among normotensive women [105]. Increased sFlt-1 and sEng levels were linked to PE regardless of HIV infection [106]. This study also elucidated a significant downregulation in PlGF levels in HIV-negative preeclamptic women compared to normotensive women. However, HIV infection downregulates PlGF in normotensive pregnant women compared to their HIV-negative counterparts (p = 0.02), thereby predisposing the individual to PE development [106]. TGF-β1 levels remain unchanged in HIV infection regardless of the increase in its coreceptor sEng [106,107].

In contrast, a study of HIV-associated PE women revealed that HIV/HAART is linked to a significant downregulation of IL-2, TNF-α and IL-6, with substantial decreases in IL-2 and TNF-α observed in preeclamptic women [108]. Saums *et al.* found that integrase strand transfer inhibitor-containing ARTs had a greater frequency of HDP development than protease inhibitor-containing regimens [93]. Another study concluded that HIV infection, rather than its pharmacological treatment, induces alterations in markers of endothelial function [109]. The short-term duration of treatment with HAART reduces some markers of ED, including VCAM-1, with no differences between protease inhibitors and nonnucleoside reverse transcriptase inhibitors. However, SELP remained elevated upon exposure to both treatments [109].

The repurposing of various antiviral drugs (Table 1) has gained momentum as a desperate measure to prevent the deleterious effects of COVID-19 [110].

Table 1: Antiviral drug repurposing for COVID-19 therapeutics highlighted in this review

Drugs	Mechanism of action	Safety in pregnancy	Effectiveness in treatment of COVID-19	Placental transfer	Clinicaltrials.gov: COVID-19 (including pregnant women)
Lopinavir/ Ritonavir	Antiretroviral (Protease inhibitor) SARS-CoV 3-chymotrypsin-like cysteine *protease* inhibitor [111]	Considered safe in pregnancy [112,113] despite contradicting data [100,101]	Potential to reduce mortality, although no benefit beyond standard care is clinically proven [114]	Low [115,116]	NCT04364022
Dolutegravir	Antiretroviral (Integrase strand transfer inhibitor) Possibly inhibits 2′-O-ribose methyltransferase involved in coronavirus infectivity [117]	Recommended for HIV-infected pregnancies (International guidelines, 2020) [118] Potential risks of neural tube defects (initiation < 6 weeks gestation) [119]	No clinical evidence	Moderate to high [120]	None
Remdesivir	Broad-spectrum antiviral (Viral RNA-dependent RNA polymerase inhibitor)	Requires greater research	Potential to improve clinical improvement time [121] FDA approved for compassionate use (22/10/2020) [122]	Unknown [116]	NCT04292899 NCT04292730 NCT04582266

ANTIRETROVIRAL THERAPY IN PREGNANCY AND CORONAVIRUS DISEASE 2019

It is plausible to assume that HIV-infected individuals receiving ARVs have a lower risk of developing complications from COVID-19 infection [123-126]. Protease inhibitor-based ARVs, such as LPV/r, have shown potential against SARS-CoV-2 infection due to their ability to bind SARS-CoV-1. Studies have shown a strong sequence homology between SARS-CoV-1 and SARS-CoV-2 [127]. However, SARS-CoV-2 binds ACE 2 with a 10-20-fold greater affinity than SARS-CoV-1, which explains the high human transmission and infectivity rates of SARS-CoV-2 [128]. Lopinavir/ritonavir lowers the risk of patients developing acute respiratory distress syndrome (ARDS) and subsequently dying from SARS-CoV-2 infection [129]. In various subsequent clinical trials comparing prenatal exposure to LPV/r and prenatal exposure to efavirenz (EFV), there were no significant differences in adverse outcomes in pregnancy [130] or other control measures [131]. These drugs, therefore, have become the preferred drugs of choice for pregnancy complicated by COVID-19 in China [132].

Randomized controlled trials (RCTs) are essential for providing standard guidance on clinical management, even in an emergency setting, since RCTs offer data without bias due to confounding factors, as seen in nonrandomized studies [133]. Randomized controlled trials of LVP/r in severe COVID-19 showed no benefit beyond standard care [114]. Other RCTs revealed that a combination of antiviral drugs (interferon beta-1b, ribavirin, and LPV/r) were more successful in symptom alleviation than LPV/r alone in mild to moderate COVID-19 cases [134]. A systemic review of RCTs of LPV/r in COVID-19 highlighted that ARVs may reduce mortality; however, this reduction varies across different risk groups [135].

The South African National ART guidelines employ LPV/r-based ARTs as the second-line therapy in HIV-infected adults [136]. The guidelines further recommend that women who become pregnant while receiving the LPV/r-containing regimen should continue treatment with monthly clinical observations [136]. Dolutegravir (DTG), the newly established ARV in South Africa, together with two NRTIs, is also recommended as a second-line ART after failing a non-NRTI-based first-line regimen since DTG is suggested to be better tolerated by HIV-infected individuals than PIs such as LPV/r [119]. South Africa

has experienced over 716,700 confirmed cases of COVID-19 as of late October 2020 [58]. Considering the slow switch from previously approved ARVs to DTG, LPV/r-containing ARTs are still readily available in SA, and clinical trials may include women in their first trimester. Notably, LPV/r has a negative influence on decidualization and placentation; therefore, the safety of these drugs in HIV-associated PE complicated by COVID-19 infection requires urgent and intensive scrutiny.

LATEST ANTIRETROVIRAL THERAPY EFFECTS ON ENDOTHELIAL DYSFUNCTION

In 2017, SA and other low/middle-income countries agreed to launch a new high-quality ART, a single-tablet regimen containing an integrase strand transfer inhibitor (INSTI), DTG, which provides rapid viral suppression [137]. Notably, the 2019 ART guidelines in SA state that the preferred first-line ART regimen is a DTG-based drug (TLD) for patients experiencing EFV side effects or those who prefer to use DTG [136]. However, the EFV-containing regimen (TEE) is recommended for use in the first 6 weeks of gestation and in women of child-bearing age due to a high risk of neural tube defects associated with TLD [136]. *In vivo* studies of human coronary artery endothelial cells (HCAECs) showed that DTG reduced inflammation and IL-6, IL-8, VCAM-1, and ICAM-1 secretion via NF-κB pathway inhibition and decreased senescence by repressing apoptotic pathways [138,139]. Dolutegravir also displayed protective properties in HCAECs, such as reduced OS, inflammation, and senescence and improved ED from an aged donor with persistently elevated levels of senescence [139]. The Stockholm pregnancy cohort showed that the PE rate was normal; however, the population size was too small to make any deductions [140]. In a study on treatment-naïve HIV-infected individuals, a significant decrease in TNF-α was observed 12 months following DTG initiation in comparison to PIs and other INSTIs, such as elvitegravir [141]. This study also revealed DTG's capacity to significantly reduce D-dimer levels [141], implicating a possible positive interaction in COVID-19-infected individuals since elevated D-dimer, a marker of clot formation is associated with increasing severity of the disease [142]. A case study of a 63-year-old HIV-infected woman with an undetectable viral load on a DTG-containing ART showed

improvement despite presenting with COVID-19 complications during admission [143]. However, the role of DTG in the treatment of COVID-19 requires further investigation.

In light of the lack of evidence that particular ARVs are clinically active against SARS-CoV-2, HIV-infected individuals are advised to refrain from changing their ART regimen in an attempt to prevent or treat COVID-19 [118].

REMDESIVIR IN COVID-19 AND PREGNANCY

Remdesivir (RDV), initially used in the treatment of Ebola, is among the top contenders against the new coronavirus [110]. Remdesivir is a broad-acting nucleoside analogue drug that has shown inhibitory effects on pathogenic animal and human coronaviruses such as SARS-CoV-2 *in vitro* and inhibits Middle East respiratory syndrome coronavirus, SARS-CoV-1 and SARS-CoV-2 replication in nonhuman primates [144]. In a randomized, double-blind, placebo-controlled trial, RDV showed no significant difference in terms of clinical benefits [145]. However, a larger study population is needed to confirm the observed reduction in clinical improvement time following RDV treatment [145]. Another RCT's final report revealed that RDV was superior to placebo in decreasing recovery time among hospitalized adults [121]. However, neither trial included pregnant women. A case report of RDV-based treatment showed the successful management of a COVID-19-positive critically ill obstetrics patient [146]. Another case report of RDV-treated COVID-19 in the third trimester of pregnancy showed no adverse outcomes apart from elevated transaminases, which is also associated with PE development [147]. Preeclampsia as a cause of transaminitis was ruled out, as the patient did not present with hypertension and proteinuria [147]. This report also noted that there was no clarity on whether the transaminitis observed was due to COVID-19 or RDV intake [147]. In a recent study, 86 pregnant, and postpartum women with severe COVID-19 who received compassionate use of remdesivir showed a high rate of recovery with a low rate of serious adverse events, such as transaminitis, hypertension and hypoxia [148]. In Ebola clinical trials, there were no adverse outcomes among pregnant women receiving RDV [80]. On October 22, 2020, the United States Food and Drug Administration (FDA) approved the emergency use of RDV

for severe cases of COVID-19 [122]. However, the safety of RDV in pregnancy has not been elucidated; therefore, the inclusion of pregnant women in clinical trials is necessary to guide risk-benefit considerations of RDV treatment in COVID-19.

LMICs such as SA have a limited capacity to accommodate the daily rise in COVID-19 infections [149]. The use of RDV may be vital for the prevention of adverse outcomes and a decrease in clinical improvement time in severe COVID-19 cases while regulating intensive care unit bed capacity [149]. Recently, Gilead Sciences Incorporated, CIPLA was granted a license to manufacture and distribute a generic form of remdesivir for compassionate use against COVID-19 in 127 countries, including SA; however, RDV is still not readily available to all citizens [150]. There is also no knowledge on the interactions of RDV with ARVs.

CONCLUSION

An imbalanced angiogenic status, inflammation and oxidative/nitrosative stress induced by placental maladaptation facilitate pervasive multi-organ ED in PE. Adverse effects associated with HIV infection and ART promote ED predisposing PE development; however, higher prevalence and mortality rates among PE cases are still associated with ART use. Pregnancies complicated by the COVID-19 exploitation of ACE 2 have a strong correlation with PE-like symptoms such as endothelial injury, implicating COVID-19 in PE onset. Despite the inconsistent data on LPV/r against COVID-19, its availability in LMICs suggests the need for further insight into its safety in HIV-associated PE complicated by COVID-19. The observed endothelial protective properties of DTG in pregnancy and its role in COVID-19 therapeutics, along with the approved compassionate use of RDV in pregnancy, have yet to be established.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

HUMAN AND ANIMAL RIGHTS AND INFORMED CONSENT

This article does not contain any studies with human or animal subjects performed by any of the authors.

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CHAPTER THREE

Original Article: The Regulation of Osteopontin and Soluble Neuropilin-1 in HIV-Associated Preeclampsia

In line with the specific objectives of this dissertation, we evaluated the concentration of angiogenic proteins, osteopontin (OPN) and soluble neuropilin-1 (sNRP-1), in the synergy of HIV-infection and PE development. This research was submitted to a high impact peer-reviewed DOHET-approved journal (citation below). Note, the format of the manuscript is consistent with the journal.

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The Regulation of Osteopontin and Soluble Neuropilin-1 in HIV-Associated Preeclampsia

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Abstract:	Objectives: Since impaired decidualization and angiogenesis may be affected by antiretroviral therapy (ART) during pregnancy, the comorbidity of preeclampsia (PE) and human immunodeficiency virus (HIV) infection remains a concern to the South African healthcare system. This study evaluated the concentration of angiogenic proteins, osteopontin (OPN) and soluble neuropilin-1 (sNRP-1), in the synergy of HIV-infection and PE development. Study design: A Bio-plex multiplex immunoassay was used to quantify serum OPN and sNRP-1 concentrations in preeclamptic vs normotensive pregnancy type stratified by HIV status (n=19 per subgroup). Results: The concentration of OPN was significantly downregulated by pregnancy type (preeclamptic vs normotensive; $p = 0.0033$) and upregulated by HIV status (HIV-positive vs HIV-negative; $p = 0.0012$). In addition, there was a significant upregulation in sNRP-1 concentration by pregnancy type (preeclamptic vs normotensive; $p = 0.0054$) and by HIV status (HIV-positive vs HIV-negative; ($p = 0.0005$). In comparison to the normotensive HIV-negative group, there was a significant difference in sNRP-1 concentrations between the sub-groups normotensive HIV-positive ($p = 0.0049$), preeclamptic HIV-negative ($p = 0.0244$), and preeclamptic HIV-positive ($p < 0.001$), respectively. Conclusion: This innovative study validates a significant downregulation of OPN in PE compared to normotensive pregnancies in contrast to an upregulation of sNRP-1, conforming to the anti-angiogenic milieu of PE. Additionally, based on HIV-status, both OPN and sNRP-1 were upregulated in HIV-positive pregnancies. This may be attributed to the HIV trans-activator of transcription protein mimicry to vascular

	endothelial growth factor and/or the immune reconstitution following ART as well as to integrin expression that mediates endothelial cell tube formation during angiogenesis.

The Regulation of Osteopontin and Soluble Neuropilin-1 in HIV-Associated Preeclampsia

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Abstract

Objectives: Since impaired decidualization and angiogenesis may be affected by antiretroviral therapy (ART) during pregnancy, the comorbidity of preeclampsia (PE) and human immunodeficiency virus (HIV) infection remains a concern to the South African healthcare system. This study evaluated the concentration of angiogenic proteins, osteopontin (OPN) and soluble neuropilin-1 (sNRP-1), in the synergy of HIV-infection and PE development.

Study design: A Bio-plex multiplex immunoassay was used to quantify serum OPN and sNRP-1 concentrations in preeclamptic *vs* normotensive pregnancy type stratified by HIV status (n=19 per subgroup).

Results: The concentration of OPN was significantly downregulated by pregnancy type (preeclamptic *vs* normotensive; $p = 0.0033$) and showed a non-significant increase by HIV status (HIV-positive vs HIV-negative; $p = 0.5099$). In addition, there was a significant upregulation in sNRP-1 concentration by pregnancy type (preeclamptic *vs* normotensive; $p = 0.0054$) and by HIV status (HIV-positive *vs* HIV-negative; ($p = 0.0005$). In comparison to the normotensive HIV-negative group, there was a significant difference in sNRP-1 concentrations between the sub-groups normotensive HIV-positive ($p = 0.0049$), preeclamptic HIV-negative ($p = 0.0244$), and preeclamptic HIV-positive ($p < 0.001$), respectively.

Conclusion: This innovative study validates a significant downregulation of systemic OPN in PE compared to normotensive pregnancies in contrast to an upregulation of sNRP-1, conforming to an anti-angiogenic milieu. Additionally, based on HIV-status, both OPN and sNRP-1 were upregulated in HIV-positive pregnancies. It is plausible that the following three assumptions may have influenced angiogenesis: (1) HIV trans-activator of transcription protein mimicry to vascular endothelial growth factor and/or (2) the immune reconstitution following ART and (3) dysregulation of integrin expression that mediates endothelial cell tube formation during angiogenesis.

Keywords: HIV infection, Preeclampsia, Osteopontin, Soluble neuropilin-1

Introduction

Preeclampsia (PE) is a human pregnancy disorder which clinically manifests with new-onset hypertension after 20 weeks of gestation [1]. Globally, PE complicates 5-7% of pregnancies often escalating to greater than 10% in low-middle income countries (LMICs) and is annually accountable for a substantial fraction of maternal and foetal mortality [2,1]. Preeclampsia accounts for the majority of hypertensive disorders of pregnancy (HDP) in South Africa (SA) and has a prevalence rate of 12% in primigravidae at a regional hospital in the province of KwaZulu-Natal (KZN) [3,4].

The etiology of PE is ill-defined; however, its pathogenesis involves deficient extravillous trophoblast (EVT) invasion and a lack of physiological conversion of myometrial spiral arteries with subsequent placental maladaptation [5,6]. Consequently, a hypoxic-ischemic microenvironment brings about an imbalance in angiogenic and anti-angiogenic homeostasis [7,8]. This phenomenon is believed to be the initiator of pervasive multi-organ endothelial dysfunction in PE.

Osteopontin (OPN), an angiogenic factor, is expressed within the epithelial cells of the endometrium during the secretory phase, endometrial glands, and within the EVT cells and the placenta [9]. Also coupled with its integrin αvβ3 receptor, it facilitates cell adhesion and signaling, thereby promoting implantation, cell migration, invasion and placentation [10]. Also, OPN enhances vascular endothelial growth factor (VEGF) expression, generating a positive feedback signal that mediates angiogenesis [11]. The expression of OPN at the maternal-fetal interface alludes to its fundamental role in maintaining the uterine-embryonic microenvironment [9].

In contrast, soluble neuropilin-1 (NRP-1) is an anti-angiogenic polypeptide that binds and sequesters VEGF [12]. Its pro-angiogenic counterpart, full-length neuropilin-1 (NRP-1), is also expressed in the human decidua and trophoblast cells [13]; however, there is a paucity of information available on the role of sNRP-1 in pregnancy. Previous studies have highlighted a significant downregulation in blood and placental tissue OPN and full-length NRP-1 expression in PE and their probable influence in the pathogenesis of the disease [10,14-17].

Globally, 37.9 million individuals are living with the human immunodeficiency virus (HIV) infection, of which, approximately 7.97 million reside in SA [18,19]. The 2019 mid-year statistics of SA revealed that 22.71% of women in their reproductive ages (15-49 years) are HIV positive [19]. In KZN, more than 40% of women

receiving ante-natal care are HIV infected [20] and receive antiretroviral therapy (ART) [21]. The province of KZN has the highest global rollout of ARTs, making it a suitable region to conduct this study.

Notably, immunosuppressive HIV infection reduces the risk of developing PE by neutralizing the exaggerated immune response [22]. However, pregnant women receiving highly active antiretroviral therapy (HAART) or other forms of HIV treatment, show an increased prevalence of PE in comparison to untreated women [22,23]. There is conflicting evidence on the regulation of OPN and surprisingly, no studies on sNRP-1 and OPN in pregnant HIV-infected women [24-26]. In SA, 87% of pregnant women are reportedly living with HIV infection and receive ART [21].

In light of the lack of data on the dual-burden of HIV infection and PE, this study aimed to determine the regulation of OPN and sNRP-1 concentrations in HIV-infected pregnancies complicated by PE.

Materials and Methods

Ethical considerations

Institutional ethical approval was obtained for the prospective use of the retrospectively collected samples (BCA 338/17). In the primary study, health authority permission, written informed consent, as well as hospital managers' approval, was sought for the use of samples in subsequent studies.

Sample Size

Sample size was determined after consultation with an institutional biostatistician (Mrs. Catherine Conolly). A sample size of 76 pregnant women was required to detect a moderate effect size of 0.66 between two groups normotensive and preeclamptic women or HIV positive and HIV negative assuming equal groups (n=38 per group). To compare four groups, normotensive (HIV+ vs HIV-) and preeclamptic (HIV+ vs HIV-), a sample size of 19 in each group was needed to detect a large effect size of 0.95. All calculations are with 80% power and 95% probability and were done using G*Power statistical software.

Study population

The study consisted of 76 pregnant women attending a large regional hospital in Durban, KZN, SA. These women were grouped into normotensive (n = 38) and preeclamptic (n = 38) pregnancy type and further stratified by HIV status into HIV-negative normotensive (n = 19), HIV-negative preeclamptic (n = 19), HIV-positive normotensive (n = 19) and HIV-positive preeclamptic (n = 19) sub-groups. Patient demographic and clinical data is included in Table 1.

Inclusion criteria

Women diagnosed with preeclampsia was based on new-onset hypertension (blood pressure of ≥ 140/90 mmHg) and/or proteinuria (average of ≥ 300mg at least 4 hours apart), known HIV status (all HIV+ pregnant women were in receipt of ARVs), and singleton pregnancy were included.

Exclusion criteria

Women with eclampsia, chronic hypertension, intrauterine death, abruptio placentae, polycystic ovarian syndrome, chorioamnionitis, pre-existing seizure disorders, gestational diabetes, chronic diabetes, systemic lupus erythematosus, chronic renal disease, sickle cell disease, thyroid disorder, antiphospholipid antibody syndrome, connective tissue disorder, cardiac disease, asthma, unknown HIV status, patients who did not consent to participation, patients who were unable to provide informed consent and women without antenatal care were excluded from this study.

Sample collection

Blood samples of eligible participants were previously collected in EDTA-coated vacutainer tubes by the research nurse and centrifuged at 1000 g for 10 minutes at 4°C. This was followed by the transfer of serum into cryovials and stored in a biofreezer at -80°C until required.

Bio-Plex multiplex immunoassay

To analyze the serum concentrations of OPN and sNRP-1, a Bio-Plex multiplex immunoassay was performed according to the manufacturer's guidelines, MILLIPLEX® Map Human Angiogenesis Magnetic Bead Panel 2 96-Well Plate Assay # HANG2MAG-12K (Merck KGaA, Germany). Serum samples were diluted 1:5 in the assay buffer. In summary, OPN and sNRP-1 capture antibody-coupled beads were added to a 96-well plate. A biotinylated detection antibody was added to the wells and then conjugated with a reporter molecule, streptavidin-phycoerythrin (SA-PE) and incubated. Following the washing of the plate three times, and the addition of drive fluid, the reaction was complete for detection. This was performed using the Bio-Plex MAGPIX Multiplex reader which detected the fluorescence of the SA-PE bound to each bead, which was proportional to the concentration of the analytes in the sample (Bio-Rad Laboratories Inc., USA).

Data Analysis

A protocol was generated on the Bio-Plex Manager^{TM} software version 6.1, to acquire the data from the multiplex analysis. The known concentration (pg/ ml) was used to generate a standard curve for OPN and sNRP-1. The

concentrations of the samples were interpolated from the standard curve for each analyte and exported to an Excel spreadsheet for statistical analysis.

Statistical Analysis

Data were analyzed using GraphPad Prism software version 8.0.1 (GraphPad Software, San Diego, California, USA). Normality tests (D'Agostino & Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov) revealed non-parametrically distributed data. A Mann-Whitney U test was utilized to determine statistical significance according to pregnancy type (normotensive *vs* preeclamptic) and HIV status (negative *vs* positive). A Kruskal-Wallis test and Dunn's multiple comparison *post hoc* test was used across all groups. The results are represented as the median and interquartile range (IQR) and a p value < 0.05 was considered statistically significant.

Results

Patients demographics and clinical characteristics

Patient demographics and clinical characteristics are represented in **Table 1** as median and IQR. Significant differences across all groups were reported in maternal age ($p = 0.0211$), gestational age ($p = 0.0004$), parity ($p = 0.0042$), systolic blood pressure ($p < 0.0001$) and diastolic blood pressure ($p < 0.0001$). However, there was no statistical significance noted in maternal weight ($p = 0.2116$).

Table 1. Patient demographics across study groups (n = 76).

	Normotensive HIV-	**Normotensive HIV+**	**Preeclamptic HIV-**	**Preeclamptic HIV+**	***p* Value**
Maternal age (years)	25.00 (9.00)	31.00 (11.00)	28.00 (16.00)	34.00 (14.50)	0.0211*
Gestational age (weeks)	37.00 (9.00)	25.00 (14.00)	24.00 (10.00)	23.00 (10.00)	0.0004***
Parity	1.00 (1.00)	2.00 (1.00)	1.00 (1.00)	2.00 (1.00)	0.0042*
Systolic BP (mmHg)	109.00 (20.00)	112.00 (16.00)	146.00 (14.00)	147.00 (20.00)	< 0.0001***
Diastolic BP (mmHg)	65.00 (13.0)	72.00 (14.0)	93.00 (10.0)	97.00 (13.0)	< 0.0001***
Maternal weight (kg)	74.00 (22.00)	81.00 (28.00)	82.00 (44.00)	79.50 (35.00)	0.2116 (ns)

Data is presented as the median (IQR), ns = non-significant, *$p < 0.05$, ***$p < 0.001$.

Osteopontin

Pregnancy Type: OPN concentration was significantly lower in the preeclamptic (median = 2033.0 pg/ml, IQR = 2397.0 pg/ml) compared to the normotensive group (median = 3539.0 pg/ml, IQR = 2733.0 pg/ml), irrespective of HIV status (Mann-Whitney U = 416.0; $p = 0.0033$; **Fig. 1A**).

HIV Status: Irrespective of pregnancy type, OPN concentrations in HIV-positive pregnancies (median = 3174.0 pg/ml, IQR = 2413.0 pg/ml) was elevated compared to HIV-negative pregnancies (median = 2988.0 pg/ml, IQR = 3130.0 pg/ml), albeit, non-significantly (Mann-Whitney U = 622.5; $p = 0.5099$; **Fig. 1B**).

Across All Groups: A statistical significance was noted between the normotensive HIV-negative (median = 3887.0 pg/ml, IQR = 5416.0 pg/ml) versus preeclamptic HIV-negative (median = 1570.0 pg/ml, IQR = 2311.8 pg/ml) pregnant women (Kruskal-Wallis H = 14.49; $p = 0.0023$; **Fig. 1C**). No significant differences were noted across all other groups.

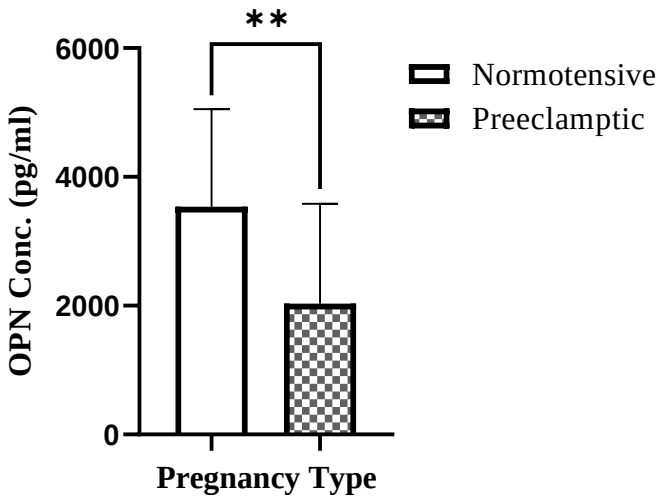

Fig. 1A Bar graph illustrating osteopontin concentration in preeclampsia *vs* normotensive pregnancy types.

**Serum concentrations of OPN are significantly different between the normotensive and preeclamptic group, $p = 0.0033$.

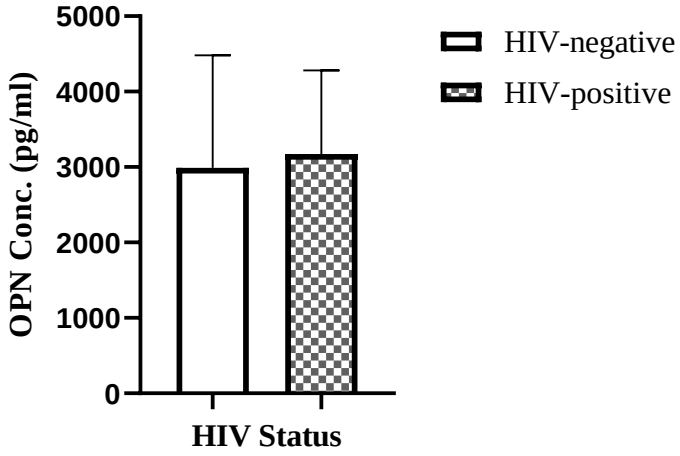

Fig. 1B Bar graph illustrating osteopontin concentration in HIV-positive *vs* HIV-negative groups.

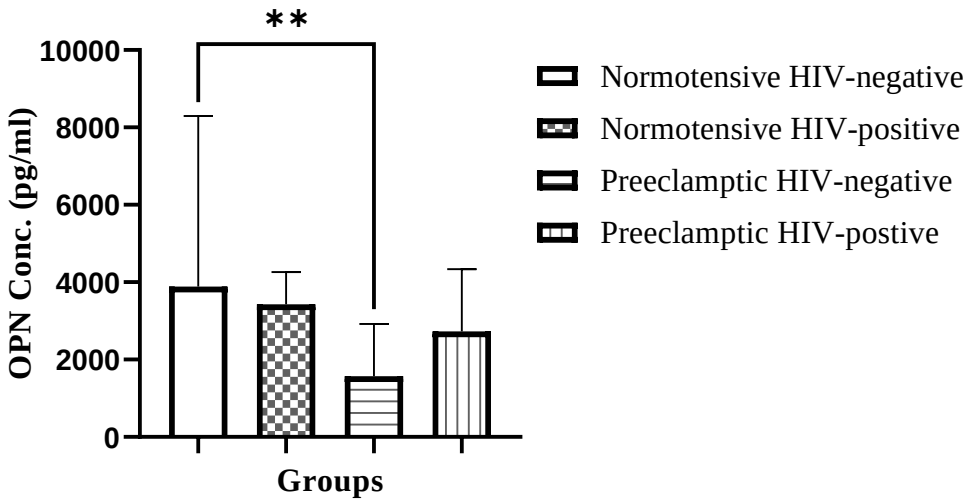

Fig. 1C Bar graph illustrating osteopontin concentration across all groups.

**Serum concentrations of OPN are statistically significant between normotensive HIV-negative and preeclamptic HIV-negative groups, $p = 0.0012$.

Soluble neuropilin-1

Pregnancy Type: The sNRP-1 concentration was significantly upregulated in the preeclamptic (median = 141355.0 pg/ml, IQR = 985811.0 pg/ml) compared to the normotensive group (median = 115961.0 pg/ml, IQR = 67229.0 pg/ml), irrespective of HIV status (Mann-Whitney U = 264.0; $p = 0.0054$; **Fig. 2A**).

HIV Status: There was a significant difference in sNRP-1 concentration between the HIV-negative (median = 114036.0 pg/ml, IQR = 69359.0 pg/ml) versus the HIV-positive (median = 153912.0 pg/ml, IQR = 981239.0 pg/ml) groups (Mann-Whitney U = 219.0; $p = 0.0005$; **Fig. 2B**).

Across All Groups: A statistically significant difference of sNRP-1 was noted between normotensive HIV-negative (median = 84517.0 pg/ml, IQR = 61885.0 pg/ml) versus normotensive HIV-positive (median = 146011.0 pg/ml, IQR = 43274.0 pg/ml) groups; normotensive HIV-negative (median = 84517.0 pg/ml, IQR = 61885.0 pg/ml) versus preeclamptic HIV-negative (median = 126795.0 pg/ml, IQR = 79576.0 pg/ml) groups; as well as between normotensive HIV-negative (median = 84517.0 pg/ml, IQR = 61885.0 pg/ml) versus preeclamptic HIV-positive (median = 179967.0 pg/ml, IQR = 976595.0 pg/ml) pregnancies (Kruskal-Wallis H = 21.05; $p = 0.0001$; **Fig. 2C**). No significant differences were noted across other groups.

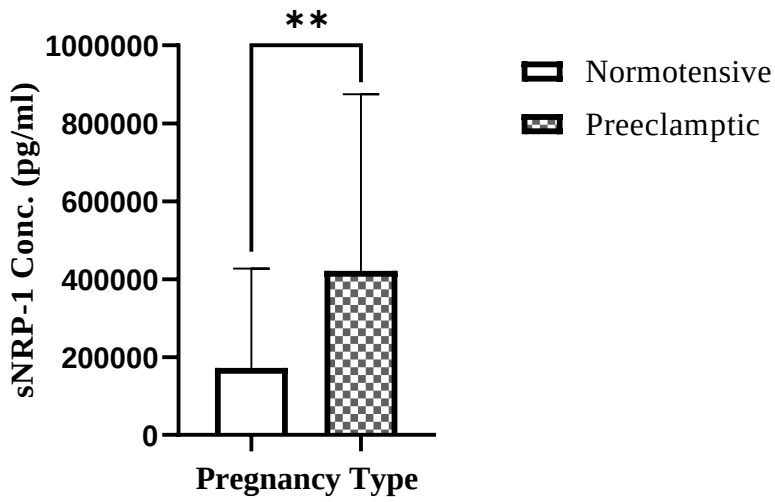

Fig. 2A Bar graph illustrating soluble neuropilin-1 concentration in preeclampsia *vs* normotensive pregnancy types.

**Serum concentrations of sNRP-1 are significantly different between the normotensive *vs* preeclamptic group, $p = 0.0054$.

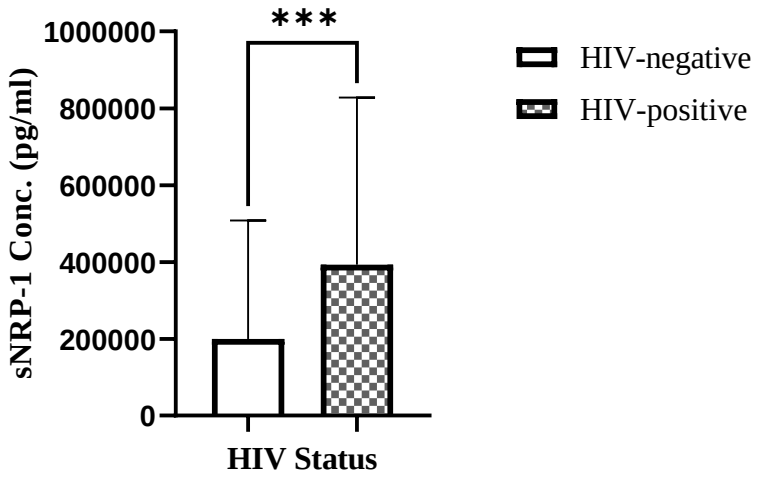

Fig. 2B Bar graph illustrating soluble neuropilin-1 concentration in HIV-positive *vs* HIV-negative groups.

***Serum concentrations of sNRP-1 are statistically significant between HIV-negative *vs* HIV-positive groups, $p = 0.0005$.

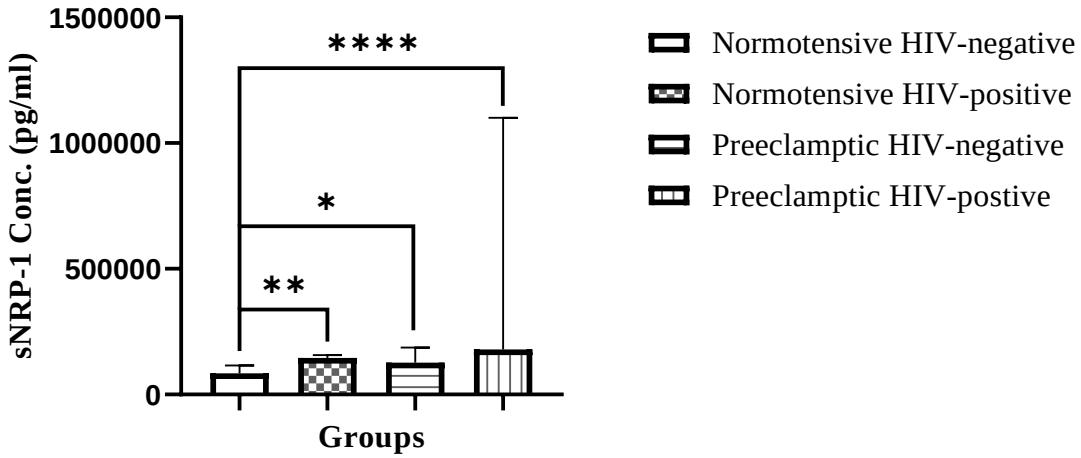

Fig. 2C Bar graph illustrating soluble neuropilin-1 concentration across all groups.

*Serum concentrations of sNRP-1 are significantly different between normotensive HIV-negative *vs* preeclamptic HIV-negative groups, $p = 0.0244$.

**Serum concentrations of sNRP-1 are statistically significant between normotensive HIV-negative *vs* normotensive HIV-positive groups, $p = 0.0049$.

****Serum concentrations of sNRP-1 are significantly different between normotensive HIV-negative vs preeclamptic HIV-positive groups, $p < 0.001$.

Discussion

Osteopontin

The main finding of our study is a significant downregulation of OPN in PE compared to normotensive pregnancies, regardless of HIV status. The multifunctional protein OPN is a class of extracellular matrix (ECM) glycoproteins that bind to integrin subunits (αv, β1, β3, and β5) via the arginine-glycine aspartate binding domain hence is reported to increase the invasion potential of cytotrophoblast cells [10,27-29]. OPN has been implicated in the progression and maintenance of healthy pregnancies [9].

Our findings are consistent with a previous study [10]; the defective trophoblast invasion in PE may be due to the decline of OPN. Modulation of the proliferative cytotrophoblast to an invasive phenotype is mediated by a signaling cascade to ensure normal placentation [14]. Notably, decreased αvβ3 integrin expression would hinder intrinsic signal transduction pathways, resulting in the abnormal trophoblast cell migration in PE [14]. Similarly, studies in mice show an ovarian estrogen surge promotes OPN expression in the uterine glandular epithelium and induces blastocyst endometrial adhesion during implantation [30]. Proceeding decidualization, OPN is highly expressed in decidual cells and is under progesterone control. More specifically, progesterone and cyclic adenosine monophosphate (cAMP), as well as heart and neural crest derivatives expressed transcript 2 (HAND 2), forkhead box O1 (FOXO 1), homebox A10 (HOXA 10), and signal transducers and activators of transcription (STAT), play a vital role in decidualization [31]. OPN thereafter mediates trophoblast cell invasion via the enzymatic activity of matrix metalloproteinases (MMPs), the decline of OPN in our study would correlate with the defective trophoblast invasion in PE.

It is well accepted that during PE development, angiogenic balance is altered in favor of high levels of anti-angiogenic factors such as soluble fms-like tyrosine kinase and soluble endoglin [32]. Since OPN is implicated in angiogenesis [30], the reduced OPN concentration in PE in our study is supported by its known pro-angiogenic function. In contrast, however, Stenczer *et al.* observed an elevation in OPN with concurrent endothelial damage in PE [33]. Surprisingly, Gabinskaya *et al.* also reported elevated OPN expression despite a concurrent reduction of αvβ3 integrin subunits in PE [14].

Furthermore, the decline of OPN concentration in PE in our study is associated with a lower median gestational age, compared to normotensive pregnancy [34]. Osteopontin activates anti-apoptosis and pro-survival pathways in

healthy pregnancy via phosphoinositide 3-kinase-Akt and nuclear factor-κB signaling molecules, modulate angiogenesis via VEGF induction and ECM proteolysis via MMPs [35]. The downregulation of OPN would favor elevated apoptosis which is a feature of PE [6].

In our study, irrespective of pregnancy type, OPN concentration exhibited an up-regulatory trend in HIV-positive compared to HIV-negative pregnancies, albeit non-significantly. This observation is corroborated by other studies that noted increased OPN levels in HIV-positive individuals receiving ART [24,25]. This elevation may emanate from the pro-inflammatory cytokine function of OPN following exposure to ART [25]. OPN is involved in CD4+ T helper (Th1) cell linage commitment [36]. Furthermore, it is an early T-lymphocyte-activating factor within the ECM [37,38] and functions in immune regulation at the maternal-fetal interface [39]. It has been suggested that the source of OPN during ART emanates from the activation of T-cells and antigen-presenting cells [25]. Notably, the opposing immune response of PE and HIV infection would indicate a neutralization [40]. However, the initiation of ART increases the prevalence of PE in HIV-infected women by facilitating immune reconstitution [41].

The trans-activator of transcription (Tat) protein is a regulatory protein of HIV-1 that promotes viral infectivity [42]. The sequential homology of Tat and VEGF allows for VEGF mimicry that promotes endothelial cell adhesion mediated by αvβ3 and α5β1 integrin expression [43,44], which bind to OPN [45]. Theoretically, the expression of integrins αvβ3 and α5β1 induced by Tat may subsequently increase OPN expression and its activity in HIV infection, as seen in our study. However, the influence of Tat on OPN regulation is unclear as all HIV-positive pregnant women recruited in our study received ART, a standard of care practice in SA.

Soluble Neuropilin-1

This study demonstrated a significant upregulation of sNRP-1 concentration in preeclamptic pregnancies compared to normotensive pregnant women, irrespective of HIV status. Neuropilin-1 binds $VEGF_{165}$ and PlGF via its b1b2 domains [46]. Since these domains also occur in sNRP-1, it would bind to and sequester $VEGF_{165}$ and PlGF [12]. Soluble neuropilin-1, however, is an anti-angiogenic molecule. An overexpression of sNRP-1 in tumor cells leads to damaged vasculature, subsequently promoting tumor cell apoptosis in prostate cancer [47]. Notably, PE has defective vasculature as well as an elevation of apoptosis [6]. In another study, sNRP-1 counteracted the upregulation in inflammation and oedema induced by VEGF overexpression in cutaneous

delayed-type hypersensitivity reactions [48]. A previous study implicated sNRP-1 in the inhibition of human breast carcinoma cell migration, suggesting the antagonistic role of sNRP-1 in angiogenesis and tumorigenesis compared to the full-length NRP-1 [49]. The latter findings have a twofold interpretation; one in inhibition of trophoblast cell migration as well as decreased angiogenesis in the pathological state of PE.

Considering the opposing functions of sNRP-1 and NRP-1, the increase of sNRP-1 observed in our study is supported by Arad *et al.* who showed a reduced expression of NRP-1 within the syncytiotrophoblast villous layer in PE compared to normotensive pregnancies [17]. Another study analyzing the expression of NRP-1 and VEGF also showed significantly lower levels of NRP-1 and VEGF in both preeclamptic women and in homocysteine-induced PE in mice which promote endothelial dysfunction [16]. Further evidence on NRP-1 downregulation in preeclamptic placental tissue correlates with foetal growth restriction where an absent end-diastolic flow in the umbilical artery suggests an anti-angiogenic state; a characteristic feature of PE [15].

Notably, NRP-1 functions as a receptor for both VEGF-A and semaphorin 3A (SEMA 3A) [50]. However, SEMA 3A exerts anti-angiogenic effects such as impaired endothelial cell adhesion, migration, and survival *in vitro* [50]. Considering that VEGF165 and SEMA 3A are competitive inhibitors, their imbalance may affect the degree of tumor angiogenesis and in turn, alter sNRP-1 levels [51].

In our study, we also report a significant increase in sNRP-1 concentrations between HIV-positive compared to HIV-negative pregnancies. The lack of literature on sNRP-1 in HIV infection prompts future research on sNRP-1 and NRP-1 regulation in viral infection. Lane *et al.* reported that pre-incubation of β-herpesvirus murine cytomegalovirus with sNRP-1 dramatically inhibits infection by reducing virus attachment [52]. Moreover, NRP-1 has been implicated in the binding of the novel severe acute coronavirus 2 to cell surface NRP-1 upon entry [53,54]. So far, studies only shed light on the anti-angiogenic ability of sNRP-1; however, knowledge on its effects in systemic inflammation and HIV-associated conditions is lacking. The upregulation of sNRP-1 in HIV-positive pregnancies may arise from ART providing evidence for the role of sNRP-1 in inflammation as well as its ability to complement antiretroviral inhibition of viral binding and infectivity. This study postulates a downregulation of sNRP-1 with an increase in NRP-1 in HIV infection; however, the opposite may be noted in HIV-positive women

receiving ART. This hypothesis is supported by Korgaonkar *et al.*, who reported an upregulation in VEGFR-2 and its co-receptor NRP-1 in HIV-infection [55]. A limitation of our study was that the origin/source of OPN and sNRP-1 were not elucidated.

In line with a novel study associating OPN-induced VEGF and full-length NRP-1 in tumor cell migration, growth, and angiogenesis [56], our study hypothesizes a physiological association between OPN and sNRP-1 in HIV-associated PE (**Fig. 3**). Antiretroviral therapies are involved in immune reconstitution, placentation, and oxidative stress. Antiretrovirals such as protease inhibitors decrease trophoblast cell secretion of OPN, thereby impairing trophoblast invasion and the physiological transformation of spiral arteries [58]. Also, nucleotide reverse transcriptase inhibitors generate reactive oxygen species hindering VEGF/VEGFR interaction and promoting PE development [59]. Similarly, soluble neuropilin-1 affects VEGF transduction [12]. Moreover, ART reconstitutes the immune state [41], thereby upregulating OPN [59] and sNRP-1.

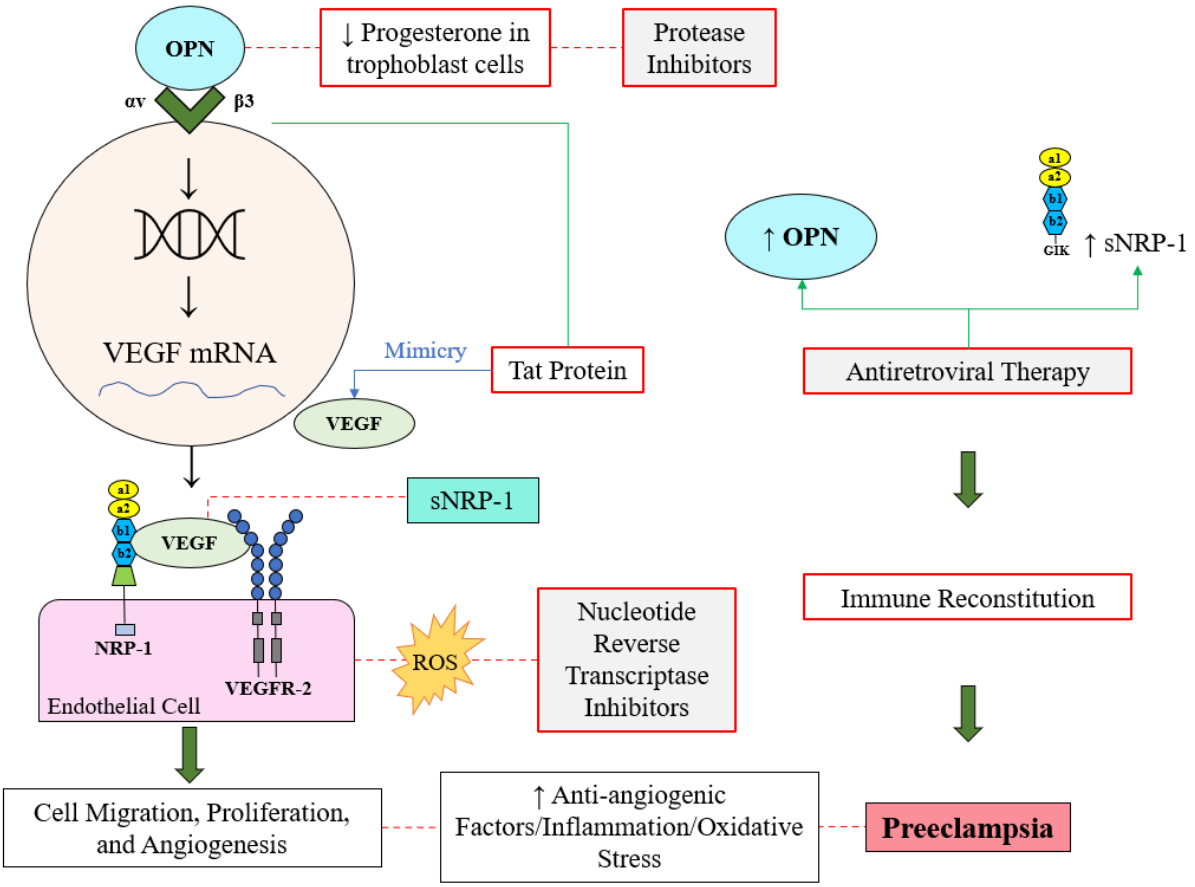

Fig. 3 Schematic representation of OPN-induced VEGF and sNRP-1 in HIV-associated PE. Upon αvβ3 binding, OPN induces VEGF messenger (mRNA) transcription and translation [56]. Through VEGFR-2 and NRP-1

reception, OPN-induced VEGF facilitates endothelial cell migration, proliferation, and angiogenesis [56]. However, increased anti-angiogenic factors, inflammation and oxidative stress in pregnancy predispose PE [23]. Furthermore, Tat protein in HIV-1 mimics VEGF and influences angiogenesis [43,44]. Antiretroviral therapy such as protease inhibitors and nucleotide reverse transcriptase inhibitors dysregulate angiogenesis in PE [56]. Protease inhibitors decrease trophoblast cell secretion of OPN, thereby impairing trophoblast cell invasion and spiral artery remodeling [59]. Also, nucleotide reverse transcriptase inhibitors induce mitochondrial oxidative stress interrupting VEGF/VEGFR subsequently leading to PE development [59]. Soluble neuropilin-1 binds and hinders VEGF signaling [12]. Moreover, ART reconstitutes the immune state [41], thereby upregulating OPN [60] and sNRP-1.

Conclusion

This novel study demonstrates a significant downregulation of OPN in PE compared to normotensive pregnancies, conforming to the defective trophoblast invasion in PE. Notably, the role of this glycoprotein is influenced by its binding to ECM integrin subunits. This study also reports a significant upregulation of sNRP-1 concentrations in preeclamptic pregnancies compared to their normotensive counterpart, irrespective of HIV status; probably linked to its anti-angiogenic functional activity in PE. This is the first study to demonstrate a significant increase in sNRP-1 and an upregulated trend in OPN concentration in HIV-positive compared to HIV-negative pregnancies. These differences may emanate from the effect of ARVs, sNRP-1 and OPN polymorphisms as well as HIV-1 Tat-VEGF mimicry. Also, OPN has a role in inflammation as well as an ability to complement ARV inhibition of viral binding, thereby preventing amplification and dissemination. Finally, both OPN and sNRP-1 are promising candidate proteins with predictor test value in HIV-associated PE.

Recommendations

Investigations on genetic polymorphisms of OPN and sNRP-1 are recommended for establishing their specific roles in conditions such as pregnancy and related disorders. Insight into the physiological association between OPN, full-length NRP-1 and sNRP-1, particularly receptor-mediated interactions and common signal transduction pathways, will demystify disorders such as PE. It may also be beneficial to correlate OPN and sNRP-1 together with other angiogenic and inflammatory factors across early-onset PE, late-onset PE, mild and moderate PE as

well as across all trimesters during gestation. Moreover, further large-scale studies are warranted to verify the role of OPN and sNRP-1 in the synergy of pregnancy, PE, and HIV infection.

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Ethical considerations

Institutional ethical approval from the University of KwaZulu-Natal was obtained for the prospective use of the retrospectively collected samples (BCA 338/17).

Declarations

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Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Consent to participate and for publication

Health authority permission, written informed consent from all participants as well as hospital managers' approval is available for this study.

Code availability:

Not applicable.

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CHAPTER FOUR

SYNTHESIS

The burden of maternal mortality in LMICs, such as SA is concerning (Lewis, 2008; Girum and Wasie, 2017). Non-pregnancy-related infections (particularly HIV infection) and hypertension account for 25.03% and 17.72% of the institutional maternal mortality ratio (iMMR), respectively, in SA (National Committee for Confidential Enquiry into Maternal Deaths, 2018). There is an urgent need to reduce iMMR (130.96 per 100 000 live births) in SA in order for the country to achieve the global Sustainable Developmental Goals 2016-2030 (World Health Organization, 2015; United Nations, 2016; National Committee for Confidential Enquiry into Maternal Deaths, 2018). South Africa has the highest ART-rollout in the world, including ARVs for the prevention of mother-to-child transmission (PMTCT) (Pattinson, 2014; Woldesenbet *et al.*, 2018). The influence of HIV-infection in the predisposition of PE is controversial (Frank *et al.*, 2004; HM Sebitloane and D Moodley, 2017; HM Sebitloane and J Moodley, 2017).

In view of the high prevalence of PE (14.8%) and HIV infection (30.7%) in pregnant women in SA, it is imperative that one examines the synergy of HIV-associated PE (Kalumba *et al.*, 2013; National Committee for Confidential Enquiry into Maternal Deaths, 2018; Woldesenbet *et al.*, 2018). Moreover, improving maternal health, reducing child mortality, and combating HIV infection are priority areas for the Sustainable Development Goals, which this study addresses (United Nations, 2016).

This dissertation follows a manuscript format. Due to the COVID-19 shutdown in SA, an appraisal of maternal endothelial dysfunction in HIV-associated PE comorbid with COVID-19 was highlighted (Naidoo *et al.*, 2021). In preeclampsia, there is defective CT cell conversion to the invasive EVT cell phenotype eventuating myometrial spiral artery maladaptation (Gabinskaya *et al.*, 1998). As a result, placental hypoxia increases circulating anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) (Moghaddas Sani *et al.*, 2019). In turn, sFlt-1 binds to and impairs signalling of VEGF and PlGF while sEng decreases transforming growth factor beta (TGF-β) activity in endothelial cells (Possomato-Vieira and Khalil, 2016; Moghaddas Sani *et al.*, 2019). Consequently, impaired VEGF/PlGF-VEGF signalling disrupts endothelial cell proliferation, migration, and angiogenesis (Naicker *et al.*, 2019). Furthermore, elevated sEng-TGF-β binding potentiates increased vasoconstriction (Touyz *et al.*, 2018). This shift in angiogenic factors also prompts the release of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukins (IL)s which induces cell injury through oxidative/nitrosative stress generating a positive feedback loop (Aouache *et al.*, 2018; Moghaddas Sani *et al.*, 2019). The sustained vasoconstriction encountered by the maternal endothelium predisposes widespread hypertension seen in PE (Maynard *et al.*, 2003).

Furthermore, we highlight an association between PE and the novel coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The SARS-CoV-2 antigen imposes endothelial dysfunction and hypertension upon binding to angiotensin-converting enzyme 2 (ACE 2), which resembles angiotensin II-mediated PE in severe cases of infection (Mendoza *et al.*, 2020). The physiological upregulation of ACE 2 in pregnancy is postulated to increase the susceptibility of SARS-CoV-2 infection and, subsequently, PE development (Phoswa and Khaliq, 2020). The higher plasma ACE2 levels in pregnancy may be correlated with an upregulation of Ang-(1-7). In women with PE, the reduced ACE2 and neprilysin levels at term, could be contributing to the reduction in Ang-(1-7) levels. These findings suggest that dysfunctional relationships between two key enzymes in the circulating RAS are involved in the pathogenesis of PE and SGA. Since soluble ACE2 can prevent binding of the novel coronavirus, SARS-CoV-2, to membrane bound ACE2, the interplay between ACE2 and the coronavirus and its impact in pregnancy requires further investigation (Tamanna *et al.*, 2020).

Moreover, our review evaluated the role of HIV infection and ART in pregnant women and their role in PE development. The sequential homology of HIV-1 accessory protein, Tat, facilitates VEGF mimicry (Albini *et al.*, 1996; Zhou *et al.*, 2013); however, its pro-angiogenic potential remains controversial as previous studies demonstrated dysregulated angiogenesis in the presence of Tat protein possibly implicating Tat in PE development despite neutralization of the opposing immune states of HIV infection and PE (Paladugu *et al.*, 2003).

There is a stronger inference of ART in endothelial dysfunction potentiating PE development (Sebitloane and D Moodley, 2017). Antiretroviral treatments containing nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) showed impaired endothelial cell proliferation, migration, and angiogenesis by hindering VEGFR signals as well as elevated trophoblast apoptosis following increased oxidative stress (Sandra Hernández *et al.*, 2017; Song *et al.*, 2018). Protease inhibitors restore the immune state (Autran *et al.*, 1999) and lower progesterone in trophoblast cells, thereby hindering spiral artery remodelling and placentation (Powis and Shapiro, 2015). Protease inhibitors also decreased pro-angiogenic proteins such as VEGF and PlGF, along with matrix metalloproteinase (Kala *et al.*, 2020). Highly active antiretroviral therapy (HAART) also impairs signal transduction pathways that promote angiogenesis (Sgadari *et al.*, 2002). Antiretrovirals also increase the sFlt-1/PlGF ratio in pregnant women denoting PE predisposition (Powis *et al.*, 2013). Antiretroviral exposure also prompts the upregulation of inflammation and oxidative stress, thereby increasing the risk of PE development (Francisci *et al.*, 2009). These observations highlight the events predisposing widespread endothelial dysfunction in PE and its associated adverse neonatal outcomes (S. Hernández *et al.*, 2017).

The potential of lopinavir/ritonavir (LPV/r), dolutegravir (DTG), and remdesivir (RDV) in drug-repurposing and their safety in pregnancy complicated by SARS-CoV-2 infection is interrogated. Although LPV/r showed potential effectiveness against COVID-19, randomized controlled trials showed no benefit beyond standard care (Cao *et al.*, 2020); furthermore, LPV/r showed defective decidualization and increased markers of endothelial dysfunction (Kala *et al.*, 2020). The new high-quality ART, a single-tablet regimen containing an integrase strand transfer inhibitor (INSTI), DTG showed endothelial protective properties (Afonso *et al.*, 2017; World Health Organization, 2017); however, there is a lack of evidence of its influence on pregnancy and SARS-CoV-2 infection. Despite the recent FDA-approval of RDV for compassionate use in COVID-19 infected patients, its effect in pregnancy requires intense perusal.

This novel study investigates the dysregulation of OPN and sNRP-1 in the synergy of HIV-infection and PE development. The main findings of our study were a significant downregulation of OPN in contrast to an upregulation of sNRP-1 in PE compared to normotensive pregnancies. In normal pregnancies, OPN induces VEGF expression (Chakraborty *et al.*, 2008). Our results are expected as PE is associated with a decline in VEGF favouring an upregulation of the anti-angiogenic sFlt-1 and sEng proteins (Moghaddas Sani *et al.*, 2019). Furthermore, OPN within the ECM facilitates EVT infiltration upon binding to its integrin receptor, αvβ3 (Xia *et al.*, 2009). Our results are corroborated by the previous study, which alludes to the inability of the CT cells to take on the invasive phenotype due to a dysregulation of OPN-αvβ3 signalling (Gabinskaya *et al.*, 1998).

The decline of OPN concentration in PE in our study is associated with a lower median gestational age than normotensive pregnancy (Dombai *et al.*, 2017). OPN mediates anti-apoptosis and pro-survival pathways in healthy pregnancy via PI3K/Akt and nuclear factor-κB signalling molecules to induce VEGF and ECM proteolysis via MMPs (Cao *et al.*, 2012). The downregulation of OPN would favour elevated apoptosis, which characterizes the EVT cell population in PE (Naicker *et al.*, 2013).

To our knowledge, this is the first study to demonstrate an upregulation of sNRP-1 in PE compared to normotensive pregnancies. Notably, in the anti-angiogenic milieu of PE, sNRP-1 binds to and sequesters $VEGF_{165}$ and PlGF via its b1b2 domains, thereby preventing VEGF-induced signal transduction pathways (Klagsbrun *et al.*, 2002; Mamluk *et al.*, 2002). Furthermore, the observation of exacerbated levels of sNRP-1 in prostate cancer cell lines resulted in endothelial injury with subsequent tumour cell apoptosis (Gagnon *et al.*, 2000), both of which are cardinal features of PE (Naicker *et al.*, 2013; Naicker *et al.*, 2019). Despite the paucity of information on sNRP-1 in pregnancy, this study

reveals the anti-angiogenic potential of sNRP-1 in pregnancy. Moreover, the pro-angiogenic full-length NRP-1 is significantly downregulated in PE in previous studies (Xu *et al.*, 2016; Arad *et al.*, 2017).

In addition, this study demonstrates a tendency of OPN concentration to increase ($p = 0.5099$) and a significant upregulation of sNRP-1 concentration ($p = 0.0005$) by HIV status (HIV-positive *vs.* HIV-negative). The HIV-1 specific Tat protein may promote the expression of integrins αvβ3 and α5β1 (Albini *et al.*, 1996; Zhou *et al.*, 2013). It is plausible to assume that the positive feedback induced by Tat-VEGF mimicry promotes αvβ3, thereby increasing OPN expression and activity in HIV infection, as seen in our study (Yokosaki *et al.*, 2005). However, the influence of ART on Tat regulation requires scrutiny. Nonetheless, all women in our study received ART; other studies also noted elevated OPN levels in HIV-positive individuals receiving ART (Chagan-Yasutan *et al.*, 2009; Bryant *et al.*, 2016), possibly emanating from the pro-inflammatory cytokine function of OPN following exposure to ART (Chagan-Yasutan *et al.*, 2009). It has been suggested that the source of OPN during ART emanates from the activation of T-cells and antigen-presenting cells (Chagan-Yasutan *et al.*, 2009).

Our study also reports a significant upregulation of sNRP-1 concentrations in HIV-positive pregnancies possibly emanating from ART, thereby implicating sNRP-1 in inflammation. Due to a general lack of literature on sNRP-1 in HIV infection, we hypothesize that it would complement ARV inhibition of viral binding. Notably, pre-incubation of β-herpesvirus murine cytomegalovirus with sNRP-1 markedly deters viral binding and subsequent infection (Lane *et al.*, 2020). Moreover, NRP-1 has been implicated in binding SARS-CoV-2 to the cell surface upon entry (Cantuti-Castelvetri *et al.*, 2020; Daly *et al.*, 2020).

LIMITATIONS

There was a general lack of knowledge on the influence of COVID-19 pregnancies complicated by HIV. Moreover, interactions between RDV and ART have not been established. A limitation of our study was that the origins of OPN and sNRP-1 were not elucidated. Also, all HIV-positive pregnant women recruited in this study were receiving ART as a standard of care for PMTCT. Furthermore, the small sample size was a limitation.

CONCLUSION

The aetiology of PE is centred around widespread endothelial dysfunction due to angiogenic imbalance, inflammation, and oxidative stress. The ACE 2 manipulation by SARS-CoV-2 infection upon entry

exhibits PE-like symptoms and may be implicated in its predisposition. Inconclusive evidence on LPV/r against COVID-19 and its availability in LMICs prompts further insight into its safety in HIV-associated PE complicated by COVID-19. Despite the observed endothelial protective properties of DTG, its safety in pregnancy and its role in COVID-19 therapeutics, along with the FDA approved compassionate use of RDV in pregnancy, is yet to be elucidated.

This novel study demonstrates a significant downregulation of OPN in PE compared to normotensive pregnancies. This may be attributed to the shallow trophoblast invasion of the myometrial spiral arteries as a result of impaired OPN- αvβ3 signalling seen in PE. We further reported a significant upregulation of sNRP-1 concentration in PE compared to normotensive pregnancies, regardless of HIV status, implicating its anti-angiogenic function in pregnancy. This is the first study demonstrating a significant increase in sNRP-1 and a tended upregulation of OPN concentration trend in HIV-positive compared to HIV-negative pregnancies. These differences may emanate from the effect of ARVs, sNRP-1, and OPN polymorphisms, as well as HIV-1 Tat-VEGF mimicry. Also, OPN has a role in inflammation and an ability to complement ARV inhibition of viral binding, thereby preventing amplification and dissemination. Finally, both OPN and sNRP-1 are promising candidate proteins with predictor test value in HIV-associated PE.

RECOMMENDATIONS FOR FUTURE RESEARCH

Remdesivir-ART interactions and the inclusion of pregnant women in antiviral drug repurposing trials are essential. Further research on the pathogenesis and molecular interactions of PE-markers in the HIV-COVID-19 syndemic is warranted. Insight into the genetic polymorphisms of OPN and sNRP-1 are proposed for verifying their influence in pregnancy and related disorders. Knowledge of the physiological link between OPN, full-length NRP-1, and sNRP-1, particularly receptor-mediated interactions and common signal transduction pathways, will help demystify disorders such as PE. It may also prove beneficial to establish a correlation between OPN and sNRP-1 and other angiogenic and inflammatory factors across early-onset PE, late-onset PE, mild and moderate PE, and across all trimesters during gestation. Moreover, further large-scale studies are warranted to validate the role of OPN and sNRP-1 in the synergy of pregnancy, PE, and HIV infection.

CHAPTER FIVE

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APPENDIX

Appendix 1

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

04 June 2020

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 20 May 2020 to append the studies below to the above study has now been approved by a sub-committee of the Biomedical Research Ethics Committee

MMedSci	Rowen Govender	215023500	The role of complement component 4B (C4B) and complement factor I (CFI) in the duality of HIV infected preeclamptic women
MMedSci	Sumeshree Govender	21351694	The role of C5a and C2 protein in pre-eclampsia complicated by HIV infection.
MMedSci	Camille Naicker	214515577	The components C5 and Mannose- binding lectin (MBL) functionality in the complement system in relation to HIV and preeclampsia pregnant women in Durban, South Africa.
MMedSci	Mikyle David	216000603	The function of Adipsin and C9 protein in the complement system with relation to HIV-associated pre-eclampsia
MMedSci	Tashlin Abel	215013948	The regulation of SLK-1 and SFLT-4 and their involvement in Pre-eclamptic woman with HIV.
MMedSci	Omeshini Naiker	215028862	The role of angiostatin and PDGF in maintaining placental health in preeclamptic patients
MMedSci	Nqobile Mdlalose	216002159	The role of HER2 and HER 3 in HIV associated preeclampsia
MMedSci	Nitalia Naidoo	216013288	The role of osteopontin and neuropilin in HIV associated preeclampsia

The committee will be notified of the above approval at its next meeting to be held on 14 July 2020.

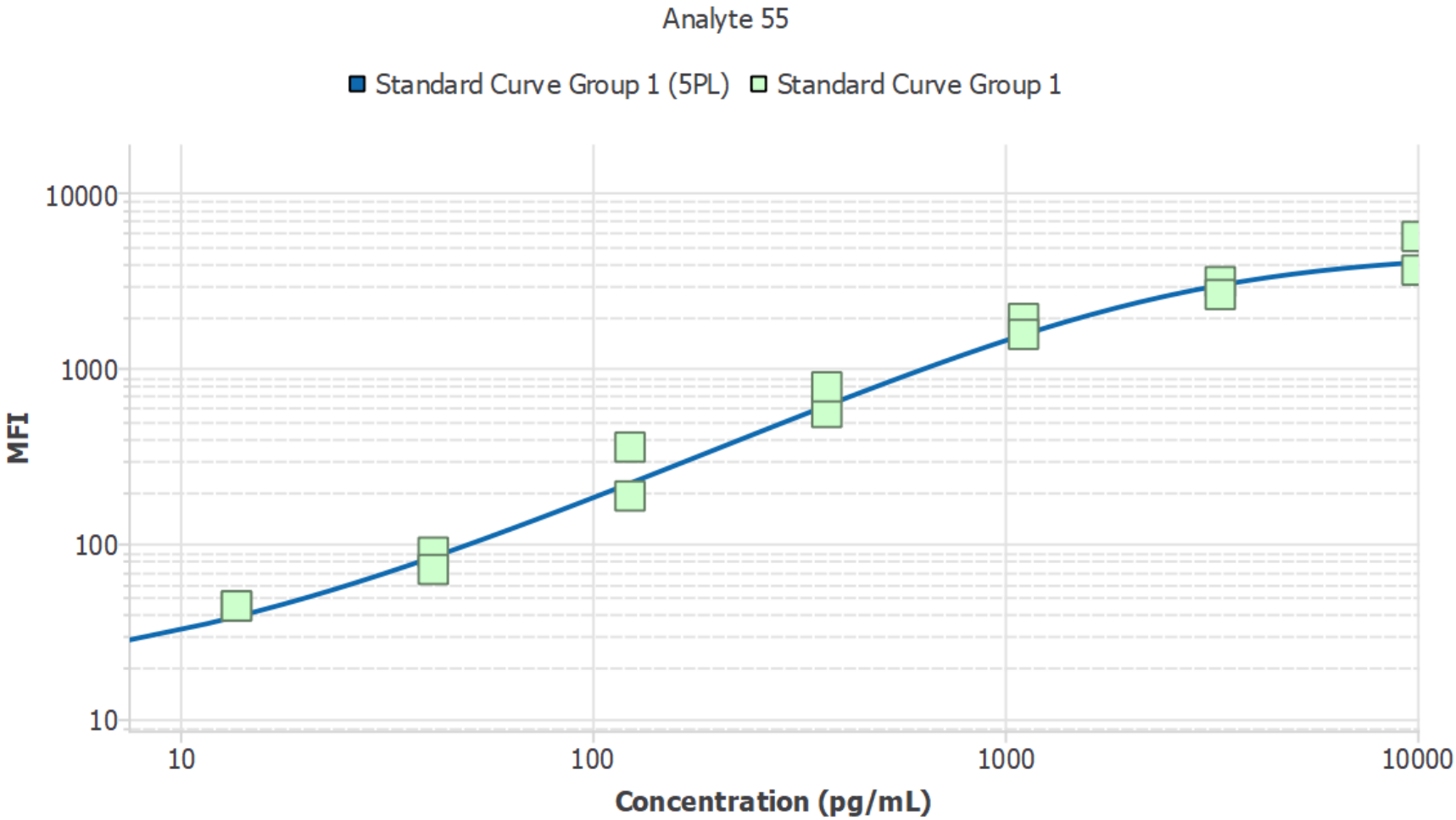
Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

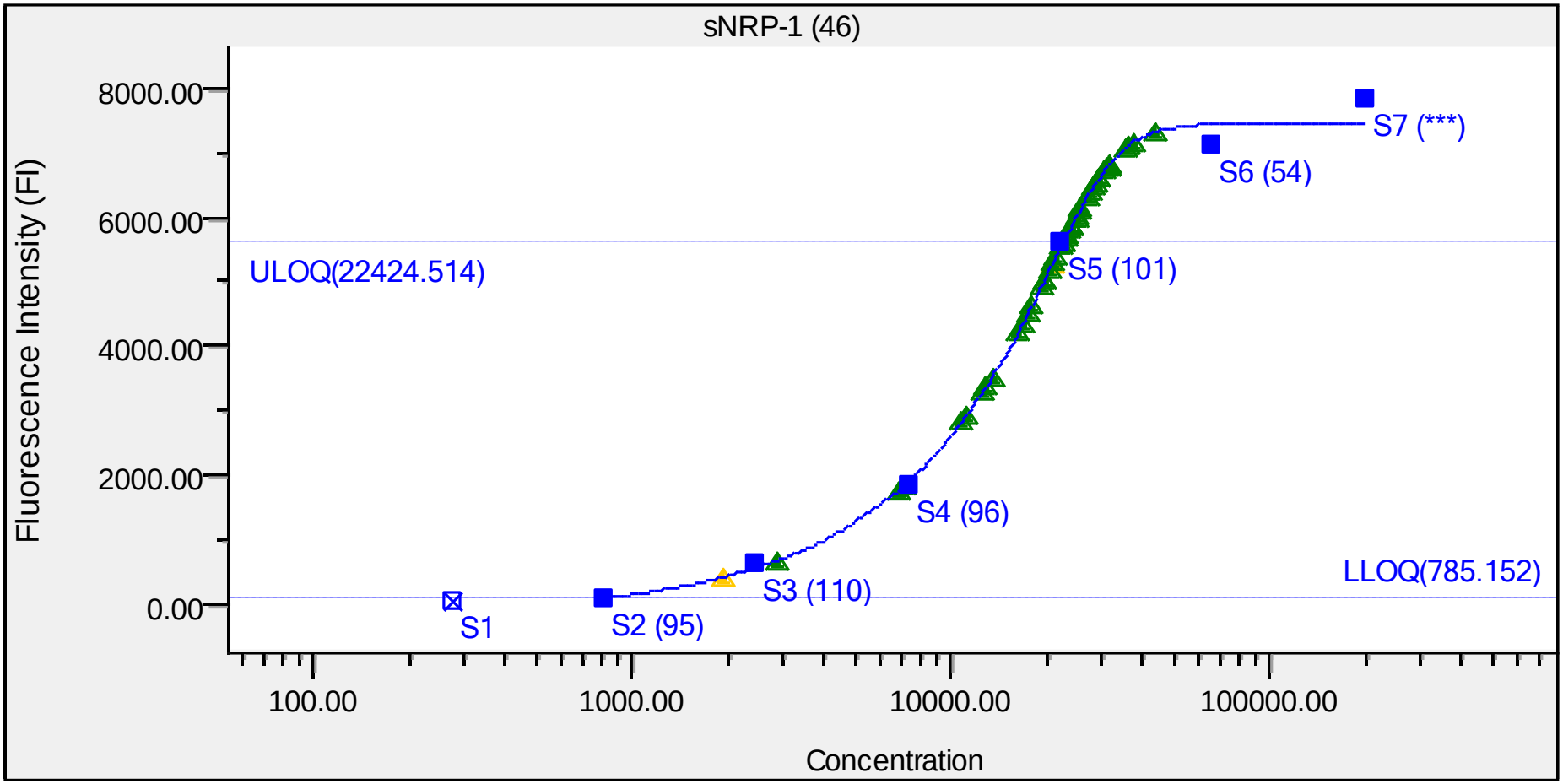
Appendix 1: Ethical approval (BCA338/17)

Appendix 2



Appendix 2: Standard curve for osteopontin

Appendix 3



Appendix 3: Standard curve for soluble neuropilin-1

Appendix 4

Publication: Naidoo, N., Moodley, J. & Naicker, T. 2021. Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19: A review. *Hypertens Res*, 1-13.

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REVIEW ARTICLE

Maternal endothelial dysfunction in HIV-associated preeclampsia comorbid with COVID-19: a review

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Abstract

This review assesses markers of endothelial dysfunction (ED) associated with the maternal syndrome of preeclampsia (PE). We evaluate the role of antiretroviral therapy (ART) in human immunodeficiency virus (HIV)-infected preeclamptic women. Furthermore, we briefly discuss the potential of lopinavir/ritonavir (LPV/r), dolutegravir (DTG) and remdesivir (RDV) in drug repurposing and their safety in pregnancy complicated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In HIV infection, the trans-activator of transcription protein, which has homology with vascular endothelial growth factor, impairs angiogenesis, leading to endothelial injury and possible PE development despite neutralization of their opposing immune states. Markers of ED show strong evidence supporting the adverse role of ART in PE development and mortality compared to treatment-naïve pregnancies. Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 infection, exploits angiotensin-converting enzyme 2 (ACE 2) to induce ED and hypertension, thereby mimicking angiotensin II-mediated PE in severe cases of infection. Upregulated ACE 2 in pregnancy is a possible risk factor for SARS-CoV-2 infection and subsequent PE development. The potential effectiveness of LPV/r against COVID-19 is inconclusive; however, defective decidualization, along with elevated markers of ED, was observed. Therefore, the safety of these drugs in HIV-positive pregnancies complicated by COVID-19 requires attention. Despite the observed endothelial protective properties of DTG, there is a lack of evidence of its effects on pregnancy and COVID-19 therapeutics. Understanding RDV-ART interactions and the inclusion of pregnant women in antiviral drug repurposing trials is essential. This review provides a platform for further research on PE in the HIV-COVID-19 syndemic.

Keyword Antiretrovirals · Endothelial dysfunction · HIV · Preeclampsia · SARS-CoV-2

Introduction

Maternal mortality is a major concern worldwide, with its prevalence being particularly high in low- and middle-income countries (LMICs) [1, 2]. Sub-Saharan Africa bears the brunt of maternal deaths, namely, 66% of the global estimate [3]. The leading direct cause of maternal mortality in South Africa (SA) is preeclampsia (PE) [3].

Hypertensive disorders of pregnancy (HDP) are classified as follows: chronic hypertension (high blood pressure predating pregnancy or present at/or before 20 weeks of gestation); gestational hypertension, which is persistent de novo hypertension that develops at/or after 20 weeks of gestation without evidence of other organ involvement; PE without severe features; and PE with severe features [4]. PE is defined as new-onset hypertension presenting after 20 weeks of gestation in conjunction with one or more characteristic features, such as proteinuria and/or acute kidney injury, persistent headache, visual disturbances, epigastric pain, HELLP syndrome (hemolysis, elevated liver enzymes, low platelets), eclampsia (hypertension-associated seizures in pregnancy), and uteroplacental dysfunction, including fetal growth restriction [4]. Maternal mortality is present in all categories of HDP with eclampsia and PE, with severe features being the most common

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diagnosis before death [5]. It has been reported that PE accounts for >70,000 maternal deaths and 500,000 fetal deaths worldwide every year [4]. Globally, PE complicates 5–7% of pregnancies, and this incidence often increases to >10% in LMICs [6].

Although the exact etiology of PE remains elusive, endothelial dysfunction (ED) initiates the maternal syndrome of PE as a result of placental hypoxia, a reduction in uterine natural killer (uNK) cells, oxidative stress (OS), angiogenic imbalance and an exaggerated inflammatory response [7]. Human immunodeficiency virus (HIV) infection and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection also impact the inflammatory response and endothelial function. It is unclear whether HIV infection increases or decreases the frequency of PE. Nonetheless, the synergistic effect of these inflammatory conditions occurring concurrently requires investigation.

Pathophysiology of preeclampsia

In a normal pregnancy, the uteroplacental vasculature undergoes a significant morphological and physiological transformation to sustain fetal development [8]. Usually, cytotrophoblast (CT) cells derived from the tips of the chorionic villi migrate into the decidua and the inner myometrium in a set-time sequence [9, 10]. Thereafter, they fuse to form the multinucleated syncytiotrophoblast (ST) layer, which encloses the floating villi of the placenta and establishes the maternal-fetal interface for efficient gaseous and nutrient exchange [9, 11]. Extravillous trophoblast cells infiltrate fibrinoid-type material that replaces the musculo-elastic media of the spiral arteries, converting them into low-resistance large flaccid sinusoidal-like arteries [11, 12]. In the decidua, uNK cells regulate the depth of placentation and spiral artery remodeling [7]. The lumen of the spiral arteries is dilated five- to tenfold, ensuring an adequate supply of blood to the developing fetus [13]. These changes are typically achieved by 20 weeks of gestation [14].

Aberrant vascular remodeling predisposes the individual to PE development. PE is considered a two-stage placental disease where stage 1, often referred to as the fetoplacental or asymptomatic stage, occurs during the first and second trimesters of pregnancy [15]. In this stage, CT cells fail to take on the invasive endothelial phenotype; hence, CT migration is deficient, and there is a lack of physiological transformation of the myometrial spiral arteries [16, 17]. The resulting small arterial lumen, surrounded by vasoactive medial cells, is unable to provide adequate blood to meet the oxygen and nutrient demands of the fetus [17, 18]. This reduction in blood flow creates a hypoxic-ischemic microenvironment that marks the second stage [7, 19]. Stage 2, also referred to as the maternal stage, prompts the release of antiangiogenic factors and other mediators that initiate systemic inflammation, OS, and endothelial cell (EC) dysfunction. These mediators pre-empt the maternal syndrome of PE (presence of hypertension, proteinuria, liver dysfunction, cerebral edema, eclampsia, etc.) [4, 20]. An imbalance in circulating angiogenic factors persists during the pathogenesis of maternal syndrome [7].

Pathogenesis of the maternal syndrome in preeclampsia

Neovascularization (new blood vessel formation) results from either vasculogenesis or angiogenesis [21]. Vasculogenesis is the de novo construction of blood vessels from precursor cells, such as angioblasts, which differentiate into ECs that shape lumens to form simple blood vessels. In contrast, angiogenesis is the formation of new capillaries from the pre-existing vasculature [21, 22]. Angiogenesis is strongly associated with female reproductive conditions such as decidualization, implantation, and embryonic development [23]. Proangiogenic factors such as vascular endothelial growth factors (VEGFs) and placental growth factor (PlGF) are released into circulation, thereby increasing vascular permeability and promoting proteolysis of the extracellular matrix via proteases, leading to EC proliferation, migration and infiltration into the lumen and subsequent endothelial maturation [24, 25]. An array of VEGF isoforms, namely, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF, are present in the blood circulatory system and are responsible for various vascular processes. VEGF-A binding to VEGFR-1 does not produce significant receptor activation (in this case, the receptor acts as a decoy), whereas VEGF-B binding to VEGFR-1 promotes cell survival [26]. VEGF-A can also bind to VEGFR-2, with a lower affinity, in the presence of NRP-1, a coreceptor of VEGF, thereby promoting EC migration and proliferation (Fig. 1) [26, 27]. However, VEGFs and their receptors are significantly downregulated in preeclamptic conditions due to the overexpression of their antiangiogenic counterparts [28].

Soluble fms-like tyrosine kinase (sFlt-1), also known as sVEGFR-1, is the soluble form of endothelial-bound VEGF receptors and functions as a VEGF antagonist to maintain angiogenic homeostasis [29]. Elevated sFlt-1 prevents VEGF and PlGF binding to VEGFR-2 on ECs, thus hindering angiogenic signal transduction leading to EC injury [30]. Concentrations of sFlt-1 are markedly elevated in pregnancy and are even higher in PE [31, 32]. Studies have demonstrated that the overexpression of sFlt-1 in rats induces PE-like syndrome early in pregnancy, supporting the role of antiangiogenic factors in PE development [33].

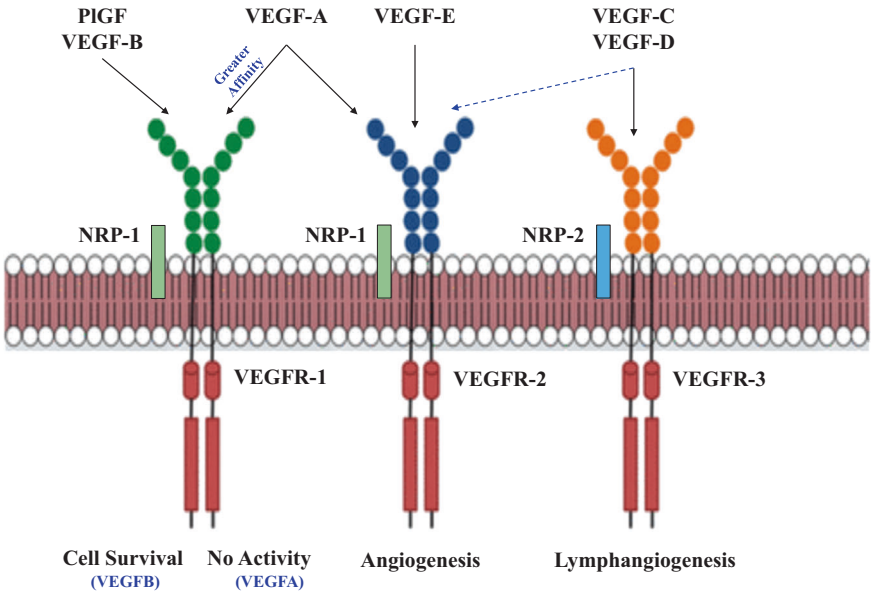

Fig. 1 Differential functions of vascular endothelial growth factor receptors. Adapted from Pandey et al. [27]. Vascular endothelial growth factor-A, B, C, D, and E (VEGF-A, B, C, D, and E), VEGF receptor-1, 2, and 3 (VEGFR-1, 2, and 3), Neuropilin-1 and 2 (NRP-1 and 2), Placental growth factor (PlGF)

Assessment of the imbalance in the sFlt-1/PlGF ratio is currently used in the diagnosis and management of PE; however, more accurate and effective modes of early detection are urgently needed [34].

Endoglin (Eng), a coreceptor for the transforming growth factor (TGF) group of factors, is involved in vascular remodeling and hemostatic events via the activation of the endothelial nitric oxide synthase (eNOS) pathway that facilitates angiogenesis. In contrast, soluble endoglin (sEng), an extracellular variant of Eng, is highly expressed by trophoblasts and opposes TGF-β interactions with its receptor, thereby preventing vasodilation [35]. Upregulation of sEng impedes potent production of the vasodilator nitric oxide (NO) in ECs via its binding with TGF-β [36]. Therefore, exaggerated levels of sEng observed in PE may be central to the characteristic hypertension encountered during the maternal syndrome of the disease [35].

OS and nitrosative stress (NS) that causes endothelial injury in PE emanates from an imbalance between pro-oxidants and their therapeutic antagonists (antioxidants) [37]. This stress includes an increase in reactive oxygen species (ROS) and reactive nitrogen species production and/or diminished availability of antioxidant mechanisms [38]. The release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6 and IL-8 from the ischemic placenta is intensified by syncytiotrophoblast microparticle (STMB) recruitment of monocytes and neutrophils to damaged EC sites [36]. These inflammatory cytokines not only decrease the bioavailability of NO and prostaglandin I2 (PGI2) but also produce ROS, which stimulates the elevation of endothelin-1 (ET-1), a potent vasoconstrictor. Vascular smooth muscle contraction results from an imbalance of endothelial vasodilators (NO and PGI2) and vasoconstrictors [Angiotensin II (Ang II), ET-1, and thromboxane A2 (TXA2)] during EC damage [39]. Vasoconstrictors decrease calcium ion efflux from smooth muscle cells through protein kinase C and Rho-kinase activation [39]. This leads to sustained vascular resistance and the hypertensive hallmark of endothelial injury observed in PE [33], depicted in Fig. 2.

Maternal antioxidant imbalance and oxidative/nitrosative stress in preeclampsia

The EC activation encountered during PE exacerbates systemic inflammation and increases the expression of leukocyte adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin (SELP), and E-selectin (SELE) [40, 41]. Leukocytes such as neutrophils and macrophages express nicotinamide adenine dinucleotide-phosphate (NADPH) oxidase, which generates superoxide (O_2^-) with subsequent production of other free radicals leading to a respiratory burst [42]. Usually, this process is tightly regulated; however, an increase in this phenomenon greatly overwhelms reducing agents such as glutathione, glutathione peroxidase, superoxide dismutase and catalase, resulting in OS and endothelial damage [43]. Elevated proinflammatory cytokines, such as TNF-α, observed in PE not only promote NO degradation leading to O_2^- generation but also induce free radical production during oxidative phosphorylation, further contributing to EC injury [44, 45]. Moreover, increased levels of proinflammatory cytokines such as IL-1 and TNF-α upregulate lectin-like oxidized low-density

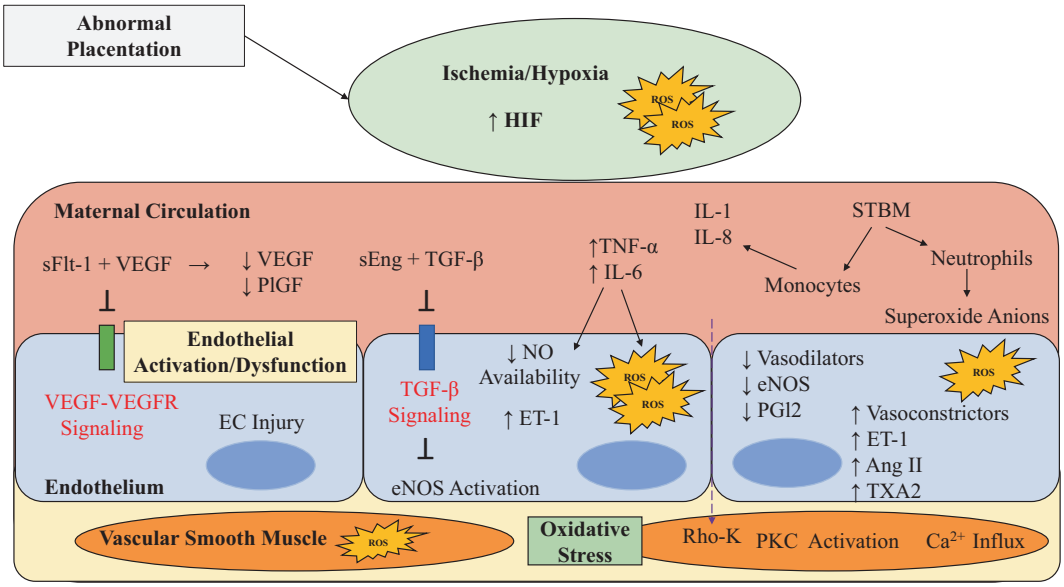

Fig. 2 Endothelial dysfunction in Preeclampsia. Adapted from Moghaddas et al. [36]. Angiotensin II (Ang II), Endothelial nitric oxide synthase (eNOS), Endothelin-1 (ET-1), Hypoxia-inducible factor (HIF), Interleukin-1, 6, and 8 (IL-1, IL-6, and IL-8), Nitric oxide (NO), Prostaglandin (PGI2), Protein kinase C (PKC), Placental growth factor (PlGF), Reactive oxygen species (ROS), Soluble endoglin (sEng), Soluble fms-like tyrosine kinase-1 (sFlt-1), Syncytiotrophoblast microparticles (STBMs), Transforming growth factor-β (TGF-β), Tumor necrosis factor-α (TNF-α), Thromboxane A2 (TXA2), Vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)

lipoprotein receptor-1 (LOX-1), which consequently elevates the receptor for oxidized low-density lipoprotein (oxLDL), thereby facilitating O_2^- production via NO degradation [46, 47]. In maternal circulation, STBMs evoke ED via the activation of LOX-1 with an increase in O_2^- and a subsequent reduction in NO-mediated vasodilation [48]. However, contradictory studies demonstrate a significant upregulation in oxLDL with reduced concentrations of LOX-1 in PE [49]. Elevated agonist autoantibodies against angiotensin receptors (AT1-AA) due to placental ischemia enhance Ang II sensitivity via angiotensin II type I receptor (AT1) in PE [50]. Higher levels of AT1-AA have demonstrated increased placental OS [51] due to superoxide production through NADPH activation [52], which may result in vascular injury, deficient trophoblast invasion, placental hypoxia, inflammation, angiogenic imbalance, and reduced bioavailability of NO [53]. In addition, free fetal hemoglobin and circulating xanthine oxidase induce ROS production through various mechanisms [38]. These pathways can lead to eNOS uncoupling, generating O_2^- [54], which may prompt NO-O_2^- interactions and the production of the potent oxidant peroxynitrite, which inevitably predisposes cells to damage and DNA fragmentation and alteration [55]. Peroxynitrite can also hinder eNOS activity and disrupt endothelium-dependent vasodilation [56]. ROS have also been shown to downregulate the calcium-activated potassium channels KCa2.3 and KCa3.1, which are vital for electrical stimulation of vascular smooth muscle to ensure effective vasodilation [38].

Severe acute respiratory syndrome coronavirus-2 and preeclampsia

The outbreak of coronavirus disease 2019 (COVID-19) was declared a pandemic in March 2020 by the World Health Organization (WHO) [57]. At present, over 43.3 million cases of COVID-19 have been confirmed, with ~1.15 million deaths in over 218 countries and territories [58]. Genetic analysis of the novel beta-coronavirus revealed that its entry mechanism exploits the renin-angiotensin system (RAS) [59, 60]. The virus thereafter induces an array of symptoms, including vasoconstriction, elevated blood pressure and profibrotic pathway activation via coagulation [61]. An observational study conducted on COVID-19-infected pregnant women revealed that severe to critical cases of COVID-19 present with PE-like symptoms exclusive to placental maladaptation [62]. PE mimicry by COVID-19 was confirmed following the alleviation of preeclamptic symptoms without delivery of the placenta, which is currently the only known method for obtaining resolution of the clinical signs and symptoms of PE [62]. This prompted further insight into COVID-19's role in PE.

During normal RAS activation, renin catalyzes the conversion of angiotensinogen into angiotensin I (Ang I). Ang I is further cleaved by angiotensin-converting enzyme (ACE) to form Ang II [63]. The physiological antagonist of ACE and Ang II, ACE 2, serves to cleave Ang I and Ang II into angiotensin 1-9 and angiotensin 1-7 [Ang (1-7)], respectively, bringing about vasodilatory, anti-inflammatory, and

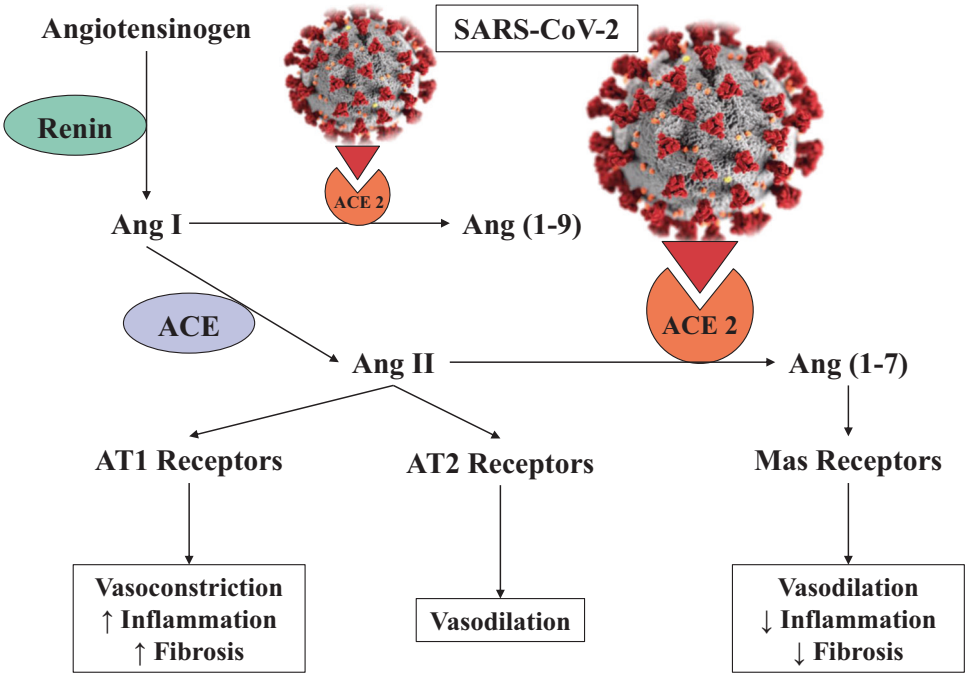

Fig. 3 Manipulation of RAS by SARS-CoV-2 in COVID-19. Angiotensin-converting enzyme (ACE), Angiotensin-converting enzyme 2 (ACE 2), Angiotensin (1-7), (1-9), I and, II [Ang (1-7), (1-9), I, and II], Angiotensin type 1 (AT1) receptors, Angiotensin type 2 (AT2) receptors, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

antifibrotic effects upon binding to its Mas receptor [61, 64]. RAS activation is, therefore, dependent on the balance between ACE and ACE 2. Pregnant women are partially unreceptive to circulating Ang II to maintain low vascular resistance. However, this adaptation is reversed in PE, leading to an angiogenic imbalance [65]. In SARS-CoV-2 infection, ACE 2 receptors are increased and exploited for effective viral infectivity, which decreases ACE 2 function, subsequently upregulating Ang II activity [66]. The decrease in ACE 2 function, along with an increase in the Ang II/Ang (1-7) ratio, may result in hypoxia-induced upregulation of sFlt-1 [67, 68], which further sensitizes ECs to Ang II [69]. Similar to PE, COVID-19 infection shows an increase in the sFlt-1/PlGF ratio due to the pathologic Ang II/Ang (1-7) imbalance [70]. Ang II acts through its receptors (AT1 and AT2) to induce vascular impairment, which is the initiator of the maternal syndrome of PE, thereby reinforcing Ang II-mediated ED [71], as depicted in Fig. 3.

The elevated expression of ACE 2 in STs, CTs, and the placental vasculature is imperative for blood pressure mediation for sufficient perfusion of the developing fetus. Therefore, SARS-CoV-2 infection and its alteration of ACE 2 expression may lead to dire adverse outcomes [72, 73]. A recent review highlighted that both normal pregnancy and COVID-19 infection show upregulation of ACE 2, IL-8, and IL-10; thus, pregnancy may be a risk factor for COVID-19 morbidity [74]. They also postulated that increased expression of ACE 2 receptors in the placenta might escalate the risk of vertical transmission of SAR-CoV-2 infection [74]. This suggestion is supported by the predominant localization of SARS-CoV-2 in STs at the maternal-fetal interface of the placenta, potentiating severe comorbidity among COVID-19-complicated pregnancies [75]. Conflicting evidence has revealed no significant differences in ACE 2 expression between normotensive pregnant women and preeclamptic women in the third trimester; however, the data are inconclusive, as PE onset occurs earlier in gestation [76]. Another study showed no significances in the prevalence rates of intrauterine growth restriction (IUGR) and PE between COVID-19-negative and COVID-19-positive pregnant women. The observed ED in this study was attributed to the 'cytokine storm' of COVID-19, similar to the proinflammatory state of PE. This is further supported by Shanes et al., who showed altered maternal vascular perfusion following placental hypoxia, conceivably due to systemic inflammation, in sixteen placentae obtained from COVID-19-infected women [77]. In contrast to PE, acute lung injury and acute respiratory distress syndrome (ARDS) have upregulated VEGF levels, which increases vascular permeability. Moreover, the same study identified VEGF-D as the most prominent indicator related to the severity of clotting in COVID-19 [78].

Soluble angiotensin-converting enzyme 2 in the therapeutic intervention of covid-19

Unlike the ACE 2 receptor, soluble angiotensin-converting enzyme 2 (sACE 2) is unable to facilitate SARS-CoV-2 entry into cells due to its lack of cell membrane interactions along with the absence of transmembrane serine protease 2

(TMPRSS2), a corequisite for SARS-CoV-2 endocytosis [79]. sACE 2 is formed through ACE 2 receptor cleavage/shedding by a disintegrin and metalloproteinase 17 (ADAM17) and is suggested to have protective effects against SARS-CoV-2 infection [80]. sACE 2 may serve as a competitive decoy for the coronavirus, thereby reducing the binding of viral particles to membrane-bound ACE 2 and consequently reducing viral infectivity [81]. Research on therapeutic interventions using sACE 2 has revealed its higher affinity for COVID-19, thereby neutralizing the virus without altering endogenous ACE 2 homeostasis [82]. However, research on the effects and safety of sACE, TMPRSS2 and ADAM17 manipulation in pregnancy and HIV-infected individuals comorbid with COVID-19 has yet to be established.

Human immunodeficiency virus infection and preeclampsia

The trans-activator of transcription (Tat) protein is a regulatory protein of HIV-1 that improves the efficiency of viral infectivity [83]. The rich arginine and lysine arrangement seen in Tat resembles the VEGF sequence [84]. Therefore, Tat mimics the role of VEGF by promoting EC adhesion and αvβ3 and α5β1 integrin expression [84, 85], which also binds osteopontin (an angiogenic factor involved in decidualization) [86]. A study conducted on HIV-1 Tat-induced angiogenesis demonstrated that Tat protein notably reduced endothelium-dependent vasorelaxation and eNOS expression and regulation in ECs of porcine coronary arteries [37]. The latter study also implicated Tat in coronary artery disease, which is associated with the long-term effect of PE [37]. In addition, Tat protein was also shown to induce the expression of ICAM-1 and VCAM-1, suggesting a possible mechanism by which HIV-1 infection contributes to endothelial injury and accelerates atherosclerosis [87, 88]. Therefore, it is plausible that Tat's homology with VEGF affects angiogenesis in PE.

In contrast to the exaggerated immune state of PE, there is significant immune suppression after HIV infection [89, 90]. Although infection has been shown to reduce the risk of developing PE, most studies show that pregnant women receiving highly active antiretroviral therapy (HAART) have an increased prevalence of PE development [90, 91]. This increase is believed to be due to immune restoration [92]. Recent studies show no difference in the risk of PE development between treated and untreated HIV-infected pregnant women [93], but others have reported findings that do not support the notion that HIV infection has protective qualities against HDP development [94].

Role of HIV therapy in maternal endothelial dysfunction

The WHO recommends that all individuals living with HIV infection receive HAART, regardless of their $CD4^+$ count and disease stage (including pregnant and breastfeeding women) [95]. HAART or antiretrovirals (ARVs) not only improve life expectancy but also decrease the risk of mother-to-baby (vertical) transmission of the infection in utero during birth and breastfeeding [95]. However, ARVs may trigger severe PE development [96]. A study conducted on nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), namely, azidothymidine, tenofovir disoproxil fumarate and lamivudine, revealed dysregulation of EC proliferation and migration [97]. The study also suggested that NRTIs induce mitochondrial OS, which hinders the activation and transduction of endothelial receptor tyrosine kinase signals and VEGFR-2 pathways in vascular ECs [97]. In addition, this adverse effect on angiogenesis may predispose the individual to PE development [98]. Excessive production of ROS is associated with increased trophoblast apoptosis, which may occur in placental-mediated disorders, such as PE and/or IUGR, overpowering antioxidant defenses with deleterious effects [98]. Protease inhibitors (PIs) deter HIV aspartyl protease, causing reconstitution of the immune microenvironment, which may predispose the individual to PE development [99]. In vivo, three PIs [atazanavir, lopinavir (LPV), and ritonavir (r)] significantly lower progesterone in trophoblast cells, thus indicating its hindrance of trophoblast proliferation and migration [100]. In a recent study, Kala et al. showed that LPV-based ART dysregulated uterine decidualization and spiral artery remodeling in both human ex vivo and mouse in vivo models [101]. Lower expression of the chemokines VEGF, PlGF, angiopoietin-2, granulocyte-macrophage colony-stimulating factor, interferon-gamma and matrix metalloproteinase (MMP) 9 was observed upon LPV exposure of primary decidual cell cultures [101]. They reported uNK cell depletion and deficient trophoblast invasion as a result of decreased expression of the transcription factor STAT3, which mediates decidualization [101]. These observations highlight the events that precede widespread ED in PE and its associated adverse neonatal outcomes. HAART impairs nuclear factor kappa B (NF-κB) transcription factors that decrease MMP and VEGF expression, which inevitably dysregulate angiogenesis, promoting ED and PE development [102]. The placentae of HIV-infected women receiving zidovudine-containing ART showed evidence of mitochondrial DNA depletion, elevated OS levels, and apoptosis, implicating secondary mitochondrial failure potentiating PE development and adverse perinatal outcomes [103].

Increased immune-expression of Flt-1 and sFlt-1 was observed within trophoblast cells during PE, regardless of HIV status, implying autocrine signaling in trophoblast invasion and differentiation [104]. This is believed to promote abnormal placentation with subsequent EC dysfunction in PE [104]. Pre-HAART exposure in HIV infection showed lower PlGF levels and increased sFlt-1 in women who developed PE compared to normotensive pregnant women [105]. Multivariate analysis demonstrated that PlGF and viral load were significantly related to PE development, and no significant shifts were observed in angiogenic factors following HAART among normotensive women [105]. Increased sFlt-1 and sEng levels were linked to PE regardless of HIV infection [106]. This study also elucidated a significant downregulation in PlGF levels in HIV-negative preeclamptic women compared to normotensive women. However, HIV infection downregulates PlGF in normotensive pregnant women compared to their HIV-negative counterparts ($p = 0.02$), thereby predisposing the individual to PE development [106]. TGF-β1 levels remain unchanged in HIV infection regardless of the increase in its coreceptor sEng [106, 107].

In contrast, a study of HIV-associated PE women revealed that HIV/HAART is linked to significant downregulation of IL-2, TNF-α and IL-6, with significant decreases in IL-2 and TNF-α observed in preeclamptic women [108]. Saums et al. found that integrase strand transfer inhibitor-containing ARTs had a greater frequency of HDP development than protease inhibitor-containing regimens [93]. Another study concluded that HIV infection, rather than its pharmacological treatment, induces alterations in markers of endothelial function [109]. The short-term duration of treatment with HAART reduces some markers of ED, including VCAM-1, with no differences between PIs and nonnucleoside reverse transcriptase inhibitors. However, SELP remained elevated upon exposure to both treatments [109].

The repurposing of various antiviral drugs (Table 1) has gained momentum as a desperate measure to prevent the deleterious effects of COVID-19 [110].

Antiretroviral therapy in pregnancy and coronavirus disease 2019

It is plausible to assume that HIV-infected individuals receiving ARVs have a lower risk of developing complications from COVID-19 infection [111–114]. Protease inhibitor-based ARVs, such as LPV/r, have shown potential against SARS-CoV-2 infection due to their ability to bind SARS-CoV-1. Studies have shown a strong sequence homology between SARS-CoV-1 and SARS-CoV-2 [115]. However, SARS-CoV-2 binds ACE 2 with a 10-20-fold

Table 1 Antiviral drug repurposing for COVID-19 therapeutics highlighted in this review

Drugs	Mechanism of action	Safety in pregnancy	Effectiveness in treatment of COVID-19[a]	Placental transfer	Clinicaltrials.gov: COVID-19 (including pregnant women)
Lopinavir/ Ritonavir	Antiretroviral (Protease inhibitor) SARS-CoV[b] 3-chymotrypsin-like cysteine protease inhibitor [144]	Considered safe in pregnancy [145, 146] despite contradicting data [100, 101]	Potential to reduce mortality, although no benefit beyond standard care is clinically proven [122]	Low [147, 148]	NCT04364022
Dolutegravir	Antiretroviral (Integrase strand transfer inhibitor) Possibly inhibits 2′-O-ribose methyltransferase involved in coronavirus infectivity [149]	Recommended for HIV[c]-infected pregnancies (International guidelines, 2020) [134] Potential risks of neural tube defects (initiation < 6 weeks gestation) [126]	No clinical evidence	Moderate to high [150]	None
Remdesivir	Broad-spectrum antiviral (Viral RNA-dependent RNA polymerase inhibitor)	Requires greater research	Potential to improve clinical improvement time [137] FDA[d] approved for compassionate use (22/10/2020) [141]	Unknown [148]	NCT04292899 NCT04292730 NCT04582266

[a]Coronavirus disease 2019

[b]Severe acute respiratory syndrome coronavirus

[c]Human immunodeficiency virus

[d]Food and drug administration

greater affinity than SARS-CoV-1, which explains the high human transmission and infectivity rates of SARS-CoV-2 [116]. LPV/r lowers the risk of patients developing ARDS and subsequently dying from SARS-CoV-2 infection [117]. In various subsequent clinical trials comparing prenatal exposure to LPV/r and prenatal exposure to efavirenz (EFV), there were no significant differences in adverse outcomes in pregnancy [118] or other control measures [119]. These drugs, therefore, have become the preferred drugs of choice for pregnancy complicated by COVID-19 in China [120].

Randomized controlled trials (RCTs) are essential for providing standard guidance on clinical management, even in an emergency setting, since RCTs offer data without bias due to confounding factors, as seen in nonrandomized studies [121]. RCTs of LPV/r in severe COVID-19 showed no benefit beyond standard care [122]. Other RCTs revealed that a combination of antiviral drugs (interferon beta-1b, ribavirin, and LPV/r) was more successful in symptom alleviation than LPV/r alone in mild to moderate COVID-19 cases [123]. A systemic review of RCTs of LPV/r in COVID-19 highlighted that ARVs may reduce mortality; however, this reduction varies across different risk groups [124].

The South African National ART guidelines employ LPV/r-based ARTs as the second-line therapy in HIV-infected adults [125]. The guidelines further recommend that women who become pregnant while receiving the LPV/r-containing regimen should continue treatment with monthly clinical observations [125]. Dolutegravir (DTG), the newly established ARV in SA, together with two NRTIs, is also recommended as a second-line ART after failing a non-NRTI-based first-line regimen since DTG is suggested to be better tolerated by HIV-infected individuals than PIs such as LPV/r [126]. SA has experienced over 716,700 confirmed cases of COVID-19 as of late October 2020 [58]. Considering the slow switch from previously approved ARVs to DTG, LPV/r-containing ARTs are still readily available in SA, and clinical trials may include women in their first trimester. Notably, LPV/r has a negative influence on decidualization and placentation; therefore, the safety of these drugs in HIV-associated PE complicated by COVID-19 infection requires urgent and intensive scrutiny.

Latest antiretroviral therapy effects on endothelial dysfunction

In 2017, SA and other low/middle-income countries agreed to launch a new high-quality ART, a single-tablet regimen containing an integrase strand transfer inhibitor (INSTI), DTG, which provides rapid viral suppression [127]. Notably, the 2019 ART guidelines in SA state that the preferred first-line ART regimen is a DTG-based drug (TLD) for patients experiencing EFV side effects or those who prefer to use DTG [125]. However, the EFV-containing regimen (TEE) is recommended for use in the first 6 weeks of gestation and in women of child-bearing age due to a high risk of neural tube defects associated with TLD [125]. In vivo studies of human coronary artery endothelial cells (HCAECs) showed that DTG reduced inflammation and IL-6, IL-8, VCAM-1, and ICAM-1 secretion via NF-κB pathway inhibition and decreased senescence by repressing apoptotic pathways [128, 129]. DTG also displayed protective properties in HCAECs, such as reduced OS, inflammation, and senescence and improved ED from an aged donor with persistently elevated levels of senescence [129]. The Stockholm pregnancy cohort showed that the PE rate was normal; however, the population size was too small to make any deductions [130]. In a study on treatment-naïve HIV-infected individuals, a significant decrease in TNF-α was observed 12 months following DTG initiation in comparison to PIs and other INSTIs, such as elvitegravir [131]. This study also revealed DTG's capacity to significantly reduce D-dimer levels [131], implicating a possible positive interaction in COVID-19-infected individuals since elevated D-dimer, a marker of clot formation, is associated with increasing severity of the disease [132]. A case study of a 63-year-old HIV-infected woman with an undetectable viral load on a DTG-containing ART showed improvement despite presenting with COVID-19 complications during admission [133]. However, the role of DTG in the treatment of COVID-19 requires further investigation.

In light of the lack of evidence that particular ARVs are clinically active against SARS-CoV-2, HIV-infected individuals are advised to refrain from changing their ART regimen in an attempt to prevent or treat COVID-19 [134].

Remdesivir in covid-19 and pregnancy

Remdesivir (RDV), initially used in the treatment of Ebola, is among the top contenders against the new coronavirus [110]. RDV is a broad-acting nucleoside analog drug that has shown inhibitory effects on pathogenic animal and human coronaviruses such as SARS-CoV-2 in vitro and inhibits Middle East respiratory syndrome coronavirus, SARS-CoV-1, and SARS-CoV-2 replication in nonhuman primates [135]. In a randomized, double-blind, placebo-controlled trial, RDV showed no significant difference in terms of clinical benefits [136]. However, a larger study population is needed to confirm the observed reduction in clinical improvement time following RDV treatment [136]. Another RCT's final report revealed that RDV was superior to placebo in decreasing recovery time among hospitalized

adults [137]. However, neither trial included pregnant women. A case report of RDV-based treatment showed the successful management of a COVID-19-positive critically ill obstetrics patient [138]. Another case report of RDV-treated COVID-19 in the third trimester of pregnancy showed no adverse outcomes apart from elevated transaminases, which is also associated with PE development [139]. PE was ruled out as a cause of transaminitis because the patient did not present with hypertension and proteinuria [139]. This report also noted that there was no clarity on whether the transaminitis observed was due to COVID-19 or RDV intake [139]. In a recent study, 86 pregnant and postpartum women with severe COVID-19 who received compassionate use of RDV showed a high rate of recovery with a low rate of serious adverse events, such as transaminitis, hypertension, and hypoxia [140]. In Ebola clinical trials, there were no adverse outcomes among pregnant women receiving RDV [80]. On October 22, 2020, the United States Food and Drug Administration (FDA) approved the emergency use of RDV for severe cases of COVID-19 [141]. However, the safety of RDV in pregnancy has not been elucidated; therefore, the inclusion of pregnant women in clinical trials is necessary to guide risk-benefit considerations of RDV treatment in COVID-19.

LMICs such as SA have a limited capacity to accommodate the daily rise in COVID-19 infections [142]. The use of RDV may be vital for the prevention of adverse outcomes and a decrease in clinical improvement time in severe COVID-19 cases while regulating intensive care unit bed capacity [142]. Recently, Gilead Sciences Incorporated, CIPLA was granted a license to manufacture and distribute a generic form of RDV for compassionate use against COVID-19 in 127 countries, including SA; however, RDV is still not readily available to all citizens [143]. There is also no knowledge of the interactions of RDV with ARVs.

Conclusion

An imbalanced angiogenic status, inflammation and OS/NS induced by placental maladaptation facilitate pervasive multiorgan ED in PE. Adverse effects associated with HIV infection and ART promote ED predisposing PE development; however, higher prevalence and mortality rates among PE cases are still associated with ART use. Pregnancies complicated by the COVID-19 exploitation of ACE 2 have a strong correlation with PE-like symptoms such as endothelial injury, implicating COVID-19 in PE onset. Despite the inconsistent data on LPV/r against COVID-19, its availability in LMICs suggests the need for further insight into its safety in HIV-associated PE complicated by COVID-19. The observed endothelial protective properties of DTG in pregnancy and its role in COVID-19 therapeutics, along with the approved compassionate use of RDV in pregnancy, have yet to be established.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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