THE REGULATION OF sVEGFR-2 AND sVEGFR-3 IN THE SERUM OF PREGNANT WOMEN WITH HIV-RELATED PREECLAMPSIA RECEIVING ANTIRETROVIRAL THERAPY

By

TASHLEN ABEL

Submitted in partial fulfilment of the requirements for the degree of

Master of Medical Science

in the

Discipline of Optics and Imaging

Nelson R Mandela School of Medicine

College of Health Sciences

University of KwaZulu-Natal

Durban, South Africa

2020

PREFACE

This study represents original work by the author and has not been submitted in any other form for any degree or diploma to another institution. 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.

Tashlen Abel
(Student Number: 215013948)

Professor Thasajvarie Naicker
(Supervisor)

DECLARATION

I, **Tashlen Abel** declare that:

i) The research reported in this dissertation, except where otherwise indicated is my original work.
ii) This dissertation has not been submitted for any degree or examination at any other university.
iii) This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
iv) This dissertation does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:
    a) Their words have been rewritten but the general information attributed by them has been referenced.
    b) Where their exact words have been used their writing has been placed inside quotation marks and referenced.
v) Where I have reproduced a publication of which I am the author, co-author, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.
vi) This dissertation does not contain text, graphics, or tables copied and pasted from the internet, unless specifically acknowledged and the source being detailed in the dissertation and the reference section.

03/11/2020

Tashlen Abel
(215013948)

Date

DEDICATION

To God Almighty

My source of wisdom, knowledge and understanding.

"The simple believeth every word: but the prudent man looketh well to his going." Proverbs 14:15

To my mother, grandparents, and little brother

To my mother and grandparents, thank you for the outstanding job you have done in raising me and for all the love, support and encouragement you have given me throughout my academic career, I am everything I am today because of you. To my little brother, thank you for all your love, support and fun times.

ACKNOWLEDGEMENTS

I would like to thank the following people, without whom I would not have been able to complete this research, and without whom I would not have made it through my Masters degree:

- My supervisor, Professor Thajasvarie Naicker whose guidance, support and encouragement has been invaluable throughout this study. Your constant motivation and care throughout this degree is greatly appreciated.
- Professor Jagidesa Moodley, for his expert guidance and support.
- Miss Nitalia Naidoo, my best friend, support-structure, and fellow colleague. Thank you for all the love, kindness, and motivation you have given me throughout this year.
- Miss Yazira Pillay and Miss Sayuri Padayachee for their laboratory assistance.
- Cathy Connolly for her expertise as a biostatistician consultant.
- Wided Kelmemi for her assistance in additional analysis of our results.
- The Optics and Imaging Center.

PUBLICATIONS

1. Abel T., Padayachee S., Naicker T. (2020). The regulation of sVEGFR-2 and sVEGFR-3 in the serum of pregnant women with HIV-related preeclampsia receiving antiretroviral therapy. **Submitted** to *Placenta*, Manuscript ID: PLAC-S-20-00962

2. Abel T., Moodley J., Naicker T. (2020). The involvement of MicroRNAs in SARS-CoV-2 infection comorbid with HIV-associated preeclampsia. **Submitted** to *Current Hypertension Reports*, Manuscript ID: HYPR-D-20-00090R1

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 2019	COVID-19
Extracellular matrix	ECM
Endothelial cell	EC
Highly active antiretroviral therapy	HAART
Human immunodeficiency virus	HIV
Interleukin	IL
KwaZulu-Natal	KZN
Matrix metalloproteinase	MMP
MicroRNA	miRNA
Placental growth factor	PlGF
Preeclampsia	PE
Protease Inhibitor	PI
Severe acute respiratory syndrome coronavirus 2	SARS-CoV-2
Soluble and/or endoglin	sEng or Eng
Soluble and/or foetal liver kinase 1	sFlk-1 or Flk-1
Soluble and/or fms-like tyrosine kinase 1	sFlt-1 or Flt-1
Soluble and/or fms-like tyrosine kinase 4	sFlt-4 or Flt-4
Soluble vascular endothelial growth factor receptor 1 to 3	sVEGFR-1 to sVEGFR-3
South Africa	SA
Trans-activator of transcription	Tat
Transforming growth factor β	TGF-β
Tumour necrosis factor	TNF-α
Vascular endothelial growth factor	VEGF
Vascular endothelial growth factor receptor 1 to 3	VEGFR-1 to VEGFR-3

LIST OF FIGURES

CHAPTER THREE

LIST OF TABLES

ABSTRACT

Background: An imbalance in the concentration of pro- and anti-angiogenic factors is evident in preeclampsia (PE). This study evaluated the expression of soluble vascular endothelial growth factor receptor 2 (sVEGFR-2) and sVEGFR-3 in the serum of preeclamptic compared to normotensive women complicated by Human Immunodeficiency Virus (HIV) infection. Additionally, in light of the coronavirus disease 2019 (COVID-19) pandemic, maternal and foetal health is a great concern; hence, we have composed a review article that provides an insight into the synergy of PE, HIV and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, as well as the involvement of epigenetic regulation.

Method: The serum expression of sVEGFR-2 and sVEGFR-3 in preeclamptic *vs* normotensive pregnancies, stratified by HIV status (n = 19) was evaluated through the utilization of a Milliplex Multiplex immunoassay.

Results: In comparison to normotensive (HIV-negative and HIV-positive), gestational age ($p = 0.0004$), systolic and diastolic blood pressure ($p<0.0001$) , and parity ($p = 0.0042$) were significantly different in preeclamptic (HIV-negative and HIV-positive) pregnancies. The serum expression of sVEGR-2 was significantly downregulated in PE compared to normotensive pregnancies ($p = 0.0025$), regardless of HIV status. A downward trend in the concentration of sVEGFR-3 was observed in preeclamptic women ($p = 0.0586$), irrespective of HIV status. Across all groups, the concentration of sVEGFR-2 was significantly downregulated in HIV-positive PE ($p = 0.0053$) and the expression of sVEGFR-3 was significantly reduced in HIV-negative PE ($p = 0.0393$), compared to HIV-negative PE.

Conclusion: This novel investigation reports a significant downregulation of serum sVEGFR-2 and a downward trend in the serum expression of sVEGFR-3 in preeclamptic compared to normotensive pregnancies. The hypoxic microenvironment of PE is associated with endothelial cell damage which greatly contributes to the decreased serum expression of sVEGFR-2 and sVEGFR-3. The use of antiretroviral therapy (ART) reconstitutes the immune response in HIV-positive preeclamptic women; hence, it significantly contributes to the risk of developing PE. Furthermore, the HIV-1 trans-activator of transcription protein mimics the behaviour of vascular endothelial growth factors (VEGF) due to their structural homology; however, this does not counterbalance the decline of VEGF in PE due to the administration of ART. In addition, the association of pregnancy with an upregulation of angiotensin converting enzyme 2 receptors increases the risk of pregnant women being infected with SARS-CoV-2. Further investigations are essential to critically evaluate the influence of HIV infection and the epigenetic regulation of these soluble anti-angiogenic factors.

OKUNGAQONDAKALI

Isendlalelo: Ukungalingani ekuqoqweni kwezici ze-pro- and anti-angiogenic kuyabonakala ku-preeclampsia (PE). Lolu cwaningo luhlole ukuvezwa kwe-soluble vascular endothelial grow factor receptor 2 (sVEGFR-2) ne-sVEGFR-3 ku-serum ye-preeclamptic kuqhathaniswa nabesifazane abajwayelekile abayinkimbinkimbi yokutheleleka nge-Human Immunodeficiency Virus (HIV). Ngaphezu kwalokho, ngokubheka ubhubhane lwesifo se-coronavirus 2019 (COVID-19), impilo kamama neye-fetus kuyinkinga enkulu; ngakho-ke, siqambe i-athikili yokubukeza enikeza ukuqonda ngokuhlangana kwe-PE, i-HIV kanye ne-acute respiratory syndrome coronavirus 2 (SARS-CoV-2), kanye nokubandakanyeka komthethonqubo we-epigenetic.

Indlela: Isichasiso se-serum se-sVEGFR-2 ne-sVEGFR-3 ekukhulelweni kwe-preeclamptic vs Normatensive, sihlukaniswe ngesimo se-HIV (n = 19) sahlolwa ngokusebenzisa iMilliplex Multiplex immunoassay.

Imiphumela: Uma uqhathanisa ne-standardotensive (i-HIV-negative ne-HIV-positive), iminyaka yokuthinta ($p = 0.0004$), umfutho wegazi we-systolic ne-diastolic ($p < 0.0001$), kanye nokulingana ($p = 0.0042$) kwehluke kakhulu ku-preeclamptic (ukukhulelwa okungekuhle ne-HIV-positive). Isichasiso se-serum se-sVEGR-2 sabhalwa phansi kakhulu ku-PE ngokuqhathaniswa nokukhulelwa okujwayelekile ($p = 0.0025$), ngaphandle kwesimo se-HIV. Umkhuba ophansi wokuhlushwa kwe-sVEGFR-3 wabonwa kwabesifazane be-preeclamptic ($p = 0.0586$), kungakhathalekile ukuthi banesimo se-HIV. Kuwo wonke amaqembu, ukuqoqwa kwe-sVEGFR-2 kwehle kakhulu kwi-HIV-positive PE ($p = 0.0053$) futhi inkulumo ye-sVEGFR-3 yehliswe kakhulu kwi-HIV-negative PE ($p = 0.0393$), uma kuqhathaniswa ne-HIV- negative PE

Isiphetho: Lolu phenyo lwenoveli lubika ukwehla okukhulu kwe-serum sVEGFR-2 kanye nokwehla kwesimo sokuvezwa kwe-serum ye-sVEGFR-3 ku-preeclamptic kuqhathaniswa nokukhulelwa okujwayelekile. I-hypoxic microenvelo ye-PE ihlotshaniswa nomonakalo weseli we-endothelial onikela kakhulu ekunciphiseni kwe-serum expression ye-sVEGFR-2 ne-sVEGFR-3. Ukusetshenziswa kwe-antiretroviral therapy (ART) kuphinda kuphenduleke ukusabela kokuzivikela kwabesifazane abane-HIV preeclamptic; ngakho-ke, kunomthelela omkhulu engcupheni yokuthuthukisa i-PE. Ngaphezu kwalokho, i-HIV-1 trans-activator ye-protein transcript ilingisa ukusebenza kwezici zokukhula kwe-vascular endothelial (VEGF) ngenxa yokuvela kwazo okuhlelekile; kodwa-ke, lokhu akuphikisi ukwehla kwe-VEGF ku-PE ngenxa yokuphathwa kwe-ART. ama-angiotensin aguqula ama-enzyme 2 receptors akhulisa ubungozi besifazane abakhulelwe abangenwa yi-SARS-CoV-2.

DISSERTATION LAYOUT

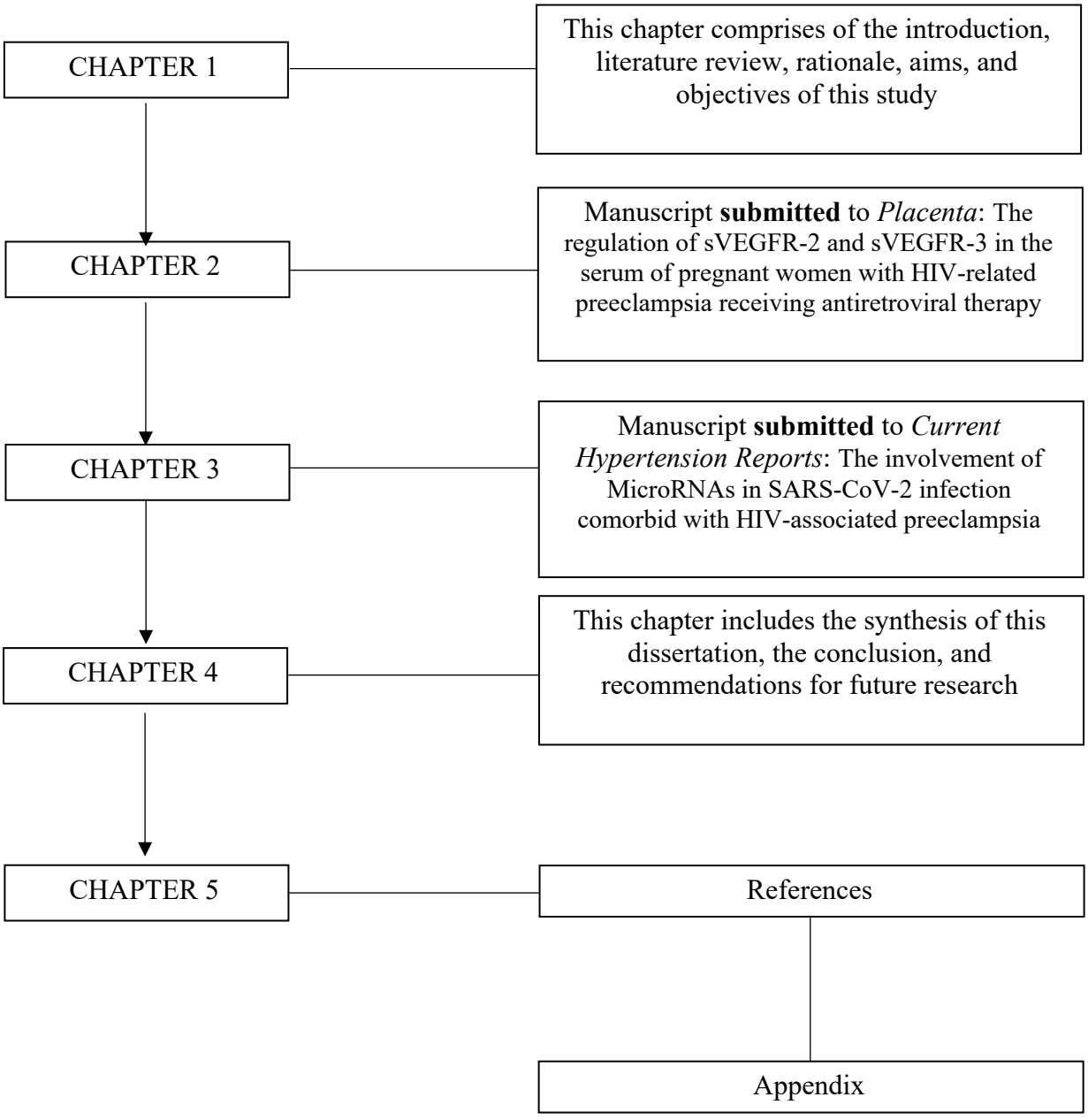


Figure 1: Schematic diagram showing the dissertation layout

CHAPTER ONE

LITERATURE AND BACKGROUND REVIEW

1.1 Hypertensive Disorders of Pregnancy

Despite extensive research, maternal mortality and morbidity remains a global issue. In 2017, almost 300 000 women died during and following pregnancy and childbirth, two-thirds of these deaths occurring within Sub-Saharan Africa (World Health Organization, 2019a). Moreover, approximately 94% of maternal deaths occurred in low-resource settings, such as South Africa (SA) (World Health Organization, 2019b). Hypertensive disorders of pregnancy (HDP) is one of the leading causes of maternal mortality and morbidity worldwide. It is responsible for 18% of maternal deaths in SA (National Committee for Confidential Enquiry into Maternal Deaths, 2018). Accordingly, for every 100 000 live births, there were 136 maternal deaths in SA (National Committee for Confidential Enquiry into Maternal Deaths, 2018). The HDP is comprised of various pregnancy-related complications including chronic hypertension, white coat hypertension, masked hypertension, gestational hypertension (GH), preeclampsia (PE), and eclampsia (Moodley *et al.*, 2019). PE and eclampsia are the most common direct cause of maternal mortality in SA (Moodley *et al.*, 2019).

1.2 Preeclampsia

Preeclampsia is a pregnancy-specific disorder, of unknown origin, that complicates 5-7% of pregnancies worldwide (Rana *et al.*, 2019) and is significantly more prevalent in low-middle income countries (LMIC) (Nathan *et al.*, 2018). The prevalence of PE in the South African province of KwaZulu-Natal (KZN) is 12% (Moodley *et al.*, 2016).

This pregnancy-related disorder is characterized by new-onset hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) with or without excessive proteinuria (average of ≥300 mg every 24 hours), presenting at or after 20 weeks' gestation in pregnant women without a history of hypertensive disorders (Brown *et al.*, 2018). Although medical practitioners still use proteinuria as a characteristic of PE, it is no longer a requirement for the diagnosis of PE. This is supported by Sperenza *et al.*, who reported no significant difference in the development of PE and eclampsia between patients who had low and high concentrations of proteinuria (Speranza *et al.*, 2019). In the event of new-onset hypertension in a pregnant woman without a history of hypertension and absence of proteinuria, PE is characterized by haemolysis, elevated liver enzymes, and low platelet count, referred to as the HELLP syndrome (Sibai *et al.*, 2005; Young *et al.*, 2010)

1.3 Classification of Preeclampsia

The classification of PE includes early-onset PE (EOPE) and late-onset PE (LOPE), entities that may manifest with or without clinical features, such as epigastric pain, visual disturbances, persistent

headaches, nausea, and vomiting (Pillay *et al.*, 2017). Importantly, each subtype is dependent on gestational age in which patients diagnosed at ≤33 weeks of gestational age and those at ≥34 weeks of gestation are diagnosed with EOPE and LOPE, respectively (Tranquilli *et al.*, 2014). Greater maternal and foetal morbidity and mortality occur in EOPE, as compared to LOPE (Redman, 2017). The disease burden is greater in LOPE and is referred to as a maternal disorder; whilst EOPE is regarded as a foetal disorder. Abnormal placentation and placental pathology occur in the EOPE subtype (Flint *et al.*, 2019).

1.3.1 Mechanism of Subclasses

Intrauterine growth restriction (IUGR), the reduced rate of growth of a foetus in the womb, is unique to EOPE (Redman and Staff, 2015). Abnormal cytotrophoblast invasion leading to insufficient spiral artery remodelling results in high vascular resistance and low blood flow; that contribute to the development of oxidative stress (Redman and Staff, 2015).

The mechanism of LOPE still requires extensive research. However, it is believed that LOPE involves the placenta outgrowing the capacity of the uterus, which results in diffuse uteroplacental dysfunction (Flint *et al.*, 2019).

1.3.2 Relationship Between Subclasses

The aetiology for the initial dysfunction of the placenta in PE has not been elucidated; nonetheless, many causes have been proposed and theorized, such as oxidative stress (Mert *et al.*, 2012), genetic (Hiby *et al.*, 2004) and immune (Yia *et al.*, 2003; Bobst *et al.*, 2005) factors. Also, angiogenic factors are circulating proteins that function in angiogenesis, the dysregulated levels of which are indicative of syncytiotrophoblast dysfunction, a characteristic feature of PE development (Karumanchi and Stillman, 2006; Flint *et al.*, 2019).

With regards to angiogenic dysregulation, research has shown that the ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) is significantly increased in PE compared to controls (Levine *et al.*, 2006; Herraiz *et al.*, 2015; Herraiz *et al.*, 2017). The anti-angiogenic, sFlt-1 produced by the placenta enters the maternal circulation and is able to act on distant targets, thereby causing endothelial dysregulation (Maynard *et al.*, 2005). Recent clinical trials that used the sFlt/PlGF ratio have proven to be successful in improving the prediction of PE in women at risk of developing the disease (Zeisler *et al.*, 2016; Perales *et al.*, 2017; Pant *et al.*, 2019).

1.4 Normal Placentation

The attachment of the blastocyst to the uterine endothelial wall occurs 4-6 days post-conception; following this, the placenta begins to develop. After implantation, the trophoblast is the first cell lineage

that begins to differentiate. The trophoblast layer surrounding the blastocyst remains whilst the daughter cells function to differentiate and proliferate, the daughter cells go on to form the cytotrophoblast. The cytotrophoblast cells have the potential to differentiate in two ways; extravillous cytotrophoblasts (EVTs) and villous cytotrophoblasts (CT), that function in invasion and fusion, respectively (Rana *et al.*, 2019). EVTs differentiate into giant cells, and endovascular cells whilst the CTs go on to form syncytiotrophoblasts (STBs) (Rana *et al.*, 2019).

Notably, STBs are incapable of replicating on their own; therefore, they recruit new mononucleated trophoblasts into the STBs. This process results in the renewal of the STBs which function in a fusion process that includes the integration of the cytoplasm content, proteins and RNA, and membranes and nuclei from cytotrophoblasts into themselves (Redman and Staff, 2015).

Extravillous trophoblasts (EVTs) function in the remodelling of the spiral artery, a process that occurs in the first 20-22 weeks of gestation. EVTs degrade the elastic and matrix tissue of the spiral artery by an enzymatic process (Haram *et al.*, 2019). The degraded material is then replaced with fibrinoid material (Haram *et al.*, 2019). This allows for penetration and invasion of the spiral arteries by the EVTs. Following the enzymatic degradation, EVTs migrate along the lumen wall of the spiral artery, gradually replacing the endothelium (Haram *et al.*, 2019). For effective remodelling of the artery, endothelial apoptosis is required which is induced by Fas/Fas ligand interactions (Redman and Staff, 2015). The expression of Fas has been identified in the spiral artery endothelium, smooth muscle cells and the decidual endothelial cells (ECs). The remodelling process of the spiral artery leads to dilation of the artery, a low-resistance flow system that enables an increase in intervillous blood circulation (Haram *et al.*, 2019).

Physiological changes during gestation include the combination of cytotrophoblasts, accumulated fibrinoid material, and loss of musculo-elastic tissue in the media of placental spiral arteries (Haram *et al.*, 2019). Any interference to these processes can result in complications in pregnancy, such as the development of PE, GH, IUGR, and vascular pathologies (Brown *et al.*, 2018). Vascular anomalies together with IUGR are characteristic of PE.

1.5 Pathogenesis of Preeclampsia

Often referred to as “the disease of theories” (Pipkin and Rubin, 1994; Higgins and Brennecke, 1998; George, 2017), PE is currently highly researched and an insidious disease (Phipps *et al.*, 2019). Currently, a direct treatment for PE is not available and the placental disease regresses following the delivery of the placenta, making early delivery of the infant the only treatment available (Valero *et al.*, 2018). Based on the regression of the disease following delivery, the placenta is seen as a causal agent in the pathogenesis of PE. The pathogenesis of PE, although not completely elucidated, is believed to

progress in two stages (Shanmugalingam *et al.*, 2019). Stage one, the foeto-placental stage occurs in the first and second trimester, followed by stage two or the maternal stage that occurs during the second and third trimester (fig. 1.1).

1.5.1 The foeto-placental stage

The first stage in the development of PE occurs in the first and second trimesters (Sircar *et al.*, 2015). This stage is referred to as the foeto-placental stage or the preclinical stage as there are no clinical signs or symptoms of PE.

The preclinical stage of PE development involves deficient EVT invasion of the spiral arteries. In this stage, EVT invasion does not progress beyond the decidual segment of the spiral artery together with reduced EVT invasion into the myometrial segment of the spiral artery (Naicker *et al.*, 2003). Insufficient invasion of the spiral artery by CTs due to elevated apoptosis leads to prevention of normal physiological changes to occur during pregnancy (Naicker *et al.*, 2013; Rana *et al.*, 2019). In normal pregnancies there is maintenance of a system with constant low-pressure flow however, in PE there is flow at a high-pressure which damages the endothelial vessels as the pregnancy progresses (Redman and Staff, 2015). Hence, there is reduced blood flow through the spiral arteries which results in hypoperfusion of the placenta. Ultimately this will cause insufficient levels of oxygen and nutrients to be delivered to the foetus throughout pregnancy.

1.5.2 The maternal stage

The maternal or clinical stage in the pathogenesis of PE occurs in the second and third trimesters of gestation. Roberts and Gammil, reported that PE is a disease that is incapable of getting better, it gets progressively worse and progresses rapidly (Roberts and Gammill, 2005). This is a statement that still holds true 15 years later as there is still no clarity on the pathogenesis of PE. Although the placenta plays a pivotal role in the progression of PE, the target organ of the disease is the maternal endothelium. Upon damage to the endothelium, placental and maternal factors such as angiogenic and vasopressive factors are released into maternal circulation (Brosens *et al.*, 2019).

Uncomplicated and successful pregnancies involve a physiological decrease in arterial blood pressure as well as peripheral resistance (Young *et al.*, 2010). However, in preeclamptic women widespread vasoconstriction is evident which causes a resultant increase in blood pressure. As the disease progresses, it injures ECs throughout the body and not just the spiral arteries. This is most evident in the kidneys where infiltration is impaired (Young *et al.*, 2010). Also, in PE there is an increase in sensitivity to vasopressors, such as angiotensin II and norepinephrine (GANT *et al.*, 1974).

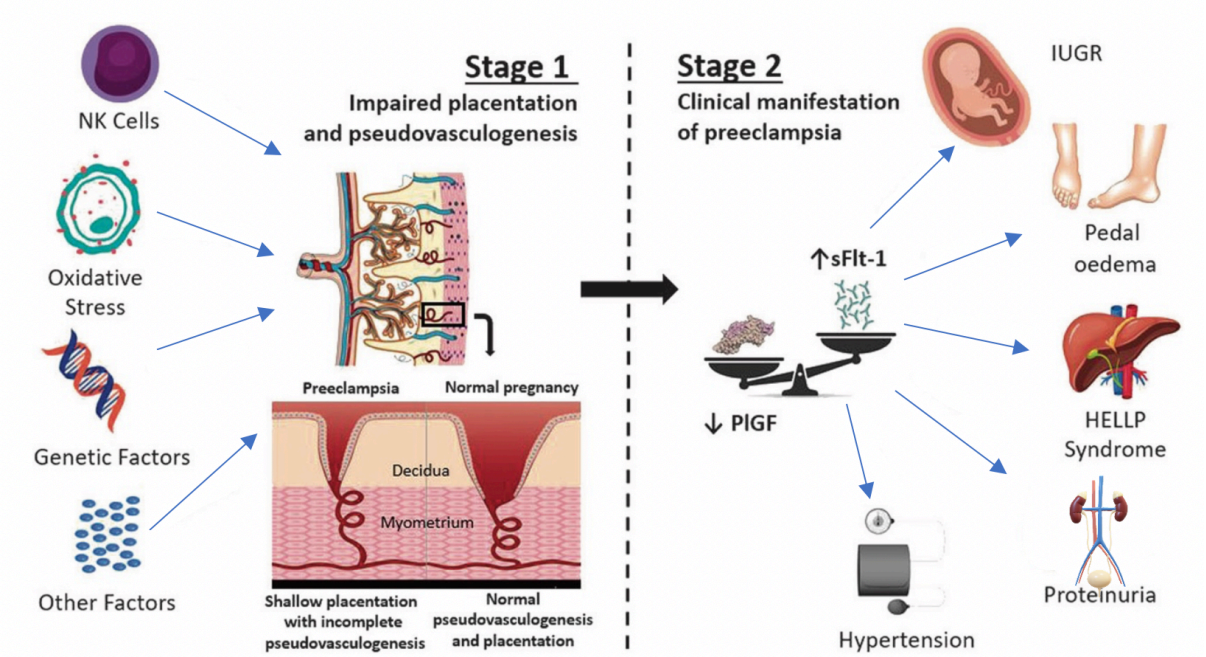


Figure 1.1: Schematic representation of the two-stage theory of preeclampsia (Adapted from Shanmugalingam *et al.*, 2019)

1.6 Vasculogenesis

Vasculogenesis is a process involving the formation of new blood vessels during the embryonic development through a *de novo* production of ECs (Patan, 2004). Vasculogenesis should not be confused with angiogenesis, it is a distinctly different process that describes the formation of new blood vessels from pre-existing blood vessels by sprouting of the ECs (Patan, 2004; Kumar *et al.*, 2009). Successful vasculogenesis requires a balance between pro-angiogenic and anti-angiogenic factors in circulation. An imbalance of angiogenic factors disrupts angiogenesis which eventuate in pathologies such as PE, IUGR, diabetes and nephropathy (Carmeliet, 2003; Wu *et al.*, 2010; Maynard and Karumanchi, 2011; Rana *et al.*, 2012).

The beginning of vasculogenesis is marked by the formation of hemangioblasts, which have the ability to proliferate and differentiate into hematopoietic cells or ECs (Patan, 2004; Azevedo Portilho and Pelajo-Machado, 2018). The Indian hedgehog (IHH) is a signalling protein that is secreted by the extra-embryonic endoderm which stimulates the formation of hemangioblasts (Kim *et al.*, 2013). The extra-embryonic mesoderm secretes fibroblast growth factors which function as signalling molecules to induce the development of further hemangioblasts (Kim *et al.*, 2013).

Hemangioblasts begin to aggregate together and form blood islands. The hemangioblasts that are located towards the periphery of the blood islands develop into ECs to form the vessel wall, whilst

hemangioblasts located towards the middle of the blood islands develop into hematopoietic cells to later form blood cells (Patan, 2004). The ECs are found to express receptors for vascular endothelial growth factor (VEGF), specifically vascular endothelial growth factor receptor-1 (VEGFR-1) and vascular endothelial growth factor receptor-2 (VEGFR-2) (Ribatti and Crivellato, 2012). Vascular endothelial growth factor-A (VEGF-A) has the affinity to bind to both receptors and upon doing so, it stimulates the formation of blood vessels (Ho and Fong, 2015). The activation of VEGFR-1 is associated with normal vascular development (Ali *et al.*, 2019) whilst the activation of VEGFR-2 regulates migration, proliferation and differentiation of ECs (Gille *et al.*, 2001).

Ultimately activation of both receptors results in the development of blood vessels therefore, disruption to the binding of VEGF to its receptors could negatively impacts vasculogenesis and angiogenesis. Following the development of *de novo* blood vessels, their growth and expansion needs to be maintained and this is achieved through the process of angiogenesis (Ferozepurwalla *et al.*, 2019).

1.7 Angiogenesis

As eluded to earlier, angiogenesis is a process that involves the development of new blood vessels from pre-existing blood vessels, thereby mediating a vascular network (Kumar *et al.*, 2009). Of note, angiogenesis is stimulated in response to hypoxic conditions or at areas where there is a deprivation of oxygen or nutrients in the tissue (Margadant, 2020). Although both achieve the same end goal, that is to develop blood vessels from pre-existing ones, it occurs in two very different processes.

1.7.1 Sprouting angiogenesis

A hypoxic environment and lack of vascularisation stimulates the release of angiogenic factors from parenchymal cells. The existing blood vessels that were created by vasculogenesis express receptors for VEGF. Following the release of VEGF from parenchymal cells, they activated and bind to their receptors on the EC. This stimulates ECs to release proteases that are able to degrade the basement membrane, which is necessary for the proliferating ECs to escape the 'parent' vessel (Ferozepurwalla *et al.*, 2019). As the ECs proliferate, they form solid sprouts which grow towards the angiogenic stimulus whilst the ECs migrate in the same direction, allowing the vessel to grow longer and across gaps in the vascular network (fig. 1.2) (Gerhardt *et al.*, 2003; Ribatti and Crivellato, 2012).

1.7.2 Intussusceptive angiogenesis

Intussusceptive angiogenesis is the formation of a new blood vessel by means of splitting an existing vessel into two, therefore it is also referred to as splitting angiogenesis (fig. 1.2). Being a process that is independent of cell proliferation and migration, intussusceptive angiogenesis is faster and more efficient as it involves the reorganization of existing ECs (Mentzer and Konerding, 2014).

In this form of angiogenesis, the endothelial walls on opposite ends of the existing blood vessel begin to migrate towards each, forming an intraluminal pillar (De Spiegelaere *et al.*, 2012). The intercellular junctions between the ECs begin to reorganize themselves, thereby forming a perforation in the core of the pillar. Following the formation of the perforation, pericytes and myofibroblasts invade and begin to create the extracellular matrix in the pillar. To conclude this process of angiogenesis, the pillars increase in size and fuse with each other, and the initial blood vessel then splits into two (De Spiegelaere *et al.*, 2012; Mentzer and Konerding, 2014).

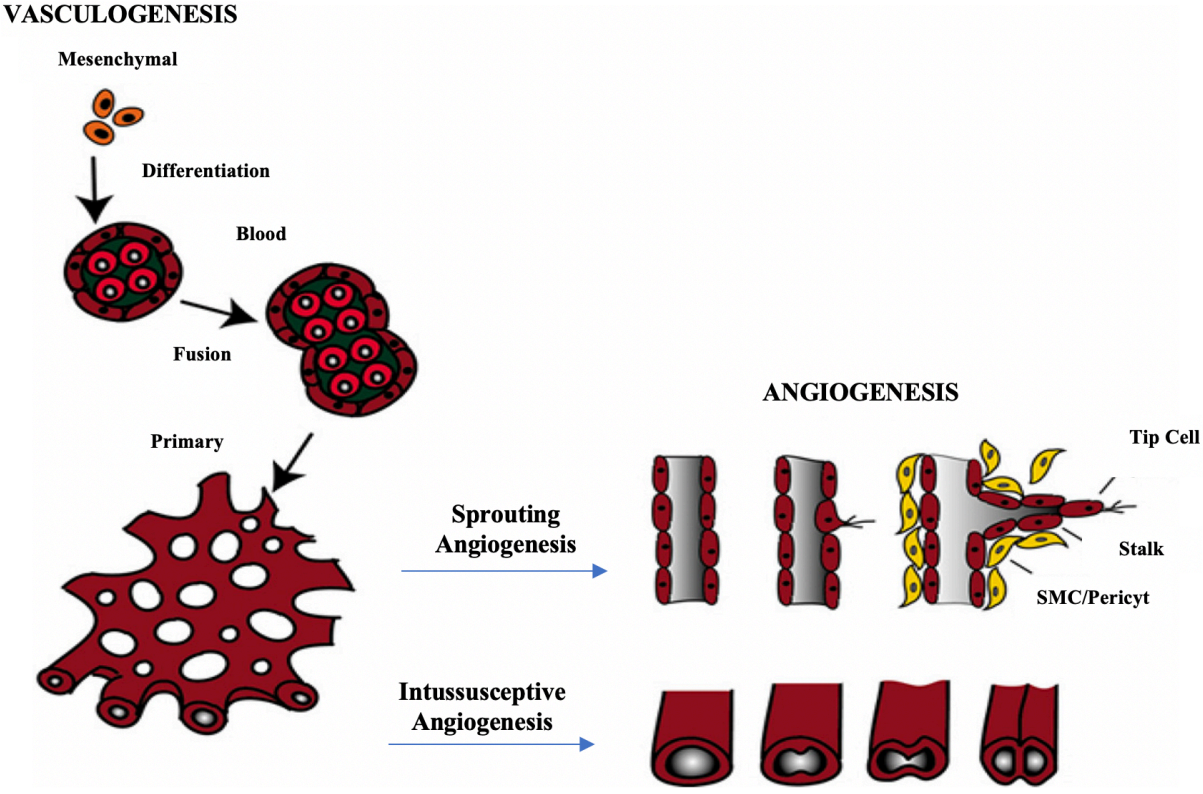


Figure 1.2: Illustration showing the difference between sprouting and intussusceptive angiogenesis (Adapted from Heinke *et al.*, 2012)

1.8 Vascular Endothelial Growth Factors and Their Receptors

An essential component of a healthy and successful pregnancy is the maintenance of a balance between pro-angiogenic and anti-angiogenic factors in maternal circulation (Rana *et al.*, 2012). The family of VEGFs are the main regulators of angiogenesis during pregnancy (Shibuya and Claesson-Welsh, 2006). VEGFs is a family that is made of up 6 members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PlGF (fig. 1.3) (Roy *et al.*, 2006; Pandey *et al.*, 2018). There are three vascular endothelial growth factor receptors (VEGFRs) found in the human body, VEGFR-1, VEGFR-2 and VEGFR-3 (Karaman

et al., 2018). The family of VEGFs display pro-angiogenic and anti-angiogenic properties however, splice variants of VEGFRs do exist and tend to exhibit anti-angiogenic effects. VEGF-C and VEGF-D bind to VEGFR-3, thereby exhibiting actions on lymphangiogenesis (Eddy *et al.*, 2018).

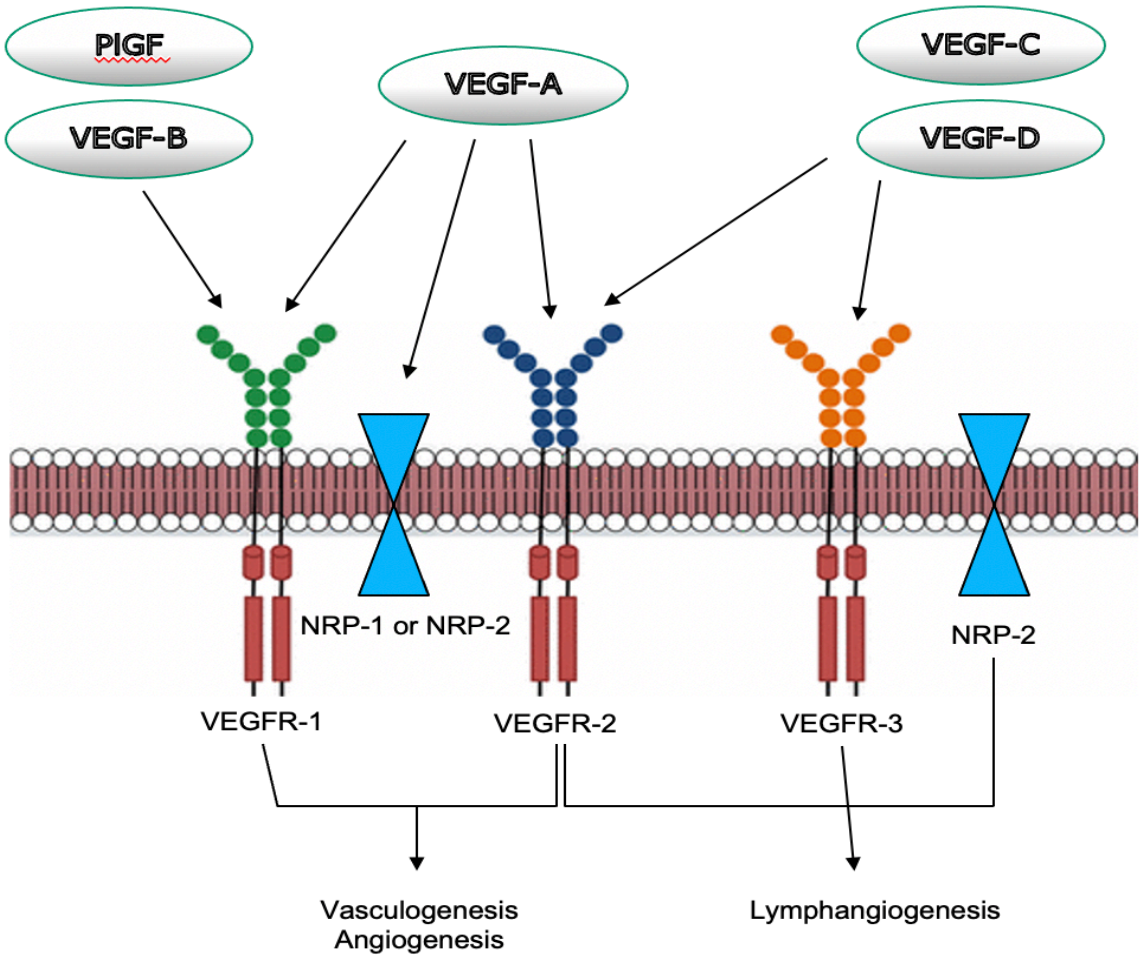


Figure 1.3: Schematic representation of the VEGF family. NRP = Neuropilin (Adapted from Pandey *et al.*, 2018)

1.8.1 Soluble VEGFR-2

The soluble form of VEGFR-2 (sVEFGR-2) or Soluble foetal liver kinase 1 (sFlk-1), is found almost only on the ECs of the placental villi and placental trophoblast (Clark *et al.*, 1996) and is considered to be one of the most important VEGFRs because of its ability to bind all VEGFs except VEGF-B and PlGF, when its co-receptor, neuropillin-1, is bound (fig. 1.3) (Tjoa *et al.*, 2010). When activated VEGFR-2 is involved in a variety of angiogenic signals that regulate mitogenic cell signalling and migratory activity of ECs (Pijnenborg *et al.*, 2010). The membrane bound VEGFR-2 possesses greater kinase activity in comparison to VEGFR-1, although the latter has a greater affinity for binding VEGFs (Naicker *et al.*, 2019).

Earlier research on VEGFRs have revealed that the circulating levels of VEGFR-2 are decreased in PE compared to normotensive pregnancies. They also found that circulating levels of sVEGFR-1 are increased in preeclamptic pregnancies (Helske et al., 2001; Maynard et al., 2003). The increase in sVEGFR-1 results in the binding of VEGF, with a concomitant decline of circulating VEGF. The synthesis of VEGFR-2 is stimulated by circulating levels of VEGF, therefore a directly proportional relationship exists between the synthesis of VEGFR-2 and circulating VEGF (Shibuya, 2013). A decrease in the synthesis of VEGFR-2 will have a direct impact and decline on the splice variant of this receptor, sVEGFR-2 (Sardar et al., 2020). Importantly, the contribution of HIV infection to EC damage may further increase the risk of developing COVID-19. Despite intensive research, there still remains a gap connecting the effects of HIV infection, ART and the duration of ART in the synergy of PE development and progression.

1.8.2 Soluble VEGFR-3

Lymphangiogenesis is the process by which lymphatic vessels develop. The lymphatic system is responsible for regulating fluid balance, lipid transport and immune cell trafficking, a defect in this pathway can lead to failure of fluid clearance, resulting accumulation of interstitial fluid and increase in pressure (Liu *et al.*, 2015). The result of a defective pathway will also present as oedema due to accumulation of fluid and increasing pressure, a condition that was a diagnostic feature of PE. Lymphangiogenesis is mediated by VEGF-C and its receptor VEGFR-3, VEGF-D and other factors such as hypoxia-inducible factor (HIF-1), the Tie/angiopoietin system, neuropillin-2 and integrin-α-9 (Naicker *et al.*, 2019). PE is characterised by decreased sVEGFR-3, slightly decreased sVEGFR-2 and increased VEGF-C (Lely *et al.*, 2013).

The signalling pathway of the soluble form of VEGFR-3 (sVEGFR-3) or fms-like tyrosine kinase-4 (sFlt-4) is initiated when stimulated by VEGF-C (Lely *et al.*, 2013). The mechanism behind the role of sVEGFR-3 in lymphangiogenesis in PE is yet to be completely elucidated. The ratio of sVEGFR-2 + sVEGFR-3/VEGF-C is significantly lower in PE compared to normotensive pregnancies and to gestational hypertension (Lely *et al.*, 2013). In fact, the expression of sVEGFR-3 was significantly lower in PE compared to normotensive pregnant women (Lely *et al.*, 2013).

There are conflicting reports on the presence of lymphatic vessels in the placenta; however, literature suggests that lymphatic vessels are absent in the placenta (Gu *et al.*, 2006; Castro *et al.*, 2011; Wang *et al.*, 2011; Liu *et al.*, 2015; Onyangunga *et al.*, 2016; Cele *et al.*, 2018). Liu et. al. found lymphatic vessels to be absent in both preeclamptic and normal pregnancies (Liu *et al.*, 2015). It was reported that the presence of lymphatic vessels were observed in the decidua (Red-Horse, 2008; Platonova *et al.*,

2013; Brown and Russell, 2014; Jerman and Hey-Cunningham, 2015; Liu *et al.*, 2015; Cele *et al.*, 2018) and the uterine wall (Cao *et al.*, 2012; Naghshvar *et al.*, 2013). Moreover, the work of Liu *et al.* verifies inhibition of lymphangiogenesis in PE group associated with a significant reduction of VEGFR-3 in the decidua PE compared to the control group (Liu *et al.*, 2015).

1.9 Human Immunodeficiency Virus

The Human Immunodeficiency Virus (HIV) infections attack the cells of the immune system, thereby making the host susceptible to other infections and diseases as the immune system is unable to fight off any foreign invaders following the infection and progression of the infection (Awi and Teow, 2018).

Following decades of research in the field, researchers are yet to create a cure for the virus, hence once a patient is infected, they will remain infected for life; however, the development of highly active antiretroviral therapy (HAART) has made major impacts in the field. The World Health Organization (WHO) has recommended that all HIV infected individuals begin and continue the use of HAART (World Health Organization, 2015). Women who are pregnant are also encouraged to continue with HAART treatment as it prevents vertical transmission of HIV, as well as during breast-feeding (World Health Organization, 2015). Nonetheless, the current treatment for HIV infection has shown to have an adverse impact on a woman's pregnancy (Sebitloane *et al.*, 2017).

1.9.1 Epidemiology

HIV infection is a global concern with almost 38 million people living with HIV at the end of 2018 (World Health Organization, 2019b). According to the UNAIDS report, there were 1.7 million new infections worldwide (Joint United Nations Programme on HIV/AIDS, 2019). In Sub-Saharan Africa, 4 out of every 5 HIV infected people between the ages 15 and 19 years are women (Joint United Nations Programme on HIV/AIDS, 2019). The total population in SA as of mid-year 2019 was 58.78 million people, of which over 51% were female (Stats SA, 2019). In 2019, 13.5% of the South African population was infected with HIV (7.97 million) (Stats SA, 2019), a country that has the highest ARV rollout in the world with almost 5 million people receiving treatment (Joint United Nations Programme on HIV/AIDS, 2019).

1.9.2 HIV-associated Preeclampsia

Preeclampsia and HIV are associated with major maternal and perinatal mortality and morbidity, this is especially observed in LMIC (Backes *et al.*, 2011). Several studies have postulated that HIV infection influences the rate of PE development (Mattar *et al.*, 2004; Hall, 2007; Kalumba *et al.*, 2013; Moodley, 2013; Landi *et al.*, 2014). HIV infection causes a decrease in CD4 T cells, indicating an effect of immunosuppression (Maartens *et al.*, 2014). Preeclampsia causes a heightened immunological response

(Redman and Sargent, 2005); therefore, HIV infection should theoretically lower the risk of developing PE (Wimalasundera *et al.*, 2002; Moodley, 2013); however, there are conflicting reports concerning this effect (Mattar *et al.*, 2004; Hall, 2007). Importantly, many recent studies have reported an increase in the risk of developing PE in HIV positive patients receiving ART (Suy *et al.*, 2006; Machado *et al.*, 2014).

1.9.2.1 sVEGFR-2 in HIV-associated Preeclampsia

The HIV-1 encodes at least nine genes in its RNA genome, including gag, pol, env, tat, rev, nef, vif, vpr, and vpu. The trans-activator of transcription or the tat protein is a polypeptide released from HIV-1 infected cells. In the process of HIV replication, the tat protein significantly enhances the efficiency of viral transcription (Debaisieux *et al.*, 2012). A significant relationship exists between the tat protein and the endothelium due to its ability to interact with several types of receptors found on the surface of endothelial cells thereby, triggering various biological responses in the endothelium (Rusnati and Presta, 2002).

The tat protein found in HIV-1 possesses an arginine- and lysine-rich sequence that is similar to several other growth factors, including fibroblast growth factor, VEGF-A, hepatocyte growth factor, and heparin-binding epidermal growth factor (Stürzl *et al.*, 1995; Albini *et al.*, 1996a; Albini *et al.*, 1996b). The similarities in the sequence enables tat to mimic the angiogenic effects of VEGF by binding to and activating the VEGFR-2/Flk-1 receptor. Albini *et al.* observed tat-stimulated endothelial cell growth and migration *in vitro*, as well as tat-induced angiogenesis *in vivo* (Albini *et al.*, 1994; Albini *et al.*, 1996b). Interestingly, it was determined that the binding of tat to VEGFR-2 was specific as no interaction was observed between the tat protein and a range of other tyrosine kinase receptors (Albini *et al.*, 1996b). The tat protein was also observed to bind to VEGFR-2 with a similar affinity as that of the receptor's endogenous ligand (Albini *et al.*, 1996b). The fact that the tat protein proved to bind to VEGFR-2 with greater specificity than that of the endogenous ligand, VEGF, indicates that the tat protein could potentially elicit more potent angiogenic properties (Noonan and Albini, 2000).

The endothelial dysfunction observed in PE has been attributed to the disruption in the levels of VEGF and its receptors (Zhou *et al.*, 2002). Despite this, VEGF expression in PE remains inconsistent. Previous studies highlighted a VEGF increase (Munaut *et al.*, 2008; Lee *et al.*, 2010; Kweider *et al.*, 2011), a VEGF decrease (Cooper *et al.*, 1996; Somerset and Kilby, 1997; Kim *et al.*, 2012) whilst other studies observed no change of VEGF expression in PE compared to controls (Sgambati *et al.*, 2004; Toft *et al.*, 2008).

The angiogenic effects of VEGF are stimulated by the binding of VEGF to VEGFR-2. The maternal circulatory levels of Flk-1 have been observed to be similar between normotensive pregnant and

normotensive non-pregnant women. Several studies investigating the expression of VEGFR-2 has identified the receptor to be decreased in early-onset PE or pregnancies with IUGR (Wallner *et al.*, 2007; Chaiworapongsa *et al.*, 2008; Tripathi *et al.*, 2009), suggesting that PE is associated with reduced levels of VEGFR-2. Although sVEGFR-2 has been investigated in the serum of preeclamptic patients and in HIV positive women, there still remains a gap establishing the expression of sVEGFR-2 in preeclamptic pregnant women who are HIV-positive.

1.9.2.2 sVEGFR-3 in HIV-associated Preeclampsia

Although HIV-1 is spread through bodily fluids such as breast milk, the most common mode of transmission is through unprotected vaginal and anal sexual intercourse. Following infiltration of the mucosal tissue by HIV, it uses lymphatic endothelial channels to disseminate infected cells to the draining lymph nodes (Zhang *et al.*, 2012).

A previous study reported that PE is characterised by a decrease in sVEGFR-3, slightly decreased sVEGFR-2, and increased VEGF-C; they also reported that the levels of sEVGFR-3 in plasma positively correlated with the levels of VEGFR-3 in the decidua (Lely *et al.*, 2013). The same group concluded that these characteristics, combined with a low ratio of (sVEGFR-2 + sVEGFR-3)/VEGF-C indicates a pro-lymphangiogenic state in preeclamptic conditions (Lely *et al.*, 2013). In theory, a decrease in sVEGFR-2 and sVEGFR-3 should result in a decrease of VEGF-C however, that does not hold true in practice. It is plausible that increased VEGF-C is a compensatory response to the oedema, hypertension and heightened inflammatory state observed in PE (Volchek *et al.*, 2010; Lely *et al.*, 2013).

Endothelial cells express secretory protein 2 (Slit2) and roundabout protein 4 (Robo4) which function to modulate EC permeability and are thus involved in the mechanism of lymphangiogenesis (Park *et al.*, 2003; Zhang *et al.*, 2012). The gp120 envelope glycoprotein of HIV-1 is able to cause the hyperpermeability of lymphatic cells *in vitro* (Zhang *et al.*, 2012). It does this by stimulating the expression of fibronectin and $\alpha5\beta1$ integrins which lead to the complexing of gp120, fibronectin, and $\alpha5\beta1$ integrins which is then able to interact with Robo4 to induce lymphatic hyperpermeability (Zhang *et al.*, 2012). The same study revealed that Slit2 inhibits the interaction, thereby inhibiting lymphatic hyperpermeability (Zhang *et al.*, 2012). Previous studies have reported that Slit2/Robo4 interactions tend to inhibit VEGF-C and block its receptor, VEGFR-3 (Yu *et al.*, 2014).

The effect of the combination of HIV-1 and PE on the levels of sVEGFR-3 warrants further research.

1.9.3 Effect of Antiretroviral Therapy on Preeclampsia

Advances that led to the development of HAART has significantly reduced the risk of vertical transmission of HIV worldwide (Aaron *et al.*, 2015); however, there are still concerns regarding the long-term effect of HAART on foetal, neonatal, and maternal outcomes (Wimalasundera *et al.*, 2002).

One of the active ingredients in HAART is nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). A study conducted by Song *et al.* reported that NRTIs inhibit the proliferation and migration of ECs, although their survival rate remained unchanged. The suppression of EC proliferation and migration led to suppressed vascular tube formation (Song *et al.*, 2018).

Moreover, NRTIs dysregulate the receptor tyrosine kinase (RTK) pathway (Song *et al.*, 2018). Disruption to angiogenic signalling leads to disruption of angiogenesis and vasculogenesis pathways. Ultimately, the dampening effect of NRTIs on angiogenic signalling leads to suppression of the development of the vascular network.

The prolonged use of NRTIs in the treatment of HIV has been shown to have negative effects on intima-media remodelling of the aorta and carotid artery in animal models (Sutliff *et al.*, 2002; Jiang *et al.*, 2010). Moreover, NRTIs have the ability to cause mitochondrial dysfunction and oxidative stress (Jiang *et al.*, 2007). Song *et al.* showed that the ability of NRTIs to induce mitochondrial oxidative stress impairs RTK signalling in ECs, which leads to the inhibition of angiogenesis and lymphangiogenesis both *in vivo* and *in vitro* (Song *et al.*, 2018). Furthermore, use of ARV that are in current use such as tenofovir disoproxil fumarate, azidothymidine, and lamivudine impacts angiogenesis and lymphangiogenesis (Song *et al.*, 2018).

In an ideal situation, PE patients comorbid with HIV infection would have a neutralisation of the immune responses. However, HAART in pregnancy reconstitutes the immune response and may predispose PE development (Maharaj *et al.*, 2017); however, the effect of the duration of HAART on immune reconstitution requires further investigation.

The biological reasoning behind patients being at higher risk of developing PE when on HAART remains to be explored; however, research has suggested that HAART causes PE by direct hepatotoxic and nephrotoxic effects, thereby mimicking PE (Wimalasundera *et al.*, 2002; Mawson, 2003). Furthermore, the administration of HAART was reported to heighten the maternal immune response to foetal antigens; thereby causing the patient to be at great risk of developing PE (Mol *et al.*, 2016).

Evidence suggests that HIV infection does play a role in the development of PE. It has been established that certain HIV proteins are associated with angiogenesis and lymphangiogenesis which are key

pathways that are imbalanced in PE; however, there is minimal research investigating the expression of soluble VEGFs in HIV-positive preeclamptic women. South Africa has one of the highest HIV infection rates globally, making it an ideal place to investigate the synergy of HIV-associated preeclampsia.

1.10 Aim

To compare soluble angiogenic factors, specifically sFlt-4 and sFlk-1 based on pregnancy type (normotensive pregnant and preeclamptic women) and by HIV status. In light of the emergence of the novel SARS-CoV-2, we aimed to highlight and investigate the synergy of PE, HIV and SARS-CoV-2.

1.10.1 Specific objectives:

- To quantify the expression of sFlk-1 and sFlt-4 in the serum of pregnant women using a Bioplex Multiplex Immunoassay.

- To compare the serum expression of sFlk-1 and sFlt-4 between normotensive and preeclamptic women regardless of HIV status using a Bioplex Multiplex Immunoassay.

- To compare the serum expression of sFlk-1 and sFlt-4 between HIV negative and HIV positive women irrespective of pregnancy type using a Bioplex Multiplex Immunoassay.

- To compare the serum expression of sFlk-1 and sFlt4 across all study types (Normotensive: HIV negative, HIV positive; Preeclamptic: HIV negative, HIV positive) using a Bioplex Multiplex Immunoassay.

- To compare the serum expression of sFlk-1 and sFlt-4 with patient demographics across all study groups.

1.11 Hypothesis

The serum expression of sVEGFR-2 and sVEGFR-3 will be dysregulated in PE compared to normotensive pregnancies complicated by HIV infection.

1.12 Research Question

Is there a dysregulation of the signalling transduction pathways of soluble angiogenic factors in the serum of preeclamptic women and comorbid HIV infection receiving HAART?

CHAPTER TWO

Original Article: The regulation of sVEGFR-2 and sVEGFR-3 in the serum of pregnant women with HIV-related preeclampsia receiving antiretroviral therapy.

This chapter explores the serum expression of sVEGFR-2 and sVEGFR-3 in preeclamptic compared to normotensive pregnancies, with associated HIV infection. The format of this chapter follows the manuscript format of a DoHET accredited peer-reviewed journal.

Citation:

Tashlen Abel, Sayuri Padayachee and Thajasvarie Naicker (2020). The regulation of sVEGFR-2 and sVEGFR-3 in the serum of pregnant women with HIV-related preeclampsia receiving antiretroviral therapy. **Submitted** to *Placenta*, Manuscript ID: PLAC-S-20-00962.

Placenta

THE REGULATION OF sVEGFR-2 AND sVEGFR-3 IN THE SERUM OF PREGNANT WOMEN WITH HIV-RELATED PREECLAMPSIA RECEIVING ANTIRETROVIRAL THERAPY

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original article
Keywords:	Human Immunodeficiency Virus, Hypertension, Placenta, Preeclampsia, Pregnancy
Corresponding Author:	Tashlen Abel University of KwaZulu-Natal Nelson R Mandela School of Medicine: University of KwaZulu-Natal College of Health Sciences SOUTH AFRICA
First Author:	Tashlen Abel
Order of Authors:	Tashlen Abel
	Sayuri Padayachee
	Thajasvarie Naicker
Abstract:	Introduction: Preeclampsia (PE) is characteristic of an angiogenic imbalance favoring anti-angiogenesis. This study investigated the serum concentrations of anti-angiogenic factors viz., soluble vascular endothelial growth factor 2 (sVEGFR-2) and 3 (sVEGFR-3), in normotensive pregnant and preeclamptic women associated with human immunodeficiency virus (HIV) infection. Methods: A Milliplex multiplex immunoassay was used to quantify the expression of serum sVEGFR-2 and sVEGFR-3 in the serum of preeclamptic vs normotensive pregnancies stratified by HIV status. Results: The expression of serum sVEGFR-2 was significantly downregulated in preeclamptic groups vs normotensive groups ($p = 0.0025$). By comparison with the HIV-negative normotensive group, we observed a significant downregulation of sVEGFR-2 in the HIV-positive preeclamptic group ($p= 0.0053$) and sVEGFR-3 in the HIV-negative preeclamptic group ($p = 0.0393$). Discussion: This novel study reports a significant downregulation of sVEGFR-2 and sVEGFR-3 in preeclamptic vs normotensive pregnancies. Endothelial cell damage significantly contributes to the downregulation of sVEGFR-2 and sVEGFR-3 in the hypoxic setting of PE. Furthermore, based on HIV status, we demonstrated a downregulation of sVEGFR-2 in HIV-positive vs HIV-negative women; in contrast, sVEGFR-3 was upregulated in HIV-positive women. The vascular endothelial growth factor mimicry effect of trans-activator of transcription protein as well as the dysregulatory effect of antiretroviral therapy treatment may account for the differential expression observed.
Suggested Reviewers:	Wendy N Phoswa phoswawendy@gmail.com Reviewer possesses a great understanding of Preeclampsia.
	Nalini Govender nalinip@dut.ac.za This reviewer is a senior lecturer in the field of maternal health.

THE REGULATION OF sVEGFR-2 AND sVEGFR-3 IN THE SERUM OF PREGNANT WOMEN WITH HIV-RELATED PREECLAMPSIA RECEIVING ANTIRETROVIRAL THERAPY

Tashlen Abel*, Sayuri Padayachee and Thajasvarie Naicker

AFFILIATIONS:

Optics and Imaging Centre, Doris Duke Medical Research Institution, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa

*** CORRESPONDING AUTHOR:**

Tashlen Abel

Optics & Imaging Centre,

Doris Duke Medical Research Institute,

College of Health Sciences,

University of KwaZulu-Natal,

Durban, South Africa.

Postal address: Private Bag 7, Congella

KwaZulu-Natal, 4013

South Africa

E-mail: **tashlen.abel@gmail.com**; **naickera@ukzn.ac.za**

Abstract

Introduction: Preeclampsia (PE)[1] is characteristic of an angiogenic imbalance favoring anti-angiogenesis. This study investigated the serum concentrations of anti-angiogenic factors *viz*., soluble vascular endothelial growth factor 2 (sVEGFR-2)[2] and 3 (sVEGFR-3)[3], in normotensive pregnant and preeclamptic women associated with human immunodeficiency virus (HIV)[4] infection. ***Methods:*** A Milliplex multiplex immunoassay was used to quantify the expression of serum sVEGFR-2 and sVEGFR-3 in the serum of preeclamptic *vs* normotensive pregnancies stratified by HIV status. ***Results:*** The expression of serum sVEGFR-2 was significantly downregulated in preeclamptic groups *vs* normotensive groups (p = 0.0025). By comparison with the HIV-negative normotensive group, we observed a significant downregulation of sVEGFR-2 in the HIV-positive preeclamptic group (p = 0.0053) and sVEGFR-3 in the HIV-negative preeclamptic group (p = 0.0393). ***Discussion:*** This novel study reports a significant downregulation of sVEGFR-2 and a trend towards a decline of sVEGFR-3 in preeclamptic *vs* normotensive pregnancies. Endothelial cell damage significantly contributes to the downregulation of sVEGFR-2 and sVEGFR-3 in the hypoxic setting of PE. Furthermore, based on HIV status, we demonstrated a downregulation of sVEGFR-2 in HIV-positive preeclamptic *vs* HIV-negative normotensive pregnancies; in contrast, sVEGFR-3 was upregulated in HIV-negative preeclamptic *vs* HIV-negative normotensive pregnancies. The vascular endothelial growth factor mimicry effect of trans-activator of transcription protein as well as the dysregulatory effect of antiretroviral therapy treatment may account for the differential expression observed.

Keywords: Human Immunodeficiency Virus, Hypertension, Placenta, Preeclampsia, Pregnancy

[1] Preeclampsia
[2] Soluble vascular endothelial growth factor 2
[3] Soluble vascular endothelial growth factor 3
[4] Human Immunodeficiency Virus

Introduction

Both preeclampsia (PE), a multifactorial hypertensive disorder of pregnancy (HDP), and human immunodeficiency virus (HIV) infection are major contributors to maternal and perinatal morbidity and mortality in low-middle-income countries (LMIC), such as South Africa (SA) [1, 2]. In 2019, over 7 million South Africans were living with HIV infection [3]. The South African province of KwaZulu-Natal (KZN) accounted for 20% of the overall maternal deaths in 2017, whilst 18% of maternal deaths in South Africa were directly linked to hypertension [1].

Preeclampsia is a disease of the placenta that is characterized by new-onset hypertension (systolic ≥140 and diastolic ≥90 mmHg) with or without proteinuria (≥300 mg) presenting at or after 20 weeks' gestation [4]. The etiology of PE has not been fully elucidated however, it is believed to occur in two stages [5]. The preclinical stage involves deficient extravillous trophoblast (EVT) invasion and defective spiral artery remodeling, predisposing placental hypoxia with a subsequent shift in angiogenic homeostasis [6]. This results in widespread damage to the maternal endothelium in the clinical stage of PE that presents with hypertension, proteinuria, and intrauterine growth restriction (IUGR) [7]. Delivery of the placenta resolves the disease, making early delivery of the fetus the only available treatment [8].

The family of vascular endothelial growth factors [VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF)] regulate various physiological responses that maintain angiogenic and lymphangiogenic homeostasis [9, 10]. Dependent on the receptor they bind, these factors may exhibit either anti-angiogenic or pro-angiogenic properties. Key vascular endothelial growth factor receptors (VEGFR) belong to the receptor tyrosine kinase (RTK) family and include VEGFR-1 or Fms-like tyrosine kinase receptor-1 (Flt-1), VEGFR-2 also known as kinase insert domain receptor (KDR) or fetal liver kinase-1 (Flk-1), and VEGFR-3 [11].

In PE, soluble isoforms of these angiogenic receptors are found within the maternal circulation [12]. Soluble VEGFR-2 (sVEGFR-2) and VEGFR-3 (sVEGFR-3) are involved in angiogenesis and lymphangiogenesis, respectively [11, 13].

VEGFR-2 binds VEGF-A and its splice variants, VEGF-C and VEGF-D whilst it does not bind VEGF-B and PlGF [14]. Activation of VEGFR-2 stimulates mitogenic cell signalling and the migratory activity of endothelial cells (ECs) [11, 14]. There is a paucity of data regarding the expression of sVEGFR-2 in PE and its exact role in the disease. Soluble VEGFR-2 is reported to be decreased in PE in comparison to pregnancies with IUGR [15-17].

Lymphangiogenesis is mediated by VEGF-C and its receptor, VEGFR-3 [13]. Reports have shown that a decline of both sVEGFR-2 and sVEGFR-3, and increased VEGF-C are characteristic of PE development [18]. The mechanism by which sVEGFR-3 is involved in lymphangiogenesis is not fully elucidated. The presence of lymphatic vessels has been observed in the decidua [19-21] and the uterine wall [22, 23]; however, recent data suggests an absence of lymphatic vessels within the placenta [20, 24]. Membranous VEGFR-3 is significantly reduced in the decidua of preeclamptic patients, indicating substantial damage to the lymphangiogenesis pathway [20].

In South Africa, PE comorbid with HIV infection significantly contributes to the high rates of pregnancy-related deaths; however, reports on the relationship between PE and HIV infection are conflicting [25-27]. It is believed that PE and HIV infection share opposing immunological and inflammatory responses [28]. Furthermore, evidence indicates that HIV-positive pregnant women receiving highly active antiretroviral therapy (HAART) are more susceptible to PE development [29, 30]. In an ideal situation, PE patients comorbid with HIV infection would have a neutralisation of the immune response [27, 31]; however, HAART in pregnancy reconstitutes the immune response thereby predisposing PE development [30, 32, 33].

Limited research on the expression of angiogenic factors in HIV infection has associated HIV infection with an increase in anti-angiogenic factors in patients receiving antiretroviral therapy (ART). The accessory trans-activator of transcription (tat) protein of HIV-1 is a polypeptide released from HIV infected cells that significantly enhances the efficiency of HIV viral transcription [34]. Interestingly, tat

protein shares a similar structural sequence to VEGF-A [35]. The tat protein can act as a soluble mediator that activates VEGFR-2 on the surface of ECs inducing EC proliferation, migration, and the release of proteolytic enzymes *in vivo* [36]. Additionally, tat protein can regulate VEGFR-2 by phosphorylating integrin subunits and neuropilin-1 (NRP-1) which is a co-receptor for VEGFR-2 [37]. Moreover, tat protein has been reported to stimulate VEGFR-3 thereby increasing endothelial nitric oxide synthase (eNOS) levels [38]. However, VEGFR-3 is reported to be absent in placental conducting and exchange villi in both HIV-negative and HIV-positive women [39]. Regrettably, there is a paucity of data on the functional role of soluble angiogenic factors in HIV infection.

The involvement of sVEGFR-2 and sVEGFR-3 in the synergy of PE and HIV infection warrants further investigating. Furthermore, there is a paucity of data on the expression of sVEGFR-2 and sVEGFR-3 in HIV-positive pregnant women receiving HAART. Based on the high prevalence of HIV infection in pregnancy in KZN and the high prevalence of PE in SA, this study attempts to elucidate the expression of soluble angiogenic factors in the serum of pregnant women, based on pregnancy type (normotensive *vs* PE) and HIV status (HIV-positive *vs* HIV-negative).

Methods and materials

Ethical approval

All participants were required to submit an informed consent form and the study was approved by the hospital CEO. This study was granted regulatory and ethical approval by the Biomedical Research Ethics Committee of the College of Health Sciences, University of KwaZulu-Natal (BCA 338/17).

Study population

This project recruited 76 pregnant women between 25 and 37 weeks of gestation from Prince Mysheni Memorial Hospital in KwaZulu-Natal, South Africa. The study population (N = 76) was divided into preeclamptic (n = 38) and normotensive groups (n = 38). The study groups were further stratified by HIV status, resulting in 4 subgroups (n = 19).

Preeclampsia was defined as having a systolic BP ≥140 mmHg and diastolic BP ≥90 mmHg with a proteinuria dipstick test of at least +2 in a 24-hour urine sample, after 20 weeks' of gestation. Gestational age was determined by ultrasonographic examination and date of last period. Information regarding maternal age and parity were self-reported. Participants' HIV status were determined via bed-side tests and CD4 cell counts, all HIV-positive patients were on treatment with antiretroviral therapy. Patients who did not provide informed consent were excluded from the study. Pregnant women with the following conditions were excluded from our study: chronic diabetes, gestational diabetes, chronic hypertension, connective tissue disorder, chronic renal disease, cardiac disease, sickle cell disease, polycystic ovarian syndrome, abruption placentae, intrauterine death, unknown HIV status, active asthma, chorio-amnionitis, systemic lupus erythematous, antiphospholipid antibody syndrome, and pre-existing seizure disorders. A qualified research nurse registered with HPCSA collected all samples taking into account the exclusion criteria. The patient demographics data was anonymised and collated into a data file.

Sample collection

Following informed consent, 10 ml of blood was extracted from the medial cubital vein, irrespective of fasting status, and collected in EDTA tubes (Becton Dickinson and Company, South Africa). The venous blood was then centrifuged, and 2 ml of serum was aliquoted into cryovials and stored at -80˚C until required.

Bio-plex Multiplex immunoassay

A Milliplex multiplex immunoassay was performed following the manufacturer's guidelines (Merck KGaA, Germany) to analyse serum samples for the expression of sVEGFR-2 and sVEGFR-3. The samples were prepared using a 1:5 dilution series.

In brief, the sVEGFR-2 and sVEGFR-3 capture antibody-coupled magnetic beads were added to a 96-well plate and washed twice. Standards, samples and blanks were then added into their respective wells and allowed to incubate; this was followed by washing the plate three times. A biotinylated detection antibody was added to the wells and allowed to incubate before washing the plate three times. Streptavidinphycoerythrin conjugate (SA-PE) was added to each well. The plate was then washed three times and each well was resuspended in assay buffer. This was performed using the Bio-Plex® MAGPIX™ Multiplex reader (Bio-Red Laboratories Inc., USA). The reader detected the fluorescence of the SA-PE bound to each bead, which was proportional to the concentration of each analyte in the sample.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 (GraphPad software, San Diego California, USA). Data normality and distribution were assessed by the D'Agostino and Pearson, Shapiro-Wilk, and Kolmogorov Smirnov tests. For group analysis, a one-way ANOVA and Bonferroni *post hoc* test were used for parametric data while the Kruskal-Wallis and Dunn's *post hoc* test were used for non-

parametric data. For individual analysis *i.e.* pregnancy type (normotensive *vs* preeclamptic) and HIV status (negative *vs* positive), a Mann-Whitney *U* test was performed. Similarly, an unpaired *t*-test was used for parametric data. Parametrically distributed data was represented as mean ± standard deviation whilst non-parametrically distributed data was represented as median and interquartile range. A p value of <0.05 was considered statistically significant.

Results

Demographics

The patient demographics of our sample population are summarized in Table 1. Gestational age, parity, maternal age, systolic and diastolic BP were significantly different between normotensive (HIV-negative and HIV-positive) pregnant and preeclamptic (HIV-negative and HIV-positive) groups ($p <$ 0.05). There were no significant differences in maternal weight between the study groups. Proteinuria was detected in all PE samples by the use of a rapid proteinuria dipstick test.

Table 1: Patient demographics (N=76). Data is represented as the median (IQR).

	Normotensive HIV-	Normotensive HIV+	Preeclamptic HIV-	Preeclamptic HIV+	P Value
Gestational age (weeks)	37.00 (9.00)	25.00 (14.00)	24.00 (10.00)	23.00 (10.00)	0.0004***
Systolic BP (mmHg)	109.0 (20.0)	112.0 (13.0)	146.0 (14.0)	147.0 (20.0)	<0.0001***
Diastolic BP (mmHg)	65.00 (13.00)	72.00 (14.00)	93.00 (10.00)	97.00 (13.00)	<0.0001***
Parity	1.000 (1.000)	2.000 (1.000)	1.000 (1.000)	2.000 (1.000)	0.0042*

Maternal age (years)	25.00 (9.00)	31.00 (11.00)	28.00 (16.00)	34.00 (14.50)	0.0211*
Maternal weight (kg)	74.00 (22.00)	81.00 (28.00)	82.00 (44.00)	79.50 (35.00)	0.2116

*p<0.05, **p<0.001, ***p<0.0001

Serum levels of sVEGFR-2

Pregnancy type

Irrespective of HIV status, there was a significant difference of sVEGFR-2 detected between normotensive and preeclamptic pregnancies [F(4.398) = 1.904; p = 0.0025]. Significantly reduced levels of sVEGFR-2 were observed in PE (mean = 13649 ±5574 pg/ml) compared to normotensive pregnancies (mean = 18480 ±7691 pg/ml) (*fig. 1A*).

HIV status

Serum sVEGFR-2 did not significantly differ [F(4.398) = 2.846; p =. 0.1022] between HIV-positive (mean = 17397 ±8540 pg/ml) and HIV-negative (mean = 14732 ±5062 pg/ml) groups, regardless of pregnancy type (*fig. 1B*).

Across all groups

Albeit non-significantly across all groups, sVEGFR-2 was significantly different [F(4.398) = 4.398; p = 0.053] between HIV-negative normotensive and HIV-positive PE groups. The concentration of sVEGFR-2 was significantly downregulated in HIV-positive PE (mean = 12006 ±4046 pg/ml) in comparison to HIV-negative normotensive pregnancies (mean = 19501 ±9939 pg/ml) (*fig. 1C*). There were no significant differences between all other groups recorded.

Serum levels of sVEGFR-3

Pregnancy type

There was no significance difference in the concentration of sVEGFR-3 between normotensive (median = 4080 pg/ml; IQR = 5459 pg/ml) and preeclamptic (median = 2610 pg/ml; IQR = 6623.18 pg/ml) pregnancies (Mann-Whitney U = 540.5; $p = 0.0586$) (*fig. 2A*).

HIV status

Regardless of pregnancy type, there was no statistically significant difference of sVEGFR-3 between the HIV-negative (median = 2512 pg/ml; IQR = 6508.7 pg/ml) and HIV-negative (median = 4052 pg/ml; IQR = 5441 pg/ml) groups (Mann-Whitney U = 564; $p = 0.1004$) (*fig. 2B*).

Across all groups

A level of significance (Kruskal-Wallis=10.11; p=<0.05) for sVEGFR-3 concentrations was detected between HIV-negative normotensive and HIV-negative pregnancies (*fig. 2C*). Notably, sVEGFR-3 was elevated in the HIV-negative normotensive (median=4143 pg/ml; IQR=5948 pg/ml) *vs* HIV-negative PE (median=875 pg/ml; IQR=4196 pg/ml) groups. There was no evidence of statistically significant differences between all other groups.

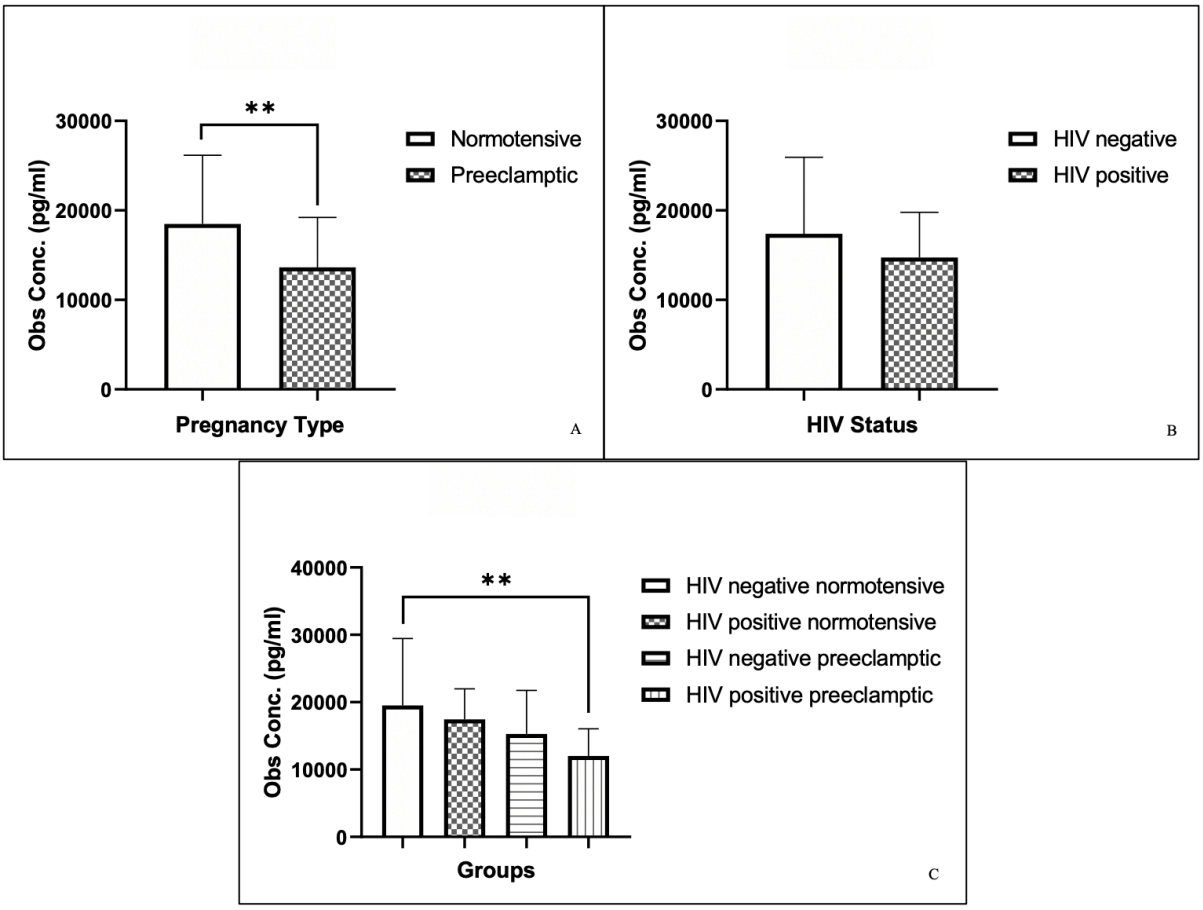


Figure 1: Serum concentrations of soluble vascular endothelial growth factor 2 (sVEGFR-2) with respect to: (A) Normotensive *vs* Preeclampsia groups, (B) HIV-negative *vs* HIV-positive, (C) Across all groups. **Serum concentrations of sVEGFR-2 are significantly different between normotensive and preeclamptic pregnancies, p = 0.0025. **Serum concentrations of sVEGFR-2 are significantly different between HIV-negative normotensive and HIV-positive preeclamptic pregnancies, p = 0.0053. Data represented as mean and standard deviation.

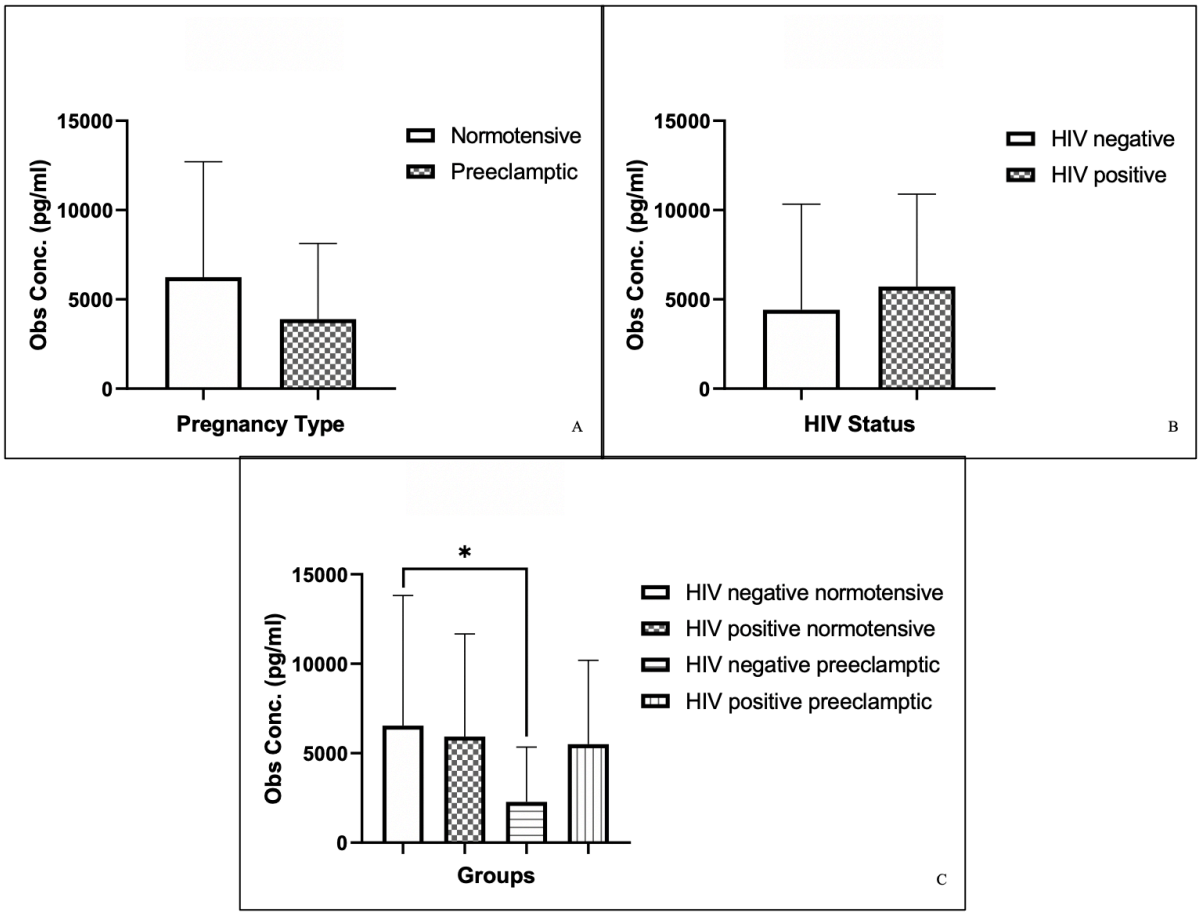


Figure 2: Serum concentrations of soluble vascular endothelial growth factor 3 (sVEGFR-3) with respect to: (A) Normotensive *vs* Preeclampsia groups, (B) HIV-negative *vs* HIV-positive, (C) Across all groups. *Serum concentrations of sVEGFR-3 are significantly different between HIV-negative normotensive and HIV-negative preeclamptic pregnancies, $p = 0.0393$.

Discussion

sVEGFR-2

The main finding of our study was the significant downregulation of serum sVEGFR-2 in preeclamptic compared to normotensive pregnancies, irrespective of HIV status. Our findings are corroborated by several investigations [15, 17]. Chaiworapongsa *et al.* reported that the expression of sVEGFR-2 is downregulated in PE pregnancies compared to normotensive pregnancies [17]. The same group reported a downregulation of sVEGFR-2 as early as 6-10 weeks prior to the onset of the clinical characteristics of PE [17]. The VEGF/VEGFR-2 signaling pathway plays an important role in angiogenesis and its dysregulation in PE highlights its potential to be used as an early biomarker for the detection of possible PE development [15].

Under certain physiological conditions such as hypoxia, the bioavailability of VEGF is decreased [40]. Hypoxia inducible factor-1-α (HIF-1-α) is a key mediator of hypoxic response [41]. In a recent study, we demonstrated increased placental expression of HIF-1-α in PE compared to normotensive pregnancies that correlate to their increased syncytiotrophoblast microvesicles concentration in maternal circulation [42]. These findings suggest that due to the HIF-1-α rich microenvironment of PE, there may be an insufficient availability of VEGFR-2 for VEGF binding. Additionally, VEGF mRNA dysregulation and its association with growth factors may control the release of VEGF available to bind VEGFR-2 [43].

Activation of VEGFR-2 through VEGF stimulation via an autocrine/paracrine loop is essential for EC proliferation, function and survival [44]. Tripathi *et al.* reported a significant reduction in the expression of VEGFR-2 in syncytiotrophoblasts, cytotrophoblasts, ECs, which is associated with a subsequent decrease in circulating sVEGFR-2 [15]. During the third trimester of pregnancy, VEGFR-2 is primarily expressed on the vascular ECs of the placenta [45]. Preeclamptic patients are reported to express decreased levels of free VEGF [46]; this combined with a decrease in VEGFR-2 may be associated with the systemic endothelial dysfunction evident in PE. Furthermore, the decreased availability of free

VEGF could interfere with the function and survival of ECs; whereby it prevents the stimulation of VEGFR-2 in ECs, leading to downregulation of sVEGFR-2 (*fig. 3*) [47]. Inhibition of retinal neovascularization after local administration of recombinant sVEGFR-2, supports the role of sVEGFR-2 as an anti-angiogenic factor [48]. Additionally, it has been suggested that the administration of adenovirus encoding murine sVEGFR-2 to non-pregnant rats induces hypertension and proteinuria, further supporting the anti-angiogenic role of sVEGFR-2 [46]. Since PE is characterized by an anti-angiogenic state, there should be an increase in sVEGFR-2; however, the expression sVEGFR-2 has been reported to be downregulated in PE. In accordance with this, our study revealed the downregulation of sVEGFR-2 in PE [15]. A possible explanation for these observations can be attributed to the early-in-pregnancy decline of sVEGFR-2; which leads to wide-spread endothelial damage and might indicate a low regenerative capacity of ECs [49]. This prevents the stabilization of sVEGFR-2 expression and is possibly responsible for the downregulation of sVEGFR-2 observed in PE.

A decreased concentration of sVEGFR-2 was observed in HIV-positive compared to HIV-negative groups, albeit non-significantly. A structural homology is evident between tat protein and VEGF-A [35]. It was reported that VEGF-A is able to bind to sVEGFR-2 [50] hence, it is possible that tat protein is able to bind to sVEGFR-2 due to their structural homology. The reduced levels of sVEGFR-2 in HIV-positive women could be due to the binding of tat protein to sVEGFR-2 which leads to a decrease of sVEGFR-2 in maternal circulation (*fig. 3*). Chronic HIV infection is associated with chronic arterial injury and subsequent EC damage [51]. Therefore, it is plausible to assume that chronic HIV infections leads to the inability of ECs to produce VEGFR-2 which leads to a decrease of sVEGFR-2 expression in HIV-positive pregnant women, irrespective of pregnancy type. Furthermore, we observed a significant decrease of sVEGFR-2 in HIV-positive preeclamptic pregnancies in comparison to HIV-negative normotensive pregnancies. Preeclampsia and HIV infection share opposing immune responses [51]. However, administration of ART reconstitutes the immune response in HIV infection, initiating an immuno-inflammatory state [51]. Additionally, Suy *et al.* reported that women exposed to HAART prior to pregnancy are at a greater risk of developing PE compared to treatment-naïve pregnant women

[33]. The ART-induced immuno-inflammatory state, along with the immuno-inflammatory state of PE may result in chronic inflammation. Endothelial dysfunction is a consequence of chronic inflammation [52]; hence, it is plausible that the heightened endothelial dysfunction leads to severely reduced sVEGFR-2 expression in HIV-associated PE.

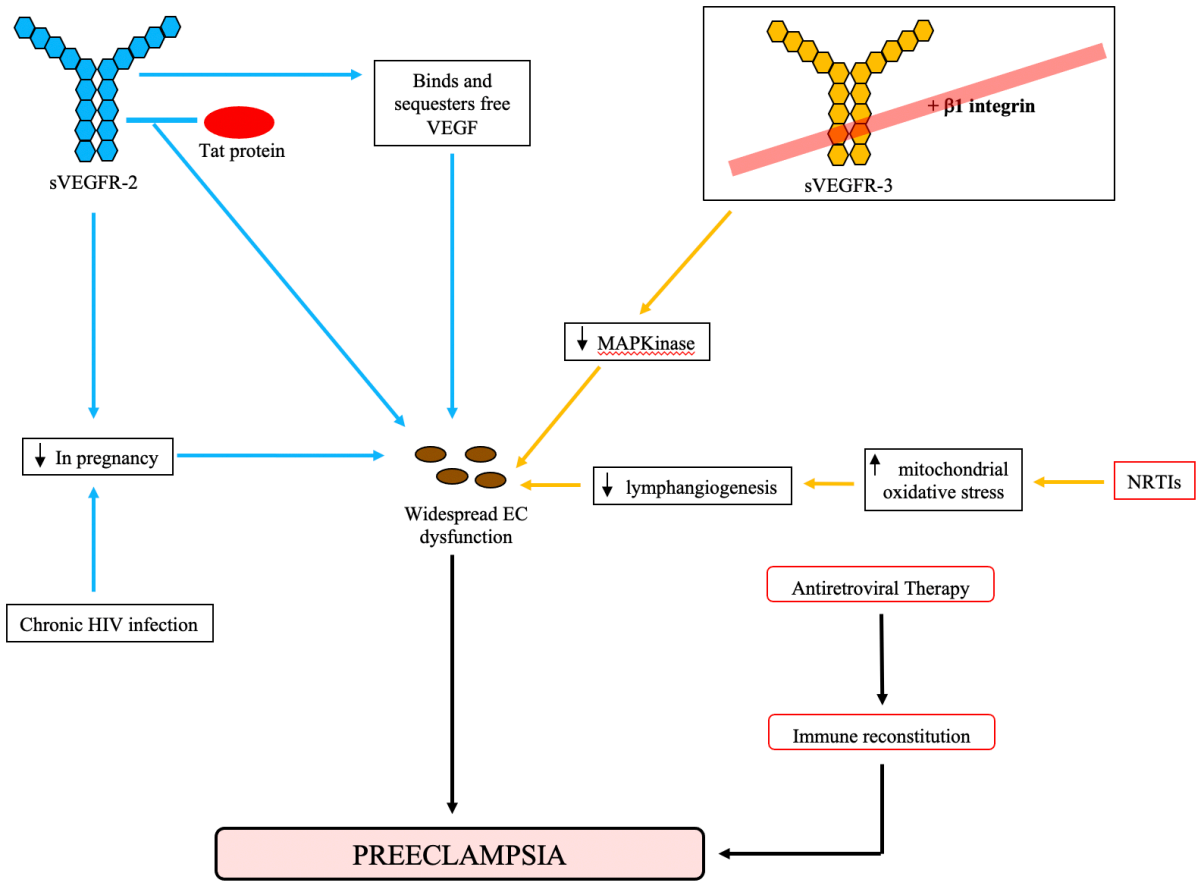


Figure 3: Schematic representation of sVEGFR-2 and sVEGFR-3, HIV infection and ART in PE development.

sVEGFR-3

This study also reports a downregulation of sVEGFR-3 in preeclamptic pregnancies compared to normotensive pregnant women, albeit non-significantly. Our observations are supported by Lely *et al.* who reported a significant decrease of sVEGFR-3 in preeclamptic compared with normotensive pregnancies; an anti-angiogenic and anti-lymphangiogenic factor that binds and sequesters VEGF-C [18]. However, it is important to note that the origins of the circulatory factors were unknown [18]. Both VEGF-C and VEGF-D bind to membranous VEGFR-3, thus this receptor is associated with

lymphangiogenesis [53]. Preeclampsia is characterized as expressing decreased sVEGFR-3, slightly decreased sVEGFR-2 and increased VEGF-C [18]. The lower ratio of sVEGFR-2 + sVEGFR-3/VEGF-C is descriptive of a pro-lymphangiogenic state in PE. Lely *et al.* attributed the pro-lymphangiogenic state of PE to a compensatory mechanism in response to the exacerbated inflammatory state of PE [18]. The involvement of the lymphangiogenic pathway in the synergy of PE and HIV infection is yet to be elucidated; due to a paucity of available literature exploring the functional role of sVEGFR-3 in hypertension-related disorders as well as in HIV infection. Nevertheless, Machnik *et al.* showed that dysregulation of the VEGF-C/VEGFR-3 pathway in rats promotes the development of salt-sensitive hypertension [54]. It is plausible to assume that sVEGFR-3 has a greater affinity for binding VEGF-C than VEGFR-3 based on the trends observed in soluble isoforms of VEGFR. In this scenario, it is possible that sVEGFR-3 binds and sequesters a significant concentration of VEGF-C leading to dysregulation of the lymphangiogenic pathway, whilst the increased levels of VEGF-C can be attributed to a compensatory mechanism to the dysregulation.

Activation of the VEGF-C/VEGFR-3 pathway attenuates the production of proinflammatory cytokines [55]; however, the increase of proinflammatory cytokines, TNF-α and IL-6 [56], in PE indicate an inhibition of this pathway. Vascular remodeling of the uterus to increase blood flow during pregnancy is regulated by VEGFR-3 [57] which is possibly downregulated in PE due to the evidence of incomplete spiral artery remodeling in PE. Downregulation of VEGFR-3 signaling in lymphatic ECs may prevent the activation of the mitogen-activated protein kinase (MAPKinase) pathway that is essential for the survival of ECs [58]. Thus, inhibition of the MAPKinase pathway can promote PE development due to dysregulation of EC survival. Additionally, activation of signal transduction pathways of both β1 integrins and VEGFR-3 are essential for EC migration, growth, differentiation, and adhesion [59]. Due to the anti-angiogenic state of PE, we speculate that there is a lack of β1 integrin stimulation and subsequent inactivation of the MAPKinase pathway as well as the function of VEGFR-3 (*fig. 3*). The molecular interactions of sVEGFR-3 in PE and HIV infection remains to be thoroughly investigated, however current data shows a link between sVEGFR-3 and the pathophysiology of PE.

Furthermore, our results showed a trend of higher sVEGFR-3 concentration in HIV-positive compared to HIV-negative women, regardless of pregnancy type. Angiogenesis and lymphangiogenesis are downregulated during HIV infection treatment with nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), due to their induction of mitochondrial oxidative stress that leads to damage of the RTK signaling in ECs (*fig. 3*) [60]. Protease inhibitors (PIs) are also reported to decrease progesterone levels in trophoblast cells which leads to dysregulated trophoblast cell proliferation and migration [61]. Kala *et al.* reported that ART incorporating PIs disrupts the normal physiology of uterine decidualization and spiral artery remodeling in animal and human models [62]. Additionally, Conroy *et al.* reported an increase in anti-angiogenic factors in HIV-positive women receiving ART [63]. Since all HIV-positive patients in our study received ART treatment, it is plausible that the increase in sVEGFR-3 during HIV infection is due to ART and its potential to inhibit lymphangiogenesis. In further support of this, we also observed a trend of sVEGFR-3 elevation in HIV-positive PE groups compared to HIV-negative PE groups.

The exclusion of the severity of PE and viral load on sample collection was a limitation in our study. It is plausible that the expression of soluble angiogenic factors and their receptors will differ in different severities of PE and HIV infection. Also, viral load was not conducted as this is not a standard of care in SA. Furthermore, this study did not identify the origin of sVEGFR-2 and sVEGFR-3 in maternal circulation during normotensive and complicated pregnancies. Nonetheless, the strength of this study is the analysis of soluble VEGFR rather than membrane bound VEGFR. The concentration of soluble VEGFR is easier to grasp in a clinical location and is less affected by preanalytical variation risks such as risk of platelet activation during serum sample preparation.

The epigenetic regulatory mechanism of microRNAs in pregnancy-related disorders warrants further investigation in order to establish an understanding of the post-transcriptional evolution of sVEGFR-2 and sVEGFR-3. We recommend investigating the genetic polymorphisms of sVEGFR-2 and sVEGFR-3 in an attempt to elucidate the regulation of anti-angiogenic genes. Further research investigating

expression of these factors with regards to the severity of the diseases, as well as the effect of HAART on their expressions are recommended. Additionally, it will be beneficial to conduct a large-scale study investigating the functional role of sVEGFR-2 and sVEGFR-3 in pregnancy, PE, and HIV infection.

Conclusion

This novel study demonstrates a downregulation of both sVEGFR-2 and sVEGFR-3 in preeclamptic compared to normotensive pregnancies, irrespective of HIV status. The decline of sVEGFR-2 early in pregnancy can cause EC damage which further downregulates the expression of sVEGFR-2 in PE. It is possible that sVEGFR-3 has a greater affinity for binding VEGF-C compared to VEGFR-3; thus, we speculate that sVEGFR-3 binds and sequesters VEGF-C, leading to the dysregulation of the lymphatic pathway in PE. Additionally, based on HIV status, this study reports a downregulation of sVEGFR-2 in HIV-positive women; whilst sVEGFR-3 was upregulated in HIV-positive pregnant women. The decline of sVEGFR-2 is possibly due to the EC damage that arises from chronic HIV infection. The tat protein shares similar structural sequences with the mRNA of VEGF-A; therefore, it can bind to sVEGFR-2; hence, it is possible to assume that the binding of tat protein significantly contributed to the downregulation of sVEGFR-2 in HIV-positive women. Antiretroviral therapy significantly impacts trophoblast proliferation and migration; and has been associated with impaired uterine decidualization and vascular remodeling, and lymphangiogenesis. Future investigations should focus on the differential expression of anti-angiogenic factors at the level of gene expression in pregnancy and related disorders.

References

[1] National Committee for Confidential Enquiry into Maternal Deaths, Saving Mothers Report 2017, Pretoria: South African Department of Health, 2018.

[2] C.H. Backes, K. Markham, P. Moorehead, L. Cordero, C.A. Nankervis, P.J. Giannone, Maternal preeclampsia and neonatal outcomes, J Pregnancy 2011 (2011) 214365.

[3] Stats SA, Mid-year Population Estimates 2019, Pretoria, South Africa, 2019.

[4] M.A. Brown, L.A. Magee, L.C. Kenny, S.A. Karumanchi, F.P. McCarthy, S. Saito, D.R. Hall, C.E. Warren, G. Adoyi, S. Ishaku, Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice, Hypertension (Dallas, Tex. : 1979) 72(1) (2018) 24-43.

[5] R. Shanmugalingam, A. Hennessy, A. Makris, Aspirin in the prevention of preeclampsia: the conundrum of how, who and when, Journal of human hypertension 33(1) (2019) 1-9.

[6] S. Rana, E. Lemoine, J. Granger, S.A. Karumanchi, Preeclampsia, Circ Res 124(7) (2019) 1094-1112.

[7] R. Akolekar, A. Syngelaki, R. Sarquis, M. Zvanca, K.H. Nicolaides, Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks, Prenat Diagn 31(1) (2011) 66-74.

[8] L. Valero, K. Alhareth, S. Gil, E. Lecarpentier, V. Tsatsaris, N. Mignet, T. Fournier, K. Andrieux, Nanomedicine as a potential approach to empower the new strategies for the treatment of preeclampsia, Drug discovery today 23(5) (2018) 1099-1107.

[9] S. Rana, C.E. Powe, S. Salahuddin, S. Verlohren, F.H. Perschel, R.J. Levine, K.-H. Lim, J.B. Wenger, R. Thadhani, S.A. Karumanchi, Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia, Circulation 125(7) (2012) 911-919.

[10] A.C. Eddy, G.L. Bidwell, E.M. George, Pro-angiogenic therapeutics for preeclampsia, Biology of sex differences 9(1) (2018) 1-11.

[11] R. Pijnenborg, I. Brosens, R. Romero, Placental bed disorders: basic science and its translation to obstetrics, Cambridge University Press2010.

[12] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nature medicine 9(6) (2003) 669-76.

[13] T. Naicker, W.N. Phoswa, O.A. Onyangunga, P. Gathiram, J. Moodley, Angiogenesis, Lymphangiogenesis, and the Immune Response in South African Preeclamptic Women Receiving HAART, International journal of molecular sciences 20(15) (2019) 3728.

[14] M.L. Tjoa, E.V. Khankin, S. Rana, S.A. Karumanchi, Angiogenic factors and preeclampsia, Placent Bed Disord Basic Sci its Transl to Obstet 31(1) (2010) 229-42.

[15] R. Tripathi, G. Rath, R. Ralhan, S. Saxena, S. Salhan, Soluble and membranous vascular endothelial growth factor receptor-2 in pregnancies complicated by pre-eclampsia, Yonsei Med J 50(5) (2009) 656-66.

[16] C. Munaut, S. Lorquet, C. Pequeux, C. Coulon, J. Le Goarant, F. Chantraine, A. Noël, F. Goffin, V. Tsatsaris, D. Subtil, Differential expression of Vegfr-2 and its soluble form in preeclampsia, PloS one 7(3) (2012).

[17] T. Chaiworapongsa, R. Romero, A.L. Tarca, J.P. Kusanovic, F. Gotsch, P. Mittal, S.K. Kim, E. Vaisbuch, S. Mazaki-Tovi, O. Erez, Z. Dong, C.J. Kim, L. Yeo, S.S. Hassan, A decrease in maternal plasma concentrations of sVEGFR-2 precedes the clinical diagnosis of preeclampsia, Am J Obstet Gynecol 202(6) (2010) 550.e1-10.

[18] A.T. Lely, S. Salahuddin, K.M. Holwerda, S.A. Karumanchi, S. Rana, Circulating lymphangiogenic factors in preeclampsia, Hypertension in pregnancy 32(1) (2013) 42-49.

[19] S.B. Cele, F. Odun-Ayo, O.A. Onyangunga, J. Moodley, T. Naicker, Analysis of hepatocyte growth factor immunostaining in the placenta of HIV-infected normotensive versus preeclamptic pregnant women, European journal of obstetrics, gynecology, and reproductive biology 227 (2018) 60-66.

[20] H. Liu, Y. Li, J. Zhang, M. Rao, H. Liang, G. Liu, The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia, Placenta 36(3) (2015) 279-286.

[21] H.M. Brown, D.L. Russell, Blood and lymphatic vasculature in the ovary: development, function and disease, Hum Reprod Update 20(1) (2014) 29-39.

[22] R. Cao, H. Ji, N. Feng, Y. Zhang, X. Yang, P. Andersson, Y. Sun, K. Tritsaris, A.J. Hansen, S. Dissing, Y. Cao, Collaborative interplay between FGF-2 and VEGF-C promotes lymphangiogenesis and metastasis, Proceedings of the National Academy of Sciences of the United States of America 109(39) (2012) 15894-9.

[23] F. Naghshvar, Z. Torabizadeh, N. Moslemi Zadeh, H. Mirbaha, P. Gheshlaghi, Investigating the Relationship between Serum Level of s-Met (Soluble Hepatic Growth Factor Receptor) and Preeclampsia in the First and Second Trimesters of Pregnancy, ISRN Obstet Gynecol 2013 (2013) 925062.

[24] Y. Wang, J. Sun, Y. Gu, S. Zhao, L.J. Groome, J.S. Alexander, D2-40/podoplanin expression in the human placenta, Placenta 32(1) (2011) 27-32.

[25] K.A. Frank, E.J. Buchmann, R.C. Schackis, Does human immunodeficiency virus infection protect against preeclampsia-eclampsia?, Obstetrics and gynecology 104(2) (2004) 238-42.

[26] R. Mattar, A.M. Amed, P.C. Lindsey, N. Sass, S. Daher, Preeclampsia and HIV infection, European journal of obstetrics, gynecology, and reproductive biology 117(2) (2004) 240-1.

[27] R.C. Wimalasundera, N. Larbalestier, J.H. Smith, A. de Ruiter, G.T.S.A. Mc, A.D. Hughes, N. Poulter, L. Regan, G.P. Taylor, Pre-eclampsia, antiretroviral therapy, and immune reconstitution, Lancet (London, England) 360(9340) (2002) 1152-4.

[28] C.W. Redman, I.L. Sargent, Immunology of pre-eclampsia, Am J Reprod Immunol 63(6) (2010) 534-43.

[29] B.W.J. Mol, C.T. Roberts, S. Thangaratinam, L.A. Magee, C.J.M. de Groot, G.J. Hofmeyr, Pre-eclampsia, Lancet (London, England) 387(10022) (2016) 999-1011.

[30] N.R. Maharaj, A. Phulukdaree, S. Nagiah, P. Ramkaran, C. Tiloke, A.A. Chuturgoon, Pro-inflammatory cytokine levels in HIV infected and uninfected pregnant women with and without preeclampsia, PloS one 12(1) (2017).

[31] J. Moodley, Impact of HIV on the incidence of pre-eclampsia, Cardiovasc J Afr 24(2) (2013) 5.

[32] E.S. Machado, M.R. Krauss, K. Megazzini, C.M. Coutinho, R. Kreitchmann, V.H. Melo, J.H. Pilotto, M. Ceriotto, C.B. Hofer, G.K. Siberry, D.H. Watts, Hypertension, preeclampsia and eclampsia

among HIV-infected pregnant women from Latin America and Caribbean countries, J Infect 68(6) (2014) 572-80.

[33] A. Suy, E. Martínez, O. Coll, M. Lonca, M. Palacio, E. de Lazzari, M. Larrousse, A. Milinkovic, S. Hernández, J.L. Blanco, J. Mallolas, A. León, J.A. Vanrell, J.M. Gatell, Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy, AIDS (London, England) 20(1) (2006) 59-66.

[34] S. Debaisieux, F. Rayne, H. Yezid, B. Beaumelle, The ins and outs of HIV-1 Tat, Traffic 13(3) (2012) 355-363.

[35] A. Albini, R. Benelli, M. Presta, M. Rusnati, M. Ziche, A. Rubartelli, G. Paglialunga, F. Bussolino, D. Noonan, HIV-tat protein is a heparin-binding angiogenic growth factor, Oncogene 12(2) (1996) 289-297.

[36] A. Albini, G. Barillari, R. Benelli, R.C. Gallo, B. Ensoli, Angiogenic properties of human immunodeficiency virus type 1 Tat protein, Proceedings of the National Academy of Sciences of the United States of America 92(11) (1995) 4838-42.

[37] S. Soker, S. Takashima, H.Q. Miao, G. Neufeld, M. Klagsbrun, Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor, Cell 92(6) (1998) 735-45.

[38] T.A. Kim, H.K. Avraham, Y.H. Koh, S. Jiang, I.W. Park, S. Avraham, HIV-1 Tat-mediated apoptosis in human brain microvascular endothelial cells, J Immunol 170(5) (2003) 2629-37.

[39] S. Pillay, T. Naicker, Morphometric image analysis of vascular endothelial growth factor receptor-3 in preeclamptic, HIV infected women, European journal of obstetrics, gynecology, and reproductive biology 253 (2020) 304-311.

[40] K.M. Oltmanns, H. Gehring, S. Rudolf, B. Schultes, C. Hackenberg, U. Schweiger, J. Born, H.L. Fehm, A. Peters, Acute hypoxia decreases plasma VEGF concentration in healthy humans, Am J Physiol Endocrinol Metab 290(3) (2006) E434-9.

[41] J.S. Possomato-Vieira, R.A. Khalil, Mechanisms of Endothelial Dysfunction in Hypertensive Pregnancy and Preeclampsia, Advances in pharmacology (San Diego, Calif.) 77 (2016) 361-431.

[42] S. Verma, P. Pillay, T. Naicker, J. Moodley, I. Mackraj, Placental hypoxia inducible factor -1α & CHOP immuno-histochemical expression relative to maternal circulatory syncytiotrophoblast micro-vesicles in preeclamptic and normotensive pregnancies, European journal of obstetrics, gynecology, and reproductive biology 220 (2018) 18-24.

[43] G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, Vascular endothelial growth factor (VEGF) and its receptors, The FASEB journal 13(1) (1999) 9-22.

[44] G. Rath, R. Tripathi, VEGF and its soluble receptor VEGFR-2 in hypertensive disorders during pregnancy: the Indian scenario, J Hum Hypertens 26(3) (2012) 196-204.

[45] D. Clark, S. Smith, A. Sharkey, D. Charnock-Jones, Localization of VEGF and expression of its receptors flit and KDR in human placenta throughout pregnancy, Human Reproduction 11(5) (1996) 1090-1098.

[46] S.E. Maynard, J.-Y. Min, J. Merchan, K.-H. Lim, J. Li, S. Mondal, T.A. Libermann, J.P. Morgan, F.W. Sellke, I.E. Stillman, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute

to endothelial dysfunction, hypertension, and proteinuria in preeclampsia, The Journal of clinical investigation 111(5) (2003) 649-658.

[47] C.J. Robinson, S.E. Stringer, The splice variants of vascular endothelial growth factor (VEGF) and their receptors, J Cell Sci 114(Pt 5) (2001) 853-65.

[48] H. Agostini, K. Boden, A. Unsöld, G. Martin, L. Hansen, U. Fiedler, N. Esser, D. Marmé, A single local injection of recombinant VEGF receptor 2 but not of Tie2 inhibits retinal neovascularization in the mouse, Curr Eye Res 30(4) (2005) 249-57.

[49] S.-Y. Kim, S.-Y. Park, J.-W. Kim, Y.-M. Kim, J.-H. Yang, M.-Y. Kim, H.-K. Ahn, J.-S. Shin, J.-O. Kim, H.-M. Ryu, Circulating endothelial progenitor cells, plasma VEGF, VEGFR-1, and VEGFR-2 levels in preeclampsia, American Journal of Obstetrics & Gynecology 193(6) (2005) S74.

[50] H. Pavlakovic, J. Becker, R. Albuquerque, J. Wilting, J. Ambati, Soluble VEGFR-2: an antilymphangiogenic variant of VEGF receptors, Ann N Y Acad Sci 1207 Suppl 1(Suppl 1) (2010) E7-15.

[51] N. Govender, T. Naicker, J. Moodley, Maternal imbalance between pro-angiogenic and anti-angiogenic factors in HIV-infected women with pre-eclampsia, Cardiovasc J Afr 24(5) (2013) 174-9.

[52] X. Castellon, V. Bogdanova, Chronic Inflammatory Diseases and Endothelial Dysfunction, Aging Dis 7(1) (2016) 81-9.

[53] N. Singh, M. Tiem, R. Watkins, Y.K. Cho, Y. Wang, T. Olsen, H. Uehara, C. Mamalis, L. Luo, Z. Oakey, B.K. Ambati, Soluble vascular endothelial growth factor receptor 3 is essential for corneal alymphaticity, Blood 121(20) (2013) 4242-9.

[54] A. Machnik, W. Neuhofer, J. Jantsch, A. Dahlmann, T. Tammela, K. Machura, J.K. Park, F.X. Beck, D.N. Müller, W. Derer, J. Goss, A. Ziomber, P. Dietsch, H. Wagner, N. van Rooijen, A. Kurtz, K.F. Hilgers, K. Alitalo, K.U. Eckardt, F.C. Luft, D. Kerjaschki, J. Titze, Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism, Nature medicine 15(5) (2009) 545-52.

[55] Y. Zhang, Y. Lu, L. Ma, X. Cao, J. Xiao, J. Chen, S. Jiao, Y. Gao, C. Liu, Z. Duan, D. Li, Y. He, B. Wei, H. Wang, Activation of vascular endothelial growth factor receptor-3 in macrophages restrains TLR4-NF-κB signaling and protects against endotoxin shock, Immunity 40(4) (2014) 501-14.

[56] B.D. LaMarca, M.J. Ryan, J.S. Gilbert, S.R. Murphy, J.P. Granger, Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia, Curr Hypertens Rep 9(6) (2007) 480-5.

[57] Y.G. Park, J. Choi, H.K. Jung, I.K. Song, Y. Shin, S.Y. Park, J.W. Seol, Fluid shear stress regulates vascular remodeling via VEGFR-3 activation, although independently of its ligand, VEGF-C, in the uterus during pregnancy, Int J Mol Med 40(4) (2017) 1210-1216.

[58] M. Shakibaei, G. Schulze-Tanzil, A. Mobasheri, T. Beichler, J. Dressler, W. Schwab, Expression of the VEGF receptor-3 in osteoarthritic chondrocytes: stimulation by interleukin-1 beta and association with beta 1-integrins, Histochem Cell Biol 120(3) (2003) 235-41.

[59] J.F. Wang, X.F. Zhang, J.E. Groopman, Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration, The Journal of biological chemistry 276(45) (2001) 41950-7.

[60] L. Song, S. Ding, Z. Ge, X. Zhu, C. Qiu, Y. Wang, E. Lai, W. Yang, Y. Sun, S.A. Chow, L. Yu, Nucleoside/nucleotide reverse transcriptase inhibitors attenuate angiogenesis and lymphangiogenesis

by impairing receptor tyrosine kinases signalling in endothelial cells, British journal of pharmacology 175(8) (2018) 1241-1259.

[61] K.M. Powis, R.L. Shapiro, Protease inhibitors and adverse birth outcomes: is progesterone the missing piece to the puzzle?, J Infect Dis 211(1) (2015) 4-7.

[62] S. Kala, C. Dunk, S. Acosta, L. Serghides, Periconceptional exposure to lopinavir, but not darunavir, impairs decidualization: a potential mechanism leading to poor birth outcomes in HIV-positive pregnancies, Hum Reprod 35(8) (2020) 1781-1796.

[63] A.L. Conroy, C.R. McDonald, J.L. Gamble, P. Olwoch, P. Natureeba, D. Cohan, M.R. Kamya, D.V. Havlir, G. Dorsey, K.C. Kain, Altered angiogenesis as a common mechanism underlying preterm birth, small for gestational age, and stillbirth in women living with HIV, Am J Obstet Gynecol 217(6) (2017) 684.e1-684.e17.

Acknowledgements

- Miss Yazira Padayachee for her laboratory assistance.
- Cathy Connolly for her expertise as a biostatistician consultant.
- Wided Kelmemi for her assistance in additional analysis of our results.
- *Funding:* The College of Health Sciences, University of KwaZulu-Natal.

CHAPTER THREE

Review Article: The involvement of MicroRNAs in SARS-CoV-2 infection comorbid with HIV-associated preeclampsia.

In light of the COVID-19 pandemic, the South African COVID-19 lockdown and the institutional shutdown a review paper was written and submitted to a DoHET peer-reviewed journal, herein presented in the manuscript format.

Citation:

Tashlen Abel, Jagidesa Moodley and Thajasvarie Naicker (2020). The involvement of MicroRNAs in SARS-CoV-2 infection comorbid with HIV-associated preeclampsia. **Submitted** to *Current Hypertension Reports*, Manuscript ID: HYPR-D-20-00090R1.

Current Hypertension Reports

The involvement of MicroRNAs in SARS-CoV-2 infection comorbid with HIV-associated Preeclampsia

--Manuscript Draft--

Manuscript Number:	HYPR-D-20-00090R1
Full Title:	The involvement of MicroRNAs in SARS-CoV-2 infection comorbid with HIV-associated Preeclampsia
Article Type:	Review
Section/Category:	Preeclampsia
Corresponding Author:	Tashlen Abel, MSc University of KwaZulu-Natal Nelson R Mandela School of Medicine: University of KwaZulu-Natal College of Health Sciences Durban, KwaZulu-Natal SOUTH AFRICA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of KwaZulu-Natal Nelson R Mandela School of Medicine: University of KwaZulu-Natal College of Health Sciences
Corresponding Author's Secondary Institution:	
First Author:	Tashlen Abel, MSc
First Author Secondary Information:	
Order of Authors:	Tashlen Abel, MSc
	Jagidesa Moodley
	Thajasvarie Naicker
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Purpose of review: This review investigated the potential role of microRNAs (miRNAs) in the synergy of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, preeclampsia (PE), and Human Immunodeficiency Virus (HIV) infection. Maternal health is a great concern when treating pregnant women fighting this triad of diseases that is highly prevalent in South Africa. MicroRNAs (miRNAs) are involved in fine-tuning of physiological processes. Disruptions to the balance of this minute protein can lead to various physiological changes that are sometimes pathological. Recent findings: MicroRNAs have recently been implicated in PE and have been linked to the anti-angiogenic imbalance evident in PE. Recent in silico studies have identified potential host miRNAs with anti-viral properties against SARS-CoV-2 infection. Studies have demonstrated dysregulated expression of several miRNAs in HIV-1 infection along with the ability of HIV-1 to downregulated anti-viral host microRNAs. Summary: This review has highlighted the significant gap in literature the potential of miRNAs in HIV-associated PE women in synergy with the novel SARS-CoV-2 infection. In addition, this review has provided evidence of the critical role that the epigenetic regulatory mechanism of miRNA play in viral infections and PE; thereby providing a foundation for further research investigating the potential of therapeutic miRNA development with fewer side-effects for pregnant women.

THE INVOLVEMENT OF MicroRNAs IN SARS-CoV-2 INFECTION COMORBID WITH HIV-ASSOCIATED PREECLAMPSIA

Tashlen Abel[1*], Jagidesa Moodley[2] and Thajasvarie Naicker[1]

AFFILIATIONS:

[1] Optics and Imaging Centre, Doris Duke Medical Research Institution, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa

[2] Women's Health and HIV Research Group, Department of Obstetrics & Gynaecology, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

*** CORRESPONDING AUTHOR:**

Tashlen Abel

Optics & Imaging Centre,

Doris Duke Medical Research Institute,

College of Health Sciences,

University of KwaZulu-Natal,

Durban, South Africa.

Postal address: Private Bag 7, Congella

KwaZulu-Natal, 4013

South Africa

E-mail: tashlen.abel@gmail.com; naickera@ukzn.ac.za

Abstract

Purpose of review: This narrative review investigated the potential role of microRNAs (miRNAs) in the synergy of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, preeclampsia (PE), and Human Immunodeficiency Virus (HIV) infection. Maternal health is a great concern when treating pregnant women fighting this triad of diseases that is highly prevalent in South Africa. MicroRNAs (miRNAs) are involved in fine-tuning of physiological processes. Disruptions to the balance of this molecule can lead to various physiological changes that are sometimes pathological.

Recent findings: MicroRNAs have recently been implicated in PE and have been linked to the anti-angiogenic imbalance evident in PE. Recent *in silico* studies have identified potential host miRNAs with anti-viral properties against SARS-CoV-2 infection. Studies have demonstrated dysregulated expression of several miRNAs in HIV-1 infection along with the ability of HIV-1 to downregulated anti-viral host microRNAs.

Summary: This review has highlighted the significant gap in literature the potential of miRNAs in HIV-associated PE women in synergy with the novel SARS-CoV-2 infection. In addition, this review has provided evidence of the critical role that the epigenetic regulatory mechanism of miRNA play in viral infections and PE; thereby providing a foundation for further research investigating the potential of therapeutic miRNA development with fewer side-effects for pregnant women.

Keywords: Human Immunodeficiency Virus, Hypertension, MicroRNA, Preeclampsia, Pregnancy, SARS-CoV-2 infection

Declarations

Funding

The authors appreciate the funding provided by the College of Health Sciences, UKZN.

Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest.

Availability of data and material

All articles reviewed in this review paper are available online.

Availability of data and material

Not applicable

Authors Contributions

Not applicable

Introduction

The novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in late November 2019 and has led to the Coronavirus Disease 2019 (COVID-19) pandemic [1]. It is believed that SARS-CoV-2 originated from a wild meat market in Wuhan, Hubei, China [2]. Severe Acute Respiratory Syndrome Coronavirus 2 transmission occurs across humans regardless of age and sex; however, it is more prevalent amongst the elderly, the overweight, and those with asthma, diabetes, and other immunocompromised conditions [3]. According to the World Health Organization (WHO), South Africa (SA) has the highest COVID-19 prevalence in Africa. Despite an "early hard lockdown" by the country, more than 700 000 South Africans have been infected with SARS-CoV-2 as of October 2020 [4]. Considered to be a low- and middle-income-country (LMIC), it seems unlikely that SA will avoid a fall in the local economy. Hence, it is of utmost important to rapidly discover solutions to overcome the COVID-19 pandemic.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that are able to post-transcriptionally regulate the expression of proteins through modulation of the protein's mRNA. MicroRNAs are approximately 22 nucleotides long and possess a long half-life and stability that is 10 times stronger than messenger RNAs (mRNAs), even in extracellular fluids like urine and plasma [5]. MicroRNAs are able to degrade mRNA and suppress protein translation when the 5' terminal of miRNA pairs with the 3'-untranslated region (3'-UTR) of mRNA [6, 7]. When miRNAs are incompletely complementary to multiple sites in the 3'-UTR, protein synthesis is inhibited [8]. In comparison, when completely base-paired, a single phosphodiester bond is cleaved leading to degradation of the target mRNA [8].

Host miRNAs have been reported to be involved in cell proliferation, angiogenesis, immune cell development, and apoptosis [9]. Differential expression of miRNAs have been implicated in several viral diseases [10], cancer [9], diabetes [11], schizophrenia [12], and cardiovascular diseases [13]. The diverse role of miRNAs ignites the curiosity of its role in contemporary diseases and associated conditions.

Hypertensive disorders in pregnancy (HDP) are one of the commonest direct causes of mortality and morbidity worldwide; approximately 94% of maternal deaths occur in LMIC [14, 15]. Furthermore, it is responsible for 18% of all maternal deaths in SA [14].

Preeclampsia is an HDP of unknown origin that complicates 5-8% of pregnancies worldwide [16] and occurs more frequently in LMIC compared to high income countries [17, 15]. Preeclampsia is characterized by new-onset hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) with or without excessive proteinuria (≥300 mg every 24 hours); the disorder presents with the clinical signs of hypertension at or after 20 weeks' gestation [18]. The diagnosis of PE is also made in the absence of proteinuria when there is evidence of multi-organ involvement such as acute kidney injury, neurologic signs, liver disease and intrauterine foetal growth restriction. In addition, evidence of haemolysis, elevated liver enzymes and low platelet counts, leads to a diagnosis of HELLP syndrome [19, 20].

The Human Immunodeficiency Virus (HIV) attack cells of the immune system thereby weakening immunity which leads to the host being susceptible to other infections and diseases. [21]. HIV infection is a global concern with over 30 million people living with HIV at the end of 2019 [22]. In 2019, 13.5% of the South African population was infected with HIV (7.97 million) [23]. South Africa has the highest antiretroviral (ARV) "rollout program" in the world

with 4.7 million citizens receiving treatment [24]. The world health organization (WHO) has recommended that all infected humans initiate and continue the life-long use of HAART as a treatment for HIV [25]. Pregnant and breast-feeding women are also encouraged to continue with HAART treatment as it was shown to markedly reduce mother to child transmission [25]. However, antiretrovirals (ARVs) may be associated with PE predisposition [26]. Maternal deaths from HIV infection is high (>34%) in SA followed by obstetric haemorrhage and HDP [15]. Several studies have postulated that HIV infection influences the rate of PE development [27-31].

In light of the high maternal mortality emanating from HIV infection and PE it is of paramount importance that one examines their interaction with this new deadly COVID-19 pandemic. This review will address the missing gaps in literature concerning the effects of microRNAs in HIV-associated PE comorbid with COVID-19; thereby providing a foundation for further research investigating the triad of inflammatory-related conditions.

Severe Acute Respiratory Syndrome Coronavirus 2

Severe Acute Respiratory Syndrome Coronavirus 2 belongs to the subfamily of Beta coronaviruses, similar to SARS-CoV-1 and MERS-CoV [32]. SARS-CoV-2 is an enveloped virus with positive-sense single-stranded RNA (+ssRNA). Beta coronavirus have been attributed to be the most fatal subfamilies of coronaviruses [32]. Based on current knowledge, SARS-CoV-2 is composed of four structural and functional proteins which include the spike, membrane, envelope, and nucleocapsid proteins, together with RNA viral genome [33].

The route of COVID-19 spread is similar to other coronaviruses via human to human contact. Humans have a basic biological imperative to connect with other people, making human to

human contact a very efficient way to amplify viral dissemination. However, it is also spread through the oral-fecal route [34, 35]. SARS-CoV-2 infection occurs in three stages [36]. Stage one includes the incubation period which lasts for approximately 5 days. The virus becomes detectable in stage two and the patient displays mild flu-like symptoms. Stage three presents with severe symptoms which include acute respiratory distress syndrome (ARDS), multi-organ involvement and subsequent death [36].

Upon entry of the virus into the host, SARS-CoV-2 attaches to angiotensin converting enzyme 2 (ACE 2) receptors of pneumocytes, thereby infecting host cells [37]. Current literature suggests that the receptor binding domain of SARS-CoV-2 spike protein is activated via cleavage by transmembrane serine protease 2 (TMPRSS2) [38, 39]. Severe Acute Respiratory Syndrome Coronavirus 2 is then able to follow normal trends in viral infection such as replication, maturation, and release of virions. Since ACE 2 receptors are involved in pregnancy [40], it is plausible that SARS-CoV-2 infection predispose pregnancy complications.

Soluble Angiotensin Converting Enzyme 2 in SARS-CoV-2 infection

ACE 2 is a membrane bound protein (surface protein) that is used by SARS-CoV-2. A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10) and ADAM 17 are ectodomain sheddases that are able to cleave the extracellular domain of ACE 2 between amino acids 716 and 741; producing the soluble form of ACE 2 (sACE 2) that is released into maternal circulation [41].

Individuals with metabolic conditions have a higher expression of angiotensin II, whereas healthy individuals express angiotensin (1-7) [42]. SARS-CoV-2 has a greater affinity for

sACE 2 in comparison to the membrane-bound form, indicative of potential therapeutic properties [43]. Soluble ACE 2 can potentially neutralize SARS-CoV-2, thereby reducing viral pathogenicity [42, 43]. In light of the dire pandemic, it is vital that we investigate the properties of sACE 2 and its potential therapeutic benefits in HIV-positive preeclamptic women comorbid with COVID-19.

The role of Angiotensin Converting Enzyme 2 in pregnancy and Preeclampsia

In a normal physiological environment, the juxtaglomerular cells of the kidney secrete renin, which enzymatically converts angiotensinogen to angiotensin I [44]. Angiotensin Converting Enzyme (ACE) converts angiotensin I to angiotensin II [45]. Angiotensin II functions to increase blood pressure by acting on the kidney, brain, arterioles, and adrenal cortex, via its receptors – angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R), shown in **fig.1** [46]. Angiotensin Converting Enzyme 2 serves as a regulatory mechanism by degrading angiotensin II to angiotensin-(1-7) and angiotensin I to angiotensin-(1-9), which have opposing effects to that of angiotensin II [47]. Thus, ACE 2 maintains a balance in the renin-angiotensin system (RAS).

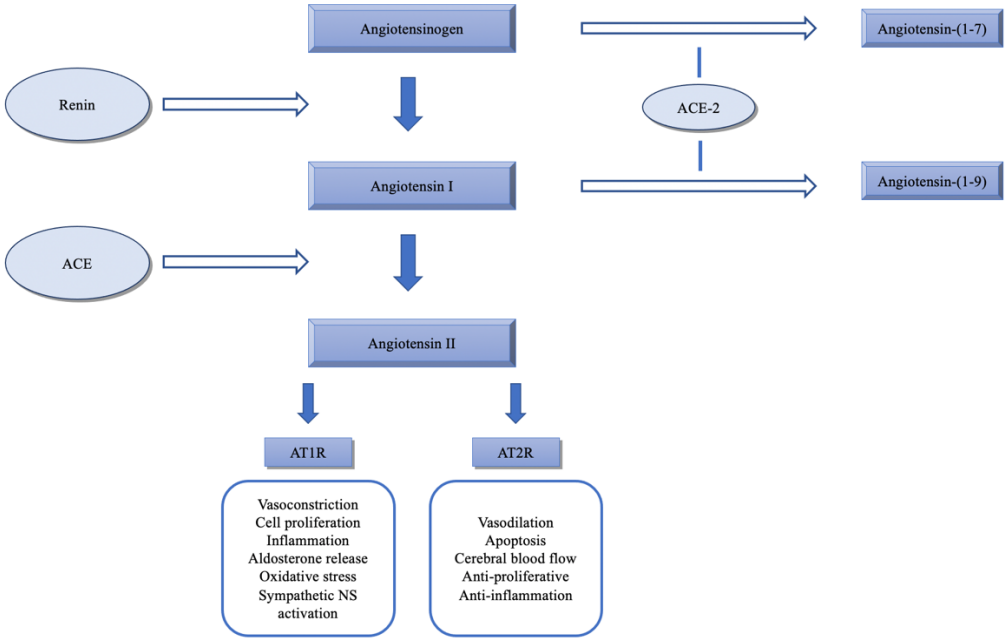


Fig. 1 Schematic representation of the Renin-angiotensin system and the physiological role of ACE-2 receptors

Pregnancies begin along various psychological, physical and physiological changes in the body. It is critical that salt-balance and blood pressure (BP) are maintained during pregnancy, which is a principle function of the RAS. From week 6 of gestation all components of the classical RAS are found in placental tissue, with the potential to regulate villous and extravillous cytotrophoblast (EVT) proliferation, extravillous cytotrophoblast migration, invasion and placental angiogenesis [48]. Placental RAS is a vital component for the suboptimal regulation of blood flow at the maternal-foetal interface, hence its dysregulation may predispose HDP such as PE [49, 50]. ACE 2 is expressed in human placenta within syncytiotrophoblasts (ST), cytotrophoblasts (CT), endothelium, and vascular smooth muscle of conducting villi [51]. Interestingly, ACE 2 is also expressed in the invasive interstitial and intravascular trophoblast cell populations, as well as within decidual cells [51]. This highlights the potential for COVID-19 to induce, mimic or accelerate PE as the SARS-CoV-2 infection

exploits ACE 2. The exploitation of ACE 2 by SARS-CoV-2 highlights its ability to induce or accelerate PE development.

In normal pregnancies, there is a slight increase in the expression of angiotensin II albeit without vasoconstriction or rise in systemic BP because of the development of a refractoriness to the effect of angiotensin II [52, 53]. In contrast, pregnancies complicated by PE are highly sensitized to angiotensin II [54]. This correlates with the clinical findings of PE, which include evidence of elevated BP. Studies by Merrill *et al.* and Valdés *et al.* provide evidence of angiotensin 1-7 downregulated in the plasma of PE compared to normotensive healthy pregnancies [55, 56]. These studies confirm potential of ACE 2 suppression in PE.

Pathophysiology of Preeclampsia

The etiology of PE has not been fully elucidated however, it is believed to occur in two stages [57]. The preclinical stage of PE development involves deficient EVT invasion of the uterine spiral arterioles. In this stage, endovascular trophoblast invasion does not progress beyond the decidual segment of the spiral artery, additionally there is reduced interstitial myometrial invasion [58]. Defective spiral artery remodeling causes placental hypoxia, leading to a shift in the balance of antiangiogenic and proangiogenic factors [58]. Soluble endoglin (sEng) is an antiangiogenic factor that was found to be overexpressed in the serum of preeclamptic women [59]. Endoglin (Eng), a transmembrane glycoprotein that is highly expressed on vascular endothelium, functions as a co-receptor for transforming growth factor beta (TGF- ß) [60]. In contrast, sEng inhibits the normal physiology of TGF-ß by binding to circulating TGF-beta, which leads to dysregulation of TGF-ß signalling in ECs [59]. Transforming growth factor receptor I (TGFR-I), otherwise referred to as activin receptor-like kinase 5 (ALK5), and transforming growth factor receptor II (TGFR-II) function as native receptors of TGF-ß [61].

It was reported that sEng can potentially inhibit the downstream signalling of TGF-ß, including effects on activation of endothelial nitric oxide synthase (eNOS) and vasodilation [59].

Angiogenic imbalance leads to the clinical stage in which an increase in antiangiogenic factors causes widespread damage to the maternal endothelium [62]. This stage presents the clinical features of PE, including hypertension, proteinuria, and intrauterine growth restriction (IUGR) [63]. Delivery of the placenta usually causes rapid resolution of the clinical signs of the disease, making it the only treatment available, which often includes premature delivery of the fetus [64].

The expression of microRNAs in pregnancy

Pregnancy is a time of significant changes in the body in order to prepare for and accommodate the developing fetus. MicroRNAs are able to regulate many of these changes through its control over the expression of mRNA. MicroRNAs have been implicated in the earliest stages of pregnancy, including embryo implantation [65]. After implantation, the trophoblast cell lineage is the first to begin differentiating [66]. Cuman *et al.* noted miR-661 and miR-372 upregulation in blastocysts that failed to implant [67], the expression of miR-372 was supported by Rosenbluth *et al.* as they found a similar expression [68]. In contrast, miR-142-3p is highly expressed in blastocysts that successfully implanted according to a pilot study conducted by Borges *et al.* [69]. This suggests an involvement of miRNA in ectopic pregnancies and miscarriages. Although differential expression profiling of miRNAs is achievable, the results are not easily reproducible, as evident in significant variations between similar investigations. The difficulty in reproducing results may be explained due to differences in laboratory conduct of the study, methodological differences, differences in miRNA array panels, as well as the use

of either stored or fresh samples [65]. MicroRNA expression is a very dynamic process and varies greatly with the requirements needed at different times [65].

The endometrium is essential for successful embryo implantation. Kresowik *et al.* identified miR-31 to be overexpressed in endometrium in the mid-secretory phase [70]. MicroRNA-31 is a potent miRNA that inversely regulates forkhead box P3 (FOXP3), a transcription factor for T regulatory cells and CXCL12, a homeostatic chemokine. CXCL12 is a chemoattractant for uterine NK cells, with the potential to be involved in providing a suitable environment that is immune-tolerant in the secretory phase [65]. Tochigi *et al.* and Estella *et al.* investigated the miRNA expression profiles between decidualized human endometrial stem cells (hESC) and control hESC, only miR-155 was commonly expressed in both studies [71, 72].

The attachment of the blastocyst to the uterine endothelial wall occurs 4-6 days post-conception; following this, the placenta begins to develop [73]. MicroRNAs are highly expressed in the human placenta which undergoes physiological changes throughout pregnancy [74, 75]. The precise role of miRNAs in the placenta are yet to be identified. However, the placenta releases placental miRNAs into the maternal circulation, hence is found in maternal serum and plasma and placental tissue. The expression of placental miRNAs are associated with HDPs, such as PE [76]. Previous studies have highlighted the presence of hypoxic conditions in PE compared to healthy controls [77, 78, 58]. MicroRNA-210 is upregulated in trophoblast cells cultured in hypoxic environments, and importantly, in PE [79]. Additionally, miRNAs that are involved in angiogenesis and immune cell developments are dysregulated in trophoblastic cells cultured in hypoxic conditions [80-83]. Thus, there exists a possible influence of miRNAs in the progression of normal pregnancies, and in pathological pregnancies.

MicroRNAs in pregnancies complicated by Preeclampsia

There are significant gaps in the investigation of miRNAs in pregnancy-related complications and there is a paucity of data on the miRNA regulation of sEng. Importantly, the miRNA regulation of sFlt-1 is yet to be elucidated as no miRNA has been directly correlated with the regulation of sFlt-1 [84]. Nevertheless, Shyu *et al.* reported that miR-208a is responsible for the activation of Eng and collagen I in the stimulation of myocardial fibrosis [85]. This was supported by similar studies [86, 87]. Furthermore, several miRNAs have been suggested to play a role in trophoblast proliferation and invasion, including direct effect on TGF-β signalling. An investigation analysing the HTR-8/SVneoplacental cell line concluded that miR-376c inhibits ALK5 [88]. Also, miR-29b directly binds to the 3'-UTRs of myeloid cell leukaemia sequence 1, matrix metalloproteinase 2, VEGF-A and integrin-β1 [89]. When miR-29b is upregulated in the placenta, it causes trophoblastic apoptosis and inhibition of trophoblast invasion and angiogenesis [89]. MicroRNA-193b is increased in preeclamptic patients [90]. Zhou *et al.* showed that miR-193b-3p decreases migration and invasion of HTR-8/SVneoplacental cells [90]. Interestingly, inhibition of miR-126 in mouse embryos led to abnormal vasculogenesis, haemorrhage, and loss of vascular integrity [91]. This indicated that miR-126 is necessary for proper vessel formation.

Placental hypoxia

Abnormal trophoblast invasion of the placenta in PE leads to hypoperfusion of the placenta and ultimately accelerates the placenta into a hypoxic state. The hypoxic state that is associated with PE correlates with the decrease of eNOS and nitric oxide (NO) in preeclamptic patients. MicroRNA-222 was reported to induce the production of eNOS [92] yet was found to be downregulated in the placenta of PE patients [93]. Furthermore, miR-155 was identified to

negatively regulate the expression of eNOS in trophoblastic cells [94]. It was also found to be increased in PE placenta, suggesting a negative regulatory role of miR-155 in the migratory behaviour of trophoblasts through the regulation of eNOS [94]. Sun *et al.* showed that miR-155 exerts its inhibitory effects on eNOS by binding to the 3'-UTR of eNOS mRNA and suggested that silencing of this miRNA can lead to improvement of endothelial dysfunction [95]. Dai *et al.* reported that miR-155 may inhibit trophoblast invasion and proliferation by downregulating cyclin D1; furthermore another investigative group reported that miR-155 can inhibit trophoblast invasion by decreasing eNOS expression [96]. This can lead to an exaggerated hypoxic state of the placenta in PE.

Many studies have highlighted the overexpression of miR-210 in preeclamptic placentae and plasma [97]. MicroRNA-210, believed to be a miRNA that is induced by hypoxia, is one of the most studied miRNAs [98]. The hypoxic state of the placenta in PE causes oxidative stress which leads to the upregulation of hypoxia inducing factor 1-α (HIF-1-α) in placental tissue [98]. Research has revealed that miR-210 is regulated by HIF-1-α, thereby creating a positive feedback loop inducing hypoxia.

Angiogenesis

There is evidence of abnormal angiogenesis in PE. Vascular endothelial growth factor (VEGF) is a potent proangiogenic factor, that plays a pivotal role in angiogenesis, particularly in endothelial cell proliferation, invasion, and migration [99]. It promotes the production of NO and prostacyclin in the maternal vascular system [100]. Phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) and sprout-related drosophilia enabled/vasodilator-stimulated phosphoprotein homology 1 (EVH1) domain are a part of the VEGF signalling pathway and are targets of miR-126 [101, 91]. It was reported that miR-126 was downregulated in PE

patients and the expression of miR-126 is directly proportional to the expression of VEGF mRNA [101]. VEGF is also targeted by miR-29b, miR-16, and miR-155 as they inhibit the expression of VEGF-A [89, 102, 103]. They also inhibit trophoblast cell invasion and tube formation apart from suppressing VEGF-A, thus they are involved in placental angiogenesis. Ephrin-B2 (EFNB2) has been identified to influence angiogenesis. MicroRNA-126, miR-20a, miR-17, and miR-20b have been identified as miRNA regulators of EFNB2 and interestingly, they were shown to be differentially expressed in PE [104, 101]. These miRNAs can indirectly regulate the expression of VEGF through the inactivation of EFNB2. The microRNAs greatly involved in PE are summarised in **table 1**.

Placental miRNAs are also released into the maternal circulation, contributing the maternal stage of PE, interestingly, miRNAs have also been found to be contained within exosomes, nanoparticle carrier proteins, in the maternal circulation [105]. The release of antiangiogenic factors and other inflammatory mediators into the maternal circulation lead to the systemic endothelial cell inflammation and endothelial cell dysfunction that are characteristic of PE.

Table 1 A summary of microRNAs and their roles in Preeclampsia

MicroRNA	**Expression**	**Target Gene**	**Reference**
miR-29b	Upregulated	MCL1, MMP2, VEGF-A, ITGB1	[89]
miR-126	Downregulated	VEGFA, EFNB2, CRK	[101]
miR-222	Downregulated	eNOS, PTEN, BCL2L11,	[93]
miR-155	Upregulated	eNOS, Cyclin D1	[94]
miR-210	Upregulated	HIF1-alpha, NF-kBp50	[97, 79]

The role of MicroRNAs in modulating HIV-1 infection

HIV infection is one of the more prevalent viral infections in SA [23]. Currently HIV-infected individuals are treated with HAART [25]. Although HAART is the most effective treatment at present, it is associated with various side-effects. All pregnant women who are HIV-positive are required to adopt the HAART treatment in SA, as it reduces mother-to-child transmission [25]. However, studies have shown that HAART could exhibit a negative influence during pregnancy. Furthermore, there is evidence that the administration of highly active retroviral therapy (HAART) to pregnant HIV-positive patients predisposes the development of PE [106, 107]. In an ideal situation, PE patients comorbid with HIV infection would have a neutralisation of immune response [31, 108]. However, HAART in pregnancy reconstitutes immune response thereby influencing PE development [107, 109, 110]. In light of this, it is essential to thoroughly investigate key regulators in HIV-1-infection in order to identify alternative avenues in the fight against HIV-infection globally. Epigenetic regulatory mechanisms, specifically miRNAs, have been shown to play a significant role in HIV-infection, as well as other RNA and DNA viral infections [111].

Moreover, miRNAs may be partially responsible for the latency period of the HIV [112]. Huang *et al.* reported that several miRNAs were differentially expressed in resting $CD4^+$ T cells and activated $CD4^+$ T cells, including miR-28, miR-125b, miR-150, miR-223 and miR-382. These miRNAs have also been shown to target the 3' ends of HIV-1 mRNAs. Additionally, the group showed that inhibition of these miRNAs can stimulate virus production in resting $CD4^+$ T cells isolated from HIV-positive individuals receiving HAART [10]. It is therefore plausible that these differentially expressed miRNAs can inhibit HIV-1 expression in resting $CD4^+$ T cells, thereby contributing to the viral latency observed in HIV infection.

Apart from direct targeting of the HIV-1 mRNAs by miRNAs, cellular miRNAs can indirectly affect HIV-infection through modulating factors that are essential for HIV-1 expression. Cyclin T1 protein is responsible for efficient transcription of the viral genome [113]. A study in 2012 reported that the expression of cyclin T1 is reduced in resting $CD4^+$ T cells however, it is induced upon activation of $CD4^+$ T cells [114]. A similar investigation identified miR-198 to be downregulated during monocyte to macrophage differentiation and reported that miR-198 is able to suppress HIV-1 replication by downregulating cyclin T1 [115].

Houzet *et al.* reported that miR-29a and miR-29b are downregulated in HIV-1-infected patients and infected peripheral blood mononuclear cells (PBMCs) [116]. It was reported that the host miRNA, miR-29a targets the *nef* gene of HIV-1. The *nef* protein serves as an accessory protein of HIV and influences viral pathogenesis [117]. The group suggested that expression of miR-29a leads to a reduction of *nef* mRNA and a decrease in viral levels was observed [118]. A study conducted by Nathans *et al*. observed miR-29a to suppress infectivity of HIV through direct targeting of HIV-1 transcripts to processing bodies (P bodies) [119]. Chable-Bessia *et al.* demonstrated that major components of P bodies are able to negatively regulate HIV-1 gene expression via blocking of viral mRNA association with polysomes. They also showed that deletion of these components reactivates the virus in PBMCs isolated from HIV-1 patients receiving HAART [120]. Thus, the downregulation of miR-29a in HIV infected humans could serve as a mechanism for the maintenance of a latent state of infection. The miR-29 family is composed of miR-29a, miR-29b, and miR-29c. It is important to underline that miR-29a and miR-29b share highly similar sequences [118]. Above and beyond the regulation of *nef* expression by miR-29a, Ahluwalia *et al.* suggested that miR-29a and miR-29b are able to suppress virus replication in HEK293T cells and Jurkat T cells [118]. An *in vivo* study revealed that a cytokine-microRNA pathway could potentially impact HIV-1 replication. Specifically,

the group identified the IL-21/miR-29a pathway to be associated with HIV-1 replication and infectivity [121]. Adoro *et al.* reported that the IL-21/miR-29a pathway suppresses viral replication since IL-21 stimulated $CD4^+$ T cells upregulate the expression of miR-29a, and IL-21 reverses the downregulation of miR-29a induced by HIV-1-infection [122]. This reiterates the plausibility of the IL-21/miR-29a axis influencing HIV-1 replication and infectivity.

As important as host miRNAs are, viruses bring along with it a set of its own miRNAs, referred to as viral miRNAs (v-miRNAs). The existence of v-miRNAs has been controversial to a degree due to the failure of reproducing findings [123]. The first v-miRNA that was isolated from HIV-1 was discovered in 2004 and was termed miR-N367 [124]. However, subsequent studies that attempted to reproduce the discovery were unsuccessful in their attempts [125-127].

The transactivation-responsive (TAR) element of HIV-1 is an RNA hairpin structure found at the 5' end of all HIV-1 transcripts [128]. Dominique L Ouellet at al. reported that TAR is a source of miRNAs in cultured HIV-1-infected cell lines and in HIV-1 infected human $CD4^+$ T lymphocytes [128]. TAR has been shown to be involved in cell survival and displays anti-apoptotic properties [129]. HIV-1 TAR miRNAs have been identified to downregulate ERCC1 (excision repair cross complementation group 1) and IER3 (intermediate early response gene 3) which are components involved in apoptosis and cell survival [130]. Therefore, HIV-1 infected cells may be able to evade death and maintain the virulence of HIV-1.

The novel microRNAs have proven to have highly intricate regulatory roles in the human genomes. However, evidence also supports their existence in both RNA and DNA viruses which can potentially be involved in epigenetic regulation, by both direct and indirect

mechanisms. It is thus of paramount importance that miRNAs and v-miRNAs are investigated more thoroughly utilizing newer sequencing technology. The significant impact of miRNAs in viruses and hosts highlights the possibility of their role in other viral infections threatening mankind.

MicroRNAs in HIV-associated Preeclampsia and COVID-19

There are numerous reports suggesting an interaction of miRNAs in viral infections. A study investigating the expression of miRNAs in HIV infection found differentially expressed miRNAs between resting $CD4^+$ T cells and activated $CD4^+$ T cells. Specifically, they found miR-28, miR-125b, miR-150, miR-223, and miR-382 to be differentially expressed [10]. Nersisyan *et al.* conducted an *in silico* analysis of potential host miRNAs that can bind coronavirus and identified miR-21, miR-195-5p, miR-16-5p, miR-3065-5p, miR-424-5p, and miR-421 to exhibit this potential [131].

MicroRNAs in Angiotensin converting enzyme 2 Receptors

ACE 2 receptors are predominantly found on the endothelial cells [132], heart, blood vessels, and the kidneys [133]. According to several studies, miRNAs are indeed regulators of ACE 2 [134, 135]. ACE 2 abnormalities have been implicated in disorders such as hypertension [136], cardiovascular disease [13], diabetes [11], and old age [13]. MicroRNA-125b is reported to directly target the mRNA of ACE 2 [137]. The same miRNA is found to be downregulated in HIV infected $CD4^+$ T cells and exhibits anti-viral properties [10]. Thus, it is plausible to hypothesise that HIV-positive individuals could be at an increased risk of being infected with SARS-CoV-2 because the host will be experiencing a decline in the expression of miR-125b due to HIV infection. Since miR-125b is a negative regulator of ACE 2 [10], under HIV-positive conditions the patients will have an increase in the expression of ACE 2, potentially

leading to greater viral entry. Supporting this is the work of Batlle *et al.* who highlighted the fact that healthier people are at a lower risk of developing severe COVID-19 due to lower membrane bound ACE 2 expression [42]. MicroRNA-125 is also associated with blocking of apoptosis when downregulated [138]. This possibly allows for the virus to replicate without interruption.

Recently, miR-155 was reported to be associated with ACE 2 modulation by regulating the expression of AT1R by silencing AT1R mRNA [139]. This receptor is involved in cardiovascular homeostasis mechanisms including vasoconstriction, release of catecholamines, and blood pressure evaluation [140]. Vasoconstriction and elevated blood pressure are characteristics that are evident in PE. MicroRNA-155 was observed to be upregulated in the placenta of PE [94] where it negatively regulates the expression of eNOS in trophoblasts. There is a lack of research investigating miR-155 expression in COVID-19. Nevertheless, miR-155 has been described to exhibit anti-viral properties. Silencing of miR-155 led to an approximate 50% increase in the replication of Rhinovirus [141]. In a case-control study, miR-155 was found to be upregulated in patients infected with Respiratory syncytial virus (RSV), a condition associated with bronchial inflammation [142]. The overexpression of miR-155 shows a correlation with acute inflammatory responses [142]. Theoretically, a preeclamptic patient would be at a greater risk of experiencing severe symptoms of COVID-19, due to the effect of miR-155. Although the miRNA is unlikely to cause a pregnant woman to be at risk of being infected, the endothelial dysfunction seen in PE will be compounded by the dysregulation effects of miR-155 following SARS-CoV-2 infection. Although there is a paucity of data regarding the expression of miR-155 in COVID-19, it is possible to assume an initial downregulation in order to evade immune detection, followed by overexpression when the host develops an inflammatory response to the infection. Research investigating PE patients with

SARS-CoV-2 infection will greatly aid in illuminating the effects of miR-155 both in COVID-19 and PE, which can lead to possible therapeutic actions from antagomirs (antagonistic microRNAs).

A geographical study including the USA, Wuhan, Italy, India, and Nepal found several anti-viral host miRNAs that were specific to SARS-CoV-2, one of which was miR-126 [143]. MicroRNA-126 has been identified to target the nucleocapsid of the SARS-CoV-2 [143]. Interestingly, miR-126 is downregulated in PE [101]. The inhibition of miR-126 in mouse embryos was assessed and it was found that it led to abnormal vessel formation and loss of vascular integrity [91]. Since miR-126 is decreased in PE, pregnant women with PE could be at risk of infection due to the loss of an anti-viral miRNA that targets SARS-CoV-2. Furthermore, it is plausible to expect the further downregulation of miR-126 following infection, this can lead to further endothelial cell damage in preeclamptic women and hence contribute to worsening the effects of PE, possibly inducing death. Additionally, miR-126-3p was found to be downregulated in HIV-1-positive patients receiving HAART. Interestingly, miR-126-3p was upregulated in patients with HAART resistance in comparison to patients without resistance [144]. It was indicated that this is suggestive of miR-126 being linked with HIV treatment failure [144]. This evidence has possible detrimental results for HIV-associated PE women as both conditions exhibit a decrease in miR-126. Hence, patients with HIV-associated PE could be at a greater risk of both contraction of SARS-CoV-2 infection and the experiencing of severe COVID-19. Furthermore, Li *et al.* found several miRNAs to be differentially expressed in the peripheral blood of patients with COVID-19 [145]. There is a great need to investigate the expression of miRNAs in COVID-19; which is yet to be achieved.

Conclusion

Currently there exists a wide gap in literature interrelating miRNAs and SARS-CoV-2 infection. Analysis of the differential expression of miRNAs in COVID-19 can help identify those at risk as well as aid in the development of therapeutic approaches. An inflammatory response is a common characteristic shared between SARS-CoV-2 infection, pregnancy, PE, and HIV infection. Maternal health should be of utmost importance when SARS-CoV-2 infection arises in HIV-positive preeclamptic women. Thus, further research investigating the functionality of microRNAs on the synergy of SARS-CoV-2 infection, PE, and HIV infection could provide significant breakthroughs that will enhance the treatment in pregnant women. Understanding how miRNAs are affected and identifying which miRNAs are aberrantly expressed will accelerate the development of a vaccine that will also be safe for pregnant women diagnosed with HIV-associated PE.

References

1. Wang H, Li X, Li T, Zhang S, Wang L, Wu X et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis. 2020;39(9):1629-35. doi:10.1007/s10096-020-03899-4.

2. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nature medicine. 2020;26(4):450-2. doi:10.1038/s41591-020-0820-9.

3. Zhu F, Cao Y, Xu S, Zhou M. Co-infection of SARS-CoV-2 and HIV in a patient in Wuhan city, China. J Med Virol. 2020;92(6):529-30. doi:10.1002/jmv.25732.

4. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. https://covid19.who.int. Accessed 02 November 2020 2020.

5. Sayed AS, Xia K, Salma U, Yang T, Peng J. Diagnosis, prognosis and therapeutic role of circulating miRNAs in cardiovascular diseases. Heart Lung Circ. 2014;23(6):503-10. doi:10.1016/j.hlc.2014.01.001.

6. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet. 2008;9(2):102-14. doi:10.1038/nrg2290.

7. Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell. 2008;132(1):9-14. doi:10.1016/j.cell.2007.12.024.

8. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev. 2016;96(4):1297-325. doi:10.1152/physrev.00041.2015.

9. Bhaskaran M, Mohan M. MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. Vet Pathol. 2014;51(4):759-74. doi:10.1177/0300985813502820.

10. Huang J, Wang F, Argyris E, Chen K, Liang Z, Tian H et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nature medicine. 2007;13(10):1241-7. doi:10.1038/nm1639.

11. Tikellis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J et al. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. Hypertension (Dallas, Tex : 1979). 2003;41(3):392-7. doi:10.1161/01.Hyp.0000060689.38912.Cb.

12. Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA et al. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol. 2007;8(2):R27. doi:10.1186/gb-2007-8-2-r27.

13. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD et al. The emerging role of ACE2 in physiology and disease. J Pathol. 2007;212(1):1-11. doi:10.1002/path.2162.

14. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers Report 2017. Pretoria: South African Department of Health2018.

15. World Health O. Trends in maternal mortality 2000 to 2017: estimates by WHO, UNICEF, UNFPA, World Bank Group and the United Nations Population Division. Geneva: World Health Organization; 2019.

16. Hutcheon JA, Lisonkova S, Joseph K. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best practice & research Clinical obstetrics & gynaecology. 2011;25(4):391-403.

17. Nathan HL, Seed PT, Hezelgrave NL, De Greeff A, Lawley E, Conti-Ramsden F et al. Maternal and perinatal adverse outcomes in women with pre-eclampsia cared for at facility-level in South Africa: a prospective cohort study. Journal of global health. 2018;8(2).

18. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S et al. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension (Dallas, Tex : 1979). 2018;72(1):24-43. doi:10.1161/hypertensionaha.117.10803.

19. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. The Lancet. 2005;365(9461):785-99.

20. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of preeclampsia. Annual Review of Pathology: Mechanisms of Disease. 2010;5:173-92.

21. Awi NJ, Teow SY. Antibody-Mediated Therapy against HIV/AIDS: Where Are We Standing Now? Journal of pathogens. 2018;2018:8724549. doi:10.1155/2018/8724549.

22. World Health Organization. Number of people (all ages) living with HIV: Estimates by WHO region. 2019. http://apps.who.int/gho/data/view.main.22100WHO?lang=en. Accessed 30 October 2020 2020.

23. Stats SA. Mid-year Population Estimates 2019. Pretoria, South Africa2019.

24. Joint United Nations Programme on HIV/AIDS. Global HIV & AIDS statistics — 2019 fact sheet. 2019. https://www.unaids.org/en/resources/fact-sheet. Accessed 30 October 2020 2020.

25. World Health Organization. Maternal Health. 2015. https://www.afro.who.int/health-topics/maternal-health. Accessed 30 October 2020 2020.

26. Sebitloane HM, Moodley J, Sartorius B. Associations between HIV, highly active anti-retroviral therapy, and hypertensive disorders of pregnancy among maternal deaths in South Africa 2011–2013. International Journal of Gynecology & Obstetrics. 2017;136(2):195-9.

27. Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. European journal of obstetrics, gynecology, and reproductive biology. 2004;117(2):240-1. doi:10.1016/j.ejogrb.2004.04.014.

28. Hall DR. Is pre-eclampsia less common in patients with HIV/AIDS? J Reprod Immunol. 2007;76(1-2):75-7. doi:10.1016/j.jri.2007.04.005.

29. Landi B, Bezzeccheri V, Guerra B, Piemontese M, Cervi F, Cecchi L et al. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. World Journal of Cardiovascular Diseases. 2014;2014.

30. Kalumba VM, Moodley J, Naidoo TD. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. Cardiovasc J Afr. 2013;24(2):24-7. doi:10.5830/cvja-2012-078.

31. Moodley J. Impact of HIV on the incidence of pre-eclampsia. Cardiovasc J Afr. 2013;24(2):5.

32. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020;5(4):562-9. doi:10.1038/s41564-020-0688-y.

33. Morse JS, Lalonde T, Xu S, Liu WR. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. Chembiochem. 2020;21(5):730-8. doi:10.1002/cbic.202000047.

34. Hu Y, Shen L, Yao Y, Xu Z, Zhou J, Zhou H. A report of three COVID-19 cases with prolonged viral RNA detection in anal swabs. Clin Microbiol Infect. 2020;26(6):786-7. doi:10.1016/j.cmi.2020.04.010.

35. Jones DL, Baluja MQ, Graham DW, Corbishley A, McDonald JE, Malham SK et al. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. Sci Total Environ. 2020;749:141364. doi:10.1016/j.scitotenv.2020.141364.

36. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. Jama. 2020;323(11):1061-9. doi:10.1001/jama.2020.1585.

37. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. Journal of virology. 2020;94(7). doi:10.1128/jvi.00127-20.

38. Hoffmann M, Kleine-Weber H, Pöhlmann S. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol Cell. 2020;78(4):779-84.e5. doi:10.1016/j.molcel.2020.04.022.

39. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications. 2020;11(1):1620. doi:10.1038/s41467-020-15562-9.

40. Levy A, Yagil Y, Bursztyn M, Barkalifa R, Scharf S, Yagil C. ACE2 expression and activity are enhanced during pregnancy. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2008;295(6):R1953-R61.

41. Mulangu S, Dodd LE, Davey RT, Jr., Tshiani Mbaya O, Proschan M, Mukadi D et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. The New England journal of medicine. 2019;381(24):2293-303. doi:10.1056/NEJMoa1910993.

42. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? Clin Sci (Lond). 2020;134(5):543-5. doi:10.1042/cs20200163.

43. Mostafa-Hedeab G. ACE2 as Drug Target of COVID-19 Virus Treatment, Simplified Updated Review. Rep Biochem Mol Biol. 2020;9(1):97-105. doi:10.29252/rbmb.9.1.97.

44. Zhuo JL, Ferrao FM, Zheng Y, Li XC. New frontiers in the intrarenal Renin-Angiotensin system: a critical review of classical and new paradigms. Front Endocrinol (Lausanne). 2013;4:166. doi:10.3389/fendo.2013.00166.

45. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical Renin-Angiotensin system in kidney physiology. Compr Physiol. 2014;4(3):1201-28. doi:10.1002/cphy.c130040.

46. Alifano M, Alifano P, Forgez P, Iannelli A. Renin-angiotensin system at the heart of COVID-19 pandemic. Biochimie. 2020;174:30-3. doi:10.1016/j.biochi.2020.04.008.

47. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/Angiotensin 1-7 Axis of the Renin-Angiotensin System in Heart Failure. Circulation research. 2016;118(8):1313-26. doi:10.1161/circresaha.116.307708.

48. Pringle KG, Tadros MA, Callister RJ, Lumbers ER. The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis? Placenta. 2011;32(12):956-62. doi:10.1016/j.placenta.2011.09.020.

49. Moritz KM, Cuffe JS, Wilson LB, Dickinson H, Wlodek ME, Simmons DG et al. Review: Sex specific programming: a critical role for the renal renin-angiotensin system. Placenta. 2010;31 Suppl:S40-6. doi:10.1016/j.placenta.2010.01.006.

50. Ito M, Itakura A, Ohno Y, Nomura M, Senga T, Nagasaka T et al. Possible activation of the renin-angiotensin system in the feto-placental unit in preeclampsia. The Journal of clinical endocrinology and metabolism. 2002;87(4):1871-8. doi:10.1210/jcem.87.4.8422.

51. Valdés G, Neves LA, Anton L, Corthorn J, Chacón C, Germain AM et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta. 2006;27(2-3):200-7. doi:10.1016/j.placenta.2005.02.015.

52. Shah DM. Role of the renin-angiotensin system in the pathogenesis of preeclampsia. Am J Physiol Renal Physiol. 2005;288(4):F614-25. doi:10.1152/ajprenal.00410.2003.

53. Massicotte G, St-Louis J, Parent A, Schiffrin E. Decreased in vitro responses to vasoconstrictors during gestation in normotensive and spontaneously hypertensive rats. Canadian journal of physiology and pharmacology. 1987;65(12):2466-71.

54. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest. 1973;52(11):2682-9. doi:10.1172/jci107462.

55. Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. Endocrine. 2002;18(3):239-45. doi:10.1385/endo:18:3:239.

56. Valdés G, Germain AM, Corthorn J, Berrios C, Foradori AC, Ferrario CM et al. Urinary vasodilator and vasoconstrictor angiotensins during menstrual cycle, pregnancy, and lactation. Endocrine. 2001;16(2):117-22. doi:10.1385/endo:16:2:117.

57. Shanmugalingam R, Hennessy A, Makris A. Aspirin in the prevention of preeclampsia: the conundrum of how, who and when. Journal of human hypertension. 2019;33(1):1-9.

58. Rana S, Lemoine E, Granger J, Karumanchi SA. Preeclampsia. Circ Res. 2019;124(7):1094-112. doi:10.1161/circresaha.118.313276.

59. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. New England Journal of Medicine. 2006;355(10):992-1005.

60. Rossi E, Bernabeu C, Smadja DM. Endoglin as an Adhesion Molecule in Mature and Progenitor Endothelial Cells: A Function Beyond TGF-β. Front Med (Lausanne). 2019;6:10. doi:10.3389/fmed.2019.00010.

61. Cheifetz S, Bellón T, Calés C, Vera S, Bernabeu C, Massagué J et al. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. The Journal of biological chemistry. 1992;267(27):19027-30.

62. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. Am J Obstet Gynecol. 2015;213(4 Suppl):S9.e1, S9-11. doi:10.1016/j.ajog.2015.08.003.

63. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn. 2011;31(1):66-74. doi:10.1002/pd.2660.

64. Valero L, Alhareth K, Gil S, Lecarpentier E, Tsatsaris V, Mignet N et al. Nanomedicine as a potential approach to empower the new strategies for the treatment of preeclampsia. Drug discovery today. 2018;23(5):1099-107.

65. Paul ABM, Sadek ST, Mahesan AM. The role of microRNAs in human embryo implantation: a review. J Assist Reprod Genet. 2019;36(2):179-87. doi:10.1007/s10815-018-1326-y.

66. Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M et al. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest. 2004;114(6):744-54. doi:10.1172/jci22991.

67. Cuman C, Van Sinderen M, Gantier MP, Rainczuk K, Sorby K, Rombauts L et al. Human Blastocyst Secreted microRNA Regulate Endometrial Epithelial Cell Adhesion. EBioMedicine. 2015;2(10):1528-35. doi:10.1016/j.ebiom.2015.09.003.

68. Rosenbluth EM, Shelton DN, Wells LM, Sparks AE, Van Voorhis BJ. Human embryos secrete microRNAs into culture media--a potential biomarker for implantation. Fertil Steril. 2014;101(5):1493-500. doi:10.1016/j.fertnstert.2014.01.058.

69. Borges E, Jr., Setti AS, Braga DP, Geraldo MV, Figueira RC, Iaconelli A, Jr. miR-142-3p as a biomarker of blastocyst implantation failure - A pilot study. JBRA Assist Reprod. 2016;20(4):200-5. doi:10.5935/1518-0557.20160039.

70. Kresowik JD, Devor EJ, Van Voorhis BJ, Leslie KK. MicroRNA-31 is significantly elevated in both human endometrium and serum during the window of implantation: a potential biomarker for optimum receptivity. Biol Reprod. 2014;91(1):17. doi:10.1095/biolreprod.113.116590.

71. Tochigi H, Kajihara T, Mizuno Y, Mizuno Y, Tamaru S, Kamei Y et al. Loss of miR-542-3p enhances IGFBP-1 expression in decidualizing human endometrial stromal cells. Sci Rep. 2017;7:40001. doi:10.1038/srep40001.

72. Estella C, Herrer I, Moreno-Moya JM, Quiñonero A, Martínez S, Pellicer A et al. miRNA signature and Dicer requirement during human endometrial stromal decidualization in vitro. PloS one. 2012;7(7):e41080. doi:10.1371/journal.pone.0041080.

73. Rana S, Lemoine E, Granger J, Karumanchi SA. Preeclampsia: pathophysiology, challeneges, and perspectives. Circulation research. 2019;124(7):1094-112. doi:10.1161/circresaha.118.313276.

74. Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. 2007;8:166. doi:10.1186/1471-2164-8-166.

75. Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K et al. Identification of pregnancy-associated microRNAs in maternal plasma. Clin Chem. 2010;56(11):1767-71. doi:10.1373/clinchem.2010.147660.

76. Arthurs AL, Lumbers ER, Pringle KG. MicroRNA mimics that target the placental renin-angiotensin system inhibit trophoblast proliferation. Mol Hum Reprod. 2019;25(4):218-27. doi:10.1093/molehr/gaz010.

77. Tong W, Giussani DA. Preeclampsia link to gestational hypoxia. J Dev Orig Health Dis. 2019;10(3):322-33. doi:10.1017/s204017441900014x.

78. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125(7):911-9.

79. Lee DC, Romero R, Kim JS, Tarca AL, Montenegro D, Pineles BL et al. miR-210 targets iron-sulfur cluster scaffold homologue in human trophoblast cell lines: siderosis of interstitial trophoblasts as a novel pathology of preterm preeclampsia and small-for-gestational-age pregnancies. Am J Pathol. 2011;179(2):590-602. doi:10.1016/j.ajpath.2011.04.035.

80. Bidarimath M, Khalaj K, Wessels JM, Tayade C. MicroRNAs, immune cells and pregnancy. Cell Mol Immunol. 2014;11(6):538-47. doi:10.1038/cmi.2014.45.

81. Heusschen R, van Gink M, Griffioen AW, Thijssen VL. MicroRNAs in the tumor endothelium: novel controls on the angioregulatory switchboard. Biochim Biophys Acta. 2010;1805(1):87-96. doi:10.1016/j.bbcan.2009.09.005.

82. Suárez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. Circulation research. 2009;104(4):442-54. doi:10.1161/circresaha.108.191270.

83. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. Faseb j. 2010;24(6):2030-9. doi:10.1096/fj.09-149724.

84. Harapan H, Yeni CM. The role of microRNAs on angiogenesis and vascular pressure in preeclampsia: The evidence from systematic review. Egyptian Journal of Medical Human Genetics. 2015;16(4):313-25.

85. Shyu KG. The Role of Endoglin in Myocardial Fibrosis. Acta Cardiol Sin. 2017;33(5):461-7. doi:10.6515/acs20170221b.

86. Wang BW, Wu GJ, Cheng WP, Shyu KG. MicroRNA-208a increases myocardial fibrosis via endoglin in volume overloading heart. PloS one. 2014;9(1):e84188. doi:10.1371/journal.pone.0084188.

87. Shyu KG, Wang BW, Cheng WP, Lo HM. MicroRNA-208a Increases Myocardial Endoglin Expression and Myocardial Fibrosis in Acute Myocardial Infarction. Can J Cardiol. 2015;31(5):679-90. doi:10.1016/j.cjca.2014.12.026.

88. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y et al. MicroRNA-376c impairs transforming growth factor-β and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension (Dallas, Tex : 1979). 2013;61(4):864-72. doi:10.1161/hypertensionaha.111.203489.

89. Li P, Guo W, Du L, Zhao J, Wang Y, Liu L et al. microRNA-29b contributes to pre-eclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. Clin Sci (Lond). 2013;124(1):27-40. doi:10.1042/cs20120121.

90. Zhou X, Li Q, Xu J, Zhang X, Zhang H, Xiang Y et al. The aberrantly expressed miR-193b-3p contributes to preeclampsia through regulating transforming growth factor-β signaling. Sci Rep. 2016;6:19910. doi:10.1038/srep19910.

91. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell. 2008;15(2):272-84. doi:10.1016/j.devcel.2008.07.008.

92. Suárez Y, Fernández-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circulation research. 2007;100(8):1164-73. doi:10.1161/01.Res.0000265065.26744.17.

93. Cronqvist T, Saljé K, Familari M, Guller S, Schneider H, Gardiner C et al. Syncytiotrophoblast vesicles show altered micro-RNA and haemoglobin content after ex-vivo perfusion of placentas with haemoglobin to mimic preeclampsia. PloS one. 2014;9(2):e90020. doi:10.1371/journal.pone.0090020.

94. Li X, Li C, Dong X, Gou W. MicroRNA-155 inhibits migration of trophoblast cells and contributes to the pathogenesis of severe preeclampsia by regulating endothelial nitric oxide synthase. Molecular medicine reports. 2014;10(1):550-4. doi:10.3892/mmr.2014.2214.

95. Sun HX, Zeng DY, Li RT, Pang RP, Yang H, Hu YL et al. Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension (Dallas, Tex : 1979). 2012;60(6):1407-14. doi:10.1161/hypertensionaha.112.197301.

96. Dai Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H et al. MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. Placenta. 2012;33(10):824-9. doi:10.1016/j.placenta.2012.07.012.

97. Bounds KR, Chiasson VL, Pan LJ, Gupta S, Chatterjee P. MicroRNAs: New Players in the Pathobiology of Preeclampsia. Frontiers in cardiovascular medicine. 2017;4:60. doi:10.3389/fcvm.2017.00060.

98. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L et al. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. J Cell Mol Med. 2012;16(2):249-59. doi:10.1111/j.1582-4934.2011.01291.x.

99. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. Reproduction. 2009;138(6):895-902. doi:10.1530/rep-09-0092.

100. Wang A, Rana S, Karumanchi SA. Preeclampsia: the role of angiogenic factors in its pathogenesis. Physiology (Bethesda). 2009;24:147-58. doi:10.1152/physiol.00043.2008.

101. Hong F, Li Y, Xu Y. Decreased placental miR-126 expression and vascular endothelial growth factor levels in patients with pre-eclampsia. J Int Med Res. 2014;42(6):1243-51. doi:10.1177/0300060514540627.

102. Wang Y, Fan H, Zhao G, Liu D, Du L, Wang Z et al. miR-16 inhibits the proliferation and angiogenesis-regulating potential of mesenchymal stem cells in severe pre-eclampsia. Febs j. 2012;279(24):4510-24. doi:10.1111/febs.12037.

103. Liu Q, Yang J. Expression and significance of miR155 and vascular endothelial growth factor in placenta of rats with preeclampsia. Int J Clin Exp Med. 2015;8(9):15731-7.

104. Wang W, Feng L, Zhang H, Hachy S, Satohisa S, Laurent LC et al. Preeclampsia up-regulates angiogenesis-associated microRNA (i.e., miR-17, -20a, and -20b) that target ephrin-B2 and EPHB4 in human placenta. The Journal of clinical endocrinology and metabolism. 2012;97(6):E1051-9. doi:10.1210/jc.2011-3131.

105. Salomon C, Guanzon D, Scholz-Romero K, Longo S, Correa P, Illanes SE et al. Placental Exosomes as Early Biomarker of Preeclampsia: Potential Role of Exosomal MicroRNAs Across Gestation. The Journal of clinical endocrinology and metabolism. 2017;102(9):3182-94. doi:10.1210/jc.2017-00672.

106. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet (London, England). 2016;387(10022):999-1011. doi:10.1016/s0140-6736(15)00070-7.

107. Maharaj NR, Phulukdaree A, Nagiah S, Ramkaran P, Tiloke C, Chuturgoon AA. Pro-inflammatory cytokine levels in HIV infected and uninfected pregnant women with and without preeclampsia. PloS one. 2017;12(1).

108. Wimalasundera RC, Larbalestier N, Smith JH, de Ruiter A, Mc GTSA, Hughes AD et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. Lancet (London, England). 2002;360(9340):1152-4. doi:10.1016/s0140-6736(02)11195-0.

109. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. J Infect. 2014;68(6):572-80. doi:10.1016/j.jinf.2013.12.018.

110. Suy A, Martínez E, Coll O, Lonca M, Palacio M, de Lazzari E et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS (London, England). 2006;20(1):59-66. doi:10.1097/01.aids.0000198090.70325.bd.

111. Mishra R, Kumar A, Ingle H, Kumar H. The Interplay Between Viral-Derived miRNAs and Host Immunity During Infection. Frontiers in immunology. 2019;10:3079. doi:10.3389/fimmu.2019.03079.

112. Romani B, Allahbakhshi E. Underlying mechanisms of HIV-1 latency. Virus Genes. 2017;53(3):329-39. doi:10.1007/s11262-017-1443-1.

113. Couturier J, Orozco AF, Liu H, Budhiraja S, Siwak EB, Nehete PN et al. Regulation of cyclin T1 during HIV replication and latency establishment in human memory CD4 T cells. Virol J. 2019;16(1):22. doi:10.1186/s12985-019-1128-6.

114. Chiang K, Sung TL, Rice AP. Regulation of cyclin T1 and HIV-1 Replication by microRNAs in resting CD4+ T lymphocytes. Journal of virology. 2012;86(6):3244-52. doi:10.1128/jvi.05065-11.

115. Sung TL, Rice AP. miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1. PLoS pathogens. 2009;5(1):e1000263. doi:10.1371/journal.ppat.1000263.

116. Houzet L, Yeung ML, de Lame V, Desai D, Smith SM, Jeang KT. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. Retrovirology. 2008;5:118. doi:10.1186/1742-4690-5-118.

117. Geyer M, Fackler OT, Peterlin BM. Structure--function relationships in HIV-1 Nef. EMBO Rep. 2001;2(7):580-5. doi:10.1093/embo-reports/kve141.

118. Ahluwalia JK, Khan SZ, Soni K, Rawat P, Gupta A, Hariharan M et al. Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. Retrovirology. 2008;5:117. doi:10.1186/1742-4690-5-117.

119. Nathans R, Chu CY, Serquina AK, Lu CC, Cao H, Rana TM. Cellular microRNA and P bodies modulate host-HIV-1 interactions. Mol Cell. 2009;34(6):696-709. doi:10.1016/j.molcel.2009.06.003.

120. Chable-Bessia C, Meziane O, Latreille D, Triboulet R, Zamborlini A, Wagschal A et al. Suppression of HIV-1 replication by microRNA effectors. Retrovirology. 2009;6:26. doi:10.1186/1742-4690-6-26.

121. Ortega PAS, Saulle I, Mercurio V, Ibba SV, Lori EM, Fenizia C et al. Interleukin 21 (IL-21)/microRNA-29 (miR-29) axis is associated with natural resistance to HIV-1 infection. AIDS (London, England). 2018;32(17):2453-61. doi:10.1097/qad.0000000000001938.

122. Adoro S, Cubillos-Ruiz JR, Chen X, Deruaz M, Vrbanac VD, Song M et al. IL-21 induces antiviral microRNA-29 in CD4 T cells to limit HIV-1 infection. Nature communications. 2015;6:7562. doi:10.1038/ncomms8562.

123. Cullen BR. Is RNA interference involved in intrinsic antiviral immunity in mammals? Nat Immunol. 2006;7(6):563-7. doi:10.1038/ni1352.

124. Omoto S, Ito M, Tsutsumi Y, Ichikawa Y, Okuyama H, Brisibe EA et al. HIV-1 nef suppression by virally encoded microRNA. Retrovirology. 2004;1:44. doi:10.1186/1742-4690-1-44.

125. Lin J, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. Journal of virology. 2007;81(22):12218-26. doi:10.1128/jvi.01390-07.

126. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer FA et al. Identification of microRNAs of the herpesvirus family. Nat Methods. 2005;2(4):269-76. doi:10.1038/nmeth746.

127. Swaminathan S, Murray DD, Kelleher AD. The role of microRNAs in HIV-1 pathogenesis and therapy. AIDS (London, England). 2012;26(11):1325-34. doi:10.1097/QAD.0b013e328352adca.

128. Ouellet DL, Plante I, Landry P, Barat C, Janelle ME, Flamand L et al. Identification of functional microRNAs released through asymmetrical processing of HIV-1 TAR element. Nucleic Acids Res. 2008;36(7):2353-65. doi:10.1093/nar/gkn076.

129. Pilakka-Kanthikeel S, Saiyed ZM, Napuri J, Nair MP. MicroRNA: implications in HIV, a brief overview. J Neurovirol. 2011;17(5):416-23. doi:10.1007/s13365-011-0046-1.

130. Klase Z, Winograd R, Davis J, Carpio L, Hildreth R, Heydarian M et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. Retrovirology. 2009;6:18. doi:10.1186/1742-4690-6-18.

131. Nersisyan S, Engibaryan N, Gorbonos A, Kirdey K, Makhonin A, Tonevitsky A. Potential role of cellular miRNAs in coronavirus-host interplay. PeerJ. 2020;8:e9994. doi:10.7717/peerj.9994.

132. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circulation research. 2000;87(5):E1-9. doi:10.1161/01.res.87.5.e1.

133. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271-80.e8. doi:10.1016/j.cell.2020.02.052.

134. Zhang R, Su H, Ma X, Xu X, Liang L, Ma G et al. MiRNA let-7b promotes the development of hypoxic pulmonary hypertension by targeting ACE2. Am J Physiol Lung Cell Mol Physiol. 2019;316(3):L547-l57. doi:10.1152/ajplung.00387.2018.

135. Nersisyan S, Shkurnikov M, Turchinovich A, Knyazev E, Tonevitsky A. Integrative analysis of miRNA and mRNA sequencing data reveals potential regulatory mechanisms of ACE2 and TMPRSS2. PloS one. 2020;15(7):e0235987. doi:10.1371/journal.pone.0235987.

136. Yu J, Wu Y, Zhang Y, Zhang L, Ma Q, Luo X. [Role of ACE2-Ang (1-7)-Mas receptor axis in heart failure with preserved ejection fraction with hypertension]. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2018;43(7):738-46. doi:10.11817/j.issn.1672-7347.2018.07.007.

137. Huang YF, Zhang Y, Liu CX, Huang J, Ding GH. microRNA-125b contributes to high glucose-induced reactive oxygen species generation and apoptosis in HK-2 renal tubular epithelial cells by targeting angiotensin-converting enzyme 2. Eur Rev Med Pharmacol Sci. 2016;20(19):4055-62.

138. Yu G, Zhan X, Zhang Z, Li Y. [Overexpression of miR-125b promotes apoptosis of macrophages]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2016;32(7):958-62.

139. Wang G, Kwan BC, Lai FM, Chow KM, Li PK, Szeto CC. Elevated levels of miR-146a and miR-155 in kidney biopsy and urine from patients with IgA nephropathy. Dis Markers. 2011;30(4):171-9. doi:10.3233/dma-2011-0766.

140. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(12):5003-8. doi:10.1073/pnas.1019055108.

141. Bondanese VP, Francisco-Garcia A, Bedke N, Davies DE, Sanchez-Elsner T. Identification of host miRNAs that may limit human rhinovirus replication. World J Biol Chem. 2014;5(4):437-56. doi:10.4331/wjbc.v5.i4.437.

142. Inchley CS, Sonerud T, Fjærli HO, Nakstad B. Nasal mucosal microRNA expression in children with respiratory syncytial virus infection. BMC Infect Dis. 2015;15:150. doi:10.1186/s12879-015-0878-z.

143. Sardar R, Satish D, Birla S, Gupta D. Comparative analyses of SAR-CoV2 genomes from different geographical locations and other coronavirus family genomes reveals unique features potentially consequential to host-virus interaction and pathogenesis. bioRxiv. 2020.

144. Marquez-Pedroza J, Cárdenas-Bedoya J, Morán-Moguel MC, Escoto-Delgadillo M, Torres-Mendoza BM, Pérez-Ríos AM et al. Plasma microRNA expression levels in HIV-1-positive patients receiving antiretroviral therapy. Biosci Rep. 2020;40(5). doi:10.1042/bsr20194433.

145. Li C, Hu X, Li L, Li JH. Differential microRNA expression in the peripheral blood from human patients with COVID-19. J Clin Lab Anal. 2020:e23590. doi:10.1002/jcla.23590.

CHAPTER FOUR

Synthesis

In SA, maternal morbidity and mortality, and HIV infection is of great concern since the prevalence of HIV infections in pregnant women remains stable at 30% since 2017 (Woldesenbet *et al.*, 2018). The latest maternal mortality audits in SA revealed that approximately 18% of maternal deaths in SA in 2017 were directly associated with HDP, with PE being one of the most clinically diagnosed HDP (National Committee for Confidential Enquiry into Maternal Deaths, 2018). The synergy of PE and HIV infection are major contributors to maternal and perinatal morbidity and mortality in SA. HIV infection and ART have been reported to predispose PE development; however, there are conflicting reports concerning the involvement of HIV infection and ART in PE (Mol *et al.*, 2016; Maharaj *et al.*, 2017). An imbalance of pro- and anti-angiogenic factors is characteristic of PE. Interestingly, ART has been reported to upregulate the expression of anti-angiogenic factors in pregnant women receiving ART (Song *et al.*, 2018). In light of the angiogenic imbalance evident in PE and its association with HIV infection, we investigated the expression of soluble angiogenic factors, sVEGFR-2 and sVEGFR-3, in preeclamptic women infected with HIV.

Furthermore, the novel SARS-CoV-2 infection has caused the COVID-19 pandemic (Wang *et al.*, 2020). By entering the host via mucous and saliva droplets in the air, SARS-CoV-2 enters the hosts' cells by binding to ACE 2 receptors that are located on epithelial tissue (Hoffmann *et al.*, 2020). Pregnancy includes great physiological changes such as an increase in ACE 2 receptors and the desensitisation to the effects of angiotensin II (Levy *et al.*, 2008). Additionally, SARS-CoV-2 infection was reported to induce hypertension and preeclampsia-like symptoms in pregnant women (Mendoza *et al.*, 2020). Based on the strong association of pregnancy and SARS-CoV-2 infection with the renin-angiotensin system (RAS), it may be plausible that pregnant women are at a greater risk of being infected with SARS-CoV-2.

This novel study demonstrates a significant downregulation of sVEGFR-2 in preeclamptic compared to normotensive pregnant women. These results are in accordance with other studies (Chaiworapongsa et al., 2010). The concentration of the soluble isoform of VEGFR-2 is proportional to the concentration of the membrane bound form of VEGFR-2, which is expressed on the surface of endothelial cells (ECs) (Shibuya, 2013). During the third trimester of gestation, VEGFR-2 is primarily expressed on placental vascular ECs (Clark et al., 1996). This receptor regulates EC proliferation, function, and survival when stimulated by the binding of VEGF-A, VEGF-C, and VEGF-D (Pijnenborg et al., 2010). In addition to regulating angiogenesis, VEGFR-2 is also involved in the regulation of lymphangiogenesis which arises when stimulated by VEGF-C (Pijnenborg et al., 2010). The VEGF-induced stimulation of VEGFR-2 is essential for mitogenic cell signalling and the migratory activity of ECs (Rath and Tripathi, 2012).

Endothelial cell damage is a major characteristic of the clinical stage of PE development. The expression of sVEGFR-2 is downregulated as early as 6-10 weeks' gestation (Chaiworapongsa *et al.*, 2010), indicating a possible downregulation of VEGFR-2 as well. The decline of VEGFR-2 and its soluble isoform significantly early in pregnancy can lead to systemic EC damage before the onset of the clinical characteristics of PE due to the lack of VEGFR-2 stimulation. It is plausible to speculate that the systemic EC damage exacerbates the downregulation of sVEGFR-2, which enhances the clinical stage of PE. In a recent study, we demonstrated a HIF-1-α rich microenvironment in preeclamptic placentae (Verma *et al.*, 2018). A decrease in the biological availability of VEGF mRNA is associated with an increase in HIF-1-α (Robinson and Stringer, 2001). Due to the hypoxic state evident in PE, it is plausible to assume that there is reduced VEGF-mediated stimulation of VEGFR-2 which contributes to EC damage and the significant decline of sVEGFR-2 as observed in our study.

Moreover, our study reports a significant decrease of sVEGFR-2 in HIV-positive preeclamptic in comparison to HIV-negative normotensive pregnancies; and a trend indicating its downregulation in HIV-positive versus HIV-negative pregnancies. The HIV-1 encodes several genes in its RNA genome, including the tat protein which possesses an arginine and lysine rich sequence, similar to the VEGF amino acid sequence (Albini *et al.*, 1996a). As a consequence of the structural homology between tat protein and VEGF, tat protein is able to bind to VEGF native receptors, VEGFR-1 and VEGFR-2 (Albini *et al.*, 1996b). Therefore, it is plausible that the downregulation of sVEGFR-2 in HIV infected women in our study emanates from tat binding and sequestering sVEGFR-2. Upon binding of tat to sVEGFR-2, it is plausible to assume that the stimulation will lead to the activation of anti-angiogenic properties of sVEGFR-2.

Chronic HIV infection leads to chronic arterial injury, of which EC damage is a subsequent consequence (Govender *et al.*, 2013). We speculate that EC damage stemming from chronic HIV infection can cease the ability of EC to activate the VEGFR-2 signalling pathway, leading to the downregulation of sVEGFR-2 in HIV infected women and the enhanced downregulation in HIV-positive preeclamptic pregnant women. Additionally, administration of ART reconstitutes the opposing immune responses of PE and HIV infection, thereby inducing an immune-inflammatory state (Maharaj *et al.*, 2017). The heightened immune-inflammatory state in preeclamptic women infected with HIV may promote the development of chronic inflammation, a consequence of which is endothelial dysfunction (Castellon and Bogdanova, 2016). Therefore, we speculate that the endothelial dysfunction evident in PE may be worsened by the administration of ART which leads to severely reduced levels of sVEGFR-2 in PE.

Furthermore, this investigation shows a trend indicating the decrease of sVEGFR-3 in preeclamptic compared to normotensive pregnancies. These outcomes are corroborated by another study (Lely *et al.*, 2013). The membrane bound form of sVEGFR-3 is a regulator of the lymphangiogenic pathway which is stimulated following the binding of VEGF-C to VEGFR-3 (Singh *et al.*, 2013). In contrast, sVEGFR-3 exhibits anti-angiogenic and anti-lymphangiogenic properties and is also able to bind VEGF-C (Lely *et al.*, 2013). Preeclamptic women reflect a pro-lymphangiogenic state as deciphered from a lower sVEGFR-2 + sVEGFR-3/VEGF-C ratio in comparison to normotensive pregnant women (Lely *et al.*, 2013). The imbalance of this ratio may be a compensatory mechanism in response to the exacerbated inflammatory state of PE (Lely *et al.*, 2013). Studies on animal models showed that inhibition of the VEGFR-3/VEGF-C can lead to development of hypertension (Machnik *et al.*, 2009). Stimulation of the VEGFR-3/VEGF-C signalling pathway decreases the expression of pro-inflammatory cytokines (Machnik *et al.*, 2009); hence, an increase of TNF-α and IL-6 pro-inflammatory cytokines in PE (LaMarca *et al.*, 2007) indicates the inactivity of the VEGFR-3/VEGF-C pathway in preeclamptic women.

Vascular remodelling of the uterus during pregnancy to accommodate for an increase in the blood flow is a process regulated by VEGFR-3 (Park *et al.*, 2017); and is dysregulated in PE as evident by incomplete spiral artery remodelling (Rana *et al.*, 2019). Hence, we speculate that VEGFR-3 is downregulated in PE which leads to incomplete spiral artery remodelling. Simultaneous stimulation of VEGFR-3 and integrin β1 signal transduction pathways, and subsequent activation of the mitogen-activated protein kinase (MAPKinase) pathway are essential for EC survival, migration, growth, differentiation, and adhesion (Wang *et al.*, 2001; Shakibaei *et al.*, 2003). Due to the dysregulation of vascular remodelling in PE, these signalling pathways are dysregulated in PE, which promotes EC damage and subsequently decreased sVEGFR-3.

In addition, our observations revealed a significant decrease of sVEGFR-3 in HIV-negative PE compared to HIV-negative normotensive pregnancies; along with a trend indicating increased expression of sVEGFR-3 in HIV-positive compared to HIV-negative pregnant women. Currently, ART is the gold standard in HIV treatment. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) is one of several key components that form highly active antiretroviral therapy (HAART) (Song *et al.*, 2018). The use of NRTIs in HIV treatment promotes the production of mitochondrial oxidative stress that leads to damage of the RTK signalling in ECs; this may result in EC damage due to the dysregulation of VEGFR signalling pathways (Song *et al.*, 2018). Protease inhibitors is one more of the components that are incorporated into HAART and has been reported to dysregulate uterine decidualization and spiral artery remodelling due to its effect of decreasing progesterone levels in trophoblast cells and subsequent decrease in trophoblast cell proliferation and migration (Kala *et al.*,

2020). Importantly, all HIV-positive women in our study were undergoing ART treatment at the time of venous blood collection, as it is a standard of care for HIV infection in SA. Based on the anti-angiogenic and anti-lymphangiogenic properties, we speculate that administration of HAART may significantly contribute to the decline of sVEGFR-3 in preeclamptic women infected with HIV undergoing HAART treatment.

MicroRNAs are noncoding post-transcriptional regulators of gene expression and minute changes in a particular miRNA could impart significant changes to its respective gene (Sayed *et al.*, 2014). The maintenance of maternal health when treating diseases during pregnancies still remains a challenge in SA, in light of this we analytically examined the potential role of miRNAs in the synergy of PE, SARS-CoV-2 and HIV infection. Recent studies have identified several host miRNAs to be differentially expressed in PE and HIV infection (Huang *et al.*, 2007; Bhaskaran and Mohan, 2014). Computational analysis identified host miRNAs with anti-viral effects against the novel SARS-CoV-2 infection (Nersisyan *et al.*, 2020). This review highlights a major gap in research on miRNAs in pregnancy, PE, and viral infections. In addition, evidence was provided indicating that the epigenetic regulatory mechanism of miRNA may play a pivotal role in PE and viral infections.

Research on miRNAs in pregnancy-related disorders is significantly lacking. Nonetheless, the differential expression of VEGF receptors are regulated by miRNAs, whilst the expression of certain miRNAs are directly proportional to the expression of the mRNA of their respective gene (Hong *et al.*, 2014).

Placental hypoperfusion that stems from insufficient trophoblast invasion of the placenta propels the placenta into a hypoxic state with an HIF-1-α rich microenvironment in PE (Possomato-Vieira and Khalil, 2016; Verma *et al.*, 2018). MicroRNA-210 (miR-210) is proportional to the expression of HIF-1-α (Oltmanns *et al.*, 2006); hence, we speculate that a positive feedback loop inducing hypoxia is formed due to abnormal placental trophoblast invasion. Additionally, miR-10 was shown to regulate angiogenesis (Hassel *et al.*, 2012). The downregulation of miR-10 leads to a decrease in the EC signalling pathway by antagonising VEGFR-2 stimulation (Hassel *et al.*, 2012). Furthermore, miR-10 regulates the expression of VEGFR-1 as well as its soluble isoform, sVEGFR-1 (Hassel *et al.*, 2012). Therefore, it is plausible to conclude that there may be an association between miR-10 and sVEGFR-2. The clinical stage of PE is characterised by widespread EC dysfunction. The expression of miR-126 is downregulated in PE and the expression of this miRNA is directly proportional to the expression of VEGF mRNA (Hong *et al.*, 2014). The survival, proliferation and migration of ECs is dependent on VEGF-mediated activation of VEGFR signalling pathways (Shakibaei *et al.*, 2003); hence, a downregulation of miR-126 may be involved in the dysregulation of EC signalling in PE. In addition,

EC damage emanating from decreased miR-126 may cause the downregulation of sVEGFR-2 and sVEGFR-3 observed in our study. MicroRNA-126 has been highlighted to target the nucleocapsid of the SARS-CoV-2, thereby exhibiting anti-viral properties (Sardar *et al.*, 2020). The downregulated levels of miR-126 in PE may facilitate SARS-CoV-2 infection in pregnant women. Furthermore, SARS-CoV-2 infection may exacerbate the EC damage already present in PE.

The epigenetic regulatory ability of miRNAs plays a major role in viral infections, including HIV infection (Huang *et al.*, 2007). Several miRNAs are differentially expressed between resting and activated CD4 T cells (Huang *et al.*, 2007). HIV has adapted the ability to dysregulate certain miRNAs in an attempt to prevent detection of the virus and extend the latency period of HIV infection. The nef protein of HIV-1 is involved in viral pathogenesis and is regulated by host miR-29a and miR-29b (Geyer *et al.*, 2001; Houzet *et al.*, 2008). Moreover, miR-29a and miR-29b suppresses HIV replication (Ahluwalia *et al.*, 2008; Nathans *et al.*, 2009). Due to its downregulation in HIV infected humans, this may be a mechanism to prolong the latent state of HIV infection. The expression of miR-126 is downregulated in HIV infected patients receiving ART; however, miR-126 is upregulated in HIV-positive patients resistant to HAART compared to patients without resistance (Marquez-Pedroza *et al.*, 2020). Hence, it is plausible to speculate that HIV infected patients receiving ART will be at a greater risk of being infected with SARS-CoV-2. Furthermore, the downregulation of miR-126 induced by ART administration in the synergy of PE, HIV and SARS-CoV-2 infections is particularly concerning as EC damage may be severe, leading to the worsening of PE.

Limitations

The limitations of our study were the small sample size, heterogeneity of the PE population by gestational age and severity. Furthermore, viral load tests are not a standard of care in SA; therefore, it was excluded from this study together with the severity of PE. Lastly, this study did not identify the origin of detected concentration of sVEGFR-2 and sVEGFR-3.

Conclusion

In conclusion, this study demonstrates a significant decrease of sVEGFR-2 and a downregulated trend of sVEGFR-3 concentrations in preeclamptic compared to normotensive pregnancies. The decline of these anti-angiogenic factors contributes to EC dysfunction which eventuates dysregulation of angiogenic and lymphangiogenic pathways in PE. Additionally, we demonstrate a similar expression of both sVEGFR-2 and sVEGFR-3 in HIV infected compared to HIV-negative pregnancies, irrespective of pregnancy type. The expressional similarities of sVEGFR-2 and sVEGFR-3 is attributed to ART. Notably, the VEGF-mimicry of the HIV-1 tat protein is counterbalanced by the administration of ART. The observations in this study provides an insight into the role of soluble isoforms of VEGF receptors

and warrants further investigations with a focus on soluble angiogenic factors at the level of gene expression.

Future recommendations

We recommend conducting a large-scale study investigating the functional role of sVEGFR-2 and sVEGFR-3 in pregnancy, PE, and HIV infection. The focus of future investigations should be directed towards the epigenetic regulation, as well as possible genetic polymorphisms of sVEGFR-2 and sVEGFR-3.

CHAPTER FIVE

REFERENCES

AARON, E., COHAN, D. & WEBER, S. 2015. The changing landscape for HIV-serodifferent couples desiring a child. *Am J Obstet Gynecol,* 213**,** 746.

AHLUWALIA, J. K., KHAN, S. Z., SONI, K., RAWAT, P., GUPTA, A., HARIHARAN, M., SCARIA, V., LALWANI, M., PILLAI, B., MITRA, D. & BRAHMACHARI, S. K. 2008. Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. *Retrovirology,* 5**,** 117.

ALBINI, A., BENELLI, R., PRESTA, M., RUSNATI, M., ZICHE, M., RUBARTELLI, A., PAGLIALUNGA, G., BUSSOLINO, F. & NOONAN, D. 1996a. HIV-tat protein is a heparin-binding angiogenic growth factor. *Oncogene,* 12**,** 289-297.

ALBINI, A., FONTANINI, G., MASIELLO, L., TACCHETTI, C., BIGINI, D., LUZZI, P., NOONAN, D. M. & STETLER-STEVENSON, W. G. 1994. Angiogenic potential in vivo by Kaposi's sarcoma cell-free supernatants and HIV-1 tat product: inhibition of KS-like lesions by tissue inhibitor of metalloproteinase-2. *Aids,* 8**,** 1237-1244.

ALBINI, A., SOLDI, R., GIUNCIUCLIO, D., GIRAUDO, E., BENELLI, R., PRIMO, L., NOONAN, D., SALIO, M., CAMUSSI, G. & ROCK, W. 1996b. The angiogenesis induced by HIV–1 Tat protein is mediated by the Flk–1/KDR receptor on vascular endothelial cells. *Nat Med,* 2**,** 1371-1375.

ALI, Z., KHALIQ, S., ZAKI, S., AHMAD, H. U. & LONE, K. P. 2019. Altered expression of vascular endothelial growth factor, vascular endothelial growth factor receptor-1, vascular endothelial growth factor receptor-2, and Soluble Fms-like Tyrosine Kinase-1 in peripheral blood mononuclear cells from normal and preeclamptic pregnancies. *Chin J Physiol,* 62**,** 117-122.

AWI, N. J. & TEOW, S. Y. 2018. Antibody-Mediated Therapy against HIV/AIDS: Where Are We Standing Now? *J Pathog,* 2018**,** 8724549.

AZEVEDO PORTILHO, N. & PELAJO-MACHADO, M. 2018. Mechanism of hematopoiesis and vasculogenesis in mouse placenta. *Placenta,* 69**,** 140-145.

BACKES, C. H., MARKHAM, K., MOOREHEAD, P., CORDERO, L., NANKERVIS, C. A. & GIANNONE, P. J. 2011. Maternal preeclampsia and neonatal outcomes. *J Pregnancy,* 2011**,** 214365.

BHASKARAN, M. & MOHAN, M. 2014. MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. *Vet Pathol,* 51**,** 759-74.

BOBST, S. M., DAY, M.-C., GILSTRAP, L. C., XIA, Y. & KELLEMS, R. E. 2005. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human mesangial cells and induce interleukin-6 and plasminogen activator inhibitor-1 secretion. *American journal of hypertension,* 18**,** 330-336.

BROSENS, I., BROSENS, J. J., MUTER, J., PUTTEMANS, P. & BENAGIANO, G. 2019. Preeclampsia: the role of persistent endothelial cells in uteroplacental arteries. *American Journal of Obstetrics and Gynecology,* 221**,** 219-226.

BROWN, H. M. & RUSSELL, D. L. 2014. Blood and lymphatic vasculature in the ovary: development, function and disease. *Hum Reprod Update,* 20**,** 29-39.

BROWN, M. A., MAGEE, L. A., KENNY, L. C., KARUMANCHI, S. A., MCCARTHY, F. P., SAITO, S., HALL, D. R., WARREN, C. E., ADOYI, G. & ISHAKU, S. 2018. Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. *Hypertension,* 72**,** 24-43.

CAO, R., JI, H., FENG, N., ZHANG, Y., YANG, X., ANDERSSON, P., SUN, Y., TRITSARIS, K., HANSEN, A. J., DISSING, S. & CAO, Y. 2012. Collaborative interplay between FGF-2 and VEGF-C promotes lymphangiogenesis and metastasis. *Proc Natl Acad Sci U S A,* 109**,** 15894-9.

CARMELIET, P. 2003. Angiogenesis in health and disease. *Nat Med,* 9**,** 653-660.

CASTELLON, X. & BOGDANOVA, V. 2016. Chronic Inflammatory Diseases and Endothelial Dysfunction. *Aging Dis,* 7**,** 81-9.

CASTRO, E., PARKS, W. T. & GALAMBOS, C. 2011. Neither normal nor diseased placentas contain lymphatic vessels. *Placenta,* 32**,** 310-316.

CELE, S. B., ODUN-AYO, F., ONYANGUNGA, O. A., MOODLEY, J. & NAICKER, T. 2018. Analysis of hepatocyte growth factor immunostaining in the placenta of HIV-infected normotensive versus preeclamptic pregnant women. *Eur J Obstet Gynecol Reprod Biol,* 227**,** 60-66.

CHAIWORAPONGSA, T., ROMERO, R., GOTSCH, F., ESPINOZA, J., NIEN, J. K., GONCALVES, L., EDWIN, S., KIM, Y. M., EREZ, O., KUSANOVIC, J. P., PINELES, B. L., PAPP, Z. & HASSAN, S. 2008. Low maternal concentrations of soluble vascular endothelial growth factor receptor-2 in preeclampsia and small for gestational age. *J Matern Fetal Neonatal Med,* 21**,** 41-52.

CHAIWORAPONGSA, T., ROMERO, R., TARCA, A. L., KUSANOVIC, J. P., GOTSCH, F., MITTAL, P., KIM, S. K., VAISBUCH, E., MAZAKI-TOVI, S., EREZ, O., DONG, Z., KIM, C. J., YEO, L. & HASSAN, S. S. 2010. A decrease in maternal plasma concentrations of sVEGFR-2 precedes the clinical diagnosis of preeclampsia. *Am J Obstet Gynecol,* 202**,** 550.e1-10.

CLARK, D., SMITH, S., SHARKEY, A. & CHARNOCK-JONES, D. 1996. Localization of VEGF and expression of its receptors flit and KDR in human placenta throughout pregnancy. *Human Reproduction,* 11**,** 1090-1098.

COOPER, J. C., SHARKEY, A. M., CHARNOCK-JONES, D. S., PALMER, C. R. & SMITH, S. K. 1996. VEGF mRNA levels in placentae from pregnancies complicated by pre-eclampsia. *Br J Obstet Gynaecol,* 103**,** 1191-6.

DE SPIEGELAERE, W., CASTELEYN, C., VAN DEN BROECK, W., PLENDL, J., BAHRAMSOLTANI, M., SIMOENS, P., DJONOV, V. & CORNILLIE, P. 2012. Intussusceptive angiogenesis: a biologically relevant form of angiogenesis. *Journal of vascular research,* 49**,** 390-404.

DEBAISIEUX, S., RAYNE, F., YEZID, H. & BEAUMELLE, B. 2012. The ins and outs of HIV-1 Tat. *Traffic,* 13**,** 355-363.

EDDY, A. C., BIDWELL, G. L. & GEORGE, E. M. 2018. Pro-angiogenic therapeutics for preeclampsia. *Biology of sex differences,* 9**,** 1-11.

FEROZEPURWALLA, Z., MERZAH, J., THIELEMANS, L. & BIRDSEY, G. 2019. Molecular and Cellular Mechanisms of Angiogenesis. *Heart of the Matter.* Springer.

FLINT, E. J., CERDEIRA, A. S., REDMAN, C. W. & VATISH, M. 2019. The role of angiogenic factors in the management of preeclampsia. *Acta Obstet Gynecol Scand,* 98**,** 700-707.

GANT, N. F., CHAND, S., WHALLEY, P. J. & MACDONALD, P. C. 1974. The nature of pressor responsiveness to angiotensin II in human pregnancy. *Obstetrics & Gynecology,* 43**,** 854-860.

GEORGE, E. M. 2017. The disease of theories: unravelling the mechanisms of pre-eclampsia. *The Biochemist,* 39**,** 22-25.

GERHARDT, H., GOLDING, M., FRUTTIGER, M., RUHRBERG, C., LUNDKVIST, A., ABRAMSSON, A., JELTSCH, M., MITCHELL, C., ALITALO, K. & SHIMA, D. 2003. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *The Journal of cell biology,* 161**,** 1163-1177.

GEYER, M., FACKLER, O. T. & PETERLIN, B. M. 2001. Structure--function relationships in HIV-1 Nef. *EMBO Rep,* 2**,** 580-5.

GILLE, H., KOWALSKI, J., LI, B., LECOUTER, J., MOFFAT, B., ZIONCHECK, T. F., PELLETIER, N. & FERRARA, N. 2001. Analysis of biological effects and signaling properties of Flt-1 (VEGFR-1) and KDR (VEGFR-2) A reassessment using novel receptor-specific vascular endothelial growth factor mutants. *Journal of Biological Chemistry,* 276**,** 3222-3230.

GOVENDER, N., NAICKER, T. & MOODLEY, J. 2013. Maternal imbalance between pro-angiogenic and anti-angiogenic factors in HIV-infected women with pre-eclampsia. *Cardiovasc J Afr,* 24**,** 174-9.

GU, B., ALEXANDER, J. S., GU, Y., ZHANG, Y., LEWIS, D. F. & WANG, Y. 2006. Expression of lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) in the human placenta. *Lymphatic research and biology,* 4**,** 11-17.

HALL, D. R. 2007. Is pre-eclampsia less common in patients with HIV/AIDS? *J Reprod Immunol,* 76**,** 75-7.

HARAM, K., MORTENSEN, J. H., MYKING, O., ROALD, B., MAGANN, E. F. & MORRISON, J. C. 2019. Early development of the human placenta and pregnancy complications. *J Matern Fetal Neonatal Med***,** 1-8.

HASSEL, D., CHENG, P., WHITE, M. P., IVEY, K. N., KROLL, J., AUGUSTIN, H. G., KATUS, H. A., STAINIER, D. Y. & SRIVASTAVA, D. 2012. MicroRNA-10 regulates the angiogenic behavior of zebrafish and human endothelial cells by promoting vascular endothelial growth factor signaling. *Circ Res,* 111**,** 1421-33.

HELSKE, S., VUORELA, P., CARPÉN, O., HORNIG, C., WEICH, H. & HALMESMÄKI, E. 2001. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. *Molecular human reproduction,* 7**,** 205-210.

HERRAIZ, I., SIMÓN, E., GÓMEZ-ARRIAGA, P. I., MARTÍNEZ-MORATALLA, J. M., GARCÍA-BURGUILLO, A., JIMÉNEZ, E. A. L. & GALINDO, A. 2015. Angiogenesis-related biomarkers (sFlt-1/PLGF) in the prediction and diagnosis of placental dysfunction: an approach for clinical integration. *International journal of molecular sciences,* 16**,** 19009-19026.

HERRAIZ, I., SIMÓN, E., TOLDOS, Ó., RODRÍGUEZ, Y., GÓMEZ-ARRIAGA, P. I. & GALINDO, A. 2017. Angiogenesis-related biomarkers (sFlt-1/PlGF) in placental mesenchymal dysplasia. *The Journal of Maternal-Fetal & Neonatal Medicine,* 30**,** 958-961.

HIBY, S. E., WALKER, J. J., O'SHAUGHNESSY, K. M., REDMAN, C. W., CARRINGTON, M., TROWSDALE, J. & MOFFETT, A. 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med,* 200**,** 957-965.

HIGGINS, J. R. & BRENNECKE, S. P. 1998. Pre-eclampsia-still a disease of theories? *Current Opinion in Obstetrics and Gynecology,* 10**,** 129-133.

HO, V. C. & FONG, G.-H. 2015. Vasculogenesis and angiogenesis in VEGF receptor-1 deficient mice. *VEGF Signaling.* Springer.

HOFFMANN, M., KLEINE-WEBER, H., SCHROEDER, S., KRÜGER, N., HERRLER, T., ERICHSEN, S., SCHIERGENS, T. S., HERRLER, G., WU, N. H., NITSCHE, A., MÜLLER, M. A., DROSTEN, C. & PÖHLMANN, S. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell,* 181**,** 271-280.e8.

HONG, F., LI, Y. & XU, Y. 2014. Decreased placental miR-126 expression and vascular endothelial growth factor levels in patients with pre-eclampsia. *J Int Med Res,* 42**,** 1243-51.

HOUZET, L., YEUNG, M. L., DE LAME, V., DESAI, D., SMITH, S. M. & JEANG, K. T. 2008. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. *Retrovirology,* 5**,** 118.

HUANG, J., WANG, F., ARGYRIS, E., CHEN, K., LIANG, Z., TIAN, H., HUANG, W., SQUIRES, K., VERLINGHIERI, G. & ZHANG, H. 2007. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. *Nat Med,* 13**,** 1241-7.

JERMAN, L. F. & HEY-CUNNINGHAM, A. J. 2015. The role of the lymphatic system in endometriosis: a comprehensive review of the literature. *Biol Reprod,* 92**,** 64.

JIANG, B., HEBERT, V. Y., LI, Y., MATHIS, J. M., ALEXANDER, J. S. & DUGAS, T. R. 2007. HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis. *Toxicology and applied pharmacology,* 224**,** 60-71.

JIANG, B., KHANDELWAL, A. R., ROGERS, L. K., HEBERT, V. Y., KLEINEDLER, J. J., ZAVECZ, J. H., SHI, W., ORR, A. W. & DUGAS, T. R. 2010. Antiretrovirals induce endothelial dysfunction via an oxidant-dependent pathway and promote neointimal hyperplasia. *Toxicological Sciences,* 117**,** 524-536.

JOINT UNITED NATIONS PROGRAMME ON HIV/AIDS. 2019. *Global HIV & AIDS statistics — 2019 fact sheet* [Online]. Available: https://www.unaids.org/en/resources/fact-sheet [Accessed 30 October 2020 2020].

KALA, S., DUNK, C., ACOSTA, S. & SERGHIDES, L. 2020. Periconceptional exposure to lopinavir, but not darunavir, impairs decidualization: a potential mechanism leading to poor birth outcomes in HIV-positive pregnancies. *Hum Reprod,* 35**,** 1781-1796.

KALUMBA, V. M., MOODLEY, J. & NAIDOO, T. D. 2013. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. *Cardiovasc J Afr,* 24**,** 24-7.

KARAMAN, S., LEPPÄNEN, V.-M. & ALITALO, K. 2018. Vascular endothelial growth factor signaling in development and disease. *Development,* 145**,** dev151019.

KARUMANCHI, S. A. & STILLMAN, I. E. 2006. In vivo rat model of preeclampsia. *Placenta and Trophoblast.* Springer.

KIM, P. G., ALBACKER, C. E., LU, Y.-F., JANG, I.-H., LIM, Y., HEFFNER, G. C., ARORA, N., BOWMAN, T. V., LIN, M. I. & LENSCH, M. W. 2013. Signaling axis involving Hedgehog, Notch, and Scl promotes the embryonic endothelial-to-hematopoietic transition. *Proceedings of the National Academy of Sciences,* 110**,** E141-E150.

KIM, S. C., PARK, M. J., JOO, B. S., JOO, J. K., SUH, D. S. & LEE, K. S. 2012. Decreased expressions of vascular endothelial growth factor and visfatin in the placental bed of pregnancies complicated by preeclampsia. *J Obstet Gynaecol Res,* 38**,** 665-73.

KUMAR, I., STATON, C., CROSS, S., REED, M. & BROWN, N. 2009. Angiogenesis, vascular endothelial growth factor and its receptors in human surgical wounds. *British Journal of Surgery: Incorporating European Journal of Surgery and Swiss Surgery,* 96**,** 1484-1491.

KWEIDER, N., FRAGOULIS, A., ROSEN, C., PECKS, U., RATH, W., PUFE, T. & WRUCK, C. J. 2011. Interplay between vascular endothelial growth factor (VEGF) and nuclear factor erythroid 2-related factor-2 (Nrf2): implications for preeclampsia. *J Biol Chem,* 286**,** 42863-72.

LAMARCA, B. D., RYAN, M. J., GILBERT, J. S., MURPHY, S. R. & GRANGER, J. P. 2007. Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. *Curr Hypertens Rep,* 9**,** 480-5.

LANDI, B., BEZZECCHERI, V., GUERRA, B., PIEMONTESE, M., CERVI, F., CECCHI, L., MARGARITO, E., GIANNUBILO, S. R., CIAVATTINI, A. & TRANQUILLI, A. L. 2014. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. *World Journal of Cardiovascular Diseases,* 2014.

LEE, G. S., JOE, Y. S., KIM, S. J. & SHIN, J. C. 2010. Cytokine-related genes and oxidation-related genes detected in preeclamptic placentas. *Arch Gynecol Obstet,* 282**,** 363-9.

LELY, A. T., SALAHUDDIN, S., HOLWERDA, K. M., KARUMANCHI, S. A. & RANA, S. 2013. Circulating lymphangiogenic factors in preeclampsia. *Hypertens Pregnancy,* 32**,** 42-49.

LEVINE, R. J., LAM, C., QIAN, C., YU, K. F., MAYNARD, S. E., SACHS, B. P., SIBAI, B. M., EPSTEIN, F. H., ROMERO, R. & THADHANI, R. 2006. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *New England Journal of Medicine,* 355**,** 992-1005.

LEVY, A., YAGIL, Y., BURSZTYN, M., BARKALIFA, R., SCHARF, S. & YAGIL, C. 2008. ACE2 expression and activity are enhanced during pregnancy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 295**,** R1953-R1961.

LIU, H., LI, Y., ZHANG, J., RAO, M., LIANG, H. & LIU, G. 2015. The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia. *Placenta,* 36**,** 279-286.

MAARTENS, G., CELUM, C. & LEWIN, S. R. 2014. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet,* 384**,** 258-71.

MACHADO, E. S., KRAUSS, M. R., MEGAZZINI, K., COUTINHO, C. M., KREITCHMANN, R., MELO, V. H., PILOTTO, J. H., CERIOTTO, M., HOFER, C. B., SIBERRY, G. K. & WATTS, D. H. 2014. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. *J Infect,* 68**,** 572-80.

MACHNIK, A., NEUHOFER, W., JANTSCH, J., DAHLMANN, A., TAMMELA, T., MACHURA, K., PARK, J. K., BECK, F. X., MÜLLER, D. N., DERER, W., GOSS, J., ZIOMBER, A., DIETSCH, P., WAGNER, H., VAN ROOIJEN, N., KURTZ, A., HILGERS, K. F., ALITALO, K., ECKARDT, K. U., LUFT, F. C., KERJASCHKI, D. & TITZE, J. 2009. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med,* 15**,** 545-52.

MAHARAJ, N. R., PHULUKDAREE, A., NAGIAH, S., RAMKARAN, P., TILOKE, C. & CHUTURGOON, A. A. 2017. Pro-inflammatory cytokine levels in HIV infected and uninfected pregnant women with and without preeclampsia. *PLoS One,* 12.

MARGADANT, C. 2020. Positive and negative feedback mechanisms controlling tip/stalk cell identity during sprouting angiogenesis. *Angiogenesis***,** 1-3.

MARQUEZ-PEDROZA, J., CÁRDENAS-BEDOYA, J., MORÁN-MOGUEL, M. C., ESCOTO-DELGADILLO, M., TORRES-MENDOZA, B. M., PÉREZ-RÍOS, A. M., GONZÁLEZ-

ENRIQUEZ, G. V. & VÁZQUEZ-VALLS, E. 2020. Plasma microRNA expression levels in HIV-1-positive patients receiving antiretroviral therapy. *Biosci Rep,* 40.

MATTAR, R., AMED, A. M., LINDSEY, P. C., SASS, N. & DAHER, S. 2004. Preeclampsia and HIV infection. *Eur J Obstet Gynecol Reprod Biol,* 117**,** 240-1.

MAWSON, A. R. 2003. Effects of antiretroviral therapy on occurrence of pre-eclampsia. *Lancet,* 361**,** 347-8.

MAYNARD, S. E. & KARUMANCHI, S. A. Angiogenic factors and preeclampsia. Seminars in nephrology, 2011. Elsevier, 33-46.

MAYNARD, S. E., MIN, J.-Y., MERCHAN, J., LIM, K.-H., LI, J., MONDAL, S., LIBERMANN, T. A., MORGAN, J. P., SELLKE, F. W. & STILLMAN, I. E. 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *The Journal of clinical investigation,* 111**,** 649-658.

MAYNARD, S. E., VENKATESHA, S., THADHANI, R. & KARUMANCHI, S. A. 2005. Soluble Fms-like tyrosine kinase 1 and endothelial dysfunction in the pathogenesis of preeclampsia. *Pediatric research,* 57**,** 1-7.

MENDOZA, M., GARCIA-RUIZ, I., MAIZ, N., RODO, C., GARCIA-MANAU, P., SERRANO, B., LOPEZ-MARTINEZ, R. M., BALCELLS, J., FERNANDEZ-HIDALGO, N., CARRERAS, E. & SUY, A. 2020. Pre-eclampsia-like syndrome induced by severe COVID-19: a prospective observational study. *Bjog,* 127**,** 1374-1380.

MENTZER, S. J. & KONERDING, M. A. 2014. Intussusceptive angiogenesis: expansion and remodeling of microvascular networks. *Angiogenesis,* 17**,** 499-509.

MERT, I., SARGIN ORUC, A., YUKSEL, S., CAKAR, E. S., BUYUKKAGNICI, U., KARAER, A. & DANISMAN, N. 2012. Role of oxidative stress in preeclampsia and intrauterine growth restriction. *Journal of Obstetrics and Gynaecology Research,* 38**,** 658-664.

MOL, B. W. J., ROBERTS, C. T., THANGARATINAM, S., MAGEE, L. A., DE GROOT, C. J. M. & HOFMEYR, G. J. 2016. Pre-eclampsia. *Lancet,* 387**,** 999-1011.

MOODLEY, J. 2013. Impact of HIV on the incidence of pre-eclampsia. *Cardiovasc J Afr,* 24**,** 5.

MOODLEY, J., ONYANGUNGA, O. A. & MAHARAJ, N. R. 2016. Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis. *Hypertens Pregnancy,* 35**,** 529-535.

MOODLEY, J., SOMA-PILLAY, P., BUCHMANN, E. & PATTINSON, R. 2019. Hypertensive disorders in pregnancy: 2019 National guideline. *South African Medical Journal,* 109.

MUNAUT, C., LORQUET, S., PEQUEUX, C., BLACHER, S., BERNDT, S., FRANKENNE, F. & FOIDART, J. M. 2008. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. *Hum Reprod,* 23**,** 1407-15.

NAGHSHVAR, F., TORABIZADEH, Z., MOSLEMI ZADEH, N., MIRBAHA, H. & GHESHLAGHI, P. 2013. Investigating the Relationship between Serum Level of s-Met (Soluble Hepatic Growth Factor Receptor) and Preeclampsia in the First and Second Trimesters of Pregnancy. *ISRN Obstet Gynecol,* 2013**,** 925062.

NAICKER, T., DORSAMY, E., RAMSURAN, D., BURTON, G. J. & MOODLEY, J. 2013. The role of apoptosis on trophoblast cell invasion in the placental bed of normotensive and preeclamptic pregnancies. *Hypertens Pregnancy,* 32**,** 245-56.

NAICKER, T., KHEDUN, S. M., MOODLEY, J. & PIJNENBORG, R. 2003. Quantitative analysis of trophoblast invasion in preeclampsia. *Acta Obstet Gynecol Scand,* 82**,** 722-9.

NAICKER, T., PHOSWA, W. N., ONYANGUNGA, O. A., GATHIRAM, P. & MOODLEY, J. 2019. Angiogenesis, Lymphangiogenesis, and the Immune Response in South African Preeclamptic Women Receiving HAART. *International journal of molecular sciences,* 20**,** 3728.

NATHAN, H. L., SEED, P. T., HEZELGRAVE, N. L., DE GREEFF, A., LAWLEY, E., CONTI-RAMSDEN, F., ANTHONY, J., STEYN, W., HALL, D. R. & CHAPPELL, L. C. 2018. Maternal and perinatal adverse outcomes in women with pre-eclampsia cared for at facility-level in South Africa: a prospective cohort study. *Journal of global health,* 8.

NATHANS, R., CHU, C. Y., SERQUINA, A. K., LU, C. C., CAO, H. & RANA, T. M. 2009. Cellular microRNA and P bodies modulate host-HIV-1 interactions. *Mol Cell,* 34**,** 696-709.

NATIONAL COMMITTEE FOR CONFIDENTIAL ENQUIRY INTO MATERNAL DEATHS 2018. Saving Mothers Report 2017. Pretoria: South African Department of Health.

NERSISYAN, S., ENGIBARYAN, N., GORBONOS, A., KIRDEY, K., MAKHONIN, A. & TONEVITSKY, A. 2020. Potential role of cellular miRNAs in coronavirus-host interplay. *PeerJ,* 8**,** e9994.

NOONAN, D. & ALBINI, A. 2000. From the outside in: extracellular activities of HIV Tat. *Advances in pharmacology,* 48**,** 229-250.

OLTMANNS, K. M., GEHRING, H., RUDOLF, S., SCHULTES, B., HACKENBERG, C., SCHWEIGER, U., BORN, J., FEHM, H. L. & PETERS, A. 2006. Acute hypoxia decreases plasma VEGF concentration in healthy humans. *Am J Physiol Endocrinol Metab,* 290**,** E434-9.

ONYANGUNGA, O. A., MOODLEY, J., MERHAR, V., OFUSORI, D. A. & NAICKER, T. 2016. Lymphatic vascular endothelial hyaluronan receptor-1 immunoexpression in placenta of HIV infected pre-eclamptic women. *J Reprod Immunol,* 117**,** 81-8.

PANDEY, A. K., SINGHI, E. K., ARROYO, J. P., IKIZLER, T. A., GOULD, E. R., BROWN, J., BECKMAN, J. A., HARRISON, D. G. & MOSLEHI, J. 2018. Mechanisms of VEGF (vascular endothelial growth factor) inhibitor–associated hypertension and vascular disease. *Hypertension,* 71**,** e1-e8.

PANT, V., YADAV, B. K. & SHARMA, J. 2019. A cross sectional study to assess the sFlt-1: PlGF ratio in pregnant women with and without preeclampsia. *BMC pregnancy and childbirth,* 19**,** 266.

PARK, K. W., MORRISON, C. M., SORENSEN, L. K., JONES, C. A., RAO, Y., CHIEN, C. B., WU, J. Y., URNESS, L. D. & LI, D. Y. 2003. Robo4 is a vascular-specific receptor that inhibits endothelial migration. *Dev Biol,* 261**,** 251-67.

PARK, Y. G., CHOI, J., JUNG, H. K., SONG, I. K., SHIN, Y., PARK, S. Y. & SEOL, J. W. 2017. Fluid shear stress regulates vascular remodeling via VEGFR-3 activation, although independently of its ligand, VEGF-C, in the uterus during pregnancy. *Int J Mol Med,* 40**,** 1210-1216.

PATAN, S. 2004. Vasculogenesis and angiogenesis. *Angiogenesis in brain tumors.* Springer.

PERALES, A., DELGADO, J., DE LA CALLE, M., GARCÍA-HERNÁNDEZ, J., ESCUDERO, A., CAMPILLOS, J., SARABIA, M., LAIZ, B., DUQUE, M. & NAVARRO, M. 2017. sFlt-1/PlGF for prediction of early-onset pre-eclampsia: STEPS (Study of Early Pre-eclampsia in Spain). *Ultrasound in Obstetrics & Gynecology,* 50**,** 373-382.

PHIPPS, E. A., THADHANI, R., BENZING, T. & KARUMANCHI, S. A. 2019. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol,* 15**,** 275-289.

PIJNENBORG, R., BROSENS, I. & ROMERO, R. 2010. *Placental bed disorders: basic science and its translation to obstetrics*, Cambridge University Press.

PILLAY, P., MOODLEY, K., MOODLEY, J. & MACKRAJ, I. 2017. Placenta-derived exosomes: potential biomarkers of preeclampsia. *Int J Nanomedicine,* 12**,** 8009-8023.

PIPKIN, F. B. & RUBIN, P. 1994. Pre-eclampsia—the 'disease of theories'. *British medical bulletin,* 50**,** 381-396.

PLATONOVA, N., MIQUEL, G., REGENFUSS, B., TAOUJI, S., CURSIEFEN, C., CHEVET, E. & BIKFALVI, A. 2013. Evidence for the interaction of fibroblast growth factor-2 with the lymphatic endothelial cell marker LYVE-1. *Blood,* 121**,** 1229-37.

POSSOMATO-VIEIRA, J. S. & KHALIL, R. A. 2016. Mechanisms of Endothelial Dysfunction in Hypertensive Pregnancy and Preeclampsia. *Adv Pharmacol,* 77**,** 361-431.

RANA, S., LEMOINE, E., GRANGER, J. & KARUMANCHI, S. A. 2019. Preeclampsia. *Circ Res,* 124**,** 1094-1112.

RANA, S., POWE, C. E., SALAHUDDIN, S., VERLOHREN, S., PERSCHEL, F. H., LEVINE, R. J., LIM, K.-H., WENGER, J. B., THADHANI, R. & KARUMANCHI, S. A. 2012. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation,* 125**,** 911-919.

RATH, G. & TRIPATHI, R. 2012. VEGF and its soluble receptor VEGFR-2 in hypertensive disorders during pregnancy: the Indian scenario. *J Hum Hypertens,* 26**,** 196-204.

RED-HORSE, K. 2008. Lymphatic vessel dynamics in the uterine wall. *Placenta,* 29 Suppl A**,** S55-9.

REDMAN, C. 2017. Early and late onset preeclampsia: two sides of the same coin. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health,* 7**,** 58.

REDMAN, C. W. & SARGENT, I. L. 2005. Latest advances in understanding preeclampsia. *Science,* 308**,** 1592-4.

REDMAN, C. W. & STAFF, A. C. 2015. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol,* 213**,** S9.e1, S9-11.

RIBATTI, D. & CRIVELLATO, E. 2012. "Sprouting angiogenesis", a reappraisal. *Developmental biology,* 372**,** 157-165.

ROBERTS, J. M. & GAMMILL, H. S. 2005. Preeclampsia: recent insights. *Hypertension,* 46**,** 1243-9.

ROBINSON, C. J. & STRINGER, S. E. 2001. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. *J Cell Sci,* 114**,** 853-65.

ROY, H., BHARDWAJ, S. & YLÄ-HERTTUALA, S. 2006. Biology of vascular endothelial growth factors. *FEBS letters,* 580**,** 2879-2887.

RUSNATI, M. & PRESTA, M. 2002. HIV-1 Tat protein and endothelium: from protein/cell interaction to AIDS-associated pathologies. *Angiogenesis,* 5**,** 141-151.

SARDAR, R., SATISH, D., BIRLA, S. & GUPTA, D. 2020. Comparative analyses of SAR-CoV2 genomes from different geographical locations and other coronavirus family genomes reveals unique features potentially consequential to host-virus interaction and pathogenesis. *bioRxiv*.

SAYED, A. S., XIA, K., SALMA, U., YANG, T. & PENG, J. 2014. Diagnosis, prognosis and therapeutic role of circulating miRNAs in cardiovascular diseases. *Heart Lung Circ,* 23**,** 503-10.

SEBITLOANE, H. M., MOODLEY, J. & SARTORIUS, B. 2017. Associations between HIV, highly active anti-retroviral therapy, and hypertensive disorders of pregnancy among maternal deaths in South Africa 2011–2013. *International Journal of Gynecology & Obstetrics,* 136**,** 195-199.

SGAMBATI, E., MARINI, M., ZAPPOLI THYRION, G. D., PARRETTI, E., MELLO, G., ORLANDO, C., SIMI, L., TRICARICO, C., GHERI, G. & BRIZZI, E. 2004. VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. *Bjog,* 111**,** 564-70.

SHAKIBAEI, M., SCHULZE-TANZIL, G., MOBASHERI, A., BEICHLER, T., DRESSLER, J. & SCHWAB, W. 2003. Expression of the VEGF receptor-3 in osteoarthritic chondrocytes: stimulation by interleukin-1 beta and association with beta 1-integrins. *Histochem Cell Biol,* 120**,** 235-41.

SHANMUGALINGAM, R., HENNESSY, A. & MAKRIS, A. 2019. Aspirin in the prevention of preeclampsia: the conundrum of how, who and when. *Journal of human hypertension,* 33**,** 1-9.

SHIBUYA, M. 2013. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. *The Journal of Biochemistry,* 153**,** 13-19.

SHIBUYA, M. & CLAESSON-WELSH, L. 2006. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Experimental cell research,* 312**,** 549-560.

SIBAI, B., DEKKER, G. & KUPFERMINC, M. 2005. Pre-eclampsia. *The Lancet,* 365**,** 785-799.

SINGH, N., TIEM, M., WATKINS, R., CHO, Y. K., WANG, Y., OLSEN, T., UEHARA, H., MAMALIS, C., LUO, L., OAKEY, Z. & AMBATI, B. K. 2013. Soluble vascular endothelial growth factor receptor 3 is essential for corneal alymphaticity. *Blood,* 121**,** 4242-9.

SIRCAR, M., THADHANI, R. & KARUMANCHI, S. A. 2015. Pathogenesis of preeclampsia. *Curr Opin Nephrol Hypertens,* 24**,** 131-8.

SOMERSET, D. A. & KILBY, M. D. 1997. VEGF mRNA levels in placentae from pregnancies complicated by pre-eclampsia. *Br J Obstet Gynaecol,* 104**,** 972.

SONG, L., DING, S., GE, Z., ZHU, X., QIU, C., WANG, Y., LAI, E., YANG, W., SUN, Y., CHOW, S. A. & YU, L. 2018. Nucleoside/nucleotide reverse transcriptase inhibitors attenuate angiogenesis and lymphangiogenesis by impairing receptor tyrosine kinases signalling in endothelial cells. *Br J Pharmacol,* 175**,** 1241-1259.

SPERANZA, R., GREINER, K., RINCON, M., BEERAKA, S. & BURWICK, R. 2019. A population-based definition for severe proteinuria and its association with adverse outcomes in preeclampsia. *American Journal of Obstetrics and Gynecology,* 220.

STATS SA 2019. Mid-year Population Estimates 2019. Pretoria, South Africa.

STÜRZL, M., BRANDSTETTER, H., ZIETZ, C., EISENBURG, B., RAIVICH, G., GEARING, D. P., BROCKMEYER, N. H. & HOFSCHNEIDER, P. 1995. Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined in vitro and in vivo analysis. *Oncogene,* 10**,** 2007-2016.

SUTLIFF, R. L., DIKALOV, S., WEISS, D., PARKER, J., RAIDEL, S., RACINE, A. K., RUSS, R., HAASE, C. P., TAYLOR, W. R. & LEWIS, W. 2002. Nucleoside reverse transcriptase inhibitors impair endothelium-dependent relaxation by increasing superoxide. *American Journal of Physiology-Heart and Circulatory Physiology,* 283**,** H2363-H2370.

SUY, A., MARTÍNEZ, E., COLL, O., LONCA, M., PALACIO, M., DE LAZZARI, E., LARROUSSE, M., MILINKOVIC, A., HERNÁNDEZ, S., BLANCO, J. L., MALLOLAS, J., LEÓN, A., VANRELL, J. A. & GATELL, J. M. 2006. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. *Aids,* 20**,** 59-66.

TJOA, M. L., KHANKIN, E. V., RANA, S. & KARUMANCHI, S. A. 2010. Angiogenic factors and preeclampsia. *Placent Bed Disord Basic Sci its Transl to Obstet,* 31**,** 229-42.

TOFT, J. H., LIAN, I. A., TARCA, A. L., EREZ, O., ESPINOZA, J., EIDE, I. P., BJØRGE, L., DRAGHICI, S., ROMERO, R. & AUSTGULEN, R. 2008. Whole-genome microarray and targeted analysis of angiogenesis-regulating gene expression (ENG, FLT1, VEGF, PlGF) in placentas from pre-eclamptic and small-for-gestational-age pregnancies. *J Matern Fetal Neonatal Med,* 21**,** 267-73.

TRANQUILLI, A. L., DEKKER, G., MAGEE, L., ROBERTS, J., SIBAI, B. M., STEYN, W., ZEEMAN, G. G. & BROWN, M. A. 2014. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens,* 4**,** 97-104.

TRIPATHI, R., RATH, G., RALHAN, R., SAXENA, S. & SALHAN, S. 2009. Soluble and membranous vascular endothelial growth factor receptor-2 in pregnancies complicated by pre-eclampsia. *Yonsei Med J,* 50**,** 656-66.

VALERO, L., ALHARETH, K., GIL, S., LECARPENTIER, E., TSATSARIS, V., MIGNET, N., FOURNIER, T. & ANDRIEUX, K. 2018. Nanomedicine as a potential approach to empower the new strategies for the treatment of preeclampsia. *Drug discovery today,* 23**,** 1099-1107.

VERMA, S., PILLAY, P., NAICKER, T., MOODLEY, J. & MACKRAJ, I. 2018. Placental hypoxia inducible factor -1α & CHOP immuno-histochemical expression relative to maternal circulatory syncytiotrophoblast micro-vesicles in preeclamptic and normotensive pregnancies. *Eur J Obstet Gynecol Reprod Biol,* 220**,** 18-24.

VOLCHEK, M., GIRLING, J. E., LASH, G. E., CANN, L., KUMAR, B., ROBSON, S. C., BULMER, J. N. & ROGERS, P. A. 2010. Lymphatics in the human endometrium disappear during decidualization. *Hum Reprod,* 25**,** 2455-64.

WALLNER, W., SENGENBERGER, R., STRICK, R., STRISSEL, P. L., MEURER, B., BECKMANN, M. W. & SCHLEMBACH, D. 2007. Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clin Sci (Lond),* 112**,** 51-7.

WANG, H., LI, X., LI, T., ZHANG, S., WANG, L., WU, X. & LIU, J. 2020. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur J Clin Microbiol Infect Dis,* 39**,** 1629-1635.

WANG, J. F., ZHANG, X. F. & GROOPMAN, J. E. 2001. Stimulation of beta 1 integrin induces tyrosine phosphorylation of vascular endothelial growth factor receptor-3 and modulates cell migration. *J Biol Chem,* 276**,** 41950-7.

WANG, Y., SUN, J., GU, Y., ZHAO, S., GROOME, L. J. & ALEXANDER, J. S. 2011. D2-40/podoplanin expression in the human placenta. *Placenta,* 32**,** 27-32.

WIMALASUNDERA, R. C., LARBALESTIER, N., SMITH, J. H., DE RUITER, A., MC, G. T. S. A., HUGHES, A. D., POULTER, N., REGAN, L. & TAYLOR, G. P. 2002. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet,* 360**,** 1152-4.

WOLDESENBET, S., KUFA, T., LOMBARD, C., MANDA, S., AYALEW, K., CHEYIP, M. & PURE, A. 2018. The 2017 National antenatal sentinel HIV survey. *South Africa, National Department of Health.[cited 2019 July 29] Available from: http://www.nicd.ac.za/wp-content/uploads/2019/07/Antenatal_survey-report_24July19. pdf*.

WORLD HEALTH ORGANIZATION. 2015. *Maternal Health* [Online]. Available: https://www.afro.who.int/health-topics/maternal-health [Accessed 30 October 2020 2020].

WORLD HEALTH ORGANIZATION. 2019b. *Number of people (all ages) living with HIV: Estimates by WHO region* [Online]. Available: http://apps.who.int/gho/data/view.main.22100WHO?lang=en [Accessed 30 October 2020 2020].

WORLD HEALTH ORGANIZATION 2019a. Trends in maternal mortality 2000 to 2017: estimates by WHO, UNICEF. UNFPA, World Bank Group and the United Nations Population Division.

WU, F. T., STEFANINI, M. O., GABHANN, F. M., KONTOS, C. D., ANNEX, B. H. & POPEL, A. S. 2010. A systems biology perspective on sVEGFR1: its biological function, pathogenic role and therapeutic use. *Journal of cellular and molecular medicine,* 14**,** 528-552.

YIA, Y., WNE, H., BOBST, S., DAY, M.-C. & KELLEMS, R. E. 2003. Maternal autoantibodies from preeclamptic patients active angiotensin receptors on human trophoblast cells. *Journal of the Society for Gynecologic Investigation,* 10**,** 82-93.

YOUNG, B. C., LEVINE, R. J. & KARUMANCHI, S. A. 2010. Pathogenesis of preeclampsia. *Annual Review of Pathology: Mechanisms of Disease,* 5**,** 173-192.

YU, J., ZHANG, X., KUZONTKOSKI, P. M., JIANG, S., ZHU, W., LI, D. Y. & GROOPMAN, J. E. 2014. Slit2N and Robo4 regulate lymphangiogenesis through the VEGF-C/VEGFR-3 pathway. *Cell Commun Signal,* 12**,** 25.

ZEISLER, H., LLURBA, E., CHANTRAINE, F., VATISH, M., STAFF, A. C., SENNSTRÖM, M., OLOVSSON, M., BRENNECKE, S. P., STEPAN, H. & ALLEGRANZA, D. 2016. Predictive value of the sFlt-1: PlGF ratio in women with suspected preeclampsia. *N Engl J Med,* 374**,** 13-22.

ZHANG, X., YU, J., KUZONTKOSKI, P. M., ZHU, W., LI, D. Y. & GROOPMAN, J. E. 2012. Slit2/Robo4 signaling modulates HIV-1 gp120-induced lymphatic hyperpermeability. *PLoS Pathog,* 8.

ZHOU, Y., MCMASTER, M., WOO, K., JANATPOUR, M., PERRY, J., KARPANEN, T., ALITALO, K., DAMSKY, C. & FISHER, S. J. 2002. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *The American journal of pathology,* 160**,** 1405-1423.

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.

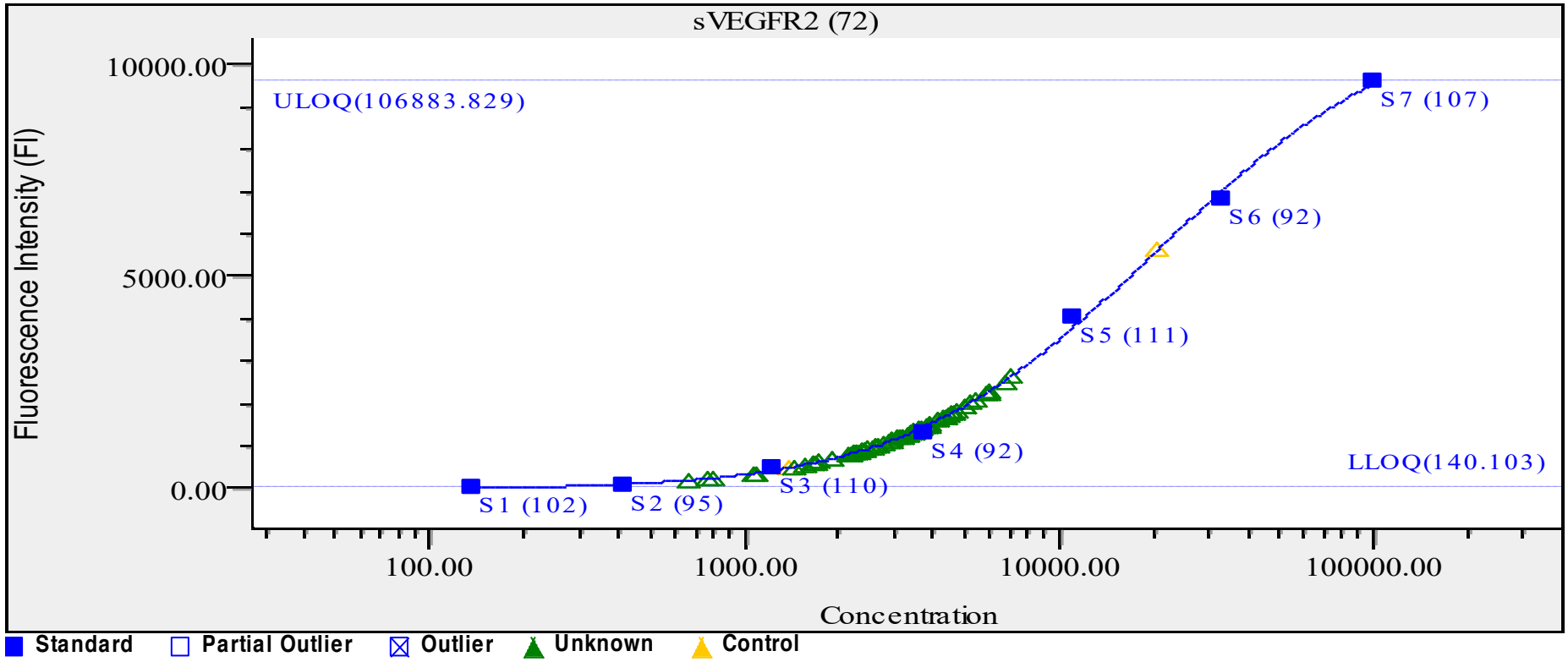
Yours sincerely

[redacted]

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

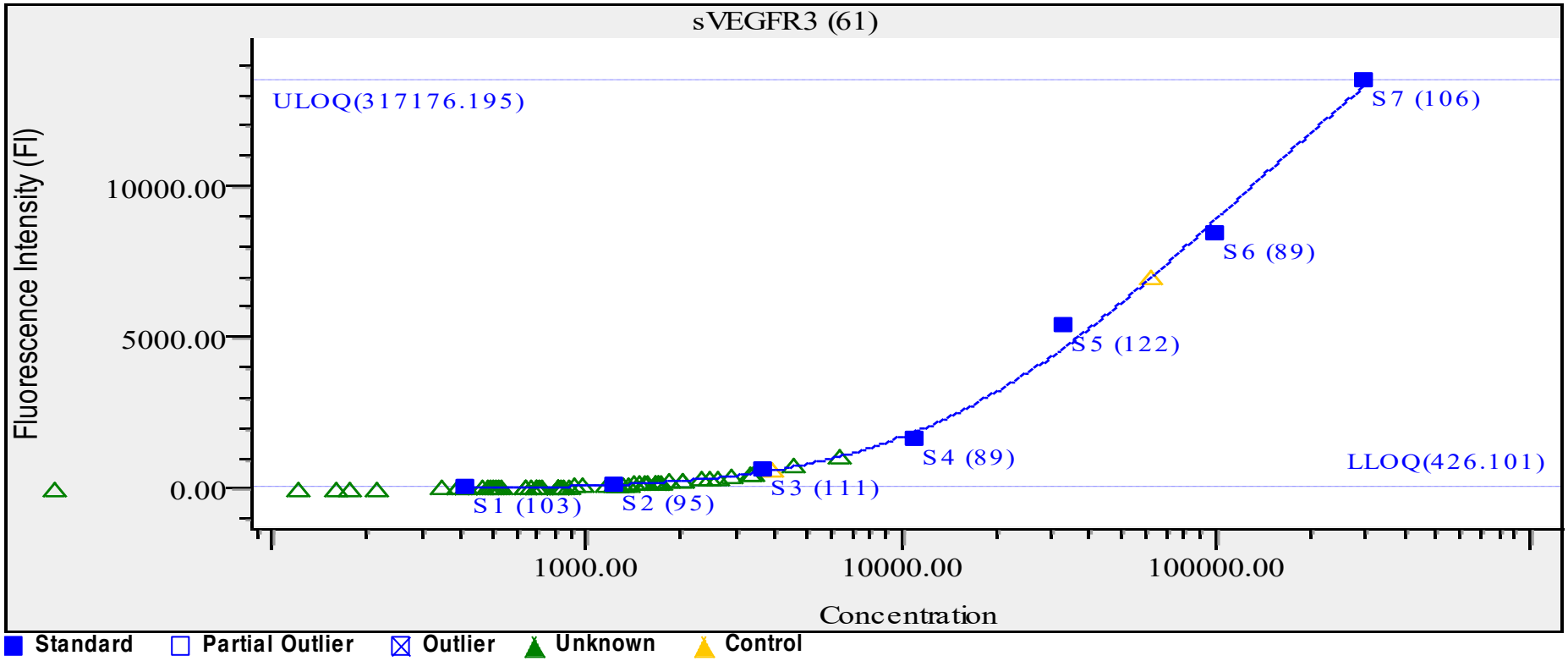
Appendix 1: Ethical Approval (BCA338/17)

Appendix 2



Appendix 2: Standard curve of sVEGFR-2

Appendix 3

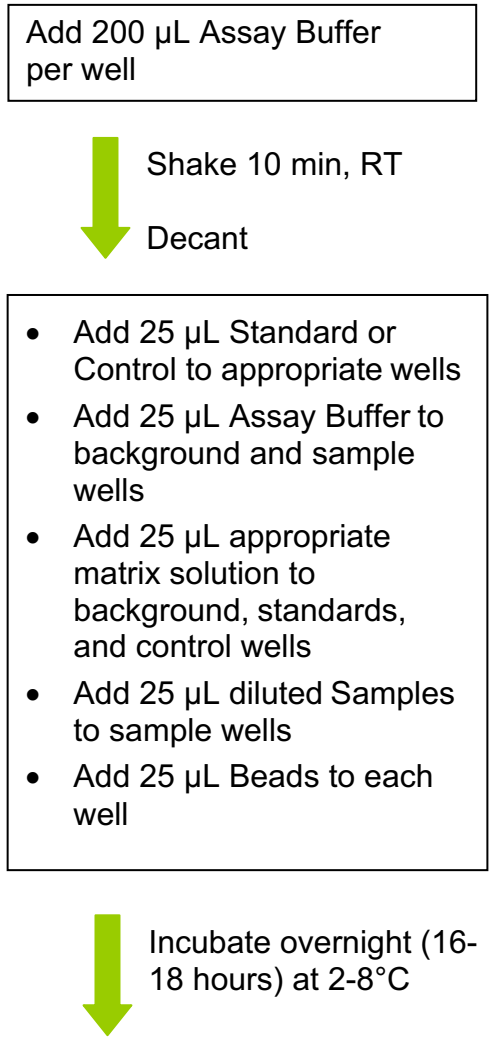


Appendix 3: Standard curve of sVEGFR-3

IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), Standard 1 through 7], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will read only the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Add 200 µL of Assay Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of Sample (diluted) into the appropriate wells.
7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8°C.



9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
10. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
11. Seal, cover with foil and incubate with agitation on a plate shaker for one hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**
12. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
15. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT® software.
17. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. (Note: For diluted samples, final sample concentrations should be multiplied by the dilution factor. For samples diluted as per protocol instructions, multiply by 5. If using another dilution factor, multiple by the appropriate dilution factor.)

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 hour at RT

Do Not Aspirate

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 100 µL Sheath Fluid or Drive Fluid per well

Read on Luminex® (50 µL, 50 beads per bead set)

Current Hypertension Reports (2021) 23:20
https://doi.org/10.1007/s11906-021-01138-5

PREECLAMPSIA (V GAROVIC, SECTION EDITOR)

The Involvement of MicroRNAs in SARS-CoV-2 Infection Comorbid with HIV-Associated Preeclampsia

Tashlen Abel[1] · Jagidesa Moodley[2] · Thajasvarie Naicker[1]

Accepted: 18 March 2021

Abstract

Purpose of Review This review investigated the potential role of microRNAs (miRNAs) in the synergy of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, preeclampsia (PE), and human immunodeficiency virus (HIV) infection. Maternal health is a great concern when treating pregnant women fighting this triad of diseases, which is highly prevalent in South Africa. MicroRNAs are involved in fine-tuning of physiological processes. Disruptions to the balance of this minute protein can lead to various physiological changes that are sometimes pathological.

Recent Findings MicroRNAs have recently been implicated in PE and have been linked to the anti-angiogenic imbalance evident in PE. Recent in silico studies have identified potential host miRNAs with anti-viral properties against SARS-CoV-2 infection. Studies have demonstrated dysregulated expression of several miRNAs in HIV-1 infection along with the ability of HIV-1 to downregulate anti-viral host microRNAs.

Summary This review has highlighted the significant gap in literature on the potential of miRNAs in women with HIV-associated PE in synergy with the novel SARS-CoV-2 infection. In addition, this review has provided evidence of the critical role that the epigenetic regulatory mechanism of miRNA plays in viral infections and PE, thereby providing a foundation for further research investigating the potential of therapeutic miRNA development with fewer side-effects for pregnant women.

Keywords Human immunodeficiency virus · Hypertension · MicroRNA · Preeclampsia · Pregnancy · SARS-CoV-2 infection

Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late November 2019 and has led to the coronavirus disease 2019 (COVID-19) pandemic [1•]. It is believed that SARS-CoV-2 originated from a wild meat market in Wuhan, Hubei, China [2]. Severe acute respiratory syndrome coronavirus 2 transmission occurs across humans regardless of age and sex; however, it is more prevalent amongst the elderly, the overweight, and those with asthma, diabetes, and other immunocompromised conditions [3]. According to the World Health Organization (WHO), South Africa (SA) has the highest COVID-19 prevalence in Africa. Despite an "early hard lockdown" by the country, more than 700,000 South Africans have been infected with SARS-CoV-2 as of October 2020 [4]. Considered to be a low- and middle-income country (LMIC), it seems unlikely that SA will avoid a fall in the local economy. Hence, it is of utmost importance to rapidly discover solutions to overcome the COVID-19 pandemic.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that are able to post-transcriptionally regulate the expression of proteins through modulation of the protein's messenger RNA. MicroRNAs are approximately 22 nucleotides long and possess a long half-life and stability that is 10 times stronger than mRNAs, even in extracellular fluids like urine and plasma [5]. MicroRNAs are able to degrade mRNA and

This article is part of the Topical Collection on *Preeclampsia*

✉ Tashlen Abel
tashlen.abel@gmail.com

Jagidesa Moodley
jmog@ukzn.ac.za

Thajasvarie Naicker
naickera@ukzn.ac.za

[1] Optics and Imaging Centre, Doris Duke Medical Research Institution, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa

[2] Women's Health and HIV Research Group, Department of Obstetrics & Gynaecology, School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Published online: 13 April 2021

suppress protein translation when the 5′ terminal of miRNA pairs with the 3′-untranslated region (3′-UTR) of mRNA [6, 7]. When miRNAs are incompletely complementary to multiple sites in the 3′-UTR, protein synthesis is inhibited [8]. In comparison, when completely base-paired, a single phosphodiester bond is cleaved leading to degradation of the target mRNA [8].

Host miRNAs have been reported to be involved in cell proliferation, angiogenesis, immune cell development, and apoptosis [9]. Differential expression of miRNAs has been implicated in several viral diseases [10], cancer [9], diabetes [11], schizophrenia [12], and cardiovascular diseases [13]. The diverse role of miRNAs ignites the curiosity of its role in contemporary diseases and associated conditions.

Hypertensive disorders in pregnancy (HDP) are one of the commonest direct causes of mortality and morbidity worldwide; approximately 94% of maternal deaths occur in LMIC [14, 15]. Furthermore, it is responsible for 18% of all maternal deaths in SA [14].

Preeclampsia (PE) is an HDP of unknown origin that complicates 5–8% of pregnancies worldwide [16] and occurs more frequently in LMIC compared to high-income countries [15, 17]. Preeclampsia is characterized by new-onset hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) with or without excessive proteinuria (≥300 mg every 24 h); the disorder presents with the clinical signs of hypertension at or after 20-week gestation [18]. The diagnosis of PE is also made in the absence of proteinuria when there is evidence of multi-organ involvement such as acute kidney injury, neurologic signs, liver disease, and intrauterine foetal growth restriction. In addition, evidence of haemolysis, elevated liver enzymes, and low platelet counts leads to a diagnosis of HELLP syndrome [19, 20].

The human immunodeficiency virus (HIV) attack cells of the immune system thereby weakening immunity which leads to the host being susceptible to other infections and diseases. [21]. HIV infection is a global concern with over 30 million people living with HIV at the end of 2019 [22]. In 2019, 13.5% of the South African population was infected with HIV (7.97 million) [23]. South Africa has the highest antiretroviral (ARV) "rollout program" in the world with 4.7 million citizens receiving treatment [24]. The world health organization (WHO) has recommended that all infected humans initiate and continue the life-long use of highly active antiretroviral therapy (HAART) as a treatment for HIV [25]. Pregnant and breast-feeding women are also encouraged to continue with HAART treatment as it was shown to markedly reduce mother to child transmission [25]. However, ARVs may be associated with PE predisposition [26••]. Maternal deaths from HIV infection is high (>34%) in SA followed by obstetric haemorrhage and HDP [15]. Several studies have postulated that HIV infection influences the rate of PE development [27–31].

In light of the high maternal mortality emanating from HIV infection and PE, it is of paramount importance that one examines their interaction with the new deadly COVID-19 pandemic. This review will address the missing gaps in literature concerning the effects of microRNAs in HIV-associated PE comorbid with COVID-19; thereby providing a foundation for further research investigating the triad of inflammatory-related conditions.

Severe Acute Respiratory Syndrome Coronavirus 2

Severe acute respiratory syndrome coronavirus 2 belongs to the subfamily of Beta coronaviruses, similar to SARS-CoV-1 and MERS-CoV [32]. SARS-CoV-2 is an enveloped virus with positive-sense single-stranded RNA (+ssRNA). Beta coronavirus have been attributed to be the most fatal subfamilies of coronaviruses [32]. Based on current literature, SARS-CoV-2 is composed of four structural and functional proteins which include the spike, membrane, envelope, and nucleocapsid proteins, together with RNA viral genome [33].

The route of COVID-19 spread is similar to other coronaviruses via human-to-human contact. Humans have a basic biological imperative to connect with other people, making human-to-human contact a very efficient way to amplify viral dissemination. However, it is also spread through the oral-faecal route [34, 35]. SARS-CoV-2 infection occurs in three stages [36]. Stage one includes the incubation period which lasts for approximately 5 days. The virus becomes detectable in stage two and the patient displays mild flu-like symptoms. Stage three presents with severe symptoms which include acute respiratory distress syndrome (ARDS), multi-organ involvement, and subsequent death [36].

Upon entry of the virus into the host, SARS-CoV-2 attaches to angiotensin-converting enzyme 2 (ACE 2) receptors of pneumocytes, thereby infecting host cells [37]. Current literature suggests that the receptor-binding domain of SARS-CoV-2 spike protein is activated via cleavage by transmembrane serine protease 2 (TMPRSS2) [38, 39]. SARS-CoV-2 is then able to follow normal trends in viral infection such as replication, maturation, and release of virions. Since ACE 2 receptors are involved in pregnancy [40], it is plausible that SARS-CoV-2 infection predispose pregnancy complications.

Soluble Angiotensin-Converting Enzyme 2 in SARS-CoV-2 Infection

ACE 2 is a membrane-bound protein (surface protein) that is used by SARS-CoV-2. A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10) and ADAM 17 are ectodomain sheddases that are able to cleave the extracellular

domain of ACE 2 between amino acids 716 and 741; producing the soluble form of ACE 2 (sACE 2) that is released into maternal circulation [41].

Individuals with metabolic conditions have a higher expression of angiotensin II, whereas healthy individuals express angiotensin (1-7) [42]. SARS-CoV-2 has a greater affinity for sACE 2 in comparison to the membrane-bound form, indicative of potential therapeutic properties [43]. Soluble ACE 2 can potentially neutralize SARS-CoV-2, thereby reducing viral pathogenicity [42, 43]. In light of the dire pandemic, it is vital that we investigate the properties of sACE 2 and its potential therapeutic benefits in HIV-positive preeclamptic women comorbid with COVID-19.

The Role of Angiotensin-Converting Enzyme 2 in Pregnancy and Preeclampsia

In a normal physiological environment, the juxtaglomerular cells of the kidney secrete renin, which enzymatically converts angiotensinogen to angiotensin I [44]. Angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II [45]. Angiotensin II functions to increase blood pressure by acting on the kidney, brain, arterioles, and adrenal cortex, via its receptors—angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R), shown in Fig. 1 [46]. Angiotensin-converting enzyme 2 serves as a regulatory mechanism by degrading angiotensin II to angiotensin-(1-7) and angiotensin I to angiotensin-(1-9), which have opposing effects to that of angiotensin II [47]. Thus, ACE 2 maintains a balance in the renin-angiotensin system (RAS).

Pregnancies begin along various psychological, physical, and physiological changes in the body. It is critical that salt-balance and blood pressure (BP) are maintained during pregnancy, which is a principle function of the RAS. From week 6 of gestation, all components of the classical RAS are found in placental tissue, with the potential to regulate villous and extravillous cytotrophoblast (EVT) proliferation, extravillous cytotrophoblast migration, invasion, and placental angiogenesis [48]. Placental RAS is a vital component for the suboptimal regulation of blood flow at the maternal-foetal interface; hence, its dysregulation may predispose HDP such as PE [49, 50]. ACE 2 is expressed in human placenta within syncytiotrophoblasts (ST), cytotrophoblasts (CT), endothelium, and vascular smooth muscle of conducting villi [51]. Interestingly, ACE 2 is also expressed in the invasive interstitial and intravascular trophoblast cell populations, as well as within decidual cells [51]. This highlights the potential for COVID-19 to induce, mimic, or accelerate PE as the SARS-CoV-2 infection exploits ACE 2.

In normal pregnancies, there is a slight increase in the expression of angiotensin II albeit without vasoconstriction or rise in systemic BP because of the development of a refractoriness to the effect of angiotensin II [52, 53]. In contrast, pregnancies complicated by PE are highly sensitized to angiotensin II [54]. This correlates with the clinical findings of PE, which include evidence of elevated BP. Studies by Merrill et al. and Valdés et al. provide evidence of angiotensin 1-7 downregulated in the plasma of PE compared to normotensive

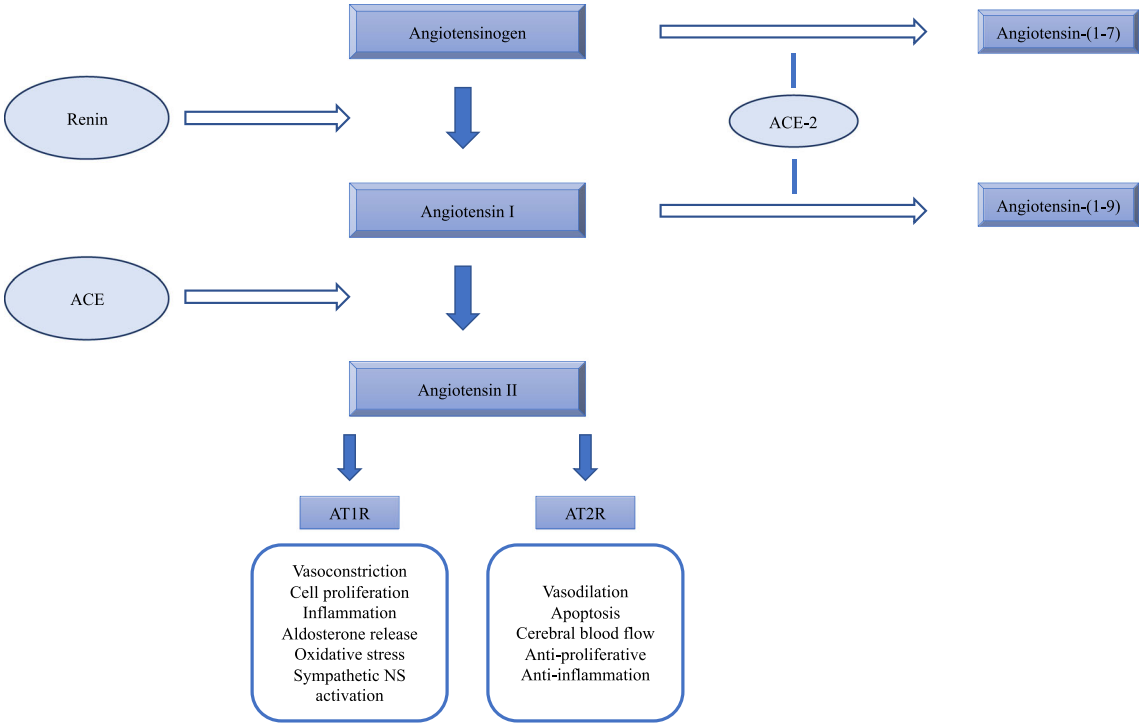


Fig. 1 Schematic representation of the renin-angiotensin system and the physiological role of ACE-2 receptors

healthy pregnancies [55, 56]. These studies confirm potential of ACE 2 suppression in PE.

Pathophysiology of Preeclampsia

The etiology of PE has not been fully elucidated; however, it is believed to occur in two stages [57]. The preclinical stage of PE development involves deficient EVT invasion of the uterine spiral arterioles. In this stage, endovascular trophoblast invasion does not progress beyond the decidual segment of the spiral artery; additionally, there is reduced interstitial myometrial invasion [58]. Defective spiral artery remodeling causes placental hypoxia, leading to a shift in the balance of antiangiogenic and proangiogenic factors [58]. Soluble endoglin (sEng) is an antiangiogenic factor that was found to be overexpressed in the serum of preeclamptic women [59]. Endoglin (Eng), a transmembrane glycoprotein that is highly expressed on vascular endothelium, functions as a co-receptor for transforming growth factor beta (TGF-β) [60]. In contrast, sEng inhibits the normal physiology of TGF-β by binding to circulating TGF-β, which leads to dysregulation of TGF-β signalling in ECs [59]. Transforming growth factor receptor I (TGFR-I), otherwise referred to as activin receptor–like kinase 5 (ALK5), and transforming growth factor receptor II (TGFR-II) function as native receptors of TGF-β [61]. It was reported that sEng can potentially inhibit the downstream signalling of TGF-β, including effects on activation of endothelial nitric oxide synthase (eNOS) and vasodilation [59].

Angiogenic imbalance leads to the clinical stage in which an increase in antiangiogenic factors causes widespread damage to the maternal endothelium [62]. This stage presents the clinical features of PE, including hypertension, proteinuria, and intrauterine growth restriction (IUGR) [63]. Delivery of the placenta usually causes rapid resolution of the clinical signs of the disease, making it the only treatment available, which often includes premature delivery of the fetus [64].

The Expression of microRNAs in Pregnancy

Pregnancy is a time of significant changes in the body in order to prepare for and accommodate the developing fetus. MicroRNAs are able to regulate many of these changes through its control over the expression of mRNA. MicroRNAs have been implicated in the earliest stages of pregnancy, including embryo implantation [65]. After implantation, the trophoblast cell lineage is the first to begin differentiating [66]. Cuman et al. noted miR-661 and miR-372 upregulation in blastocysts that failed to implant [67]; the expression of miR-372 was supported by Rosenbluth et al. as they found a similar expression [68]. In contrast, miR-142-3p is highly expressed in blastocysts successfully implanted according to a pilot study conducted by Borges et al. [69]. This suggests an involvement of miRNA in ectopic pregnancies and miscarriages. Although differential expression profiling of miRNAs is achievable, the results are not easily reproducible, as evident in significant variations between similar investigations. The difficulty in reproducing results may be explained due to differences in laboratory conduct of the study, methodological differences, and differences in miRNA array panels, as well as the use of either stored or fresh samples [65]. MicroRNA expression is a very dynamic process and varies greatly with the requirements needed at different times [65].

The endometrium is essential for successful embryo implantation. Kresowik et al. identified miR-31 to be overexpressed in endometrium in the mid-secretory phase [70]. MicroRNA-31 is a potent miRNA that inversely regulates forkhead box P3 (FOXP3), a transcription factor for T regulatory cells, and CXCL12, a homeostatic chemokine. CXCL12 is a chemoattractant for uterine natural killer (NK) cells, with the potential to be involved in providing a suitable environment that is immune-tolerant in the secretory phase [65]. Tochigi et al. and Estella et al. investigated the miRNA expression profiles between decidualized human endometrial stem cells (hESC) and control hESC; only miR-155 was commonly expressed in both studies [71, 72].

The attachment of the blastocyst to the uterine endothelial wall occurs 4–6 days post-conception; following this, the placenta begins to develop [73]. MicroRNAs are highly expressed in the human placenta which undergoes physiological changes throughout pregnancy [74, 75]. The precise role of miRNAs in the placenta is yet to be identified. However, the placenta releases placental miRNAs into the maternal circulation, hence is found in maternal serum and plasma and placental tissue. The expression of placental miRNAs is associated with HDPs, such as PE [76]. Previous studies have highlighted the presence of hypoxic conditions in PE compared to healthy controls [58, 77, 78]. MicroRNA-210 is upregulated in trophoblast cells cultured in hypoxic environments, and importantly, in PE [79]. Additionally, miRNAs that are involved in angiogenesis and immune cell development are dysregulated in trophoblastic cells cultured in hypoxic conditions [80–83]. Thus, there exists a possible influence of miRNAs in the progression of normal pregnancies, and in pathological pregnancies.

MicroRNAs in Pregnancies Complicated by Preeclampsia

There are significant gaps in the investigation of miRNAs in pregnancy-related complications and there is a paucity of data on the miRNA regulation of sEng. Importantly, the miRNA

regulation of sFlt-1 is yet to be elucidated as no miRNA has been directly correlated with the regulation of sFlt-1 [84]. Nevertheless, KG Shyu (2017) reported that miR-208a is responsible for the activation of Eng and collagen I in the stimulation of myocardial fibrosis [85]. This was supported by similar studies [86, 87]. Furthermore, several miRNAs have been suggested to play a role in trophoblast proliferation and invasion, including direct effect on TGF-β signalling. An investigation analyzing the HTR-8/SVneoplacental cell line concluded that miR-376c inhibits ALK5 [88]. Also, miR-29b directly binds to the 3′-UTRs of myeloid cell leukaemia sequence 1, matrix metalloproteinase 2, VEGF-A, and integrin-β1 [89]. When miR-29b is upregulated in the placenta, it causes trophoblastic apoptosis and inhibition of trophoblast invasion and angiogenesis [89]. MicroRNA-193b is increased in preeclamptic patients [90]. Zhou et al. showed that miR-193b-3p decreases migration and invasion of HTR-8/SVneoplacental cells [90]. Interestingly, inhibition of miR-126 in mouse embryos led to abnormal vasculogenesis, haemorrhage, and loss of vascular integrity [91]. This indicated that miR-126 is necessary for proper vessel formation.

Placental Hypoxia

Abnormal trophoblast invasion of the placenta in PE leads to hypoperfusion of the placenta and ultimately accelerates the placenta into a hypoxic state. The hypoxic state that is associated with PE correlates with the decrease of eNOS and nitric oxide (NO) in preeclamptic patients. MicroRNA-222 was reported to induce the production of eNOS [92] yet was found to be downregulated in the placenta of PE patients [93]. Furthermore, miR-155 was identified to negatively regulate the expression of eNOS in trophoblastic cells [94]. It was also found to be increased in PE placenta, suggesting a negative regulatory role of miR-155 in the migratory behaviour of trophoblasts through the regulation of eNOS [94]. Sun et al. showed that miR-155 exerts its inhibitory effects on eNOS by binding to the 3′-UTR of eNOS mRNA and suggested that silencing of this miRNA can lead to improvement of endothelial dysfunction [95]. Dai et al. reported that miR-155 may inhibit trophoblast invasion and proliferation by downregulating cyclin D1; furthermore, another investigative group reported that miR-155 can inhibit trophoblast invasion by decreasing eNOS expression [96]. This can lead to an exaggerated hypoxic state of the placenta in PE.

Many studies have highlighted the overexpression of miR-210 in preeclamptic placentae and plasma [97•]. MicroRNA-210, believed to be a miRNA that is induced by hypoxia, is one of the most studied miRNAs [98]. The hypoxic state of the placenta in PE causes oxidative stress which leads to the upregulation of hypoxia inducing factor 1-α (HIF-1-α) in placental tissue [98]. Research has revealed that miR-210 is regulated by HIF-1-α, thereby creating a positive feedback loop inducing hypoxia.

Angiogenesis

There is evidence of abnormal angiogenesis in PE. Vascular endothelial growth factor (VEGF) is a potent proangiogenic factor that plays a pivotal role in angiogenesis, particularly in endothelial cell proliferation, invasion, and migration [99]. It promotes the production of NO and prostacyclin in the maternal vascular system [100]. Phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) and sprout-related drosophilia enabled/vasodilator-stimulated phosphoprotein homology 1 (EVH1) domain are a part of the VEGF signalling pathway and are targets of miR-126 [91, 101]. It was reported that miR-126 was downregulated in PE patients and the expression of miR-126 is directly proportional to the expression of VEGF mRNA [101]. VEGF is also targeted by miR-29b, miR-16, and miR-155 as they inhibit the expression of VEGF-A [89, 102, 103]. They also inhibit trophoblast cell invasion and tube formation apart from suppressing VEGF-A; thus, they are involved in placental angiogenesis. Ephrin-B2 (EFNB2) has been identified to influence angiogenesis. MicroRNA-126, miR-20a, miR-17, and miR-20b have been identified as miRNA regulators of EFNB2 and interestingly, they were shown to be differentially expressed in PE [101, 104]. These miRNAs can indirectly regulate the expression of VEGF through the inactivation of EFNB2. The microRNAs greatly involved in PE are summarized in Table 1.

Placental miRNAs are also released into the maternal circulation, contributing the maternal stage of PE; interestingly, miRNAs have also been found to be contained within exosomes, nanoparticle carrier proteins, in the maternal circulation [105]. The release of antiangiogenic factors and other inflammatory mediators into the maternal circulation leads to the systemic endothelial cell inflammation and endothelial cell dysfunction that are characteristic of PE.

The Role of MicroRNAs in Modulating HIV-1 Infection

HIV infection is one of the more prevalent viral infections in SA [23]. Currently, HIV-infected individuals are treated with HAART [25]. Although HAART is the most effective treatment at present, it is associated with various side-effects. All pregnant women who are HIV-positive are required to adopt the HAART treatment in SA, as it reduces mother-to-child transmission [25]. However, studies have shown that HAART could exhibit a negative influence during pregnancy. Furthermore, there is evidence that the administration of HAART to pregnant HIV-positive patients predisposes the development of PE [106, 107]. In an ideal situation, PE

Table 1 A summary of microRNAs and their roles in preeclampsia

MicroRNA	Expression	Target gene	Reference
miR-29b	Upregulated	MCL1, MMP2, VEGF-A, ITGB1	[89]
miR-126	Downregulated	VEGFA, EFNB2, CRK	[101]
miR-222	Downregulated	eNOS, PTEN, BCL2L11,	[93]
miR-155	Upregulated	eNOS, Cyclin D1	[94]
miR-210	Upregulated	HIF1-alpha, NF-kBp50	[97•, 79]

patients comorbid with HIV infection would have a neutralization of immune response [31, 108]. However, HAART in pregnancy reconstitutes immune response thereby influencing PE development [107, 109, 110]. In light of this, it is essential to thoroughly investigate key regulators in HIV-1 infection in order to identify alternative avenues in the fight against HIV infection globally. Epigenetic regulatory mechanisms, specifically miRNAs, have been shown to play a significant role in HIV infection, as well as other RNA and DNA viral infections [111].

Moreover, miRNAs may be partially responsible for the latency period of the HIV [112]. Huang et al. reported that several miRNAs were differentially expressed in resting $CD4^+$ T cells and activated $CD4^+$ T cells, including miR-28, miR-125b, miR-150, miR-223, and miR-382. These miRNAs have also been shown to target the 3′ ends of HIV-1 mRNAs. Additionally, the group showed that inhibition of these miRNAs can stimulate virus production in resting $CD4^+$ T cells isolated from HIV-positive individuals receiving HAART [10]. It is therefore plausible that these differentially expressed miRNAs can inhibit HIV-1 expression in resting $CD4^+$ T cells, thereby contributing to the viral latency observed in HIV infection.

Apart from direct targeting of the HIV-1 mRNAs by miRNAs, cellular miRNAs can indirectly affect HIV infection through modulating factors that are essential for HIV-1 expression. Cyclin T1 protein is responsible for efficient transcription of the viral genome [113]. A study in 2012 reported that the expression of cyclin T1 is reduced in resting $CD4^+$ T cells; however, it is induced upon activation of $CD4^+$ T cells [114]. A similar investigation identified miR-198 to be downregulated during monocyte to macrophage differentiation and reported that miR-198 is able to suppress HIV-1 replication by downregulating cyclin T1 [115].

Houzet et al. reported that miR-29a and miR-29b are downregulated in HIV-1-infected patients and infected peripheral blood mononuclear cells (PBMCs) [116]. It was reported that the host miRNA, miR-29a targets the *nef* gene of HIV-1. The *nef* protein serves as an accessory protein of HIV and influences viral pathogenesis [117]. The group suggested that expression of miR-29a leads to a reduction of *nef* mRNA and a decrease in viral levels was observed [118]. A study conducted by Nathans et al. observed miR-29a to suppress infectivity of HIV through direct targeting of HIV-1 transcripts to processing bodies (P bodies) [119]. Chable-Bessia et al. demonstrated that major components of P bodies are able to negatively regulate HIV-1 gene expression via blocking of viral mRNA association with polysomes. They also showed that deletion of these components reactivates the virus in PBMCs isolated from HIV-1 patients receiving HAART [120]. Thus, the downregulation of miR-29a in HIV-infected humans could serve as a mechanism for the maintenance of a latent state of infection. The miR-29 family is composed of miR-29a, miR-29b, and miR-29c. It is important to underline that miR-29a and miR-29b share highly similar sequences [118]. Above and beyond the negative regulation of *nef* expression by miR-29a, Ahluwalia et al. suggested that miR-29a and miR-29b are able to suppress virus replication in HEK293T cells and Jurkat T cells [118]. An in vivo study revealed that a cytokine-microRNA pathway could potentially impact HIV-1 replication. Specifically, the group identified the IL-21/miR-29a pathway to be associated with HIV-1 replication and infectivity [121]. Adoro et al. reported that the IL-21/miR-29a pathway suppresses viral replication since IL-21-stimulated $CD4^+$ T cells upregulate the expression of miR-29a, and IL-21 reverses the downregulation of miR-29a induced by HIV-1 infection [122]. This reiterates the plausibility of the IL-21/miR-29a axis influencing HIV-1 replication and infectivity.

As important as host miRNAs are, viruses bring along with it a set of its own miRNAs, referred to as viral miRNAs (v-miRNAs). The existence of v-miRNAs has been controversial to a degree due to the failure of reproducing findings [123]. The first v-miRNA that was isolated from HIV-1 was discovered in 2004 and was termed miR-N367 [124]. However, subsequent studies that attempted to reproduce the discovery were unsuccessful in their attempts [125–127].

The transactivation-responsive (TAR) element of HIV-1 is an RNA hairpin structure found at the 5′ end of all HIV-1 transcripts [128]. Dominique L Ouellet at al. reported that TAR is a source of miRNAs in cultured HIV-1-infected cell lines and in HIV-1-infected human $CD4^+$ T lymphocytes [128]. TAR has been shown to be involved in cell survival and displays anti-apoptotic properties [129]. HIV-1 TAR miRNAs have been identified to downregulate ERCC1 (excision repair cross complementation group 1) and IER3 (intermediate early response gene 3) which are components

involved in apoptosis and cell survival [130]. Therefore, HIV-1-infected cells may be able to evade death and maintain the virulence of HIV-1.

The novel microRNAs have proven to have highly intricate regulatory roles in the human genomes. However, evidence also supports their existence in both RNA and DNA viruses which can potentially be involved in epigenetic regulation, by both direct and indirect mechanisms. It is thus of paramount importance that miRNAs and v-miRNAs are investigated more thoroughly utilizing newer sequencing technology. The significant impact of miRNAs in viruses and hosts highlights the possibility of their role in other viral infections threatening mankind.

MicroRNAs in HIV-Associated Preeclampsia and COVID-19

There are numerous reports suggesting an interaction of miRNAs in viral infections. A study investigating the expression of miRNAs in HIV infection found differentially expressed miRNAs between resting $CD4^+$ T cells and activated $CD4^+$ T cells. Specifically, they found miR-28, miR-125b, miR-150, miR-223, and miR-382 to be differentially expressed [10]. Nersisyan et al. [131] conducted an in silico analysis of potential host miRNAs that can bind coronavirus and identified miR-21, miR-195-5p, miR-16-5p, miR-3065-5p, miR-424-5p, and miR-421 to exhibit this potential.

MicroRNAs in Angiotensin-Converting Enzyme 2 Receptors

ACE 2 receptors are predominantly found on the endothelial cells [132], heart, blood vessels, and the kidneys [133]. According to several studies, miRNAs are indeed regulators of ACE 2 [134, 135]. ACE 2 abnormalities have been implicated in disorders such as hypertension [136], cardiovascular disease [13], diabetes [11], and old age [13]. MicroRNA-125b is reported to directly target the mRNA of ACE 2 [137]. The same miRNA is found to be downregulated in HIV-infected $CD4^+$ T cells and exhibits anti-viral properties [10]. Thus, it is plausible to hypothesize that HIV-positive individuals could be at an increased risk of being infected with SARS-CoV-2 because the host will be experiencing a decline in the expression of miR-125b due to HIV infection. Since miR-125b is a negative regulator of ACE 2 [10], under HIV-positive conditions, the patients will have an increase in the expression of ACE 2, potentially leading to greater viral entry. Supporting this is the work of Batlle et al. who highlighted the fact that healthier people are at a lower risk of developing severe COVID-19 due to lower membrane-bound ACE 2 expression [42]. MicroRNA-125 is also associated with blocking of apoptosis when downregulated [138]. This possibly allows for the virus to replicate without interruption.

Recently, miR-155 was reported to be associated with ACE 2 modulation by regulating the expression of AT1R by silencing AT1R mRNA [139]. This receptor is involved in cardiovascular homeostasis mechanisms including vasoconstriction, release of catecholamines, and blood pressure evaluation [140]. Vasoconstriction and elevated blood pressure are characteristics that are evident in PE. MicroRNA-155 was observed to be upregulated in the placenta of PE [94] where it negatively regulates the expression of eNOS in trophoblasts. There is a lack of research investigating miR-155 expression in COVID-19. Nevertheless, miR-155 has been described to exhibit anti-viral properties. Silencing of miR-155 led to an approximate 50% increase in the replication of rhinovirus [141]. In a case-control study, miR-155 was found to be upregulated in patients infected with respiratory syncytial virus (RSV), a condition associated with bronchial inflammation [142]. The overexpression of miR-155 shows a correlation with acute inflammatory responses [142]. Theoretically, a pre-eclamptic patient would be at a greater risk of experiencing severe symptoms of COVID-19, due to the effect of miR-155. Although the miRNA is unlikely to cause a pregnant woman to be at risk of being infected, the endothelial dysfunction seen in PE will be compounded by the dysregulation effects of miR-155 following SARS-CoV-2 infection. Although there is a paucity of data regarding the expression of miR-155 in COVID-19, it is possible to assume an initial downregulation in order to evade immune detection, followed by overexpression when the host develops an inflammatory response to the infection. Research investigating PE patients with SARS-CoV-2 infection will greatly aid in illuminating the effects of miR-155 both in COVID-19 and PE, which can lead to possible therapeutic actions from antagomirs (antagonistic microRNAs).

A geographical study including the USA, Wuhan, Italy, India, and Nepal found several anti-viral host miRNAs that were specific to SARS-CoV-2, one of which was miR-126 [143]. MicroRNA-126 has been identified to target the nucleocapsid of the SARS-CoV-2 [143]. Interestingly, miR-126 is downregulated in PE [101]. The inhibition of miR-126 in mouse embryos was assessed and it was found that it led to abnormal vessel formation and loss of vascular integrity [91]. Since miR-126 is decreased in PE, pregnant women with PE could be at risk of infection due to the loss of an anti-viral miRNA that targets SARS-CoV-2. Furthermore, it is plausible to expect the further downregulation of miR-126 following infection; this can lead to further endothelial cell damage in pregnant women and hence contribute to worsening the effects of PE, possibly inducing death. Additionally, miR-126-3p was found to be downregulated in HIV-1-positive patients receiving HAART. Interestingly, miR-126-3p was upregulated in patients with HAART resistance in comparison to

patients without resistance [144••]. It was indicated that this is suggestive of miR-126 being linked with HIV treatment failure [144••]. This evidence has possible detrimental results for HIV-associated PE women as both conditions exhibit a decrease in miR-126. Hence, patients with HIV-associated PE could be at a greater risk of both contraction of SARS-CoV-2 infection and the experiencing of severe COVID-19. Furthermore, Li et al. found several miRNAs to be differentially expressed in the peripheral blood of patients with COVID-19 [145••]. There is a great need to investigate the expression of miRNAs in COVID-19, which is yet to be achieved.

Conclusion

Currently, there exists a wide gap in literature interrelating miRNAs and SARS-CoV-2 infection. Analysis of the differential expression of miRNAs in COVID-19 can help identify those at risk as well as aid in the development of therapeutic approaches. An inflammatory response is a common characteristic shared between SARS-CoV-2 infection, pregnancy, PE, and HIV infection. Maternal health should be of utmost importance when SARS-CoV-2 infection arises in HIV-positive preeclamptic women. Thus, further research investigating the functionality of microRNAs on the synergy of SARS-CoV-2 infection, PE, and HIV infection could provide significant breakthroughs that will enhance the treatment in pregnant women. Understanding how miRNAs are affected and identifying which miRNAs are aberrantly expressed will accelerate the development of a vaccine that will also be safe for pregnant women diagnosed with HIV-associated PE.

Author Contribution Not applicable

Funding The authors appreciate the funding provided by the College of Health Sciences, UKZN.

Availability of Data and Material All articles reviewed in this review paper are available online.

Declarations

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.

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• Wang H, Li X, Li T, Zhang S, Wang L, Wu X, et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis. 2020;39(9):1629–35. https://doi.org/10.1007/s10096-020-03899-4 **Wang et al. published the genetic sequence of the novel SARS-CoV-2. Furthermore, they highlighted the origin of the virus.**
2. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020;26(4):450–2. https://doi.org/10.1038/s41591-020-0820-9.
3. Zhu F, Cao Y, Xu S, Zhou M. Co-infection of SARS-CoV-2 and HIV in a patient in Wuhan city, China. J Med Virol. 2020;92(6):529–30. https://doi.org/10.1002/jmv.25732.
4. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. https://covid19.who.int. Accessed 02 November 2020 2020.
5. Sayed AS, Xia K, Salma U, Yang T, Peng J. Diagnosis, prognosis and therapeutic role of circulating miRNAs in cardiovascular diseases. Heart Lung Circ. 2014;23(6):503–10. https://doi.org/10.1016/j.hlc.2014.01.001.
6. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet. 2008;9(2):102–14. https://doi.org/10.1038/nrg2290.
7. Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell. 2008;132(1):9–14. https://doi.org/10.1016/j.cell.2007.12.024.
8. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev. 2016;96(4):1297–325. https://doi.org/10.1152/physrev.00041.2015.
9. Bhaskaran M, Mohan M. MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. Vet Pathol. 2014;51(4):759–74. https://doi.org/10.1177/0300985813502820.
10. Huang J, Wang F, Argyris E, Chen K, Liang Z, Tian H, et al. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+ T lymphocytes. Nat Med. 2007;13(10):1241–7. https://doi.org/10.1038/nm1639.
11. Tikellis C, Johnston CI, Forbes JM, Burns WC, Burrell LM, Risvanis J, et al. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. Hypertension (Dallas, Tex : 1979). 2003;41(3):392–7. https://doi.org/10.1161/01.Hyp.0000060689.38912.Cb.
12. Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, et al. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol. 2007;8(2):R27. https://doi.org/10.1186/gb-2007-8-2-r27.
13. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, et al. The emerging role of ACE2 in physiology and disease. J Pathol. 2007;212(1):1–11. https://doi.org/10.1002/path.2162.
14. National Committee for Confidential Enquiry into Maternal Deaths. Saving Mothers Report 2017. Pretoria: South African Department of Health 2018.
15. World Health O. Trends in maternal mortality 2000 to 2017: estimates by WHO, UNICEF, UNFPA, World Bank Group and the

United Nations Population Division. Geneva: World Health Organization; 2019.
16. Hutcheon JA, Lisonkova S, Joseph K. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best Pract Res Clin Obs Gynaecol. 2011;25(4):391–403.
17. Nathan HL, Seed PT, Hezelgrave NL, De Greeff A, Lawley E, Conti-Ramsden F, et al. Maternal and perinatal adverse outcomes in women with pre-eclampsia cared for at facility-level in South Africa: a prospective cohort study. J Glob Health. 2018;8(2): 020401.
18. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension (Dallas, Tex : 1979). 2018;72(1):24–43. https://doi.org/10.1161/hypertensionaha.117.10803.
19. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005;365(9461):785–99.
20. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of pre-eclampsia. Ann Rev Pathol: Mechanisms of Disease. 2010;5: 173–92.
21. Awi NJ, Teow SY. Antibody-mediated therapy against HIV/AIDS: where are we standing now? J Pathogens. 2018;2018: 8724549. https://doi.org/10.1155/2018/8724549.
22. World Health Organization. Number of people (all ages) living with HIV: estimates by WHO region. 2019. http://apps.who.int/gho/data/view.main.22100WHO?lang=en. Accessed 30 October 2020 2020.
23. Stats SA. Mid-year Population Estimates 2019. Pretoria, South Africa2019.
24. Joint United Nations Programme on HIV/AIDS. Global HIV & AIDS statistics — 2019 fact sheet. 2019. https://www.unaids.org/en/resources/fact-sheet. Accessed 30 October 2020 2020.
25. World Health Organization. Maternal Health. 2015. https://www.afro.who.int/health-topics/maternal-health. Accessed 30 October 2020 2020.
26.•• Sebitloane HM, Moodley J, Sartorius B. Associations between HIV, highly active anti-retroviral therapy, and hypertensive disorders of pregnancy among maternal deaths in South Africa 2011–2013. Int J Gynecol Obstet. 2017;136(2):195–9 **This paper discusses the association between HIV, HIV treatment and hypertensive disorders of pregnancy. The authors indicate that antiretroviral therapy may be associated with preeclampsia predisposition.**
27. Mattar R, Amed AM, Lindsey PC, Sass N, Daher S. Preeclampsia and HIV infection. Eur J Obstet Gynecol Reprod Biol. 2004;117(2):240–1. https://doi.org/10.1016/j.ejogrb.2004.04.014.
28. Hall DR. Is pre-eclampsia less common in patients with HIV/AIDS? J Reprod Immunol. 2007;76(1-2):75–7. https://doi.org/10.1016/j.jri.2007.04.005.
29. Landi B, Bezzeccheri V, Guerra B, Piemontese M, Cervi F, Cecchi L, et al. HIV infection in pregnancy and the risk of gestational hypertension and preeclampsia. World J Cardiovasc Dis. 2014;2014.
30. Kalumba VM, Moodley J, Naidoo TD. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. Cardiovasc J Afr. 2013;24(2):24–7. https://doi.org/10.5830/cvja-2012-078.
31. Moodley J. Impact of HIV on the incidence of pre-eclampsia. Cardiovasc J Afr. 2013;24(2):5.
32. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020;5(4):562–9. https://doi.org/10.1038/s41564-020-0688-y.
33. Morse JS, Lalonde T, Xu S, Liu WR. Learning from the past: possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. Chembiochem. 2020;21(5):730–8. https://doi.org/10.1002/cbic.202000047.
34. Hu Y, Shen L, Yao Y, Xu Z, Zhou J, Zhou H. A report of three COVID-19 cases with prolonged viral RNA detection in anal swabs. Clin Microbiol Infect. 2020;26(6):786–7. https://doi.org/10.1016/j.cmi.2020.04.010.
35. Jones DL, Baluja MQ, Graham DW, Corbishley A, McDonald JE, Malham SK, et al. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. Sci Total Environ. 2020;749:141364. https://doi.org/10.1016/j.scitotenv.2020.141364.
36. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. Jama. 2020;323(11):1061–9. https://doi.org/10.1001/jama.2020.1585.
37. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020;94(7):e00127–0. https://doi.org/10.1128/jvi.00127-20.
38. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Mol Cell. 2020;78(4):779–84.e5. https://doi.org/10.1016/j.molcel.2020.04.022.
39. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11(1): 1620. https://doi.org/10.1038/s41467-020-15562-9.
40. Levy A, Yagil Y, Bursztyn M, Barkalifa R, Scharf S, Yagil C. ACE2 expression and activity are enhanced during pregnancy. Am J Phys Regul Integr Comp Phys. 2008;295(6):R1953–R61.
41. Mulangu S, Dodd LE, Davey RT Jr, Tshiani Mbaya O, Proschan M, Mukadi D, et al. A randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med. 2019;381(24):2293–303. https://doi.org/10.1056/NEJMoa1910993.
42. Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? Clin Sci (Lond). 2020;134(5):543–5. https://doi.org/10.1042/cs20200163.
43. Mostafa-Hedeab G. ACE2 as drug target of COVID-19 virus treatment, simplified updated review. Rep Biochem Mol Biol. 2020;9(1):97–105. https://doi.org/10.29252/rbmb.9.1.97.
44. Zhuo JL, Ferrao FM, Zheng Y, Li XC. New frontiers in the intrarenal renin-angiotensin system: a critical review of classical and new paradigms. Front Endocrinol (Lausanne). 2013;4:166. https://doi.org/10.3389/fendo.2013.00166.
45. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical renin-angiotensin system in kidney physiology. Compr Physiol. 2014;4(3):1201–28. https://doi.org/10.1002/cphy.c130040.
46. Alifano M, Alifano P, Forgez P, Iannelli A. Renin-angiotensin system at the heart of COVID-19 pandemic. Biochimie. 2020;174:30–3. https://doi.org/10.1016/j.biochi.2020.04.008.
47. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. Circ Res. 2016;118(8):1313–26. https://doi.org/10.1161/circresaha.116.307708.
48. Pringle KG, Tadros MA, Callister RJ, Lumbers ER. The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis? Placenta. 2011;32(12):956–62. https://doi.org/10.1016/j.placenta.2011.09.020.
49. Moritz KM, Cuffe JS, Wilson LB, Dickinson H, Wlodek ME, Simmons DG, et al. Review: Sex specific programming: a critical role for the renal renin-angiotensin system. Placenta.

2010;31(Suppl):S40–6. https://doi.org/10.1016/j.placenta.2010.01.006.
50. Ito M, Itakura A, Ohno Y, Nomura M, Senga T, Nagasaka T, et al. Possible activation of the renin-angiotensin system in the feto-placental unit in preeclampsia. J Clin Endocrinol Metab. 2002;87(4):1871–8. https://doi.org/10.1210/jcem.87.4.8422.
51. Valdés G, Neves LA, Anton L, Corthorn J, Chacón C, Germain AM, et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta. 2006;27(2-3):200–7. https://doi.org/10.1016/j.placenta.2005.02.015.
52. Shah DM. Role of the renin-angiotensin system in the pathogenesis of preeclampsia. Am J Physiol Ren Physiol. 2005;288(4):F614–25. https://doi.org/10.1152/ajprenal.00410.2003.
53. Massicotte G, St-Louis J, Parent A, Schiffrin E. Decreased in vitro responses to vasoconstrictors during gestation in normotensive and spontaneously hypertensive rats. Can J Physiol Pharmacol. 1987;65(12):2466–71.
54. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest. 1973;52(11):2682–9. https://doi.org/10.1172/jci107462.
55. Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. Endocrine. 2002;18(3):239–45. https://doi.org/10.1385/endo:18:3:239.
56. Valdés G, Germain AM, Corthorn J, Berrios C, Foradori AC, Ferrario CM, et al. Urinary vasodilator and vasoconstrictor angiotensins during menstrual cycle, pregnancy, and lactation. Endocrine. 2001;16(2):117–22. https://doi.org/10.1385/endo:16:2:117.
57. Shanmugalingam R, Hennessy A, Makris A. Aspirin in the prevention of preeclampsia: the conundrum of how, who and when. J Hum Hypertens. 2019;33(1):1–9.
58. Rana S, Lemoine E, Granger J, Karumanchi SA. Preeclampsia. Circ Res. 2019;124(7):1094–112. https://doi.org/10.1161/circresaha.118.313276.
59. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006;355(10):992–1005.
60. Rossi E, Bernabeu C, Smadja DM. Endoglin as an adhesion molecule in mature and progenitor endothelial cells: a function beyond TGF-β. Front Med (Lausanne). 2019;6:10. https://doi.org/10.3389/fmed.2019.00010.
61. Cheifetz S, Bellón T, Calés C, Vera S, Bernabeu C, Massagué J, et al. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. J Biol Chem. 1992;267(27):19027–30.
62. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. Am J Obstet Gynecol. 2015;213(4 Suppl):S9.e1–11. https://doi.org/10.1016/j.ajog.2015.08.003.
63. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. Prenat Diagn. 2011;31(1):66–74. https://doi.org/10.1002/pd.2660.
64. Valero L, Alhareth K, Gil S, Lecarpentier E, Tsatsaris V, Mignet N, et al. Nanomedicine as a potential approach to empower the new strategies for the treatment of preeclampsia. Drug Discov Today. 2018;23(5):1099–107.
65. Paul ABM, Sadek ST, Mahesan AM. The role of microRNAs in human embryo implantation: a review. J Assist Reprod Genet. 2019;36(2):179–87. https://doi.org/10.1007/s10815-018-1326-y.
66. Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, et al. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. J Clin Invest. 2004;114(6):744–54. https://doi.org/10.1172/jci22991.
67. Cuman C, Van Sinderen M, Gantier MP, Rainczuk K, Sorby K, Rombauts L, et al. Human blastocyst secreted microRNA regulate endometrial epithelial cell adhesion. EBioMedicine. 2015;2(10):1528–35. https://doi.org/10.1016/j.ebiom.2015.09.003.
68. Rosenbluth EM, Shelton DN, Wells LM, Sparks AE, Van Voorhis BJ. Human embryos secrete microRNAs into culture media–a potential biomarker for implantation. Fertil Steril. 2014;101(5):1493–500. https://doi.org/10.1016/j.fertnstert.2014.01.058.
69. Borges E Jr, Setti AS, Braga DP, Geraldo MV, Figueira RC, Iaconelli A Jr. miR-142-3p as a biomarker of blastocyst implantation failure - a pilot study. JBRA Assist Reprod. 2016;20(4):200–5. https://doi.org/10.5935/1518-0557.20160039.
70. Kresowik JD, Devor EJ, Van Voorhis BJ, Leslie KK. MicroRNA-31 is significantly elevated in both human endometrium and serum during the window of implantation: a potential biomarker for optimum receptivity. Biol Reprod. 2014;91(1):17. https://doi.org/10.1095/biolreprod.113.116590.
71. Tochigi H, Kajihara T, Mizuno Y, Mizuno Y, Tamaru S, Kamei Y, et al. Loss of miR-542-3p enhances IGFBP-1 expression in decidualizing human endometrial stromal cells. Sci Rep. 2017;7:40001. https://doi.org/10.1038/srep40001.
72. Estella C, Herrer I, Moreno-Moya JM, Quiñonero A, Martínez S, Pellicer A, et al. miRNA signature and Dicer requirement during human endometrial stromal decidualization in vitro. PLoS One. 2012;7(7):e41080. https://doi.org/10.1371/journal.pone.0041080.
73. Rana S, Lemoine E, Granger J, Karumanchi SA. Preeclampsia: pathophysiology, challeneges, and perspectives. Circ Res. 2019;124(7):1094–112. https://doi.org/10.1161/circresaha.118.313276.
74. Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics. 2007;8:166. https://doi.org/10.1186/1471-2164-8-166.
75. Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K, et al. Identification of pregnancy-associated microRNAs in maternal plasma. Clin Chem. 2010;56(11):1767–71. https://doi.org/10.1373/clinchem.2010.147660.
76. Arthurs AL, Lumbers ER, Pringle KG. MicroRNA mimics that target the placental renin-angiotensin system inhibit trophoblast proliferation. Mol Hum Reprod. 2019;25(4):218–27. https://doi.org/10.1093/molehr/gaz010.
77. Tong W, Giussani DA. Preeclampsia link to gestational hypoxia. J Dev Orig Health Dis. 2019;10(3):322–33. https://doi.org/10.1017/s204017441900014x.
78. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125(7):911–9.
79. Lee DC, Romero R, Kim JS, Tarca AL, Montenegro D, Pineles BL, et al. miR-210 targets iron-sulfur cluster scaffold homologue in human trophoblast cell lines: siderosis of interstitial trophoblasts as a novel pathology of preterm preeclampsia and small-for-gestational-age pregnancies. Am J Pathol. 2011;179(2):590–602. https://doi.org/10.1016/j.ajpath.2011.04.035.
80. Bidarimath M, Khalaj K, Wessels JM, Tayade C. MicroRNAs, immune cells and pregnancy. Cell Mol Immunol. 2014;11(6):538–47. https://doi.org/10.1038/cmi.2014.45.
81. Heusschen R, van Gink M, Griffioen AW, Thijssen VL. MicroRNAs in the tumor endothelium: novel controls on the angioregulatory switchboard. Biochim Biophys Acta. 2010;1805(1):87–96. https://doi.org/10.1016/j.bbcan.2009.09.005.

82. Suárez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. Circ Res. 2009;104(4):442–54. https://doi.org/10.1161/circresaha.108.191270.
83. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J. 2010;24(6):2030–9. https://doi.org/10.1096/fj.09-149724.
84. Harapan H, Yeni CM. The role of microRNAs on angiogenesis and vascular pressure in preeclampsia: the evidence from systematic review. Egypt J Med Human Gen. 2015;16(4):313–25.
85. Shyu KG. The Role of Endoglin in Myocardial Fibrosis. Acta Cardiol Sin. 2017;33(5):461–7. https://doi.org/10.6515/acs20170221b.
86. Wang BW, Wu GJ, Cheng WP, Shyu KG. MicroRNA-208a increases myocardial fibrosis via endoglin in volume overloading heart. PLoS One. 2014;9(1):e84188. https://doi.org/10.1371/journal.pone.0084188.
87. Shyu KG, Wang BW, Cheng WP, Lo HM. MicroRNA-208a increases myocardial endoglin expression and myocardial fibrosis in acute myocardial infarction. Can J Cardiol. 2015;31(5):679–90. https://doi.org/10.1016/j.cjca.2014.12.026.
88. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, et al. MicroRNA-376c impairs transforming growth factor-β and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension (Dallas, Tex : 1979). 2013;61(4):864–72. https://doi.org/10.1161/hypertensionaha.111.203489.
89. Li P, Guo W, Du L, Zhao J, Wang Y, Liu L, et al. microRNA-29b contributes to pre-eclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. Clin Sci (Lond). 2013;124(1):27–40. https://doi.org/10.1042/cs20120121.
90. Zhou X, Li Q, Xu J, Zhang X, Zhang H, Xiang Y, et al. The aberrantly expressed miR-193b-3p contributes to preeclampsia through regulating transforming growth factor-β signaling. Sci Rep. 2016;6:19910. https://doi.org/10.1038/srep19910.
91. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell. 2008;15(2):272–84. https://doi.org/10.1016/j.devcel.2008.07.008.
92. Suárez Y, Fernández-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res. 2007;100(8):1164–73. https://doi.org/10.1161/01.Res.0000265065.26744.17.
93. Cronqvist T, Saljé K, Familari M, Guller S, Schneider H, Gardiner C, et al. Syncytiotrophoblast vesicles show altered micro-RNA and haemoglobin content after ex-vivo perfusion of placentas with haemoglobin to mimic preeclampsia. PLoS One. 2014;9(2):e90020. https://doi.org/10.1371/journal.pone.0090020.
94. Li X, Li C, Dong X, Gou W. MicroRNA-155 inhibits migration of trophoblast cells and contributes to the pathogenesis of severe preeclampsia by regulating endothelial nitric oxide synthase. Mol Med Rep. 2014;10(1):550–4. https://doi.org/10.3892/mmr.2014.2214.
95. Sun HX, Zeng DY, Li RT, Pang RP, Yang H, Hu YL, et al. Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension (Dallas, Tex : 1979). 2012;60(6):1407–14. https://doi.org/10.1161/hypertensionaha.112.197301.
96. Dai Y, Qiu Z, Diao Z, Shen L, Xue P, Sun H, et al. MicroRNA-155 inhibits proliferation and migration of human extravillous trophoblast derived HTR-8/SVneo cells via down-regulating cyclin D1. Placenta. 2012;33(10):824–9. https://doi.org/10.1016/j.placenta.2012.07.012.
97.• Bounds KR, Chiasson VL, Pan LJ, Gupta S, Chatterjee P. MicroRNAs: new players in the pathobiology of preeclampsia. Front Cardiovasc Med. 2017;4:60. https://doi.org/10.3389/fcvm.2017.00060 ***Importance:*** **Bounds et al. described the potential roles of microRNAs in preeclampsia. The group also highlighted the upregulation of miR-210 in plasma and placental tissue of preeclamptic women.**
98. Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, et al. Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. J Cell Mol Med. 2012;16(2):249–59. https://doi.org/10.1111/j.1582-4934.2011.01291.x.
99. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. Reproduction. 2009;138(6):895–902. https://doi.org/10.1530/rep-09-0092.
100. Wang A, Rana S, Karumanchi SA. Preeclampsia: the role of angiogenic factors in its pathogenesis. Physiology (Bethesda). 2009;24:147–58. https://doi.org/10.1152/physiol.00043.2008.
101. Hong F, Li Y, Xu Y. Decreased placental miR-126 expression and vascular endothelial growth factor levels in patients with preeclampsia. J Int Med Res. 2014;42(6):1243–51. https://doi.org/10.1177/0300060514540627.
102. Wang Y, Fan H, Zhao G, Liu D, Du L, Wang Z, et al. miR-16 inhibits the proliferation and angiogenesis-regulating potential of mesenchymal stem cells in severe pre-eclampsia. FEBS J. 2012;279(24):4510–24. https://doi.org/10.1111/febs.12037.
103. Liu Q, Yang J. Expression and significance of miR155 and vascular endothelial growth factor in placenta of rats with preeclampsia. Int J Clin Exp Med. 2015;8(9):15731–7.
104. Wang W, Feng L, Zhang H, Hachy S, Satohisa S, Laurent LC, et al. Preeclampsia up-regulates angiogenesis-associated microRNA (i.e., miR-17, -20a, and -20b) that target ephrin-B2 and EPHB4 in human placenta. J Clin Endocrinol Metab. 2012;97(6):E1051–9. https://doi.org/10.1210/jc.2011-3131.
105. Salomon C, Guanzon D, Scholz-Romero K, Longo S, Correa P, Illanes SE, et al. Placental exosomes as early biomarker of preeclampsia: potential role of exosomal MicroRNAs across gestation. J Clin Endocrinol Metab. 2017;102(9):3182–94. https://doi.org/10.1210/jc.2017-00672.
106. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet (London, England). 2016;387(10022):999–1011. https://doi.org/10.1016/s0140-6736(15)00070-7.
107. Maharaj NR, Phulukdaree A, Nagiah S, Ramkaran P, Tiloke C, Chuturgoon AA. Pro-inflammatory cytokine levels in HIV infected and uninfected pregnant women with and without preeclampsia. PLoS One. 2017;12(1):e0170063.
108. Wimalasundera RC, Larbalestier N, Smith JH, de Ruiter A, Mc GTSA, Hughes AD, et al. Pre-eclampsia, antiretroviral therapy, and immune reconstitution. Lancet (London, England). 2002;360(9340):1152–4. https://doi.org/10.1016/s0140-6736(02)11195-0.
109. Machado ES, Krauss MR, Megazzini K, Coutinho CM, Kreitchmann R, Melo VH, et al. Hypertension, preeclampsia and eclampsia among HIV-infected pregnant women from Latin America and Caribbean countries. J Inf Secur. 2014;68(6):572–80. https://doi.org/10.1016/j.jinf.2013.12.018.
110. Suy A, Martínez E, Coll O, Lonca M, Palacio M, de Lazzari E, et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. AIDS (London, England). 2006;20(1):59–66. https://doi.org/10.1097/01.aids.0000198090.70325.bd.
111. Mishra R, Kumar A, Ingle H, Kumar H. The interplay between viral-derived miRNAs and host immunity during infection. Front Immunol. 2019;10:3079. https://doi.org/10.3389/fimmu.2019.03079.
112. Romani B, Allahbakhshi E. Underlying mechanisms of HIV-1 latency. Virus Genes. 2017;53(3):329–39. https://doi.org/10.1007/s11262-017-1443-1.

113. Couturier J, Orozco AF, Liu H, Budhiraja S, Siwak EB, Nehete PN, et al. Regulation of cyclin T1 during HIV replication and latency establishment in human memory CD4 T cells. Virol J. 2019;16(1):22. https://doi.org/10.1186/s12985-019-1128-6.
114. Chiang K, Sung TL, Rice AP. Regulation of cyclin T1 and HIV-1 Replication by microRNAs in resting CD4+ T lymphocytes. J Virol. 2012;86(6):3244–52. https://doi.org/10.1128/jvi.05065-11.
115. Sung TL, Rice AP. miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1. PLoS Pathog. 2009;5(1): e1000263. https://doi.org/10.1371/journal.ppat.1000263.
116. Houzet L, Yeung ML, de Lame V, Desai D, Smith SM, Jeang KT. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. Retrovirology. 2008;5: 118. https://doi.org/10.1186/1742-4690-5-118.
117. Geyer M, Fackler OT, Peterlin BM. Structure–function relationships in HIV-1 Nef. EMBO Rep. 2001;2(7):580–5. https://doi.org/10.1093/embo-reports/kve141.
118. Ahluwalia JK, Khan SZ, Soni K, Rawat P, Gupta A, Hariharan M, et al. Human cellular microRNA hsa-miR-29a interferes with viral nef protein expression and HIV-1 replication. Retrovirology. 2008;5:117. https://doi.org/10.1186/1742-4690-5-117.
119. Nathans R, Chu CY, Serquina AK, Lu CC, Cao H, Rana TM. Cellular microRNA and P bodies modulate host-HIV-1 interactions. Mol Cell. 2009;34(6):696–709. https://doi.org/10.1016/j.molcel.2009.06.003.
120. Chable-Bessia C, Meziane O, Latreille D, Triboulet R, Zamborlini A, Wagschal A, et al. Suppression of HIV-1 replication by microRNA effectors. Retrovirology. 2009;6:26. https://doi.org/10.1186/1742-4690-6-26.
121. Ortega PAS, Saulle I, Mercurio V, Ibba SV, Lori EM, Fenizia C, et al. Interleukin 21 (IL-21)/microRNA-29 (miR-29) axis is associated with natural resistance to HIV-1 infection. AIDS (London, England). 2018;32(17):2453–61. https://doi.org/10.1097/qad.0000000000001938.
122. Adoro S, Cubillos-Ruiz JR, Chen X, Deruaz M, Vrbanac VD, Song M, et al. IL-21 induces antiviral microRNA-29 in CD4 T cells to limit HIV-1 infection. Nat Commun. 2015;6:7562. https://doi.org/10.1038/ncomms8562.
123. Cullen BR. Is RNA interference involved in intrinsic antiviral immunity in mammals? Nat Immunol. 2006;7(6):563–7. https://doi.org/10.1038/ni1352.
124. Omoto S, Ito M, Tsutsumi Y, Ichikawa Y, Okuyama H, Brisibe EA, et al. HIV-1 nef suppression by virally encoded microRNA. Retrovirology. 2004;1:44. https://doi.org/10.1186/1742-4690-1-44.
125. Lin J, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. J Virol. 2007;81(22):12218–26. https://doi.org/10.1128/jvi.01390-07.
126. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer FA, et al. Identification of microRNAs of the herpesvirus family. Nat Methods. 2005;2(4):269–76. https://doi.org/10.1038/nmeth746.
127. Swaminathan S, Murray DD, Kelleher AD. The role of microRNAs in HIV-1 pathogenesis and therapy. AIDS (London, England). 2012;26(11):1325–34. https://doi.org/10.1097/QAD.0b013e328352adca.
128. Ouellet DL, Plante I, Landry P, Barat C, Janelle ME, Flamand L, et al. Identification of functional microRNAs released through asymmetrical processing of HIV-1 TAR element. Nucleic Acids Res. 2008;36(7):2353–65. https://doi.org/10.1093/nar/gkn076.
129. Pilakka-Kanthikeel S, Saiyed ZM, Napuri J, Nair MP. MicroRNA: implications in HIV, a brief overview. J Neuro-Oncol. 2011;17(5):416–23. https://doi.org/10.1007/s13365-011-0046-1.
130. Klase Z, Winograd R, Davis J, Carpio L, Hildreth R, Heydarian M, et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. Retrovirology. 2009;6:18. https://doi.org/10.1186/1742-4690-6-18.
131. Nersisyan S, Engibaryan N, Gorbonos A, Kirdey K, Makhonin A, Tonevitsky A. Potential role of cellular miRNAs in coronavirus-host interplay. PeerJ. 2020;8:e9994. https://doi.org/10.7717/peerj.9994.
132. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res. 2000;87(5):E1–9. https://doi.org/10.1161/01.res.87.5.e1.
133. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271–80.e8. https://doi.org/10.1016/j.cell.2020.02.052.
134. Zhang R, Su H, Ma X, Xu X, Liang L, Ma G, et al. MiRNA let-7b promotes the development of hypoxic pulmonary hypertension by targeting ACE2. Am J Phys Lung Cell Mol Phys. 2019;316(3): L547–l57. https://doi.org/10.1152/ajplung.00387.2018.
135. Nersisyan S, Shkurnikov M, Turchinovich A, Knyazev E, Tonevitsky A. Integrative analysis of miRNA and mRNA sequencing data reveals potential regulatory mechanisms of ACE2 and TMPRSS2. PLoS One. 2020;15(7):e0235987. https://doi.org/10.1371/journal.pone.0235987.
136. Yu J, Wu Y, Zhang Y, Zhang L, Ma Q, Luo X. Role of ACE2-Ang (1-7)-Mas receptor axis in heart failure with preserved ejection fraction with hypertension. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2018;43(7):738–46. https://doi.org/10.11817/j.issn.1672-7347.2018.07.007.
137. Huang YF, Zhang Y, Liu CX, Huang J, Ding GH. microRNA-125b contributes to high glucose-induced reactive oxygen species generation and apoptosis in HK-2 renal tubular epithelial cells by targeting angiotensin-converting enzyme 2. Eur Rev Med Pharmacol Sci. 2016;20(19):4055–62.
138. Yu G, Zhan X, Zhang Z, Li Y. Overexpression of miR-125b promotes apoptosis of macrophages. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2016;32(7):958–62.
139. Wang G, Kwan BC, Lai FM, Chow KM, Li PK, Szeto CC. Elevated levels of miR-146a and miR-155 in kidney biopsy and urine from patients with IgA nephropathy. Dis Markers. 2011;30(4):171–9. https://doi.org/10.3233/dma-2011-0766.
140. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A. 2011;108(12):5003–8. https://doi.org/10.1073/pnas.1019055108.
141. Bondanese VP, Francisco-Garcia A, Bedke N, Davies DE, Sanchez-Elsner T. Identification of host miRNAs that may limit human rhinovirus replication. World J Biol Chem. 2014;5(4): 437–56. https://doi.org/10.4331/wjbc.v5.i4.437.
142. Inchley CS, Sonerud T, Fjærli HO, Nakstad B. Nasal mucosal microRNA expression in children with respiratory syncytial virus infection. BMC Infect Dis. 2015;15:150. https://doi.org/10.1186/s12879-015-0878-z.
143. Sardar R, Satish D, Birla S, Gupta D. Comparative analyses of SAR-CoV2 genomes from different geographical locations and other coronavirus family genomes reveals unique features

potentially consequential to host-virus interaction and pathogenesis. bioRxiv. 2020.

144.•• Marquez-Pedroza J, Cárdenas-Bedoya J, Morán-Moguel MC, Escoto-Delgadillo M, Torres-Mendoza BM, Pérez-Ríos AM, et al. Plasma microRNA expression levels in HIV-1-positive patients receiving antiretroviral therapy. Biosci Rep. 2020;40(5): BSR20194433. https://doi.org/10.1042/bsr20194433 **Marquez-Pedroza et al. investigated the expression of microRNAs in HIV-positive patients receiving treatment. This was one of very few studies that examined the role of microRNAs in HIV infection.**

145.•• Li C, Hu X, Li L, Li JH. Differential microRNA expression in the peripheral blood from human patients with COVID-19. J Clin Lab Anal. 2020;34(10):e23590. https://doi.org/10.1002/jcla.23590 **There is a greatly significant lack of research investigating microRNAs in COVID-19. This was a rare group of authors who indicated that the expression of microRNAs is dysregulated in COVID-19 patients.**