THE ROLE OF ENDOTHELIN-1 IN HIV-ASSOCIATED PREECLAMPSIA

by

MBUSO HERALD MTHEMBU

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PREFACE

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

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

_____ _____

Mbuso. H. Mthembu Professor Thajasvarie Naicker

(Student number: 214556782) (Supervisor)

DECLARATION

I, **Mbuso .H. Mthembu** declare that:

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

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

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DEDICATION

To God the Father, the Son and the Holy Spirit, He who provides everything.

My lovely parents – You are amazing! You have been with me at every step of this journey. Your love and prayers have kept me going till this far. Each day you continue to inspire me. I hope I have made you proud. I love you.

To Aphiwe – Thank you for understanding that daddy has to be away from home for a long time. I am trying a better future for us. I hope my perseverance will be an example to you. I love you.

My family and friends - I express my deepest love and appreciation for your unending support, motivation and for always believing in me. I love you

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LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
ET-1	Endothelin-1
AIDS	Acquired Immunodeficiency Syndrome
ECE	Endothelin converting enzyme
PE	Preeclampsia
EOPE	Early-onset preeclampsia
LOPE	Late-onset preeclampsia
ART	Antiretroviral therapy
HAART	Highly active antiretroviral therapy
RAAS	Renin angiotensin aldosterone system
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
MDG	Millennium Development Goals
SDG	Sustainable Development Goals
SA	South Africa
KZN	KwaZulu-Natal
HELLP	Hemolysis elevated liver enzyme, low platelet count
NO	Nitric oxide
NOS	Nitric oxide synthase
MMR	Maternal mortality ratio
MTCT	Mother-to-child transmission
WHO	World Health Organization
Pg/mL	Picogram per milliliter
mmHg	Millimeters of Mercury

Abstract

Introduction and Background: Preeclampsia (PE), a hypertensive disorder specific to human pregnancy, remains a major cause of maternal mortality and morbidity globally. Endothelin-1 (ET-1) is a powerful vasoconstrictor that plays a crucial role in endothelial cell dysfunction, a characteristic feature of preeclampsia development. As a result, this study assessed the role of ET-1 in an HIV-infected preeclamptic cohort. Ethics approval was obtained BCA/17.

Method: The study population (n = 72) was grouped according to pregnancy type *i.e.*, normotensive (n = 36) and preeclamptic (n = 36) further stratified by human immunodeficiency virus (HIV) status. ET-1 levels were quantified using the Bioplex Immunoassay.

Results: Gravidity, gestational age, systolic and diastolic blood pressure were significant across the study groups ($p < 0.05$). The concentration of ET-1 was significantly elevated in preeclamptic vs normotensive pregnancies regardless of HIV status ($p \leq 0.0418$).

Conclusion: This study observed a non-significant increase in ET-1 in the HIV positive pre-eclampsia group compared to the HIV negative pre-eclampsia group. ET-1 was significantly increased in pre-eclampsia compared to normotensive pregnancy.

ABSTRACT (IsiZulu)

Isendlalelo nesingeniso: I-preeclampsia (PE), isifo sokunyuka kokugijima kwegazi esihlasela umuntu wesifazane ngesikhathi ekhulelwa, iyimbangela enkulu yokushona kukamama kanye nokugula komzimba emhlabeni wonke jikelele. I-Endothelin-1 (ET-1) iyi-vasoconstrictor enamandla ebamba iqhaza elibalulekile ekungasebenzi kahle kwamaseli we-endothelial, ibuye ibe nomthelela ekuthuthukeni kwe Preeclampsia. Lolu cwaningo beluhlola iqhaza le-ET-1 kubantu besifazane abanegciwane lesandulela ngculaza Kanye ne Preeclampsia ngesikhathi esisodwa.

Indlela yokwenza: Inani labantu abacwaninguyo lingama-72, lihlukaniswa ngokwamaqembu ezinhlobo zokukhulelwa i.e., i-Abangenaso isifo (Normatensive) abangama-36 kanye nabanesifo se Pre-eclampsia abangama-36-, abuye aphindwa yahlukaniswa ngesimo seegciwane lesandulela ngculaza. Amazinga we-ET-1 asehlaziywa ngokusebenzisa iBioplex Immunoassay.

Imiphumela: Ukuthola amandla wobudala, iminyaka yokuthomba, umfutho wegazi ne-diastolic kubonakale kwenza umehluko kuwo amaqembu ocwaningo ($p < 0.05$). Amazinga we-ET-1 akhuphuke kakhulu ekukhulelweni kwe-PE uma kuqhathaniswa nokukhulelwa okujwayelekile kungakhathalekile ukuthi sinjani isimo segciwane lesandulelaNgculaza ($p \leq 0.0418$).

Isiphetho: Lolu cwaningo lubone ukwanda okungabalulekanga kwe-ET-1 eqenjini labanepre-eclampsia Kanye negciwane lesandulela ngculaza kuqhathaniswa nabane Preeclampsiankodwa bengenalo igciwane lesandulela ngculaza. I-ET-1 ibonakale yanda kakhulu ekukhuliseni i-pre-eclampsia uma kuqhathaniswa nokukhulelwa okujwayelekile

CHAPTER ONE

INTRODUCTION AND BACKGROUND LITERATURE

1.0 PROBLEM IDENTIFICATION

Maternal mortality and morbidity.

In September 2000, the eight United Nations (UN) Millennium Development Goals (MDGs) was accepted worldwide. The fifth MDG, a global health priority, targeted a decrease in the maternal mortality ratio (MMR) by 75% between 1990 and 2015 (WHO, 2014). Despite non-attainment of MDG5 in 2015 by many developing countries, a positive change was reported. In the sub-Saharan Africa region there was a slow regression in the MMR due to the effect of HIV/AIDS (Alkema *et al*., 2016). There was the introduction of the Sustainable Development Goals (SDGs) in 2015, which also targeted to reduce the global MMR to less than 70 deaths per 100 000 livebirths by the year 2030 (WHO, 2015).

In South Africa, obstetric haemorrhage is the most common direct cause of maternal death accounting for 16.9 % of maternal deaths, followed by hypertensive disorders in pregnancy (14.8 %) (Saving Mothers Report, 2018). Maternal deaths due to hypertension have dropped by 18% in the last 3 trienniums (Saving Mothers Report, 2015). Non-pregnancy related infections account for 34.7 % of all maternal deaths (Saving Mothers Report, 2015). Two- thirds of all maternal deaths are attributed to HIV infection (Moodley, 2018).

1.1 HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The Human Immunodeficiency Virus (HIV) is a lentivirus that causes infection and immune suppression resulting in an increased susceptibility to opportunistic diseases (Bennett *et al*., 2013). The genetic material of the lentivirus is carried in the form of RNA which is then reverse transcribed to DNA by reverse transcriptase, a viral encoded enzyme. Following reverse transcription, viral DNA is integrated into the host cell nucleus which is further translated into viral proteins (Brik *et al*., 2003). HIV infection is predominantly caused by HIV-1 whilst HIV-2 has lower virulence and transmission and is mostly localized in West Africa (Bull *et al*., 2015).

HIV attacks T cells that exhibit the CD4 antigen on their surface. HIV infection requires cellular and viral membranes fusion, a process mediated by viral envelope glycoproteins (gp41 and gp120) and receptors (CD4 and co-receptors CCR5 or CXCR4) on the target cell (Brik *et al*., 2003; Maartens *et al*., 2014). The virus infects cells bearing CD4 and chemokine receptors including monocytes and macrophages, dendritic cells and resting CD T cells.

HIV is transmitted across the mucosal membranes through receptors CCR5 or CXCR4 for entry and is resistant to interaction with dendritic cells as well as interferon-α (Maartens *et al*., 2014). HIV infection causes a gradual decrease in CD4 lymphocytes which lowers immunity (Vidya Vijayan *et al*., 2017), leading to a variety of opportunistic diseases or eventually the risk of AIDS (Bull *et al*., 2015).

Central nervous system complications associated with HIV-1 infection manifests as neuronal dysfunction associated with monocyte infiltration into the brain, myelin pallor and the formation of multinucleated giant cells (Didier *et al*., 2002). These morbid attributes result in the synthesis and accumulation of nitric oxide (NO), arachidonic acid metabolites, and soluble factors such as endothelin-1 (ET-1) (Didier *et al*., 2002). HIV infection is also linked with a chronic inflammatory process (Feijoo *et al*., 2014).

The routine use of highly active antiretroviral therapy (HAART) has resulted in a drastic and sustained decrease in mortality and morbidity from HIV infection and the risk of mother-to-child transmission (Suy *et al*., 2006). HIV can be transmitted through sexual, perinatal (mother-to-child) or blood transmission (exposure to contaminated blood) (Karim *et al*., 2007). Over 80% of new infections in women are sexually transmitted with the majority of affected women living in sub-Saharan Africa (UNAIDS, 2015).

1.1.1 Prevalence of HIV

.South Africa (SA) has the highest HIV/AIDS prevalence in the world (13.5%) with 7.97 million of the population in 2019 being HIV positive (Stats SA, 2019). Provincial variation of HIV-prevalence exists with KwaZulu-Natal (KZN) been the highest and is considered the epicenter of the global HIV/AIDS pandemic with a prevalence of 18.1% (Simbayi *et al*., 2019). Over a fifth of South African women in their reproductive ages (15–49 years) are HIV positive (Stats SA, 2019). Approximately 4.2 million South African women of ages (15+) are infected with HIV (UNAIDS, 2018).

In 2018, UNAIDS reported that 4.4 million people were receiving antiretroviral therapy (ART) in SA. Due to the success of the ART rollout, the South African National Department of Health drafted a new policy regarding an improved ART rollout in 2016 (Mukumbang *et al*., 2019). Provision of ART to women before and during pregnancy and breastfeeding prevents mother-to-child transmission (MTCT) and improves health and survival of mothers (WHO, 2016a), which itself benefits the health of their children (Newell *et al*., 2004). Seventy six percent of pregnant women living with HIV (over 1 million women) receive ART annually to prevent MTCT of HIV (Bailey *et al*., 2018).

1.2 PREECLAMPSIA

Preeclampsia (PE) is a multi-system disorder of vascular dysfunction that manifests as hypertension (>140/90 mm Hg) with/without proteinuria (>300mg/ 24-hour urine) during pregnancy (Brown *et al.*, 2018). PE is also associated with coagulation abnormalities and decreased uteroplacental flow (Walsh, 2007) and can result in fetal growth restriction (FGR), stillbirth, abruptio placentae, and preterm labor with intact membranes (Nakimuli *et al.*, 2014). Maternal organ dysfunction associated with PE manifests as eclampsia, HELLP syndrome (elevated liver enzymes, haemolysis) and thrombocytopenia (low platelet count) (WHO, 2016b; Rana *et al.*, 2019). Pulmonary oedema and myocardial ischaemia or infarction are part of the cardiorespiratory complications resulting from PE (Mol *et al.*, 2016; Rana *et al.*, 2019). Women with severe PE might experience symptoms of visual disturbances (including blindness), headaches, epigastric pain, or nausea and vomiting (Mol *et al.*, 2016).

1.2.1 Epidemiology of Preeclampsia

PE is a global concern, complicating approximately 10% of all pregnancies and is a major cause of maternal and perinatal morbidity and mortality (Mattar *et al.*, 2004; George and Granger, 2011; Kalumba *et al.*, 2013; Craici *et al.*, 2014; English *et al.*, 2015; Herse and LaMarca, 2013). According to the World Health Organization (WHO), nearly one-tenth of all maternal deaths are associated with hypertensive disorders of pregnancy in Asia and Africa, whereas in Latin America one-quarter of all maternal deaths are related to hypertensive complications. In South Africa (SA), maternal deaths due to hypertension in pregnancy is approximately 14%, of which 83% is due to PE development (Saving Mothers Report, 2017). In KZN, the incidence of PE is 12% (Gathiram and Moodley, 2016).

1.2.2 Classification of Preeclampsia

Based on gestational age, PE may be clinically divided into two sub-types: early-onset preeclampsia (EOPE) <34 gestational weeks, and late-onset preeclampsia (LOPE) >34 gestational weeks (Raymond *et al.*, 2011; Lisonkova *et al.*, 2014).

EOPE is considered a fetal disorder associated with a plethora of outcomes including placental dysfunction, low birth weight, perinatal death, multiorgan dysfunction, reduction in placental volume, abnormal uterine and umbilical artery Doppler evaluation and intrauterine growth restriction (Aksornphusitaphong *et al.*, 2013).

LOPE is regarded as a maternal disorder with minimal changes to placental pathophysiology, however induced maternal inflammatory response leads to PE development, eclampsia and maternal death

(Lisonkova *et al*., 2014). LOPE presents more favourable maternal and neonatal outcomes to EOPE (Raymond *et al*., 2011).

Severe PE may be described as the maternal systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg confirmed in 15 minutes; eclampsia, proteinuria ≥5 g/24hours, HELLP syndrome, acute pulmonary oedema, neurological disorders, polykinetic tendon reflexes, oliguria <500 cc/day, creatinine >120 µmol/L, intrauterine growth retardation and fetal death in utero (Brown *et al*., 2018), however, there are different views on the definition of severe PE (ACOG, 2019).

1.2.3 Preeclampsia aetiology

The exact aetiology underlying PE remains elusive however, it is believed to originate from placental deficiency (George and Granger, 2011), emanating from an abnormal cytotrophoblast invasion with resultant non-physiological transformation of the maternal spiral arteries within the myometrium (Naicker *et al*., 2013; Hod *et al*., 2015).

Normal placental development begins with cytotrophoblast invasion of the maternal spiral arteries. These invasive cytotrophoblasts originate in the fetus. Following vascular invasion, in a process called "vascular mimicry" or "pseudovasculogenesis" the cytotrophoblasts differentiate into an endothelial phenotype. In pre-eclampsia, invasion of the spiral arteries is shallow due to failure of cytotrophoblasts differentiation from an epithelial to endothelial phenotype (Wang *et al*., 2009). The resultant invasion transforms the spiral arteries from small-caliber resistance vessels to high-caliber capacitance vessels to give apposite support for the growing fetus (Figure 1.1) (Lam *et al*., 2005).

PE is characterised by increased oxidative stress and decreased antioxidants. Levels of glutathione, iron binding capacity, superoxide dismutase and vitamin A, C, E are altered in the maternal circulation (Rumbold *et al*., 2008). Due to the decrease in superoxide dismutase activity and vitamin E, the iron concentration and superoxide anion concentration increases resulting in significant oxidative stress (Siddiqui *et al*., 2010). PE pathophysiology includes an imbalance between thromboxane (a potent vasoconstrictor) and prostacyclin (a potent vasodilator); thromboxane is favored by the imbalance, oxidative stress, activation of circulating leukocytes and vascular cell dysfunction (Walsh, 2007). Endothelial dysfunction is caused by increased oxidative stress and enhanced maternal inflammatory response (Margaritis, 2019).

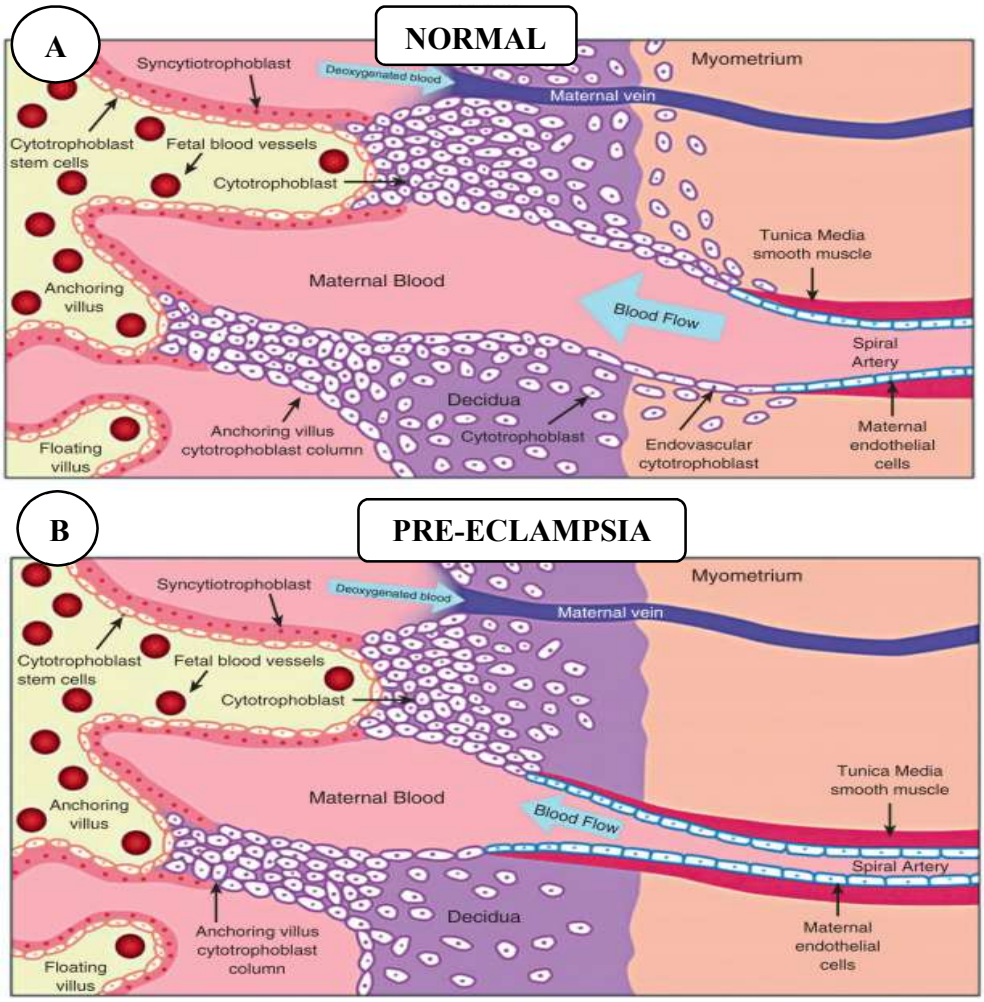

Figure 1.1: Abnormal placentation in preeclampsia. In normal placentation (a), invasion of maternal spiral arteries by invasive cytotrophoblasts transforms their capacitance from small to high caliber resistance vessels to pertinently support the growing fetus. Cytotrophoblasts differentiate from an epithelial to endothelial phenotype. In preeclampsia (b), cytotrophoblasts fail to differentiate into an invasive endothelial phenotype instead have a shallow invasion of spiral arteries and remain small caliber resistance vessels [adapted from (Wang *et al*., 2009)].

1.2.4 Renin-Angiotensin Aldosterone System

The renin-angiotensin aldosterone system (RAAS) regulates extracellular fluid volume, electrolyte balance and arterial pressure to maintain vascular tonicity (Irani and Xia, 2008, Patel *et al*., 2017). Agitation of the RAAS can interrupt normal blood pressure, resulting in chronic or acute diseases, or even sudden death (Patel *et al*., 2017). Renin cleaves angiotensinogen to 10- amino acid peptide called angiotensin-l (Ang-1) in a rate-limiting step of the RAAS cascade (Irani and Xia, 2008). Ang-1 is converted to angiotensin-ll

(Ang-ll) in a reaction catalysed by the angiotensin converting enzyme in the RAAS (Aung *et al*., 2017). Ang-ll constricts smooth muscles as it exhibit its vasoactive role in increasing the blood pressure (Patel *et al*., 2017). High renin levels causes endothelial impairment and results in high Ang-ll activity (Patel *et al*., 2017). PE is marked by the increase in systemic vascular resistance with hypovolemia and a low cardiac output which is followed by the suppression of the RAAS (Saleh *et al*., 2016b).

Alternatively, suppression of the RAAS results in increased production of auto-antibodies to the Ang-ll type 1 receptor from the placenta of preeclamptic patients (Saleh *et al*., 2016b). PE is also affected by a dysfunctional RAAS with an expanded Ang-ll sensitivity before disease onset characterized by endothelial dysfunction (Saleh *et al*., 2016b). Ang-ll type 2 receptor is implicated in the increased vasoconstrictor response to Ang-ll in PE (Saleh *et al*., 2016b).

It has been hypothesized that the activity of Ang-II affects ET-1 synthesis, similar to the effect of ET-1 on the RAAS (Rossi *et al*., 1999). The final pathway leading to hypertension, renal toxicity and RAAS suppression in PE appears to be the endothelin system (Saleh *et al*., 2016b).

1.2.5 Risk factors for Preeclampsia development

The risk of PE is markedly increased in women who are genetically predisposed to the disease by mothers who were previously affected by PE (Mogren *et al*., 1999; Mol *et al*., 2016). PE also substantially increases the risk of post-partum depression and decreases health-related quality later on in life (Mol *et al*., 2016). Obesity is a risk factor of PE although the involved mechanisms are still unknown. An increase in pregnancy body mass index (BMI) leads to an increased risk of PE development (Walsh, 2007). Moreover, the risk of PE development is greater for women who have partners that previously fathered a preeclamptic pregnancy (Hawfield and Freedman, 2009).

Chronic kidney disease, hypertension in pregnancy, previous PE, diabetes (type 1 and type 2), and autoimmune disorders, including antiphospholipid syndrome and lupus erythematosus are strong risk factors of PE (Creasy and Resnik, 2004; Duckitt and Harrington, 2005). Moderate risk factors of PE include (i). first pregnancy, (ii). a pregnancy interval greater than 10 years, (iii) age of 40 years or more, (iv). body mass index of 35 kg/m^2 or more, (v). polycystic ovarian syndrome and (vi). multiple pregnancy (Mol *et al*., 2016). Kidney donors are more likely to develop PE than matched women who had not donated a kidney (Deshpande *et al*., 2011).

1.2.6 Prevention of Preeclampsia

Termination of pregnancy/ delivery of the fetus and placenta is the only ultimate treatment for PE, although some women with PE also exhibit a transitory aggravation of the disease in the postpartum period (Hawfield and Freedman, 2009; WHO, 2016b). As a result, preventive measures for PE development has become a key research focus and has shown promise in recent years. Based on the findings of individual patient data (IPD), aspirin was found to be a more suitable drug to prevent PE (Mol *et al*., 2016). PE is associated with low serum calcium concentration and low dietary calcium. As, a result, women with low dietary calcium require high dose calcium supplementation especially in the second half of pregnancy to reduce PE (WHO, 2016b). L-arginine, a nitric oxide precursor, reduces the risk of PE when given in combination with antioxidants (Noris *et al*., 2005).

1.2.7 Preeclampsia complicated by HIV infection

It is plausible to assume that preeclamptic pregnancies complicated by HIV infection are a representation of opposing immune responses (Haffejee *et al*., 2013). Kalumba *et al*. (2013) stated that the rate of PE development is lower in HIV-positive pregnant women that the general population. HIV infected women have a higher risk of PE compared to women without HIV and the risk is increased in women receiving highly active antiretroviral therapy HAART therapy (Sansone *et al*., 2016). HIV infection could possibly inhibit or block factors that may play a vital role in PE development (Mattar *et al*., 2004).

PE was an uncommon complication of pregnancy in HIV-infected women prior to the administration of HAART (Suy *et al*., 2006). Nonetheless, HAART restores immunity in the infected individual and results in an improved response which contributes to increased susceptibility to PE development (Kalumba *et al*., 2013; Maharaj *et al*., 2017).

1.3 ENDOTHELIN-1

Endothelin-1 (ET-1) is a 21-amino acid peptide isolated from endothelial cells and is regarded as the most potent vasoconstrictor peptide in the cytokine family (Didier *et al*., 2002; Freeman *et al*., 2014). It forms part of the three known endothelin isoforms, specifically encoded by a distinct peptide, but results from an identical two-step metabolic pathway (Freeman *et al*., 2014). ET-1 is the most abundant isoform *in vivo,* derived from a longer 203-amino acid precursor known as preproendothelin-1, which is converted into the intermediate precursor big ET-1 (Figure 1.2). The endothelin converting enzyme (ECE) generates ET-1 by

cleaving the bond between Trp^{21} and Val^{22} of big ET-1 (George and Granger, 2011; Freeman *et al*., 2014; Davenport *et al*., 2016; Yanagisawa *et al*., 1988; D'Orleans-Juste *et al*., 2003). ECE is a zinc-dependent metallo-endopeptidase found in several cell types such as smooth muscle cells, endothelial cells, macrophages and cardiomyocytes (Takahashi *et al*., 1995; Barnes and Turner, 1997; Pelayo *et al*., 2011).

ET-1 has two endothelin receptors *viz*., ET-1 type A (ET_A) and ET-1 type B (ET_B). ET_A receptor found on vascular smooth muscle and are essential regulators of ET-1 dependant vasoconstriction and cellular proliferation, and ET_B is located on renal epithelial and vascular endothelial cells (George and Granger, 2011). ET_A receptor activation by ET-1 mediates vasoconstriction through stimulation of phospholipase C with resultant formation of inositol triphosphate (IP_3) that induces the release of Ca^{2+} from endoplasmic reticulum stores. Binding of ET-1 to ET_A promotes calcium influx, proliferation and contraction of smooth muscle cells (Freeman *et al*., 2014). In contrast, the binding of ET-1 to ET_B receptor mediates vasodilation through activation of the Phosphatidylinositol 3-kinase/Protein B kinase (PI3/Akt) pathway, followed by activation of the endothelial nitric oxide (NO) synthase generating NO (Figure 1.3) (Khimji and Rockey, 2010).

Hypoxia induces the synthesis of ET-1 in the endothelium (Freeman *et al*., 2014). The vasoconstrictive properties of ET-1 stimulates DNA synthesis and proliferation in pulmonary arterial smooth muscle cells (Sandoval-Gutierrez *et al*., 2015). Additionally, ET-1 has neuroregulatory and physiologic functions. ET-1 is further implicated as a mediator of cerebrovascular responses linked with subarachnoid haemorrhage and ischemic strokes (Didier *et al*., 2002).

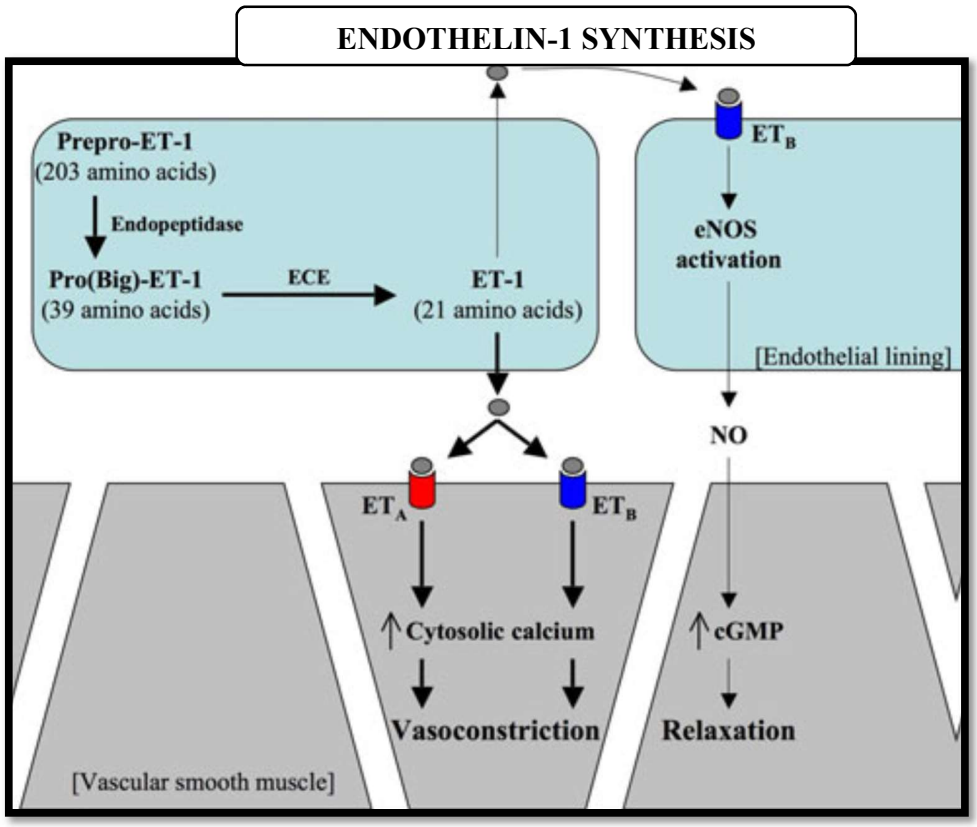

Figure 1.2: Endothelin-1 development from prepro-endothelin (adapted from (Kawanabe *et al*., 2011)).

1.3.1 The role of endothelin-1 in Preeclampsia

The mRNA of ET-1 has been found in the human placenta (Fiore *et al*., 2005). ET-1 dysregulation occurs in PE, specifically through the increase of circulating ET-1 levels (George and Granger, 2011; Zhou *et al*., 2011). The altered ET-1 system in PE causes an increased ECE activity in the maternal circulation with elevated tissue production of preproET-1 mRNA in comparison to normal ET-1 functioning in healthy pregnant or non-pregnant women (George and Granger, 2011).

Endothelial dysfunction induced by soluble fms-like tyrosine kinase-1 and soluble endoglin results in ET-1 overproduction thereby leading to hypertension and proteinuria (Aggarwal *et al*., 2012). ET-1 causes oxidative stress in placental and human endothelial cells and has cytotoxic effects on trophoblast cells, thereby suggesting that ET-1 is the key link between the primary placental cause and the secondary systemic maternal disorder of PE (Fiore *et al*., 2005; Aggarwal *et al*., 2012).

The vitality and proliferation rate of trophoblast cells is decreased by ET-1 (Fiore *et al*., 2005). Moreover, proliferation, function and migration of trophoblasts are deficient in PE (McNally *et al*., 2017). However during PE, placental ischemia resulting from reduced uterine perfusion pressure enhances formation of ET-1, which plays a role in mediating hypertension as a rescue mechanism to enhance maternal blood flow through the placenta by increasing maternal blood pressure (Fiore *et al*., 2005).

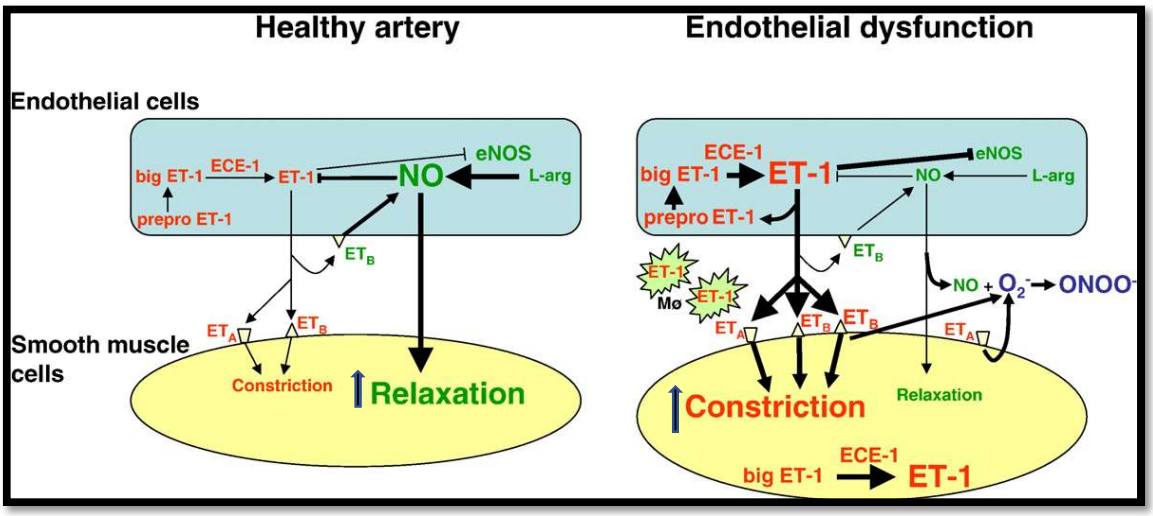

Figure 1.3: Schematic figure representing conditions of a healthy artery and endothelial dysfunction. In healthy arteries, ET-1 production is reduce, and NO is preserved resulting in vasorelaxation. In endothelial dysfunction however, an overproduction of ET-1 exists, resulting in an over-expression in smooth muscles and macrophages. ET_B is expressed in the smooth muscles and mediates vasoconstriction [adapted from (Böhm and Pernow, 2007)].

1.3.2 Endothelin-1 in HIV infection

HIV viral antigens found on pulmonary endothelium have been reported to stimulate abnormal growth, apoptosis and proliferation (Mette *et al*., 1992). In HIV infection, ET-1 production from endothelial cells and inflammatory cells are affected by HIV-related proteins (Humbert, 2008). Glycoprotein 120 (gp120) is a viral protein that targets human lung endothelial cells, assists in the binding and entry of HIV into macrophages, stimulates the secretion of ET-1, and increases apoptosis (Sandoval-Gutierrez *et al*., 2015). Additionally, the HIV viral protein *Tat*, boosts ET-1 production in astrocytes indicating enhanced ET-1 synthesis during infection (Freeman *et al*., 2014). ET-1 enhances neuropathology of HIV through the facilitation of monocyte transmigration into the brain (Didier *et al*., 2002, Freeman *et al*., 2014).

1.4 AIM OF STUDY

In light of the problem identification statement, the aim of this study is to investigate the role of the vasoconstrictor, endothelin-1 in the pathogenesis of HIV associated pre-eclampsia.

1.4.1 Objectives

- To determine the effect of pregnancy type (normotensive *vs* preeclamptic) on the concentration of endothelin-1, irrespective of HIV status (HIV positive and HIV negative) utilizing BioPlex Multiplex Immunoassay.
- To establish the effect of HIV status (HIV positive *vs* HIV negative) on the concentration of endothelin-1, irrespective of pregnancy type (normotensive *vs* preeclamptic).
- To compare and contrast the concentration of endothelin-1 across all study population based on pregnancy type (normotensive *vs* preeclamptic) and HIV status (HIV positive *vs* HIV negative).
- To correlate maternal clinical findings with endothelin-1 across study population.

CHAPTER TWO

MANUSCRIPT

Original Article: Is endothelin-1 concentration altered in HIV-associated preeclampsia?

Chapter 2 is presented as an original article which has been submitted to a DoHET accredited international journal.

The first author is the author of this thesis (MH Mthembu) contributing to the literature and protocol review, experimental procedures and interpretation of the results.

This article investigates the concentrations of Endothelin-1 in serum samples of South African women with African ancestry. This study investigates concentration of this vasoconstrictor in the duality of HIV and Preeclampsia.

THE ROLE OF ENDOTHELIN-1 IN HIV ASSOCIATED PRE-ECLAMPSIA

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THE ROLE OF ENDOTHELIN-1 IN HIV ASSOCIATED PREECLAMPSIA

Authors: Mbuso Herald Mthembu, Louansha Nandlal, Sayuri Padayachee, Thajasvarie Naicker

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

***Corresponding author:**

Mbuso H. Mthembu

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

Email address: mbusomthembu7@gmail.com; naickera@ukzn.ac.za

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ABSTRACT

Objective: South Africa has the fourth-highest HIV/AIDS prevalence rate in the world. Maternal mortality emanating from HIV infection and preeclampsia is unacceptably high. Endothelin-1 (ET-1) is a powerful vasoconstrictor that plays a crucial role in endothelial cell dysfunction, a characteristic feature of preeclampsia development. In light of the paucity of data on ET-1 in HIV infection comorbid with preeclampsia, this study assessed the role of ET-1 in HIV infected preeclamptic women.

Method: The study population (n = 72) was grouped according to pregnancy type *i.e.*, normotensive (n = 36) and preeclamptic (n = 36) and was further stratified by HIV status. ET-1 levels were quantified using the Bioplex immunoassay.

Results: Gravidity, gestational age, systolic and diastolic blood pressure were significant across the study groups ($p < 0.05$). The concentration of ET-1 was significantly elevated in preeclamptic *vs* normotensive pregnancies, regardless of HIV status ($p = 0.0418$). The expression of ET-1 was similar between HIV positive versus HIV negative groups, irrespective of pregnancy type ($p = 0.8512$). There was no significant difference detected across all study groups ($p = 0.2349$).

Conclusion: HIV status did not influence ET-1 in HIV associated preeclampsia. In light of the fact that the HIV accessory protein, *Tat* is a potent angiogenic factor hence would neutralize the antiangiogenic effect of ET-1. Notably, HAART may have also influenced ET-1 levels in HIV infected women.

Keywords: Endothelin-1, Hypertension, HIV infection, Preeclampsia

Running title: Endothelin-1 in HIV associated preeclampsia

INTRODUCTION

Hypertensive disorders of pregnancy remain a global threat to maternal mortality. Developing countries are faced with the greatest burden of hypertension in pregnancy (1), with preeclampsia (PE) among the leading causes of maternal deaths (2). In South Africa, PE accounts for approximately 83% of all maternal deaths (3).

Furthermore in South Africa, 20.4% of non-pregnancy related deaths are attributed to HIV infection (4). In the province of KwaZulu-Natal (KZN), the prevalence of HIV infection in pregnancy is approximately 41.1% (5). It is controversial whether the co-existence of HIV infection and PE leads to a neutralization, increase or decrease of the immune response (6-8). Some studies suggest HIV-positive women receiving highly active antiretroviral therapy (HAART) are at an increased risk of developing PE than untreated women due to the immune reconstitution effects of HAART (8, 9)

In PE, the failure to remodel vascular uteroplacental arteries due to inadequate trophoblast invasion and non-conversion of maternal spiral arteries leads to placental ischaemia and a state of under perfusion (10). In response, the placenta secretes soluble factors into the maternal circulation resulting in an exacerbated maternal inflammatory response due to an increase of anti-angiogenic factors (11). Generally, a balance between pro- and anti-angiogenic factors are necessary for placental development in normal pregnancy, however in PE, a paradigm shift in angiogenesis as a result of dysfunctional syncytiotrophoblast leads to maternal vascular endothelial dysfunction and resultant clinical symptoms of the disease (12).

These endothelial abnormalities leads to the enhanced reproduction of vasoconstrictors *viz*., endothelin and superoxide, an increased sensitivity to the hormone angiotensin II and decreased formation of the vasodilator, nitric oxide (13).

Endothelins belong to a family of three 21-amino acid peptides, of which endothelin-1 (ET-1) is the most prominent and potent vasoconstrictor, produced by endothelial cells and placental syncytiotrophoblasts. ET-1 activity is mediated by the endothelin receptors, ET_A and ET_B. ET-1 exerts its vasoconstriction via the ET_A receptor, contributing to high blood pressure in PE (14). ET-1 is elevated in PE (15).

The secretion of other soluble factors in the maternal circulation including soluble FMS-like tyrosine kinase-1 (sFlt-1), inflammatory cytokines and angiotensin II type-1 receptor auto-antibodies have been shown to induce hypertension in PE through the production of ET-1 (16, 17). The production of these causative agents of endothelial cell injury reduces the synthesis of vasorelaxing agents with a resultant increase in vasoconstriction (14). This provides a potential mechanism through which ET-1 activity

promotes blood pressure elevation in PE. Notably in PE, there is an increased vascular resistance with hypovolemia and a low cardiac output preceded by the suppression of RAAS (18).

HIV infection is associated with a chronic inflammatory process, similar to PE. The release of ET-1 and other inflammatory markers may directly contribute to endothelial damage. The HIV accessory protein *Tat* mimics VEGF and is a potent angiogenic factor.

In light of the synergistic effect of ET-1 in HIV infection and PE, the aim of this study is to investigate the role of ET-1 in HIV-associated preeclampsia within the KZN province.

METHODS AND MATERIALS

Study Population

This is a prospective experimental study utilizing retrospectively collected samples from a large regional hospital in eThekwini, KZN. Institutional, regulatory approval and patient informed consent was obtained (BCA 338/17). The study population consisted of 72 subjects, preeclamptic (n=36) and normotensive (n=36) pregnant women, further stratified by HIV status into HIV-negative (n=18) and HIV-positive (n=18) subgroups. Preeclampsia was defined as sustained systolic blood pressure ≥ 140 mmHg and diastolic blood pressure 90 mmHg or greater, taken at least 4 hours apart, after 20 weeks' gestation in a previously normotensive patient (19). Proteinuria was defined as urine protein concentration of ≥300 mg/dl or 1+ on a urine dipstick in at least two random specimens collected at least 4 hours apart. HIV status was determined by a rapid test and CD4 count was carried out for all HIV-positive women. Exclusion criteria for the PE group was chorioamnionitis, chronic hypertension, eclampsia, and abruptio placentae; intrauterine death, pregestational diabetes, gestational diabetes, and chronic renal disease; systemic lupus erythematosus, sickle cell disease, and antiphospholipid antibody syndrome; and thyroid disease, cardiac disease, and active asthma requiring medication during pregnancy and pre-existing seizure disorders.

Bio-Plex Multiplex Immunoassay

Serum samples were stored at -80°C until use. The concentration of ET-1 was quantified using the Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 (catalogue number HAGP1MAG-12K, EMD Millipore Corporation, USA) following the manufacturer's instructions. Serum samples were diluted in 1:3 using Assay Buffer. Assay buffer (200µl) was added to a 96 well plate, followed by the addition of the

standards, control, assay buffer, matrix solution, samples and antibody-coupled magnetic beads to the appropriate wells. The plate was incubated overnight at 2-8°C. Thereafter the plate was washed three times with 10X wash buffer. Biotinylated detection antibodies were added to each well and incubated for 1 hour at room temperature. The reaction was completed by the addition of streptavidin-phycoerythrin (SA-PE) and incubated for 30 minutes at room temperature. Post final washing (X3) drive fluid was added and the plate was read using the Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad Laboratories Inc., USA) and data was analyzed using the MILLIPLEX® Analyst 5.1 software.

Statistical analysis

Data was analysed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California USA). The Kolmogorov Smirnov normality test indicated non-parametrically distributed data. A Mann-Whitney *U* test was to determine significance based on pregnancy type (normotensive *vs*. preeclamptic) and HIV status (negative *vs*. positive). One-way ANOVA analysis of variance test along with Dunn's *post hoc* test (for multiple comparisons) was used to determine statistical significance across all study groups. The non-parametric data was presented as median and interquartile range. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical findings and patient demographics

Table 1 outlines the patient clinical and demographic data across the study group. A significant difference was detected for gestational age, systolic and diastolic BP ($p < 0.0001$) as well as gravidity (p=0.0125). Maternal age, systolic BP, diastolic BP and maternal weight were higher in preeclamptic vs normotensive pregnancies. There was no significant difference for maternal age (p=0.7859) and parity (p=0.5188) across all groups. Similarly, maternal weight showed no significant difference across all groups (p=0.1478).

Table 1: Clinical demographics and patient data across all study groups (n=72)

	Preeclamptic HIV-positive (PE+) (n=18)	Preeclamptic HIV-negative (PE-) (n=18)	Normotensive HIV-positive (N+) (n=18)	Normotensive HIV-negative (N-) (n=18)	*p* value
Maternal age (years)	27.50 (34.50-24.00)	25.50 (38.50-19.00)	29.00 (30.25-25.75)	25.00 (32.00-20.00)	0.8046
Parity	2 (4-0)	1 (4-0)	1 (3-0)	1 (4-0)	0.5188
Gravidity	3 (5-1)	1 (2-1)	2 (4-0)	2 (4-1)	0.0125*
Gestational age (weeks)	32 (39-24)	35 (40-25)	39 (42-26)	40 (41-37)	<0.0001***
Systolic blood pressure (mmHg)	167.50 (190-149)	168 (206-146)	120.50 (134-99)	119 (132-92)	<0.0001***
Diastolic blood pressure (MmHg)	103.50 (146-89)	105.50 (130-72)	76.50 (90-32)	70 (82-52)	<0.0001***
Maternal weight (kg)	69.50 (106.80-54)	75.50 (120-50.60)	68 (81-55.20)	75 (94-58)	0.1478

Data represented as median and interquartile range. * *p value* < 0.05 was considered statistically significant. #Parity and Gravidity represented as median and range.

Concentration of serum ET-1

Pregnancy type

Regardless of HIV status, there was a significant increase of serum ET-1 in PE (median = 14.02 pg/mL, IQR = 23.47 – 0.19 pg/mL) compared to normotensive (median = 3.94 pg/mL; 95% CI: 11.66 – 4.68) group (Mann-Whitney U=468.50; *p*=0.0418) (Figure 1a).

HIV status

There was no significance difference in the expression of ET-1 between HIV positive (median = 11.31 pg/mL, IQR = 18.14 – 0.13 pg/mL) versus HIV negative (median = 6.04 pg/mL; 95% CI: 15.76 – 6.94 pg/mL) groups irrespective of pregnancy type (Mann-Whitney U=631.00; *p* = 0.8512) (Figure 1b).

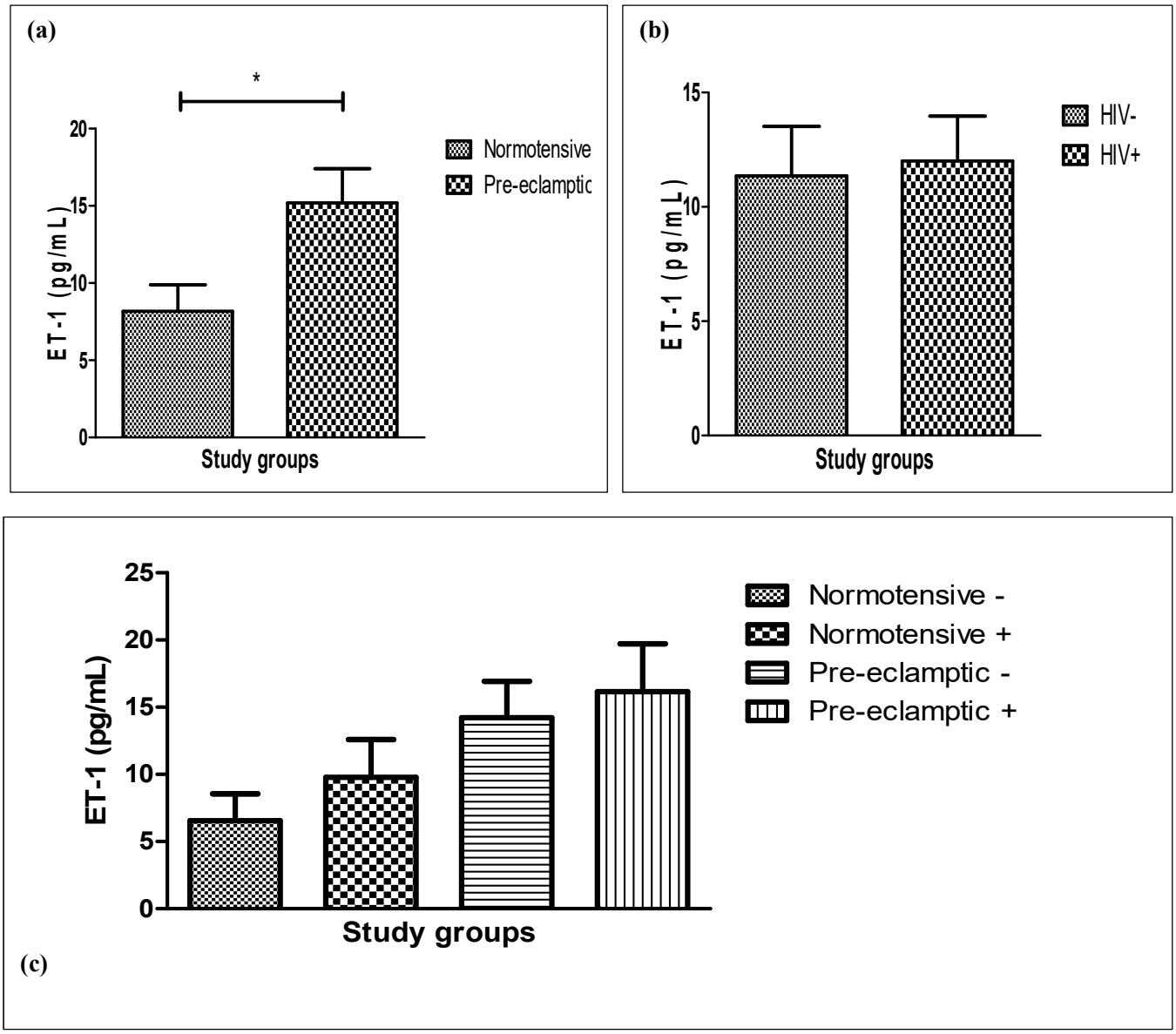
Across all groups

There was no significant difference detected across all study groups (p = 0.2349) as illustrated in Table 2 and Figure 1c.

Table 2: Concentration of ET-1 (pg/ml) observed across all groups

	Preeclamptic pregnancies		**Normotensive pregnancies**		
	Preeclamptic HIV positive (n=18)	**Preeclamptic HIV negative (n=18)**	**Normotensive HIV positive (n=18)**	**Normotensive HIV negative (n=18)**	***p*- value**
ET-1 (pg/ml)	15.24 (0.13 -25.33)	12.67 (2.99-23.94)	6.04 (0.13-14.63)	3.94 (0.38-11.99)	p = 0.2349

Value are represented as median and interquartile range



ET-1: endothelin-1; N-: normotensive negative; N+: normotensive positive; PE-: preeclamptic negative; PE+: preeclamptic positive

Figure 1: Endothelin-1 concentrations, (a) normotensive vs preeclamptic, * ET-1 concentrations are significantly increased in preeclamptic vs normotensive ($p = 0.0418$); (b) HIV positive *vs* HIV negative, no significant difference was noted ($p = 0.8512$); (c) across all groups, no significant difference was observed ($p = 0.2349$).

DISCUSSION

This study demonstrates an upregulation of serum ET-1 concentration in PE compared to normotensive pregnant women, regardless of their HIV status. This finding is corroborated by various other studies (20-

24). Our findings were predicted as ET-1 is a potent vasoconstrictor which plays a role in the regulation of the vascular tone in PE (25). The peptide advances ventricular and vascular remodeling, acute and chronic increases in vascular resistance and inflammation in models of heart failure (26). Existence of endothelial damage linked with increased ET-1 is crucial in PE development. ET-1 upregulation in the hyperinflammatory state of PE may be due to leakage to the systemic circulation caused by endothelial damage, increase in placental production and feedback mechanism between NO and ET-1 (27, 28).

Pathophysiological conditions such as hypoxia as well as hormones such as thrombin, adrenaline, vasopressin, angiotensin II (Ang II), insulin and cytokines upregulate ET-1 production (26, 29). (30) demonstrated increased expression of hypoxia-inducible factor-1 (HIF-1α) in PE implicating placental hypoxia and endoplasmic reticulum stress in the development of PE.

Shear stress regulates the production and release of ET-1 from endothelial cells. The shear stress receptor of endothelial cells triggers an increase in the rate of blood flow eliciting vasodilation (31). However, failure of shear stress-mediated dilation in myometrial arteries may contribute to the defective trophoblast cell invasion and consequential decreased blood flow to the neonate, as observed in PE (32). As a result of shear stress receptor activation, NO is produced and released from the endothelium (33).

Endothelial dysfunction also affects the action of ET-1 through the bioavailability of nitric oxide (NO) which opposes the vasoconstrictive nature of ET-1 (33). A study by (34) supports the hypothesis that a decrease in NO production inhibits endothelium-dependent vasorelaxation which disturbs vascular homeostasis and elicits endothelial dysfunction.

In our study, based on HIV status, ET-1 was slightly increased albeit non-significantly in HIV positive compared to HIV negative participants, regardless of pregnancy type. Similar to our findings, Feijoo *et al*. (2014) reported higher plasma levels of ET-1 in HIV infected patients compared to non-infected patients. The mechanism attributed to the increase in ET-1 in the HIV infected population is not clearly elucidated. Presumably, ET-1 release is a product of endothelial damage caused by chronic inflammation emanating from the viral infection (35).

Notably, HIV-related proteins such as gp120 stimulate the production of ET-1 from endothelial cells whilst the accessory protein *Tat* boosts ET-1 production in astrocytes (36). The HIV envelope protein gp120 is suggested to cause pulmonary vascular damage (37).

The increase in ET-concentrations in our HIV-infected cohort may also be attributed to the usage of antiretroviral drugs *viz*., AZT, indinavir and highly active antiretroviral therapy (HAART) (38, 39). These nucleos(t)ide reverse transcriptase inhibitors (NRTIs) inhibit the action of DNA polymerase γ, responsible

for mitochondrial DNA replication (40). The decreased mitochondrial respiration leads to increased production of reactive oxygen species (ROS), which in turn stimulates the release of circulatory ET-1 (38). Moreover, HAART induces a dramatic increase in the release of ET-1 from human umbilical vein endothelial cells (38).

(41) stated that HIV associated PE represent opposing immune responses. (42) supports this statement suggesting that HIV immune-depressive effects there is a link between HIV and PE. Additionally, HIV infection could inhibit factors that impacts the development of PE (43). The risk of developing PE is increased in HIV positive women compared to uninfected women and even higher in women receiving HAART therapy (7). In the pre-HAART period, PE was an uncommon complication of pregnancy in HIV-infected women compared to the general population (44). HAART induces immunocompetence in HIV infected pregnant women predisposing them to PE development compared to a failing immune system where PE will fail to thrive on (8). Similar findings were observed in a study conducted in SA by Maharaj *et al*., (2017) who reported that HAART rebuilds the immune response thereby exacerbating the already enhanced inflammatory response in PE.

CONCLUSION

This study demonstrates a significant upregulation of serum ET-1 levels in PE compared to normotensive pregnancy, validating its potent vasoconstrictor effect. We also report an elevated trend in ET-1 expression in HIV positive compared to HIV negative pregnant women. This finding is novel and mirrors the effect of HIV envelope gp 120 and accessory proteins, *Tat* on ET-1 production in the inflammatory ambiance of PE. Notable ET-1 levels remained unchanged despite the synergy of PE and HIV infection in our study population emanating from HAART induced oxidative stress. However, more studies on the immune rejuvenation induced by HAART should be done to further understand the impact it has on the duad HIV associated PE. Assessing ET-1 activity in PE and HIV infection may lead to potential therapeutic strategies manage hypertension in pregnancy.

DECLARATION OF INTEREST

There are no conflicts of interest.

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CHAPTER THREE

SYNTHESIS

Maternal Mortality as described by World Health Organization (WHO) is the death of a pregnant women or within 42 days following termination of pregnancy, irrespective of the site and duration of pregnancy, from any cause aggravated by pregnancy, but not from accidental causes (Alkema *et al*., 2016). According to WHO, an estimated 800+ women die per day due to pregnancy or obstetric complications with most deaths recorded in sub-Saharan Africa and Asia (Kassebaum *et al*., 2014). HIV infection in the sub-Saharan Africa region is responsible for 6.4% of all maternal deaths when compared to other regions across the globe (Say *et al*., 2014).

At the Millennium Summit in 2000, world leaders pledged to reduce maternal mortality ratio (MMR) by 75% from 1990 to 2015 as part of the Millennium Development Goals (MDGs) (Say *et al*., 2014), however the progress of the MDG5 was slower than required (Alkema *et al*., 2016). There was the introduction of the Sustainable Development Goals (SDGs) in 2015, which also targeted to reduce the global MMR to less than 70 deaths per 100 000 livebirths by the year 2030 (WHO, 2015).

Preeclampsia (PE), an obstetric disorder that complicates 3-8% of global pregnancies and a major cause of maternal mortality and morbidity worldwide (Carty *et al*., 2010). Approximately 16% of all maternal deaths in developed countries and 9% in Africa is attributed to PE (Khan *et al*., 2006, Alkema *et al*., 2016). The prevalence of PE in KwaZulu-Natal is 12% (Gathiram and Moodley, 2016) and HIV infection in pregnant women is estimated at 41.1% (Woldesenbet *et al*., 2017).

Endothelin-1 (ET-1) is a peptide from the cytokine family and a potent vasoconstrictor. It is secreted by endothelial cells (Yanagisawa *et al*., 1988) and placental syncytiotrophoblasts (Saleh *et al*., 2016b). ET-1 acts through two receptors; ET_A and ET_B, these receptors are responsible for vasoconstriction and vasodilation respectively (Schiffrin, 1995; Deng *et al*., 1996). Endothelial cell dysfunction is a primary pathophysiological complication linked with PE development (Maynard *et al*., 2003).

This study demonstrates an upregulation in endothelin-1 concentration in PE compared to normotensive pregnancy, irrespective of HIV status. This elevation is attributed to the increased activity of endothelin converting enzyme (ECE) found in the circulation of PE (Ajne *et al*., 2003; Ouellette and Hazelzet, 2011). About 60% of circulating ET-1 is removed when the plasma passes through the pulmonary circulation the first time which leads to an increase in ET-1 in hypertensive pregnancy (De Nucci *et al*., 1988). Endothelial damage and endotheliosis arising from the imbalance between vasoconstrictor and vasodilator factors may increase the levels of ET-1 in circulation leading to PE development (Boura *et al*., 1994). The increase in circulating levels of tumor necrosis factor-α (TNF-α) and the agonistic angiotensin 11 type-1 receptor auto-antibody (AT1-AA) results in the increase of ET-1 production which leads to maternal hypertension

(LaMarca *et al*., 2011). Moreover, excessive production of ET-1 is linked to pulmonary arterial hypertension (PAH) and endothelial dysfunction (Humbert *et al*., 2004). It is therefore plausible that the elevation of ET-1 expression in PE noted in our study may be associated with the widespread endothelial dysfunction that characterises PE. However, a study by Mastrogiannis *et al*., (1991) reported that intravenous incorporation of magnesium sulfate significantly decreases ET-1 levels in PE women.

Based on HIV status, the serum concentration of ET-1 was slightly elevated (non-significant) in HIV positive compared to HIV negative participants, regardless of pregnancy type. Mechanisms attributed to the increase of ET-1 in the HIV infected population are not clearly expounded. However, chronic inflammation from viral infection causes endothelial damage which results in ET-1 production (Mazzuca *et al*., 2018). Notably, viral glycoprotein gp120 stimulates the secretion of ET-1 from endothelial cells (Sandoval-Gutierrez *et al*., 2015). ET-1 production in astrocytes is enhanced by the active viral accessory protein *Tat* (Freeman *et al*., 2014).

Importantly, the ET-1 increase noted in our study may be attributed to the usage of antiretroviral drugs such as HAART (Yuhki *et al*., 2001). Additionally, across all study groups we demonstrate an increase of ET-1 in HIV positive PE versus HIV positive normotensive pregnant women. Increased levels in the HIV-positive PE group are suggestive of the restoration of immune response in PE induced by HAART.

CONCLUSION

Our study demonstrated the upregulation of ET-1 in PE compared to normotensive pregnant women irrespective of HIV status emanating from its powerful vasoconstrictive nature. This is the first time that increased trend of ET-1 albeit non-significant expression in HIV positive compared to HIV negative pregnant women has been demonstrated. This elevation was attributed to the increased production of ET-1 triggered by viral proteins *Tat* and gp120. It is also plausible that HAART may have influenced ET-1 expression. Additionally, these results highlight the need for further investigation on the immune reconstitution effects of HAART during pregnancy, and the potential of immune inflection therapy for the supervision of PE, where treatment remains elusive.

CHAPTER FOUR

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CHAPTER FIVE

APPENDICES

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

24 May 2019

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

Dear Prof Naicker

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

We wish to advise that your request dated 30 April 2019 to add the studies below to the above study has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee.

Studies added to the above study:

STUDENT	TITLE	DEGREE
Seke Nzau MAfuika	The role of Tenascin C in HIV associated preeclampsia	MMedSci
Samukelisiwe Sibiya	The role of human complement proteins C3b/iC3b and C4 in HIV associated preeclampsia	MMedSci
Phumelelle Kikine	The role of human complement proteins Factor B and Factor P/Properdin in HIV associated preeclampsia	MMedSci
Zinhle Pretty Mlambo	The role of Apolipoprotein A1 and A2 in HIV associated preeclampsia	MMedSci
Saieshni Pillay	The role of VEGFR-3 in the placenta and placental bed in HIV associated preeclampsia	MMedSci
Girija Naidoo	The role of soluble E-selectin and Thrombospondin-2 in HIV associated preeclampsia	MMedSci
Mbuso Herald Mthembu	The role of Endothelin-1 in HIV associated preeclampsia	MMedSci

The committee will be notified of the above approval at its next meeting to be held on 11 June 2019.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee