CELL SIGNALING EXPRESSION OF STAT-3 AND MEK-1 IN HIV-ASSOCIATED PRE-ECLAMPSIA

by

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PREFACE

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

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

Sayuri Padayachee **Professor Thajasvarie Naicker**

(212511676) **(Supervisor)**

DECLARATION

I, Sayuri Padayachee 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,

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Signed: _____ Date: _____24/01/2018_____

DEDICATION

To my grandparents,

You have taught me through prayer and perseverance, the world is yours.

The person I am today, I owe all to you.

I love you, Amma and Appa, Ma and Dada.

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- I have undoubtedly reached this pinnacle of success through the encouragement of my parents. The morals and values which they have instilled in me, as well as their firm affirmation of the impact of education on one's life, continuously inspires me to strive for the best;
- Finally, it is through the continuous support and guidance of the rest of my family that I have been able to further my academic studies. Words will not suffice to express my appreciation.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

HIV……	Human immunodeficiency virus
AIDS……..	Acquired immunodeficiency virus
PE…….....	Pre-eclampsia
EOPE…….	Early onset pre-eclampsia
LOPE…….	Late onset pre-eclampsia
WHO……	World Health Organization
EVT……	Extravillous trophoblast cells
vs……	Versus
ROS……..	Reactive oxygen species
IL…….	Interleukin
AART…….	Active antiretroviral therapy
HAART……..	Highly active antiretroviral therapy
JAK-STAT…….	Janus activated kinase-signal transducer and activator of transcription pathway
DNA……	Deoxyribonucleic acid
HGF……	Hepatocyte growth factor
LIF……..	Leukemia inhibitory factor
MMP…….	Matrix metalloproteinase
SOCS……..	Suppressor of cytokine signaling
PIAS…….	Protein inhibitor of activated STAT
PTPs…….	Protein tyrosine phosphates
MAPK…….....	Mitogen-activated protein kinase

MAPKKK……	Mitogen-activated protein kinase kinase kinase
MAPKK……..	Mitogen-activated protein kinase kinase
ERK…….....	Extracellular regulated kinase
MEK-1…….	Mitogen-activated kinase/ERK kinase 1/2
S1P……	Sphingosine-1-phosphate
S1PR2……...	Sphingosine-1-phosphate receptor 2
CDKs…….	Cyclin dependant kinases
VEGF…….	Vascular endothelial growth factor
PIGF…….....	Placental growth factor
ELISA……...	Enzyme-linked immunosorbent assay
SA-PE……	Streptavidin-phycoerythrin conjugate
MFI…….	Median fluorescent intensity
BP…….....	Blood pressure
DC……..	Dendritic cell
mRNA……..	Messenger ribonucleic acid
MHC……...	Major histocompatibility complex
Egr-1……...	Early growth response protein 1
JNK…….	JUN N-terminal kinase

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ABSTRACT

Background: Sub-Saharan Africa remains the epicenter of the HIV pandemic. In South Africa 35.8% of maternal deaths are ascribed to non-pregnancy related infections, namely HIV infection. The prevalence rate of HIV in pregnancy in KwaZulu-Natal is an estimated 37.7%. Furthermore, in women of reproductive age, the incidence of HIV infection is high (18.8%). The shallow trophoblast invasion and the lack of physiological conversion of the spiral arteries into small bore low resistance conduits is the primary cause behind the pathogenesis of pre-eclampsia. HIV-positive patients on HAART are predisposed to pre-eclampsia development. Trophoblast cells are stimulated to invade the maternal decidua by interacting cytokines and growth factors in the local environment through signal transduction pathways. Aberrant cell signaling and signaling molecule function impedes trophoblast invasion necessary for a healthy pregnancy, leading to endothelial dysfunction. As a result, the expression of cell signaling analytes may potentially be a predictive biomarker for patients at risk to pre-eclamptic development. This study investigated expression of STAT-3 and MEK-1 in HIV-associated pre-eclampsia, as a diagnostic tool of abnormal placentation in pre-eclampsia.

Method: Venous blood samples of 40 normotensive and 40 pre-eclamptic patients further stratified by HIV status were collected at a large regional hospital for buffy coat extraction, followed by analysis of cell signalling analytes by the Bio-Plex Multiplex immunoassay.

Results: The downregulation of both STAT-3 and MEK-1 expression was observed in pre-eclamptic pregnancies irrespective of HIV status, respectively ($p < 0.05$; $p = 0.1526$). No significance for STAT-3 ($p = 0.0859$) was detected between HIV-positive and HIV-negative groups irrespective of pregnancy type, although a lowered trend was observed in the HIV-positive group. Contrary to expected outcomes, MEK-1 expression was significantly decreased ($p = 0.0102$) in the HIV-positive group, irrespective of pregnancy type.

Conclusion: This study demonstrated the downregulation of STAT-3 and MEK-1 expression in pre-eclampsia, corroborating a defective trophoblast invasion. The decreased expression of STAT-3 in HIV-infection may be attributed to HAART and/or to the reduced expression of IL-6 that stimulates STAT-3 phosphorylation. It is also plausible to assume that the downregulation of MEK-1 arises from non-phosphorylation of HIV-regulatory proteins. Additional studies, are required to further investigate the role of signal transduction pathways and its effect on HAART in pre-eclampsia development.

CHAPTER ONE

LITERATURE AND BACKGROUND REVIEW

1.1 HIV/AIDS

The Human Immunodeficiency Virus causes immunosuppression and is the leading global health crisis. Research on the pathogenesis, transmission and prevention strategies of the virus have evolved, yet a cure to the disease remains elusive (Simon *et al*., 2006).

1.1.1 Epidemiology of HIV/AIDS

Globally, there are an estimated 36.7 million people living with HIV-1 infection, with approximately 35 million people having died from AIDS related diseases. Antiretroviral therapy has been administered to 19.5 million people (UNAIDS, 2016). Statistics reveal that in 2016, approximately 1.8 million new HIV-1 infections emerged (UNAIDS, 2016). These estimates indicate the vigorous nature of this rapidly evolving epidemic, in relation to epidemiology, magnitude, viral diversity and modes of transmission (Inciardi and Williams, 2005).

1.1.2 HIV/AIDS in Southern Africa

Southern Africa is the epicentre of the HIV/AIDS pandemic with new HIV-1 infections continuously emerging (Hayes and Weiss, 2006). South Africa has the highest prevalence (12.7%) of HIV infection/AIDS in the world, with 7.03 million HIV-positive individuals (Statistics, 2016). Contributing factors to the spread of HIV include poverty, inequality and an unstable social environment, increasing rates of sexually transmitted infections, limited access to quality medical care and poor control and guidance in response to the disease (Zuma *et al.*, 2016). KwaZulu-Natal is considered the epicentre of this burden, with the highest prevalence (19.8%) amongst the provinces (Statistics, 2016).

1.1.3 HIV/AIDS amongst adolescent females

Approximately 70% of infected women live in sub-Saharan Africa. In general, 25-30% of all new HIV-1 infections occur in young adults (<25 years) (Dellar *et al*., 2015). In South Africa, this percentage is a translation of 113 000 new infections in young women annually, which is four-times more than the infection occurring in men (Dellar *et al*., 2015). However, HIV prevalence among the youth aged 15-24 yrs has declined to 5.6% in 2016. The greatest difference exists in this 15-24 age group, where for every one man infected, 3 women become infected. This is a serious dilemma for obstetricians as one-fifth of South African women in their reproductive age are HIV positive (Statistics, 2016).

The driving force behind the age-sex disparity in HIV acquisition is the increasing prevalence of young girls engaging in sexual activities with older men (Dellar *et al*., 2015). In addition to the age-sex disparity in HIV acquisition, the biological factors which also contribute to HIV acquisition includes large surface area of the genital mucosa, single layer of the immature cervical mucosa; increasing levels of activating immune cells in the female genital tract, increasing expression of co-receptors of HIV in cervical cells in comparison to foreskin cells and a mucosal surface with an increased likelihood of acquiring micro-abrasions during sexual practices (Dellar *et al*., 2015).

Furthermore, research has shown a link between HIV infection and hypertensive disorders in pregnancy such as pre-eclampsia (PE), as a result of the immune-depressive effects of HIV (Browne *et al.*, 2015).

1.2 Pre-eclampsia

Hypertensive disorders in pregnancy, contributes a large proportion of morbidity and mortality rates worldwide (Uzan *et al*., 2011). Pre-eclampsia is the development of hypertension and proteinuria during the second half of gestation in a woman who was previously healthy and normotensive (Huppertz, 2008).

The set of criteria that defines PE is characterized by new onset high blood pressure (≥140/90 mmHg) together with excessive protein (≥300mg protein) in urine during the second trimester (20 weeks) of pregnancy with/without oedema (Magnussen *et al*., 2007). In South Africa, hypertensive disorders of pregnancy account for 14% of maternal deaths (Saving Mothers Report, 2013).

Pre-eclampsia may also be classified according to gestational period. Early onset pre-eclampsia (EOPE) develops before 34 weeks of pregnancy and late onset pre-eclampsia (LOPE) develops at or after 34 weeks of pregnancy (Raymond and Peterson, 2011). EOPE is regarded as the more severe form and is considered as a fetal disorder. LOPE on the other hand, is considered as a maternal disorder and is associated with a normal functioning placenta, an increasing placental volume, normal growth of the fetus, normal birth weight and more favourable maternal and neonatal outcomes (Raymond and Peterson, 2011).

1.2.1 Epidemiology of pre-eclampsia

Approximately 7 – 10% of pregnancies worldwide are complicated by PE. The primary cause of maternal mortality in developing countries is PE exacerbated by limited health care accessibility. The only solution to PE is premature delivery of the placenta (Kalumba *et al*., 2013). According to the World Health Organization (WHO), the burden of PE in developing countries is approximately seven times greater than that in developed countries (Osungbade and Ige, 2011). African countries such as Ethiopia, Tanzania and Egypt have a pre-eclamptic incidence rate that ranges from 1.8% - 7.1% (Osungbade and Ige, 2011). In South Africa, 83% of maternal deaths emanating from hypertension (14.8%) is attributed to PE development. In KwaZulu-Natal the prevalence of pre-eclampsia is 12% (Saving Mothers Report, 2013).

There are a number of predisposing factors to PE development such as bacterial infection, immune maladaptation, inflammatory response and cytokine dysregulation (Perez-Sepulveda *et al.*, 2014). A mothers' risk of developing pre-eclampsia is influenced by age, primigravidae, primi-paternity, obesity, a

medical history of chronic conditions including hypertension; diabetes; kidney disease; frequent migraines; lupus; thrombophilia, and characteristics of pregnancy such as a molar or twin pregnancy, previous history of PE or fetal abnormality (Uzan *et al.*, 2011). Women aged above 40 years are at a higher risk of developing PE. The risk of PE also increases as the interval between pregnancies increase (English *et al.*, 2015). Furthermore, chronic conditions and obesity, specifically hypertension, predisposes the mother to a higher risk of PE development (English *et al.*, 2015).

Pre-eclampsia development can be attributed to a strong familial disposition of the disorder. Research has shown that normotensive women related to first-degree relatives with PE, are 5 times more likely to develop PE than second-degree relatives (Valenzuela *et al*., 2012). Additionally, paternal genes are also thought to play a role in developing PE, as evidenced by an increased risk of PE in women involved with men that have previously been involved in pre-eclamptic pregnancies (Valenzuela *et al*., 2012). Paternal genes in PE are of significant importance from a genetic aspect, as these genes play a role in controlling trophoblast invasion and placental growth (Valenzuela *et al*., 2012).

1.2.2 Pathogenesis of pre-eclampsia

The placenta is the fundamental organ to a healthy pregnancy. To effectively achieve healthy growth and development of the fetus, the placenta is required to bring the circulation of the fetus and mother into close contact, thus normal placentation is required for a successful pregnancy (Gude *et al*., 2004).

Extravillous trophoblast cells (EVT) proliferate from the tips of the anchoring chorionic villi, and after the first 2 weeks of gestation in a precisely timed sequence, columns of EVT cells invade the maternal decidua and into the inner myometrium. These invasive EVT cells are termed interstitial EVTs. Some EVT cells merge into the walls of the spiral arteries and are referred to as intramural trophoblasts (Van Der Spuy, 2002).

In another pathway, some trophoblast cells migrate through the lumen of the spiral artery, a process called endovascular invasion. This process of remodeling includes replacing the endothelium and media smooth muscle cells, replacing internal elastic lamina resulting in loss of elasticity, dilation of the arteries to wide bore sinusoids and the loss of vasomotor control (Raymond and Peterson, 2011). Therefore, the invasion of EVT cells influences the transformation of the high resistant muscular spiral arteries into distended, flaccid vessels of high capacitance and low resistance, enabling sufficient blood perfusion thereby sustaining the nutrient and oxygen demands of the fetus (Raymond and Peterson, 2011).

In PE however, placentation is inadequate (Naicker *et al*., 2003) as seen in figure 1, when comparing normal and abnormal pregnancies. Spiral artery remodeling is limited to the decidua. The myometrial artery lumen remains of small calibre and of high resistance. A state of under-perfusion persists, resulting in placental hypoxia and localised oxidative stress. This leads to a systemic inflammatory response causing endothelial dysfunction, culminating in the clinical manifestations of the syndrome (Valenzuela *et al*., 2012).

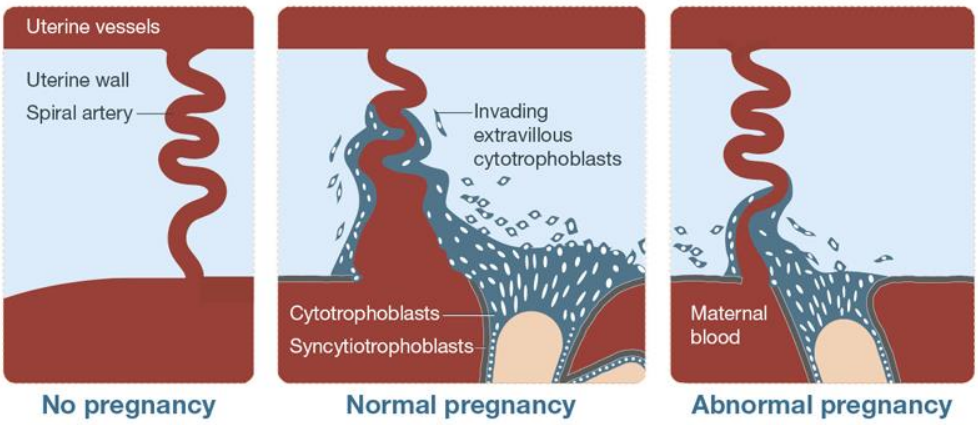

Figure 1: Normal placentation *vs* abnormal placentation (adapted from The Eunice Kennedy Shriver National Institute of Child Health and Human Development, 2017).

The enhanced maternal inflammatory response and dysfunction of endothelial cells is caused by the release of secretory factors from the maternal circulation including anti-angiogenic factors, apoptotic debris and inflammatory cytokines (Cindrova-Davies, 2009). Evidence has shown that the secretion of these factors is

induced by hypoxia and oxidative stress which have significant influence in the pathology of PE (Cindrova-Davies, 2009). The clinical manifestations of PE presented after 20 weeks of gestation occur as a result of the failure of spiral artery remodeling by trophoblast invasion (Hladunewich *et al*., 2007).

1.2.3 Aetiology of pre-eclampsia

One of the key elements in the pathogenesis of PE is oxidative stress, which causes the activation of signaling pathways hence the release of toxic and soluble factors into the maternal circulation (Cindrova-Davies, 2009). Reactive oxygen species (ROS) produced by the mitochondria are formed as a result of partially reduced molecular oxygen under normal physiological conditions. Oxidative stress usually results from excessively produced ROS, dysfunctional mitochondria or an impaired antioxidant system (Nita and Grzybowski, 2016). The overproduction of ROS intimidates the anti-oxidant defense mechanisms of the placenta, leading to a reduced anti-oxidant potential, as the maternal circulation of pre-eclamptic women contains a decreased concentration of ascorbic acid (Hubel, 1999). Despite the role of ROS in oxidative stress, strong evidence indicates that the defective conversion of the spiral arteries, subsequently results in placental insufficiency, the primary initiator of oxidative stress (Cindrova-Davies, 2009).

Moreover, the deficient trophoblast invasion in PE may be triggered by a modified maternal immune response or a defect in the maternal tolerance to the semi-allogeneic fetus (Perez-Sepulveda *et al*., 2014). Many groups have indicated that in the decidua, pre-eclamptic patients demonstrate an excessive activation of monocytes and neutrophils. Upon activation, these monocytes are spontaneously involved in the increased synthesis of pro-inflammatory cytokines including IL-1b, IL-6, and IL-8. Dendritic cells, $CD4^+$, $CD8^+$ T-lymphocytes and natural killer cells have a pro-inflammatory response in PE, rather than an anti-inflammatory and immuno-tolerant response in normal pregnancies (Perez-Sepulveda *et al*., 2014).

1.2.4 HIV associated pre-eclampsia

HIV infection is associated with immune suppression (Kalumba *et al*., 2013) whilst in normal pregnancies there is a slight elevation in the maternal immune response to the fetal allograft. In PE however, there is an exacerbation of this response (Perez-Sepulveda *et al*., 2014). Conflicting data on whether HIV infection increases, decreases or neutralises PE development exists (Hall, 2007). Also, highly active antiretroviral therapy (HAART) seems to influence pre-eclampsia development (Kalumba *et al*., 2013). HAART re-establishes immunocompetence in the infected individual, thus resulting in an active and improved immune response which leads to an increased susceptibility to PE, in comparison to a failing immune system in HIV-negative women which pre-eclampsia cannot thrive on (Kalumba *et al*., 2013; Maharaj *et al*., 2017). In light of the fact that both HIV infection and PE is high in South Africa, it is urgent that the relationship of this duality be investigated.

1.3 Cell signaling pathways

Cells and tissues express a variety of distinct receptor combinations that activate signaling pathways involved in regulating key biological processes such as cell proliferation, migration, invasion and apoptosis (Yang *et al*., 2014). Cell signaling begins through the binding of a signaling molecule to a cell surface receptor, and ends when DNA of the cell expresses a protein resulting in cell modulation (Rébé *et al*., 2013). A contributing factor relating to the modulation of apoptosis and invasion of trophoblast cells are cell signaling pathways, hence it is important elucidate their role in HIV-associated pre-eclampsia.

1.3.1 JAK-STAT signal transducing pathway

The migratory and invasive properties of EVT cells are functionally controlled by a surplus of cytokines and growth factors that commonly signal through the Janus kinase-signal transducer and activator of

transcription (JAK-STAT) pathway (Fitzgerald *et al*., 2008). The mammalian JAK family is constituted of four members, JAK-1, JAK-2, JAK-3 and TYK2. The mammalian STAT family comprises seven members, STAT-1, STAT-2, STAT-3, STAT-4, STAT-5A, STAT-5B AND STAT-6 (Shuai and Liu, 2003). STAT-3 exists as an inactive cytoplasmic form in cells (Rébé *et al*., 2013). Upon activation, STAT-3 translocates from the cytoplasm to the nucleus where it binds to cognate sequences of DNA, here it serves as a transcription activator for a vast array of genes (Zhang *et al*., 2015).

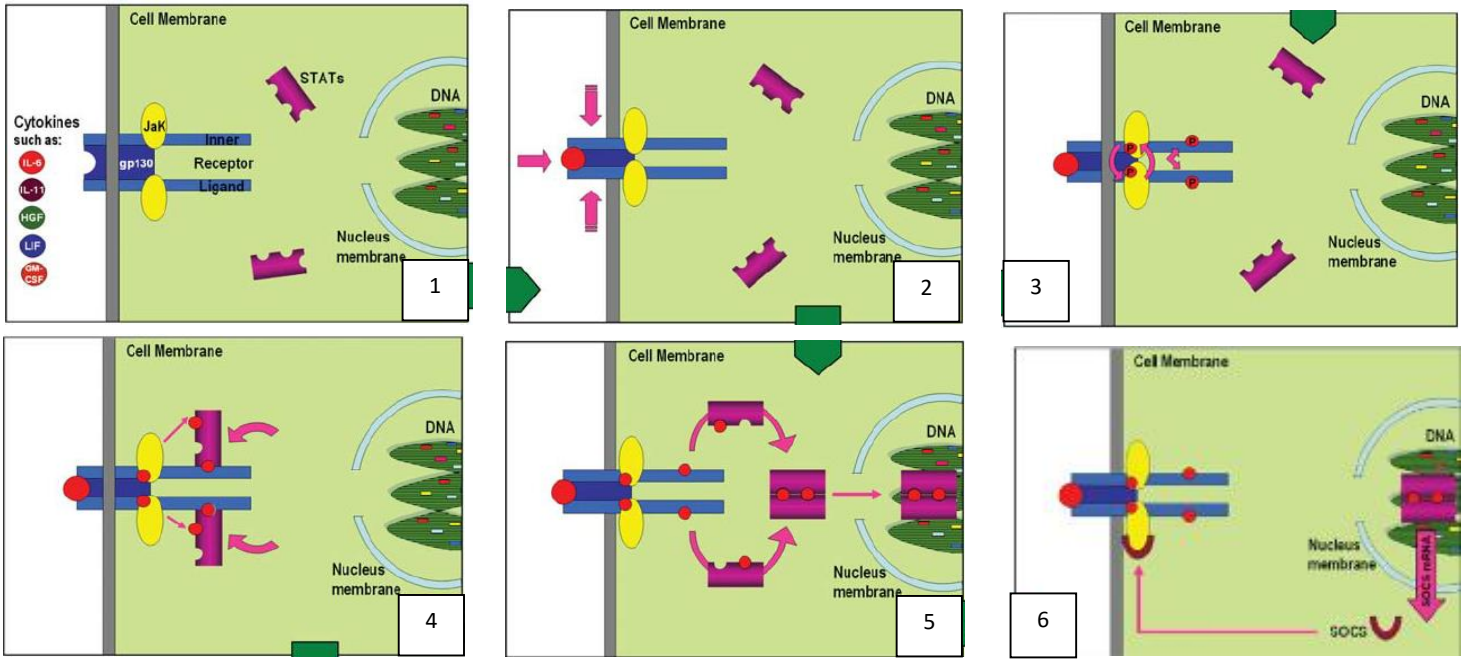

Figure 2: JAK-STAT activation (adapted from Fitzgerald *et al*., 2008).

Upon cytokine binding to inner receptor ligands in figure 2, JAKs activate and undergo phosphorylation resulting in activation of inner receptor ligands. Inactive STATs bind to inner receptor ligands to be phosphorylated by JAKs, resulting in their activation. Activated STATs dissociate and dimerize, to transcolate to the nucleus to begin transcription of target genes.

1.3.1.1 The role of STAT-3

During placentation, cytokines produced by trophoblast cells influence their migration and invasion. Such cytokines include the Hepatocyte growth factor (HGF), Interleukin-6 (IL-6), Interleukin-11 (IL-11) and the Leukemia inhibitory factor (LIF) that signal through the JAK-STAT pathway (Fitzgerald *et al*., 2008). The STAT-3 activating subunit plays a fundamental role in trophoblast invasion. In fact, phosphorylated STAT-3 enhances the invasive capabilities of trophoblast cells *in vitro,* which is necessary for a successful pregnancy (Zhang *et al*., 2015).

Expression of HGF promotes the invasive potential of trophoblast cells, as invasion is directly proportional to HGF dosage (Fitzgerald *et al*., 2008). It has been suggested that a lack of HGF production causes PE, as a study by Kauma *et al.,* 1999 revealed pre-eclamptic women had been associated with decreased production of HGF (Fitzgerald *et al.,* 2008). The IL-11 receptor located on interstitial trophoblast cells are known to boost their migratory capabilities without influencing their role in proliferation, and this is accomplished by the stimulation of phosphorylated STAT-3 (Fitzgerald *et al*., 2008). Cytotrophoblasts express high levels of IL-6, leading to the increased activity of proteins *viz.*, MMP-9 and MMP-2 that are significantly involved in invasion and implantation in pregnancy (Fitzgerald *et al*., 2008).

1.3.1.2 Inhibitors of STAT-3 activity

Negative regulators of the JAK-STAT pathway include the suppressor of cytokine signaling (SOCS) proteins, the protein inhibitor of activated STAT (PIAS) and protein tyrosine phosphates (PTPs) (Shuai and Liu, 2003). SOC proteins are immunolocalized to the syncytiotrophoblasts and it is postulated that a decrease in SOC activity may influence the overexpression of SOCS in pre-term labour (Fitzgerald *et al*., 2008). It is also possible that inflammatory cytokines occurring in infections during pre-term labour, could be responsible for the elevated levels of SOC expression (Fitzgerald *et al*., 2008).

The shortcomings of STAT-3 activity in trophoblast invasion may subsequently provoke the development of PE during placental development, because as placental apoptosis is increased, trophoblast cell invasion is concurrently decreased within the placental bed (Naicker *et al*., 2013; Zhang *et al*., 2015).

1.3.2 The MAPK/ERK pathway

The mitogen activated protein kinase (MAPK) pathway consists of the MAP kinase kinase kinase (MAP3K), MAP kinase kinase (MAPKK) and MAPK tier cascades involved in transmitting signals to target molecules to initiate the required physiological process (Figure 3; Shaul and Segar, 2007). Each tier of the pathway is composed of various protein kinases that phosphorylate from an upstream receptor, to a downstream target (Shaul and Seger, 2007). The extracellular regulated kinase (ERK) cascade is the main downstream component of the MAPK pathway (Shaul and Seger, 2007). ERK is activated by dual specific kinases MEK-1 and MEK-2. Activated ERK induces cell proliferation, differentiation and development and under certain conditions, cell survival, migration and apoptosis (Shaul and Seger, 2007).

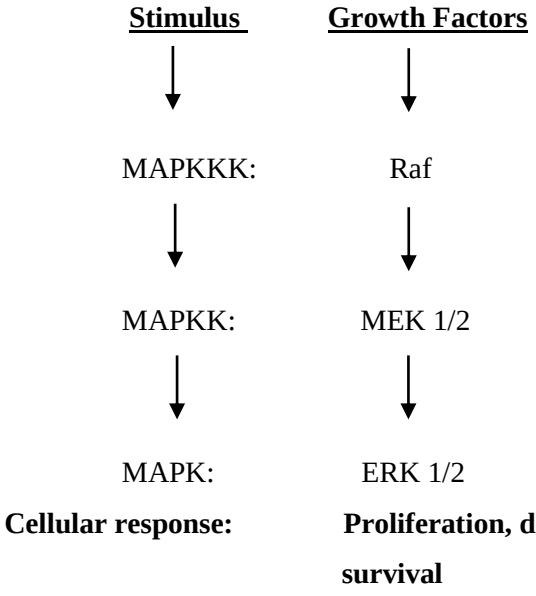

Figure 3: Schematic of the MAPK tier cascade and respective kinases for each pathway (adapted from Cowan & Storey, 2013).

1.3.2.1 The role of MEK-1

The MEK groups are three mammalian isoforms (MEK-1, MEK-1b and MEK-2) that share an 85% homology. MEK-1 is activated through the phosphorylation of residues SER218 and SER222 in the MEK-1 activation loop. In addition to being ERK activators, MEKs are stimulated to translocate to the nucleus of resting cells (Zheng and Guan, 1994).

MEK-1 plays a role in trophoblast invasion as a result of the invasive capabilities of EVT cells being tightly regulated throughout pregnancy, by various growth and regulatory factors within the microenvironment of the uterine endometrium and decidua (Graham and Lala, 1991). In most cells, sphingosine kinases play a role in the phosphorylation of sphingosine-1-phosphate (S1P) (Liu *et al.*, 2012). S1P is a signaling molecule phosphorylated from sphingosine, and binds to either one of five specific G protein-coupled receptors. These coupled receptors are responsible for the activation of the ERK pathway. Previous reports have revealed EVT cells promote the expression of S1P receptors, thereby permitting the regulation and migration of trophoblast cells via S1P receptors. Nevertheless, a lack of information exists on the influence of S1P on EVT cell invasion (Yang *et al*., 2014).

1.3.2.2 Inhibitors of MEK-1

EVT cell migration is inhibited by S1P via S1PR2, sphingosine-1-phosphate receptor 2 (Yang *et al*., 2014). S1PR2 is one of the G-protein-coupled receptors that are fundamentally involved in inhibiting angiogenesis and maintaining vascular stability. An earlier study has shown that activated S1PR1 can promote trophoblast invasion (Harapan and Andalas, 2015).

Cyclin dependant kinases (CDKs) play a significant role in regulating the cycle of cell progression (Shaul and Seger, 2007). Many studies have postulated that CDK1 and CDK5 are able to phosphorylate MEK-1,

thereby having an influence on MEK-1 activity, indicating that CDK phosphorylation inhibits MEK-1 (Shaul and Seger, 2007).

The predisposing factors to PE development as illustrated in figure 4 include immune maladaptation, inflammatory response and cytokine dysregulation. However, regarding HIV-associated PE, the innate immune response is the most significant (Bounds *et al*., 2015).

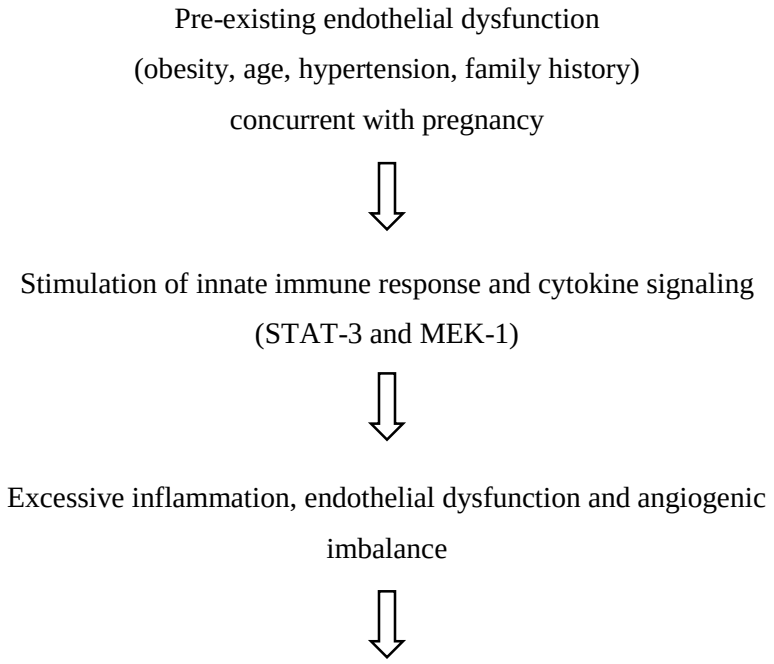

Figure 4: Schematic of factors involved in pre-eclampsia development (adapted from Bounds *et al*., 2015)

1.4 Aims and objectives

Research on serum markers with decent detection rates can pave the way for prevention strategies; and therefore improved overall antenatal and perinatal care. Therefore, this study targeted how stress and signaling pathways influence villous trophoblastic function in distinct subforms of pre-eclampsia by the

investigation of kinases involved in the regulation of cell survival, proliferation, differentiation, apoptosis, and the release of inflammatory mediators and antiangiogenic factors.

Since apoptosis is elevated in pre-eclampsia, the aim of this study is to investigate the role of cell signalling analytes *viz.*, STAT-3 and MEK-1 in HIV-associated pre-eclampsia and normotensive patients using the Bio-Plex Multiplex Immunoassay.

Objectives of this study include:

- To investigate STAT-3 and MEK-1 expression across all study groups using BioPlex Multiplex Immunoassay.
- To compare STAT-3 and MEK-1 expression based on pregnancy type (normotensive *vs* pre-eclamptic), regardless of HIV status.
- To compare STAT-3 and MEK-1 expression based on HIV status (positive *vs* negative), regardless of pregnancy type.

CHAPTER 2

HYPERTENSION
IN
PREGNANCY

THE ROLE OF THE PROTEIN KINASES STAT-3 AND MEK-1 IN HIV-ASSOCIATED PRE- ECLAMPSIA

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Keywords:	Pre-eclampsia, HIV, STAT-3, MEK-1, HAART

THE ROLE OF THE PROTEIN KINASES STAT-3 AND MEK-1 IN HIV-ASSOCIATED PRE-ECLAMPSIA

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Abstract:

Objective: Regulation of trophoblast invasion is mediated through cell signaling, therefore this study investigated protein kinases STAT-3 and MEK-1 in HIV-associated pre-eclampsia. *Method:* STAT-3 and MEK-1 expression were analysed by Bioplex immunoassays in 80 normotensive and pre-eclamptic women stratified by HIV status. *Results:* STAT-3 expression differed by pregnancy type (p=0.001) and not HIV status (p=0.0859), while MEK-1 differed by HIV status (p=0.0102) and not pregnancy type (p=0.1526). *Conclusion:* This study demonstrates a downregulation of STAT-3 and MEK-1 in pre-eclampsia, corroborating limited trophoblast invasion. HAART and non-phosphorylation of HIV regulatory proteins may account for the downregulation of STAT-3 and MEK-1, respectively

Keywords: Pre-eclampsia, HIV, STAT-3, MEK-1, HAART

Running title: Signal analytes in HIV-associated pre-eclampsia

Introduction

South Africa has the highest prevalence (12.7%) of HIV/AIDS in the world, with 7.03 million of the population being HIV positive (1). The HIV prevalence amongst antenatal women is 29.5%, hence an obstetric predicament. KwaZulu-Natal Province is regarded as the epicentre of this health burden, with an HIV prevalence rate of > 30% (2).

Research has shown a possible link between HIV and pre-eclampsia (PE) probably because of the immuno-depressive effects of HIV infection (3). Pre-eclampsia is a multisystem disorder complicating 5-10% of all pregnancies globally and accounts for considerable maternal and neonatal morbidity and mortality worldwide (4). Pre-eclampsia is characterized by sustained new onset high blood pressure (BP) (≥140/90 mmHg) and proteinuria (≥300mg protein) after 20 weeks gestational age (5). Because the exact aetiology of PE is unknown, clinical management is empiric and cure is achieved by delivery of the baby and the placenta (4).

Given the high frequencies of HIV infection and PE in South Africa, and the fact that both disorders are associated with changes to the immune system mainly a decreased cell mediated immunity and an exaggerated inflammatory response respectively, it seems reasonable to assume that opposing immune responses maybe neutralised (6). This decrease in cell mediated immunity in HIV infection can largely be contributed to the widespread use of highly active antiretroviral therapy (HAART) (7). HAART is successfully involved in the reduction of plasma HIV-1 viral load, rates of vertical transmission and delayed response of viral resistance in pregnancy (8, 9). Also, studies have shown that HIV-positive pregnant women not receiving antiretroviral therapy (ART), are less inclined to develop PE compared to those on HAART (10). According to Wimalasundera *et al,* 2002 a suppressed immune response in HIV-1 infection associated with a decreased CD4 cell count, may elucidate the low rate of pre-eclampsia development in untreated HIV-positive pregnant women. The association between PE and HIV/AIDS in South Africa provides the perfect backdrop for studies in this disease duality.

Pre-eclampsia is associated with poor placentation and uterine spiral artery remodeling which results in placental hypoxia and localized oxidative stress due to a state of under-perfusion (11). This leads to an exacerbated systemic inflammatory response as a result of defective trophoblast invasion to promote spiral artery remodeling (12). Defective trophoblast invasion leads to the release of excretory factors from the maternal circulation culminating in an enhanced maternal inflammatory response and of the pathognomonic endothelial cell lesion (13).

The migratory and invasive properties of extravillous trophoblast cells (EVT) are functionally controlled by many cytokines and growth factors that signal through the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway (14). The STAT-3 activating subunit, a member of the STAT mammalian family, plays a fundamental role in trophoblast invasion *in vitro* as phosphorylated STAT-3 enhances the invasive capabilities of trophoblast cells, a significant requirement for a successful pregnancy (15).

The mitogen activated protein kinase (MAPK) pathway is constituted of three protein kinase tier cascades (16). The extracellular regulated kinase (ERK) cascade is the main downstream component of the MAPK pathway (17). ERK is activated by dual specificity kinases MEK-1 and MEK-2. Activated ERK induces cell proliferation, differentiation and development and under certain conditions, cell survival, migration and apoptosis (18). MEK-1 regulates EVT invasive capabilities by various growth and regulatory factors within the microenvironment of the uterine endometrium and decidua (19).

Since STAT-3 AND MEK-1 are contributing factors in the invasion of trophoblast cells it is vital that their expression levels are examined in HIV-associated PE (13). Hence this study will focus on cell signalling analytes *viz.*, STAT-3 and MEK-1 in women based on pregnancy type (normotensive *vs* pre-eclamptic) and HIV status (HIV+ve *vs* HIV–ve).

Methods and materials

Study population

Ethics approval (BE210/17) and regulatory health authority permission were obtained. Pregnant women were purposively recruited from the antenatal clinic at a large district hospital in Durban, SA. The study population (n=80) determined by Fischer's test, consisted of a normotensive pregnant group (n=40) and a PE group (n=40). Each group was further stratified by HIV status into (1) HIV-positive normotensive (n=20); (2) HIV-negative normotensive (n=20); (3) HIV-positive PE (n=20) and (4) HIV-negative PE (n=20). Pre-eclampsia was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg with at least a two plus of proteinuria on dipstick testing after 20 weeks of pregnancy. The HIV status was determined by two rapid bed-side tests and CD4 counts were performed if the woman was HIV-positive. All newly diagnosed HIV-positive women were initiated on combined anti-retroviral therapy. Exclusion criteria was based on patients that declined participation and those with chorioamnionitis, eclampsia, polycystic ovarian syndrome, abruption placentae, intrauterine death, sickle cell disease, chronic renal disease, cardiac disease, unknown HIV status, pre-existing seizure disorders, asthma and patients that are not booked into the hospital.

Following written informed consent, venous blood samples were collected in K3-EDTA tubes, buffy coat extracted and stored in cryovials at -80°C until immunoassay.

Bio-Plex Multiplex immunoassay

The buffy coat (leucocyte suspension) was used to assess differences in median fluorescent intensity (MFI) of phosphorylated STAT-3 (S727) (1:4 dilution) and phosphorylated MEK-1 (S217/221) (1:4 dilution) using the Bio-Plex® Pro™ cell signaling MAPK Panel 2-Plex assay (catalogue #LQ0-0000S6KL81S; Bio-Rad Laboratories Inc., South Africa). This immunoassay was performed according to the manufacturer's instructions.

This assay follows a similar technique to that of a sandwich ELISA, following incubation of antigen samples in the study *viz.,* STAT-3 and MEK-1 with the capture antibody-coupled magnetic beads. Biotinylated detection antibodies were added to the reaction, along with a reporter conjugate, streptavidin-phycoerythrin conjugate (SA-PE). After washing, detection of the reaction was carried out using Bio-Plex®MAGPIX™ Multiplex Reader (Bio-Rad Laboratories Inc., USA). The fluorescence of the SA-PE bound to each bead is measured by the reader. Data from the Bio-plex multiplex analysis was obtained using the Bio-Plex Manager™ software version 4.1.

The measurement of analyte expression for a given bead population in each study group was provided as the median fluorescent intensity (MFI). The relative concentration of analyte bound to each bead is equal to the MFI.

Statistical analysis

Data was analysed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego, California USA). Statistical analysis across all groups of the sample population were based on HIV status and pregnancy type, using a One-way ANOVA and the Bonferroni post hoc multiple comparison test. The level of significance was considered as a probability level of $p < 0.05$. For individual analysis, a student's t-test was performed.

Results

Clinical characteristics and demographic data

As seen in table 1 across the study groups, a significant difference was detected for gestational age ($p < 0.001$) and systolic and diastolic BP ($p < 0.001$). Maternal age, gestational age, parity, systolic and diastolic BP and maternal weight were similar between HIV-positive *vs* HIV-negative groups ($p < 0.05$). Albeit non-significant, parity (2.0±0.21 *v*s 1.6±0.15; $p > 0.2370$); systolic BP (131±4.5 *vs* 130±5.4mmHg; $p > 0.6543$) and diastolic BP (85±2.9 *vs* 83±4.0mmHg; $p > 0.9005$) were elevated in HIV-positive *vs* HIV-negative women.

Statistical significance was detected for gestational age (p = 0.0009), parity (p = 0.0401) and systolic and diastolic BP (p < 0.0001). Maternal age (30±2.5 *vs* 28±0.91years; p = 0.8105); parity (2.0±0.17 *vs* 1.5±0.19; p = 0.0410); systolic BP (154±2.4 *vs* 109±3.7mmHg; p < 0.0001); diastolic BP (100±1.3 *vs* 69±2.8mmHg; p < 0.0001) and maternal weight (85±2.9 *vs* 80±2.0kg; p = 0.1931) were higher in pre-eclamptic *vs* normotensive pregnancies.

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

	HIV –ve normotensive	HIV +ve normotensive	HIV –ve pre - eclamptic	HIV +ve pre - eclamptic	p-value
Maternal Age (years)	28±1.2	28±1.4	30±4.4	31±1.8	0.8534
Gestational Age (weeks)	38±0.25	38±0.27	36±0.77	34±1.1	0.0003 (***)
Parity	1.4±0.22	1.6±0.3	1.8±0.2	2.4±0.27	0.0815
Systolic BP (mmHg)	108±6.8	111±1.9	154±3.1	154±3.8	<0.0001(***)
Diastolic BP (mmHg)	66±5.1	72±1.3	100±1.8	100±2.1	<0.0001(***)
Weight (kg)	80±2.5	80±3.2	88±3.4	81±4.9	0.3322

Mean ± SEM are presented; One-way ANOVA and the Bonferroni post hoc multiple comparison test was used for statistical analysis, *n* = 80 ***p < 0.001

Cell signaling analytes

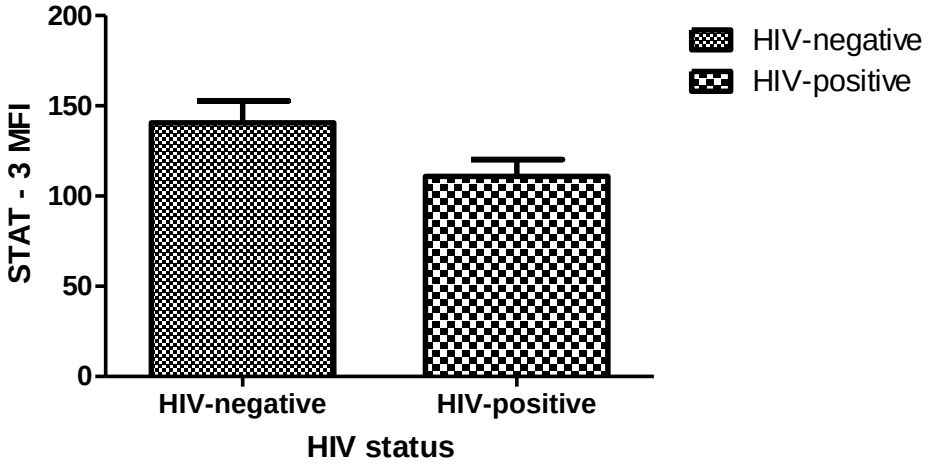
STAT-3 expression:

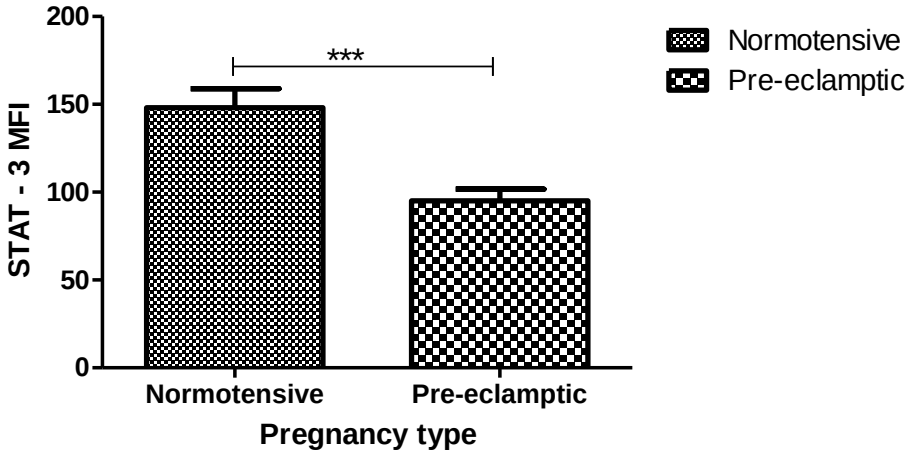
HIV status: As seen in figure 1A, based on HIV status, no significant difference (p = 0.09) was detected for STAT-3 expression between HIV-positive and HIV-negative groups (110 ± 9.3 *vs* 140 ± 12 MFI).

Pregnancy type: A level of significance ($p < 0.05$) was detected for STAT-3 expression between pre-eclamptic and normotensive (95 ± 6.5 *vs* 150 ± 11 MFI) pregnancies (Figure 1B).

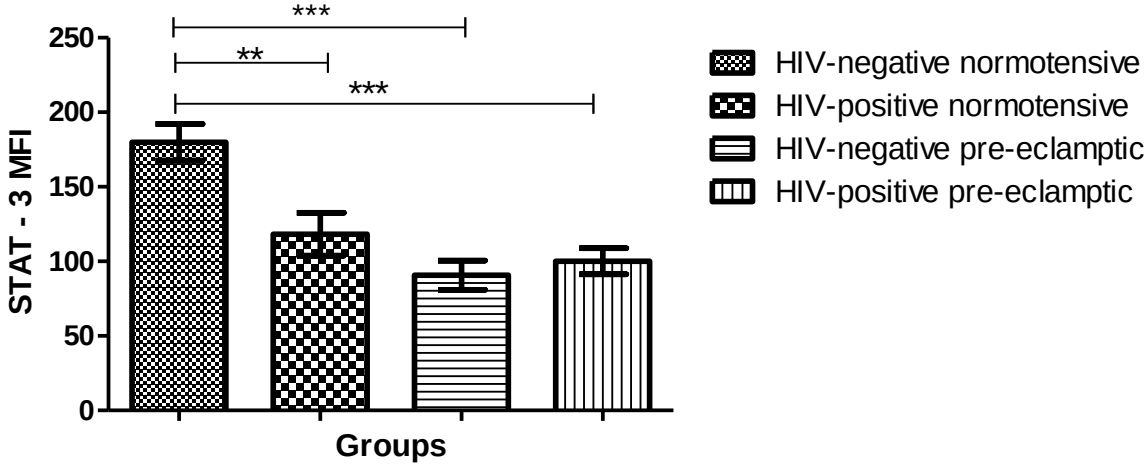
Across study groups: Figure 1C illustrates STAT-3 expression across all study groups; been significantly different ($p < 0.05$) between HIV-positive normotensive *vs* HIV-negative normotensive participants (118 ± 14 *vs* 180 ± 12 MFI); HIV-negative pre-eclamptic *vs* HIV-negative normotensive participants (91 ± 9.8 *vs* 180 ± 12 MFI) and HIV-positive pre-eclamptic *vs* HIV-negative normotensive (100 ± 8.8 *vs* 180 ± 12 MFI). The level of STAT-3 were similar between HIV-negative pre-eclamptic *vs* HIV-positive normotensive participants, HIV-positive pre-eclamptic *vs* HIV-positive normotensive participants and HIV-positive pre-eclamptic *vs* HIV-negative pre-eclamptic participants.

1A





1B



1C

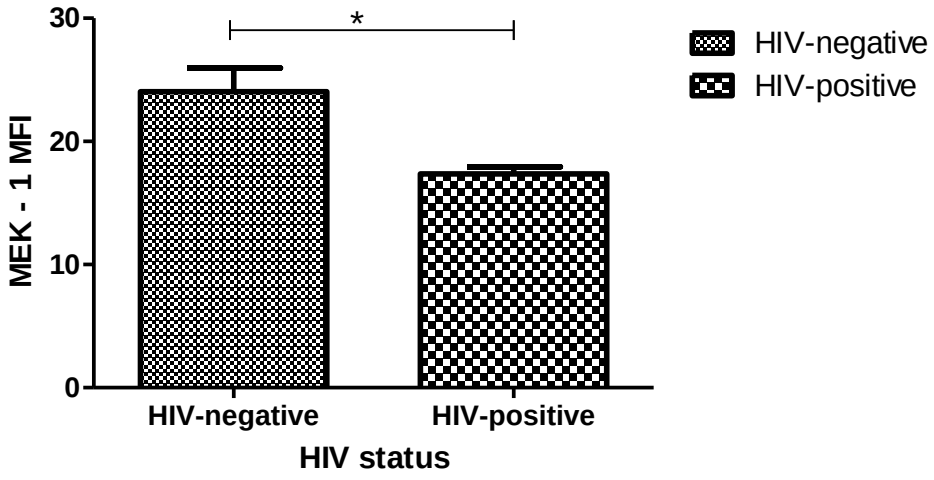
Figure 1: STAT-3 expression: (A) based on HIV status; no significance (ns) (B) based on pregnancy type; ***p < 0.01 and (C) across all study groups; **p < 0.01 ***p < 0.001. Results are represented as mean ± SEM.

MEK-1 expression:

HIV status: In figure 2A, based on HIV status and regardless of pregnancy type, a significant difference ($p = 0.0102$) in MEK-1 expression was noted between HIV-positive and HIV-negative groups (17 ± 0.58 *vs* 24 ± 1.9 MFI).

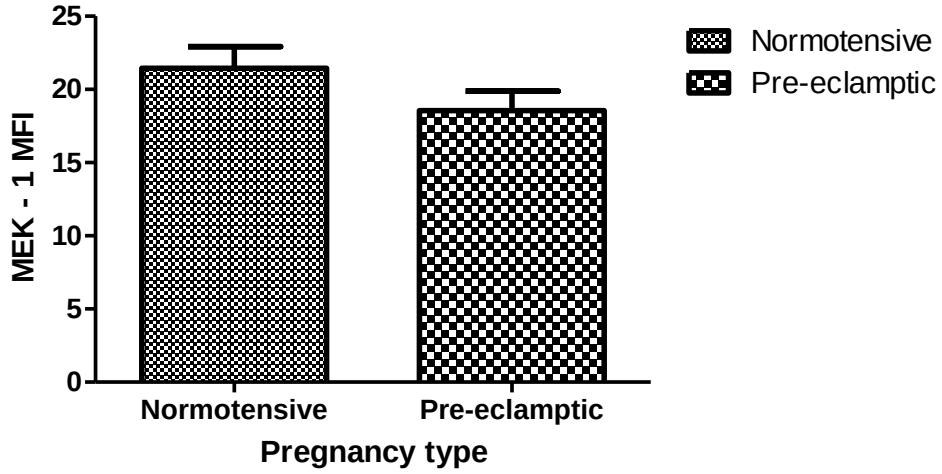
Pregnancy type: Based on pregnancy type as seen in figure 2B, no significant difference ($p = 0.1526$) was detected for MEK-1 between pre-eclamptic and normotensive pregnancies (19 ± 1.3 *vs* 21 ± 1.5 MFI).

Across study groups: Figure 2C illustrates MEK-1 expression across all study groups; been significantly different ($p < 0.001$) between HIV-positive normotensive *vs* HIV-negative normotensive participants (17 ± 0.61 *vs* 30 ± 2.7 MFI); HIV-positive pre-eclamptic *vs* HIV-negative normotensive participants (17 ± 1.3 *vs* 30 ± 2.7 MFI). A level of significance ($p < 0.05$) was detected between HIV-negative pre-eclamptic participants *vs* HIV-negative normotensive participants (20 ± 2.0 *vs* 30 ± 2.7 MFI). No significance was detected between HIV-negative pre-eclamptic *vs* HIV-positive normotensive participants, HIV-positive pre-eclamptic *vs* HIV-positive normotensive participants and HIV-positive pre-eclamptic *vs* HIV-negative pre-eclamptic participants.



2A

2B



2C

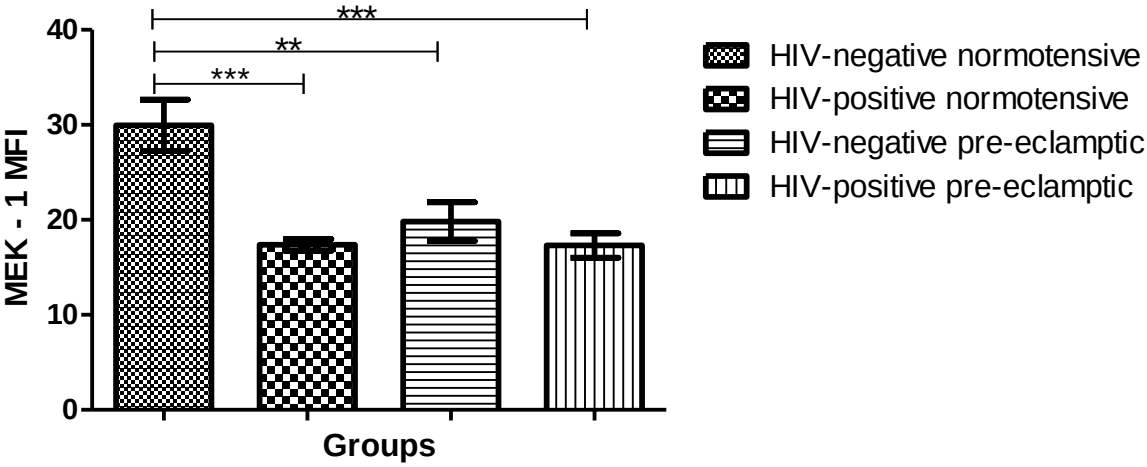


Figure 2: MEK-1 expression: (A) based on HIV status; *p < 0.05 (B) based on pregnancy type; no significance (ns) and (C) across all study groups; **p < 0.01; ***p < 0.001. Results are represented as mean ± SEM.

Discussion

The expression of protein kinases STAT-3 and MEK-1 are responsible for cytokine signaling hence vital in the controlling trophoblast differentiation, invasion and vascular remodeling (20-22).

In our study, there was an upregulation of STAT-3 expression based on pregnancy type irrespective of HIV status in normotensive participants compared to pre-eclamptic participants. These results were similar to the findings of (Zhang *et al*, 2015). In normal pregnancy, the influential role of cytokines in the modulation of trophoblast cell proliferation and migration, are dependent on the STAT-3 subunit of the JAK-STAT pathway for their signaling to facilitate normal placentation (14).

It is therefore plausible to assume that a defect of STAT-3 activity caused by negative regulators of the JAK-STAT pathway contribute to the defective trophoblast invasion in pre-eclampsia. Some of these regulators include the suppressor of cytokine signaling (SOCS) proteins, the protein inhibitor of activated STAT (PIAS) and protein tyrosine phosphates (PTPs) (23). SOC proteins are immunolocalized in the syncytiotrophoblasts (24) and it is postulated that a decrease in SOC activity in gestation may influence the overexpression of SOCS in pre-term labour (25).

In our study, STAT-3 expression was similar between HIV-positive *vs* HIV-negative groups, irrespective of pregnancy type. It is important to note that all infected participants in our study were on dual therapy for HIV infection, namely nevirapine and HAART. Our results however, did indicate a downregulation trend of STAT-3 expression in HIV-positive *vs* HIV-negative participants, as it has been established that STAT-3 plays a significant role in the regulation of the development, activation and physiology of dendritic cells (DC) (26, 27). Upon recognition of pathogens such as the HIV virus, dendritic cells are involved in connecting the innate and adaptive immune system to initiate an appropriate immune response (28). However, once HIV infection is initiated, the virus negatively influences DC function, thereby interfering the mechanisms linking innate and adaptive immunity (29, 30).

This downregulatory trend of STAT-3 in HIV-positive patients may also be attributed to HAART as studies have shown an increased predisposition to pre-eclampsia development in infected patients receiving HAART (6). Regardless of the conflicting data between HIV and pre-eclampsia, HIV-infected women without HAART have a weakened immune response and are therefore less likely to uphold the exacerbated

immune response in pre-eclampsia (10), due to the consensus that both conditions are linked to an exaggerated inflammatory response (31).

Previous studies have reported that a contributing factor to the systemic inflammatory response in pre-eclampsia are elevated levels of IL-6, which signals through the JAK-STAT pathway. HIV-positive individuals overproduce IL-6 and other pro-inflammatory cytokines (32). In HIV-positive pregnant participants receiving ARVs, the expression of IL-6 messenger (m)RNA (33) and IL-6 production are reduced, thereby supporting immune reconstitution with an increased predisposition to pre-eclampsia development. Also, STAT-3 can mediate the regulation of DC to assist in minimizing the immunosuppressive effects of HIV and further opportunistic infections, despite lower levels of STAT-3 in influencing trophoblast invasion.

Some early studies have demonstrated that an absence of STAT-3 expression in hematopoietic progenitors led to a major deficiency of DC cells (34) and DC differentiation and maturation from hematopoietic precursors were also affected (35). Therefore, in HIV-negative individuals, STAT-3 expression is vital to effectively influence DC function in comparison to the immunocompetence in the failing immune system of an HIV-positive individual, as HIV infection results in the progressive decrease of $CD4^+$ T lymphocytes and the deteriorating response of $CD4^+$ and $CD8^+$ T-cells towards HIV and other invading pathogens (36).

The lower STAT-3 expression in HIV-positive women on HAART in our study suggests that despite the minimal viremia in patients receiving HAART, persistence of the HIV virus continues as a result of latent provirus replication in resting memory $CD4^+$ T cells in the peripheral blood (37). A defect in STAT-3 and its role in DC function can be further attributed to the persistence of HIV infection.

In the current study, similar to STAT-3, MEK-1 expression was not different between the normotensive and pre-eclamptic cohorts. The lower MEK-1 expression in pre-eclamptic *vs* normotensive participants corroborates its role in promoting trophoblast invasion (19).

Extravillous (EVT) cells promote the expression of sphingosine-1-phosphate (S1P) receptors, a signaling molecule phosphorylated from sphingosine, which binds to one of five specific G protein-coupled receptors (38, 39). These coupled receptors are responsible for the activation of the ERK pathway (40, 41). Inhibition of the MEK-1 constituent by S1P via S1PR2, sphingosine-1-phosphate receptor 2, can hinder EVT cell migration (19) a major contributing factor to the defective trophoblast invasion that characterizes pre-eclampsia.

In our study, based on HIV status, MEK-1 expression was downregulated in HIV-positive *vs* HIV-negative participants regardless of pregnancy type. In contrast to our findings, an upregulation of MEK-1 expression has been reported in HIV-positive HAART naïve patients (Yang and Gabuzda, 1999 and Alhetheel *et al*, 2013). The activation of the MAPK pathway by mitogens and additional extracellular stimuli contribute to the activation of HIV-1 replication, by enhancing the pathogenic abilities of HIV-1 virions (42). This suggests that untreated HIV-positive patients have elevated levels of MEK-1 expression.

Furthermore, activated MAPKs result in the phosphorylation of HIV regulatory proteins *viz.,* Vif, Rev, Tat, Nef, and p17, thereby enhancing viral replication (43). These viral proteins activate a variety of other cell signaling molecules to support replication of the virus (44). Nef and Tat mimic T-cell signaling pathways in the early stages of infection, sustaining replication of the virus in infected T-cells (44). Nef also contributes to the downregulation of CD4 (45) and CD28 cell surface receptors (46); and class I, A and B of the major histocompatibility complex (MHC) (47). Tat viral protein plays a role in viral transcription (48) and has been shown to upregulate IL-10, resulting in the increased production of IL-10 in HIV-positive patients (49).

Furthermore, MAPK modulates the function of monocytes, lymphocytes and apoptosis during HIV infection (50), thus phosphorylation of the MAPK pathway is less heightened in treated HIV-positive patients adhering to HAART, due to improvement in the recovery of $CD4^+$ T cell function, reduced HIV-1 replication with an increase in $CD4^+$ T cell count (51).

Additionally, the inhibition of MEK-1 by a MAPK inhibitor hinders MAPK activation, as well as MAPK substrate phosphorylation, subsequently resulting in diminished viral replication (52). The inhibition of HIV-1 infection is mediated by obstructing activation of the MAPK pathway, rather than a reduced expression of MAPKs (43). These results collectively suggest that MAPKs and their constituents modulate the infective capabilities of HIV-1 virus particles (43).

Conclusion

This study demonstrates a significant downregulation of STAT-3 and MEK-1 in pre-eclampsia compared to normotensive pregnancies. These results account for the decreased trophoblast migration that characterizes PE. In our study, the HAART regimen supports the decline of STAT-3 expression in HIV-positive women. However, contrary to expected outcomes, MEK-1 expression was downregulated in HIV infection, possibly due to the non-phosphorylation of the HIV accessory proteins. However, more studies need to be conducted on the effect of signal transduction pathways to further elucidate the effect of HAART on PE development. Additionally, the detection of STAT-3 and MEK-1 cell analytes can serve as predictive biomarkers for the development of this hypertensive disorder.

Declaration of interest

There are no conflicts of interest.

Acknowledgments

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CHAPTER 3

SYNTHESIS

In sub-Saharan Africa, HIV infection and maternal mortality is a serious public health concern, since more than one third (34.7%) of maternal deaths emanate from HIV infection in pregnancy (Saving Mothers Report, 2013). In contrast, the global proportion of maternal deaths caused by HIV/AIDS ranges from 7%-21% (Zaba *et al.*, 2013). Current studies have debated whether the progression of HIV infection may be accelerated by pregnancy or if HIV-positive women have a higher pre-disposition to obstetric complications such as pre-eclampsia (Mattar *et al*., 2004). Pre-eclampsia is a multisystem hypertensive disorder and some epidemiological findings favour a genetic and immunological etiology (Uzan *et al*., 2011). In KwaZulu-Natal, South Africa, the incidence of pre-eclampsia is 12% (Saving Mothers Report, 2013).

This study demonstrates a decrease of STAT-3 and MEK-1 in pre-eclampsia compared to normotensive patients irrespective of HIV status, corroborating the roles of STAT-3 and MEK-1 in trophoblast invasion and subsequent arterial remodelling of the maternal spiral arteries.

Activation of STAT-3 occurs at tyrosine 705 in the C-terminal domain and its phosphorylation is caused by 40 different polypeptide ligands (Siveen *et al*., 2014). Phosphorylation of MEK-1 is activated by ERK and occurs on Ser218 and Ser222 in the MEK-1 activation loop within a Ser–Xaa–Ala– Xaa–Ser/Thr motif (Alessi *et al*., 1994). The consequences of phosphorylation inhibition support aberrant protein activation thus accounting for the trophoblast invasion that occurs in our PE group.

It is well known that both STAT-3 and MEK-1 enhance trophoblast invasion through their respective signal transduction pathways in normal pregnancies (Suman *et al*., 2009), however a downregulation of both analytes contribute to pre-eclamptic development as a result of defective transformation of the maternal spiral arteries by shallow trophoblast invasion. Apart from extracellular stimuli, trophoblast migration and invasion are influenced by cytokines, oxygen tension, growth factors and immune cells including decidual and uterine natural killer cells (Whitley and Cartwright, 2010). The underlying cause of the pathophysiology of pre-eclampsia is defective placentation, attributed to decreased myometrial

cytotrophoblast invasion with consequential non-physiological conversion of the spiral arteries. The resultant ischaemic microenviroment causes oxidative stress with an expulsion of specific molecules into the maternal circulation, that leads to the development of symptomatic systemic inflammation and endothelial dysfunction (Yeh *et al*., 2012).

In our study, the decreased expression of STAT-3 and MEK-1 in pre-eclamptic pregnancies may be attributed to the reduced expression of an upstream gene or protein unable to optimize phosphorylation. The cytokine IL-6 is able to increase STAT-3 phosphorylation (Zhang *et al*., 2015). Our results implicate a low expression of IL-6 in HIV-positive patients on HAART, suggesting non-phosphorylation of STAT-3. On the contrary, restoration of the immune response through the benefits of HAART provides a thriving opportunity for pre-eclampsia development, in counteracting the physiology of T-cells in the progression of HIV infection.

Based on HIV status, both STAT-3 and MEK-1 were downregulated in HIV-positive women, possibly due to HAART and non-phosphorylation of HIV regulatory proteins. The elevated STAT-3 expression in HIV-negative women, supports the influence of STAT-3 in combatting HIV infection due to its role in dendritic cell (DC) function.

In response to HIV-1 infection and HAART, STAT-3 plays a significant role in the adaptive immune response, in mediating the physiology of DCs. In spite of CD4+ T-cells being the prominent target for HIV infection, DCs are highly influential in the transmission of the virus, infection of target cells and HIV antigen presentation (Manches *et al.*, 2014). The potential deficit of DCs in HIV-1 infection may correlate with the decreased T-cell response and type-1 cytokine secretion that contributes to the increased susceptibility to opportunistic infections (Chehimi *et al*., 2002). Noteworthy, the STAT-3 expression in infected patients on HAART confers immune reconstitution and moderates viral progression via DC function. However, a defect in STAT-3 could impede DC function, supporting persistence of viral infection

(Nefedova *et al*., 2005). In untreated patients, it is presumed that STAT-3 expression is lower compared to those on HAART (Chehimi *et al*., 2002).

Depending on the type of virus, gene transcription mediated by STAT-3 can either be promoted or disrupted. Inhibition of STAT-3 signaling by suppressors of cytokine signaling (SOCS) appears to be a strategic mechanism of viruses to circumvent host immune responses, as a result of SOCS overexpression (Mohan *et al*., 2007). SOCS3 transcription is activated by STAT-3, preventing STAT-3 signaling by JAKs inhibition (Carow and Rottenberg, 2014). The presence of SOCs in the JAK/STAT pathway can further validate the downregulation of STAT-3 in HIV-positive women. SOCS3 and STAT-3 compete for pY-binding sites on activated receptor chains, or through the binding of signaling proteins for proteasomal degradation (Robinson *et al.*, 2013; Koskela *et al*., 2012).

Moreover, an overexpression of STAT-3 inhibits cell apoptosis and in turn promotes cell growth and survival (Yin *et al*., 2011), thereby promoting regular trophoblast invasion within the placental bed (Zhang *et* al., 2015). Both STAT-3 and a downstream component of the MAPK pathway, the early growth response protein 1 (Egr-1) concomitantly regulate cell growth and apoptosis (Chatterjee *et al*., 2007).

Each cascade of the activated MAPK pathway *viz*., ERK, JNK and p38 enhance the gene expression level of the HIV virion, replication level of the viral genome and infection activity (Krens *et al*., 2006; Yang and Gabuzda, 1998; Seger and Krebs, 1995). Moreover, HIV-1 infection activates the MAPK signaling pathway by phosphorylation of HIV viral proteins, Tat, Nef and gp120.

HIV-1 viral proteins also modify various transcription factors and infected CD4+ T cell surface receptors to facilitate HIV-1 infection and pathogenesis (Abbas and Herbein, 2013). As a result, low MEK-1 expression may be expected in HIV-positive patients on HAART due to the improvement of CD4 counts, control of viremia and a declined systemic immune response to HIV (Mkhize *et al*., 2010). HAART hinders MEK-1 functioning thus suppressing HIV replication and promoting restoration of CD4 T-cell counts in HIV infected patients (Tincati et al., 2009).

Moreover, in untreated HIV-1 infection, the exaggerated immune response through an increasing T-cell turnover, elevated levels of pro-inflammatory cytokines and polyclonal activation of B-cells (Sodora and Silvestri, 2008) provides opportunity for overexpression of MEK-1 through activation of the MAPK pathway. As such, the over expression of MEK-1 in HIV-infection is critical in enhancing viral replication. Inhibition of MEK-1 phosphorylation by MAPK inhibitors, and subsequently ERK1/2 activation suggests suppressed viral replication (Dudley *et al*., 1995), although further insight is required into MAPK inhibitors.

All HIV-positive women in our study adhered to the HAART regimen, as part of the standard of care treatment regimen in South Africa. This may be considered one of the limitations of our study, as differences in analyte expression based on HIV status is confounded by HAART regimen.

Conclusion:

Our study demonstrated the downregulation of STAT-3 and MEK-1 in pre-eclampsia compared to normotensive pregnant women, verifying the significance of these analytes in trophoblast invasion and non-conversion of the maternal spiral arteries for a successful pregnancy. The downregulation of STAT-3 in HIV-positive women may be attributed to low levels of IL-6 responsible for non-phosphorylation of STAT-3. In addition, HAART may be a contributing factor of STAT-3. Contrary to expected outcomes, the decreased expression of MEK-1 in HIV-positive women could be attributed to the non-phosphorylation of HIV regulatory proteins or the inhibition of the MAPK pathway by MAPK inhibitors. Although a consensus has yet to be elucidated between the role of antiretroviral therapy and HAART towards pre-eclampsia development, the emergence of novel non-invasive markers for early detection are useful to provide an improved patient management for both mother and fetus, thus potentially reducing maternal and fetal mortality and morbidity rates worldwide.

CHAPTER 4

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APPENDIX

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 May 2017

Ms S Padayachee (212511676)
Discipline of Optics and Imaging
School of Laboratory Medicine and Medical Sciences
Sayurip16@gmail.com

Dear Ms Padayachee

Protocol: The role of STAT-3 and MEK-1 in HIV associated preeclampsia.
Degree: MMedSc
BREC reference number: BE210/17

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 10 March 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 16 May 2017 to BREC correspondence dated 05 May 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 23 May 2017.

This approval is valid for one year from **23 May 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **13 June 2017.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor: naickera@ukzn.ac.za
cc administrator: dudhrajhp@ukzn.ac.za