CELL SIGNALLING OF THE EPIDERMAL GROWTH FACTOR (EGF) / EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AXIS IN HIV ASSOCIATED PREECLAMPSIA

BY

ARISHA LALDEO

Submitted in partial fulfilment for the degree of

MASTER OF MEDICAL SCIENCE

in the

Discipline of Optics and Imaging

Doris Duke Medical Research Institute

Collage of Health Sciences

University of KwaZulu-Natal

Durban, South Africa

2021

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 T. Naicker.

Arisha Laldeo

(Student number: 217000912)

Shoohana Singh

(Co-Supervisor)

Professor Thajasvarie Naicker

(Supervisor)

DECLARATION

I, **Arisha Laldeo** declare that:

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

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

iii. This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

iv. This dissertation does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:

   a. Their words have been rewritten but the general information attributed by them has been referenced.

   b. Where their exact words have been used their writing has been placed inside quotation marks and referenced.

v. Where I have reproduced a publication of which I am the author, co-author, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

vi. This dissertation does not contain text, graphics, or tables copied and pasted from the internet, unless specifically acknowledged and the source being detailed in the dissertation and the reference sections.

Signed: _  _____ **Date:** 15 November 2021

DEDICATION

To Goddess Durga, for giving me the strength

and

courage to pursue and achieve my dreams and goals.

ACKNOWLEDGEMENTS

I would like to sincerely express my gratitude to each individual who guided and supported me throughout my Master of Medical Science research endeavour:

- I would firstly like to thank God for guiding me and constantly awarding me with strength, perseverance and courage in order to achieve my goals.

- I would like to express my most humble appreciation to my supervisor, Professor Thajasvarie Naicker for allowing me the opportunity to join the Placental Research Group. I want to thank you for constantly guiding and supporting me along the way. Your continuous support, patience and guidance will always be appreciated. You are indeed a role model to me.

- I would also like to thank my co-supervisor, Mrs Shoohana Singh for her constant guidance and support throughout the year. Your assistance, dedication and caring nature will always be cherished. I am indeed indebted to both my supervisors for sharing their wisdom, thoughts and never failing to solve a problem on hand.

- I would like to express my most heartfelt thanks to my parents; Mr and Mrs Laldeo, both of you have continued to support and fund my dreams and goals throughout my life. Thank you for always being encouraging parents and believing in me, I would not be where I am today without your constant support.

FUNDING

This study was funded by the productivity award of Professor T. Naicker.

Table of Contents

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

LIST OF TABLES

CHAPTER 2

LIST OF ABBREVIATIONS

Acquired Immunodeficiency Syndrome	AIDS
ADAM metallopeptidase domain 17	ADAM17
Alpha fetoprotein	AFP
Adrenomedullin	ADM
AMP-activated kinase	AMPK
Angiotensin II	ANGII
Antiretroviral treatment	ART
Body mass index	BMI
Carboxyl-terminal tail	CT
Catalytic tyrosine kinase	TK
CC chemokine receptor 5	CCR5
Chronic kidney disease	CKD
Cluster of differentiation 4	CD4
Connexion 43	Cx43
CXC chemokine receptor type 4	CXCR4
Cytosine rich region 1	CR1
Cytosine rich region 2	CR2
Deoxyribonucleic acid	DNA
Early- onset of preeclampsia	EOPE
Endoplasmic reticulum	ER
Epidermal growth factor	EGF
Epidermal growth factor receptor	EGFR
Epidermal growth factor like domain 7	EGFL7
Extracellular signal related kinases	ERK
Focal adhesion kinase	FAK
Fibroblast growth factor 2	FGF2
FlK-1/kinase insert domain receptor	Flk-1/KDR
Growth factor receptor-bound protein 2	GRB2
Guanosine diphosphate	GDP
Guanosine triphosphate	GTP
Highly active antiretroviral therapy	HAART
Heparin-binding epidermal growth factor	HB-EGF
Human Immunodeficiency virus	HIV

Human immunodeficiency virus type 1	HIV-1
Hyaluronan	HA
Hypertensive disorders of pregnancy	HDPs
Hypoxia-inducible factor	HIF-1
Interquartile range	IQR
In vitro fertilization	IVF
Janus kinase	JAK
Late-onset of Preeclampsia	LOPE
leukemia inhibitory factor	LIF
Ligand binding domain 1	LD1
Ligand binding domain 2	LD2
Mitogen-activated protein kinase or MAPK kinase	MEK
Mammalian target of rapamycin	mTOR
Mean platelet volume	MPV
Messenger ribonucleic acid	mRNA
Non-nucleoside reverse transcriptase inhibitor	NNRTI
Non-small-cell lung cancer	NSCLC
Phosphorylation	P
Phosphinositide 3-kinase	P13K
Phospholipase C-γ	PLC-γ
Placental growth factor	PIGF
Platelet distribution width	PDW
PPAR-γ co-activator 1	PGC1α
Protein kinase B	AKT
protein kinase C-β	PKC-β
Preeclampsia	PE
Rapidly accelerated fibrosarcoma	RAF
Rat sarcoma	RAS
Ribonucleic acid	RNA
Severe acute respiratory syndrome coronavirus	SARS-CoV-2
Short transmembrane spanning sequences	TM
Soluble endoglin	sEng
Soluble fms-like tyrosine kinase 1	sFlt-1
Son of Sevenless	SOS
South Africa	SA

Signal transducer and activator of transcription	STAT
Sirtuin 1	SIRT1
Trans-activator of transcription	Tat
Transforming growth factor-β1	TGF- β1
Unconjugated estriol	UE3
Urokinase plasminogen activator	uPA
Vascular endothelial growth factor	VEGF
Vascular endothelial growth factor A	VEGF-A
Versus	*vs.*
World Health Organization	WHO

ABSTRACT

Background: Epidermal growth factor is a protein which, when bound to epidermal growth factor receptor, facilitates cell proliferation and differentiation, hence is vital for a successful normal pregnancy. In preeclampsia (PE), EGFR signalling is dysregulated leading to decline in blood flow to the fetus. Furthermore, aberrant EGF/EGFR signalling leads to deficient trophoblast development. The Trans-Activator of transcription (Tat) protein, displayed in HIV inhibits EGF related processes. Since PE and HIV infection are the leading causes of both maternal morbidity and mortality in South Africa; this study focuses on examining EGF/EGFR signalling in HIV associated PE and their effect on downstream targets.

Methods: Post ethics approval; this study selected 80 pregnant women from an archive of retrospectively stored serum samples. The samples were stratified by type of pregnancy and HIV status into the following groups: a) HIV negative preeclamptic women (n=20), b) HIV positive preeclamptic women (n=20), c) HIV negative normotensive pregnant women (n=20) and d) HIV positive normotensive pregnant women (n=20). Both EGF and EGFR were multiplexed in a Bioplex immunoassay technique to determine their serum concentration across study groups at term.

Results: Based on the clinical data, statistically significant differences were obtained across groups for gestational age ($p < 0.001$), birth weight ($p < 0.001$), systolic BP ($p < 0.001$), BMI ($p = 0.048$), diastolic BP ($p < 0.001$) and maternal weight ($p = 0.002$). However, no statistical significance was observed for maternal age across all study groups ($p = 0.065$).

A significant decline in EGF levels were observed in PE compared to normotensive pregnancy, regardless of HIV status ($p = 0.0214$). Based on HIV status, no statistical significance in EGF concentration was observed ($p = 0.6593$).

EGFR was significantly elevated in normotensive pregnant compared to preeclamptic women ($p < 0.0001$) (Figure 2A). In contrast, there were no significant differences of EGFR based on HIV status alone ($p = 0.2092$) (Figure 2B). However, a significant up-regulation of EGFR was observed between normotensive HIV positive compared to PE HIV positive women. Normotensive HIV negative was also significantly up-regulated compared to PE HIV positive ($p < 0.0001$). Normotensive HIV positive was significantly higher than PE HIV negative. Also, of note within the PE group, HIV negative EGFR levels were significantly up-regulated compared to the HIV positive group.

Conclusion: This novel study outlines a significant down-regulation of EGF and EGFR in PE compared to normotensive pregnant women; regardless of HIV status. No significant differences were observed based on HIV status alone for serum EGF levels. This could be due to immune reconstruction as all HIV infected patients received HAART, hence may have neutralised EGF levels. Furthermore, these findings may be attributed to the Trans-Activator of transcription (Tat) protein which prevents EGF related function. However, for serum EGFR concentration there were significant differences within PE group based on HIV status. Significant differences were observed between normotensive and preeclamptic based on HIV status, except for normotensive HIV negative *vs* PE HIV negative. Furthermore, there was no significance in the normotensive group based on HIV status. The decreased levels of serum EGF/ EGFR could possibly be used as a biomarker for PE development during pregnancy.

OKUFINGQIWE

Isendlalelo: I-Epidermal growth factor iyiphrotheni okuthi uma ihlangene ne-epidermal growth factor receptor, isiza ngokwandisa amaseli nokuwehlukanisa, yingakho ibalulekile ekukhulelweni okujwayelekile okuphumelelayo. Ku-preeclampsia (PE), ukusayinda kwe-EGFR kuyaphazamiseka okuholela ekwehleni kokugeleza kwegazi ku-fetus. Ngaphezu kwalokho, ukusayina kwe-EGF / EGFR ngendlela eyehlukile kunalena eyejwayelekile kuholela ekushodeni kwe-trophoblast. I-Trans-Activator of transcription (Tat) protein ivimbela izinqubo ezihlobene ne-EGF uma ihlangene neHIV. Njengoba izifo ze-PE kanye ne-HIV kuyizimbangela ezihamba phambili zokugula nokufa komama eNingizimu Afrika; lolu cwaningo lugxile ekuhloleni ukusayina kwe-EGF / EGF-R ku-PE ehlobene ne-HIV kanye nomphumela wazo uma zixhuma endaweni eziyihlosile maphansi neseli.

Izindlela: Ngemva kokugunyazwa kokuziphatha; lolu cwaningo lukhethe abesifazane abakhulelwe abangama-80 kungobo yomlando yamasampula e-serum agcinwe ngokudlule. Amasampula ahlukaniswa ngohlobo lokukhulelwa kanye nesimo se-HIV emaqenjini alandelayo: a) abesifazane abakhulelwe abangenayo i-HIV kodwa abanomfutho wegazi ophakeme (n=20), b) abesifazane abakhulelwe abane-HIV nomfutho wegazi ophakeme (n=20), c) abesifazane abakhulelwe abangenayo i-HIV futhi abanomfutho wegazi ojwayelekile (n=20), kanye d) nabesifazane abakhulelwe abane-HIV kodwa banomfutho wegazi ojwayelekile (n=20). Kokubili i-EGF ne-EGF-R zacwaningwa ngendlela ye-Bioplex immunoassay ukuze kutholwe inani lweserum yazo kuwo wonke amaqembu ocwaningo ngesikhathi.

Imiphumela: Ngokubuka idatha esungulwe yimtholampilo, umehluko obalulekile ngokwezibalo watholwa kuwo wonke amaqembu eminyaka yobudala (p <0.001), isisindo sokuzalwa (p <0.001), i-systolic BP (p <0.001), BMI (p = 0.048), i-diastolic BP (p <0.001) kanye nesisindo sikamama (p = 0.002). Kodwa-ke awukho umehluko omkhulu ngokwezibalo owabonwa ngeminyaka yobudala bomama kuwo wonke amaqembu ocwaningo (p = 0.065).

Ukwehla ngokwezibalo okubalulekile kwe-EGF kwabonwa i-PE uma kuqhathaniswa nokukhulelwa kwe-normotensive, kungakhathaliseki isimo se-HIV (p = 0.0214). Ngokubuka isimo se-HIV, akukho ukubaluleka kwezibalo kwe-EGF okuphawulwe (p = 0.6593).

Kungakhathalekile ukuthi ngabe i-HIV ithini, i-EGFR iphakanyiswe kakhulu kuma-normotensive abakhulelwe ngokuqhathaniswa nabesifazanebe-preeclamptic (p < 0.0001). Ngokuphambene, kwakungekho umehluko ophawulekayo we-EGFR ngokusekelwe esimweni se-HIV (p = 0.2092). Kodwa-ke, ukulawulwa okubalulekile kwe-EGFR kwabonwa phakathi kwe-HIV positive normotensive uma kuqhathaniswa nabesifazane abakhulelwe abangenayo i-

HIV (p = 0.001); ngaleyo nkathi ukwehla okubalulekile kwe-EGFR kwaboniswe kuwo wonke amaqembu ocwaningo (p = 0.001).

Isiphetho: Lolu cwaningo lwenoveli luveza ukwehla okubalulekile kwe-EGF ne-EGFR ku-PE uma kuqhathaniswa nabesifazane abakhulelwe abajwayelekile; kungakhathaliseki isimo se-HIV. Awukho mehluko omkhulu obonwe ngokusekelwe esimweni se-HIV. Lokhu kungase kube ngenxa yokwakhiwa kabusha kwamasosha omzimba njengoba zonke iziguli ezine-HIV zithole i-HAART, yingakho kungase kulinganise amazinga e-EGF/EGFR. Ngaphezu kwalokho, lokhu okutholakele kungase kubalulwe ku-Trans-Activator of transcription (Tat) protein evimbela umsebenzi ohlobene ne-EGF. I-down-regulation ye-EGF/EGFR ingase isetshenziswe njengokuhlola inkomba yokubikezela ukuthuthukiswa kwe-PE ngesikhathi sokukhulelwa.

CHAPTER 1

1. BACKGROUND AND LITERATURE REVIEW

1.1 HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME (HIV/AIDS)

The Human Immunodeficiency virus (HIV) belongs to the Retroviridae family and is the causative agent of a life threatening disease (Seitz, 2016). The virus is transmitted through unprotected sexual intercourse, perinatal transmission, sharing of needles and by direct contact with HIV infected blood (Cohen *et al*., 2008). The virus degrades the human immune system by attacking white blood cells known as cluster of differentiation 4 (CD4) T helper cells. These cells protect the body from infection; hence, individuals become susceptible to the acquisition of new infections. If left untreated, HIV infection leads to Acquired Immunodeficiency Syndrome (AIDS) (Meulendyke *et al*., 2014). Acquired immunodeficiency syndrome is regarded as the final stage of HIV infection, as the infected individual is unable to fight off any infection due to a compromised immune system; which is too weak to function.

Once HIV makes contact with a CD4 T cell, the glycoprotein spikes on its envelope (gp120) lock onto a CD4 receptor and a co-receptor, CC chemokine receptor 5 (CCR5). The gp41 protein of HIV is used to fuse the HIV envelope with the cell wall. This process of fusion allows the HIV capsid to enter the CD4 T cell (Wilen *et al*., 2012). Viral ribonucleic acid (RNA) and cellular enzymes are then released into the cytoplasm of the cell. The viral RNA is then synthesized by reverse transcriptase, which generates one strand of viral deoxyribonucleic acid (DNA), followed by a complementary strand of DNA. Integration of viral DNA occurs in the host cell's DNA (Meulendyke *et al.*, 2014). Viral RNA is then formed via transcription within the host cell. Replication of HIV subsequently occurs whereby long chains of HIV proteins are produced in the cell. The gene (gp120) begins to mutate, hence changing the co-receptor legions from CCR5 to CXC chemokine receptor type 4 (CXCR4). The new viruses ultimately assemble at the surface of the cell where they bud out of the host CD4 T cell. Figure 1.1 below depicts these nine stages of the HIV life cycle (Herrera-Carrillo *et al*., 2015).

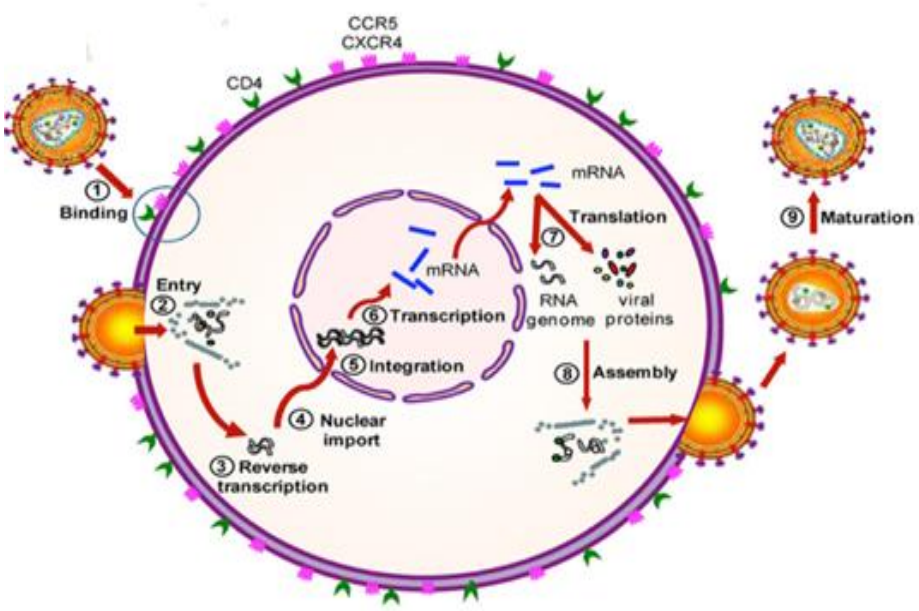


Figure 1.1: HIV life cycle depicting the nine stages of the virus. 1) Binding, 2) Entry, 3) Reverse transcription, 4) Nuclear import, 5) Integration, 6) Transcription, 7) Translation, 8) Assembly, and 9) Maturation Adapted from (Herrera-Carrillo *et al*., 2015)

There are three stages of the progression of HIV infection; which is similar to the stages of all diseases. The first stage is infection whereby the virus enters the body and begins to rapidly replicate whilst the body begins to generate antibodies. During this time individuals usually acquire flu-like symptoms, including headaches, rashes or a fever during the first few weeks of infection (Vargas and Middleton, 2013). The CD4 cell count in infected individuals begins to drop during this stage and is approximately 200 cells/mm² of blood whilst healthy individuals contain between 500 -1600 cells/mm² (Gracia and Guzman, 2020). The second stage of infection is described as the asymptomatic period; during this stage viral load begins to decrease. Symptoms during this stage are generally latent however; the virus continues to affect new cells in the body. The final stage is identified as the stage of which the virus progresses into AIDS. This is the most severe stage and is identified by a rapid increase of viral load and is followed simultaneously with a dramatic drop of CD4 cells bringing the $CD4^{+}$T cell count to below 200 cells/mm² (Vargas and Middleton, 2013). Figure 1.2 illustrates CD4 cells and viral load during HIV infection over time.

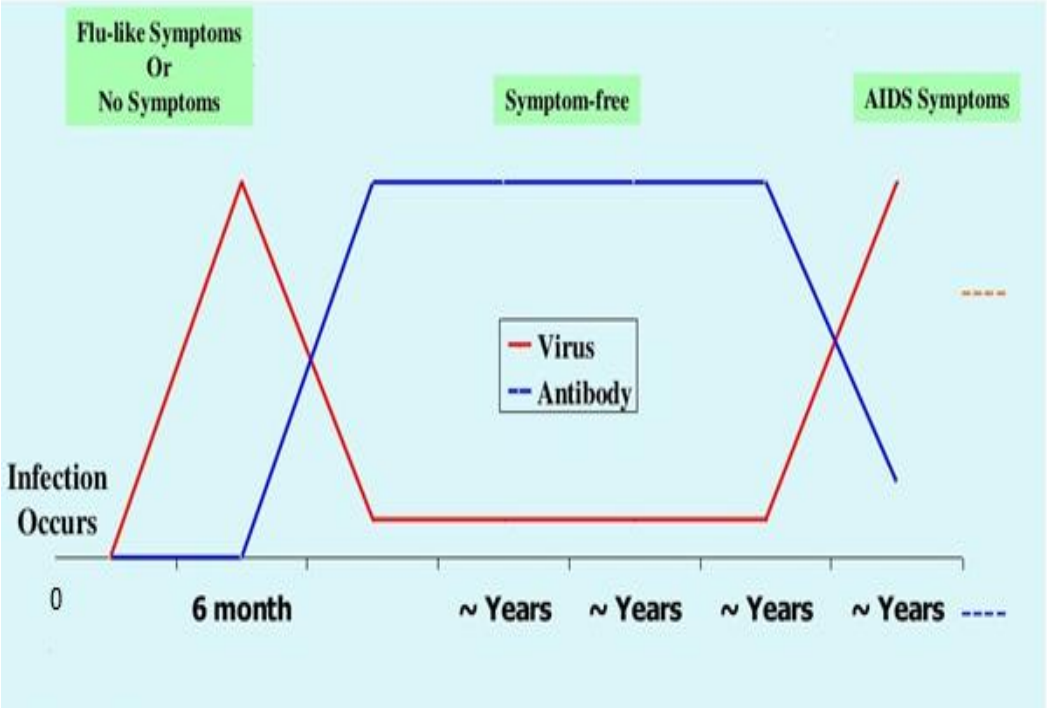


Figure 1.2: Comparison of HIV infection and antibody response in human blood during the progression of HIV infection throughout years of exposure. (Red graph indicates HIV infection whilst blue represents antibody response). Adapted from (Oza, 2015)

1.1.1. HIV Epidemiology

The World Health Organization (WHO) African Region has over 25.7 million HIV infections making it the global HIV epicentre. The mid-year 2021 population estimates of South Africa (SA) reports an overall HIV prevalence rate of 13.7 % across its population. The total number of people living with HIV infection is 8.2 million. Moreover, almost 19.5% of the population in their reproductive age (15–49 years) are HIV positive (Stats SA, 2021; Clouse *et al*., 2020). Furthermore, women are disproportionately affected and are susceptible to HIV infection especially adolescent girls and young women (aged 15-24 years). Moreover, SA has the world's largest antiretroviral treatment (ART) programme. This has increased life expectancy in SA, with males having an estimated 59.3 years and 64.6 years in females for 2021 (Stats SA, 2021).

1.2 HIV ASSOCIATED PREECLAMPSIA

Globally, approximately 37.7 million people were affected by HIV infection in 2020, with more than 53% been women and girls (UNAIDS, 2021). HIV is categorized as the leading immunosuppressive disorder in South Africa, of which 30% of antenatal woman are HIV positive (Kalumba *et al*., 2013). South Africa is therefore classified as the appropriate location for this study as maternal morbidity and mortality from HIV infection/AIDS and hypertensive disorders are high (Kalumba *et al*., 2013). Highly active antiretroviral therapy (HAART) is the prescribed standard of care treatment for HIV infected pregnant women (Eggleton and Nagalli, 2020). The HAART treatment regimen is designed to improve the quality of life of infected individuals by reducing the morbidity and mortality rate thereby improving immunity and reducing the plasma viral RNA load (Oguntibeju, 2012). Prevention of HIV transmission is by far the most important benefit of the HAART treatment as this reduces the level of HIV type-1 (HIV-1) RNA, which has proven to reduce the risk of sexual transmission to almost zero (Eggleton and Nagalli, 2020). All HIV positive pregnant women are urged to undergo HAART treatment, which drastically reduces the vertical *in-utero* transmission of HIV from mother to child. Moreover, it is debatable whether women with preeclampsia (PE), a hypertensive disorder of pregnancy have a lower/higher/neutral prevalence rate of HIV infection/AIDS compared to normotensive pregnancies (Kalumba *et al*., 2013; Mattar *et al*., 2004; Suy *et al*., 2006 and Frank *et al*., 2004).

1.3 MATERNAL MORTALITY

Hypertensive disorders of pregnancy (HDPs) are considered the leading direct cause of maternal deaths globally. It complicates 3-10% of all pregnancies, whilst accounting for 18% of maternal deaths globally (Mersha *et al*., 2019). In 2017, it was reported that approximately 86% of maternal deaths predominate in Sub-Saharan Africa and Southern Asia region. Moreover, in SA approximately 260 000 HIV infected woman give birth annually (Woldesenbet *et al*., 2019). The province of KwaZulu-Natal in SA has an astonishing 41.1% prevalence rate of HIV infection in pregnancy (Woldesenbet *et al*., 2019). Preeclampsia prevalence rate in KwaZulu-Natal is 12% (Moodley *et al*., 2016).

1.4 PREECLAMPSIA

1.4.1 Definition, signs and symptoms

Preeclampsia (PE) is a hypertensive disorder, which occurs in pregnant woman around the 20th week of gestation. Patients with an abnormal blood pressure exceeding 140/90mmHg on two accounts at approximately four hours apart, in the presence/absence of proteinuria (urine excretion greater than 300 mg protein in a 24-hour urine collection) are considered at high risk for the occurrence of PE development (Brown *et al*., 2018). The disorder may be accompanied by neurological complications such as eclampsia, stroke or visual scotomata; haematological complications and utero-placental dysfunction (Brown *et al*., 2018). Manifestation of severe headaches, vision changes, nausea and shortness of breath may also occur however, these symptoms may also be expressed throughout normotensive pregnancies; consequently, it is fundamental for patients to monitor their blood pressure throughout the duration of their pregnancy (Fondjo *et al*., 2019).

Preeclampsia may lead to severe complications if left untreated, including fatality of both mother and child; hence early detection is vital (Salam *et al*., 2015). Pregnant woman should therefore timeously attend antenatal clinics in order to facilitate a healthy pregnancy.

1.4.2 Pathogenesis

Angiogenesis is the development of new blood vessels that facilitates efficient maternofetal blood exchange. Angiogenesis supports the development and survival of the fetus (Chen and Zheng, 2013). Failure of successful formation of these blood vessels may lead to complications in pregnancy. Angiogenesis is initiated by the vascular endothelial growth factor (VEGF) as well as the placental growth factor (PIGF). These growth factors are defined as pro-angiogenic growth factors (Naicker *et al*., 2019). The process of angiogenesis can be disrupted by a shift in the balance of anti-angiogenic and angiogenic factors which causes endothelial dysfunction, hence leading to PE development (Wallace *et al*., 2014). Moreover, in HIV-1 associated pregnancy angiogenesis is dysregulated (Paydas *et al*., 2009). Additionally, the administration of anti-retroviral drugs during pregnancy leads to adverse birth outcomes (Chen *et al*., 2012).

During normal implantation, trophoblast cells migrate from the tip of the anchoring villi into the decidua and myometrium, this occurs in a set timed sequence. During this process of invasion, they physiologically convert decidual and myometrial spiral arteries into large bore flaccid conduits (diameter increases 5- to 10-fold). As the progression of pregnancy continues, utero-placental blood flow increases from 45 mL/min to 750 mL/min at term. This vast increase in

blood flow is essential to sustain adequate placental function. Furthermore, increased blood flow is required to meet the high demands of the growing fetus (Staff *et al*., 2020).

In PE however, placentation is shallow and defective (Lyall *et al*., 2013). There is reduced migration of interstitial trophoblast cells with an absence of physiological transformation of spiral arteries in the myometrium (Lyall *et al*., 2013; Naicker *et al*., 2003). As a result, the lumen is of small calibre and blood flow is reduced, hence leading to a hypoxic microenvironment. This reduced blood flow does not supply adequate oxygen and nutrients to the growing fetus, and ultimately leads to fetal complication such as growth restriction/retardation (Norwitz, 2006). Figure 1.3 illustrates the spiral artery modelling during normal pregnancy versus preeclampsia. In a normal pregnancy, the spiral artery is distensile permitting increased flow of blood and oxygen compared to a preeclamptic pregnancy.

Primary cilium which is formed in trophoblastic cells enables the stimulation of cell signalling pathways which is essential for cellular activities (Ritter *et al*., 2020). Ritter *et al*. reported an implication of primary cilium during PE which plays a vital role in the progression of PE as it enables communication between cell-cell and cell-environment in the human placenta (Ritter *et al*., 2020).

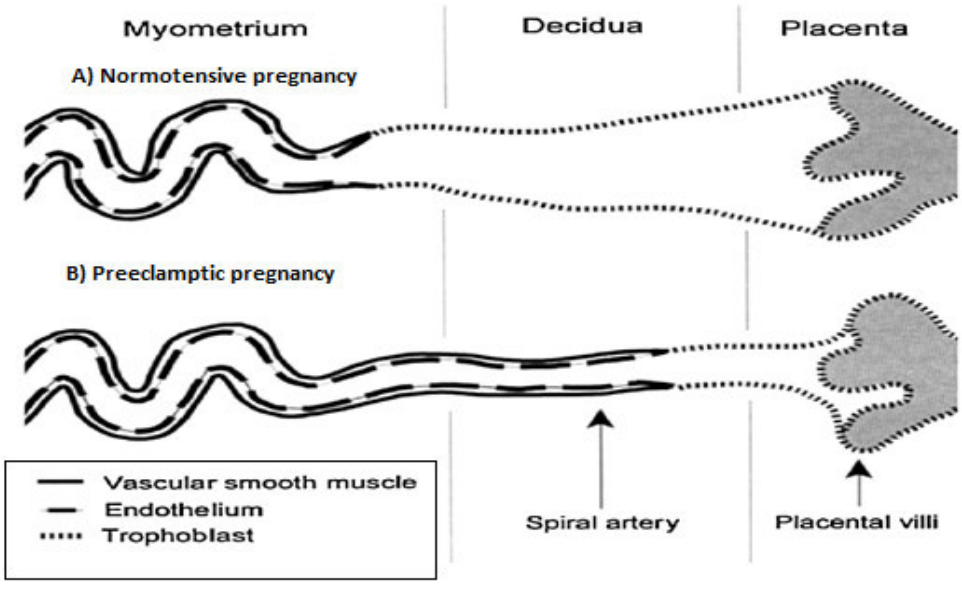


Figure 1.3: Physiological transformation of the spiral artery in a) normal pregnancy *vs.* b) preeclampsia. Adapted from (Pratt *et al*., 2014)

Anti-angiogenic factors play a vital role for the pathogenesis of PE. The elevated levels of soluble fms-like tyrosine kinase 1 (sFlt1) is elevated in PE. Therefore, overexpression of sFlt1 is a key mechanism which enables placental dysfunction. Soluble endoglin (sEng) is noted as an anti-angiogenic biomarker which is also elevated in PE (Maynard and Karumanchi, 2011). A study by Maynard *et al*. identified sEng which amplifies vascular damage and is mediated by sFlt1 in pregnant rats (Maynard *et al*., 2011). Placental growth factor (PIGF) enables the stimulation of angiogenesis when under inflammation and is vital for treatment of PE as PIGF has pro-angiogenic effects on feto-placental circulation (Chau *et al*., 2017).

1.4.3 Classification of Preeclampsia

Based on gestational age, PE may be classified as early-onset (EOPE) and late-onset (LOPE) type. Early-onset PE occurs during the first 33 weeks of pregnancy whilst LOPE begins to develop after 34 weeks of gestation (Raymond *et al*., 2011). Notably, EOPE has more adverse outcomes compared to LOPE (Weitzner *et al*., 2020). The majority of maternal and fetal deaths are linked with EOPE development emanating from inadequate placentation (You *et al*., 2018). The incidence of EOPE (27.6%) is lower than LOPE (72.4%) (Gomathy *et al*., 2018). In addition Weitzner *et al.* reported increased levels of alpha fetoprotein (AFP) and unconjugated estriol (UE3) in EOPE; hence acting as an indicator of EOPE (Weitzner *et al*., 2020).

1.4.4 Risk Factors Associated with Preeclampsia

Factors which contribute to PE development include genetic factors (Williams and Pipkin, 2011), as well as autoimmune disorders (Duckitt and Harrington, 2005). Other main risk factors that may lead to the onset of PE include being over the age of 35 years (Lamminpää *et al*., 2012), obesity (Sibai *et al*., 1997) and a pregnancy in early teenage years (Pingel *et al*., 2017).

Factors such as obesity, familial history, smoking, a history of PE in a previous pregnancy, sperm exposure, *in vitro* fertilization (IVF), age, race and co-morbid conditions increase an individual's chance of acquiring PE. Obese individuals with a body mass index (BMI> 34 kg/m^2) display a higher risk level of developing PE (Sibai *et al*., 1997). Young pregnant women such as teenagers are at high-risk of PE development; whilst maternally advanced pregnant women (over the age of 35) are also high risk candidates. Women of Black ancestry have a higher chance of experiencing a PE related pregnancy compared to other race groups (Ghosh *et al*., 2015).

Individuals with a familial history of PE are identified as high-risk candidates for the condition. Females are more likely to experience PE during pregnancy if their mother's had a preeclamptic pregnancy; whilst male individuals who had fathered a previous PE related pregnancy are more likely to undergo another PE related pregnancy with a different woman (Lie *et al*., 1998). Moreover, women who have experienced a previous PE related pregnancy, have a high possibility of undergoing PE in their subsequent pregnancies (Lie *et al*., 1998).

Unlike various other external environmental factors, smoking during a pregnancy may lead to detrimental effects to the fetus such as stillbirth, fetal growth restriction and preterm labour (Karumanchi and Levine, 2010). However, women who smoke throughout her pregnancy or smoke during later stages of pregnancy have a reduced risk of developing PE (Wikström *et al*., 2010).

Sperm exposure has been proven to affect the onset of PE development. Women who had partner's sperm exposure for more than 12 months before conception have a lower risk of developing PE during pregnancy; whilst individuals who used barrier contraceptive methods for approximately 4 months undergo a higher chance of the development of PE during pregnancy (Einarrson *et al*., 2003). *In vitro* fertilization (IVF) is regarded as a potential treatment for infertility however; IVF is associated with the development of PE compared to natural methods of conception. Woman induced with IVF were found to have a 2.6 fold increase in the development of PE (Gui *et al*., 2020). Maternal pre-existing comorbidities such as hypertension, gestational diabetes, renal disease and autoimmune diseases are at a greater risk of developing PE (Duckitt and Harrington, 2005). Other factors such as infertility issues, thyroid disorders, sickle cell disease and hypoglycaemia also contribute to the onset of PE development. Figure 1.4 below highlights factors that decrease/increase the risk of a preeclamptic pregnancy.

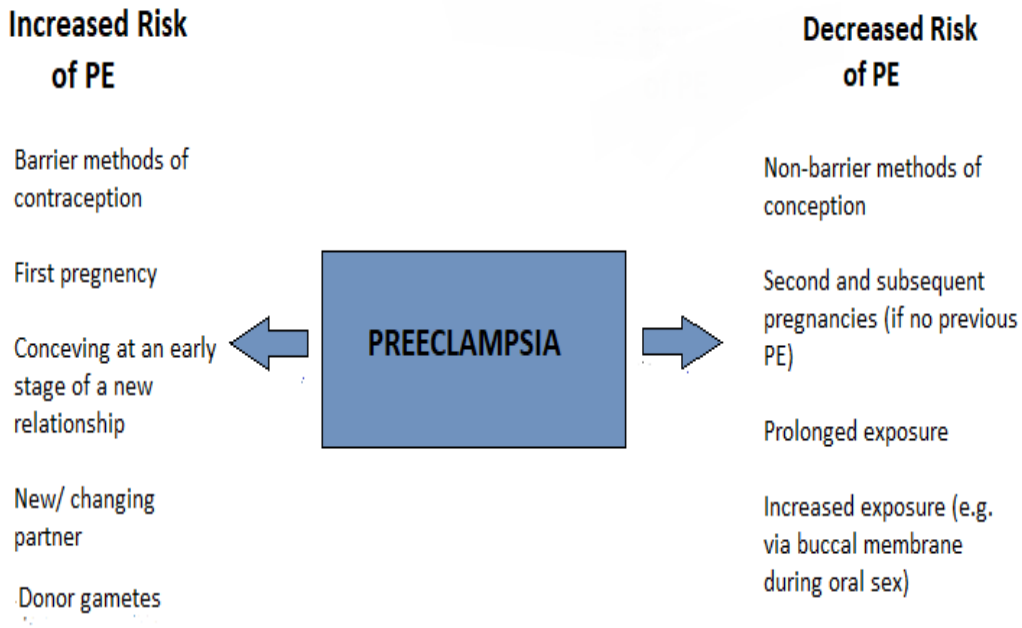


Figure 1.4: Diagram displaying the decreased and increased risk factors of PE development. Adapted from (Kenny and Kell, 2018)

1.4.5 The Progression of Preeclampsia into Eclampsia

The continuous progression of PE ultimately evolves into eclampsia, with brain injuries and seizures, which may result in a coma (Spradley, 2020). Seizures can occur up to 4 weeks post-delivery of the baby. Both PE and eclampsia are hypertensive disorders of pregnancy and are the leading causes of maternal and perinatal deaths across the world (Peres *et al*., 2018). Deterioration of the placenta can also occur at an accelerated rate during eclampsia (Peres *et al*., 2018). During more severe cases, the placenta detaches from the uterine wall prior to delivery, which leads to fetal distress. Infants who undergo preeclamptic births have a high possibility of developing cardiovascular related diseases during their lifetime (Peres *et al*., 2018).

1.5 EPIDERMAL GROWTH FACTOR

Epidermal growth factor (EGF) is a protein that stimulates cell growth and proliferation by attaching to its receptor, the epidermal growth factor receptor (EGFR) (Kupsamy, 2019). In 1962, EGF was discovered by Stanley Cohen, whilst conducting research based on a nerve growth factor (Cohen, 1986). New-born mice were injected with a crude extract of EGF, causing their eyes and teeth to develop at an earlier stage compared to normal mice (Navis, 2007). This growth factor acts as a primary messenger molecule in order to facilitate the EGF signal transduction pathway.

1.5.1. Structure of Epidermal Growth Factor

The molecular weight of EGF is 6045-Da and the crystal structure of EGF occurs at a pH 8.1. Epidermal growth factor is found in epidermal cells located in the dermis of the skin and within epithelial cells that line the entire interior of the human body. It is also present in saliva, urine, milk, blood as well as tears (Zeng and Harris, 2014).

Epidermal growth factor is a mitogenic factor in humans and it encompasses a 53-amino-acid cytokine with three disulfide bridges, whereby six cysteines generate the formation of three intramolecular disulfide bonds (C1 and C3, C2 and C4, C5 and C6) (Figure 1.5) (Schneider and Wolf, 2008). The formation of these disulfide bonds enables the production of three structural loops (A, B and C loops) (Schneider and Wolf, 2008).These loops generate high-affinity binding further enabling binding of EGF to its receptor.

Once bound to the receptor, the molecule stimulates an intrinsic protein-tyrosine kinase activity, which facilitates a cascade of events, allowing for the signal transduction to occur (Chen *et al*., 2016). This event results in the cell undergoing various biochemical changes. These biochemical changes result in an increase of protein synthesis, an excess of intracellular calcium levels as well as increased glycolysis levels. These changes consequently enable DNA synthesis, cell proliferation, angiogenesis and cell signalling of proteins (Iwakura and Nawa, 2013).

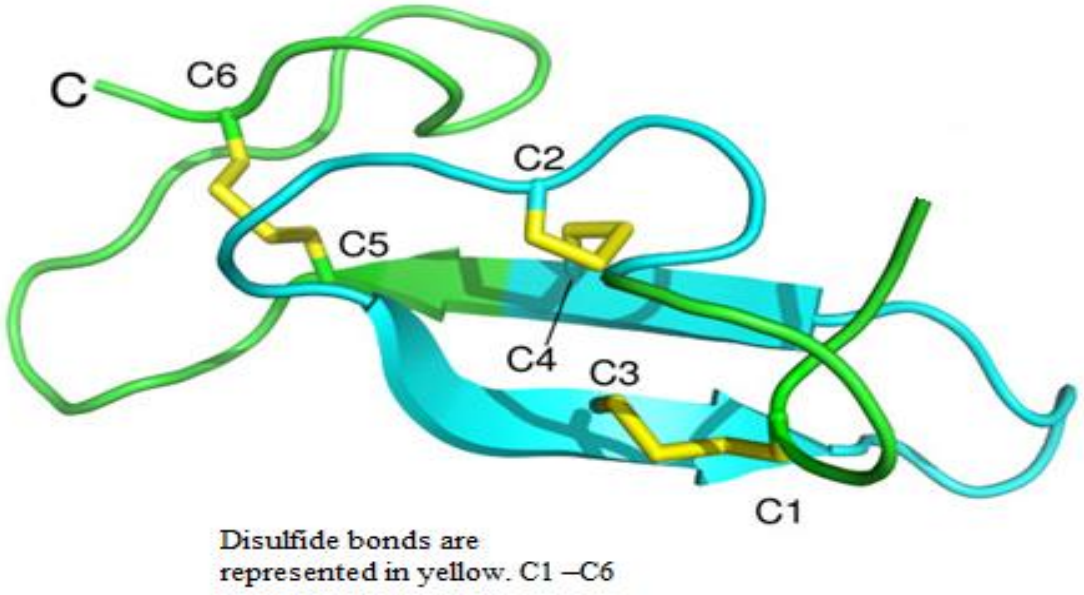


Figure 1.5: Animated image representing the structure of EGF. Disulfide bonds are represented in yellow. C1 –C6 represents the six cysteines which generate the formation of three intramolecular disulfide bonds. Adapted from (Silva *et al*., 2011)

1.5.2. Function of Epidermal Growth Factor

Binding of EGF to its receptor (EGFR) enables the facilitation of cell proliferation, differentiation as well as cell survival. Epidermal growth factor is a vital protein in the human body as it stimulates epidermal cells and fibroblasts; enabling rapid healing and regeneration of cells upon injury. Its therapeutic effects in skin healing are mediated by promotion of the growth of collagen, whilst keratinocytes enhance skin healing and endotheliocytes enhances vascular growth (Kim *et al*., 2015). Salivary EGF plays a vital role in healing gastroesophageal and oral ulcers; whilst also providing mucosal protection (Konturek *et al*., 1991).

In pregnancy, EGF plays an essential role in placental growth acting as an autocrine factor by regulating the growth and function of the placenta (Lessey, 2002). Epidermal growth factor occurs at high concentrations during the early gestational period, whilst there is regulation in later trimesters (Ances, 1973).

1.6. THE EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor receptor (EGFR) is a tyrosine-kinase receptor, which stimulates various biological key processes in the human body such as proliferation, tumorigenesis and homeostasis (Schneider and Wolf, 2008).

1.6.1. Structure of the Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) belongs to the family of epithelial tyrosine kinases, which includes three distinct receptors: EGFR (ErbB1), HER3 (ErbB3), and HER4 (ErbB4) (Iwakura and Nawa, 2013).

EGFR (ErbB1) is a 185 kDa transmembrane glycoprotein encoded by the HER1 oncogene located on chromosome 7p12. It contains ligands which are composed of type ı transmembrane tyrosine kinases (Schneider and Wolf, 2008). These proteins contain five regions or domains; the hydrophobic transmembrane domain, a juxtamembrane stalk, the carboxy-terminal fragment, the N-terminal extension and the EGF module (Schneider and Wolf, 2008; Nagy *et al*., 2010; Figure 1.6). This activity assists the cell during cell growth and proliferation, for the secretion of proteins, apoptosis and organ repair (Iwakura and Nawa, 2013). Figure 1.7 represents the basic structure of EGFR and indicates the transmembrane proteins found inside the receptor. The receptor compromises of two domains; the extracellular domain and the intracellular domain. The extracellular domain is further divided into the ligand binding domains (LD1, LD2) and cytosine rich regions (CR1, CR2); whilst the intracellular domain consists of a catalytic tyrosine kinase (TK) and a carboxyl-terminal tail (CT) (Lv *et al*., 2016). The phosphorylation sites are located within the TK and CT regions. The short transmembrane spanning sequences divide the receptor into equal domains (Lv *et al*., 2016).

The activation of EGFR occurs upon binding to the EGF ligand, the receptor then initiates a change known as dimerization. The inactive monomeric molecule changes into an active homodimer (Yarden and Schlessinger, 1987). As a result of dimerization, signals are sent to the nucleus of the cell from the cytoplasm via the binding of cytoplasmic messenger proteins to phosphorylated tyrosine residues. This in-turn triggers various biological responses such as apoptosis, cell survival, invasion and proliferation (Harari and Huang, 2001).

Additionally, EGFR has approximately five domains, namely; the C-terminal tail, a tyrosine kinase domain, the extracellular domain, the juxtamembrane and the transmembrane alpha helix domain (Figure 1.6) (Nagy *et al*., 2010). Mutations caused around the tenth day of pregnancy,

occurring on the short arm of chromosome 7 can lead to fatalities of the development of an embryo; hence highlighting the importance of this receptor.

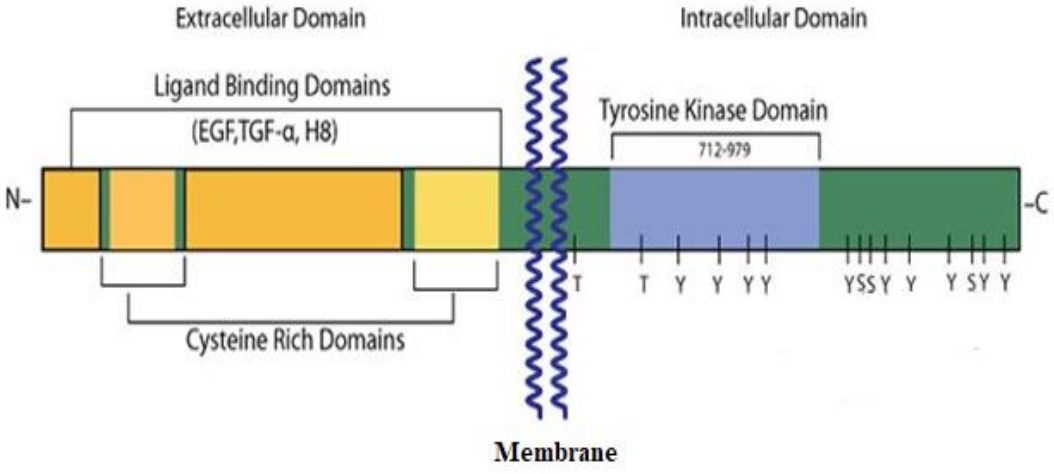


Figure 1.6: Schematic diagram displaying the 5 domains of the EGFR protein. Adapted from (Thermofisher Scientific, 2017)

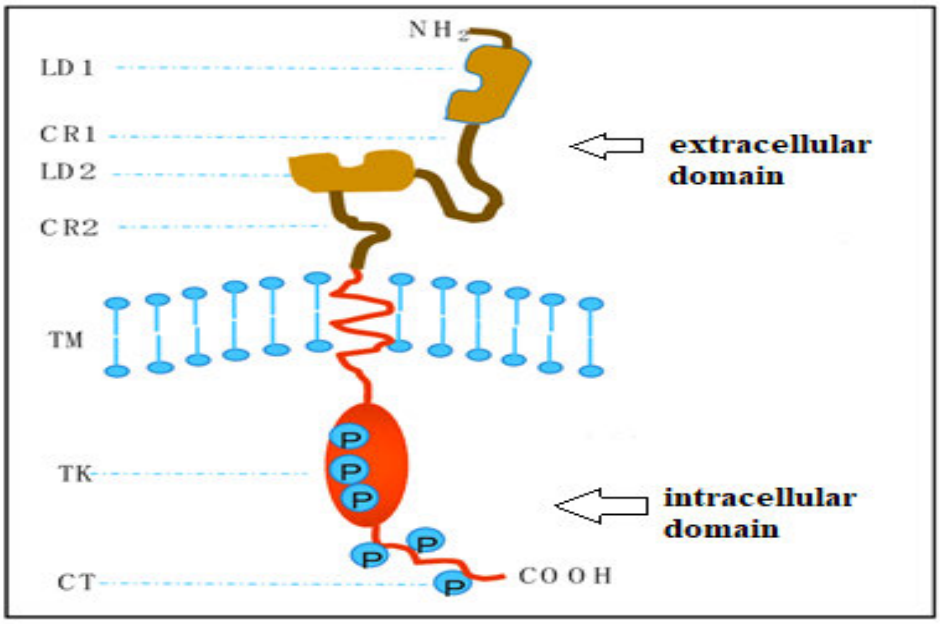


Fig 1.7 Basic structure indicating transmembrane proteins found in the epidermal growth factor receptor (EGFR). LD1 and LD2 in the extracellular domain represent the ligand binding domains, whilst CR1 and CR2 found in the extracellular domain represents cytosine rich regions. TM represents short transmembrane spanning sequences, whilst TK which is located in the intracellular domain represents a catalytic tyrosine kinase. CT which is also located in the intracellular domain is the carboxyl-terminal tail. The phosphorylation sites are indicated by the blue circled P's and are located within the TK and CT regions. (Adapted from Lv *et al*., 2016)

1.6.2 Epidermal Growth Factor Receptor and Signal Transduction

Epidermal growth factor receptor is a type one single-pass tyrosine kinase transmembrane protein, which is activated by binding to its respective binding ligands (either EGF or the transforming growth factor α). Notably, a kinase protein has the ability to phosphorylate other proteins thereby leading to their activation. Once EGF is bound to EGFR, EGFR is activated thereby causing the initiation of a cascade of downstream signalling proteins.

Growth factor receptor-bound protein 2 (GRB2) interacts with EGFR, which then activates the Son of Sevenless (SOS) protein; followed by the interaction of SOS with a rat sarcoma (RAS) protein (Orton *et al*., 2005). Thereafter; RAS is bound to guanosine diphosphate (GDP) which is replaced with guanosine triphosphate (GTP), this enables the protein to become active (Margolis and Skolnik, 1994). Mutations which arise in RAS can trigger severe complications such as cancer, rapidly accelerated fibrosarcoma (RAF) protein then becomes activated, which in turn activates the mitogen-activated protein kinase kinase (MEK) pathway (Fernàndez-Medarde and Santos, 2011). Finally, MEK activates a mitogen-activated protein kinase (MAPK) (Riesco *et al*., 2017) (Figure 1.8). The entire process is facilitated in the cytoplasm, once all proteins are active; the signal is then transferred to transcription factors which then enter the nucleus in order to enable genes responsible for growth and division responses.

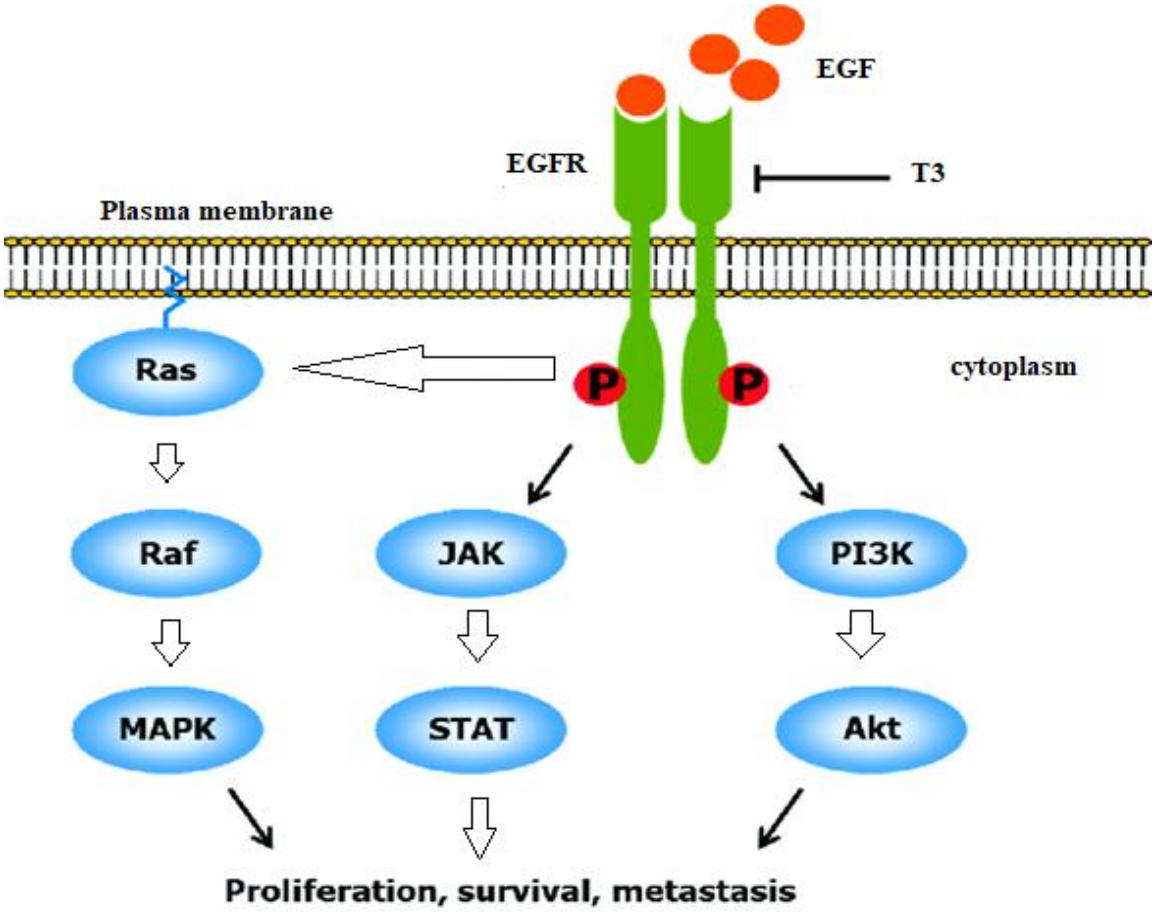


Figure 1.8: Epidermal growth factor receptor (EGFR) and its downstream signalling proteins. EGF - epidermal growth factor; EGFR - epidermal growth factor receptor; PI3K,- phosphatidylinositol 3-kinase;STAT- signal transducer and activator of transcription; P-phosphorylation; MAPK - mitogen-activated protein kinase; JAK - Janus kinase; Akt - protein kinase B. Arrows indicate activation whilst perpendicular lines indicate inhibition. Adapted from (Eitsuka *et al*., 2016)

1.6.3 The Epidermal Growth Factor Receptor in Pregnancy

The human placenta expresses high concentrations of EGFR; and contains high levels of EGF in comparison with other human non-malignant tissue (Hastie *et al*., 2019; Magid *et al*., 1985). Therefore, EGFR plays a vital role in placental development and aberrant EGFR signalling transduction is associated with PE development as well as other trophoblastic disorders (Hatsie *et al*., 2019). Also, the heparin-binding-epidermal growth factor (HB-EGF) enables the implantation of a blastocyst during the early stages of pregnancy (Lim and Dey, 2009). Failure of successful implantation results in miscarriage.

1.6.4 The Function of Epidermal Growth Factor Receptor in Human Diseases

EGFR plays a major role in human diseases, overexpression of EGFR results in tumor formation and progression to cancer. The most common places for tumour formation are the head and neck (Parkin *et al*., 2005). Lung and anal cancers have also been identified as a result of overexpression of EGFR (Walker *et al*., 2009).

EGFR has also been identified as a vital component in the progression of wound healing. The process of wound healing facilitates the differentiation of fibroblasts into myofibroblasts and is regulated by the cytokine transforming growth factor-β1 (TGF- β1) (Midgley *et al*., 2013). EGFR is a key regulator for the facilitation of this process as the receptor interacts with other receptors [hyaluronan (HA) and CD44] in order to facilitate a cellular response (Midgley *et al*., 2013). Other human diseases caused by EGFR include; malignant and inflammatory diseases such as eczema and psoriasis. These disorders are further caused by repeated deregulation of EGFR, hence resulting in the stimulation of malignant as well as benign skin disorders (Jost *et al*., 2000). Off note; EGFR stimulates the regulation of the endometrial function during the implantation stage of pregnancy. EGFR signalling in the endometrium is critical for successful progression of early pregnancy based on studies performed in EGFR knockout mice (Large *et al*., 2014).

1.6.5 The Epidermal Growth Factor in HIV associated Preeclampsia

The Trans-Activator of transcription (tat) protein is a regulatory protein which is found in HIV type 1 (HIV-1) and is encoded by the tat gene (Jiang *et al*., 2018). The protein is involved in the facilitation of viral transcription and plays a role in the pathogenesis of complications caused by HIV-1 (Jiang *et al*., 2018). Furthermore, the protein is also involved in other biological processes such as the stimulation of cell proliferation, entering cells and changing the gene expression of host cells (Nabell *et al*., 1994). The tat protein inhibits EGF related processes such as RAS/AKT and P13K/AKT pathways, hence inhibiting cellular responses (cell migration, adhesion, survival and differentiation) (Kim *et al*., 2011). The down-regulation of EGF in the presence of tat protein during HIV related pregnancy, may lead to negative trophoblastic development. This in-turn could enhance the development of PE. In addition, Tat contains an arginine and lysine rich sequence, which mimics the vascular endothelial growth factor-A (VEGF-A), Tat thereafter binds to FlK-1/kinase insert domain receptor (Flk-1/KDR), hence affecting angiogenesis (Albini *et al*., 1996).

Kamath *et al*. indicated the importance of platelets for the pathogenesis of PE, as platelet function analysis can serve as sensitive PE biomarkers. Platelets prevent bleeding during early stages of clot formation and enable haemostasis to occur during pregnancy (Kamath *et al*., 2021). Placental vascular under perfusion or maternal endothelial damage are reported as the main causative agents of PE. This results in increased platelet consumption during PE. Bone marrow is then triggered to produce more platelets which lead to an increase of platelet indices such as mean platelet volume (MPV) and platelet distribution width (PDW). It is therefore noted that platelet indices can be used as a tool for the prediction of PE (Kamath *et al*., 2021).

1.7. ALTERNATE PATHWAYS ASSOCIATED WITH EGF

The epidermal growth factor and EGFR plays a role in tumorigenesis by promoting tumor cell proliferation and inhibiting apoptosis. Hypoxia- inducible factor (HIF-1) is an essential transcription factor which stimulates cellular response to hypoxia (Swinson and O'Byrne, 2006). Notably, PE is a hypoxic and oxidative stressed microenvironment (Swinson and O'Byrne, 2006). The stimulation of EGFR is induced by hypoxia, enabling EGFR to increase the cellular response to hypoxia. This is accomplished by increasing HIF-1α, which acts as a survival factor (Swinson and O'Byrne, 2006). Previously, our group has demonstrated increased placental expression of HIF-1α in PE compared to normotensive pregnancies indicating that placental hypoxia and endoplasmic reticulum (ER) stress are interrelated contributory factors to the pathogenesis of PE (Verma *et al*., 2017).

Notably EGFR is also associated with the endoplasmic reticulum (ER) stress pathway. ER stress occurs during hypertension where EGFR enables the activation of ER stress, this occurs via angiotensin II (AngII) which induces vascular remodelling (Liu *et al*., 2006). This is facilitated by the activation of EGFR and ER stress through an ADAM metallopeptidase domain 17 (ADAM17) signal mechanism (Liu *et al*., 2006).

In addition, Malik *et al*. reported reduced levels of EGF in preeclamptic women. Furthermore, a correlation was observed between leukemia inhibitory factor (LIF) and EGF. The study also highlighted the ability of EGF to stimulate invasion and motility of trophoblastic cells by activating MAPK, Akt and urokinase plasminogen activator (uPA) (Malik et *al.,* 2017). The study additionally reported decreased levels of STAT3 in preeclamptic patients. The importance of STAT3 is further highlighted as it plays a crucial role in the invasion of trophoblast cells as well as in the activation of the JAK-STAT pathway (Malik *et al.,* 2017). The significant phosphorylation of STAT1 and STAT3 at Ser 727 residues lead to a decline in total STAT1,

whilst inhibition of ERK1/2 phosphorylation which is caused by U0126 leads to a significant decline in EGF-mediated invasion. Hence, stimulating reduced phosphorylated forms of STAT3 and STAT1 (Malik *et al.*, 2017).

Li *et al*. reported decreased levels of adrenomedullin (ADM) in PE due to a poor response to EGF (Li *et al*., 2003). This could be due to a failed compensatory increase in maternal serum ADM which occurs during PE. Furthermore, the study highlighted poor EGF response resulting from impaired spontaneous placental syncytialization and ADM reduction in PE (Li *et al*., 2003).

Based on a study by Malik *et al*. a decline of miR-92a-1-5p was observed in EGF which may be regulated by STAT1/ STAT3 and controls HTR-8/SVneo cell invasion by targeting MAPK8 and FAS. This leads to the increase of MMP-2/ MMP-9 expression (Malik *et al*., 2020).

A study by Feng *et al*. reported poor trophoblast cell invasion which is caused by the increase of KISS-1 expression and leads to the decline of EGF. This therefore results in the pathogenesis of PE. Kisspeptin and KISS-1 are placental derived hormones and are elevated during pregnancy (Feng *et al*., 2021). In addition EGF stimulates human trophoblast cell invasion. This occurs by decreasing ID3 mediated KISS-1 expression since the ID3 protein mediates the effect of PE which is done by reducing EGF and KISS-1 levels (Feng *et al*., 2021).

1.8. AIM

To determine the serum concentrations of epidermal growth factor (EGF) and its receptor (EGFR) in HIV associated preeclampsia compared to normotensive pregnancies.

1.8.1 Objectives

- To investigate the effect of HIV status (HIV positive *vs.* HIV negative) on EGF and EGFR levels, irrespective of pregnancy type using a Bioplex immunoassay
- To investigate the effect of pregnancy type (preeclampsia vs. normotensive pregnancy) on the concentration of EGF and EGFR levels, irrespective of HIV status using a Bioplex immunoassay
- To investigate the effect of the synergy of HIV infection and pregnancy type on the level of EGF and EGFR across all study groups using a Bioplex immunoassay
- To correlate EGF and EGFR levels with patient demographics
- To correlate the concentration of EGF on EGFR and *vice versa* with regards to signal transduction in PE and normotensive pregnancies.

CHAPTER 2

THE JOURNAL OF
Obstetrics and Gynaecology Research

THE EPIDERMAL GROWTH FACTOR/ EPIDERMAL GROWTH FACTOR RECEPTOR AXIS IN HIV ASSOCIATED PREECLAMPSIA

Journal:	*The Journal of Obstetrics and Gynaecology Research*
Manuscript ID	JOGR-2021-2440
Manuscript Type:	Original Article
Date Submitted by the Author:	08-Nov-2021
Complete List of Authors:	Laldeo, Arisha; University of KwaZulu-Natal Nelson R Mandela School of Medicine, Optics and Imaging Singh, Shoohana; University of KwaZulu-Natal Nelson R Mandela School of Medicine, Optics and Imaging Centre Naicker, Thajasvarie ; University of KwaZulu-Natal College of Health Sciences, School of Laboratory Medicine and Medical Sciences
Keywords:	2.510 Preeclampsia/Eclampsia < 2.500 Obstetric Complications < 2 Obstetrics, 2.115 Vascular Biology < 2.100 Obstetrics:Basic Science < 2 Obstetrics

THE EPIDERMAL GROWTH FACTOR/ EPIDERMAL GROWTH FACTOR RECEPTOR AXIS IN HIV ASSOCIATED PREECLAMPSIA

(EGF/EGFR IN HIV ASSOCIATED PE)

Arisha Laldeo; Shoohana Singh and Thajasvarie Naicker

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

Corresponding author details:

Arisha Laldeo

Optics and Imaging Centre,

Doris Duke Medical Research Institute

College of Health Sciences

University of KwaZulu-Natal,

P Bag 7, Congella, 4013

South Africa

Telephone: (27) 628088414

E-mail: arisha.laldeo@gmail.com; naickera@ukzn.ac.za

Abstract

Aim - To determine the concentration of epidermal growth factor (EGF) and its receptor (EGFR) in HIV associated preeclampsia compared to normotensive pregnancies. In addition, this study investigates the effect of HIV status (HIV positive *vs.* HIV negative) and pregnancy type (normotensive *vs.* preeclamptic) on the expression of EGF and EGFR, using a Bio-Plex immunoassay.

Methods and Materials – Serum samples were obtained from 80 pregnant women at term. The study groups were divided into normotensive HIV positive (n =20), normotensive HIV negative (n = 20), preeclamptic HIV negative (n = 20) and preeclamptic HIV positive (n = 20). The Bio-Plex multiplex immunoassay method was used to analyse serum EGF and EGFR concentration.

Results – A significant decline in EGF and EGFR levels were observed in preeclamptic compared to normotensive pregnancy at term. Based on HIV status, both EGF and EGFR, were similar between infected and uninfected women. However, for serum EGFR concentration there were significant differences within PE group based on HIV status. Significant differences were observed between normotensive and preeclamptic based on HIV status, except for normotensive HIV negative *vs* PE HIV negative. Furthermore, there was no significance in the normotensive group based on HIV status.

Conclusion – This study indicates that the regulation of EGF and EGFR plays a vital role in HIV and preeclampsia (PE). Aberrant trophoblast survival and migration possibly emanates from dysregulation of EGF/EGFR signalling axis. In HIV associated PE both the ligand and its receptor are dysregulated in PE possibly due to immune restoration from antiretroviral therapy and/or the effect of the HIV-1 trans-activator of transcription protein.

Keywords – EGF, EGFR, HIV, Hypertension, Preeclampsia

Introduction

The global human immunodeficiency virus (HIV) pandemic affects 38 million people.[1] More than 70% of HIV infections and acquired immunodeficiency syndrome (AIDS) related deaths occur in sub-Saharan Africa.[2] In South Africa (SA), the HIV prevalence rate is approximately 13.7% affecting 8.2 million people; of which 25% are in their reproductive age (15–49 years).[3] Clinical surveillance data from 37 countries report that 23.1% of people living with HIV who were hospitalized with COVID-19 demised.[4] Pregnancy involves immunological and physiological adaptations that make women susceptible to viral infections.[5] In light of the fact that hypertensive disorders of pregnancy (HDPs) account for 18% of all maternal deaths in SA; research into HIV associated preeclampsia (PE) is relevant, as approximately 30% of antenatal attendees are HIV infected.[6, 7]

Preeclampsia (PE) is a hypertensive disorder of pregnancy (HDP) characterised by blood pressure ≥ 140/90 mmHg and presence/absence of proteinuria (≥ 300mg/d) after 20 weeks of gestation.[8] Moreover, HIV infected preeclamptic women be susceptible to PE development however, anti-retroviral therapy (ART) predisposes PE due to immune reconstitution.[7] Off note, SA has the largest ART rollout in the world.[9]

Angiogenic mediators have been implicated in viral infection as well as in PE development.[10] Activation of the Epidermal Growth Factor (EGF) and its receptor, Epidermal Growth Factor Receptor (EGFR) is known to increase angiogenesis.[11] Moreover, early reports have associated both EGF and EGFR with an up-regulation of cytotrophoblast invasion via downstream signalling networks.[12]

The binding of EGF to EGFR enables EGFR to dimerize which in-turn raises its intracellular tyrosine kinase activity. This activates a cascade of cell signalling events such as the rat sarcoma (RAS)-rapidly accelerated fibrosarcoma (RAF)-mitogen activated protein kinase (MEK)-extracellular signal regulated kinases (ERK)-mitogen activated protein kinase (MAPK) (RAS-

RAF-MEK-ERK MAPK), Janus associated kinases (JAK)-signal transducer and activator of transcription (STAT) (JAK/STAT) and protein Kinase-B (AKT)-phosphoinositide 3-kinases (P13K)-mammalian target of rapamycin (mTOR) (AKT-P13K-mTOR) pathways.[13, 14] These signalling events decrease apoptosis, permit cell proliferation as well as angiogenesis.[15]

The HIV-1 accessory protein, trans-activator of transcription (Tat) inhibits the RAS/AKT and P13K/AKT pathways.[16] Moreover, since both EGF and EGFR are integral to placentation and angiogenesis, this study aims to compare the serum levels of EGF and EGFR, in HIV associated preeclamptic and normotensive pregnant women.

Methods and Materials

Ethical Approval

Institutional ethics and postgraduate approval, hospital manager's support as well as informed consent were obtained (BREC/00002899/2021) prior to collection of samples. This study uses archived serum samples collected from a large regional hospital in eThekwini, KwaZulu-Natal, SA.

Study Population

A total of 80 samples were used to generate the study population. This was stratified by pregnancy type into normotensive pregnant (n=40) and preeclamptic pregnant (n=40) women. The groups were further divided into four sub-categories a) normotensive HIV negative (n=20), b) normotensive HIV positive (n=20), c) preeclamptic HIV negative (n=20), d) preeclamptic HIV positive (n=20). All HIV positive patients received ART.

Inclusion criteria for PE included new onset high blood pressure (systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg) which occurs at or after 20 weeks of

gestation including one or more of the following conditions; proteinuria (urinary protein ≥ 300mg per 24 hours) and/or maternal organ dysfunction. Exclusion criteria for the PE group included those with unknown HIV status, eclampsia, a previous hypertension history, chronic hypertension, chronic diabetes, polycystic ovarian syndrome, placental abruption, sickle cell disease, thyroid disease, asthma, chorioamnionitis, chronic renal disease and intrauterine death.

Demographics and clinical data for the study group were collated into a spreadsheet.

Sample Type

Serum samples were stored in an ultra-freezer at -80°C until analysis.

Immunoassay Method

The Human Angiogenesis Growth Factor Panel 1 and the Human Angiogenesis Panel 2 (Milliplex® Map Kit, catalogue no: HAGP1MAG-12K and HANG2MAG-12K) were respectively performed according to the manufacturer's instructions. The standards were serially diluted. Samples for panel 1 and 2 were diluted 1:3 and 1:5 with assay buffer respectively. The controls and serum matrix for both panels were diluted with deionized water.

Two separate 96 well plates were used, the first for EGF and the second for EGFR immunoassay. Assay buffer, standards and controls were added to respective wells. Matrix solution and diluted serum samples were added to designated wells. Antibody-immobilized beads for EGF and EGFR were added to each well and incubated overnight at 2-8˚C. Well contents were then removed and washed three times with wash buffer. Detection antibodies (1 hour at room temperature) were added followed by Streptavidin-phycoerythrin (30 minutes at room temperature). Well contents were then removed and plates were washed three times with wash buffer. Drive fluid was then added to all wells and the immunoassay plates were both read by the Bio-Plex®MAGPIX™ Multiplex Reader (Bio-Rad Laboratories Inc., USA). The data was

then extrapolated from the multiplex analysis using the Bio-Plex Manager ™ software version 4.1.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 5.00 software for Windows (GraphPad Software, San Diego California USA). The Kolmogorov Smirnov test was used to test for normality. The Mann-Whitney U test was used to identify the statistical significance of the data. All results obtained are represented as the median and interquartile range (IQR). Kruskal-Wallis test was used together with the Dunn's Multiple comparison *post hoc* test in order to determine the significance across all groups; whereby $p < 0.05$ was considered statistically significant.

Results

All data were non-parametrically distributed. Table 1 represents patient demographics and clinical characteristics across study groups. Gestational age ($p < 0.001$), birth weight ($p < 0.001$), systolic BP ($p < 0.001$), BMI ($p = 0.048$), diastolic BP ($p < 0.001$) and maternal weight ($p = 0.002$) were significantly different across groups. No statistical significance was observed for maternal age ($p = 0.065$).

Table 1: Patient Demographics across study groups (n=80)

	Normotensive HIV Negative	Normotensive HIV Positive	Preeclamptic HIV Negative	Preeclamptic HIV Positive	*p*-value
Maternal Age (years)	24 (21 - 31)	25 (23 – 29)	22 (19 -29)	28 (25 -32)	0.065
Gestational Age (weeks)	39 (38- 40)	38 (38 -40)	33 (27 - 37.5)	36 (31 - 38)	0.001***
Birth Weight (kg)	3.2 (3.0 - 3.8)	3.3 (2.9 - 3.5)	2.5 (1.5 - 2.9)	2.5 (1.8 – 3.0)	0.001***
Systolic BP (mmHg)	120 (111.5 - 125.5)	123.5 (111.5 - 130)	160.5 (153.5 - 179)	153.5 (146 - 169)	0.001***
BMI (Kg/m²)	30.5 (27.6 - 34)	27.8 (24.3 - 31.6)	28.5 (24.3 - 32.2)	33.6 (27.1 - 38.1)	0.048*
Diastolic BP (mmHg)	71.5 (62.5 - 78)	74.5 (70 - 82)	105.5 (97 - 109)	96.5 (91 - 100)	0.001***
Maternal Weight (kg)	75.2 (70 - 84.2)	69.5 (63.9 - 80.8)	68.5 (60.6 - 79.3)	86.9 (70.8 - 93.4)	0.002*

Results are represented as the median (IQR); *$p < 0.05$; ***$p < 0$ .001.Kruskal-Wallis equality of populations rank test was used followed by the pairwise Dunn test where KW is <0.05. Gestational age was significantly lower in PE compared to normotensive pregnancy, birth weight was significantly lower

in PE compared to normotensive pregnancy, systolic BP was significantly higher in PE than in normotensive pregnancy, BMI was significantly higher in PE than in normotensive pregnancy, diastolic BP was significantly higher in PE compared to normotensive pregnancy and maternal weight was significantly higher in PE compared to normotensive pregnancy.

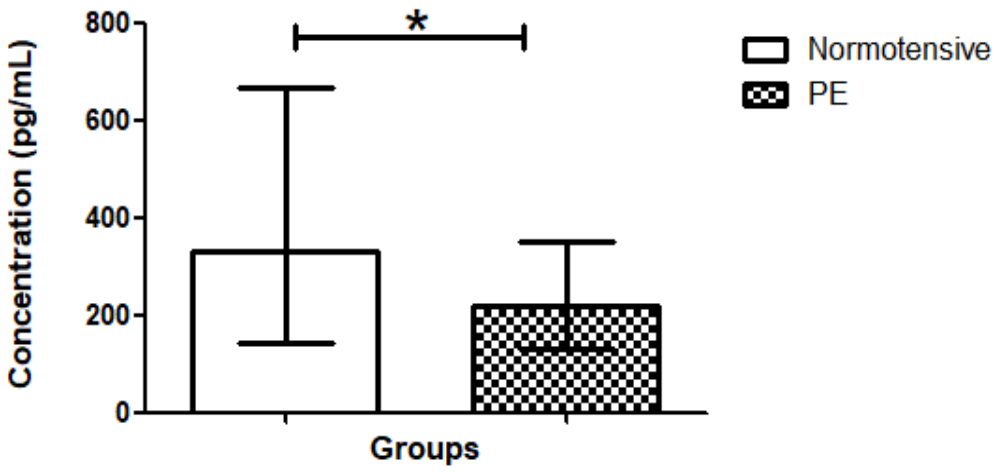
Serum Concentration of Epidermal Growth Factor

Pregnancy Type: Serum EGF levels were significantly decreased in the preeclamptic (median = 218pg/mL, IQR = 131 - 350pg/mL) compared to normotensive pregnant (median =329pg/mL, IQR = 143 - 667pg/mL) women, irrespective of HIV status (Mann-Whitney U = 1454; p = 0.0214; Figure 1A).

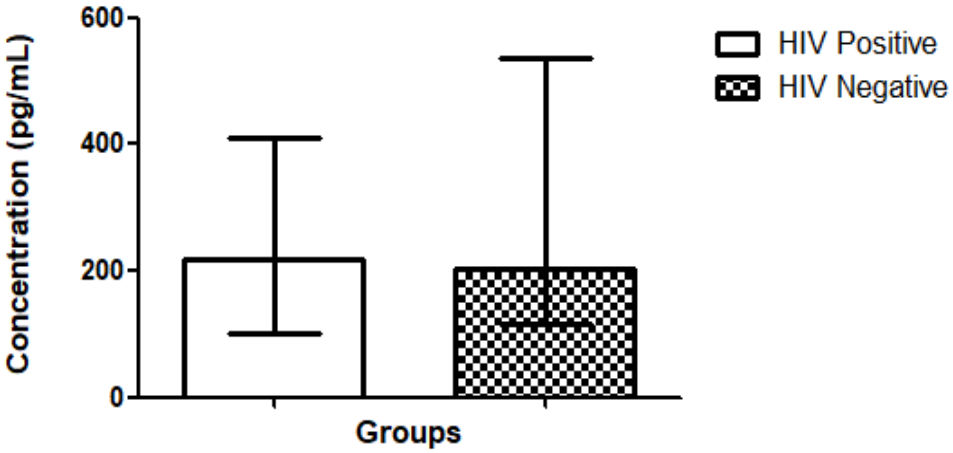
HIV Status: In contrast, serum EGF concentration did not differ between HIV positive (median = 218pg/mL; IQR = 101 - 410pg/mL) versus HIV negative (median = 203pg/mL, IQR = 115 - 537pg/mL) pregnant women, regardless of pregnancy type (Mann-Whitney U = 2516; p = 0.6593; Figure 1B).

Across all groups: EGF expression did not differ across all study groups *i*.e., normotensive HIV negative (median = 301pg/mL, IQR = 90 - 675pg/mL) *vs.* normotensive HIV positive (median = 277pg/mL, IQR = 120 - 558pg/mL) *vs.* preeclamptic HIV negative (median = 210pg/mL, IQR = 131 - 377pg/mL) *vs.* preeclamptic HIV positive (median = 277pg/mL, IQR = 89 - 418pg/mL) (Kruskal-Wallis H = 0.3254; p = 0.9552; Figure 1C)

1A


800
600
400
200
0
Concentration (pg/mL)
*
Groups
Normotensive
PE

1B


600
400
200
0
Concentration (pg/mL)
Groups
HIV Positive
HIV Negative

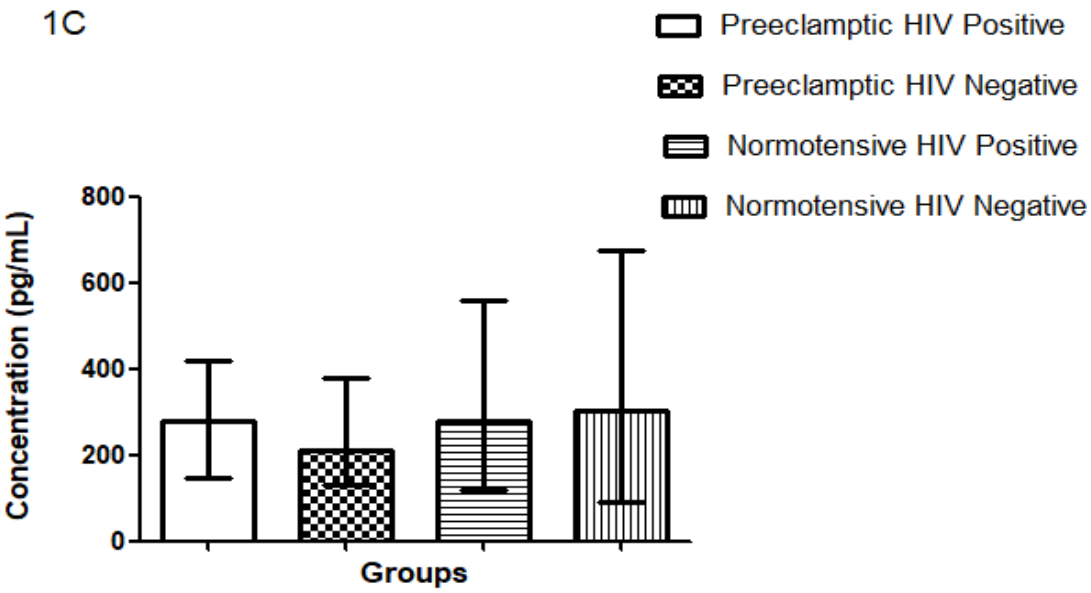


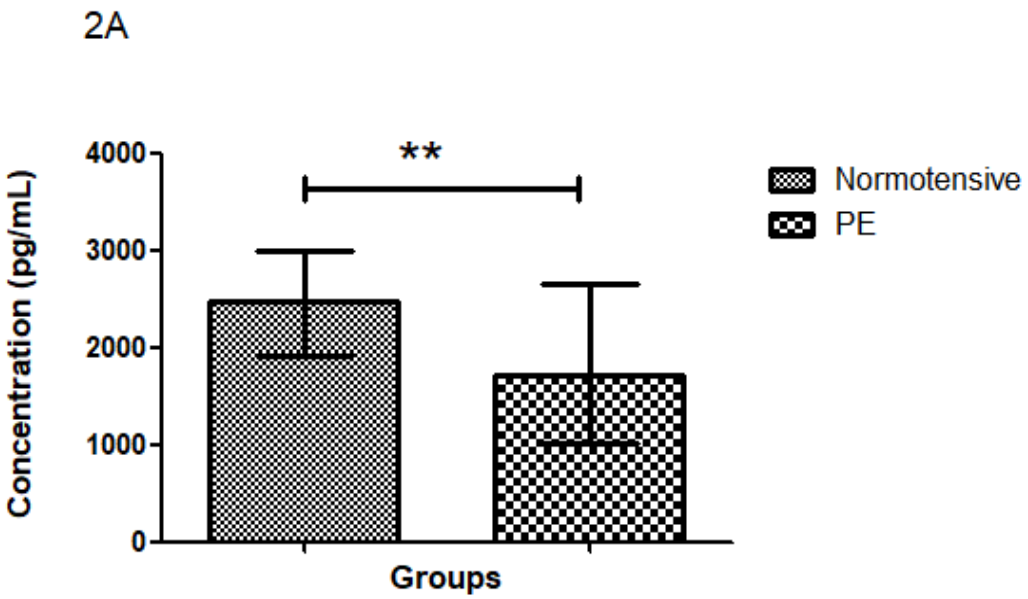
Figure 1: Serum Epidermal Growth Factor Levels (A) Serum concentrations of EGF was statistically significant between the normotensive *vs.* preeclamptic group, (p = 0.0214). (B) Serum concentrations of EGF were not statistically significant between the HIV positive and HIV negative groups, regardless of pregnancy type, (p = 0.6593) and (C) Serum concentrations of EGF were not statistically significant across all groups (normotensive HIV negative *vs.* normotensive HIV positive *vs.* preeclamptic HIV negative *vs.* preeclamptic HIV positive) (p = 0.9552).

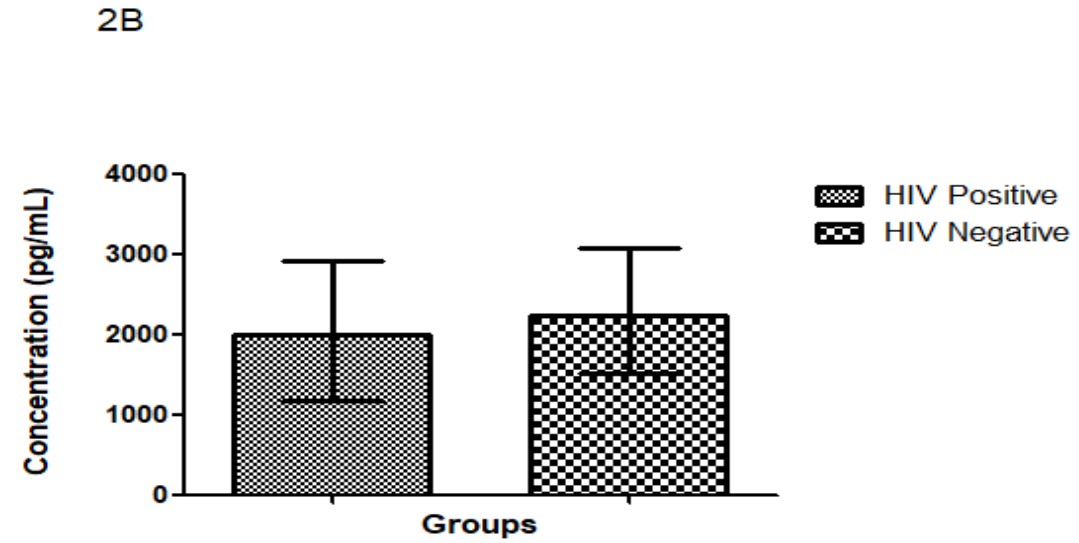
Serum Concentrations of the Epidermal Growth Factor Receptor

Pregnancy type: Based on pregnancy type, serum EGFR was statistically different between normotensive pregnant (median = 2466pg/mL, IQR = 1915 - 2993pg/mL) women *vs.* preeclamptic (median = 1715pg/mL, IQR = 1010 - 2657pg/mL) women, regardless of HIV status (Mann-Whitney U = 1792; $p < 0.0001$; Figure 2A).

HIV Status: EGFR concentration was not statistically different between HIV positive (median = 1993pg/mL, IQR= 1164 - 2917pg/mL) *vs.* HIV negative (median = 2237pg/mL, IQR = 1512 - 3073pg/mL) women, irrespective of pregnancy type (Mann-Whitney U = 2616; p = 0.2092; Figure 2B).

Across all groups: Serum EGFR concentration differed across the groups *i.*e., normotensive HIV negative (median = 2402pg/mL, IQR = 1665 - 2867pg/mL) *vs.* normotensive HIV positive (median = 2588pg/mL, IQR = 1969 - 3156pg/mL) *vs.* preeclamptic HIV negative (median = 2112pg/mL, IQR = 1277 - 3349pg/mL) *vs.* preeclamptic HIV positive women (median = 1352pg/mL, IQR = 886 - 2307pg/mL) (Kruskal-Wallis H = 58.23; $p < 0.0001$; Figure 2C).





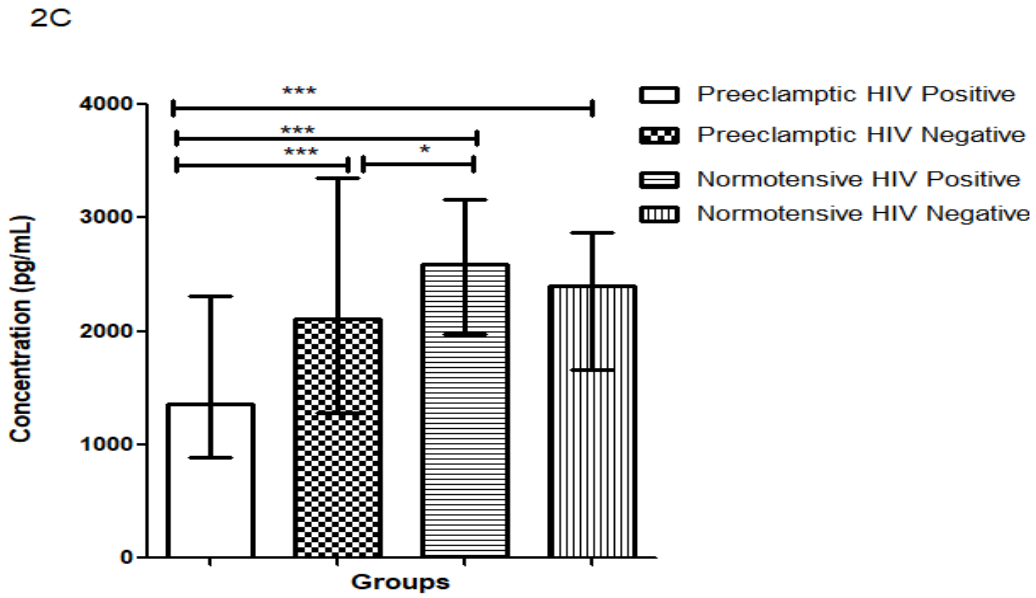


Figure 2: Serum Epidermal Growth Factor Receptor Levels: (A) Serum concentrations of EGFR were statistically significant between the normotensive *vs.* preeclamptic group, ($p < 0.0001$), (B) Serum concentrations of EGFR were not statistically significant between the HIV positive and HIV negative groups, regardless of pregnancy type, ($p = 0.2092$), (C). EGFR concentrations were significantly different within PE group based on HIV status, showing an elevated level in PE HIV negative

women. Significant differences were observed between normotensive and preeclamptic pregnancy showing elevated EGFR levels in normotensive HIV positive and negative groups compared to PE HIV positive and PE HIV negative. However, there was no significance observed for normotensive HIV negative *vs* PE HIV negative. Furthermore, there was no significance in the normotensive group based on HIV status.

Table 2 describes the serum concentration (pg/mL) of the Epidermal Growth Factor and Epidermal Growth Factor Receptor across all groups for normotensive and preeclamptic pregnancy. EGFR was significant between preeclamptic HIV positive and preeclamptic HIV negative ($p = 0.001$) pregnancy.

Table 2: Serum concentration (pg/mL) of the Epidermal Growth Factor and Epidermal Growth Factor Receptor across all groups

	Normotensive			**Preeclampsia**		
	HIV Negative (n=20)	**HIV Positive (n=20)**	***p* value**	**HIV Negative (n=20)**	**HIV Positive (n=20)**	***p* value**
EGF (pg/mL)	301 (90 -675)	277 (120 - 558)	0.559	210 (131- 377)	277 (148- 418)	0.798
EGFR (pg/mL)	2402 (1665 - 2867)	2588 (1969 - 3156)	0.911	2112 (1277 - 3349)	1352 (886.1- 2307)	0.001*

Results are indicated as median and IQR; *$p < 0.05$ is considered statistically significant (Mann Whitney test).

Abbreviations: EGF, Epidermal Growth Factor; EGFR, Epidermal Growth Factor Receptor.

Discussion

This study demonstrates a significant down-regulation of serum EGF levels in preeclampsia compared to normotensive pregnant women, regardless of HIV status. This finding is analogous to studies conducted by Armant *et al*.; who also reported a down-regulation of EGF in PE.[12]Notably aberrant signalling of EGF may account for the deficient trophoblast invasion in PE since receptor-ligand binding of these growth factors facilitates activation of the RAS-RAF-MEK-ERK MAPK and AKT-P13K-mTOR signalling pathways which regulate cell proliferation and invasion.[14]

Similar to our study, Keating *et al.* also reported no differences in serum EGF concentration between HIV negative and HIV positive women.[17] In PE, extravillous trophoblast apoptosis is increased; hindering placentation and causing systemic endothelial damage.[18, 19] Endogenous heparin-binding EGF-like growth factor (HB-EGF) is apoptosis protective. However, metalloproteinases remove the exodomain of HB-EGF and induce autocrine signalling via human EGF receptor 1 and 4 (HER1 and HER4), in a positive-feedback loop, to restrict apoptosis.[12] Also in PE, both HB-EGF and EGF are down-regulated supportive of poor EGF signalling with resultant defective extravillous trophoblast migration that is associated with elevated apoptosis.[12, 20] Off note, plasma EGF levels are also significantly decreased in PE patients ($p < 0.05$).[12]

In this study, we report low birth weight infants in PE compared to normotensive pregnant women ($p < 0.001$). It is widely accepted that a reduction of uteroplacental blood flow, results in low birth weight in PE.[21] Moreover low birth weights may also reflect reduced gestational age in PE.[22] Additionally; in our study a high body mass index was found to be associated with PE, notably obesity is a risk factor for the onset of PE development.[23]

In our study, we report a significant down-regulation of EGFR in PE compared to normotensive pregnancy, regardless of HIV status. Our finding is corroborated by the study of Zaho and Jiang

who attributed the decline to an inhibition of the JAK/STAT signalling pathway.[24] Furthermore, a related study by Shu *et al*. links the down-regulation of EGFR in PE to placental dysfunction, perinatal death as well as multi-organ dysfunction.[25] In contrast to our finding; enhanced messenger ribonucleic acid (mRNA) levels were reported in the hypoxic state of PE.[26] It is possible that polymorphisms of the EGFR gene occurs in PE, however EGFR tyrosine kinase inhibitors are promising therapeutic agents, albeit still requires extensive research for use in pregnancy.[27]

Based on HIV status, there was no significant difference for both EGF and its receptor, irrespective of pregnancy type. Notably, the HIV accessory protein, Tat has a similar arginine- and lysine-rich sequence to Vascular Endothelial Growth Factor (VEGF), hence making the Tat protein a powerful angiogenic factor.[28] The Tat protein however reduces EGF related processes such as RAS/AKT and P13K/AKT pathways.[16] Moreover; the Tat protein enables the activation of increased apoptosis in HIV infected individuals.[29] A retrospective study by Okuma *et al.* reported a high incidence of EGFR (35.7 %) mutations in HIV-infection.[27]

Additionally; HIV-1 Gag modulates EGF induced down-regulation of EGFR. This decline occurs via its internalization by clathrin-dependent endocytosis and its transport and degradation within lysosomes.[30] Furthermore; EGF has been shown to augment the endocytosis of HIV-1 virions via binding to human protein POB1.[31] The similarity of EGF and EGFR between the HIV infected and non-infected groups in our study may be attributed to the use of ART. In a paediatric study of HIV associated nephrotic syndrome, urinary EGF levels were down-regulated.[32] Also since EGF initiates the phosphorylation of p38 and MAPKs, and not ERK1/2 signifying that EGFR signalling augments infection in oral epithelial cells herpes infection of Kaposi sarcoma patients.[33] However another report indicates that the matrix protein p17 of HIV-1 elevates phosphorylation of EGFR with resultant effects on ERK1/2, focal adhesion kinase (FAK), phospholipase C-γ (PLC-γ) and protein kinase C-β (PKC-β) pathways. Concurrently, an EGFR inhibitor would impede angiogenesis and cell signalling.[34]

All HIV positive individuals in our study received highly active anti-retroviral treatment (HAART), to reduce HIV replication and to restore immune competence.[7, 35] Furthermore, HIV infection could possibly act as a protective agent against the onset of PE development; however patients receiving HAART display a higher risk of developing PE.[7, 36, 37] Notably HIV infected patients with advanced lung cancer and with EGFR mutations who receive EGFR-tyrosine kinase inhibitors with ART show extended survival.[27] A synthetic antisense sequence of deoxyribonucleic acid (DNA) in EGFR has been shown to inhibit EGFR expression.

Angiogenesis is dysregulated in PE and in HIV infected individuals receiving ARTs and is associated with adverse birth outcomes.[38] A study by Conroy *et al*., highlights altered angiogenesis in HIV infected individuals on ARV treatment by gestational age particularly in the second and third trimesters of pregnancy.[39] Moreover ARVs are associated with the development of metabolic disorders and endothelial dysfunction; leading to the onset of PE.[40,41] In addition, the non-nucleoside reverse transcriptase inhibitor (NNRTI) targets the protease of severe acute respiratory syndrome coronavirus (SARS-CoV-2), thus preventing the replication of the virus.[42] Furthermore; gefitinib which is an EGFR inhibitor was observed to display a high receptor-ligand binding affinity to the main protease receptor.[42]

It is noted that EGF is a prime candidate for the therapeutic progression in diseases. In non-small-cell lung cancer (NSCLC), EGFR is highly expressed. The development of EGFR inhibitors (gefitinib and erloyinib) were found to reduce tumour regeneration; however responders to treatment were observed to have mutations in the EGFR coding gene.[43]

The limitations of our study were that all HIV positive women received ARTs and the small sample size of sub-groups. Furthermore; the study population was not stratified into late onset PE and early onset PE, as PE is confounded by gestational age.

Conclusion

Our study demonstrates a significant down-regulation of the expression of the ligand EGF and its receptor, EGFR in preeclamptic compared to normotensive pregnancy, regardless of HIV status. It is plausible that genetic mutations of either the ligand and/or its receptor affect trophoblast survival and/or aberrant signalling of EGF may account for the deficient trophoblast invasion in PE. Our study also shows similar EGF and EGFR expression in HIV infected compared to uninfected pregnancies which may emanate from the effect of the HIV-1 Tat, p17 and the gag protein in inhibiting EGF- induced signalling processes. Moreover, the use of HAART, and its reestablishment of immune function may have altered EGF and EGFR levels in HIV infection.

Future recommendation

Serum and plasma EGF and its receptor levels are required in a large cohort study that includes the stratification of PE by gestational age and across the trimesters of pregnancy. Moreover it is important that evaluation of both EGF and EGFR genes elucidate the presence of single nucleotide polymorphisms in woman of African ancestry. Since both EGF tyrosine kinase inhibitor, and EGFR tyrosine kinase inhibitors are used to treat human cancers; they may in the long term require extensive study for their use in PE development.

Acknowledgement

This study was fully funded by the productivity award of Professor T. Naicker.

Disclosure

There are no financial conflicts of interest to disclose.

References

1. UNAIDS. UNAIDS data 2020.Joint United Nations Programme on HIV/AIDS. 2020; [Cited 2020.] Available from URL: https://www.unaids.org/sites/default/files/media_asset/2020_aids-data-book_en.pdf
2. Kharsany AB, Karim QA. HIV infection and AIDS in sub-Saharan Africa: current status, challenges and opportunities. *Open AIDS J* 2016;10:34-48.
3. Stastics South Africa. Mid-year population estimates 2021. National Department of Health. (P03022021.pdf) 2021; [Cited 09 February 2021.] Available from URL: http://www.statssa.gov.za/publications/P0302/P03022021.pdf
4. World Health Organization. WHO warns that HIV infection increases risk of severe and critical COVID-19. 2021 [Cited 15 July 2021.] Available from URL: https://www.who.int/news/item/15-07-2021-who-warns-that-hiv-infection-increases-risk-of-severe-and-critical-covid-19
5. Vale AJM, Fernandes ACL, Guzen FP, Pinheiro FI, de Azevedo EP, Cobucci RN,. Susceptibility to COVID-19 in pregnancy, labor and postpartum period: Immune system, vertical transmission and breastfeeding. *Front Glob Womens Health* . Published Online: 17 February 2021; DOI:10.3389/fgwh.2021.602572
6. Moodley J, Soma-Pillay P, Buchmann E, Pattinson RC. Hypertensive disorders in pregnancy: 2019 National guideline. *SAMJ* 2019;109: S3-S16
7. Kalumba V, Moodley J, Naidoo T. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study: cardiovascular topics. *CVJA* 2013;24:24-27.
8. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and

management recommendations for international practice. *Hypertension* 2018;72:24-43.

9. Meyer-Rath G, Johnson LF, Pillay Y, et al. Changing the South African national antiretroviral therapy guidelines: the role of cost modelling. *PLoS One*.Published Online: 30 October 2017; DOI: 10.1371/journal.pone.0186557.

10. Naicker T, Govender N, Abel T, et al. HIV Associated Preeclampsia: A Multifactorial Appraisal. *Int J Mol Sci* 2021;22:9157.

11. van Cruijsen H, Giaccone G, Hoekman K. Epidermal growth factor receptor and angiogenesis: Opportunities for combined anticancer strategies. *Int J Cancer* 2005; 117: 883-888

12. Armant DR, Fritz R, Kilburn BA, et al. Reduced expression of the epidermal growth factor signaling system in preeclampsia. *Placenta* 2015;36:270-278.

13. Liu Q, Yu S, Zhao W, Qin S, Chu Q, Wu K. EGFR-TKIs resistance via EGFR-independent signaling pathways. *Mol Cancer* 2018;17:1-9.

14. Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers*. Published Online: 17 May 2017;DOI:10.3390/cancers9050052.

15. Pache, JC. Epidermal growth factors. *Encyclopedia of Respiratory Medicine* 2. Published Online: 2006; DOI:10.1016/B0-12-370879-6/00138-1

16. Kim Y, Hollenbaugh JA, Kim D-H, Kim B. Novel PI3K/Akt inhibitors screened by the cytoprotective function of human immunodeficiency virus type 1 Tat. *PloS one*. Published Online: 12 July 2011; DOI: 10.1371/journal.pone.0021781.

17. Keating SM, Golub ET, Nowicki M, et al. The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of US women. *AIDS* 2011; 25:1823-1832.

18. Levy R. The role of apoptosis in preeclampsia. *Isr Med Assoc J* 2005; 7:178-181.

19. Naicker T, Dorsamy E, Ramsuran D, Burton GJ, Moodley J. The role of apoptosis on trophoblast cell invasion in the placental bed of normotensive and preeclamptic pregnancies. *Hypertens pregnancy* 2013; 32:245-256.

20. Moll SJ, Jones CJ, Crocker IP, Baker PN, Heazell AE. Epidermal growth factor rescues trophoblast apoptosis induced by reactive oxygen species. *Apoptosis* 2007;12:1611-1622.

21. Xiong X, Demianczuk NN, Saunders LD, Wang F-L, Fraser WD. Impact of preeclampsia and gestational hypertension on birth weight by gestational age. *Am J Epidemiol* 2002; 155:203-209

22. Gunnarsdottir J, Cnattingius S, Lundgren M, Selling K, Högberg U, Wikström A-K. Prenatal exposure to preeclampsia is associated with accelerated height gain in early childhood. *Plos one*. Published Online: 13 February 2018; DOI: 10.1371/journal.pone.0192514.

23. Robillard P-Y, Dekker G, Scioscia M, et al. Increased BMI has a linear association with late-onset preeclampsia: A population-based study. *PloS one*. Published Online: 17 October 2019; DOI: 10.1371/journal.pone.0223888.

24. Zhao Z, Jiang J. Lowly expressed EGFR-AS1 promotes the progression of preeclampsia by inhibiting the EGFR-JAK/STAT signaling pathway. *Eur Rev Med Pharmacol Sci* 2018; 22:6190-6197.

25. Shu C, Liu Z, Cui L, et al. Protein profiling of preeclampsia placental tissues. PLoS One. Published Online: 13 November 2014; DOI: 10.1371/journal.pone.0112890.

26. Hastie R, Brownfoot FC, Pritchard N, et al. EGFR (epidermal growth factor receptor) signaling and the mitochondria regulate sFlt-1 (soluble FMS-like tyrosine kinase-1) secretion. *Hypertension* 2019; 73:659-670.

27. Okuma Y, Hosomi Y, Imamura A. Lung cancer patients harboring epidermal growth factor receptor mutation among those infected by human immunodeficiency virus. *OncoTargets Ther* 2014; 8:111-115.

28. Zhou F, Xue M, Qin D, et al. HIV-1 Tat promotes Kaposi's sarcoma-associated herpesvirus (KSHV) vIL-6-induced angiogenesis and tumorigenesis by regulating PI3K/PTEN/AKT/GSK-3β signaling pathway. *PloS one*. Published Online: 2 January 2013; doi.org/10.1371/journal.pone.0053145.

29. Chen D, Wang M, Zhou S, Zhou Q. HIV-1 Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim. *EMBO J* 2002; 21:6801-6810.

30. Valiathan RR, Resh MD. Expression of human immunodeficiency virus type 1 gag modulates ligand-induced downregulation of EGF receptor. *J Virol* 2004; 78:12386-12394.

31. Tan Y, Wang J, Su S, et al. Enhancement of endocytic uptake of HIV-1 virions into CD4-negative epithelial cells by HIV-1 gp41 via its interaction with POB1. *Cell Mol Immunol* 2017;14:568-571.

32. Soler-García ÁA, Rakhmanina NY, Mattison PC, Ray PE. A urinary biomarker profile for children with HIV-associated renal diseases. *Kidney Int* 2009;76:207-214.

33. Chen L, Feng Z, Yuan G, et al. Human immunodeficiency virus-associated exosomes promote Kaposi's sarcoma-associated herpesvirus infection via the epidermal growth factor receptor. *J Virol* 2020;94:1-18.

34. Liu D, Zeinolabediny Y, Caccuri F, et al. p17 from HIV induces brain endothelial cell angiogenesis through EGFR-1-mediated cell signalling activation. *Lab Invest*. 2019; 99:180-190.

35. Oguntibeju OO. Quality of life of people living with HIV and AIDS and antiretroviral therapy. *Hiv/Aids-Research and Palliative Care* 2012;4:117-124.

36. Sansone M, Sarno L, Saccone G,et al. Risk of preeclampsia in human immunodeficiency virus–infected pregnant women. *Obstet Gynecol* 2016;127:1027-1032.

37. Maharaj M, Korowlay N. The association of human immunodeficiency virus and skeletal metastases in breast cancer using Tx-99m methyl diphosphonate bone scan. *World J Nucl Med* 2018; 17: 178-181.

38. Paydas S, Ergin M, Seydaoglu G, Erdogan S, Yavuz S. Pronostic significance of angiogenic/lymphangiogenic, anti-apoptotic, inflammatory and viral factors in 88 cases with diffuse large B cell lymphoma and review of the literature. *Leuk Res* 2009; 33:1627-1635.

39. Conroy AL, McDonald CR, Gamble JL, et al. Altered angiogenesis as a common mechanism underlying preterm birth, small for gestational age, and stillbirth in women living with HIV. *Am J Obstet Gynecol*. Published Online: December 2017; DOI: 10.1016/j.ajog.2017.10.003.

40. Fiala M, Murphy T, MacDougall J, et al. HAART drugs induce mitochondrial damage and intercellular gaps and gp 120 causes apoptosis. *Cardiovasc Toxicol* 2004; 4:327-337.

41. Filardi PP, Paolillo S, Marciano C, et al. Cardiovascular effects of antiretroviral drugs: clinical review. *Cardiovasc Haematol Disord Drug Targets* 2008;8:238-244.

42. Jordaan MA, Oluwakemi E, Damoyi N, Shapi M. Virtual screening, molecular docking studies and DFT calculations of FDA approved compounds similar to the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz. *Heliyon.* Published Online: 1 August 2020; DOI:10.1016/j.heliyon.2020.e04642.

43. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007; 7:169-181

CHAPTER 3

3. SYNTHESIS

3.1 PROBLEM IDENTIFICATION

Hypertensive disorders of pregnancy (HDP) contribute to approximately 14% of maternal deaths globally and are the leading causes of maternal deaths worldwide (Machano and Joho, 2020). Preeclampsia is categorized as a HDP and affects nearly 2-8% of pregnancies worldwide (Machano and Joho, 2020). Severe cases of PE are more frequent in poor developing countries, with Sub-Saharan Africa accounting for nearly 16% of maternal deaths caused by HDPs (Hounkpatin *et al*., 2021). In SA, 18% of maternal deaths arise from HDP, the majority of which emanates from PE (Moodley *et al*., 2019). In SA, non-pregnancy related infections such as HIV/AIDS and hypertensive disorders are the commonest cause of maternal mortality and morbidity (Saving Mothers Report, 2017). It is a challenge that approximately 30% of antenatal cases are HIV positive (Kalumba *et al*., 2013).

Moreover the onset of PE and HIV infection also leads to adverse maternal and fetal outcomes, preterm birth, fetal distress as well as placental abruption (Fox *et al.,* 2019). Hence, South Africa represents an ideal site for a study involving HIV and preeclampsia.

This novel study investigates the expression of EGF and EGFR serum concentrations and their role in HIV associated PE. EGF binds to its tyrosine kinase receptor EGFR causing its activation. This activation drives cell cycle progression, enhances cell migration, and affects cell differentiation hence would be involved in trophoblast cell invasion in pregnancy. In addition EGF and EGFR act as protective agents against hypoxia, ensuring the development of adequate spiral arteries for the facilitation of nutrient and gaseous exchange between mother and fetus (Armant *et al*., 2015).

3.2 PREGNANCY TYPE

This novel study reports a significant down-regulation of serum EGF and its receptor (EGFR) in PE compared to normotensive pregnancies, regardless of HIV status. This finding is analogous to a study by Fang *et al*. who reported the down-regulation of EGF/EGFR signalling in PE and was correlated with poor trophoblast invasion (Fang *et al*., 2021). Signalling of EGF enables the RAS-RAF-MEK-ERK MAPK and AKT-P13K-mTOR signalling pathways which stimulates cell proliferation (Wee and Wang, 2017). Furthermore, the study identified an increase of the KISS1 human gene which encodes kisspeptin, with a concomitant down-regulation of EGF expression (Fang *et al*., 2021).

It is generally accepted that EGF/EGFR interaction promotes cell proliferation; yet EGF may paradoxically also inhibit proliferation and induces apoptosis in a transfected cell line expressing EGF receptor on its membrane (Zaho *et al*., 2006). Previous studies have shown that EGF may up-regulate EGFR protein expression via stabilizing EGFR mRNA and increasing EGFR mRNA transcription (Imai *et al*., 1982; McCulloch *et al*., 1998).

In addition, a study by Jain *et al*., reports that HB-EGF stimulates trophoblast survival and invasion during the early stages of pregnancy (Jain *et al*., 2017). Preeclamptic pregnancies display a reduction of HB-EGF, hence having adverse effects during pregnancy. In contrast, a related study reported the increase of EGF like domain 7 (EGFL7) in preeclamptic pregnancies. The increase of EGFL7 levels is associated with the expression of PE (Massimiani *et al*., 2018).

Deviant ligand expression and/or subsequent EGFR signalling is associated with fetal growth restriction, gestational trophoblastic diseases, and preeclampsia. Given the high expression of EGFR in the placenta, it is therefore not surprising that aberrant signal transduction pathways may trigger these placental diseases (Faxén *et al*., 1998; Fondacci *et al*, 1994; Balaram *et al*., 1997; Armant *et al*., 2015). Additionally; low birth weight is observed in preeclamptic pregnancy compared to normotensive pregnancy. The reduction of placental blood flow results in the occurrence of PE; hence leading to reduced fetal growth (Xiong *et al*., 2002). Furthermore; gestational duration in conjunction with fetal growth rate account for birth weight of new-borns. It is evident that the gestational period is reduced in preeclamptic pregnancies hence accounting for low birth weight of new-borns (Xiong *et al*., 2002). High body mass index can additionally be used as an indicator of PE as obesity is associated with an increased risk for PE (Mrema *et al*., 2018).

In addition, Clemente *et al*. reported increased levels of endothelial connexion 43 (Cx43) during pregnancy (Clemente *et al*., 2020). This enables vascular vasodilation; however during PE there is a decline in Cx43. Furthermore, the study highlights placenta derived exosomes positive for EGFR which may lead to endothelial dysfunction in women with PE (Clemente *et al*., 2020).

Cigarette smoking is associated with a reduced risk for the development of PE during pregnancy. Jeyabalan *et al*. reported reduced maternal sFlt-1 concentrations which are associated with smoking during pregnancy (Jeyabalan *et al*., 2008). Furthermore, the risk of PE development is reduced from the exposure of cigarette smoke by moderating the anti-angiogenic phenotype which is located in the syndrome. Nitric Oxide; commonly found in tobacco acts as a protective agent against PE as it promotes vascular relaxation (Karumanchi and Levine, 2010). Lower circulating concentrations of anti-angiogenic proteins such as soluble fms-like tyrosine

kinase 1 (sFlt-1) and soluble endoglin (sEng) are reported from smoking during pregnancy, whilst higher concentrations of pro-angiogenic proteins such as the placental growth factor are observed (Karumanchi and Levine, 2010).

In addition Weitzner *et al.* reported increased levels of alpha fetoprotein (AFP) and unconjugated estriol (UE3) in EOPE; hence acting as an indicator of EOPE (Weitzner *et al*., 2018). Lan *et al*. reported increased serum concentration of sex steroid hormones in pregnant women, which act as regulators for placental function (Lan *et al*., 2020). The study highlighted that women with PE displayed decreased levels of steroid hormones such as estrogen. Estradiol and estrogen receptor-α were down-regulated whilst testosterone and estrogen receptor-β were elevated in preeclamptic pregnancy, hence playing a role in the pathogenesis of PE (Lan *et al*., 2020).

Additionally, Thomopoulos *et al*. reported the use of assisted reproductive technologies for the management of fertility issues (Thomopoulos *et al*., 2013). The study reported techniques such as *in-vitro* fertilization; however they are accompanied by an increased risk of PE development during pregnancy. Single embryo technologies may be used as a step which can reduce the rate of hypertensive complications during pregnancy (Thomopoulos *et al*., 2013).

3.3 HIV STATUS

Our study demonstrates an absence of a statistical significant difference of both EGF and EGFR between HIV infected and uninfected individuals, irrespective of pregnancy status. Similar to our study, Keating *et al*. also reported no differences in serum EGF concentration between HIV negative and HIV positive women (Keating *et al*., 2011). Interestingly they noted a positive correlation between EGF and CD4 T cell count. Adherence to HAART increases survival by successful viral suppression and concomitant elevation in CD4 counts (Grabar *et al*., 2000).

Notably all HIV patients in our study received HAART. It is possible that this similarity may emanate immune reconstitution from the use of ART. In SA, it is mandatory for all HIV infected individuals to receive HAART to prevent mother to child transmission of HIV (Wimalasundera *et al*., 2002). The usage of ART's such as HAART is used to prevent vertical and sexual transmission of HIV. In addition the HAART regimen enables HIV positive individuals to have a manageable and nearly normal lifespan (Sansone *et al*., 2016). During HAART patients are administered with different drugs which inhibit viral replication. In addition HAART overall improves the quality of life of HIV infected individuals, prevents the transmission of HIV to others and improves immune function (Eggelton and Nagalli, 2020). It is

also possible that the HIV accessory Tat protein may have affected EGF levels. The effects of Tat and EGF are dose dependent (Nabell *et al*., 1994).The Tat protein enables transcription of HIV-1. Additionally, the Tat protein inhibits EGF/EGFR induced processes hence leading to adverse trophoblastic survival. Also, Tat stimulates apoptosis in HIV infected individuals (Chen *et al*., 2002). Numerous studies have reported increased incidence of EGFR mutations in HIV infected lung cancer patients (Okuma *et al*., 2015; Okuma *et al*., 2014; Créquit *et al*., 2016).

The Gag polyprotein precursor of HIV-1 drives viral assembly and budding of virus-like particles (Freed, 1998). HIV-1 Gag reduces the decline of EGFR (Valiathan and Resh, 2004), thereby affecting cell signalling via hyperactivation of the ERK/MAP kinase pathway; this may account for the similar concentration of EGFR by HIV status in our study. The lack of difference in EGFR concentration in our study could also be attributed to ART that would neutralize the hyperactivation of the signalling pathways.

Since HIV-positive persons are predisposed to chronic kidney disease (CKD), Muiru *et al*. assessed recognized CKD risk factors with urine biomarkers and found that age correlated with a decline in EGF levels (Muiru *et al*., 2019).

Furthermore, HIV-1 gp41 facilitates attachment to CD4+T cells. Human POB1 facilitates receptor-mediated EGF entry (Chao *et al*., 2013). HIV-1 gp41 promotes endocytosis of HIV-1 by directly binding with human POB1 or indirectly via its internalization of EGF independently of CD4. This mechanism involves binding of EGF with its receptor with consequential signal transduction, and elevated POB1-mediated clathrin-dependent endocytosis (Tan *et al*., 2017). Therefore, HIV-1 gp41 promotes viral endocytosis by interacting with the host protein POB1.

Naicker *et al*. reported the dysregulation of RAAS, angiogenesis, vascular dysfunction and apoptosis pathways in PE. The study outlines HIV-1 accessory and matrix proteins as protagonists which stimulate the increase of angiogenesis and oxidative stress (Naicker *et al*., 2021). The study further highlights PE as an oxidatively stressed microenvironment with elevated NETosis and apoptosis; however angiogenesis is decreased.

3.4 EPIDERMAL GROWTH FACTOR RECEPTOR ACROSS ALL GROUPS

Normotensive HIV positive vs. Normotensive HIV negative – This study reports a significant increase of EGFR in normotensive HIV positive pregnant women compared to normotensive HIV negative pregnant women ($p = 0.001$). Elevated levels of EGFR in HIV positive individuals could be due to confounding EGFR levels due to HAART, as all HIV positive individuals were on the treatment. In addition HAART restores immune competence therefore leading to varying EGFR levels (Kupsamy, 2018).

Preeclamptic HIV positive vs. Preeclamptic HIV negative – A statistically significant up-regulation of EGFR was observed in preeclamptic HIV negative pregnancies, compared to preeclamptic HIV positive pregnancy (p = 0.001). This observation is due to the presence of hypoxia which enables shallow trophoblast invasion, leading to PE during pregnancy (Zhou *et al*., 2013). In HIV, the Tat protein inhibits EGF related processes; hence EGFR expression is elevated in HIV negative individuals

Additionally, mitochondrial dysfunction has being associated with preeclampsia development (Wang and Walsh, 1998). Notably Hastie *et al.* reported that inhibiting the mitochondrial electron transport chain or activating downstream energy-sensing molecules AMP-activated kinase (AMPK), sirtuin 1 (SIRT1), and PPAR-γ co-activator 1 (PGC1α) which significantly downregulates sFlt-1 release from cytotrophoblast cells, without affecting downstream EGFR signalling (Bos *et al*., 2005; *Hatsie et al*.,2019; Nevo *et a*l., 2006; Semenza, 2003; Wang and Walsh, 1998). This advocates that aberrant EGFR signalling or mitochondrial pathology underlies the pathophysiology of PE. Therefore targeting these cellular pathways may be novel therapeutic approaches to prevent PE development.

3.5 LIMITATIONS

The limitations of this study includes small sample size per study group, all HIV positive individuals received ARV treatment which may have confounded the expression of EGF and EGFR. Furthermore, the duration of ARV treatment was not provided. Lastly, the heterogeneity of the study population (late onset and early onset PE) and across trimesters of pregnancy should be considered in future evaluation of EGF/EGFR

3.6 CONCLUSION

This novel study demonstrates a significant decline in serum levels of EGF and EGFR in preeclamptic compared to normotensive pregnant women, regardless of HIV status. A decline in serum concentration of EGF and EGFR levels may not be indicative of their down-regulation since no placenta specific gene expression analysis was performed. This observation may be attributed to aberrant EGF/EGFR signalling in PE which accounts for shallow trophoblast invasion. Compound mechanisms may be involved in the activation of EGFR signalling such as hypoxia, the production of EGFR-ligands by the hypoxic environmental milieu of PE, dysregulated EGF/EGFR expression and mutations. Additionally, similar concentrations of EGF and EGFR were observed in HIV infected and HIV uninfected individuals, regardless of

pregnancy type. This finding may be due to the HIV-1 accessory protein Tat, p17 and the gag protein which inhibits EGF/EGFR signalling. Furthermore, all HIV infected patients received HAART which may have confounded analyte expression for both EGF and EGFR. The expression of EGF/EGFR could therefore be used as a potential biomarker for the indication of PE.

Future studies should be conducted on a larger sample cohort. The study population should be stratified by gestational age into late onset and early onset PE as well as across the trimesters of pregnancy. Furthermore, both EGF and EGFR inhibitors are of great importance in therapeutic studies such as cancer treatment but will require extensive research for their use in preventing PE development.

CHAPTER 4

4. REFERENCES

Albini, A., Soldi, R., Giunciuglio, D.,Giraudo, E., Benelli, R.,Primo, L., Noonan, D., Salio, M., Camussi,G., Rocki, W., Bussolino, F. (1996) The angiogenesis induced by HIV-1 Tat protein is mediated by the FlK-1/KDR receptor on vascular endothelial cells. *Nature Medicin*e **2** (12): 1371-1375

Ances, I. G. (1973) Serum concentrations of epidermal growth factor in human pregnancy. *American Journal of Obstetrics and Gynecology* **115** (3): 357-362

Armant, D. R., Fritz, R., Kilburn, B. A., Kim, Y. M., Nien, J. K., Maihle, N. J., Romero, R., Leach, R. E. (2015) Reduced expression of the epidermal growth factor signalling system in preeclampsia.*Placenta* **36**: 270–278

Balaram. P., John, M., Rajalekshmy, T.N., Nair, B., Schultz, G., Nair, K. (1997) Expression of epidermal growth factor receptor in gestational trophoblastic diseases.*Journal of Cancer Research and Clinical Oncology* 123:161–166.

Bos, R., van Diest, P. J., de Jong, J. S., van der Wall, E. (2005) Hypoxia inducible factor-1 alpha is associated with angiogenesis and expression of bFGF, PDGF-BB, and EGFR in invasive breast cancer. *Histopathology* **46**: 31-35

Brown, M., Magee, L., Kenny, L., Karumanchi, S. A., Mccarthy, F., Saito, S., Hall, D. R., Warren, C., Adoyi, G., Ishaku, S. (2018) The hypertensive disorders of pregnancy: ISSHP classification, diagnosis and management recommendations for international practice. *Hypertension* **72** (1): 24-43

Chao, L., Lu, L., Yang, H., Zhu, Y., Li, Y., Wang, Q., Yu,X., Jiang, S., Chen, Y. H. (2013) Identification of a human protein derived HIV-1 fusion inhibitor targeting the gp41 fusion core structure. *PLoS One* **8**: 1-11

Chau, K., Hennessy, A., Makris, A. (2017) Placental growth factor and pre-eclampsia. *J Hum Hypertens* **31**(12):782-786

Chen, D., Wang, M., Zhou, S., Zhou, Q. (2002) HIV-Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim. *The European Molecular Biology Organization Journal* **21** (24): 6801-6810

Chen, J. T., Ribaudo, H. J., Souda, S., Parekh, N., Ogwu, A., Lockman, S., Powis, K., Dryden-Peterson, S., Creel, T., Jimbo, W., Madidimalo, T., Makhema, J., Essex, M., Shapiro, R. L. (2012) HAART and adverse birth outcomes among HIV-infected women in Botswana. *The Journal of Infectious Diseases* **206**: 1695-1705

Chen, D., Zheng, J. (2013) Regulation of placental angiogenesis. *Microcirculation* **21**(1): 15-25

Chen, J., Zeng, F., Forrester, S. J., Eguchi, S., Zhang, M. Z., Harris, R. C. (2016) Expression and function of the epidermal growth factor receptor in physiology and disease. *Physiological Reviews* **96**: 1025-1069

Clemente, L., Boeldt, D. S., Grummer, M. A., Morita, M., Morgan, T. K., Wiepz, G. J., Bertics, P. J., Bird, I. M. (2020) Adenoviral transduction of EGFR into pregnancy-adapted uterine artery endothelial cells remaps growth factor induction of endothelial dysfunction. *Molecular and Cellular Endocrinology* **499**: 1-14

Clouse, K., Malope-Kgokong, B., Bor, J., Nattey., C, Mudau, M., Maskew, M. (2020) The South African national HIV pregnancy cohort: evaluating continuity of care among women living with HIV. *BMC Public Health* **20** (1): 1-11

Cohen, S. (1986) Epidermal growth factor. *Physiology or Medicine*: 333-345

Cohen, M. S., Hellmann, N., Levy, J. A., DeCock, K., Lange, J. (2008) The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *The Journal of Clinical Investigation* **118** (4): 1244-1254

Créquit, P., Ruppert, A. M., Rozensztajn, N., Gounant, V., Vieira, T., Poulot, V., Antoine, M., Chouaid, C., Wislez, M., Cadranel, J., Lavole, A. (2016) EGFR and KRAS mutation status in non-small-cell lung cancer occurring in HIV-infected patients. *Lung Cancer* **96**:74-77

Duckitt, K., Harrington, D. (2005) Risk factors for pre-clampsia at antenatal booking: systematic review of controlled studies. *BMJ* **330**: 565-572

Eggleton, J. S., Nagalli, S. (2020) Highly active antiretroviral therapy (HAART). *StatPearls*. 5-7

Einarsson, J. L., Sangi-Haghpeykar, H., Gardner, M. O. (2003) Sperm exposure and the development of preeclampsia. *Am J Obstet Gynecol* **188** (5): 1241-1243

Eitsuka, T., Tatewaki, N., Nishida, H., Nakagawa, K., Miyazawa, T. (2016) Synergistic anticancer effect of tocotrienol combined with chemotherapeutic agents or dietary components: A review. *International Journal of Molecular Sciences* **17**: 1-18

Fang, L., Gao, Y., Wang, Z., Li, Y., Yan, Y., Wu, Z., Cheng, J. C., Sun, Y. P. (2021) EGF stimulates human trophoblast cell invasion by downregulating ID3-mediated KISS1 expression. *Cell Communication and signalling* **19**: 1-13

Faxén, M., Nasiell, J., Blanck, A., Nisell, H., Lunell, N. O. (1998) Altered mRNA expression pattern of placental epidermal growth factor receptor (EGFR) in pregnancies complicated by preeclampsia and/or intrauterine growth retardation. *American Journal of Perinatology* **15**: 9-13

Feng, J., Xu, R., Li, Y., Zhou, Q., Song, G., Deng, Y., Yan, Y. (2021) The effect of high-fat diet and exercise on KISS-1/GPR54 expression in testis of growing rats. *Nutrition and Metabolism* **18**: 1-10

Fernàndez-Medarde, A., Santos, E. (2011) Ras in cancer and developmental diseases. *Genes and Cancer* **2** (3): 344-358

Fondacci ,C., Alsat, E., Gabriel, R., Blot, P., Nessmann, C., Evain-Brion, D. (1994) Alterations of human placental epidermal growth factor receptor in intrauterine growth retardation. .*Journal of Clinical Investigation* **93**:1149–1155

Fondjo, L. A., Boamah, V. E., Fierti, A., Gyesi, D., Owiredu, W. (2019) Knowledge of preeclampsia and its associated factors among pregnant women: a possible link to reduce related adverse outcomes. *BMC Pregnancy and Childbirth* **19**: 1-7

Fox, R., Kitt, J., Leeson, P., Aye, C. Y. L., Lewandowski, A. J. (2019) Preeclampsia: Risk factors, diagnosis, management and cardiovascular impact on the offspring. *Journal of Clinical Medicine* **8**: 1-22

Frank, K.A., Buchmann, E.J., Schackis, R.C. (2004) Does HIV infection protect against preeclampsia/eclampsia? Obstetrics and Gynecology **104**(2):238–242

Freed, E.O. (1998) HIV-1 Gag proteins: diverse functions in the virus life cycle. *Virology* **251**: 1-15

Ghosh, G., Grewal, J., Männistö, T., Mendola, P., Chen, Z., Xie, Y., Laughon, S. K. (2015) Racial/ ethnic differences in pregnancy-related hypertensive disease in nulliparous women. *NIH Public Access* **24** (3): 283-289

Gomathy, E., Lahari, A., Kondareddy, R.(2018) Early onset and late onset preeclampsia-maternal and perinatal outcomes in a rural tertiary health center. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology* **7** (6): 2266-2269

Grabar, S., Moing, V. L., Goujard, C., Leport, C., Kazatchkine, M. D., Costagliola, D., Weiss, L. (2000) Clinical outcome of patients with HIV-1 infection according to immunologic and virologic response after 6 months of highly active antiretroviral therapy. *Annals of International Medicine* **133**; 401-410

Garcia S. A., Guzman, N. Acquired Immune Deficiency Syndrome CD4+ Count. [Updated 2021 Aug 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513289/

Gui, J., Ling, Z., Hou, X., Fan, Y., Xie, K., Shen, R. (2020) In vitro fertilization is associated with the onset and progression of preeclampsia. *Placenta* **89**: 50-57

Harari, R. M., Huang, S. M. (2001) Radiation response modification following molecular inhibition of epidermal growth factor receptor signalling. *Seminars in Radiation Oncology* **11** (4): 281-289

Hatsie, R.,Brownfoot F. C., Pritchard, N., Hannan, N. J., Cannon P., Nguyen V., Palmer, K., Beard S., Tong, S., Kaitu'u-Lino, T. J. (2019) EGFR (Epidermal growth factor receptor) signalling and the mitochondria regulate sFlt-1 (soluble FMS-like tyrosine kinase-1) secretion. *Hypertension* **73**: 659-670

Herrera-Carrillo, E., Berkhout, B. (2015) Bone marrow gene therapy for HIV/AIDS. *Viruses* **7**: 3910-3936

Hounkpatin, O. I., Amidou, S. A., Houehanou, Y. C., Lacroix, P., Preux, P. M., Houinato, D. S., Bezanahary, H. (2021) Systematic review of observational studies of the impact of cardiovascular risk factors on preeclampsia in sub-saharan Africa. BMC Pregnancy and Birth 21: 1-9

Imai,Y., Leung, C.K., Friesen, H.G., Shiu, R.P. (1982) Epidermal growth factor receptors and effect of epidermal growth factor on growth of human breast cancer cells in long term tissue culture. *Cancer Research* **42:** 4394-4398

Iwakura, Y., Nawa, H. (2013) ErbB1-4 dependent EGF/ neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and parkinson's disease. *Frontiers in Cellular Neuroscience* **7** (4): 1-13

Jain, C. V., Jessmon, P., Barrak, C. T., Bolnick, A. D., Kilburn, B. A., Hertz, M., Armant, D. R. (2017) Trophoblast survival signalling during human placentation requires HSP70 activation of MMP2-mediated HBEGF shedding. *Cell Death and Differentiation 24*: 1772-1783

Jeyabalan, A., Powers, R. W., Durica, A. R., Harger, G. F., Roberts, J. M., Ness, R. B. (2008) Cigarette smoke exposure and angiogenic factors in pregnancy and preeclampsia. *Am J Hypertens* **8**: 943-947

Jost, M., Kari, C., Rodeck, U. (2000) The EGF receptor- an essential regulatorof multiple epidermal functions. European Journal of Dermatology 10 (7): 505-510

Jiang, Yanan., Chai, L., Fasae, M. B., Bai, Y. (2018) The role of HIV Tat protein in HIV-related cardiovascular diseases. *Journal of Translational Medicine* **16** (121): 1-7

Kamath, K., GOmathy, E. (2021) Platelet indices in preeclampsia and normotensive pregnancy in a tertiary care centre: A cross sectional study. *Journal of Clinical and Diagnostic Research* **15:** 1-6

Kalumba, V. M. S., Moodley, J., Naidoo, T. D. (2013) Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study. *Cardiovascular Journal of Africa* **24** (2): 24-27

Karumanchi, S. A., Levine, R. J. (2010) How does smoking reduce the risk of preeclampsia? *Hypertension* **55**: 1100-1101

Kenny, L. C., Kell, D. (2018) Immunological tolerance, pregnancy and preeclampsia: The roles of semen microbes and the father. *Frontiers in Medicine* **239** (4): 1-39

Keating, S. M., Golub, E. T., Nowicki, M., Young, M., Anastosh, K., Crystal, H., Cohen, M. H., Zhange, J., Greenblatt, R. M., Desai, S., Wu, S., Landay, A. L., Gange, S. J., Norris, P. J. (2011) The effect of HIV infection and HAART on inflammatory biomarkers in a population- based cohort of women. *AIDS* **25**: 1823-1832

Konturek, S. J., Brzozowski, T., Konturek, P. K., Majka, J., Dembiński, A. (1991) Role of salivary glands and epidermal growth factor (EGF) in gastric secretion and mucosal integrity in rats exposed to stress. *Regulatory Peptides* **32** (2): 203-215

Kim, Y., Hollenbaugh, J. A., Kim, D. H., Kim, B. (2011) Novel P13K/AKT inhibitors screened by the cytoprotective function of human immunodeficiency virus type 1 tat. *PLoS One* **6** (7): 1-9

Kim, D., Kim, S. Y., Mun, S. K., Rhee, S., Kim, B. J. (2015) Epidermal growth factor improves the migration and contractility of aged fibroblasts cultured on 3D collagen matricies. *International Journal of Molecular Medicine* **35** (4): 1017-1025

Kupasamy, K. (2019). Hepatocyte growth factor and epidermal growth factor in HIV associated preeclampsia. MMEDSC dissertation, University of KwaZulu-Natal, Durban

Lamminpää, R., Julkunen, K. V., Gissker, M., Heinonen, S. (2012) Preeclampsia complicated by advanced maternal age: a registry-based study on primiparous women in Finland 1997-2008. *BMC Pregnancy and Childbirth* **12** (47): 1-5

Lan, K. C., Lai, Y. J., Cheng, H. H., Tsai, N. C., Su, Y. T., Tsai, C. C., Hsu, T. Y. (2020) Levels of sex steroid hormones and their receptors in women with preeclampsia. *Reproductive Biology and Endocrinology* **18**: 2-7

Large, M.J., Wetendorf, M., Lanz., Hartig, S.M., Creighton, C.J., Mancini, M.A., Kovanci, E., Lee, K.F., Threadgill, D.W., Lydon, J.P., Jeong, J.W., DeMayo, F.J. (2014) The epidermal growth factor receptor critically regulates endometrial function during early pregnancy. *PLOS Genetics* **10** (6): 1-18

Lessey, B. A. (2002) Adhesion molecules and implantation. *Journal of Reproductive Immunology* **55**: 101-112

Lie, R. T., Rasmussen, S., Brunborg, H., Gjessing, H. K., Lie-Nielsen, E., Irgens, L. M. (1998) Fetal and maternal contributionsto risk of pre-eclampsia: population based study. *BMJ* **316**: 1343-1457

Li, H., Dakour, J., Kaufman, S., Guilbert, L. J., Winkler-Lowen, B., Morrish, D. W. (2003) Adrenomedullin is decreased in preeclampsia because of failed response to epidermal growth factor and impaired syncytialization. *Hypertension* **42**: 895-900

Lim, H. J., Dey, S. K. (2009) HB-EGF: a unique mediator of embryo-uterine interactions during implantation. *Exp Cell Res* **315** (4): 619-626

Liu, G., Shang, Y., Yu, Y. (2006) Induced endoplasmic reticulum (ER) stress and binding of over-expressed ER specific chaperone GRP78/BiP with dimerized epidermal growth factor

receptor in mammalian cells exposed to low concentration of N-methyl-N'-nitro-N-nitrosoguanidine. *Mutat Res* **12** (21): 1-2

Lv, Q., Meng, Z., Yu, Y., Jiang, F., Guan, D., Liang, C., Zhou, W. J., Lu, A., Zhang , G. (2016) Molecular mechanisms and translational therapies for human epidermal receptor 2 positive breast cancer. *International Journal of Molecular Sciences* **17** (12): 1-21

Lyall, F., Robson, S. C., Bulmer, J. N. (2013) Spiral artery remodelling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension* **62** (6): 1046-1054

Machano, M. M., Joho, A. A. (2020) Prevalence and risk factors associated with severe pre-eclampsia among postpartum women in Zanzibar: a cross-sectional study. *BMC Public Health* **20**: 1-10

Magid, M., Nanney, L. B., Stoscheck, C. M., King, L. E. J. (1985) Epidermal growth factor binding and receptor distribution in term human placenta. *Placenta* **6** (6): 519-526

Malik, A., Pal, R., Gupta, S. K. (2017) Interdependence of JAK-STAT and MAPK signaling pathways during EGF- mediated HTR-8/SVneo cell invasion. *PLOS one* **12:**1-18

Malik, A., Pal, R., Gupta, S. K. (2020) EGF-mediated reduced miR-92a-1-5p controls HTR-8/SVneo cell invasion through activation of MAPK8 and FAS which in turn increase MMP-2/-9 expression. *Scientific Reports* **10**: 1-15

Margolis, B., Skolnik, E. Y. (1994) Activation of Ras by receptor tyrosine kinases. *Journal of the American Society of Nephrology* **5** (6): 1288-1299

Massimiani, M., Lacko, L. A., Swanson, C. S. B., Salvi, S., Argueta, L. B., Moresi, S., Ferrazzani, S., Gelber, S. E., Baergen, R.N., Toschi, N., Campagnolo, L., Stuhlmann, H. (2018) Increased circulating levels of epidermal growth factor-like Domain 7 in pregnant women affected by preeclampsia. *The Journal of Laboratory and Clinical Medicine* **207**: 19-29

Mattar R., Amed., A.M., Lindsey, P.C., Sass, N., Daher, S. (2004) Pre eclampsia and HIV infection. *European Journal of Obstetrics and Gynaecology and Reproductive Biology* **117** (2):240–241.

Maynard, S. E., Karumanchi, S. A. (2011) Angiogenic factors and preeclampsia. *Semin Nephrol* **31** (1): 33-46

McCulloch, R.K., Walker, C.E., Chakera, A., Jazayeri, J., Leedman, P.J. (1998) Regulation of EGF-receptor expression by EGF and TGF alpha in epidermoid cancer cells is cell type-specific. *International Journal of Biochemistry and Cell Biology* **30** (11):1265-1278

Mersha, A. G., Abegaz, T. M., Seid, M. A. (2019) Maternal and perinatal outcomes of hypertensive disorders of pregnancy in Ethiopia: a systematic review and meta-analysis. *BMC Pregnancy and Childbirth* **19** (458): 1-12

Meulendyke, K. A., Croteau, J. D., Zink, M. C. (2014) HIV lifecycle, innate immunity and autophagy in the central nervous system. *Curr Opin HIV AIDS* **9** (6): 565-571

Midgley, A. C., Rogers, M., Hallett, M. B., Bowen, T., Phillips, A. O., Steadman, R. (2013) Transforming growth factor-β1 (TGF-β1)- stimulated fibroblast to myofibroblast differentiation is mediated by hyaluronan (HA)- facilitated epidermal growth factor receptor (EGFR) and CD44 co-localization in lipid rafts. *Journal of Biological Chemistry* **21** (288): 14824-14838

Moodley, J., Onyangunga, O. A., Maharaj, N. R. (2016) Hypertensive disorders in primigravid black South African women: A one year descriptive analysis. *Hypertension in Pregnancy* **35** (4): 529-535

Moodley, J., Soma-Pillay, P., Buchmann, E., Pattinson, R. C. Hypertensive disorders in pregnancy: 2019 National guideline. SAMJ Guideline 109 (9): S3-S16

Mrema, D.,Lie, R.T., Østbye, T., Mahande, M. J., Daltveit, A. K. (2018) The association between pre pregnancy body mass index and risk of preeclampsia: a registry based study from Tanzania. *BMC Pregnancy and Childbirth* **18**: 1-8

Muiru, A. N., Shlipak, M. G., Scherzer, R., Zhang, W. R., Ascher, S. B., Jotwani, V., Grunfeld, G., Parikh, C. R., Nf, D., Palella, F. J., Ho, K., Kassaye, S., Sharma, A., Cohen, M., Wang, R., Qi, Q., Estrella, M.M. (2019) Kidney disease risk factors associate with urine biomarkers concentrations in HIV-positive persons; A cross-sectional study. *BMC Nephrology* **20**: 1-9

Nabell, L. M., Raja, R. H., Sayeski, P. P., Paterson, A. J., Kudlow, J. E. (1994) Human immunodeficiency virus 1 Tat stimulates transcription of the transforming growth factor alpha gene in an epidermal growth factor-dependent manner. *Cell Growth Differentiation* **5** (1): 87-93

Nagy, P., Claus, J., Jovin, T. M., Arndt-Jovin, D. J. (2010) Distribution of resting and ligand bound ErbB1 and ErbB2 receptor tyrosine kinases in living cells using number and brightness analysis. *PNAS* **107** (38): 16524-16529

Naicker, T., Khedun, S.M., Moodley, J., Pijnenberg, R. (2003) Quantitative analysis of trophoblast invasion in preeclampsia. *Acta Obsttricia et Gynecologica Scandinavica* **8** (82): 722-729

Naicker, T., Phoswa, W. N., Onyangunga, O. A., Gathiram, P., Moodley, J. (2019) Angiogenesis, lymphangiogenesis and the immune response in South African preeclamptic women receiving HAART. *International Journal of Molecular Sciences* **20** (15): 1-16

Naicker, T.,Govender, N., Abel, T., Naidoo, N., Moodley, M., Pillay, Y., Singh, S., Khaliq, O. P., Moodley, J. (2021) HIV associated preeclampsia: A multifactorial appraisal. *International Journal of Molecular Sciences* **22**: 1-16

Navis, A. R. (2007) Epidermal growth factor. *Embryo Project*: 1-3

Nevo, O., Soleymanlou, N., Wu, Y., Xu, J., Kingdom, J., Many, A., Zamudio, S., Caniggia, I. (2006) Increased expression of sFlt-1 in *in vivo* and *in vitro* models of human placental hypoxia is mediated by HIF-1.*Am J Physiol Regul Integr Comp Physiol* 291: 1085-1093

Norwitz, E. R. (2006) Defective implantation and placentation: laying the blueprint for pregnancy complications. *RBM Online* **4** (13): 591-599

Oguntibeju, O. O. (2012) Quality of life of people living with HIV and AIDS and antiretroviral therapy. *HIV/AIDS – Research and Palliative Care* **4**: 117-124

Okuma, Y., Hosomi, Y., Imamura, A.. (2014) Lung cancer patients harboring epidermal growth factor receptor mutation among those infected by human immunodeficiency virus. *OncoTargets and Therapy* **8**: 111-115.

Okuma, Y., Tanuma, J., Kamiryo, H., Kojima, Y., Yotsumoto, M., Ajisawa, A., Uehira, T., Nagai, H., Takeda, Y., Setoguchi, Y., Okada, S. (2015) A multi-institutional study of clinicopathological features and molecular epidemiology of epidermal growth factor receptor mutations in lung cancer patients living with human immunodeficiency virus infection. *J Cancer Res Clin Oncol* **14**:1669-1678

Orton, R. J., Sturm, O. E.,Vyshemirsky, V., Calder, M., Gilbert, D. R., Kolch, W. (2005) Computational modelling of the receptor-tyrosine-kinase-activated MAPK pathway. *Biochemistry Journal* **392**: 249-261

Oza, N. (2015) HIV AIDS. *Health and Medicine.* [Online] Slideshare. https://www.slideshare.net/NikhilOza2/hiv-aids-51411108

Parkin, D. M., Bray, F., Ferlay, J., Pisani, P. (2005) Global cancer statistics, 2002. CA Cancer J Clin. **2** (55): 74-108

Paydas, S., Ergin, M., Seydaoglu, G., Erdogan, S., Yavuz, S. (2009) Pronostic significance of angiogenic/ lymphangiogenic, anti-apoptotic, inflammatory and viral factors in 88 cases with diffuse large B cell lymphoma and review of the literature. *Leukemia Research* **33**: 1627-1635

Peres, G. M., Mariana, M., Cairrão, E. (2018) Pre-eclampsia and eclampsia: An update on the pharmacological treatment applied in Portugal. *Journal of Cardiovascular Development and Disease* **5** (1): 1-13

Pingel, P. E.P., Avellàn, L. A. Q., Hidalgo, L., Chedraui, P., López, F. R.P. (2017) Pregnancy outcomes in younger and older adolescent mothers with severe preeclampsia. *Adolescent Health, Medicine and Therapeutics* **8**: 81-86

Pratt, A., Costa, F. D.S., Borg, A. J., Kalionis, B., Keogh, R. J., Murthi, P. (2014) Placenta-derived angiogenic proteins and their contribution to the pathogenesis of preeclampsia. *Angiogenesis* **18** (2): 115-123

Raymond, D., Peterson, E. (2011) A critical review of early-onset and late-onset preeclampsia. *Obstet Gynecol Surv* **66** (8): 497-506

Riesco, A., Santos-Buitrago, B., Rivas, J. D.L., Knapp, M., Santos-Gracia, G, Talcott, C. (2017) Epidermal growth factor signalling towards proliferation: modelling and logic inference using forward and backward search. *BioMed Research International* **2017**:1-11

Ritter, A., Roth, S., Kreis, N. N., Friemel, A., Hoock, S. C., Souto, A. S., Eichbaum, C., Neuhoff, A., Chen, Q., Solbach, C., Louwen, F., Yuan, J. (2020) Primary cilia in trophoblastic cells: potential involvement in preeclampsia. *Hypertension* **76**: 1491-1505

Salam, R. A., Das, J. K., Ali, A., Bhaumik, S., Lassi, Z. S. (2015) Diagnosis and management of preeclampsia in community settings in low and middle-income countries. *J Family Med Prim Care* **4** (4): 501-506

Sansone, M., Sarno, L., Saccone, G., Berghella, V., Maruotti, G. M., Migliucci, A., Capone, A., Martinelli, P. (2016) Risk of Preeclampsia in Human Immunodeficiency Virus-Infected Pregnant Women. *Obstet Gynecol* **127**(6):1027-1032

Saving Mothers Report (2017) National Committee on Confidential Enquiries into Maternal Deaths in South Africa. A Short Report. [Online] Pretoria: National Department of Health.

Available at: https://www.westerncape.gov.za/gemeral-publication/saving-mothers-2017-annual-report

Semenza, G. L. (2003) Targeting HIF-1 for cancer therapy. *Nature Reviews Cancer* **3**: 721-732

Schneider, M. R., Wolf, E. (2008) The epidermal growth factor receptor ligands at a glance. *Journal of Cellular Physioology* :460-466

Seitz, R. (2016) Human immunodeficiency virus (HIV). *Transfusion Medicine and Hemotherapy* **43** (3): 203-222

Sibai, B. M., Ewell, M, Levine, R. J., Kelbanoff, M. A., Esterlitz, J, Catalano, P. M., Goldenberg, R. L., Joffe, G. (1997) Risk factors associated with preeclampsia in healthy nulliparous women. The calcium for preeclampsia prevention (CPEP) study group. *Am J Obstet Gynecol* **177** (55): 1003-1010

Silva, H. D., Saleh, S., Kovacevic, S., Black, C. G., Wang, L., Plebanski, M., Coppel, R. (2011) The antibody response to plasmodium falciparum Merozoite surface protein 4: Comparative assessment of specificity and growth inhibitory antibody activity to infection-acquired and immunization-induced epitopes. *Malaria Journal* **10** (1): 1-12

Spradley, F. T. (2020) Targeting inflammation to reduce seizure severity in an experimental model of eclampsia. *Hypertension Research* **43**: 350-353

Staff, A. C., Fjeldstad, H. E., Fosheim, I. K., Moe, K., Turowski, G., Johnsen, G. M., Katjavivi, A., Sugulle, M. (2020) Failure of physiological transformation and spiral artery atherosis: their roles in preeclampsia. *American Journal of Obstetrics and Gynecology* (20): 1-12

Stastics South Africa. (2021) Mid-year population estimates 2021. [Online] Pretoria: National Department of Health, 1- 38. Available at: http://www.statssa.gov.za/publications/P0302/P03022021.pdf

Suy, A., Martínez, E., Coll, O., Lonca, M., Palacio, M., de Lazzari, M., Milinkovic, A., Vanrell, J.A., Gatell,J.M. (2006) Increased risk of pre-eclampsia and fetal death in HIV infected women receiving HAART. *AIDS* **20**: 59–66.

Swinson, D. E. B., O'Byrne, K. J. (2006) Interactions between hypoxia and epidermal growth factor receptor in non-small-cell lung cancer. *Clinical Lung Cancer* 7 (4): 1-5

Tan, Y., Wang, J., Su, S., Wang, Q., Jiang, S., Lu, L., Chen, Y.H. (2017) Enhancement of endocytic uptake of HIV-1 virions into CD4-negative epithelial cells by HIV-1 gp41 via its interaction with POB1. *Cellular and Molecular Immunology* 14:568–571

Thermofisher Scientific. (2017) EGFR rabbit polycolonal antivody [Online] Available at: EGFR-schematic.jpg-650.jpg (650×224) (thermofisher.com)

Thomopoulos, C., Tsioufis, C., Michalopoulou, H., Makris, T., Papademetriou, V., Stefanadis, C. (2013) Assisted reproductive technology and pregnancy-related hypertensive complications: a systematic review. *J Hum Hypertens* **27**(3):148-157

UNAIDS, (2021) Global HIV and AIDS statistics. [Online] Available at: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf

Valiathan, R.R., Resh, M.D. (2004) Expression of human immunodeficiency virus type 1 gag modulates ligand-induced downregulation of EGF receptor. *Journal of Virology* **78** (22): 12386-12394

Vargas, E. A. H, Middleton, R. H. (2013) Modeling the three stages in HIV infection. *Journal of Theoretical Biology* **320**: 33-40

Verma, S., Pillay, P., Naicker, T., Moodley, J., Mackraj, I. (2017) Placental hypoxia inducible factor -1α & CHOP immuno-histochemical expression relative to maternal circulatory syncytiotrophoblast micro-vesicles in preeclamptic and normotensive pregnancies. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **220**: 18-24.

Walker, F., Abramowitz, L., Benabderrahmane, D., Duval, X., Descatorie, V., Hènin, D., Lehy, T., Aparicio, T. (2009) Growth factor receptor expression in anal squamous lesions: modifications associated with oncogenic human papillomavirus and human immunodeficiency virus. *Human Pathology* **40**: 1517-1527

Wallace, A. E., Fraser, R., Gurung, S., Goulwara, S. S., Whitley, G. S., Johnstone, A. P., Cartwright, J. E. (2014) Increased angiogenic factor secretion by decidual natural killer cells from pregnancies with high uterine artery resistance alters throphblast function. *Human Reproduction* **29**: 652-660

Wang, Y., Walsh, S. W. (1998) Placental mitochondria as a source of oxidative stress in preeclampsia. *Placenta* **19**: 581-586

Wee, P., Wang, Z. (2017) Epidermal growth factor receptor cell proliferation signalling pathways. *Cancers*, **9** (52): 1-45

Weitzner, O., Yagur, Y., Weissbach, T., Man El, G., Biron-Shental, T. (2018) Preeclampsia: risk factors and neonatal outcomes associated with early- versus late-onset diseases. J Matern Fetal Neonatal Med. **33**(5):780-784

Weitzner, O., Yagur, Y., Weissbach, T., Man El, G., Biron-Shental, T. (2020) Preeclampsia: risk factors and neonatal outcomes associated with early- versus late-onset diseases. J Matern Fetal Neonatal Med. **33**(5) 1-11

Wikström, A., Stephansson, O., Cnattingius, S. (2010) Tobacco use during pregnancy and preeclampsia risk: Effects of cigarette smoking and snuff. Hypertension. **55**(5):1254-1259

Wilen, C. B., Tilton, J.C., Doms, R. W. (2012) HIV: cell binding and entry. *Cold Spring Harb Perspect Med* **2** (8): 1-13

Williams, P. J., Pipkin, F. B. (2011) The genetics of pre-eclampsia and other hypertensive disorders of pregnancy. *Best Practice and Research Clinical Obstetrics and Gynaecology* **25** (4): 405-417

Wimalasundera, R. C., Larbalestier, N., Smith, J. H., De Ruiter, A., Thom, S. A. McG., Hughes, A. D., Poulter, N., Regan, L., Taylor, G. P. (2002) Pre-eclampsia, antiretroviral therapy, and immune reconstitution. *Lancet* 360(9340): 1152-1154

Woldesenbet, S. A., Kufa, T., Lombard, C., Manda, S., Ayalew, K, Cheyip, M., Puren, A. (2019) The 2017 national antenatal sentinel HIV survey, South Africa, National Department of Health.

Xiong, X., Demianczuk, N. N., Saunders, L. D., Wang, L. F., Fraser, W. D. (2002) Impact of preeclampsia and gestational hypertension on birth weight by gestational age. *American Journal of Epidemiology* **155**: 203-209

Yarden, Y., Schlessinger, J. (1987) Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. *Biochemistry* **26** (5): 1443-1451

You, S. H., Cheng, P. J., Chung, T. T., Kuo, C. F., Wi, H. M., Chu, P. H. (2018) Population-based trends and risk factors of early- and late-onset preeclampsia in Taiwan 2001-2014. *BMC Pregnancy and Childbirth* **18** (199): 1-11

Zeng, F. H., Harris, R. C., (2014) Epidermal growth factor, from gene organization to bedside. *Semin Cell Div Biol.* 2-11

Zhao, X., Dai, W., Zhu, H., Zhang, Y., Cao, L., Ye, Q., Lei, P., Shen, G. (2006) Epidermal growth factor (EGF) induces apoptosis in a transfected cell line expressing EGF receptor on its membrane. *Cellular Biology International* **30** (8): 653-658

Zhou, F., Xue, M., Qin, D., Zhu, X., Wang, C., Zhu, J., Hao, T., Cheng, L., Chen, X., Bai, Z., Fenf, N., Gao, S.J., Lu, C. (2013) HIV-1 Tat promotes Kaposi's sarcoma-associated herpesvirus (KSHV) vlL-6-induced angiogenesis and tumorigenesis by regulating P13K/PTEN/AKT/GSK-3β signalling pathway. *PLOS ONE* **8** (1): 1-13

APPENDIX

Appendix 1 – Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

08 July 2021

Miss Arisha Laldeo (217000912)
School of Lab Med & Medical Sc
Medical School

Dear Miss Laldeo,

Protocol reference number: BREC/00002899/2021
Project title: Cell Signalling of the Epidermal Growth Factor / Epidermal Growth Factor Receptor Axis in Human Immunodeficiency Virus Associated Preeclampsia
Degree Purposes: MMedSci

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 08 July 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_4_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 08 July 2021. 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 (2020) (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).

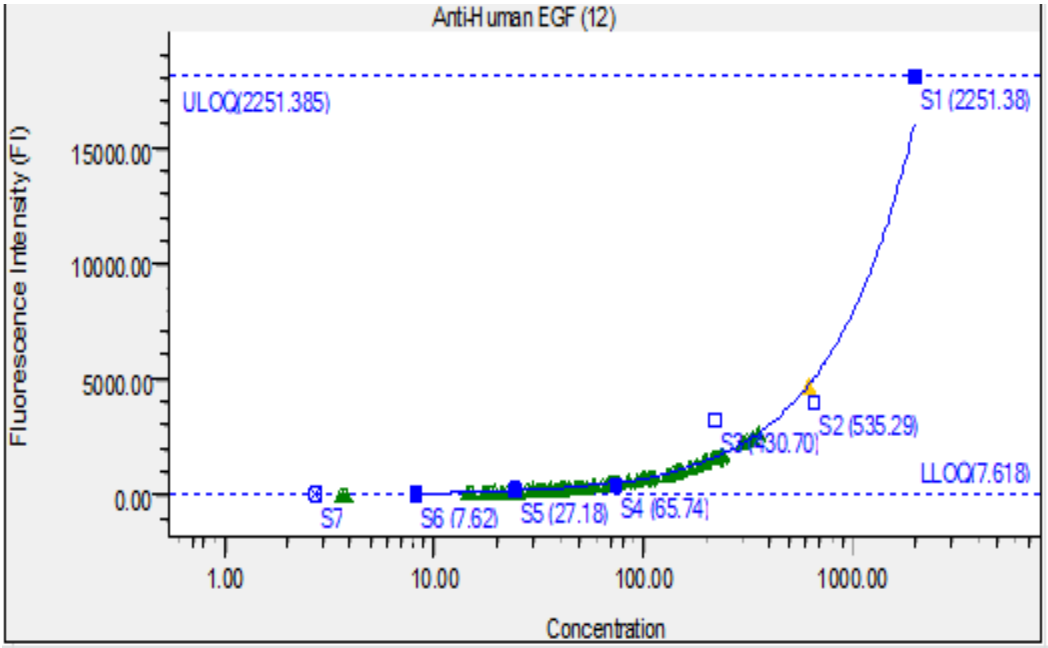
The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 10 August 2021.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Appendix 2 – Standard curve EGF

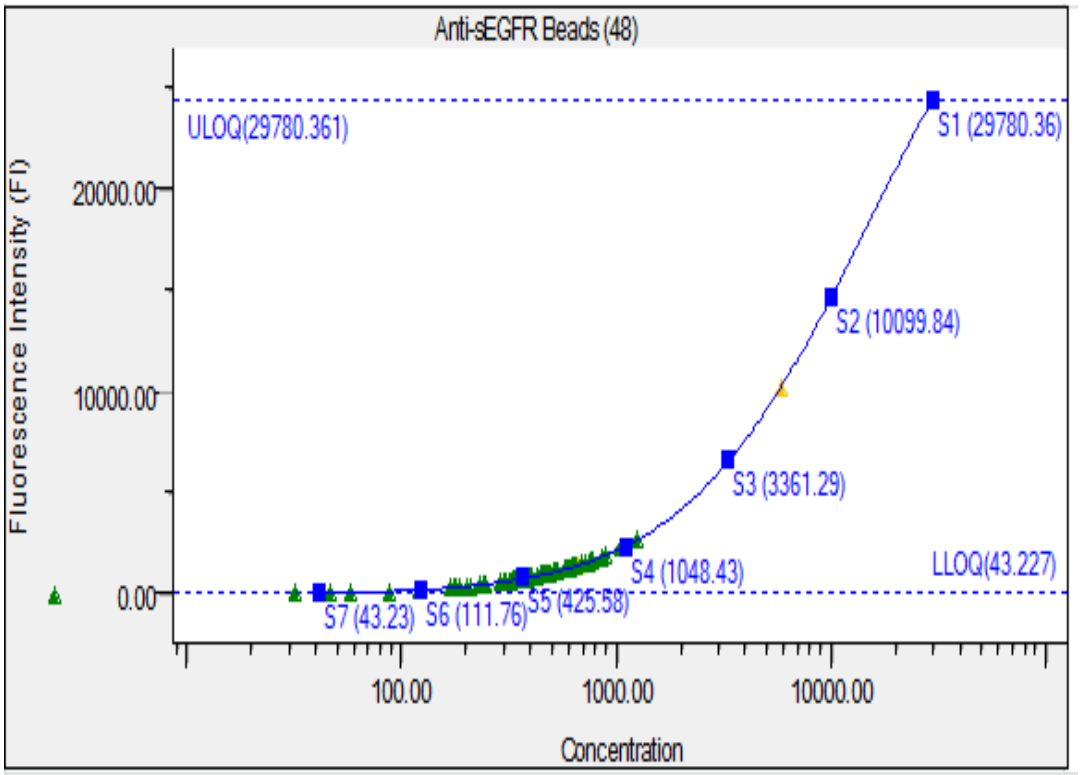


Regression Type: Logistic - 5PL

Std. Curve: FI = 99.8956 + (1.8236E+008 - 99.8956) / ((1 + (Conc / 1.11997E+007)^-0.396771))^2.70217

FitProb. = 0.0000, ResVar. = 134.7288

Appendix 3 – Standard curve EGFR



Regression Type: Logistic - 5PL

Std. Curve: FI = 55.8094 + (36830.2 - 55.8094) / ((1 + (Conc / 13756.7)^-0.979785))^1.06934

FitProb. = 0.0240, ResVar. = 3.7279

END