HEPATOCYTE GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR

IN

HIV ASSOCIATED PREECLAMPSIA

By

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PREFACE

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

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

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DECLARATION

I, **Kyle Kupsamy** declare that:

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

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

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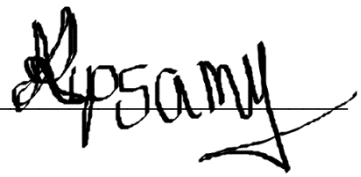
Signed:  Date: 9 November 2018

DEDICATIONS

To my Lord and Savior Jesus Christ, in all things believe in Him.

" As iron sharpens iron, so one person sharpens another."

Proverbs 27:17

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

LIST OF TABLES

CHAPTER 2

LIST OF ABBREVIATIONS

3'-5'-Cyclic adenosine monophosphate……..…….…….....cAMP
Epidermal Growth Factor……..……...…..……...……...EGF
Epidermal Growth Factor receptor……..…….....….....EGFR
Extracellular signal-regulated kinases.……...……. ERK
Hepatocyte Growth Factor ……..……...…….……..HGF
Hepatocyte Growth Factor receptor……...…..…….…..……c-Met
Highly active antiretroviral therapy.……..……..... HAART
Human Immunodeficiency Virus..……..…….……..... HIV
Interquartile range.……...…….……...…. IQR
Janus kinase……...……...…….….JAK
Mitogen-activated protein kinase……...……...……MAPK
Non-significant.……..…….....…… ns
Phosphoinositide 3-kinase……..…….....PI3K
Preeclampsia.……..…….…….... PE
Protein kinase B……..…….….AKT
Renin-angiotensin system……..……...RAS
Signal transducer and activator of transcription 3……STAT3
South Africa……SA
Trans-Activator of Transcription……..…..Tat
Transforming Growth Factor beta……...……...……TGF-β
Vascular Endothelial Growth Factor ……..……..…… VEGF

ABSTRACT

Background: The survival or death of a cell is reliant upon growth factors. Hepatocyte and Epidermal Growth Factor (HGF and EGF) promote vital cellular processes such as cell survival, proliferation, differentiation, growth, invasion and repair *via* various pathways. Hence these growth factors facilitate normal pregnancy. In complications such as preeclampsia (PE), decreased trophoblast invasion results in defective spiral artery remodeling, which leads to decreased blood flow and a hypoxic micro-environment. In South Africa (SA), HIV infection and PE are the leading causes of maternal mortality and morbidity. In light of the high prevalence of HIV infection and PE in SA, this study aimed to determine the concentrations of HGF and EGF in HIV associated PE.

Methods: Post ethics approval, serum samples were collected from normotensive HIV-negative (n = 20); normotensive HIV-positive (n = 20); preeclamptic HIV-negative (n = 20) and preeclamptic HIV-positive (n = 20) women. All HIV-positive women received Highly Active Anti-Retroviral Treatment (HAART). Quantification and analysis of HGF and EGF expression was attained by using the Bio-Plex multiplex immunoassay technique.

Results: As expected there was a statistically significant difference between gestational age, systolic and diastolic blood pressures across the study groups ($p<0.0001$). No significant difference was noted in maternal age ($p=0.16$), parity ($p=0.47$) and maternal weight ($p=0.36$) across all study groups.

Irrespective of pregnancy type, HGF was significantly increased in HIV-positive women *vs* HIV-negative women ($p=0.0225$). However, no statistical significance was found based on pregnancy type ($p=0.8890$). A significant decrease of HGF expression was noted between normotensive HIV-negative and normotensive HIV-positive women ($p=0.0022$).

Irrespective of pregnancy type, EGF was found to be significantly elevated in HIV-positive compared to HIV-negative women ($p=0.0055$). In addition, preeclamptic women displayed a higher EGF level compared to normotensive women ($p=0.003$), regardless of HIV status. The Epidermal Growth Factor was significantly down-regulated in normotensive HIV-negative group *vs* normotensive HIV-positive ($p<0.001$), preeclamptic HIV-positive ($p<0.001$) and preeclamptic HIV-negative groups ($p<0.001$).

Conclusion

This novel study displays a significant up-regulation in the expression of HGF and EGF in HIV infection during pregnancy, reflecting an immune reconstitution following HAART. These findings may be caused due to the HIV accessory protein Tat that inhibits growth factor function thereby, negatively impacting cell migration. The up-regulation of EGF expression in PE, may be responsible

for impaired trophoblast cell invasion. As anticipated in HIV associated PE, EGF expression increased in HIV infected pregnancies and PE. The expression Epidermal Growth Factor in HIV associated PE, may be used as a risk indicator, predicting PE development prior to the manifestations of clinical signs and symptoms.

CHAPTER 1

1.0. BACKGROUND AND LITERATURE REVIEW

1.1. Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS)

The Human Immunodeficiency virus (HIV) attacks the immune system, the body's natural defense system to fight diseases. Sub-Saharan Africa accounts for 56% of people living with HIV infection globally (Joint United Nations Programme on HIV/AIDS, 2018). In sub-Saharan Africa, women account for a disproportionate 59% of HIV infections among adults (aged 15 years and older) in 2017 (Joint United Nations Programme on HIV/AIDS, 2018). South Africa is home to the largest global HIV epidemic (United Nations International Children's Emergency Fund, 2018). The estimated overall HIV prevalence rate in SA, is approximately 13,1% (Statistic South Africa, 2018). The province of KwaZulu-Natal is the global "HIV hotspot" where 40.8% of adults are living with HIV (Bershteyn *et al.*, 2018).

In Africa, at least 3.28 million pregnant women infected with HIV are estimated to give birth each year, with more than 75% of these living in sub-Saharan Africa (World Health Organization, 2015). Approximately one-fifth of South African women in their reproductive ages (15–49 years) are HIV-positive (Statistic South Africa, 2018).

The latest Saving Mothers report associates the top three causes of maternal deaths with non-pregnancy related infections (such as HIV, tuberculosis, pneumonia) hemorrhages and gestational hypertension, of which 83% is attributed to preeclampsia (PE) (Department of Health, 2017).

To date the relationship between HIV infection and PE development remains contradictory. There is discord as to whether HIV-infected women are at a lesser, equivalent or advanced risk of developing PE than the general population. Some researchers debate that pregnancy may accelerate the progression of HIV to AIDS, whilst others argue that the risk of obstetric complications may be increased due to the HIV-infection (Calvert and Ronsmans, 2013).

1.2. Preeclampsia

1.2.1. Definition

Preeclampsia (PE) is a pregnancy specific syndrome characterized by high blood pressure (> 140/90 mmHg) measured on two occasions at least four hours apart and proteinuria (> 300mg/d), occurring in the 20th week of gestation (Brown *et al.*, 2018; Maynard and Karumanchi, 2011). It complicates 2-10% of pregnancies and is associated with significant maternal and neonatal morbidity and mortality (Backes *et al.*, 2011). Globally, PE is one of the main causes of maternal mortality, resulting in about 50 000–

60 000 deaths annually. In addition, the mother and her child have an increased risk of developing cardiovascular complications in the future (Gathiram and Moodley, 2016; O'Tierney-Ginn and Lash, 2014).

In contrast to a normal pregnancy where there is an altered immune sensitivity, preeclamptic pregnancies exhibit an exaggerated immune response (Cerdeira and Karumanchi, 2012). The placenta provides for the exchange of nutrients, gases and waste products between the mother and the baby. The proper formation, maturation and maintenance of the placental vasculature is critical, as failure results in outcomes such as miscarriage, small for gestational age infants (SGA) and pathological conditions such as PE (Cerdeira and Karumanchi, 2012).

1.2.2. Classification of Preeclampsia

Based on the time of inception of the disease, PE can be divided into two main sub-types, namely early-onset (EOPE) and late-onset preeclampsia (LOPE). In EOPE, the clinical signs appear at <33 gestational weeks, while in LOPE symptoms occur at ≥34 weeks. Majority of preeclamptic pregnancies (>80%) fall under the LOPE sub-type (Kovo *et al.*, 2012; Staff *et al.*, 2013).The main pathological difference between EOPE and LOPE is fetal growth restriction. Early-onset PE has a higher rate of fetal growth restriction than LOPE (Huppertz, 2008; Kovo *et al.*, 2012).

1.2.3. Pathogenesis of Preeclampsia

The structure involved to maintain and protect the fetus until birth is the placenta. Trophoblast cells differentiate into syncytiotrophoblast and cytotrophoblast cells, that connect the fetus to the mother. In PE, shallow trophoblast invasion results in incomplete physiological transformation of maternal spiral arteries which lead to hypoxic distress, placental insufficiency and fetal malnutrition (Figure 1.1) (Naicker *et al.*, 2013). Additionally, there is a decrease in the interstitial cytotrophoblast invasion of the myometrium (Gack *et al.*, 2005; Gathiram and Moodley, 2016; Lala and Chakraborty, 2003; Naicker *et al.*, 2003). Defects in spiral artery remodeling are restricted to the decidua, where they retain their smooth muscle media and elastic lamina (Staff *et al.*, 2013).

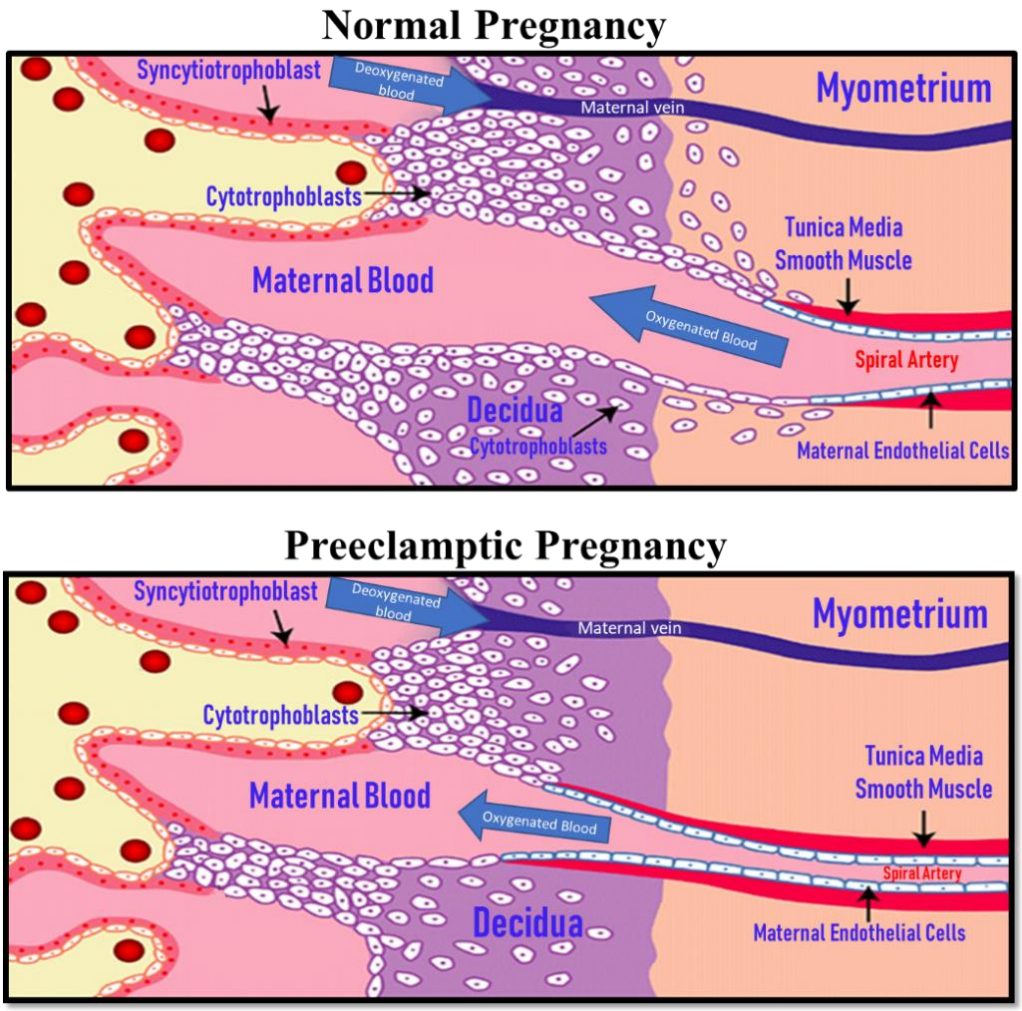


Figure 1.1. Abnormal placentation in preeclampsia *vs* normal placental development. (adapted from Lam *et al.* 2005)

Despite intensive research, the pathogenesis and pathophysiology of PE remains poorly understood. During the first trimester, the pathogenic process of PE begins long before any clinical signs are noted, making it difficult to identify a predictive diagnostic early biomarker. Moreover, there is a dearth of information in relation to bioinformatic analysis (Tejera *et al*., 2012).

Nonetheless, the defective development of placental vasculature is associated with pro-angiogenic biomarkers such as Vascular Endothelial Growth Factor (VEGF), angiopoietins and Placental Growth Factor (Cerdeira and Karumanchi, 2012). Altered levels of anti-angiogenic factors such as sFlt-1 compared to pro-angiogenic VEGF is implicated in PE development (Govender *et al*., 2015). The upregulation of sFlt-1 and sEng are directly linked with PE development (Chau *et al*., 2017; von Dadelszen and Magee, 2014). Additionally, the dysregulation of a number of growth factors such as Insulin-like Growth Factor (IGF)/Insulin-like-Growth-Factor-Binding Protein (IGFBP), Hepatocyte Growth Factor (HGF), Heparin-Binding Epidermal Growth Factor (HB-EGF) and Transforming Growth Factor beta (TGF-β) are associated with PE development. Nonetheless, it is still uncertain if

these factors cause an interference with trophoblast invasion, or if their concentration is due to the initial failure in trophoblast invasion (Gack *et al.*, 2005).

To-date, the only known treatment of PE is the delivery of the fetus and placenta (Gathiram and Moodley, 2016; Romero and Chaiworapongsa, 2013).

1.3. Hepatocyte Growth Factor

The Hepatocyte Growth Factor was initially discovered 30 years ago as a mitogen, affecting hepatocytes. Hepatocyte Growth Factor is expressed by cells of mesenchymal origin and acts as a multi-functional cytokine on cells of epithelial origin (Zarnegar and Michalopoulos, 1995). This specific cytokine is produced by stromal cells and it functions to stimulate and regulate epithelial cell growth, cell motility, proliferation, morphogenesis and angiogenesis in various tissues and organs *via* its receptor c-Met (Figure 1.2) (Mizuno and Nakamura, 2007).

During embryogenesis, this pleotropic cytokine is produced by the mesenchymal stalk of placental villi and is involved in placental growth and expansion (Naghshvar *et al.*, 2013). Trophoblast cells express both HGF and its ligand c-Met, which have a paracrine role (Naghshvar *et al.*, 2013).

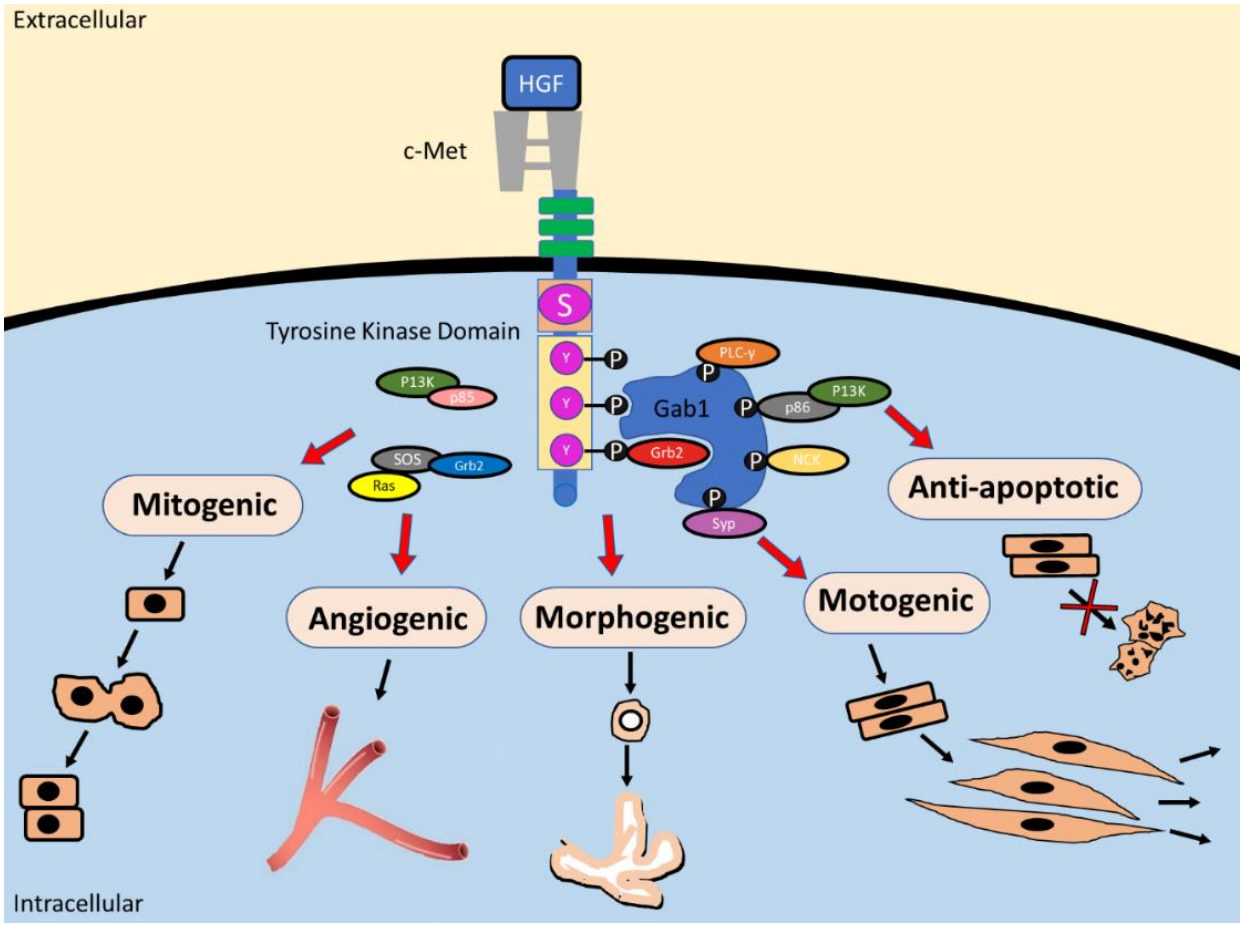


Figure 1.2. HGF binding to c-Met receptor resulting in different biological effects. (adapted from Nakamura and Mizuno, 2010)

1.3.1. Hepatocyte Growth Factor in Pregnancy

In early development, a lack of HGF or damage to the c-Met gene receptor, leads to incomplete development of many organs, indicating that HGF signalling is crucial for organ development (Mizuno and Nakamura, 2007; Mizuno *et al.*, 2008)

In a normal placenta, a serine residue site at position 985 (Ser-985) in the juxta-membrane of c-Met is constitutively phosphorylated and functions as a negative inhibitor for HGF-Met signalling (*i.e.*, switch OFF; Figure 1.3). Whilst in animal models, inhibition of HGF production causes placental insufficiency and consequential intrauterine demise (Naghshvar *et al.*, 2013). In PE, Ser-985 site is de-phosphorylated *via* recruitment of intracellular protein phosphatase 2A, resulting in the phosphorylation of c-Met tyrosine sites and the delivery of pleiotropic activities (*i.e.*, switch ON; Figure 1.3) (Nakamura and Mizuno, 2010).

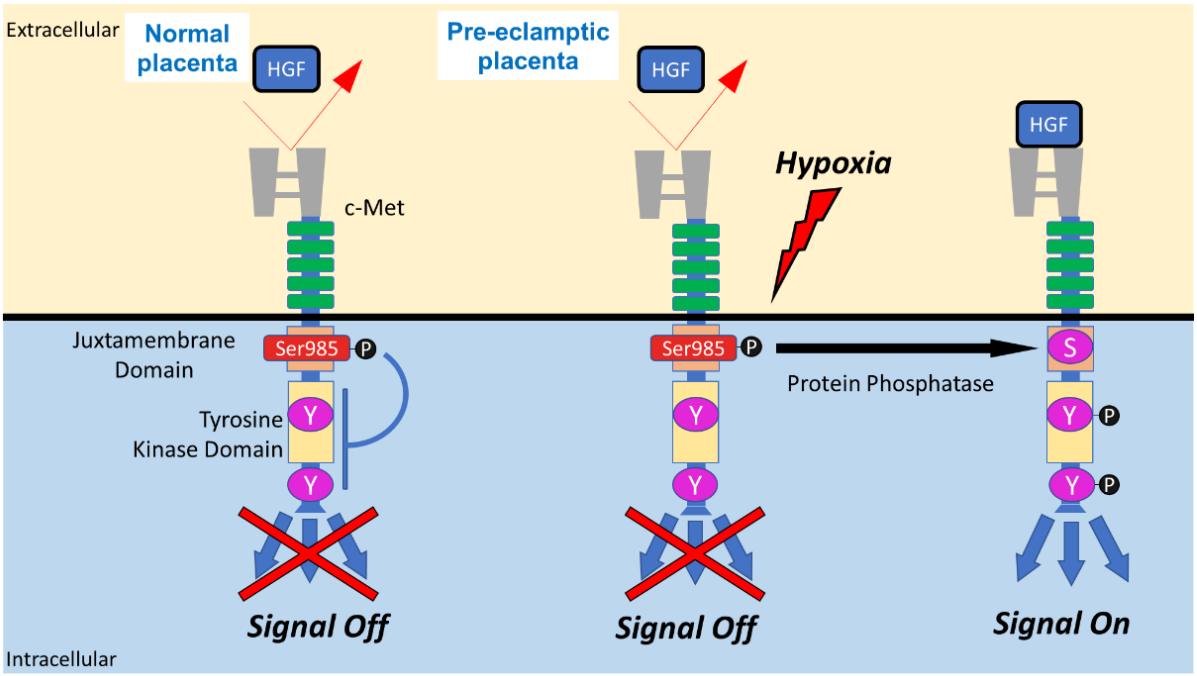


Figure 1.3. HGF and c-Met receptor, illustrating the effect of signalling in PE. (adapted from Nakamura and Mizuno, 2010)

Hepatocyte Growth Factor plasma levels are significantly increased during a multitude of organ diseases. Several *in vitro* studies report that HGF has regenerative effects on epithelium in the kidney, lung and other tissues such as skin, muscle, cartilage and mucosal repair in the intestine. Hepatocyte Growth Factor levels are noticeably increased during liver disease, indicating that HGF production occurs by distant organs such as the lung and spleen. Evidence of this is seen during a hepatectomy were liver regeneration occurs (Figure 1.4) (Mizuno and Nakamura, 2007; Mizuno *et al.*, 2008; Nakamura and Mizuno, 2010).

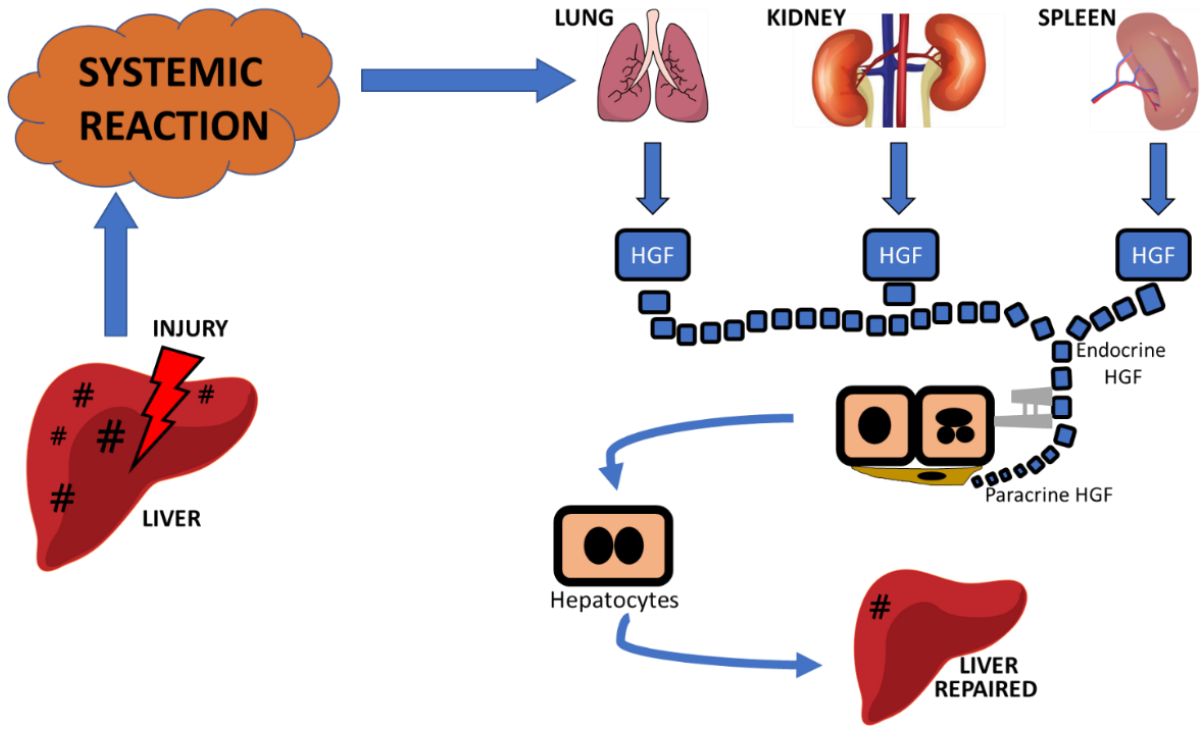


Figure 1.4. Various organs contributing to HGF production to repair liver. (adapted from Nakamura and Mizuno, 2010)

In addition, HGF and its c-Met receptor are involved in the pathogenesis of cancer, where it exerts its biological properties of invasive growth, tumor proliferation, and angiogenesis (Parikh *et al.*, 2014). From this point of view HGF displays vasoactive abilities beside stimulating growth of cells (Morishita *et al.*, 1999a).

1.3.2. Hepatocyte Growth Factor in Preeclampsia

During hypoxia there is a lack of oxygen in tissue cells due to a decrease of local vessels. Hepatocyte Growth Factor along with VEGF and basic Fibroblast Growth Factor (bFGF) are secreted to allow the formation for new blood vessel thereby, reoxygenating the tissue (Morishita *et al.*, 1999b; Nakamura *et al.*, 1996). Thus, local HGF and VEGF systems are positively associated with cardiovascular diseases.

In addition, TGF-β and angiotensin II act together to regulate blood pressure. Although, excessive TGF-β and/or angiotensin-II interacting with the HGF system, suppresses HGF gene expression *in vitro* (Morishita *et al.*, 1999b). Hence, under chronic injury or hypoxic diseases a down-regulation of HGF occurs. As the release of TGF-β or angiotensin-II gradually increases, HGF production is impaired therefore various diseases become worse with HGF downregulation (Mizuno and Nakamura, 2007; Morishita *et al.*, 1999b; Nakamura and Mizuno, 2010).

A marked reduction of local HGF production by TGF-β and angiotensin II treatment has been observed (Figure 1.5 and 1.6) (Yo *et al.*, 1998; Morishita *et al.*, 1999a).

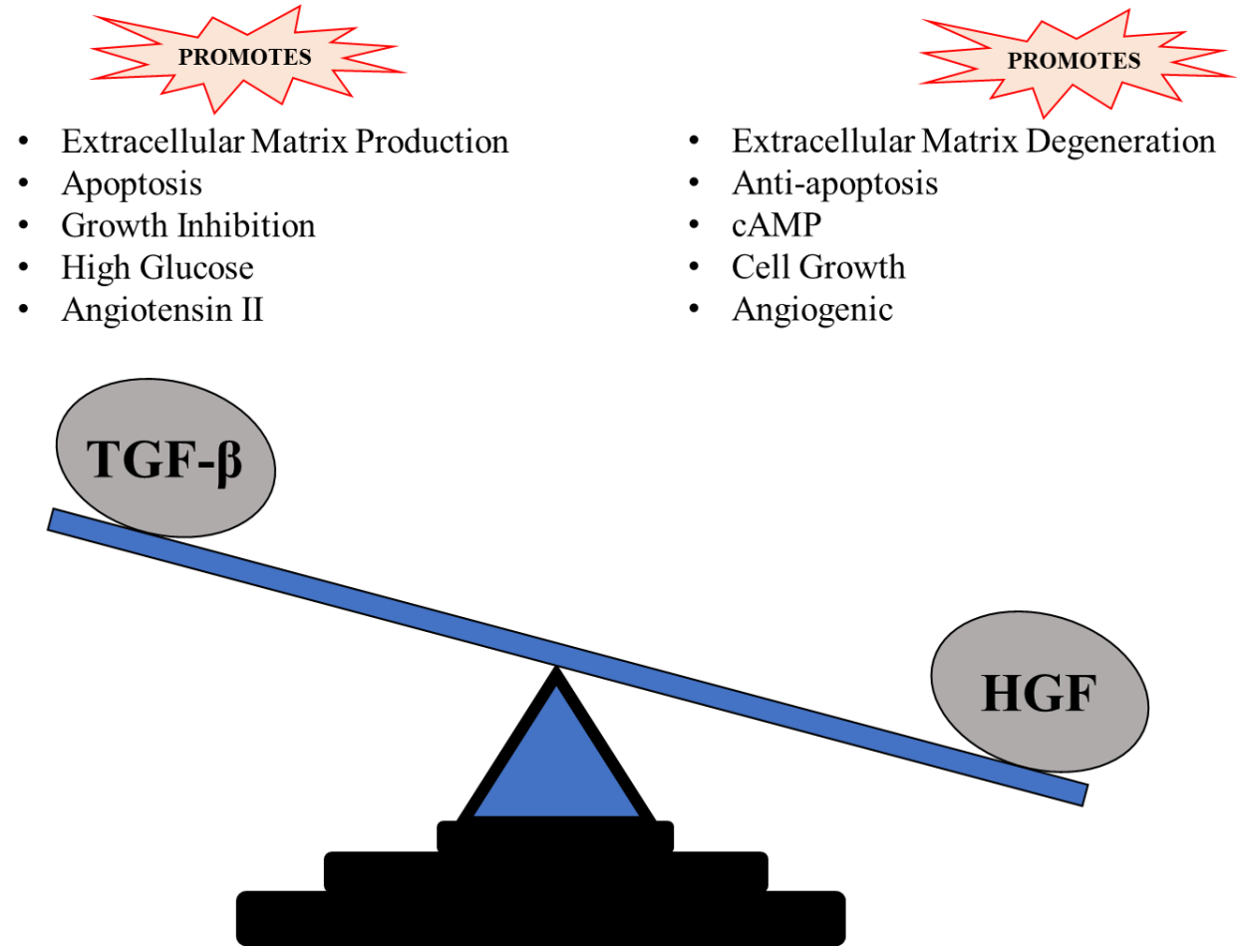


Figure 1.5. HGF regulation by TGF-β. (adapted from Nakamura and Mizuno, 2010)

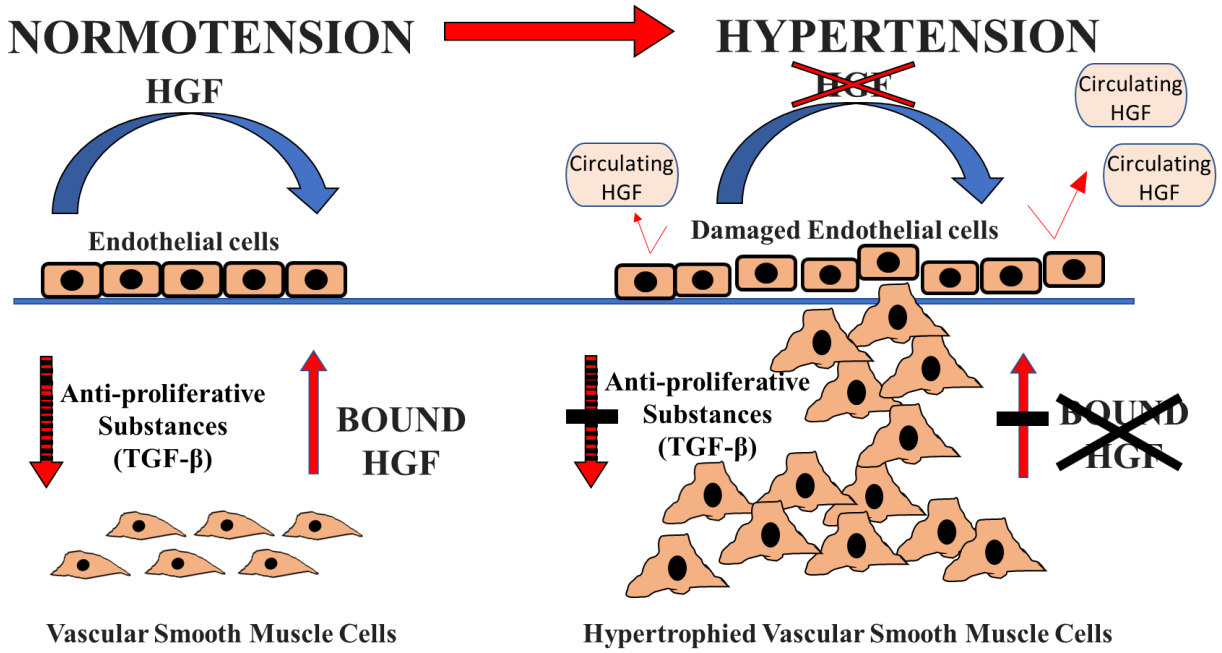


Figure 1.6. Hypertrophy due to TGF-β and Angiotensin II suppression of HGF. (adapted from Morishita *et al.*, 1999a)

Serum HGF (circulating HGF) progressively increases from normotensive to mildly hypertensive individuals. Circulating HGF in moderate and severe hypertensives are significantly higher compared to normotensive and mild hypertensive individuals. Moreover, hypertensive patients with cardiovascular complications have a higher level of serum HGF compared to healthy individuals (Figure 1.6) (Morishita *et al.*, 1999a; Morishita *et al.*, 1999b).

Therefore, it was concluded that serum HGF concentration cannot be a cause of cardiovascular diseases, but rather a potential indicator of hypertension associated with cardiovascular diseases. However, the relationship between the HGF and HIV regulatory proteins in HIV associated PE requires clarity (Cele *et al.*, 2018; Morishita *et al.*, 1999a).

1.3.3. Hepatocyte Growth Factor in HIV infection

The role of HGF in HIV infection has not been explored previously, but studies show that the HIV accessory Trans-Activator of Transcription (Tat) is involved in the rapid transcription and replication of HIV-1 (Romani *et al.*, 2010).

Poggi and Zocchi (2006) report that Tat induces TGF-β transcription, which in turn causes the immunosuppression that occurs in HIV/AIDS progression. Studies have also shown that TGF-β causes apoptosis in various human cell types during HIV-1 infection (Fittipaldi and Giacca, 2005; Licona-

Limon and Soldevila, 2007). In addition to the upregulation of TGF-β by Tat, TGF-β is also pleiotropic as it reduces anti-apoptotic proteins such as HGF, making cells more susceptible to apoptosis (Romani *et al.*, 2010).

There is a paucity of information on the association between HGF and HIV. Nonetheless, HGF influences cell proliferation and angiogenesis. In addition, HGF displays mitogenic, motogenic and anti-apoptotic properties (Ma *et al.*, 2007). Theron *et al.* (2017) reported a continuous elevation in circulating TGF-β in HIV infection. Figure 1.5 clearly outlines HGF suppression by TGF-β (Nakamura and Mizuno, 2010).

1.4. Epidermal Growth Factor

Epidermal Growth Factor (EGF) is a 53-chain amino acid polypeptide with a cytoprotective function, that improves survival in preclinical models of sepsis, through its beneficial effect on maintenance of cell integrity (Klingensmith *et al.*, 2017). The production of EGF is found at sites in salivary glands, the pancreas, and the Brunner's gland in the duodenum (Kelly *et al.*, 1997; Poulsen *et al.*, 1986). The submandibular gland is a rich source of EGF however, EGF also occurs within the general circulation. Hormones such as androgens, progestins, and adrenergic agents can increase the production of EGF in the submandibular gland and can affect its circulating level (Tsutsumi *et al.*, 1986).

A number of ligands for the EGF Receptor (EGFR) have been identified. These include EGF, Heparin Binding EGF, TGF-β, Vacinnia Virus Growth Factor and amphiregulin. Transforming Growth Factor is the most frequent ligand for the EGFR in human tissue. After EGF binds to its receptor (EGFR), it undergoes dimerization (Harari and Huang, 2001). In the cytoplasm, dimerization triggers tyrosine kinase *via* intermolecular autophosphorylation. Cytoplasmic messenger proteins bind to the phosphorylated tyrosine residues stimulating a cascade of signals from the cytoplasm to the nucleus, thus triggering a cell response. By-products of EGF binding to EGFR include motility, growth, angiogenesis, cell proliferation, invasion, metastasis, cell survival and apoptosis (Harari and Huang, 2001; Herbst, 2004; Kelly *et al.*, 1997). Due to its function, EGF receptors occur in various tissues in the body (Malhotra *et al.*, 2016). A systematic diagram (Figure 1.7) illustrates the events triggered by the EGF-EGFR binding process.

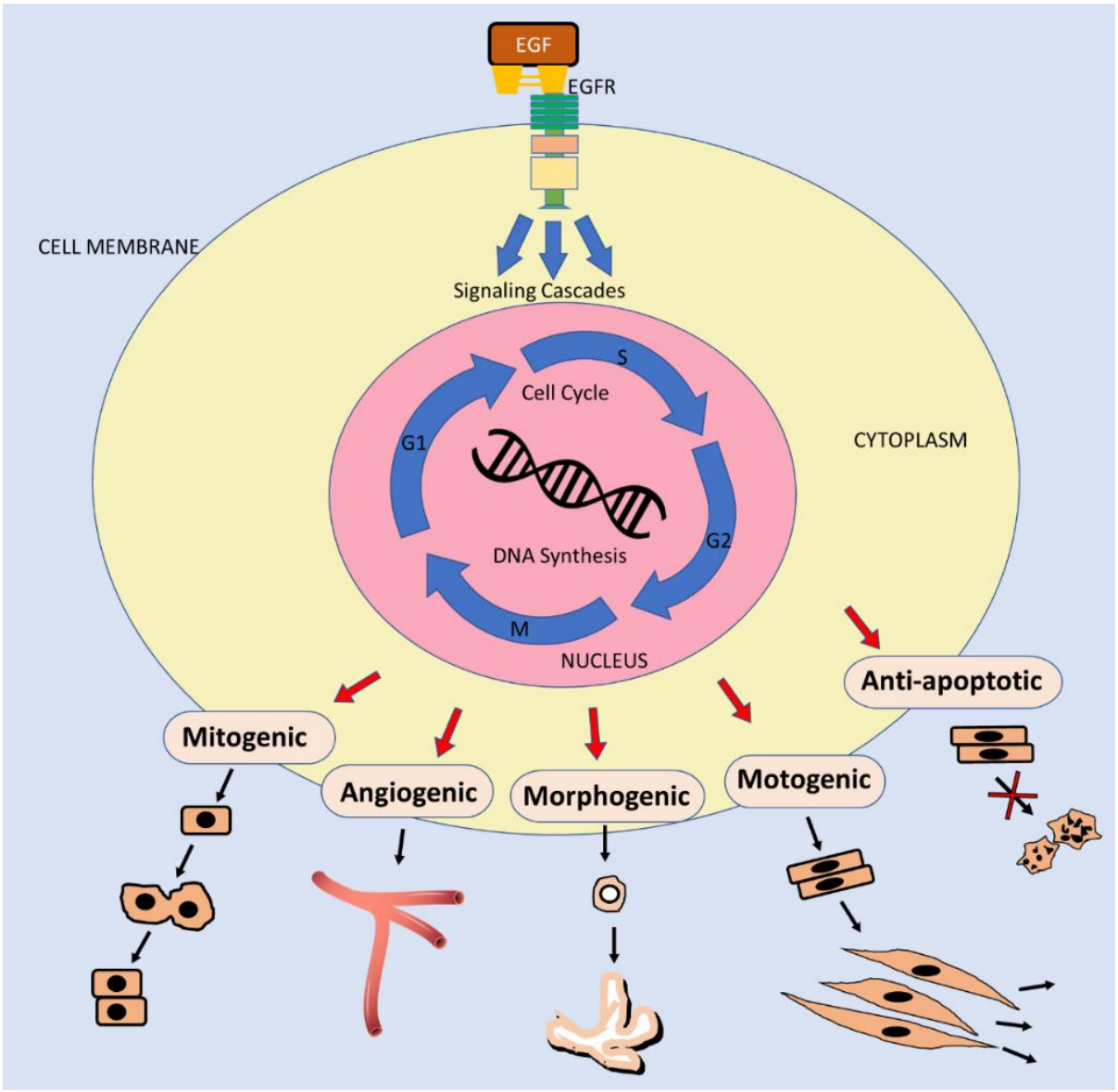


Figure 1.7. EGF binding to EGF Receptor and initiating a cell response. (adapted from Harari and Huang, 2001)

1.4.1. Epidermal Growth Factor in Pregnancy

Heparin-binding EGF plays an important role during blastocyst implantation and trophoblast invasion (Leach *et al.*, 1999). Epidermal Growth Factor also encourages the growth and maturation of different tissues and organs such as the gastrointestinal tract, kidney, bone marrow, the anterior pituitary gland and lungs (Kasselberg *et al.*, 1985; Kelly *et al.*, 1997).

Epidermal Growth Factor is mitogenic for a number of tissues. *In vitro* studies report that ethanol affects placental EGF function by EGF-induced human chorionic gonadotropin and progesterone secretion (Leach *et al.*, 1999; Wimalasena *et al.*, 1994).

The EGF family of growth factors thus play an important part of placental growth as an autocrine factor in regulating early placental growth and function (Lessey *et al.*, 2002). Epidermal Growth Factor and its receptors are expressed in the placenta (Allotey *et al.*, 2017). In addition, animal studies have demonstrated that placental growth is suppressed by maternal administration of EGF ant-iserum (Leach *et al.*, 2002; Takeda and Iwashita, 1993). The association between EGF and hypertension (preeclampsia) has not been demonstrated to-date (Imudia *et al.*, 2008; Klingensmith *et al.*, 2017; Lessey *et al.*, 2002; Shah and Catt, 2004).

During pregnancy, syncytialization occurs whereby, numerous molecules such as hormones, cytokines and growth factors in the uterine regulate the formation of syncytiotrophoblasts, *via* various signalling cascades. Epidermal Growth Factor, colony stimulating factor (CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), adiponectin and human chorionic gonadotropin (hCG) all serve to promote syncytialization whilst TGF-β and Tumor necrosis factor (TNF) inhibit syncytialization (Figure 1.8).

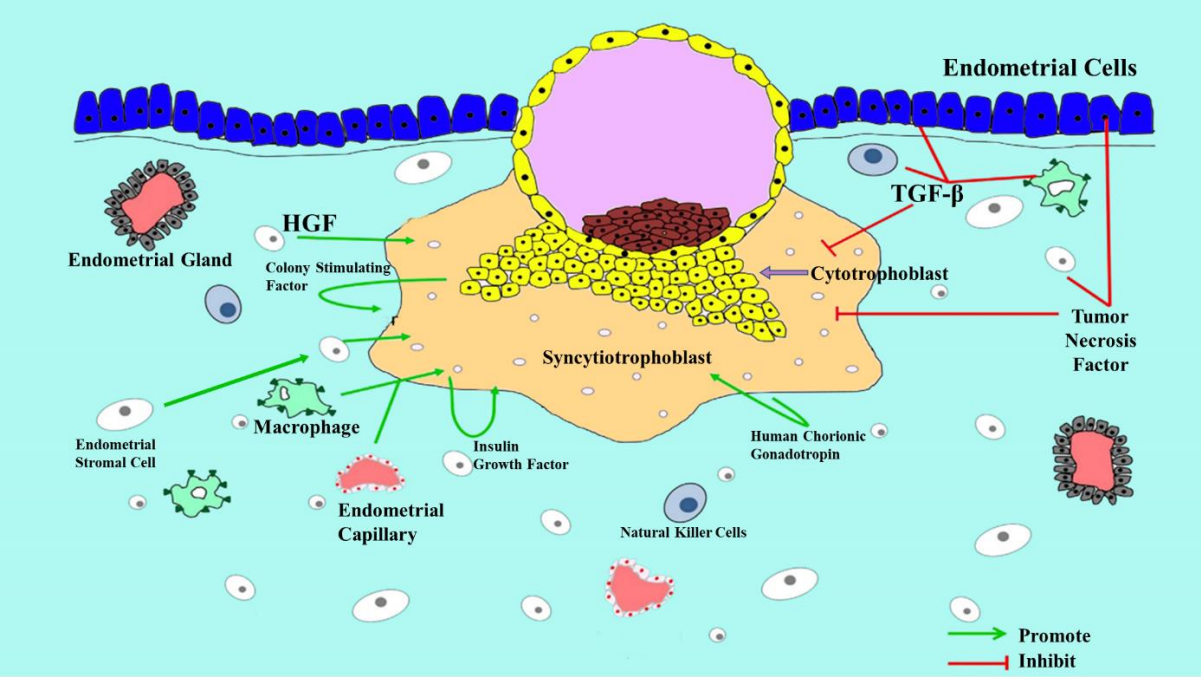


Figure 1.8. EGF secreted during placentation for placental development and further growth. (adapted from Malhotra *et al.* 2016)

1.4.2. Epidermal Growth Factor in Preeclampsia

Epidermal Growth Factor is needed for proper placentation during pregnancy. In PE, the failure of trophoblast cell differentiation and inadequate trophoblast cell migration occurs (Hall *et al.*, 2014).

A study by Armant e*t al.* (2015) observed a significant reduction of plasma and trophoblast EGF in preeclamptic compared to normotensive women. In addition, they found an overexpression of EGFR to which EGF expression is low, hence EGF plays an important role in PE development. Also, EGF downregulation can illicit apoptosis since EGF promotes cell survival and regulates cytotrophoblast invasion. Bcl-2-associated death promoter (BAD) and caspase-9 (CASP9) are two pro-apoptotic molecules that are inhibited by the binding of EGF to its receptor *via* the Phosphoinositide 3-kinase (PI3K) pathway thus, in PE low levels of EGF can cause apoptosis in trophoblast cells (Bergmann, 2002; Perkins *et al.*, 2002).

1.4.3. Epidermal Growth Factor in HIV infection

The Tat protein inhibits EGF related cell responses such as cell proliferation, migration, adhesion, cell survival, differentiation, and invasion (Fields *et al.*, 2014; Mattila *et al.*, 2005). These processes are due to the binding of EGF to EGFR and activation of the PI3K/AKT, RAS/ERK, and JAK/STAT3 pathways (Bergmann, 2002; Croker *et al.*, 2008; Sabra *et al.*, 2017; Trusolino *et al.*, 2010).

Tat is also a powerful angiogenic factor, since it mimics VEGF (Albini *et al.*, 1996). Epidermal Growth Factor and VEGF play similar physiological roles in these signalling pathways where they regulate both apoptotic and anti-apoptotic proteins, which become dysfunctional in the presence of Tat. This effectively blocks the anti-apoptotic pathways leading uncontrollable cell growth (cancer) or enabling anti-apoptotic pathways with resultant to cell death (Henson and Gibson, 2006; Li *et al.*, 2009; Limaye *et al.*, 2008; Zhang *et al.*, 2015).

1.5. Signalling

Hepatocyte and Epidermal Growth Factor are both part of the same tyrosine kinase family, therefore their signalling pathways are identical (Bos *et al.*, 2001; Zhang *et al.*, 2015). Phosphorylation of c-Met/EGFR with HGF/EGF activates and signals the following pathways, PI3K/AKT/mTOR, RAS/ERK, and JAK/STAT3 (Hallberg and Palmer, 2013). This signalling results in the regulation of a number of cellular processes such as cell proliferation, motility, angiogenesis, invasion, metastasis, cell survival, differentiation, and apoptosis (Chen and Sikic, 2012; Lin *et al.*, 2017; Mertens and Darnell, 2007).

1.6. HIV Associated Preeclampsia

Immunosuppressive condition such as HIV infection and PE have opposing immune responses (Kalumba *et al.*, 2013). In SA, about 30% of antenatal women are infected with HIV. Also, the prevalence of PE is approximately 16% (Moodley, 2013). Thus, SA is an ideal site for studying the duality of HIV associated PE. In the province of KwaZulu-Natal, the rate of HIV infection in pregnant women is 40% whilst the prevalence of PE is 12% (Panday *et al.*, 2016). Both HIV infection and PE have opposing immune responses. It is plausible to assume that PE associated with HIV infection may result in a neutral immune response. However, the prevalence of PE is reduced in untreated HIV infected patients, than in those on Highly Active Antiretroviral Therapy (HAART) (Suy *et al.*, 2006). HAART re-establishes the maternal immune response, consequently increasing one's susceptibility to developing PE (Govender *et al.*, 2013).

1.7. Aim and objectives of this investigation

To-date a gap exists in PE diagnosis. Hence, research is required to identify predictor tests for the early identification of PE development. Therefore, the use of biomarkers in the early detection of preeclampsia is promising start.

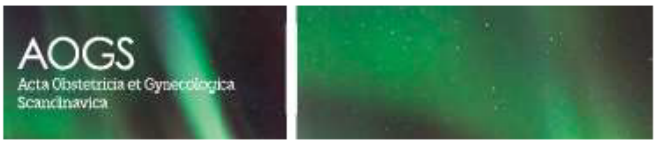
This study aims:
To determine the concentrations of HGF and EGF in the pathogenesis of HIV associated normotensive versus preeclamptic pregnancy.

The objectives will be:
-To determine the effect of pregnancy type (normotensive pregnant *vs* preeclamptic) on HGF and EGF regardless of HIV status utilizing a BioPlex Multiplex immunoassay.

- To determine the effect of HIV status (HIV-positive and HIV-negative) on the level of HGF and EGF regardless of pregnancy type utilizing a BioPlex Multiplex immunoassay.

-To compare and contrast HGF and EGF across the study population (normotensive HIV-positive, normotensive HIV-negative, preeclamptic HIV-positive and preeclamptic HIV-negative).

CHAPTER 2



HEPATOCYTE GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR IN HIV INFECTED WOMEN WITH PREECLAMPSIA

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Keywords:	Preeclampsia, HIV/AIDS, Pregnancy

HEPATOCYTE GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR IN HIV INFECTED WOMEN WITH PREECLAMPSIA

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Conflict of Interest statement

There are no conflicts of interest

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Abstract

Introduction- Hepatocyte Growth Factor (HGF) and Epidermal Growth Factor (EGF) both have tyrosine kinase receptors (c-Met and EGFR) which upon binding, activates and regulates many important cellular processes such as cell survival, growth, proliferation, differentiation, invasion, repair and so forth *via* the RAS/MAPK/ERK1/2, PI3K/AKT and JAK STAT3 pathways. These processes are crucial for the development of a placenta and other functions in order for a normal pregnancy to occur. Hence, this study determined the concentrations of HGF and EGF to find the correlation between HIV and preeclampsia (PE).

Material and methods- A total sample size of n=80 was used, n=40 preeclamptic and n=40 normotensive women these were further stratified into HIV-positive and HIV-negative women. Analysis of the growth factors were done by using the Bio-Plex multiplex immunoassay method.

Results- Irrespective of HIV status, based on pregnancy type, EGF in PE women displayed an upregulation compared to normotensive women. However, for HGF no significance was found between pregnancy type.

Based on HIV status, regardless of pregnancy type, both HGF and EGF levels were significantly increased in HIV-positive women compared to HIV-negative women.

Across all groups for HGF, significant difference was found between normotensive HIV-negative (lower) *vs* normotensive HIV-positive women (higher).

Nevertheless, for EGF across all groups, a statistically significant decrease was found in normotensive HIV-negative women compared to normotensive HIV-positive, preeclamptic HIV-positive and preeclamptic HIV-negative women.

Conclusion- The study demonstrates that there is a strong association between HIV and PE, and that HGF and EGF are promising biomarkers to use as a diagnostic tool for PE.

Keywords - HIV, Preeclampsia, HGF, EGFR, EGF, c-Met

Abbreviations – cAMP, 3'-5'-Cyclic adenosine monophosphate; EGF, Epidermal Growth Factor; EGFR, Epidermal Growth Factor receptor; ERK, Extracellular signal-regulated kinases; HGF, Hepatocyte Growth Factor; HAART, Highly active antiretroviral therapy; HIV, Human Immunodeficiency Virus; IQR, Interquartile range; JAK, Janus kinase; MAPK, Mitogen-activated protein kinase; Ns, Non-significant; PI3K, Phosphoinositide 3-kinase; PE, Preeclampsia; AKT, Protein kinase B; RAS, Renin-angiotensin system; STAT3, Signal transducer and activator of transcription 3; Tat, Trans-Activator of Transcription; TGF-β, Transforming Growth Factor beta; c-Met, Hepatocyte Growth Factor Receptor; VEGF, Vascular Endothelial Growth Factor

Key Message- Preeclampsia and HIV-1 are the two main causes of maternal mortality in South Africa. We demonstrate significant differences in HGF and EGF expression in HIV associated preeclamptic women, indicating that there is a possible association between preeclampsia and HIV.

Introduction

South Africa is home to the largest global human immunodeficiency virus (HIV) epidemic (1). In South Africa, 13.1% of the total population is HIV-positive, with one-fifth of women in their reproductive ages (15–49 years) being infected (2). Approximately 30% of South African parturients are co-infected with HIV (3) with the prevalence being the highest in the province of KwaZulu-Natal (37%) (2). Health care professionals providing maternity care are thus faced with a severe dilemma. Non-pregnancy related infections (31%), obstetric hemorrhage (24%) and hypertensive disorders of pregnancy (19%) are major contributory factors to maternal mortality with preeclampsia (PE), accounting for >83% of these deaths (4). Preeclampsia is characterized by new onset blood pressure of greater than 140/90 mmHg and proteinuria (> 300mg/d) after the 20th week of gestation. In KwaZulu-Natal, the prevalence of PE is 12%. Notably, maternal deaths from HIV infection and obstetric hemorrhage have declined over the period: 2008-2016, however, no change in mortality due to hypertensive diseases in pregnancy has occurred (4).

Whilst the exact cause of preeclampsia has not yet been elucidated, it is characterized by inadequate physiological spiral artery remodeling which causes a hypoxic and anti-angiogenic micro-environment (5).

The association between HIV infection and PE is conflicting due to opposing immune responses. Moreover, HIV-infected pregnant women treated with Highly Active Anti-Retroviral Treatment (HAART) have an increased risk for PE development due to the reconstitution of their immune system (6). The Hepatocyte Growth Factor (HGF) and Epidermal Growth Factor (EGF) belong to a family of receptor tyrosine kinases that are integral to the regulation of cell proliferation, survival, differentiation and migration (7). The Hepatocyte Growth Factor Receptor (c-Met) also known as tyrosine-protein-kinase-Met, which is a proto-oncogene located on chromosome 7q21-31 (8). Its ligand, HGF is converted from an inert precursor by a proteolytic cleavage into a bioactive mature form (9). The HGF/c-Met binding results in receptor homodimerization and phosphorylation of tyrosine residues that mediate a complex signalling network (10). Hepatocyte Growth Factor acts as a pleiotropic factor, promoting cell proliferation, survival, motility, differentiation and morphogenesis.

Since HGF promotes epithelial cell migration and invasion, it could play a pivotal role in placentation. In PE, the abnormal trophoblast invasion is associated with a downregulation of placental HGF (11). Moreover, during development, the HGF/c-Met receptor interaction is

important for organ growth (10, 12). Epidermal Growth Factor acts *via* the Epidermal Growth Factor Receptor (EGFR). Both c-Met and EGFR are transmembrane tyrosine kinases, which post binding with their ligand, initiate phosphorylation and activate transcription (13). In pregnancy, EGF and its receptor are expressed in the placenta (14). Moreover, the EGFR family activate pathways such as the Renin–Angiotensin System/Mitogen-Activated Protein Kinase/extracellular signal–regulated kinases 1/2 (RAS/MAPK/ERK1/2), Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/AKT) and Janus Kinase Signal Transducer and Activator of Transcription 3 (JAK STAT-3) (15). Additionally, EGF promotes syncytialization- an integral step in blastocyst implantation and placentation and when these processes fail to occur in a set sequence, pregnancy complication such as PE occur (5).

In light of the extensive role played by the tyrosine kinases, HGF and EGF in placentation, the aim of this study is to compare the level of serum HGF and EGF in HIV associated normotensive pregnant and preeclamptic women using the Bio-plex multiplex immunoassay.

Methods and Materials

Ethical Approval

This retrospective study received ethical approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BCA338/17; 17th April 2018). All women were recruited from the antenatal clinic of a large regional hospital in Durban, South Africa and gave their full informed consent for the participation in this study.

Study population

The study population (n=80) consisted of 40 normotensive pregnant and 40 preeclamptic women. Preeclampsia was defined as a new onset blood pressure of ≥140/90 mmHg and proteinuria > 300mg/d after the 20th week of gestation (5).Both groups were further stratified by HIV status into preeclamptic HIV-positive (n=20), preeclamptic HIV-negative (n=20), normotensive HIV-positive pregnant (n=20) and normotensive HIV-negative pregnant (n=20) women. All HIV-positive women received dual HAART and nevirapine therapy, a standard of care practice in South Africa.

Exclusion criteria for the PE group were eclampsia, chorioamnionitis, chronic diabetes, chronic hypertension, pre-existing seizure disorders, intrauterine death, abruption placenta, gestational diabetes, systemic lupus erythematous, chronic renal disease, sickle cell disease, thyroid disease, cardiac disease, asthma and an unknown HIV status.

Sample type

Maternal blood samples were collected and centrifuged at 1000g for 10 minutes at 20°C. Serum samples were stored at -80°C until required.

ProcartaPlex™ Multiplex Immunoassay method

A Growth Factor 45-Plex Human ProcartaPlex™ Panel one was performed according to manufacturer's instructions (Invitrogen by ThermoFisher Scientific, catalog no: EPX450-12171-901). The standards were prepared in a 1:4 dilution series, and no dilution of samples were required.

In a 96 well plate, HGF and EGF capture antibody-coupled magnetic beads were added to each well and washed twice. Standards, samples and blanks were then added into their designated wells and left to incubate before washing three times. Thereafter, a biotinylated detection antibody was pipetted into each well and allowed to incubate. The plate was then washed three times before adding streptavidin-phycoerythrin throughout the wells. Finally, the plate was washed for a further three times, before resuspending each well with assay buffer. The plate was then ready to be placed into the Bio-Plex system for reading.

The Bio-Plex®MAGPIX™ Multiplex Reader (Bio-Rad Laboratories Inc., USA) was utilized to read the experiment plate. Bio-Plex Manager™ software version 4.1 was used to obtain the data from the multiplex analysis.

Statistical Analysis

Data analysis was performed using Graphpad Prism 5.00 for Windows (GraphPad Software, San Diego California USA). The Kolmogorov Smirnov test for normality revealed non-parametrically distributed data. A Mann-Whitney U test was utilized to determine statistical significance according to pregnancy type (preeclamptic *vs* normotensive) and HIV status (positive *vs* negative). Results are represented as median and interquartile range (IQR). To

determine statistical significance across all groups, a Kruskal-Wallis test in combination with the Dunn's Multiple comparison *post hoc* test was used. Statistical significance was $P < 0.05$.

Results

Patient Demographics and Clinical Characteristics

Patient demographics and clinical characteristics are represented in Table 1 as median and interquartile range (IQR). No significant differences were reported in maternal age (P=0.16), parity (P=0.47) and maternal weight (P=0.36), across all study groups. However, there was a statistically significant difference between gestational age, systolic and diastolic blood pressures across the study groups (P<0.0001).

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)	24.50 (9.75)	28.50 (9)	24.00 (9.5)	29.00 (10)	0.1601 (ns)
Gestational Age (weeks)	39.00 (3)	38.00 (3)	32.00 (11)	32.00 (6)	<0.0001 ***
Parity	1.00 (2)	1.50 (1)	1.00 (1.750)	1.00 (1)	0.4707 (ns)
Systolic BP (mmHg)	121.5 (16.2)	110.0 (18.5)	158.5 (17)	157.5 (22.3)	<0.0001 ***
Diastolic BP (mmHg)	73.0 (13.75)	70.0 (7.25)	103.5 (19.5)	100.5 (13)	<0.0001 ***
Weight (kg)	76.5 (16.23)	79.5 (19.02)	70.0 (16.17)	79.0 (35.97)	0.3557 (ns)

Results are represented as the median (IQR), ns = non-significant,
* $P < 0.05$
*** $P < 0.001$

Serum concentrations of Hepatocyte Growth Factor

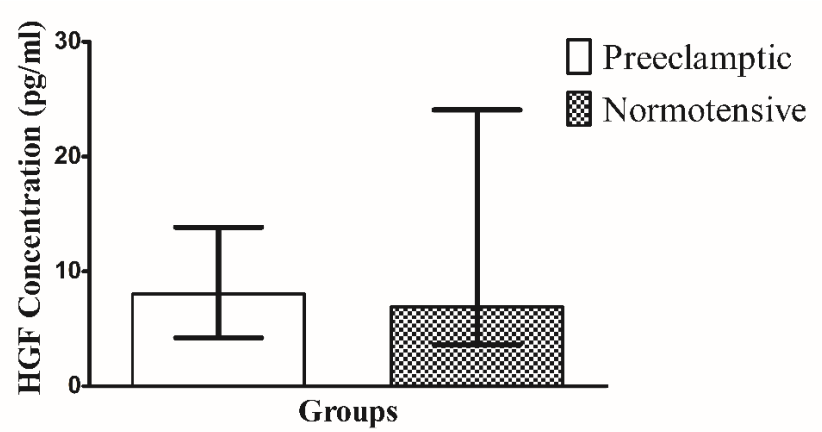
Pregnancy type- The concentration of HGF was not statistically different between the normotensive pregnant (median = 6.91pg/ml, IQR = 20.43pg/ml) *vs* preeclamptic (median = 8.03pg/ml, IQR = 9.63pg/ml) groups, regardless of HIV status (Mann-Whitney U = 382.5; P = 0.889; Figure 1A).

HIV status- However, there was a significant difference in HGF level between the HIV-positive (median = 10.68pg/ml, IQR = 21.1pg/ml) *vs* HIV-negative (median = 6.44pg/ml, IQR =

6.35pg/ml) groups, irrespective of pregnancy type, (Mann-Whitney U = 252.0; *P* = 0.0225; Figure 1B).

Across all groups- There was a significant difference in HGF concentration between normotensive HIV-negative (median = 4.17pg/ml, IQR = 5.89pg/ml) *vs* normotensive HIV-positive (median = 21.78pg/ml, IQR = 24.74pg/ml) pregnant women (Kruskal-Wallis H = 14.63; *P* = 0.0022; Figure 1C). No differences were noted across all other groups.

1A



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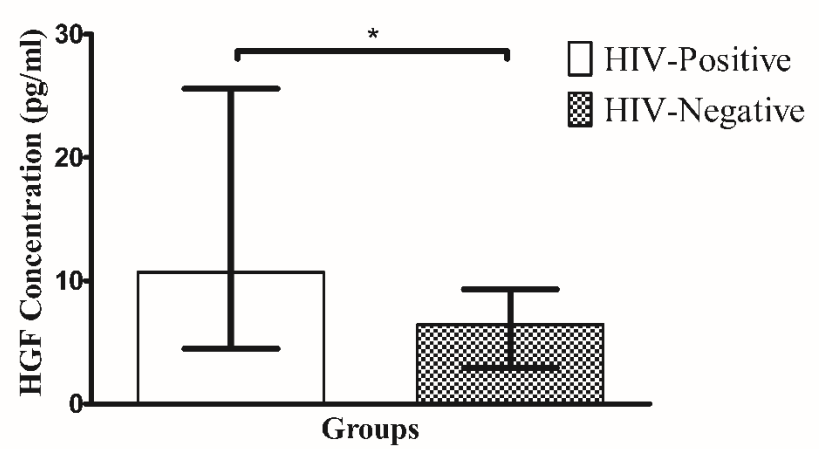

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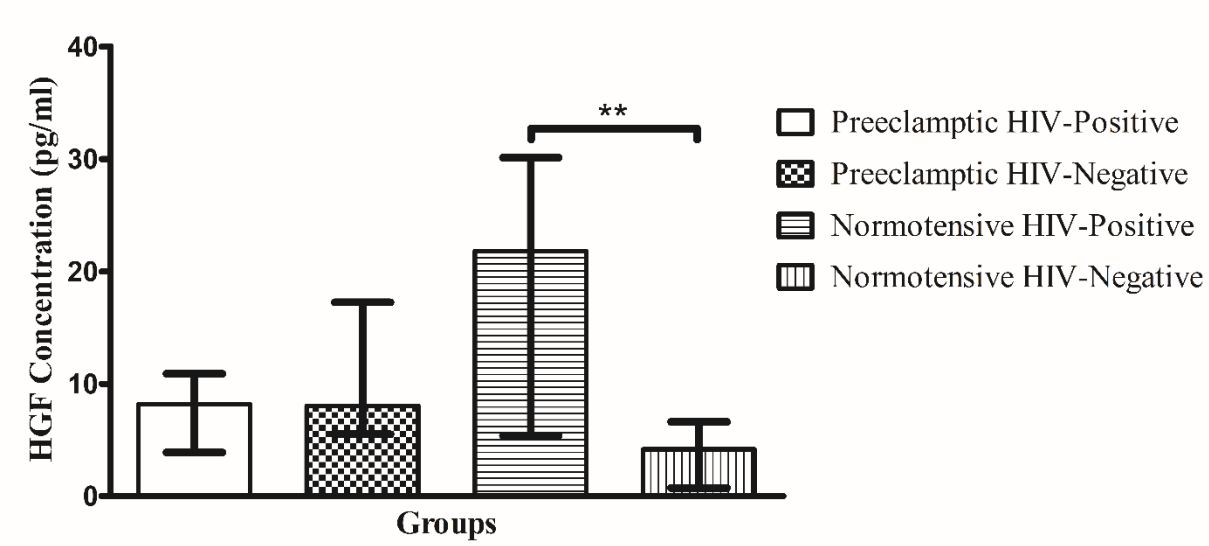


Figure 1. Hepatocyte Growth Factor: (A) Preeclamptic *vs* Normotensive Groups, (B) HIV infected *vs* HIV uninfected groups, (C) Across All Groups. *Serum concentrations of HGF are significantly different between HIV-positive and HIV-negative group, *P* = 0.0225. **Serum concentrations of HGF are significantly different between normotensive HIV-positive and normotensive HIV-negative group, *P* = 0.0022.

Serum concentrations of Epithelial Growth Factor

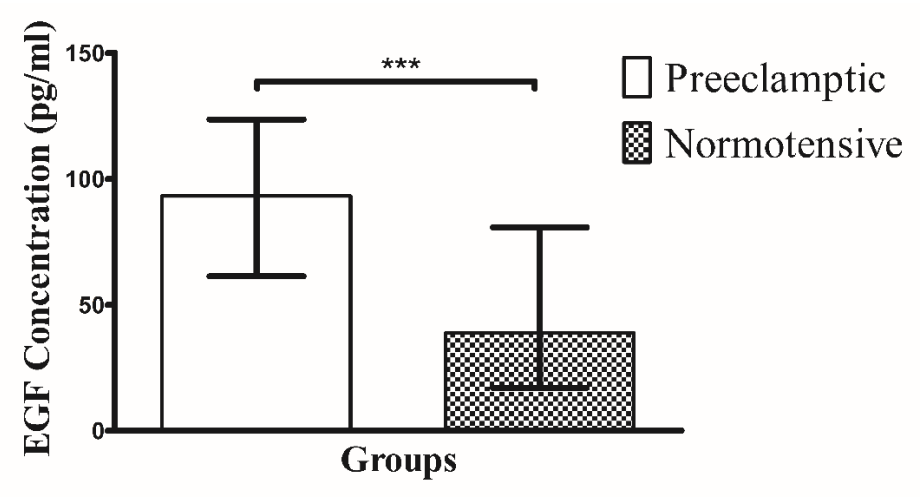
Pregnancy type- EGF expression was statistical different between the normotensive pregnant (median = 38.82pg/ml IQR = 63.63pg/ml) *vs* preeclamptic (median = 93.13pg/ml, IQR = 62.3pg/ml) groups, irrespective of HIV status (Mann-Whitney U = 186.0; *P* = 0.0003; Figure 2A).

HIV status- The concentration of EGF was significantly different between the HIV-positive (median = 88.85pg/ml, IQR = 73.14pg/ml) *vs* HIV-negative (median = 49.64pg/ml, IQR = 72.73pg/ml) groups, regardless of pregnancy type (Mann-Whitney U = 241.5; *P* = 0.0055; Figure 2B).

Across all groups- A statistically significant difference in EGF concentration was noted between: normotensive HIV-negative (median = 17.04pg/ml, IQR = 30.88pg/ml) *vs* normotensive HIV-positive (median = 72.25pg/ml, IQR = 59.88pg/ml); normotensive HIV-negative (median = 17.04pg/ml, IQR = 30.88pg/ml) *vs* preeclamptic HIV-positive (median = 96.36pg/ml, IQR = 80.81pg/ml) and normotensive HIV-negative (median =17.04pg/ml, IQR =

30.88pg/ml) *vs* preeclamptic HIV-negative groups (median =86.42pg/ml, IQR =59.50pg/ml), (Kruskal-Wallis H = 25.98; $P < 0.0001$; Figure 2C).

2A



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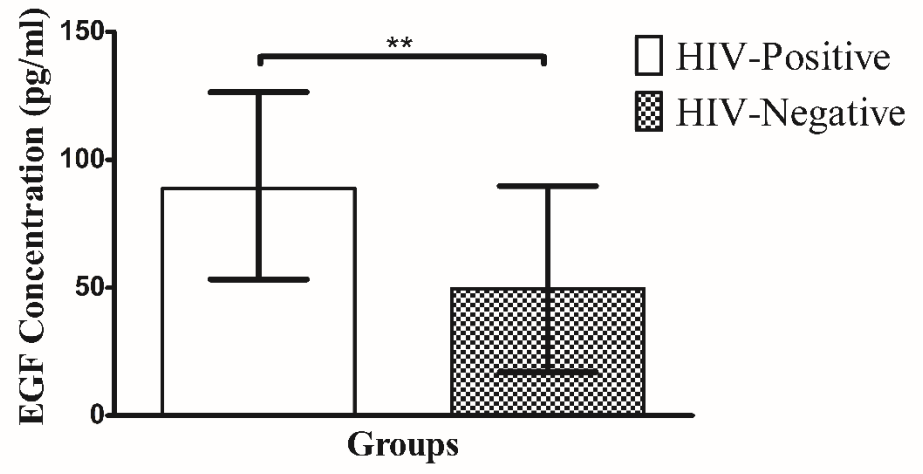

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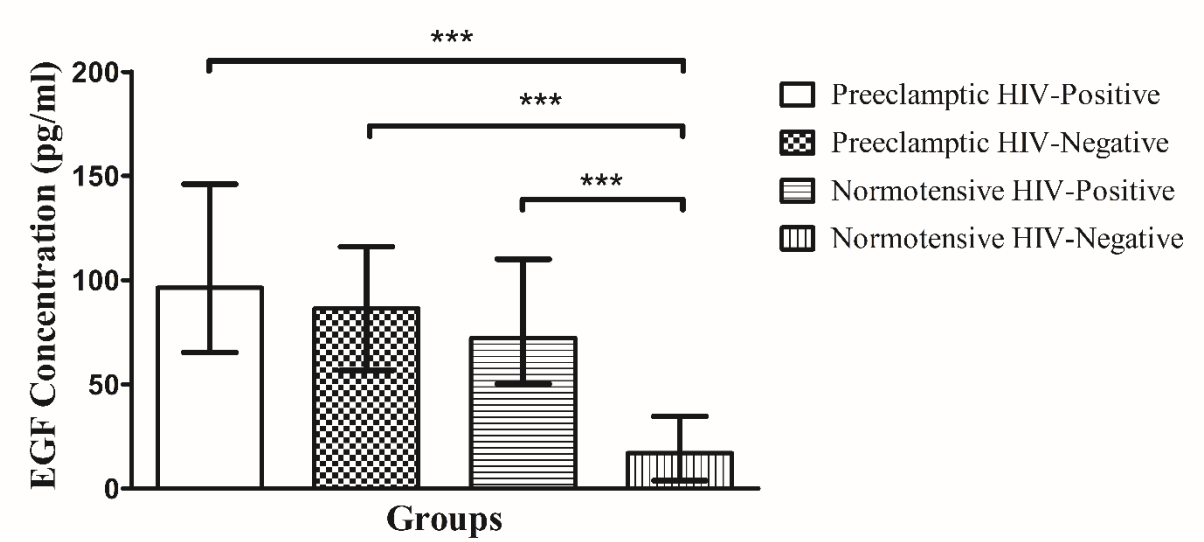

Figure 2. Epidermal Growth Factor: (A) Preeclamptic *vs* Normotensive Groups, (B) HIV infected *vs* HIV uninfected groups, (C) Across All Groups. ***Serum concentrations of EGF are significantly different between preeclamptic and normotensive group, *P* = 0.0003. **Serum concentrations of HGF are significantly different between HIV-positive and HIV-negative group, *P* = 0.0055. ***Serum concentrations of EGF are significantly different between normotensive HIV-positive and normotensive HIV-negative group, *P* < 0.0001. ***Serum concentrations of EGF are significantly different between preeclamptic HIV-negative and normotensive HIV-negative group, *P* < 0.0001. ***Serum concentrations of EGF are significantly different between preeclamptic HIV-positive and normotensive HIV-negative group, *P* < 0.0001.

Table 2. Serum concentration (pg/ml) of Growth Factors across all study groups

	Normotensive		**Preeclamptic**		***P* value**
	HIV Negative (n=20)	**HIV Positive** (n=20)	**HIV Negative** (n=20)	**HIV Positive** (n=20)	
EGF	17.04 (30.88)	72.25 (59.88)	86.42 (59.50)	96.36 (80.81)	< 0.0001
HGF	4.17 (5.89)	21.78 (24.74)	8.03 (11.75)	8.17 (7.01)	0.0022

Results are represented as median (interquartile range)

Discussion

Hepatocyte Growth Factor: This study demonstrates that serum HGF concentrations were similar between normotensive and PE pregnancies, regardless of the HIV status (Figure 1A). Several studies also report similar serum HGF levels between PE and normotensive pregnancies (16). It is plausible that sample type affects HGF as contradictory studies report a statistically significant increase in the plasma HGF concentration in PE (17). Martinez-Fierro et al. (18) noted that the urinary concentration of HGF at 20 weeks gestation were elevated in women that developed PE compared to normotensive pregnancy; suggesting that urinary HGF could possibly have predictive test value for the diagnosis of PE (18).

It is noteworthy to hypothesize that a high production of HGF causes saturation of HGF receptor (c-Met), which results in a reduced level of the free circulating HGF in blood. Nonetheless, Naghshvar et al. (19) investigated c-Met levels in both serum and plasma and found that c-Met levels correlated with severity of PE.

Both HGF and EGF are tyrosine kinases which activate signalling to mediate cell proliferation, differentiation, angiogenesis, migration and invasion *via* the JAK STAT-3 and MAPK/ERK1/2 pathways, proliferation and survival *via* PI3K/AKT cascade (12). The c-Met receptor significantly regulates the activation of cell differentiation, proliferation and self-renewal *via* the MAPK/ERK1/2 pathway (20). In PE, there is a dysregulation of the latter pathway (20). This reduced HGF/c-Met axis inhibits trophoblast cell differentiation, proliferation and repair/regeneration of trophoblast cells and may explicate the reduction in trophoblast cell migration that occurs in PE (12). A recent study by Zeng et al. (21) indicated that HGF was responsible for placental growth and that inhibition of HGF production in an animal model resulted in stillbirth.

The reduction of HGF, albeit non-significantly in PE in the current study may be attributed to the hypoxic micro-environment. Hypoxia has been shown to reduce HGF expression, thereby limiting interaction with the c-Met receptor on trophoblast cells (22). This receptor ligand binding restricts trophoblast 3′,5′-cyclic adenosine monophosphate (cAMP) expression which activates protein kinase A/ Ras-proximate-1 (PKA/RAP1). Subsequently, Serine/threonine-protein kinase (PAK) is inappropriately stimulated leading to integrin β1 down-regulation (23), eventuating in decrease trophoblast invasion (Figure 3).

Furthermore, c-Met is known for its ability to elicit a cell survival response (anti-apoptotic) *via* the PI3K/AKT pathway. Hepatocyte Growth Factor is needed to regulate this anti-apoptotic

response. In a previous report, our research group demonstrated elevated apoptosis of trophoblast cells in PE (24). Thus, it is plausible that the decline of serum HGF accounts for the increased apoptosis of trophoblast cells, with resultant decline in trophoblast invasion/migration in PE (22).

Our study also illustrated an elevated serum concentration of HGF in HIV-positive women compared to that of HIV-negative women (Figure 1B), irrespective of pregnancy type. Tulasne et al. (25) established that stress stimuli transform the anti-apoptotic function of the c-Met receptor to a pro-apoptotic function by a caspase-dependent cleavage. Both PE and HIV infection are well accepted stress stimulant conditions (26).

In an in vitro study, the HIV accessory protein, Trans-Activator of Transcription (Tat) is able to activate the intrinsic pathway of apoptosis (27). Tat induces an upregulation of Transforming Growth Factor beta (TGF-β) which inhibits HGF production, thus negating its pleiotropic actions (28). Moreover, Wiercińska-Drapalo et al. (29) noted that TGF-β levels are greater than twofold in the HIV-positive compared to HIV-negative women. Despite the lack of correlation between HAART and TGF-β in the latter study, HAART is believed to be advantageous during early stages of therapy, with positive HGF production and hepatocyte survival (30). In the current study, we noted a significantly different HGF concentration between normotensive HIV-positive *vs* normotensive HIV-negative women (Figure 1C). It is plausible that a compromised immune system elicits a compensatory response to produce HGF in order to repair tissues and organs damaged by the HIV infection (31).

Epidermal Growth Factor: Our study also demonstrated an up-regulation of serum EGF in preeclamptic compared to normotensive pregnancies (Figure 2A), regardless of HIV status. This finding is corroborated by Armant et al. (32), who reported that EGFR an EGF receptor protagonist, is also overexpressed in PE (32). Notably, EGFR initiates signal transduction cascades namely the MAPK, AKT and JAK-STAT-3 pathways. This signalling initiates DNA synthesis by modifying cell migration, adhesion, and proliferation. Thus, it is possible that the deficient trophoblast cell invasion in PE may be a result of an aberrant signal transduction cascade emanating from the dysregulation of EGFR-EGF binding (7). Ferrandina et al. (33) further hypothesized that the hypertensive disorders of pregnancy are related to increased placental EGFR concentration. In contrast to our findings, plasma EGF levels were reported to be twofold higher in normotensive pregnant compared to PE women (32).

Epidermal Growth Factor signals *via* the JAK STAT-3 pathway highlighting the fact that EGF influences proliferation and invasion of trophoblast cells (34). The PI3K/AKT pathway has been associated with many cellular processes such as regulation of the cell cycle, cell death, adhesion, migration and metabolism. Notably, a decrease in EGF reduces AKT phosphorylating of Bcl-2-associated death promoter (BAD), which leads to trophoblast cell death in PE (35). In PE, the invasive trophoblast cells exit the cell cycle in the G1 phase, leading them to apoptosis rather than passing through into the S phase and mitosis (24).

In this study, EGF was elevated in HIV-positive compared to HIV-negative women (Figure 2B), irrespective of pregnancy type. It is plausible that an immunocompromised milieu may affect EGF-EGFR interactions. Burgel and Nadel (36) correlated this upregulation of EGF with an exaggerated innate immune response.

Additionally, preeclampsia is characterized by impaired angiogenesis (24). The HIV accessory protein Tat is a powerful angiogenic factor, in that it mimics Vascular Endothelial Growth Factor (VEGF) (37). The Tat protein inhibits EGF-induced processes such as cell proliferation, differentiation, invasion, migration, adhesion and cell survival (38). Therefore, apart from Tat affecting angiogenesis, it can also affect trophoblast cell migration. In HIV-positive women, Tat would reduce the predisposition to PE development (39). Since Tat inhibits EGF function, it would negatively impact trophoblast cell migration.

An association between HGF and EGF signalling has been established by Zhang et al. (40), where upon activation, these growth factors elicit the same cellular responses (Figure 3). The binding of HGF or EGF to their respective receptors induce phosphorylation of PI3K which activates AKT, IκB kinase (IKK) and inhibits Caspase-9/BAD, thus mediating pro-apoptosis. Activation of AKT regulates mammalian target of rapamycin (mTOR) which is responsible for cell survival and proliferation. In addition, IKK promotes nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-KB) which triggers cell invasion, adhesion, proliferation and angiogenesis (41) (Figure 3).

The activation of STAT-3 *via* JAK is a byproduct of the fusion between HGF/EGF and c-Met/EGFR (Figure 3). This signalling results in the regulation of a number of cell responses, such as: cell growth, differentiation, invasion, repair and self-renewal (34).

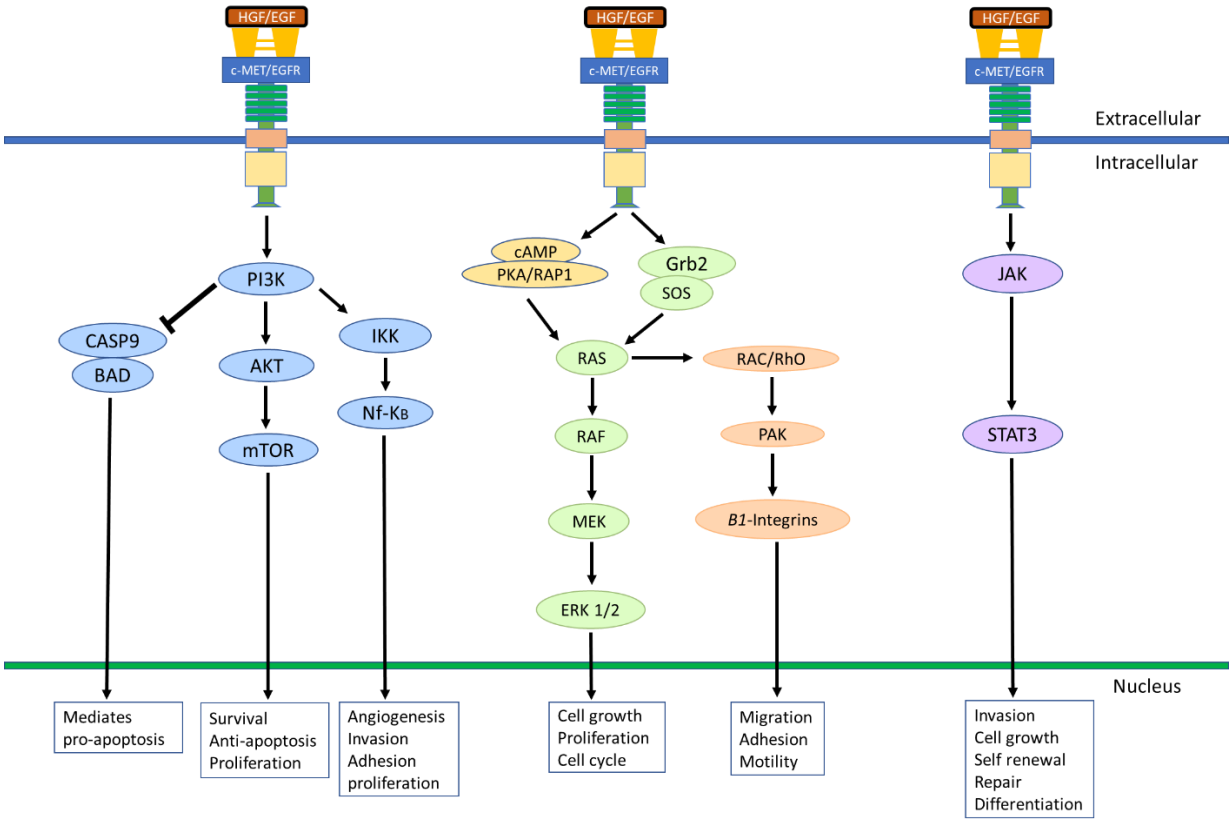

Figure 3. Cross link in PI3K/AKT, MAPK/ERK1/2 AND JAK STAT-3 pathways between HGF/EGF upon activation. (adapted from Zhang et al. (40))

The docking of HGF/EGF to their ligands results in phosphorylation of Growth factor receptor bound protein 2 /Son of Sevenless (Grb2/SOS). In addition, cAMP/PKA/RAP1 are also phosphorylated, in which both promote the RAS pathway (23). Henceforth Rapidly Accelerated Fibrosarcoma- Mitogen-activated protein kinase (RAF-MEK-ERK1/2) cascade is stimulated eliciting cell growth, proliferation and cell cycle regulation. RAS also triggers the Ras-related C- ρ - Serine/threonine-protein kinase (RAC-RhO-Pak) pathway which initiates integrin β1 upregulation for cell migration, adhesion and motility (42).

One of the limitations of this study is the absence of viral load, which subsequently resulted in no correlation between HGF/EGF based on severity of HIV infection. In addition, all HIV-positive women were on highly active anti-retroviral treatment, which may have confounded both HGF and EGF levels. Lastly, the heterogeneity of our study population, which was not stratified into early and late onset PE, could be seen as a limitation.

Conclusion

This study demonstrates a significant increase of EGF in the oxidative stressed micro-environment of PE compared to normotensive women, regardless of HIV status. In addition, a significant up-regulation of HGF and EGF was found in HIV infected women, irrespective of pregnancy type. Off-note, the HIV accessory protein affects angiogenesis, cell signalling and trophoblast cell migration. Furthermore, HAART may be implicated in the elevation of EGF and HGF in HIV infection. It is thus plausible that EGF may have possible predictor indicator test value for the early detection of PE.

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References

1. United Nations International Children's Emergency Fund. UNICEF: Eastern and Southern Africa Regional Office Annual Report 2017. Nairobi: UNICEF; 2018;90-50. (United, 2018)

2. Statistics South Africa. Mid-year population estimates 2018. Pretoria: Stats SA; 2018;1-23.

3. Kalumba V, Moodley J, Naidoo T. Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study: cardiovascular topics. *Cardiovasc J Afr*. 2013;24:24-27.

4. Department of Health. Saving mothers 2014–2016: Seventh triennial report on confidential enquiries into maternal deaths in South Africa: Short report. Republic of South Africa: National Department of Health; 2017;1-134.

5. Maynard SE, Karumanchi SA. Angiogenic factors and preeclampsia. *Semin Nephrol.* 2011;31:33-46.

6. Suy A, Martínez E, Coll O, et al. Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. *AIDS.* 2006;20:59-66.

7. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys.* 2004;59:21-26.

8. Cooper CS, Park M, Blair DG, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature.* 1984;311:29.

9. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov.* 2008;7:504.

10. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol.* 2010;11:834.

11. Kauma SW, Bae-Jump V, Walsh SW. Hepatocyte growth factor stimulates trophoblast invasion: a potential mechanism for abnormal placentation in preeclampsia. *J Clin Endocrinol Metab.* 1999;84:4092-4096.

12. Boissinot M, Vilaine M, Hermouet S. The Hepatocyte Growth Factor (HGF)/met axis: a neglected target in the treatment of chronic myeloproliferative neoplasms? *Cancers.* 2014;6:1631-1669.

13. Natarajan A, Wagner B, Sibilia M. The EGF receptor is required for efficient liver regeneration. *Proc Natl Acad Sci U S A.* 2007;104:17081-17086.

14. Takeda Y, Iwashita M. Role of growth factors on fetal growth and maturation. *Ann Acad Med Singapore.* 1993;22:134-141.

15. Harari PM, Huang SM. Radiation response modification following molecular inhibition of epidermal growth factor receptor signaling. *Semin Radiat Oncol.* 2001;11:281-289.

16. Clark D, Salvig J, Smith S, Charnock-Jones D. Hepatocyte growth factor levels during normal and intra-uterine growth-restricted pregnancies. *Placenta.* 1998;19:671-673.

17. Charkiewicz K, Jasinska E, Goscik J, et al. Angiogenic factor screening in women with mild preeclampsia–New and significant proteins in plasma. *Cytokine*. 2018;106:125-130.

18. Martinez-Fierro ML, Castruita-De La Rosa C, Garza-Veloz I, et al. Early pregnancy protein multiplex screening reflects circulating and urinary divergences associated with the development of preeclampsia. *Hypertens Pregnancy*. 2018;37:37-50.

19. Naghshvar F, Torabizadeh Z, Moslemi Zadeh N, Mirbaha H, Gheshlaghi P. Investigating the Relationship between Serum Level of s-Met (Soluble Hepatic Growth

Factor Receptor) and Preeclampsia in the First and Second Trimesters of Pregnancy. *ISRN Obstet Gynecol.* 2013;2013:1-5.

20. Ma P, Tretiakova M, Nallasura V, Jagadeeswaran R, Husain A, Salgia R. Downstream signalling and specific inhibition of c-Met/HGF pathway in small cell lung cancer: implications for tumour invasion. *Br J Cancer.* 2007;97:368.

21. Zeng X, Sun Y, Yang HX, et al. Plasma level of soluble c-Met is tightly associated with the clinical risk of preeclampsia. *Am J Obstet Gynecol.* 2009;201:618.e1-e7.

22. Chen CP. Placental villous mesenchymal cells trigger trophoblast invasion. *Cell Adh Migr.* 2014;8:94-97.

23. Bos JL, de Rooij J, Reedquist KA. Rap1 signalling: adhering to new models. *Nat Rev Mol Cell Biol.* 2001;2:369.

24. 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.
25. Tulasne D, Deheuninck J, Lourenco FC, et al. Proapoptotic function of the MET tyrosine kinase receptor through caspase cleavage. *Mol Cell Biol.* 2004;24:10328-10339.

26. Ivanov AV, Valuev-Elliston VT, Ivanova ON, et al. Oxidative stress during HIV infection: mechanisms and consequences. *Oxid Med Cell Longev.* 2016;2016:1-18.

27. Romani B, Engelbrecht S, Glashoff RH. Functions of Tat: the versatile protein of human immunodeficiency virus type 1. *J Gen Virol.* 2010;91:1-12.

28. Poggi A, Zocchi MR. HIV-1 Tat triggers TGF-β production and NK cell apoptosis that is prevented by pertussis toxin B. *J Immunol Res.* 2006;13:369-372.

29. Wiercińska-Drapalo A, Flisiak R, Jaroszewicz J, Prokopowicz D. Increased plasma transforming growth factor-β 1 is associated with disease progression in HIV-1-infected patients. *Viral Immunol.* 2004;17:109-113.

30. Theron AJ, Anderson R, Rossouw TM, Steel HC. The Role of Transforming Growth Factor Beta-1 in the Progression of HIV/AIDS and Development of Non-AIDS-Defining Fibrotic Disorders. *Front Immunol.* 2017;8:1461.

31. Soeters PB, Grimble RF. Dangers, and benefits of the cytokine mediated response to injury and infection. *Clin Nutr*. 2009;28:583-596.

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

33. Ferrandina G, Lanzone A, Scambia G, Caruso A, Panici PB, Mancuso S. Epidermal growth factor receptors in placentae and fetal membranes from hypertension-complicated pregnancies. *Hum Reprod.* 1995;10:1845-1849.

34. Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. *Semin Cell Dev Biol.* 2008;19:414-422.

35. Lin JJ, Riely GJ, Shaw AT. Targeting ALK: precision medicine takes on drug resistance. *Cancer Discov.* 2017;7:1-19

36. Burgel P, Nadel J. Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. *Eur Respir J*. 2008;32:1068-1081.

37. Albini A, Soldi R, Giunciuclio D, et al. The angiogenesis induced by HIV–1 Tat protein is mediated by the Flk–1/KDR receptor on vascular endothelial cells. *Nat Med* 1996;2:1371.

38. Fields J, Dumaop W, Langford T, Rockenstein E, Masliah E. Role of neurotrophic factor alterations in the neurodegenerative process in HIV associated neurocognitive disorders. *J Neuroimmune Pharmacol.* 2014;9:102-116.

39. Gómez-Gaviro MV, Scott CE, Sesay AK, et al. Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. *Proc Natl Acad Sci U S A.* 2012;109:1317-1322.

40. Zhang Y, Jain RK, Zhu M. Recent progress and advances in HGF/MET-targeted therapeutic agents for cancer treatment. *Biomedicines*. 2015;3:149-181.

41. Zeng Q, Chen S, You Z, et al. Hepatocyte growth factor inhibits anoikis in head and neck squamous cell carcinoma cells by activation of ERK and Akt signaling independent of NFκB. *J Biol Chem*. 2002;277:25203-25208.

42. Sabra H, Brunner M, Mandati V, et al. β1 integrin dependent Rac/group I PAK signaling mediates YAP activation of Yes associated protein 1 (YAP1) via NF2/merlin. *J Biol Chem*. 2017;292:19179-197.

CHAPTER 3

3.0. SYNTHESIS

The main cause of maternal deaths worldwide are hemorrhage, PE and infections due to poor medical infrastructure (Pasha *et al.*, 2018). Throughout the world PE complicates approximately 7% to 10% of pregnancies (Kalumba *et al.*, 2013). The repercussion of this leads to an unwanted escalation of mortality and morbidity rates globally. In Kwa-Zulu-Natal, SA the incident rate for PE is 12% (Department of Health, 2017).

South Africa also has the highest HIV prevalence in the world with 7.1 million infected individuals (Statistic South Africa, 2018). This immunosuppressant disorder is found in 30% of pregnant women in SA, such a condition may complicate a pregnancy in various ways namely PE (Moodley, 2013). The compromised immune system in HIV infection would neutralize the exaggerated immune response by PE.

Preeclampsia is categorized by having an elevated blood pressure in conjunction with proteinuria occurring within the second trimester and onwards (Brown *et al.*, 2001). The exact pathophysiology of PE requires clarity. In contrast to a normal pregnancy, the placentation process is defective in PE as cytotrophoblast cells fail to invade the endometrial wall (Romero and Chaiworapongsa, 2013). As a result of a limited decidual spiral artery remodeling blood flow to the fetus is restricted (Brosens *et al.*, 2011). Early diagnostic tests for PE development do not exist at this moment nevertheless, there have been promising biomarkers used to indicate PE such as Insulin-like Growth Factor, EGF, HGF, VEGF, TGF-β, *etc*. (Gack *et al.*, 2005). However, to date no studies have reported HGF and EGF expression in HIV associated PE.

In this study HGF and EGF were investigated due to their cell function in apoptosis, invasion, adhesion, cell proliferation, survival, motility, differentiation and morphogenesis (Boon *et al.*, 2002; Zhang *et al.*, 2015). During placentation, EGF facilitates implantation of the blastocyst and together with cytokines maintain placental function in normal pregnancy (Malhotra *et al.*, 2016).

Hepatocyte Growth Factor is also responsible for maintaining normal blood pressure *via* a chain reaction effect of TGF-β, a well-known causative component of hypertension in the renin-angiotensin-aldosterone system (RAAS) (Matsuki *et al.*, 2014). High, consistent levels of TGF-β suppress HGF production (Choi *et al.*, 2018; Morishita *et al.*, 1999b). In pregnancy, during fetal development HGF is required for differentiation of cells into tissue which is important for fetal organ development (Mizuno *et al.*, 2008). In light of the high prevalence of HIV infection and PE in SA and the role of HGF and

EGF in the cell cycle, this study evaluates serum HGF and EGF in HIV infected preeclamptic women using the Multiplex Immunoassay method (Nakamura and Mizuno, 2010).

3.1. Pregnancy type

The results of this study demonstrated no difference of HGF levels in preeclamptic compared to normotensive pregnant women, irrespective of their HIV status. This finding is in line with (Horibe *et al.*, 1995). In contrast, a later study by Charkiewicz *et al.* (2018) reported an upregulation of plasma HGF in preeclamptic *vs* normotensive women. Furthermore, Naghshvar *et al.* (2013) found that the levels of c-Met was lower in PE compared to normal pregnancies and varied with the severity of the disorder, implicating fewer receptor-ligand binding. (Naghshvar *et al.*, 2013).

In contrast to HGF, a significant up-regulation of EGF expression was observed in preeclamptic compared to normotensive pregnant women irrespective of HIV infection. However, opposing results were noted by Armant *et al.* (2015) who found a two-fold elevation of EGF in normotensive compared to PE. Epidermal Growth Factor activates the JAK STAT-3 and MEK ERK1/2 pathways and promotes trophoblast cell invasion, migration, proliferation and cell growth; vital steps that ensure a successful pregnancy (Bass *et al.*, 1994; Gupta *et al.*, 2017). Furthermore, a down-regulation of both HGF and EGF promotes trophoblast apoptosis *via* the PI3K/BAD pathway (Humphrey *et al.*, 2008). Epidermal Growth Factor and HGF down-regulation will inhibit cell adhesion, invasion and proliferation, processes essential for normal placentation (Mattila *et al.*, 2005; Mizuno *et al.*, 2008).

3.2. HIV status

In this study, HGF was enhanced in HIV-positive women, irrespective of pregnancy type. Notably, the HIV-1 accessory protein, trans-activator of transcription (Tat) favors the production of TGF-β which suppresses HGF production (Choi *et al.*, 2018; Morishita *et al.*, 1999b; Xu *et al.*, 2017). Similarly, EGF was up-regulated by HIV status regardless of pregnancy type. It is a standard of practice in SA to administer HAART to all HIV infected pregnant women. HAART promotes immune reconstitution (Wimalasundera *et al*., 2002). Nonetheless, HAART also promotes HGF production ensuring hepatocyte survival in cancer (Sonderup and Wainwright, 2017). This suggests that HAART will favour HGF and EGF production (Theron *et al.*, 2017).

3.3. Hepatocyte Growth Factor - Across the groups

Normotensive HIV-positive vs normotensive HIV-negative- Across the groups there was a significant up-regulation of HGF in normotensive HIV-positive women in comparison to normotensive HIV-negative women. The enhanced HGF level in HIV-positive women is attributed to HAART. Previous literature suggests that HIV is a stress stimulant (Chaturvedi *et al.*, 2018) and oxidative stress up-regulates HGF (Organ and Tsao, 2011; Tulasne *et al.*, 2004). The increase in HGF promotes cell survival *via* the PI3K/BAD pathway (Ozaki *et al.*, 2003).

3.4. Epidermal Growth Factor - Across the groups

Normotensive HIV-positive vs normotensive HIV-negative- We report a significant increase of EGF across the groups, in normotensive HIV-positive women compared to normotensive HIV-negative women. In HIV infected women it is found that HIV is the main cause for chronic oxidative stress. Similar to the above interrogation, stress leads to increased HIV viral replication, increased apoptosis and deteriorating immune functions (Pace and Leaf, 1995; Tang and Smit, 2000). In response to cellular stresses the JAK STAT-3 and MEK ERK1/2 pathways are activated by EGF to counteract the effect of oxidative stress (Tong *et al.*, 2014).

Preeclamptic HIV-negative vs normotensive HIV-negative- There was also a statistical upregulation of EGF in preeclamptic HIV-negative women in contrast to normotensive HIV-negative women. Preeclampsia is a result of placental hypoxia (Verma *et al.*, 2018). The presence of continuous hypoxia leads to oxidative stress because of the imbalance caused between reactive oxygen species and antioxidants (Schoots *et al.*, 2018). A study by Kothari *et al.* (2003) showed that cell death induced by hypoxia could be avoided by administration of EGF. Hence in PE, EGF production is increased due to the oxidative stress caused by the hypoxic micro-environment.

Preeclamptic HIV-positive vs normotensive HIV-negative- Furthermore, a noteworthy rise in EGF was found in preeclamptic HIV-positive women compared to normotensive HIV-negative women. The elevation of EGF may be attributed to the oxidative stress occurring both in PE and in HIV infection. HAART endorses EGF up-regulation (Theron *et al.*, 2017). Nevertheless, a patient who presents with oxidative stress becomes stress-responsive (Matsuda *et al.*, 1998) by producing EGF to promote the PI3k/AKT, JAK STAT-3 and MEK ERK1/2 pathways to protect against stress induced cell death (Trachootham *et al.*, 2008; Wang *et al.*, 2000).

3.5. Limitations

A limitation of this study is that all HIV infected women received HAART. In addition, the duration of HAART is unavailable. The lack of stratification of our groups by gestational age may have influenced analyte expression.

3.6. Conclusion

This novel study demonstrates a significant increase in the expression of HGF and EGF in HIV infection regardless of pregnancy type, reflecting an immune reconstitution following HAART. This finding may be attributed to the HIV accessory protein Tat that inhibits growth factor function thereby, negatively impacting cell migration. Hepatocyte Growth Factor was unexpectedly found to be similar between pregnancy types; it may be plausible that both HAART and TGF-β synergistically work together to neutralize HGF production. The up-regulation of EGF expression in preeclampsia may be responsible for impaired trophoblast cell invasion mediated by dysregulation of cell signalling pathways. As expected in HIV associated PE, EGF expression declined by HIV status and pregnancy type. Epidermal Growth Factor expression has potential use as a diagnostic risk indicator for preeclampsia development prior to the onset of maternal signs and symptoms.

Future direction requires studies with a larger sample size, sub-grouping by gestational age across the trimesters of pregnancies.

CHAPTER 4

4.0. REFERENCES

Albini, A., Soldi, R., Giunciuclio, D., Giraudo, E., Benelli, R., Primo, L., Noonan, D., Salio, M., Camussi, G. and Rock, W. (1996). The angiogenesis induced by HIV–1 Tat protein is mediated by the Flk–1/KDR receptor on vascular endothelial cells. *Nat Med* **2**(12): 1371.

Allotey, J., Snell, K. I., Chan, C., Hooper, R., Dodds, J., Rogozinska, E., Khan, K. S., Poston, L., Kenny, L. and Myers, J. (2017). External validation, update and development of prediction models for pre-eclampsia using an Individual Participant Data (IPD) meta-analysis: the International Prediction of Pregnancy Complication Network (IPPIC pre-eclampsia) protocol. *Diagn Progn Res* **1**(1): 16.

Armant, D. R., Fritz, R., Kilburn, B. A., Kim, Y. M., Nien, J. K., Maihle, N. J., Romero, R. and Leach, R. E. (2015). Reduced expression of the epidermal growth factor signaling system in preeclampsia. *Placenta* **36**(3): 270-278.

Backes, C. H., Markham, K., Moorehead, P., Cordero, L., Nankervis, C. A. and Giannone, P. J. (2011). Maternal preeclampsia and neonatal outcomes. *J Pregnancy* **2011**(1): 1-7.

Bass, K. E., Morrish, D., Roth, I., Bhardwaj, D., Taylor, R., Zhou, Y. and Fisher, S. J. (1994). Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. *Dev Biol* **164**(2): 550-561.

Bergmann, A. (2002). Survival signaling goes BAD. *Dev cell* **3**(5): 607-608.

Bershteyn, A., Akullian, A. N., Klein, D. J., Jewell, B. L., Vandormael, A., Cuadros, D. F., Tanser, F. and Welkhoff, P. A. (2018). *Hotspots by Random Chance: Small Community Size and Isolation Can Explain "Patchiness" in HIV Epidemics.* [Poster] Exhibited at the 22nd International AIDS Conference, 23-27 July 2018, Amsterdam, Netherlands.

Boissinot, M., Vilaine, M. and Hermouet, S. (2014). The Hepatocyte Growth Factor (HGF)/met axis: a neglected target in the treatment of chronic myeloproliferative neoplasms? *Cancers* **6**(3): 1631-1669.

Boon, E. M., van der Neut, R., van de Wetering, M., Clevers, H. and Pals, S. T. (2002). Wnt signaling regulates expression of the receptor tyrosine kinase met in colorectal cancer. *Cancer res* **62**(18): 5126-5128.

Bos, J. L., de Rooij, J. and Reedquist, K. A. (2001). Rap1 signalling: adhering to new models. *Nat Rev Mol Cell Biol* **2**(5): 369.

Brosens, I., Pijnenborg, R., Vercruysse, L. and Romero, R. (2011). The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol* **204**(3): 193-201.

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

Brown, M. A., Lindheimer, M. D., de Swiet, M., Assche, A. V. and Moutquin, J. M. (2001). The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* **20**(1): 9-14.

Burgel, P. and Nadel, J. (2008). Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. *Eur Respir J* **32**(4): 1068-1081.

Calvert, C. and Ronsmans, C. (2013). The contribution of HIV to pregnancy-related mortality: a systematic review and meta-analysis. *Aids* **27**(10): 1631.

Cele, S., Odun-Ayo, F., Onyangunga, O., Moodley, J. and Naicker, T. (2018). Analysis of hepatocyte growth factor immunostaining in the placenta of HIV-infected normotensive versus preeclamptic pregnant women. *Eur J Obstet Gynecol Reprod Biol* **227**(1): 60-66.

Cerdeira, A. S. and Karumanchi, S. A. (2012). Angiogenic factors in preeclampsia and related disorders. *Cold Spring Harb Perspect Med* **2**(11): 1-17.

Charkiewicz, K., Jasinska, E., Goscik, J., Koc-Zorawska, E., Zorawski, M., Kuc, P., Raba, G., Kluz, T., Kalinka, J. and Sakowicz, A. (2018). Angiogenic factor screening in women with mild preeclampsia–New and significant proteins in plasma. *Cytokine* **106**(1): 125-130.

Chaturvedi, S., Malik, M. Y., Azmi, L., Shukla, I., Naseem, Z., Rao, C. and Agarwal, N. K. (2018). Formononetin and biochanin A protects against ritonavir induced hepatotoxicity via modulation of NfκB/pAkt signaling molecules. *Life Sci* **213**(1): 147-182.

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

Chen, C. P. (2014). Placental villous mesenchymal cells trigger trophoblast invasion. *Cell Adh Migr* **8**(2): 94-97.

Chen, K. G. and Sikic, B. I. (2012). Molecular pathways: regulation and therapeutic implications of multidrug resistance. *Clin Cancer Res* **18**(7): 1863-1869.

Choi, W., Lee, J., Lee, J., Ko, K. R. and Kim, S. (2018). Hepatocyte Growth Factor Regulates the miR-206-HDAC4 Cascade to Control Neurogenic Muscle Atrophy following Surgical Denervation in Mice. *Mol Ther Nucleic Acids* **12**(1): 568-577.

Clark, D., Salvig, J., Smith, S. and Charnock-Jones, D. (1998). Hepatocyte growth factor levels during normal and intra-uterine growth-restricted pregnancies. *Placenta* **19**(8): 671-673.

Comoglio, P. M., Giordano, S. and Trusolino, L. (2008). Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov* **7**(6): 504.

Cooper, C. S., Park, M., Blair, D. G., Tainsky, M. A., Huebner, K., Croce, C. M. and Woude, G. F. V. (1984). Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature* **311**(5981): 29.

Croker, B. A., Kiu, H. and Nicholson, S. E. (2008). SOCS regulation of the JAK/STAT signalling pathway. *Semin Cell Dev Biol* **19**(4): 414-422.

Department of Health (2017). *Saving mothers 2014–2016: Seventh triennial report on confidential enquiries into maternal deaths in South Africa: Short report.* [Online] Republic of South Africa: National Department of Health, 1-134. Available at: https://www.sasog.co.za/Content/Docs/Saving_Mothers.pdf

Ferrandina, G., Lanzone, A., Scambia, G., Caruso, A., Panici, P. B. and Mancuso, S. (1995). Epidermal growth factor receptors in placentae and fetal membranes from hypertension-complicated pregnancies. *Hum Reprod* **10**(7): 1845-1849.

Fields, J., Dumaop, W., Langford, T., Rockenstein, E. and Masliah, E. (2014). Role of neurotrophic factor alterations in the neurodegenerative process in HIV associated neurocognitive disorders. *J Neuroimmune Pharmacol* **9**(2): 102-116.

Fittipaldi, A. and Giacca, M. (2005). Transcellular protein transduction using the Tat protein of HIV-1. *Adv Drug Deliv Rev* **57**(4): 597-608.

Gack, S., Marmé, A., Marmé, F., Wrobel, G., Vonderstraß, B., Bastert, G., Lichter, P., Angel, P. and Schorpp-Kistner, M. (2005). Preeclampsia: increased expression of soluble ADAM 12. *J Mol Med* **83**(11): 887-896.

Gathiram, P. and Moodley, J. (2016). Pre-eclampsia: its pathogenesis and pathophysiolgy: review articles. *Cardiovasc J Afr* **27**(2): 71-78.

Gómez-Gaviro, M. V., Scott, C. E., Sesay, A. K., Matheu, A., Booth, S., Galichet, C. and Lovell-Badge, R. (2012). Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. *Proc Natl Acad Sci U S A* **109**(4): 1317-1322.

Govender, N., Naicker, T. and Moodley, J. (2013). Maternal imbalance between pro-angiogenic and anti-angiogenic factors in HIV-infected women with pre-eclampsia: cardiovascular topics. *Cardiovasc J Afr* **24**(5): 174-179.

Govender, N., Naicker, T. and Moodley, J. (2015). Endoglin in HIV-associated preeclamptic placentae. *Hypertens Pregnancy* **34**(3): 342-354.

Gupta, S. K., Pal, R. and Malik, A. (2017). Interdependence of JAK-STAT and MAPK signaling pathways during EGF-mediated HTR-8/SVneo cell invasion. *PLoS One* **12**(5): 1-18.

Hall, D., Gebhardt, S., Theron, G. and Grové, D. (2014). Pre-eclampsia and gestational hypertension are less common in HIV infected women. *Pregnancy Hypertens* **4**(1): 91-96.

Hallberg, B. and Palmer, R. H. (2013). Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. *Nat Rev Cancer* **13**(10): 685-700.

Harari, P. M. and Huang, S. M. (2001). Radiation response modification following molecular inhibition of epidermal growth factor receptor signaling. *Semin Radiat Oncol* **11**(4): 281-289.

Henson, E. S. and Gibson, S. B. (2006). Surviving cell death through epidermal growth factor (EGF) signal transduction pathways: implications for cancer therapy. *Cell Signal* **18**(12): 2089-2097.

Herbst, R. S. (2004). Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* **59**(2): S21-S26.

Horibe, N., Okamoto, T., Itakura, A., Nakanishi, T., Suzuki, T., Kazeto, S. and Tomoda, Y. (1995). Levels of hepatocyte growth factor in maternal serum and amniotic fluid. *Am J Obstet Gynecol* **173**(3): 937-942.

Humphrey, R. G., Sonnenberg-Hirche, C., Smith, S. D., Hu, C., Barton, A., Sadovsky, Y. and Nelson, D. M. (2008). Epidermal growth factor abrogates hypoxia-induced apoptosis in cultured human trophoblasts through phosphorylation of BAD Serine 112. *Endocrinology* **149**(5): 2131-2137.

Huppertz, B. (2008). Placental origins of preeclampsia: challenging the current hypothesis. *Hypertension* **51**(4): 970-975.

Imudia, A. N., Kilburn, B. A., Petkova, A., Edwin, S. S., Romero, R. and Armant, D. R. (2008). Expression of heparin-binding EGF-like growth factor in term chorionic villous explants and its role in trophoblast survival. *Placenta* **29**(9): 784-789.

Ivanov, A. V., Valuev-Elliston, V. T., Ivanova, O. N., Kochetkov, S. N., Starodubova, E. S., Bartosch, B. and Isaguliants, M. G. (2016). Oxidative stress during HIV infection: mechanisms and consequences. *Oxid Med Cell Longev* **2016**(1): 1-16.

Joint United Nations Programme on HIV/AIDS (2018). *UNAIDS DATA 2018.* [Online] Switzerland: UNAIDS, 1-370. Available at: http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf

Kalumba, V., Moodley, J. and Naidoo, T. (2013). Is the prevalence of pre-eclampsia affected by HIV/AIDS? A retrospective case-control study: cardiovascular topics. *Cardiovasc J Afr* **24**(2): 24-27.

Kasselberg, A., Orth, D. N., Gray, M. E. and Stahlman, M. T. (1985). Immunocytochemical localization of human epidermal growth factor/urogastrone in several human tissues. *J Histochem Cytochem* **33**(4): 315-322.

Kauma, S. W., Bae-Jump, V. and Walsh, S. W. (1999). Hepatocyte growth factor stimulates trophoblast invasion: a potential mechanism for abnormal placentation in preeclampsia. *J Clin Endocrinol Metab* **84**(11): 4092-4096.

Kelly, E., Newell, S., Brownlee, K., Farmery, S., Cullinane, C., Reid, W., Jackson, P., Gray, S., Primrose, J. and Lagopoulos, M. (1997). Role of epidermal growth factor and transforming growth factor α in the developing stomach: These data were presented at the spring meeting of the British Society of Gastroenterology 1995, and at the annual meeting of the American Gastroenterological Association, 1995. *Arch Dis Child Fetal Neonatal Ed* **76**(3): F158-F162.

Klingensmith, N. J., Yoseph, B. P., Liang, Z., Lyons, J. D., Burd, E. M., Margoles, L. M., Koval, M., Ford, M. L. and Coopersmith, C. M. (2017). Epidermal growth factor improves intestinal

integrity and survival in murine sepsis following chronic alcohol ingestion. *Shock* **47**(2): 184-192.

Kothari, S., Cizeau, J., McMillan-Ward, E., Israels, S. J., Bailes, M., Ens, K., Kirshenbaum, L. A. and Gibson, S. B. (2003). BNIP3 plays a role in hypoxic cell death in human epithelial cells that is inhibited by growth factors EGF and IGF. *Oncogene* **22**(30): 4734.

Kovo, M., Schreiber, L., Ben-Haroush, A., Gold, E., Golan, A. and Bar, J. (2012). The placental component in early-onset and late-onset preeclampsia in relation to fetal growth restriction. *Prenat Diagn* **32**(7): 632-637.

Lala, P. and Chakraborty, C. (2003). Factors Regulating Trophoblast Migration and Invasiveness: Possible Derangements Contributing to Pre-eclampsia and Fetal Injury1. *Placenta* **24**(6): 575-587.

Lam, C., Lim, K. H. and Karumanchi, S. A. (2005). Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. *Hypertension* **46**(5): 1077-1085.

Leach, R. E., Khalifa, R., Ramirez, N. D., Das, S. K., Wang, J., Dey, S. K., Romero, R. and Armant, D. R. (1999). Multiple roles for heparin-binding epidermal growth factor-like growth factor are suggested by its cell-specific expression during the human endometrial cycle and early placentation. *J Clin Endocrinol Metab* **84**(9): 3355-3363.

Leach, R. E., Romero, R., Kim, Y. M., Chaiworapongsa, T., Kilburn, B., Das, S. K., Dey, S. K., Johnson, A., Qureshi, F. and Jacques, S. (2002). Pre-eclampsia and expression of heparin-binding EGF-like growth factor. *Lancet* **360**(9341): 1215-1219.

Lessey, B. A., Gui, Y., Apparao, K., Young, S. L. and Mulholland, J. (2002). Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium: A potential paracrine role during implantation. *Mol Reprod Dev* **62**(4): 446-455.

Li, C. F., Ma, Y., Wei, Y. Z. and Xue, Y. W. (2009). Relationship between VEGF, EGF and invasion, metastasis of gastric cancer cells. *Chin J Cancer Res* **21**(2): 122-129.

Licona-Limon, P. and Soldevila, G. (2007). The role of TGF-β superfamily during T cell development: new insights. *Immunol Lett* **109**(1): 1-12.

Limaye, P. B., Bowen, W. C., Orr, A. V., Luo, J., Tseng, G. C. and Michalopoulos, G. K. (2008). Mechanisms of hepatocyte growth factor–mediated and epidermal growth factor–mediated

signaling in transdifferentiation of rat hepatocytes to biliary epithelium. *Hepatology* **47**(5): 1702-1713.

Lin, J. J., Riely, G. J. and Shaw, A. T. (2017). Targeting ALK: precision medicine takes on drug resistance. *Cancer Discov* **7**(1): 1-19.

Ma, P., Tretiakova, M., Nallasura, V., Jagadeeswaran, R., Husain, A. and Salgia, R. (2007). Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. *Br J Cancer* **97**(3): 368.

Malhotra, S. S., Banerjee, P. and Gupta, S. K. (2016). Regulation of trophoblast differentiation during embryo implantation and placentation: Implications in pregnancy complications. *J Reprod Health Med* **2**(Supplement 2): S26-S36.

Martinez-Fierro, M. L., Castruita-De La Rosa, C., Garza-Veloz, I., Cardiel-Hernandez, R. M., Espinoza-Juarez, M. A., Delgado-Enciso, I., Castañeda-Lopez, M. E., Cardenas-Vargas, E., Trejo-Vázquez, F. and Sotelo-Ham, E. I. (2018). Early pregnancy protein multiplex screening reflects circulating and urinary divergences associated with the development of preeclampsia. *Hypertens Pregnancy* **37**(1): 37-50.

Matsuda, N., Morita, N., Matsuda, K. and Watanabe, M. (1998). Proliferation and differentiation of human osteoblastic cells associated with differential activation of MAP kinases in response to epidermal growth factor, hypoxia, and mechanical stressin vitro. *Biochem Biophys Res Commun* **249**(2): 350-354.

Matsuki, K., Hathaway, C. K., Lawrence, M. G., Smithies, O. and Kakoki, M. (2014). The role of transforming growth factor β1 in the regulation of blood pressure. *Curr Hypertens Rev* **10**(4): 223-238.

Mattila, E., Pellinen, T., Nevo, J., Vuoriluoto, K., Arjonen, A. and Ivaska, J. (2005). Negative regulation of EGFR signalling through integrin-α 1 β 1-mediated activation of protein tyrosine phosphatase TCPTP. *Nat Cell Biol* **7**(1): 78.

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

Mertens, C. and Darnell, J. E. (2007). Snapshot: Jak-stat signaling. *Cell* **131**(3): 612.

Mizuno, S., Matsumoto, K. and Nakamura, T. (2008). HGF as a renotrophic and anti-fibrotic regulator in chronic renal disease. *Front Biosci* **13**(1): 7072-7086.

Mizuno, S. and Nakamura, T. (2007). Hepatocyte growth factor: a regenerative drug for acute hepatitis and liver cirrhosis. *Regen Med* **2**(2): 161-170.

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

Morishita, R., Moriguchi, A., Higaki, J. and Ogihara, T. (1999a). Hepatocyte growth factor (HGF) as a potential index of severity of hypertension. *Hypertens Res* **22**(3): 161-167.

Morishita, R., Nakamura, S., Hayashi, S. I., Taniyama, Y., Moriguchi, A., Nagano, T., Taiji, M., Noguchi, H., Takeshita, S. and Matsumoto, K. (1999b). Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. *Hypertension* **33**(6): 1379-1384.

Naghshvar, F., Torabizadeh, Z., Moslemi Zadeh, N., Mirbaha, H. and Gheshlaghi, P. (2013). Investigating the Relationship between Serum Level of s-Met (Soluble Hepatic Growth Factor Receptor) and Preeclampsia in the First and Second Trimesters of Pregnancy. *ISRN Obstet Gynecol* **2013**(1): 1-5.

Naicker, T., Dorsamy, E., Ramsuran, D., Burton, G. J. and Moodley, J. (2013). The role of apoptosis on trophoblast cell invasion in the placental bed of normotensive and preeclamptic pregnancies. *Hypertens Pregnancy* **32**(3): 245-256.

Naicker, T., Khedun, S. M., Moodley, J. and Pijnenborg, R. (2003). Quantitative analysis of trophoblast invasion in preeclampsia. *Acta Obstet Gynecol Scand* **82**(8): 722-729.

Nakamura, T. and Mizuno, S. (2010). The discovery of hepatocyte growth factor (HGF) and its significance for cell biology, life sciences and clinical medicine. *Proc Jpn Acad Ser B Phys Biol Sci* **86**(6): 588-610.

Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Aoki, M., Moriguchi, A., Yamada, K., Hayashi, S. I., Yo, Y. and Nakano, H. (1996). Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. *J Hypertens* **14**(9): 1067-1072.

Natarajan, A., Wagner, B. and Sibilia, M. (2007). The EGF receptor is required for efficient liver regeneration. *Proc Natl Acad Sci U S A* **104**(43): 17081-17086.

O'Tierney-Ginn, P. F. and Lash, G. E. (2014). Beyond pregnancy: modulation of trophoblast invasion and its consequences for fetal growth and long-term children's health. *J Reprod Immunol* **104**(1): 37-42.

Organ, S. L. and Tsao, M. S. (2011). An overview of the c-MET signaling pathway. *Ther Adv Med Oncol* **3**(Supplement 1): S7-S19.

Ozaki, M., Haga, S., Zhang, H., Irani, K. and Suzuki, S. (2003). Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated antiapoptotic signaling: role of PI3-K and Akt kinase upon rac1. *Cell Death Differ* **10**(5): 508.

Pace, G. W. and Leaf, C. D. (1995). The role of oxidative stress in HIV disease. *Free Radic Biol Med* **19**(4): 523-528.

Panday, A., Inda, M. E., Bagam, P., Sahoo, M. K., Osorio, D. and Batra, S. (2016). Transcription factor NF-κB: an update on intervention strategies. *Arch Immunol Ther Exp* **64**(6): 463-483.

Parikh, R. A., Wang, P., Beumer, J. H., Chu, E. and Appleman, L. J. (2014). The potential roles of hepatocyte growth factor (HGF)-MET pathway inhibitors in cancer treatment. *Onco Targets Ther* **7**(1): 969-983.

Pasha, O., McClure, E. M., Saleem, S., Tikmani, S. S., Lokangaka, A., Tshefu, A., Bose, C. L., Bauserman, M., Mwenechanya, M. and Chomba, E. (2018). A prospective cause of death classification system for maternal deaths in low and middle-income countries: results from the Global Network Maternal Newborn Health Registry. *BJOG* **125**(9): 1137-1143.

Perkins, J., St John, J. and Ahmed, A. (2002). Modulation of trophoblast cell death by oxygen and EGF. *Mol Med* **8**(12): 847.

Poggi, A. and Zocchi, M. R. (2006). HIV-1 Tat triggers TGF-β production and NK cell apoptosis that is prevented by pertussis toxin B. *J Immunol Res* **13**(2-4): 369-372.

Poulsen, S. S., Nexø, E., Olsen, P. S., Hess, J. and Kirkegaard, P. (1986). Immunohistochemical localization of epidermal growth factor in rat and man. *Histochemistry* **85**(5): 389-394.

Romani, B., Engelbrecht, S. and Glashoff, R. H. (2010). Functions of Tat: the versatile protein of human immunodeficiency virus type 1. *J Gen Virol* **91**(1): 1-12.

Romero, R. and Chaiworapongsa, T. (2013). Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state. *J Clin Invest* **123**(7): 2775-2777.

Sabra, H., Brunner, M., Mandati, V., Haller, B. W., Lallemand, D., Ribba, A. S., Chevalier, G., Guardiola, P., Block, M. R. and Bouvard, D. (2017). β1 integrin dependent Rac/group I PAK signaling mediates YAP activation of Yes associated protein 1 (YAP1) via NF2/merlin. *J Biol Chem* **292**(47): 19179-19197.

Statistic South Africa (2018). *Mid-year population estimates 2018*. [Online] Pretoria: National Department of Health, 1-26. Available at: https://www.statssa.gov.za/publications/P0302/P03022017.pdf

Schoots, M. H., Gordijn, S. J., Scherjon, S. A., van Goor, H. and Hillebrands, J. L. (2018). Oxidative stress in placental pathology. *Placenta* **69**(1): 153-161.

Shah, B. H. and Catt, K. J. (2004). Matrix metalloproteinase-dependent EGF receptor activation in hypertension and left ventricular hypertrophy. *Trends Endocrinol Metab* **15**(6): 241-243.

Soeters, P. B. and Grimble, R. F. (2009). Dangers, and benefits of the cytokine mediated response to injury and infection. *Clin Nutr* **28**(6): 583-596.

Sonderup, M. W. and Wainwright, H. C. (2017). Human immunodeficiency virus infection, antiretroviral therapy, and liver pathology. *Gastroenterol Clin North Am* **46**(2): 327-343.

Staff, A. C., Benton, S. J., von Dadelszen, P., Roberts, J. M., Taylor, R. N., Powers, R. W., Charnock-Jones, D. S. and Redman, C. W. (2013). Redefining preeclampsia using placenta-derived biomarkers. *Hypertension* **61**(5): 932-942.

Suy, A., Martínez, E., Coll, O., Lonca, M., Palacio, M., de Lazzari, E., Larrousse, M., Milinkovic, A., Hernández, S. and Blanco, J. L. (2006). Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy. *Aids* **20**(1): 59-66.

Takeda, Y. and Iwashita, M. (1993). Role of growth factors on fetal growth and maturation. *Ann Acad Med Singapore* **22**(2): 134-141.

Tang, A. M. and Smit, E. (2000). Oxidative stress in HIV-1-infected injection drug users. *J Acquir Immune Defic Syndr* **25**(Supplement 1): S12-S18.

Tejera, E., Bernardes, J. and Rebelo, I. (2012). Preeclampsia: a bioinformatics approach through protein-protein interaction networks analysis. *BMC Syst Biol* **6**(1): 97.

Theron, A. J., Anderson, R., Rossouw, T. M. and Steel, H. C. (2017). The Role of Transforming Growth Factor Beta-1 in the Progression of HIV/AIDS and Development of Non-AIDS-Defining Fibrotic Disorders. *Front Immunol* **8**(1): 1461.

Tong, J., Taylor, P. and Moran, M. F. (2014). Proteomic analysis of the epidermal growth factor receptor (EGFR) interactome and post-translational modifications associated with receptor endocytosis in response to EGF and stress. *Mol Cell Proteomics* **13**(7): 1644-1658.

Trachootham, D., Lu, W., Ogasawara, M. A., Valle, N. R.-D. and Huang, P. (2008). Redox regulation of cell survival. *Antioxid Redox Signal* **10**(8): 1343-1374.

Trusolino, L., Bertotti, A. and Comoglio, P. M. (2010). MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol* **11**(12): 834.

Tsutsumi, O., Kurachi, H. and Oka, T. (1986). A physiological role of epidermal growth factor in male reproductive function. *Science* **233**(4767): 975-977.

Tulasne, D., Deheuninck, J., Lourenco, F. C., Lamballe, F., Ji, Z., Leroy, C., Puchois, E., Moumen, A., Maina, F. and Mehlen, P. (2004). Proapoptotic function of the MET tyrosine kinase receptor through caspase cleavage. *Mol Cell Biol* **24**(23): 10328-10339.

United Nations International Children's Emergency Fund (2018). *UNICEF: Eastern and Southern Africa Regional Office Annual Report 2017*. [Online] Nairobi: UNICEF, 90-50. Available at: https://www.unicef.org/about/annualreport/files/ESARO_AR_2016.pdf.

Verma, S., Pillay, P., Naicker, T., Moodley, J. and Mackraj, I. (2018). Placental hypoxia inducible factor-1α and CHOP immuno-histochemical expression relative to maternal circulatory syncytiotrophoblast micro-vesicles in preeclamptic and normotensive pregnancies. *Eur J Obstet Gynecol Reprod Biol* **220**(1): 18-24.

von Dadelszen, P. and Magee, L. A. (2014). Pre-eclampsia: an update. *Curr Hypertens Rep* **16**(8): 454.

Wang, X., McCullough, K. D., Franke, T. F. and Holbrook, N. J. (2000). Epidermal growth factor receptor-dependent Akt activation by oxidative stress enhances cell survival. *J Biol Chem* **275**(19): 14624-14631.

World Health Organization (2015). *World health statistics 2015*. [Online] Switzerland: WHO, 1-161. Available at: http://apps.who.int/iris/bitstream/handle/10665/170250/9789240694439_eng.pdf

Wiercińska-Drapalo, A., Flisiak, R., Jaroszewicz, J. and Prokopowicz, D. (2004). Increased plasma transforming growth factor-β 1 is associated with disease progression in HIV-1-infected patients. *Viral Immunol* **17**(1): 109-113.

Wimalasena, J., Beams, F. and Caudle, M. R. (1994). Ethanol modulates the hormone secretory responses induced by epidermal growth factor in choriocarcinoma cells. *Alcohol Clin Exp Res* **18**(6): 1448-1455.

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.

Xu, T. B., Li, L., Luo, X. D. and Lin, H. (2017). BMSCs protect against liver injury via suppressing hepatocyte apoptosis and activating TGF-β1/Bax singling pathway. *Biomed Pharmacother* **96**(1): 1395-1402.

Yo, Y., Morishita, R., Yamamoto, K., Tomita, N., Kida, I., Hayashi, S. I., Moriguchi, A., Kato, S. I., Matsumoto, K. and Nakamura, T. (1998). Actions of hepatocyte growth factor as a local modulator in the kidney: potential role in pathogenesis of renal disease. *Kidney Int* **53**(1): 50-58.

Zarnegar, R. and Michalopoulos, G. (1995). Mini-review 1177 The many faces of hepatocyte growth factor: From hepatopoiesis to hematopoiesis. *J cell Biol* **129**(5): 1177-1180.

Zeng, Q., Chen, S., You, Z., Yang, F., Carey, T. E., Saims, D. and Wang, C. Y. (2002). Hepatocyte growth factor inhibits anoikis in head and neck squamous cell carcinoma cells by activation of ERK and Akt signaling independent of NFκB. *J Biol Chem* **277**(28): 25203-25208.

Zeng, X., Sun, Y., Yang, H. X., Li, D., Li, Y. X., Liao, Q. P. and Wang, Y. L. (2009). Plasma level of soluble c-Met is tightly associated with the clinical risk of preeclampsia. *Am J Obstet Gynecol* **201**(6): 618. e611-618. e617.

Zhang, Y., Jain, R. K. and Zhu, M. (2015). Recent progress and advances in HGF/MET-targeted therapeutic agents for cancer treatment. *Biomedicines* **3**(1): 149-181.

APPENDIX

Appendix 1 – Ethics Approval

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17 April 2018

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

Dear Prof Naicker

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

We wish to advise you that your response received on 03 April 2018 to BREC letter dated 16 March 2018 has been noted by a sub-committee of the Biomedical Research Ethics Committee.

Your request received on 07 March 2018 to append the studies below to the above study has now been approved by a sub-committee of the Biomedical Research Ethics Committee

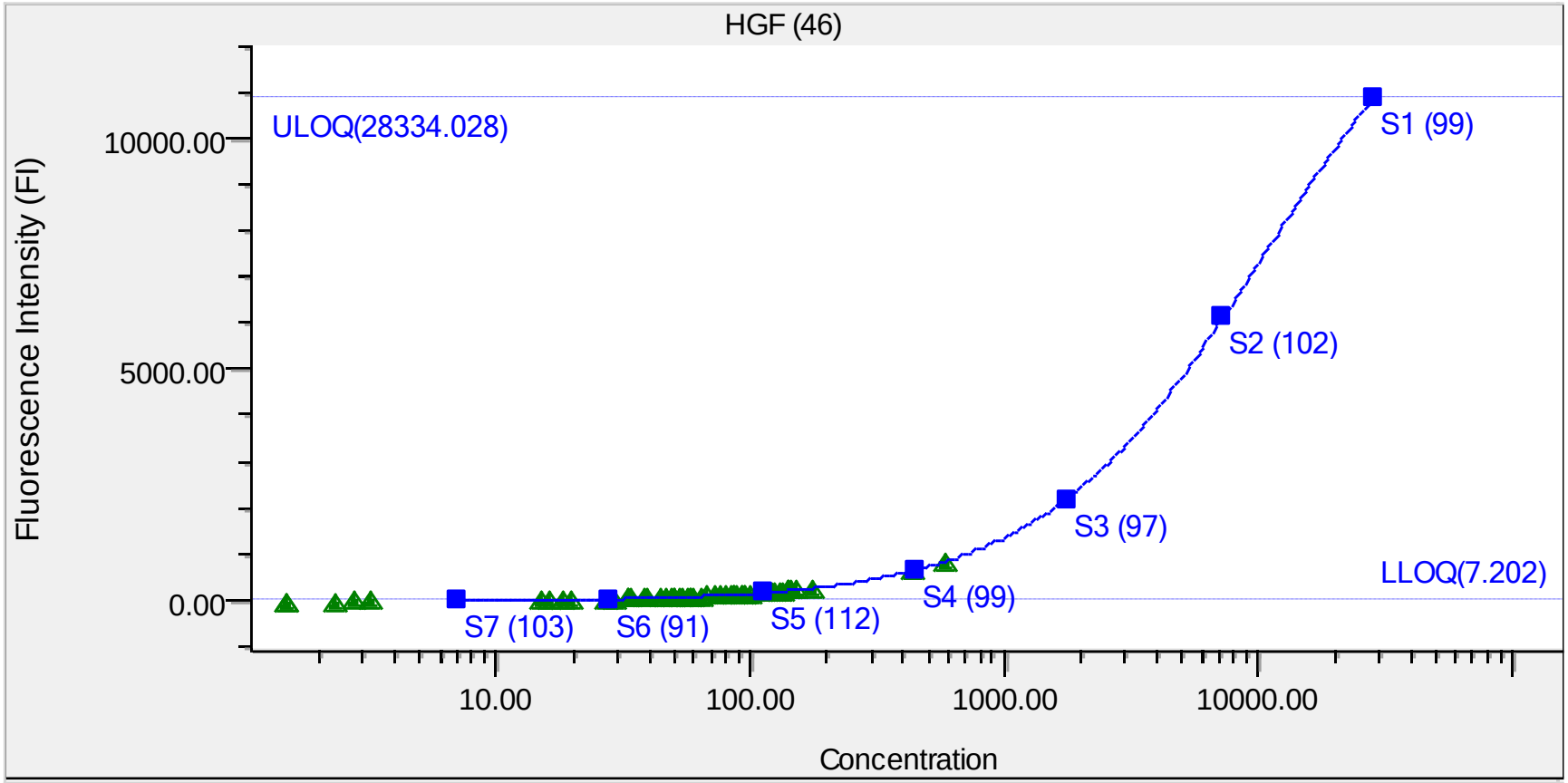
Name	Student number	Title
Deneshree Varaden	211510564	Morphometric image analysis of placental clec2d and HLA-G immunolocalization in HIV-associated pre-eclampsia
Merantha Moodley	214514757	The role of histone 2A in NETosis of HIV-associated pre-eclampsia
Kyle Kupsamy	214504430	EGF and HGF in HIV-associated pre-eclampsia
Mikaila Moodley	214558958	Immunoglobulin isotypes (IgG1, IgG2, IgG3, IgG4, IgM, IgA) in HIV-associated pre-eclampsia
Charlene Mukasa Sangany	211559876	The role of acidic and basic fibroblast growth factor in HIV-associated pre-eclampsia
Mduduzi Mazibuko	214504614	The role of sTIE2 and sHER2 in HIV-associated pre-eclampsia
Siphesihle Mdlalose	217011521	The role of follistatin and G-CSF in HIV-associated pre-eclampsia

This approval will be ratified at the next BREC meeting to be held on 08 May 2018.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix 2 – Standard curve HGF

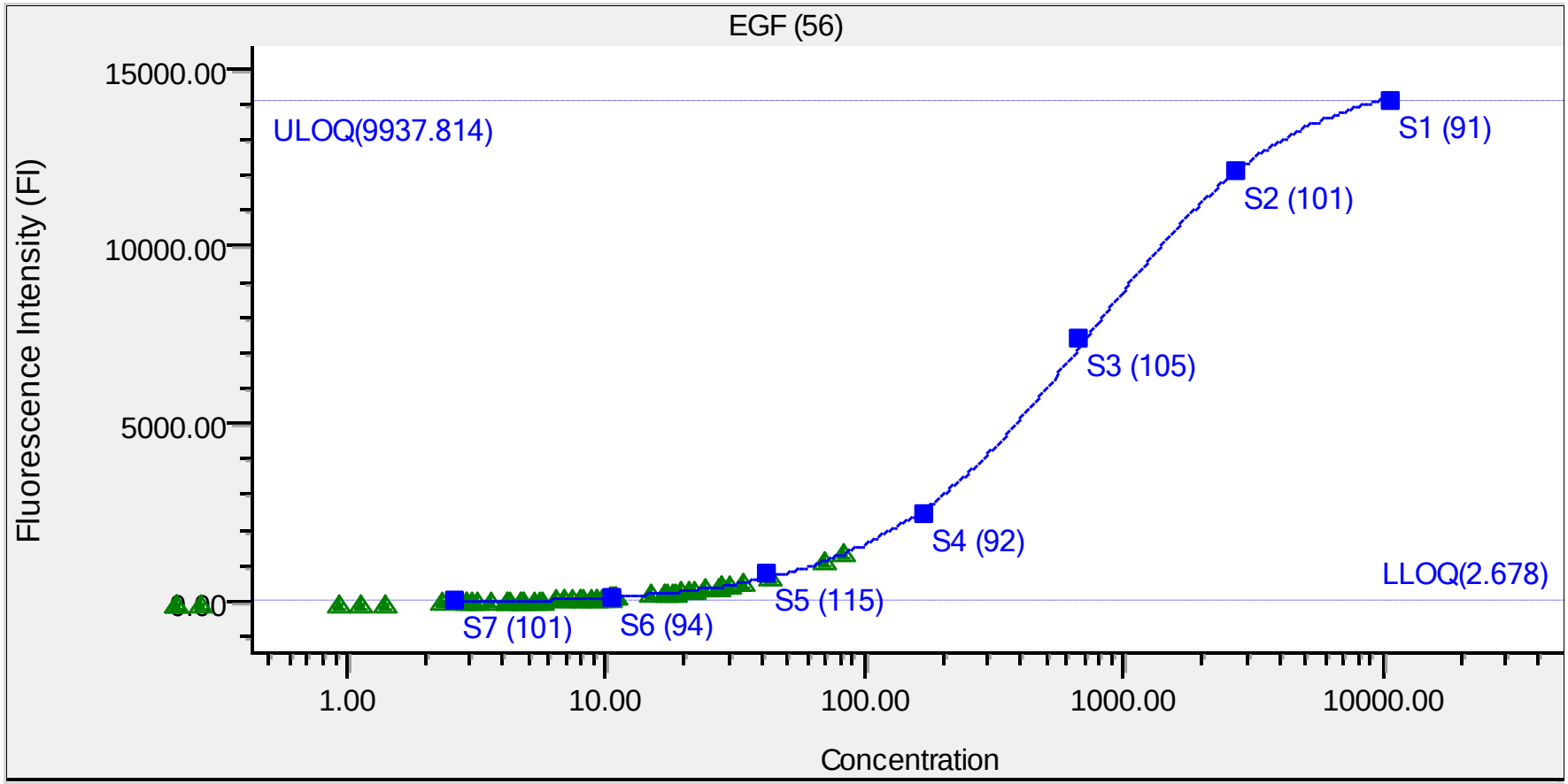


Regression Type: Logistic - 5PL

Std. Curve: FI = -4.91983 + (14469.2 + 4.91983) / ((1 + (Conc / 12445.5)^-1.10538))^0.822956

FitProb. = 0.0984, ResVar. = 2.3189

Appendix 3 – Standard curve EGF



■ Standard	□ Partial Outlier	☒ Outlier
▲ Unknown	▲ Control	

Regression Type: Logistic - 5PL

Std. Curve: FI = -4.49858 + (14899.6 + 4.49858) / ((1 + (Conc / 840.719)^-1.1409))^0.87148

FitProb. = 0.0129, ResVar. = 4.3518

END