Epidemiological Evaluation of Circulating Levels of Copeptin and Fibronectin During Pregnancy

Submitted in fulfillment of the requirements for the degree of Master in Health Sciences in the Faculty of Health Sciences at the Durban University of Technology

by

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APPROVAL

As the candidate's supervisory team, we hereby approve this dissertation for final submission.

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DECLARATION

I Kayshia Deepnarain hereby declare that this dissertation in its entirety is purely my own, original work except where acknowledged. All resources used from the work of others have been referenced in the text. This dissertation has not been submitted in any other form to any other tertiary institution prior to this date.

The research carried out for the completion of this dissertation was done in the Faculty of Health Sciences at the Durban University of Technology under the supervision of Dr. Nalini Govender and the co-supervision of Professor Poovendhree Reddy.

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DEDICATED

to

My late father, Basanth (Kemme) Deepnarain, for dedicating his entire life to his wife and children who love and miss him dearly

"In this world, people may steal everything away from you, but the one thing they cannot steal is your brain.

Always remember, education comes first, followed by success, but before that comes all the best things in life that are free and whilst you do that make sure to always see the world through the colour of our blood and not the colour of our skin."

Basanth Deepnarain

Dear dad

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until the day we meet again.

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and

My soulmate Brenton Cohen Subrayen for the continuous upliftment, love and support

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ABSTRACT

Background: Despite efforts to reduce problems associated with child and maternal health, the United Nations International Children's Emergency Fund (UNICEF) corporation reported, that low and middle-income countries such as South Africa remains burdened with this issue. Based on this premise and the recent interest in copeptin (CPP) and fibronectin (Fn) as promising biomarkers in pregnancy related disorders, this study aimed to establish baseline levels of CPP and Fn in normotensive pregnancies complicated with HIV.

Objectives: The objectives of this study were to determine circulating levels of CPP and Fn together with leucyl/cystinyl aminopeptidase (LNPEP) and cystatin C in normotensive pregnancies and to evaluate the association between these biomarkers and different demographic and clinical parameters (e.g. BMI, BP, Hb levels, maternal age and HIV status) throughout gestation.

Methodology: This study stemmed from a previous cohort study conducted between 2015 and 2016 at the Cato Manor Primary Health Care facility in KwaZulu-Natal, South Africa. A total of 41 normotensive pregnant women aged between 18-45 years were selected through convenient sampling and evaluated at 10-20 weeks, 22-30 weeks and 32-38 weeks gestation. Archived serum samples were evaluated using Enzyme Linked Immunosorbent Assays to measure circulating levels of CPP and Fn. Additionally, markers of renal function were evaluated by measuring serum levels of cystatin C and LNPEP. Biomarker profiles and epidemiological and demographic characteristics were quantitatively analysed using STATA (version 15). $P<0.05$ was considered statistically significant.

Results: Among the 41 participants, 28 were HIV positive of which 18 were on the Prevention of Mother to Child Transmission programme. A total of 23 participants were anaemic. Less than half the population were nulliparous and nulligravida. Fluctuations in biomarker concentrations were observed throughout pregnancy with CPP, LNPEP and cystatin C showing an overall decrease between 10 and 38 weeks gestation whilst Fn increased in the second gestational period and then decreased in the third gestational period. Slightly lower recordings were indicated for both systolic and diastolic blood pressures within the HIV positive group, except at gestational period 3, where systolic blood pressure was higher amongst the HIV positive group. On the other hand, haemoglobin levels were higher in the HIV positive group throughout pregnancy.

Conclusion: The baseline levels of CPP, Fn, LNPEP and cystatin C measured in this study are expected to be used for comparison or as reference values to identify the presence of pregnancy related disorders in other studies of similar design and control groups.

Keywords: Copeptin, Fibronectin, LNPEP, cystatin C, HIV, Pregnancy, Normotensive

LIST OF DEFINITIONS

Anaemia: Haemoglobin levels below 11g/dL.

Angiogenesis: The development of new blood vessels.

Angiogenic: Relating to blood vessel growth.

Antenatal: Before birth

Baroreflex: A homeostatic mechanism that helps the body maintain blood pressure at normal levels.

Biomarker: A naturally occurring molecule, gene or characteristic by which a particular pathological or physiological process, disease, etcetera can be identified.

Epidemic: The widespread of an infectious disease in a particular area at a particular time.

Equimolar: Having the same number of moles.

Exon: Any part of a gene that will encode a part of the final mature RNA produced by that gene after introns have been removed by RNA splicing. The term **exon** refers to both the DNA sequence within a gene and to the corresponding sequence in RNA transcripts.

Fetus: Unborn baby more than eight weeks after conception.

Genomics: The branch of molecular biology concerned with the structure, function, evolution and mapping of genomes.

Gestational age: The number of weeks a pregnancy is in.

Glycosylation: The controlled enzymatic modification of an organic molecule, especially a protein, by addition of a sugar molecule.

Gravidity: The number of times a female has been pregnant which includes the current pregnancy.

Haemodynamic: Relating to the flow of blood within the organs and tissues of the body.

Hemostasis: A process within the body that causes bleeding to stop.

Hepatocytes: Liver cells

Hypervolemic: A state in which the liquid portion of the blood is too high.

Isoforms: Any of two or more functionally similar proteins that have a similar but not identical amino acid sequence and are either encoded by different genes or by RNA transcripts from the same gene which have had different exons removed.

Mean: The average that is used to derive the central tendency of a data set.

Median: A simple measure of central tendency.

Morbidity: The condition of being diseased.

Morphogenesis: The biological process that causes an organism to develop its shape.

Mortality: The state of being subject to death.

Neonatal: Newborn children

Normotensive: Having or denoting a normal blood pressure.

Obstetric: Child birth and the processes associated with it.

Outlier: A point which falls more than 1.5 times of the interquartile range above the third quartile or below the first quartile that has the ability to skew data.

Pandemic: The worldwide spread of an infectious disease.

Parity: Pregnancies carried to a viable gestational age.

Perinatal: The time, usually a number of weeks, immediately before and after birth.

Placenta: An organ in the uterus of pregnant women responsible for nourishing and maintaining the fetus through the umbilical cord.

Postnatal: The period after childbirth.

Postpartum: The period after child birth.

Preterm: When the pregnancy period is less than 37 weeks.

Prevalence: The proportion of a particular population found to be affected by a medical condition.

Proteolysis: The breakdown of proteins or peptides into amino acids by the action of enzymes.

Puerperium: The period of about six weeks after childbirth during which the mother's reproductive organs return to its original non-pregnant condition.

P-value: Calculated probability

Range: A measure of statistical dispersion, being equal to the difference between 75th and 25th percentiles, or between upper and lower quartiles.

Serum: An amber-coloured, protein-rich liquid which separates from blood when it is coagulated.

Splicing: Joining or inserting a gene or gene fragment.

Standard deviation: A measure that is used to quantify the amount of variation or dispersion of a set of data values.

Source of definitions: (Pattinson, 2005 and Wheelan, 2013)

LIST OF ABBREVIATIONS

%: Percent

<: Less than

=: Equals to

≥: More than or equal to

Ab: Antibodies

ADH: Antidiuretic hormone

AIDS: Acquired Immune Deficiency Syndrome

AMANHI: Alliance for Maternal and Newborn Health Improvement

APGAR: Appearance, Pulse, Grimace, Activity and Respiration

AT: Angiotensin

AVP: Arginine Vasopressin

BM: Basement Membrane

BMI: Body Mass Index

BP: Blood Pressure

cART: combined Antiretroviral Therapy

CD4: Cluster of Differentiation

CDC: Centers for Disease Control and Prevention

cFn: cellular Fibronectin

CI: Confidence Interval

CPP: Copeptin

CVF: Cervical-Vaginal-Fluid

DNA: Deoxyribonucleic Acid

ECM: Extracellular Matrix

ELISA: Enzyme Linked Immunosorbent Assay

EP: Eppendorf

fFn: fetal Fibronectin

Fn: Fibronectin

g/dL: grams per deciliter

g: grams

GDM: Gestational Diabetes Mellitus

GH: Gestational Hypertension

GLUT: Glucose Transporter

GlyFn: Glycosylated Fibronectin

H: Hydrogen

HAART: Highly Active Antiretroviral Therapy

Hb: Haemoglobin

hCG: human Chorionic Gonadotrophic hormone

HDP: Hypertensive Disorders of Pregnancy

HIV: Human Immunodeficiency Virus

HRP: avidin-Horseradish Peroxidase

IQR: Interquartile Range

IRAP: Insulin Regulated Aminopeptidase

IREC: Institutional Research Ethical Clearance

K: Potassium

kDa: kiloDaltons

kg/m^2 : kilogram per square meter

Kg: Kilograms

KZN: KwaZulu-Natal

LMICs: Low and Middle-Income Countries

LNPEP: leucyl/cystinyl aminopeptidase

m: metre

MDGs: Millennium Development Goals

MiRNAs: MicroRNA

mmHg: millimeter of mercury

n: Number of participants

Na: Sodium

ng/mL: nanograms per milliliter

nm: nanometre

ºC: Degrees Celsius

OD: Optical Density

OECD: Organisation for Economic Co-operation and Development

OR: Odds Ratio

OT: Oxytocin

***p*:** p-value

PBS: Phosphate Buffer Solution

PD: Placental Dysfunction

PE: Preeclampsia

pFn: plasma Fn

pg/mL: pictograms per milliliter

pH: potential Hydrogen

PHC: Primary Health Care

P-LAP: Placental Leucine Aminopeptidase

PlGF: Placental Growth Factor

pmol/mL: picomoles per milliliter

PMTCT: Prevention of Mother to Child Transmission

POC: Point of Care

RAAS: Renin Angiotensin Aldosterone System

RGD: Arginine, Glycine and Aspartate

RHDC: Research Higher Degrees Committee

RNA: Ribonucleic Acid

SA: South Africa

SD: Standard Deviation

SDGs: Sustainable Development Goals

sFlt-1: Soluble fms-like tyrosine kinase-1

STATA: Statistics of data

TMB: Tetramethylbenzidine

UK: United Kingdom

UNAIDS: United Nations programme on HIV/AIDS

UNICEF: United Nations International Children's Emergency Fund

USA: United States of America

WBG: World Bank Group

WHO: World Health Organisation

WLHIV: Women Living with HIV

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CHAPTER 1:

INTRODUCTION

Although 2 of the 8 Millennium Development Goals (MDGs) focused specifically on maternal health improvement and reducing child mortality by 2015, problems associated with child and maternal health are still rife in Low and Middle-Income Countries (LMICs) such as South Africa (SA). On a daily basis, approximately 800 women die from preventable causes related to pregnancy and child birth (Centers for Disease Control and Prevention, 2017). It is estimated that 99% of these deaths occur in LMICs (Centers for Disease Control and Prevention, 2017). "In 2015 alone, 303 000 mothers died from pregnancy related causes and millions more suffered from complications related to pregnancy or childbirth including haemorrhage, infection, hypertensive disorders such as eclampsia and obstructed labour" (Maternal and Child Health, 2015).

Moreover, the unprecedented increase in Human Immunodeficiency Virus (HIV) prevalence coupled with inadequate antenatal care has contributed to the poorer uptake of HIV testing, further complicating maternal and birth outcomes (Govender, et al., 2017). Although HIV is a pandemic, SA, among other countries has become overburdened with the disease. The total number of people living with HIV in SA has increased from an estimated 4.94 million in 2002 to 7.06 million in 2017, this accounts for 12.6% of the total population of which one fifth are women in their reproductive ages (Statistics South Africa, 2018). As a result, HIV is responsible for approximately 40% of all maternal deaths in SA with KwaZulu-Natal (KZN) having the highest antenatal prevalence (Ramjee, et al., 2012; Sebitloane and Moodley, 2017).

Taking all maternal health complications into consideration it is projected that a woman's lifetime risk of maternal death is 1 in 180 in developing countries compared to 1 in 4900 in developed countries (Maternal and Child Health, 2015). Apart from maternal mortality, child mortality is equally severe. Sub-Saharan Africa has almost 12 times the average of child mortality compared to high income countries (Centers for Disease Control and Prevention, 2017). Many of these deaths occur from preventable and treatable causes which can be avoided with early detection and proper treatment. In LMICs various factors contribute to these avoidable deaths, including shortage of healthcare workers, lack of basic medical equipment, limited access to basic life-saving prevention and treatment tools, as well as poor infrastructure (Centers for Disease Control and Prevention, 2017).

It is clear that improving health systems is essential to saving the lives of mothers and children in the developing world (Maternal and Child Health, 2015). The Centers for Disease Control and Prevention (CDC) recommended that improvements in maternal and child health be a major focus of the health-related MDGs 4, 5 and 6 (Centers for Disease Control and Prevention, 2017). These goals however, were still unmet in Sub-Saharan Africa by 2015 thus the Sustainable Development Goals (SDGs) were implemented in January 2016 (Centers for Disease Control and Prevention, 2017). To assist in achieving the targets of the SDGs by 2030 the World Bank Group (WBG) has introduced several active projects focusing on antenatal care and delivery services as a support structure to improve worldwide child and maternal health (Tobar, et al., 2017). South Africa has subsequently been trying to transform and improve the country's health sector by expanding the services of primary healthcare to more than 4000 clinics across the country as well as implementing free government health services to children under the age of five and pregnant and breastfeeding women (UNICEF, 2017).

Additionally, Highly Active Antiretroviral Therapy (HAART) has been made accessible to HIV positive people which include pregnant women and has shown remarkable improvements in the prevention of mother to child transmission and maternal and perinatal outcomes (Sebitloane and Moodley, 2017). Despite these implementations however, the high death rates of new-born babies and mothers remain a concern (UNICEF, 2017). Consequently, there has been evidence showing the rates of adverse birth outcomes in HIV positive women on HAART being higher compared to HIV negative women therefore, HIV infection combined with HAART is believed to trigger the onset of pregnancy induced hypertensive diseases (Powis, et al., 2011). Furthermore, whilst a decline in child and maternal mortality rates were observed, the United Nations target for child and maternal mortality in SA remain unattained (Mulaudzi, et al., 2016).

Simple, cost-effective and reliable biomarkers however, are believed to improve maternal and child health (Maternal and Child Health, 2015). Biomarkers are proven to have clinical value in predicting the severity or possibility of the risk of a disease (Pletcher and Pigone, 2011). Serum biochemical markers have gained interest in individual-level risk prediction of pregnancy related disorders such as preeclampsia (PE) (Wataganara, et al., 2017). The need for rapid diagnosis, more accurate prognostic assessment and faster treatment decisions in various diseases has opened windows for the scrutiny of newer biomarkers such as Copeptin (CPP) and Fibronectin (Fn) (Dobsa and Edozien, 2013).

Copeptin is co-synthesised with arginine vasopressin (AVP) and is found in equimolar amounts with AVP in both healthy and disease-compromised individuals (Katan, et al., 2008). Reports on CPP being able to mirror AVP concentrations has led to its use as a replacement biomarker

of AVP release (Katan, et al., 2008). Based on these data, CPP has gained interest as a promising prognostic and diagnostic marker in various diseases, including heart disease, respiratory infections, haemorrhagic and septic shock, diabetes mellitus and insipidus, autosomal-dominant polycystic kidney disease, ischemic stroke and brain injury among others (Katan, et al., 2008; Dobsa and Edozien, 2013).

Fibronectin on the other hand, is an abundantly expressed protein of the Extracellular Matrix (ECM) in the human body (Petrini, et al., 2017). The Fn gene based on alternative splicing proteolysis, encodes a collection of isoforms that differ in sequence and length (Rasanen, et al., 2015). In humans, Fn is differentiated into plasma Fn (pFn) which is produced and secreted in a soluble form by hepatocytes and cellular Fn (cFn) which is produced by numerous cell types (Rasanen, et al., 2015). Remarkably, both pFn and cFn exhibit complex patterns of glycosylation which prompted the use of maternal serum glycosylated Fn (GlyFn) as a potential biomarker for screening of pregnancy related disorders such as PE (To and Midwood, 2011). Additionally, GlyFn has gained interest as a point of care (POC) marker for rapid detection of PE development and progression, whilst supporting its inclusion as a robust biomarker in both a standard and POC format however, its role is still being explored due to its recent introduction (To and Midwood, 2011; Rasanen et al., 2015). Copeptin and Fn are cost effective and believed to provide reliable and reproducible quantitative data of practical value especially in LMICs like SA (Strimbu and Tavel, 2010).

Based on these recent developments, this study aimed at establishing the baseline levels of CPP and Fn in normotensive pregnancies complicated by HIV, at 3 defined gestational intervals as well as evaluating the associations between both biomarkers with selected demographic and

clinical parameters. In addition, and in relation to CPP, markers of renal function, particularly cystatin C and Leucyl/cysteinyl Aminopeptidase (LNPEP) were also measured. The established baseline levels of these biomarkers may be used as reference points for comparison to identify those pregnancies that may be at risk of being complicated with pregnancy related disorders such as PE. This study is expected to provide support to the use of these biomarkers in poor resourced facilities in LMICs like SA for the benefit of pregnant women especially those complicated with HIV.

1.1 Aims and Objectives

1.1.1 Aims:

The aim of this study was to establish the circulating levels of copeptin and fibronectin in normotensive pregnancies complicated by HIV, at 3 defined gestational intervals and to evaluate the association between demographic and clinical parameters (e.g. BMI, BP, Hb levels and maternal age) to copeptin and fibronectin.

1.1.2 Objectives:

- To determine circulating levels of copeptin at 3 defined gestational intervals in normotensive pregnancies using enzyme linked immunoassays.
- To determine circulating levels of fibronectin at 3 defined gestational intervals in normotensive pregnancies using enzyme linked immunoassays.
- To evaluate any association between copeptin and fibronectin and selected demographic and clinical parameters (such as BMI, BP, Hb levels and maternal age).

CHAPTER 2:

LITERATURE REVIEW

2.1 Maternal and child health worldwide

The establishment of the 2000 MDGs committed the world to achieve a set of targets by 2015. At the core of these goals were MDGs 4 and 5 which focused on improving child and maternal health by reducing child mortality by two-thirds and maternal mortality by three-quarters through universal access to reproductive healthcare (Requejo and Bhutta, 2015). Despite the worldwide reduction of almost 44% in maternal and child mortality rates between 1990 and 2015, maternal and child health and survival was still considered a significant challenge in LMICs because the 2015 targets were not met (Maternal and Child Health, 2015). To ensure continuous improvement in child and maternal health, new targets were set with the implementation of the SDGs in January 2016 (Centers for Disease Control and Prevention, 2017).

Although child and maternal mortality and morbidity can easily be prevented, Requejo and Bhutta, (2015), have stated that most deaths occur from preventable causes, which are often pregnancy related, and are more frequent among the disadvantaged population groups in LMICs (Requejo and Bhutta, 2015). The World Health Organisation (WHO) reiterates that maternal and child mortality is most common in LMICs with Sub-Saharan Africa being most affected (Maternal Mortality, 2018). It is estimated that on a daily basis approximately 830 women die from pregnancy related complications worldwide and 99% of these cases occur in LMICs (Maternal Mortality, 2018). Accelerating the improvement of child and maternal health on a global level is necessary therefore, one specific target under SDG 3 which aims to reduce

the global maternal mortality ratio to less than 70 per 100 000 births with every country having a maternal mortality rate less than twice the global average is vital (Maternal Mortality, 2018). According to the WHO, maternal mortality is linked to several complications that may occur during pregnancy or after delivery. Major complications that account for almost 70% of maternal deaths include severe bleeding, infections such as HIV, complications from delivery and high blood pressure (BP) during pregnancy which may result in PE and eclampsia (Maternal Mortality, 2018).

Other pregnancy related complications such as preterm birth, small for gestational age infants and maternal and perinatal mortality have been associated with various hypertensive disorders of pregnancy (HDP) (Macdonald-Wallis, et al., 2012). These HDP include gestational hypertension, PE and essential hypertension, which affects more than 10% of pregnancies worldwide (Macdonald-Wallis, et al., 2012). Most HDP are maternal and perinatal complications related to high BP, endothelial dysfunction and cardiopulmonary failure (Tihtonen, 2006). Gestational hypertension is defined using BP thresholds after 20 weeks of gestation (Macdonald-Wallis, et al., 2012). In normotensive pregnancies BP levels usually decreases during the first 20 weeks followed by increased levels until delivery therefore, elevated BP during early pregnancy has been referred to as an indication for the increased risk of developing HDP (Macdonald-Wallis, et al., 2012). Normal pregnancies require extensive maternal haemodynamic changes to adjust to the increased intravascular volume loads, thus creating a balance in uteroplacental circulation for fetal development (Tihtonen, 2006). During delivery, further haemodynamic adaptation takes place to account for the termination of uteroplacental circulation which is not the case for HDP (Tihtonen, 2006). Thus, women with HDP are predisposed to increased arterial stiffness resulting in an increased risk of hypertension, renal and liver impairment, pulmonary oedema, thrombocytopenia,

coagulopathy and neurological disruptions (Tooher, et al., 2013). Whilst elevated BP measurements during pregnancy can lead to spontaneous delivery and poor birth outcomes, it is also possible that birth outcomes may not be negatively affected (Mammaro, et al., 2009). Women with pre-existing or chronic high BP are however, more susceptible to pregnancy complications than normotensive pregnant women (Mammaro, et al., 2009).

Blood pressure is reported as one of many factors that can influence pregnancy related disorders (Ota, et al., 2014). Maternal characteristics such as age, parity, smoking and Body Mass Index (BMI) can all negatively influence pregnancy, and may result in intrauterine growth restriction and low birthweight (Ota, et al., 2014; Kupers, et al., 2015). A retrospective study conducted in Israel on advanced maternal aged women (≥ 35 years**)** and a younger control group aged 24 to 27, showed that advanced aged women had a significantly higher risk of developing pregnancy complications than younger women (Schimmel, et al., 2015). These investigators also demonstrated that primiparous women were at a greater risk for the development of gestational diabetes, with the risk being higher amongst the advanced maternal aged women (Schimmel, et al., 2015).

These findings were substantiated in a population-based cohort study in Australia (Laopaiboon, et al., 2015). Laopaiboon and co-workers reported that advanced maternal aged women were more susceptible to pre-existing medical conditions, obstetric complications and adverse labour and birth outcomes (Laopaiboon, et al., 2015). Similarly, a retrospective cohort study conducted on 39763 women in Taiwan showed that advanced maternal aged women were at a greater risk for emergency pre-term delivery and stillbirth (Hsieh, et al., 2010). The study found that women older than 35 carried close to a 1.5-fold increased risk for pregnancy complications

and a 2.6-fold increased risk for adverse perinatal outcomes (Hsieh, et al., 2010). Furthermore, these women were at increased risk for operative vaginal delivery (OR = 1.6, 95% CI = 1.5-1.7) and preterm delivery (OR = 1.7, 95% CI = 1.3-2.2) (Hsieh, et al., 2010).

Another study conducted on 1030 Australian women reported an increased maternal BMI (>30 kg/m^2) as a significant risk factor for the development of Gestational Diabetes Mellitus (GDM) (Martin, et al., 2015). Body Mass Index was considered a significant risk factor because elevated body weight affects the ability of insulin to maintain blood sugar at normal levels, instead, these levels are increased in pregnancy (Martin, et al., 2015). At the same time, the study revealed that "women who were diagnosed with GDM were significantly less likely to give birth to a baby with birthweight above 4kg (OR = 0.60; 95% CI = 0.36 to 1.00; $p = 0.05$)" (Martin, et al., 2015). Pregnancy and birth outcomes are also associated with haemoglobin (Hb) levels (Jwa, et al., 2015). A retrospective Japanese study demonstrated that low reductions in Hb levels throughout pregnancy was associated with the increased risk of delivering premature babies compared to intermediate or greater Hb reductions (Jwa, et al., 2015). Results showed that women with the least reduction had a significantly increased risk of delivering babies with a low birthweight (OR = 2.0; 95% CI = 1.3-3.1) and babies that were small for gestational age (OR = 1.6; 95% CI = 1.04-2.3) (Jwa, et al., 2015).

In addition to the burden of hypertensive diseases in pregnancies, the substantial increase of HIV infection in women of reproductive age is associated with increased risks during pregnancy (Calvert and Ronsmans, 2013). Sub-Saharan Africa reports a high incidence of direct obstetric complications among HIV positive pregnant women, suggestive that HIV and maternal mortality are two intersecting epidemics (Calvert and Ronsmans, 2013). Many

pregnant women living with HIV are also susceptible to risks associated to direct pregnancy complications and delivery as well as that associated with HIV progression (Calvert and Ronsmans, 2013). The human immunodeficiency virus progresses by attacking the $CD4^+$ cells of the immune system, resulting in an immunocompromising condition such as HIV infection and Acquired Immune Deficiency Syndrome (AIDS) (Centers for Disease Control and Prevention, 2017). Highly Active Antiretroviral Therapy and combined Antiretroviral Therapy (cART) during pregnancy is therefore, essential in minimising the progression of HIV amongst pregnant women (Roth, et al., 2016). Thus, the lack of treatment, combined with delays in accessing antenatal care and undiagnosed maternal HIV infection before conception are identified as key risk factors related to obstetric complications and poor birth outcomes in HIV associated pregnancies (Roth, et al., 2016). A German study reported that most pregnant women living with HIV are between 15 and 49 years (Reitter, et al., 2014). Access to health care before and during pregnancy is therefore necessary to decrease the risk of pregnancy complications (Centers for Disease Control and Prevention, 2017).

As a result of the unprecedented increase in pregnancy related complications the search for new ways to alleviate this problem has widened. Over the years the advancement of biomarkers has increased worldwide and has shown promising results in early diagnosis and prevention of pregnancy related complications (McCormick, et al., 2017). Biobanks together with associated epidemiological studies has improved the understanding of various disease mechanisms and promoted the use of biomarkers in child and maternal health although, its use in drug development remains debatable (McCormick, et al., 2017). The Organisation for Economic Co-operation and Development (OECD), has reported that biomarkers offer new ways in understanding disease processes and treatment regimens, thus improving disease diagnosis, safety and efficacy of existing medicines as well as developing new treatment and targeted

therapies (OECD, 2011). To experience these benefits in LMICs such as SA, where access to both staff and high technology is limited, the cost effectiveness and reliability of biomarkers is essential in order to improve the diagnoses of those at risk and subsequently reduce this obstetric burden (OECD, 2011).

2.2 Prevalence of Human Immunodeficiency Virus among pregnant women

It is projected that "a woman's lifetime risk of maternal death is 1 in 180 in developing countries compared to 1 in 4900 in developed countries" (Maternal and Child Health, 2015), with child mortality being equally severe (Centers for Disease Control and Prevention, 2017). Resource poor countries particularly in Africa has almost 12 times the average of child mortality compared to high income countries worldwide (Centers for Disease Control and Prevention, 2017). This differentiation can be linked to the shortage of healthcare workers, lack of basic medical equipment or treatment tools, limited access to basic lifesaving skills and poor infrastructure in LMICs. Moreover, the advent of HIV/AIDS among the global population of women in their reproductive age is regarded as a significant contributory factor to the increase in child and maternal mortality rates (Centers for Disease Control and Prevention, 2017).

In 1986, the first case of AIDS was confirmed in India and has subsequently been identified and reported on a global scale (Nayak, et al., 2017). Despite the significant decrease in HIV prevalence among pregnant women in India, the country still falls within the top 10 countries with the highest maternal HIV prevalence, ranking third largest in the world for HIV epidemic (Nayak, et al., 2017). Furthermore, it has been estimated that out of 29 million annual pregnancies in India 35 255 occur in HIV positive women (Nayak, et al., 2017), Similarly,

significant reductions in overall HIV prevalence rates was reported in Brazil and China however, the maternal HIV prevalence rates in Brazil remain high and is a significant risk factor for maternal and child morbidity and mortality (Pereira, et al., 2016; Yang, et al., 2017). Whilst the HIV epidemic is considered stable and concentrated in key populations in Brazil, it still remains the largest HIV population in Latin America with 40 600 new cases each year (Pereira, et al., 2016).

While these countries are struggling with HIV (Figures 2.1 and 2.2), SA has the highest burden of global HIV infection prevalence despite being home to less than 1% of the global population (Kharsany, et al., 2015). The United Nations programme on HIV/AIDS (UNAIDS) describes the HIV prevalence in SA as hyper-endemic, generalised and mature however, the high prevalence rates reported in SA may be directly linked to SA having a better reporting system than other African countries (Kharsany, et al., 2015). Human Immunodeficiency Virus infection is a key contributor to maternal and neonatal morbidity and mortality in LMICs (Sebitloane and Moodley, 2017). It is estimated that 17.8 million of the total 36.7 million HIV infected people living worldwide are females within their reproductive age range (Conroy, et al., 2017). Sub-Saharan Africa carries a disproportionate burden of the HIV pandemic and accounts for almost 70% of the global HIV infected population, of which SA accounts for 25% of all cases, followed by 13% in Nigeria and 6% in Mozambique (Kharsany and Karim, 2016). Thus, HIV accounts for an estimated 40% of all maternal deaths in SA alone, with KZN having the highest antenatal prevalence (Figures 2.1 and 2.2) (Ramjee, et al., 2012; Sebitloane and Moodley, 2017).

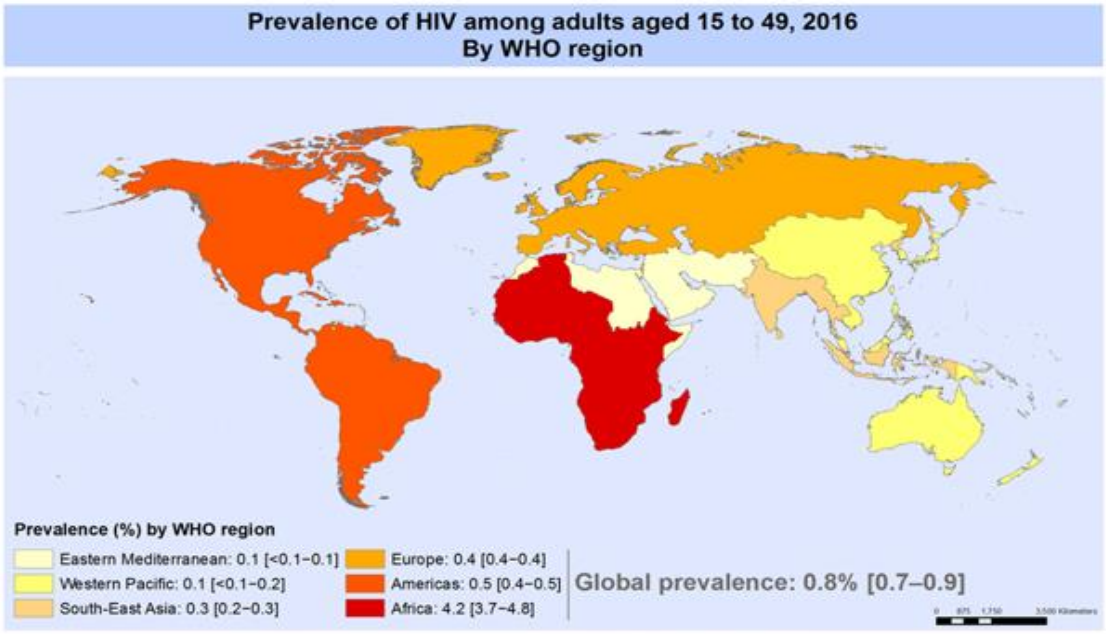

Figure 2.1: Prevalence of HIV by region among adults which include women in their reproductive age. Map indicates Africa having the highest prevalence of which SA is most affected (Adapted from World Health Organisation, 2016)

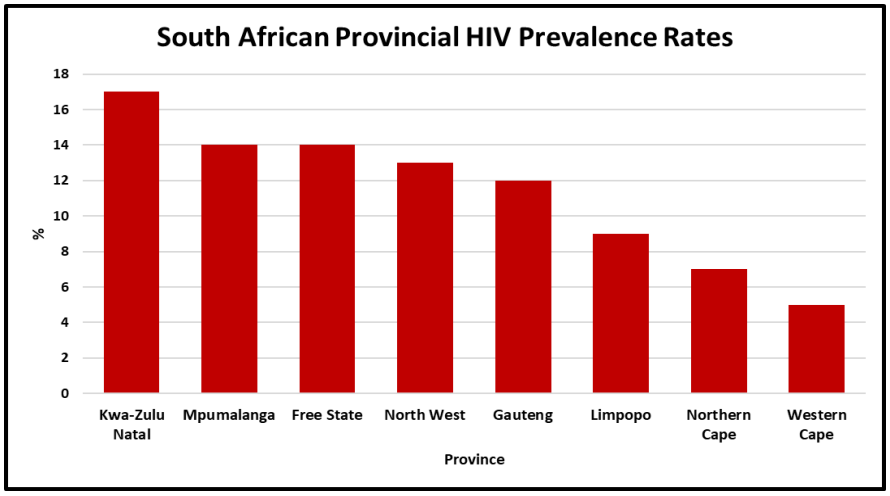

Figure 2.2: Total percentage of HIV infected people including pregnant women living in SA (Adapted from South African National HIV Prevalence, Incidence and Behaviour survey, 2017)

2.2.1 Complications associated with Human Immunodeficiency Virus and Highly Active Antiretroviral Therapy during pregnancy

The HIV pandemic in SA affects maternal health directly through opportunistic infections, postpartum sepsis and obstetric haemorrhage, and indirectly via inadequate healthcare systems and shortage of suitably qualified healthcare professionals (Sirleaf, 2012). Since the advent of the HIV outbreak and the introduction of routine antenatal HIV screening in the United Kingdom (UK) and Ireland during the early 2000s, there has been almost a 90% improvement in diagnosis of HIV among pregnant women due to the rollout of HAART administration (Townsend, et al., 2007). Whilst HAART decreases the progression of HIV, it has been associated with various pregnancy related complications and poor fetal development (Townsend, et al., 2007).

In a South African cohort, fetal complications such as preterm birth, small for gestational age and stillbirth were reported to be more common amongst women living with HIV (WLHIV) on HAART compared to those who were not (Conroy, et al., 2017). Moreover, HIV was identified as a risk factor for maternal vascular malperfusion within the study population (Conroy, et al., 2017). Maternal vascular malperfusion is characterised by hypoxic-ischemic placental damage and the consequential outcomes such as PE, systemic lupus erythematosus, fetal growth restriction, preterm delivery and spontaneous abortion (Scifres, et al., 2016; Conroy, et al., 2017). Pregnancies complicated by HIV also contributes to increased development of anaemia which subsequently leads to poor maternal and birth outcomes (Nandlal, et al., 2014). The 2012 Saving Mothers report identified HIV and anaemia as the most common contributors to maternal mortality cases during pregnancy and puerperium stages (Sirleaf, 2012). More recently, HAART was shown to prevent the vertical mother to

child HIV transmission and improves the overall health and survival of both mothers and their babies (Bailey, et al., 2018).

2.3 The Physiology of Pregnancy

Pregnancy begins with the release of an oocyte at ovulation, followed by the formation of the hormone secreting corpus luteum and thereafter conception (Pillai, 2018). Progesterone and estrogen released from the corpus luteum prepares the uterine lining for implantation (Young, 2013). Fertilisation of the ovum with a spermatozoon results in the formation of a zygote, which undergoes cleavage to produce the 16-cell morula stage (Young, 2013: Pillai, 2018). This is followed by the continuous mitotic division until the blastocyst is formed, which subsequently implants in the uterus. The inner cell mass of the blastocyst becomes the embryo whilst the outer mass develops into the chorionic sac and the fetal portion of the placenta (Young, 2013: Pillai, 2018). This allows for the division of pregnancy into three trimesters, each of which lasts approximately 12-14 weeks (American Pregnancy Association, 2016). The first trimester is characterised by conception and organ and structural development of the fetus, followed by ongoing fetal growth and development during the second and third trimesters (American Pregnancy Association, 2016: Pillai, 2018).

Following conception, there is production of the human Chorionic Gonadotrophic (hCG) hormone which maintains the strength of the endometrium lining (Pillai, 2018). The first trimester is characterised by ectodermal, mesodermal and endodermal development, which initiates the embryonic development phase of tissues, organs, limb buds and vessel circulation (Pillai, 2018). As the first trimester progresses, the embryo becomes more defined until the

fetus develops around the 10th week of pregnancy (Morris, 2008: Pillai, 2018). The second trimester allows for finer fetal development including facial expression, growth of finger and toe nails, followed by development of the eyelashes and finger and foot prints (Morris, 2008: Pillai, 2018). This period also shows an elevation in fetal heart rate and body movement. The third trimester is between weeks 27 and 40, and characterises the completion phase of fetal development, leading to labour and delivery (Pillai, 2018).

2.4 Physiological changes during pregnancy

Pregnancy is accompanied by physiological adaptation in response to hormonal changes, elevations in intravascular volumes and organ compression due to expansion of the uterus (Motosko, et al., 2017). Whilst these changes support fetal development, it is also accompanied by dermatological changes (Motosko, et al., 2017) and maternal anaemia due to increased blood volume (Datta, et al., 2010) as well as haematological and cardiovascular changes (Heidimann and McClure, 2003). Maternal plasma volume increases by 45% due to the direct action of progesterone and estrogen on the kidneys resulting in the activation of the Renin-Angiotensin Aldosterone System (RAAS) (Figure 2.3) and a non-osmotic hypothalamic release of AVP, which regulates BP, cardiac output and arterial pressure (Heidimann and McClure, 2003; Datta, et al., 2010; Soma-Pillay, et al., 2016).

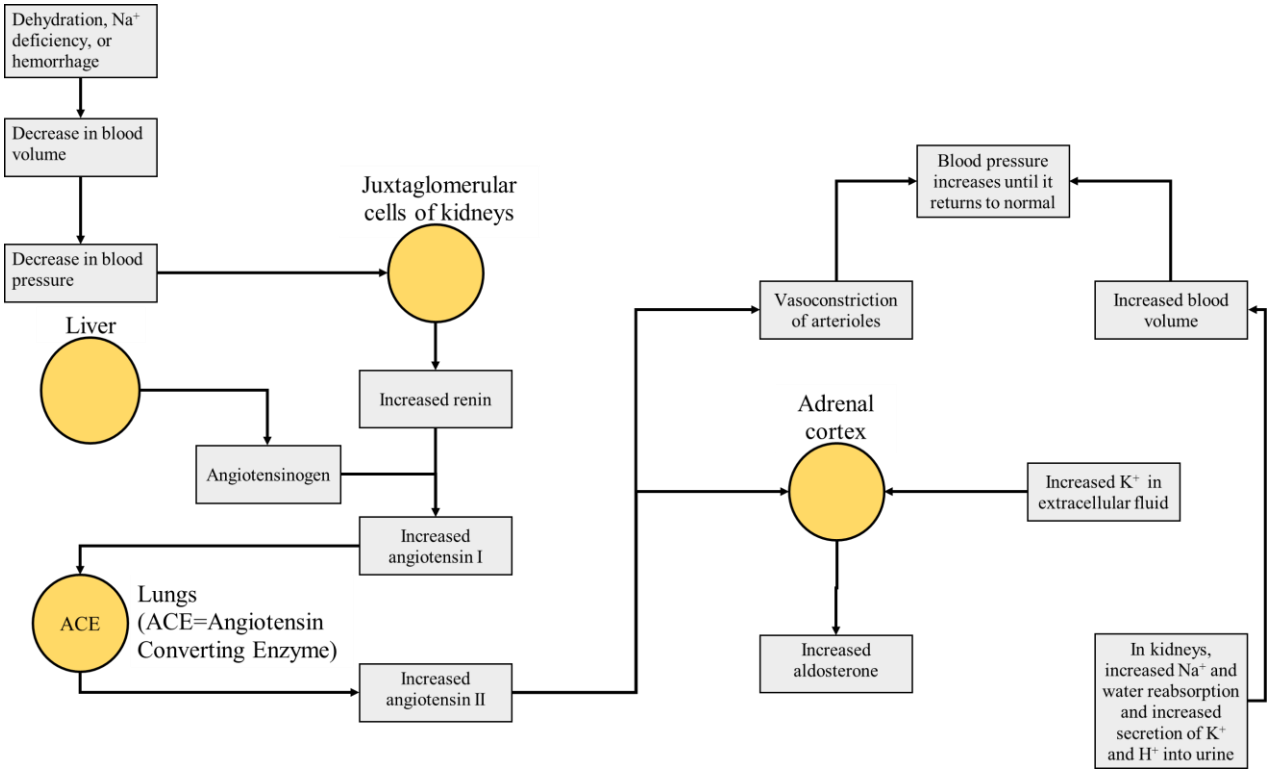

Figure 2.3: The RAAS pathway illustrating the regulation of aldosterone secretion, BP, blood volume and sodium, potassium and hydrogen levels in the body (Adapted from Klabunde, 2016)

A recent study conducted in Austria quoted "normal pregnancy is characterised by several changes of the maternal cardiovascular system to adapt BP to the needs of pregnancy in preparation for delivery" (Kolovetsiou-Kreiner, et al., 2018). These investigators demonstrated an increase in sympathetic regulation followed by the decrease of baroreflex sensitivity throughout pregnancy. These changes are controlled and regulated by the aid of biochemical reactions in the endothelium and the autonomic nervous system (Kolovetsiou-Kreiner, et al., 2018). Consequently, renal vasodilation during these haematological and cardiovascular changes result in a 40-65% increase in renal plasma flow and a 50-85% elevation in glomerular filtration rate in normal pregnancies compared to non-pregnant women (Soma-Pillay, et al., 2016). An earlier study suggested that the kidneys increase in length and volume during pregnancy (Wearne, 2014). Hence, it is possible that hydronephrosis may be experienced in

80% of all pregnancies by the third trimester, thereby predisposing these women to urinary stasis and the increased risk of urinary tract infections. Systemic blood vessels also undergo hormonal dilation and subsequently leads to a decrease in systemic vascular resistance and BP in the first and second trimesters (Wearne, 2014).

Oxygen consumption and metabolic rate also increases by 15-20% during normal pregnancy (Soma-Pillay, et al., 2016), resulting in a 50% elevation in minute ventilation in pregnant women compared to non-pregnant women (Datta, et al., 2010). These major physiological changes are also accompanied by changes in protein, glucose, lipid and calcium metabolism, variations in adrenal and pituitary secretions as well as skeletal and bone density effects, bone loss in particular (Soma-Pillay, et al., 2016). Moreover, alterations in the gastrointestinal system contribute to heartburn experienced during pregnancy. As the uterus enlarges, intra-abdominal pressure increases causing a displaced gastric axis (Heidimann and McClure, 2003). Furthermore, the physiological changes during pregnancy has significant effects on the central and peripheral nervous systems and may even cause changes in visual abilities (Datta, et al., 2010).

2.4.1 Maternal and child morbidity and mortality

The global estimation of infant deaths per annum is approximately 1.1 million, and it is estimated that almost 60% of all cases occur in LMICs (Vogel, et al., 2014). Preterm birth is classified as one of the leading causes of death during the neonatal period which increases the risk for post neonatal mortality, respiratory, infectious and metabolic and nervous system morbidities (Vogel, et al., 2014; Quinn, et al., 2016). Recent reports demonstrate that almost 289 000 maternal deaths, 6 million child deaths and 2.6 million stillbirths of the global

estimates occur in Sub-Saharan Africa and South Asia (Baqui, et al., 2017). It is possible that this increased vulnerability to child and maternal morbidity and mortality may be associated with maternal age and weight, race, parity, medical conditions, genetic predisposition, as well as lifestyle and psychological factors (Vogel, et al., 2014). Moreover, when such factors are coupled with resiliency, inadequate interfacility personnel, antenatal and post-natal care, sub optimal adherence to antenatal guidelines, delays in seeking antenatal care and insufficient equipment and transport for emergency obstetric care exacerbates the crisis in LMICs (Maredza and Chole, 2016).

According to Baqui and co-workers "the Alliance for Maternal and Newborn Health Improvement (AMANHI) group recommends a better understanding of the biological mechanisms that underlie adverse birth outcomes such as eclampsia, intrauterine birth restrictions, preterm births and stillbirths and their relationships with various phenotypic, epidemiologic and epigenetic characteristics" (Baqui, et al., 2017). It is believed that goal 3 of the SDGs which replaced the MDGs in January 2016, will intentionally improve both maternal and child health by 2030 based on implementation of new and improved interventions (Maredza, et al., 2016; Gumede, et al., 2017). Thus, the use of cost-effective biomarkers during early pregnancy for the prediction of gestational complications has gained much interest (Kane, et al., 2014). The primary goal during management of pregnancy related disorders is to prolong the pregnancy in order to deliver an adequate gestational age baby (Cuffe, et al., 2017). In LMICs however, it is difficult to predict adverse pregnancy outcomes such as PE, preterm birth and gestational diabetes via maternal history and risk factors alone, especially in nulliparous pregnancies (Kane, et al., 2014). The further investigation and implementation of the use of simple, cost effective and reliable biomarkers would be beneficial especially since biomarkers

have already proven to have clinical value in predicting the severity or possibility of the risk of a disease (Pletcher and Pigone, 2011; Maternal and Child Health, 2015).

2.5 Biomarkers in clinical medicine

Biomarkers have been used in clinical medicine for decades but, has only recently taken a new and promising direction towards contributing to universal health through early diagnosis and effective treatment decisions (Chen, et al., 2017). With the advent of genomics and additional advances in molecular biology, the study of biomarkers in medical science has grown rapidly (Barker, 2014). Biomarkers can be objectively measured and evaluated as indicators of biological, pathogenic or pharmacologic responses to a therapeutic intervention (Chen, et al., 2017). Moreover, it may indicate various health or disease characteristics including the level or type of exposure to an environmental factor, genetic susceptibility, genetic responses to environmental exposures, markers of subclinical or clinical disease or indicators of response to therapy (Barker, 2014; Chen, et al., 2017). Biomarkers are therefore, classified into five categories based on their application in different disease stages (Chen, et al., 2017). These categories include antecedent biomarkers to identify the risk of developing an illness, screening biomarkers to screen for subclinical disease, diagnostic biomarkers to recognise apparent diseases, staging biomarkers to categorise disease severity and prognostic biomarkers to predict future disease courses and outcomes (Chen, et al., 2017).

The use of non-invasive obstetric biomarkers such as circulating microRNAs, fetal specific nucleic acids and angiogenic biomarkers have been explored to monitor both maternal and child health (Tsochandaridis, et al., 2015). Gestational hypertension among US nulliparous pregnancies have increased from 12% to 28% within the last two decades (Sibai, 2014).

Hypertensive disorders of pregnancy are linked with risk factors that exist before and after pregnancy (Sibai, 2014). Risk factors before pregnancy include history of gestational or chronic hypertension, renal disease, pre-gestational diabetes, autoimmune disease, elevated BMI, maternal age, race and ethnicity whilst risk factors that develop after conception include first ongoing pregnancy, assisted reproductive technologies, multifetal pregnancy, increased blood pressures and abnormal concentrations of serum markers in the first trimester (Sibai, 2014). The discovery of circulating fetal specific nucleic acids of pregnant women has created new perspectives on non-invasive prenatal diagnosis development (Tsochandaridis, et al., 2015).

The recent advances in establishing the clinical utility of the angiogenic sFlt-1/PGIF ratio in the prediction, early diagnosis and monitoring of placental dysfunction is widely documented (Carty, et al., 2008; Herraiz, et al., 2015; Stepan, et al., 2015). Whilst biomarkers have gained popularity for its clinical application, others have scrutinised its utility in justifying its clinical application (Pletcher and Pigone, 2011). Furthermore, the use of both expensive and unreliable biomarkers as disease predictors led to various predictions that were late in its detection, inaccurate prognostic assessments and delayed treatment decisions (Strimbu and Tavel, 2010; Pletcher and Pigone, 2011). These investigators further suggested that the validity of most biomarkers is often assumed rather than allowing for a continued re-evaluation until there is sufficient information to support its clinical use (Strimbu and Tavel, 2010).

In addition, an earlier study suggested that normal distributions of biomarkers must be established based on factors such as age, weight, the extent of interindividual variation and the persistence of the biomarker in order to determine its validity and reliability (Mayeux, 2004).

Whilst biomarkers are clinically important in improving drug development and predicting various clinical anomalies, it is essential to firstly understand the correlations between measurable biological processes and clinical outcomes in expanding and supporting their clinical use (Leeflang, et al., 2007). This will support its application as measures of clinical outcomes or reference points for comparison to identify people that may be at risk of developing a disease which is beneficial for LMICs (Strimbu and Tavel, 2010). Lukaszyk and Malyszko, (2015), documented that an ideal biomarker for implementation in both developed and developing countries is one which is safe, easy to use, cost effective and easily modifiable with treatment before being translated into clinical practice. Moreover, the potential clinical use of CPP and Fn has been reported in the identification and treatment of pregnancy related disorders (Lukaszyk and Malyszko, 2015). Copeptin is a surrogate biomarker co-synthesised with AVP and has recently been identified as a promising diagnostic and prognostic marker for various diseases (Katan, et al., 2008). Similarly, Fn has also showed positive diagnostic and prognostic capabilities but, is still being explored (Rasanen, et al., 2015). Both CPP and Fn are believed to provide reliable and reproducible quantitative data of practical value (Strimbu and Tavel, 2010).

2.6 Arginine Vasopressin and Copeptin

Arginine vasopressin, also referred to as Antidiuretic Hormone (ADH) is synergistically produced and released into the bloodstream with CPP (Dobsa and Edozien, 2013). Copeptin is produced in the hypothalamus and stored in the posterior pituitary gland until it is released through stimulation of osmoreceptors or baroreceptors (Brenner and Rector, 2011; Tiwari and Ecelbarger, 2018). Pre-proAVP, a precursor protein consists of four components that include a single peptide, AVP, neurophysin II and CPP (Dobsa and Edozien, 2013). All four components

are separated during axon transport from the cell body to the axon terminal in the posterior pituitary gland (Dobsa and Edozien, 2013). Along this path, CPP and neurophysin II act as carrier proteins of AVP whilst CPP is stored in the neurohypophyseal vesicles with AVP and neurophysin II until secretion (Figure 2.4) (Dobsa and Edozien, 2013).

Arginine vasopressin exerts haemostatic, endocrine and central nervous effects by inducing water conservation by the kidneys, thus regulating urine concentration and maintaining osmotic and cardiovascular homeostasis (Bolignano, et al., 2014; Tiwari and Ecelbarger, 2018). Circulating levels of AVP in the human body during normal circumstances is usually around 1picomole (Tiwari and Ecelbarger, 2018). Arginine vasopressin also regulates glomerular filtration rates, renal blood flow, nitric oxide generation and BP as well as renal chloride, bicarbonate and potassium homeostasis (Tiwari and Ecelbarger, 2018). Furthermore, AVP is regarded as a major hypothalamic stress hormone that is stimulated at increased stress levels (Katan and Christ-Crain, 2010).

Several studies have recognised CPP as a stable and sensitive surrogate marker for AVP release and it has also been associated with renal function decline (Beljan, et al., 2017). Measuring the circulating levels of AVP is a challenge due to its unstable nature, since it abundantly attaches to platelets and dissipates rapidly from plasma (Katan and Christ-Crain, 2010; Morgenthaler, 2010). Based on its stability and reliability, circulating levels of CPP is the preferred alternative to AVP measurement (Katan and Christ-Crain, 2010). Copeptin also successfully mirrors the production of AVP and thus has the potential to be a prognostic biomarker in acute disease due to its high sensitivity and fast efficient results (Katan and Christ-Crain, 2010; Morgenthaler, 2010; Łukaszyk and Małyszko, 2015). It does not require

preanalytical procedures, has a long plasma half-life, is stable at room temperature and is easily measured in serum or plasma (Łukaszyk and Małyszko, 2015; Beljan, et al., 2017).

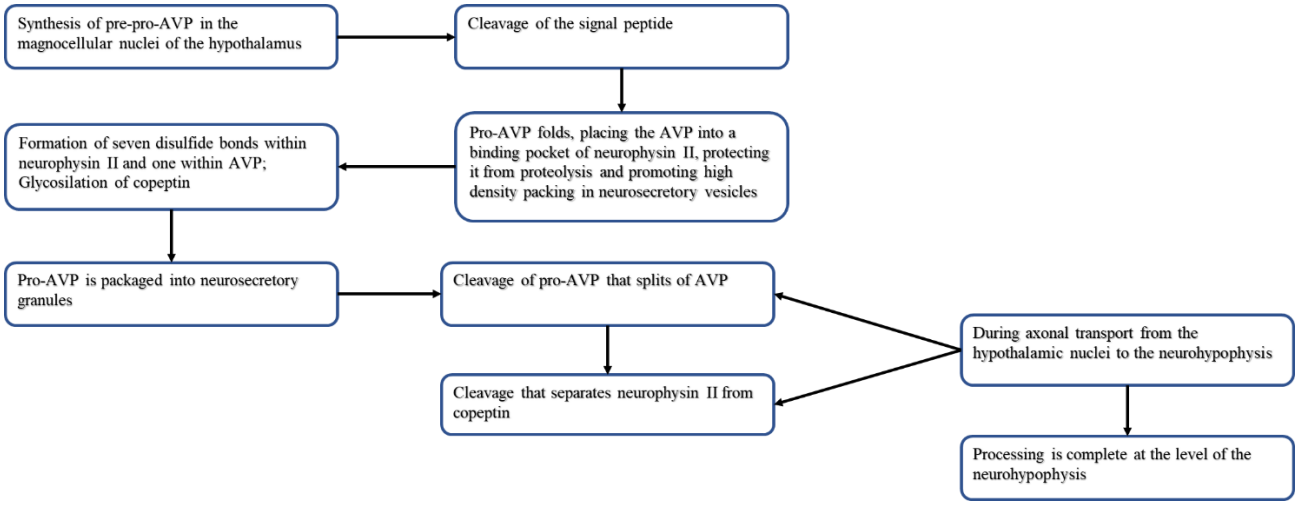

Figure 2.4: Schematic presentation of CPP and AVP generation and maturation (Adapted from Dobsa and Edozien, 2013)

2.6.1 The structure and formation of Arginine Vasopressin

Arginine vasopressin is described as a hormone derived from a 164-amino acid precursor peptide known as pre-provasopressin along with CPP which is released in equimolar ratio to AVP (Katan and Christ-Crain, 2010; Morgenthaler, 2010). Vasopressin receptors include, V1a which is responsible for stimulating vascular smooth muscle contraction, V1b, which is responsible for water retention in the kidneys and V2 which modulates corticotropin secretion in the central nervous system (Weitzel, 2011; Tiwari and Ecelbarger, 2018). Copeptin is a 39-amino acid glycopeptide that holds the C-terminal component of AVP and is released simultaneously with AVP during processing of the precursor peptide (Loughrey and Young, 2014; Jadli, et al., 2017). The sequence of pre-provasopressin contains a signal peptide, AVP, neurophysin II and CPP (Figure 2.5) (Katan and Christ-Crain, 2010).

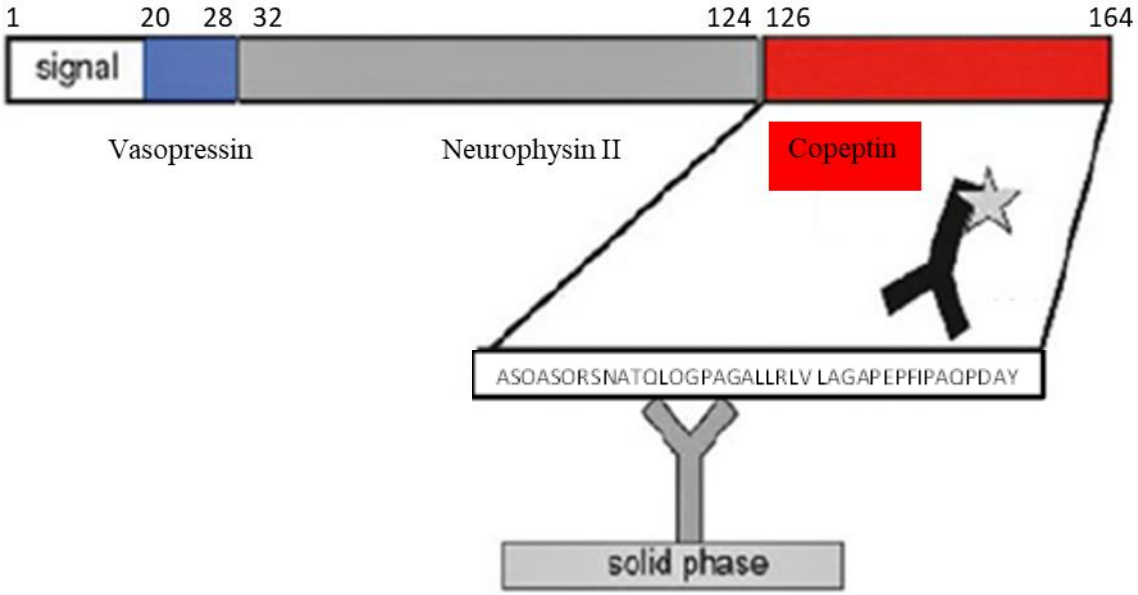

Figure 2.5: Sequence of pre-provasopressin (Adapted from Katan and Christ-Crain, 2010)

2.6.2 Clinical applications of circulating levels of Arginine Vasopressin in pregnancy

Normal AVP concentrations are known to be between 1 and 5 pg/mL in healthy people (Bolignano, et al., 2014). This was corroborated by Łukaszyk and Małyszko, (2015), who reported an average CPP plasma concentration of 4.2 pmol/L in healthy individuals however, it was reported that females have lower concentrations in contrast to males (3.7 and 5.2 pmol/L respectively, $p < 0.0001$) but, median levels may be comparable amongst different age groups (Łukaszyk and Małyszko, 2015). Furthermore, activities such as fasting or exercise usually increases CPP concentrations which tend to decrease after increasing water consumption (Lukaszyk and Malyszko, 2015). Arginine vasopressin is released from the hypothalamus during pregnancy together with sodium which causes water retention in the kidneys resulting in a hypervolaemic, hypo-osmolar state and increased extracellular and plasma volumes (Tkachenko, et al., 2014). This hypo-osmolar state is created when the thresholds for hypothalamic secretion of AVP and thirst are reset to a low plasma osmolality level (Soma-

Pillay, et al., 2016). At this point, metabolic clearance of vasopressin is enhanced to allow for the regulation of active AVP (Soma-Pillay, et al., 2016).

The clinical use of evaluating CPP in anomalies such as diabetes insipidus, sepsis and PE (Beljan, et al., 2017) and stress especially during labour in pregnancy (Lukaszyk and Malyszko, 2015) has been explored. An umbilical cord study conducted in Egypt compared the CPP levels in normotensive and preeclamptic pregnancies, taking into consideration the mode of delivery (Foda and Abdel-Aal, 2012). These investigators demonstrated increased CPP levels in PE and those who gave birth through vaginal delivery in contrast to the normotensive, caesarian section participants (Foda and Abdel-Aal, 2012). Moreover, the increased CPP levels during vaginal delivery and in preeclamptic cases were directly linked to higher levels of stress and intrauterine fetal distress (Foda and Abdel-Aal, 2012).

The findings of Foda and Abdel-Aal, (2012), were corroborated by Nissaisorakarn, et al., (2016). These investigators reported that preeclamptic cohorts experience reduced circulating activity of the RAAS which in turn increases AVP and CPP secretion (Foda and Abdel-Aal, 2012; Nissaisorakarn, et al., 2016). Gestational CPP levels are therefore increased towards the end of the second trimester or the beginning of the third trimester and at the point of delivery in patients with PE (Nissaisorakarn, et al., 2016). Katan and Christ-Crain, (2010), also suggested a positive association between CPP and disease severity and outcome, thus supporting its use as a prognostic marker for acute diseases. Copeptin levels have also been evaluated in cases of sepsis, pneumonia, lower respiratory tract infections, stroke and other acute diseases (Katan and Christ-Crain, 2010). Katan and coworkers confirm the ability of CPP to differentiate between patients with poor health outcomes and those without (Katan and

Christ-Crain, 2010). It is believed that CPP has the ability to provide accurate prognostic support to help improve patient care (Katan and Christ-Crain, 2010).

The evaluation of circulating levels of CPP has become common in studies comparing preeclamptic pregnancies and uncomplicated pregnancies (Santillan, et al, 2014; Cornelius, 2015; Joosen, et al., 2017). Reports by Santillan and co-workers substantiates the potential use of plasma AVP measurements in the prediction of PE development (Santillan, et al., 2014). The study evaluated 134 women of which 54 were non-preeclamptic pregnant women, 50 were preeclamptic pregnant women and 30 were non-pregnant women (Santillan, et al., 2014). Copeptin was measured throughout pregnancy for these women and results revealed that maternal plasma CPP was significantly higher in preeclamptic pregnancies compared to non-preeclamptic pregnancies and non-pregnant women (Santillan, et al., 2014). These concentrations still remained significant after clinical confounders such as age, BMI, chronic essential hypertension, twin pregnancies, diabetes and history of PE were controlled (Santillan, et al., 2014). Data on the non-pregnant group suggested that increased CPP levels was both pregnancy and PE specific (Santillan, et al., 2014). Similarly, a follow up study conducted by Yeung, et al., (2014), described CPP as a novel predictive biomarker specific for PE (Yeung, et al., 2014). The study analysed maternal serum CPP levels in pregnancies complicated with PE (n=169), gestational hypertension (n=101) and gestational diabetes (n=92) compared to uncomplicated pregnancies (n=136) (Yeung, et al., 2014). Results showed a positive association between CPP levels and increased risk of PE (OR = 1,61) even after accounting for confounders such as maternal age, BMI, race, insurance status, marital status, smoking status and clinical site (Yeung, et al., 2014). Associations were however, stronger amongst cases diagnosed before 37 weeks of pregnancy with no associations found for gestational diabetes or gestational hypertension (Yeung, et al., 2014).

Zulfikaroglu and colleagues have investigated CPP levels in 32 normotensive pregnant women, 32 mild preeclamptic pregnant women and 32 severe preeclamptic pregnant women (Zulfikaroglu, et al., 2011). It was reported that CPP increased in the different groups (0.31±0.09 ng/mL in the normotensive pregnant group, 0.62±0.16 ng/mL in the mild pre-eclamptic group and 0.85±0.18 ng/mL in the severe pre-eclamptic group), being significantly higher in the preeclamptic than the normotensive groups ($p<0.001$) (Zulfikaroglu, et al., 2011). Likewise, a Nigerian study demonstrated higher CPP levels in preeclamptics compared to controls (Akinlade, et al., 2015). In a different study, Tuten, et al., (2015), also reported higher levels in the preeclamptic groups compared to the control group (mean CPP levels to be 0.92±0.57 ng/mL in the early onset preeclamptic group and 1.65±0.95 ng/mL in the late onset preeclamptic group), with a statistically significant difference ($p = 0.011$) noted in the early onset preeclamptic group (Tuten, et al., 2015). In contrast, a study conducted in the Netherlands on healthy pregnant women only, concluded that overall circulating levels of CPP increased during pregnancy (Joosen, et al., 2018).

2.7 Leucyl/cystinyl Aminopeptidase during pregnancy

Vasopressinase, also known as LNPEP is an aminopeptidase produced by the placenta (Soma-Pillay, et al., 2016). It is a gene that encodes a zinc dependent aminopeptidase that splits vasopressin and other peptide hormones and is secreted in maternal serum (Gene, 2018). In addition, vasopressinase can be found in intracellular vesicles together with the insulin responsive glucose transporter (GLUT4). It may also form a type II integral membrane glycoprotein which is responsible for catalysing the final step in the conversion of angiotensinogen to angiotensin IV (AT4) in the body, hence its use as a renal marker (Gene, 2018).

Leucyl-cystinyl aminopeptidase may also be referred to as oxytocinase, placental leucine aminopeptidase (P-LAP), and insulin-regulated aminopeptidase (IRAP) (Elkins, et al., 2017). In humans, LNPEP plays a key role in the clearance of oxytocin during pregnancy (Elkins, et al., 2017). In pregnancy oxytocin (OT) is the most dominant uterotonic peptide hormone that plays a vital role in the regulation of labour (Nomura, et al., 2005). During pregnancy OT is synthesised by the mother, the fetus and the placenta where concentrations may be affected by fetal growth and environmental stressors. Usually, increased OT concentrations results in induced myometrial contractions and the onset of labour therefore, LNPEP is known to play a pivotal role in balancing degradation against production of OT in order to maintain pregnancy homeostasis (Nomura, et al., 2005).

Consequently, in cases of increased placental production of vasopressinase which occur in conditions such as PE and twin pregnancies, a temporary onset of diabetes insipidus may develop (Tkachenko, et al., 2014; Soma-Pillay, et al., 2016). A 40% increase in atrial natriuretic peptide, a polypeptide hormone secretion is usually the result of elevated volumes in the third trimester and the first week postpartum. Natriuretic peptide levels are generally higher in hypertensive pregnancies than normotensive pregnancies (Soma-Pillay, et al., 2016).

2.8 Cystatin C during pregnancy

Cystatin C is defined as “a non-glycosylated, 122 amino acid, 13.3-kDa protein belonging to cystatin protease inhibitors” (Murty, et al., 2013). It has shown promise as a replacement for serum creatinine in estimation of glomerular filtration rates (Murty, et al., 2013). Cystatin C has a low molecular weight and is a member of the cystatin superfamily of cysteine protease

inhibitors and is produced by all nucleated cells in the human body (Langlois, 2008). Furthermore, cystatin C is small in size and has a basic pH thus allowing it to be easily filtered at the glomerulus (Inker and Levey, 2014). "After filtration, approximately 99% of the filtered cystatin C is reabsorbed and catabolised by the proximal tubular cells. There is some evidence for the existence of tubular secretion as well as extrarenal elimination, which has been estimated at 15% to 21% of renal clearance" (Inker and Levey, 2014).

The role of cystatin C in pregnancy is to be used as a biomarker of kidney function in detecting renal impairment (Babay, et al., 2005). Although, the role of serum cystatin C as an endogenous marker of glomerular filtration rates has been highlighted, conflicting evidence prevails regarding its use in pregnancy (Beheiry, et al., 2015). A Sudanese study evaluated the use of serum cystatin C as an indicator of glomerular filtration rates in pregnant women (Beheiry, et al., 2015). Results of this study indicated that serum cystatin C levels was significantly higher in the preeclamptic group compared to the normotensive group (p = 0.000) and the non-pregnant group (p = 0.0001) respectively, whilst the mean glomerular filtration rate in preeclamptic cases was significantly lower than that of normal pregnant women and non-pregnant women (p = 0.0001) (Beheiry, et al., 2015). In another study, Obrenovic, et al., (2011), also concluded that cystatin C is not a reliable marker of kidney function in pregnancy because increased levels of cystatin C may be associated with a plethora of combined factors including endotheliosis, hormonal influence and alterations in glomerular filtration rates (Obrenovic, et al., 2011).

2.9 Fibronectin

Fibronectin is found in epithelial and endothelial cells and tissues (Letourneau, 2017). It is an adhesive glycoprotein and is widely distributed in the extracellular matrix (ECM) (Letourneau, 2017). The ECM is "a non-cellular three-dimensional macromolecular network made of collagens, proteoglycans, elastin, Fn, laminins and various other glycoproteins that bind to each other by adhesion receptors" (Theocharis, et al., 2015). The ECM structure of each tissue and organ undergoes constant remodeling which tends to be frequent in response to infectious agents, during wound repair and in many disease states (Daley, et al., 2007). Signals from cell surface receptors are transferred into extracellular matrices to trigger biochemical and biomechanical cues which are responsible for tissue morphogenesis, differentiation and homeostasis, thus regulating cellular functions (Frantz, et al., 2010; Theocharis, et al., 2015).

The three-dimensional structure of the ECM is divided into the basement membrane (BM) and the interstitial matrix (Kular, et al., 2014). Collagens, laminins and Fn make up the main elements of the BM and is less porous and denser than the interstitial matrix (Kular, et al., 2014). Transmembrane receptors/integrins are found between the BM and overlying cells and are responsible for cell shape and motility and facilitating ECM adhesion (Kular, et al., 2014). The interstitial matrix is found between connective tissue cells, and includes the collagens, elastin and Fn (Kular, et al., 2014). Although collagens make up most of the fibrous proteins in the ECM, Fn is responsible for the arrangement of the matrix structure (Frantz, et al., 2010; Kular, et al., 2014).

2.9.1 The structure of fibronectin

Fibronectin is arranged as a mesh like structure made of fibrils attached to integrins, which are important in attaching the intra cellular matrix to the ECM as well as transferring information to initiate cellular responses such as growth factor signaling to regulate cellular proliferation, cytoskeletal reorganisation and other responses that are necessary for cellular survival (Kular, et al., 2014). Fibronectin is produced in the form of a disulphide-bonded dimer which are broken down into subunits as Type I, II and III which are repeated within each subunit (Kular, et al., 2014). It is a dimer composed of two subunits which are folded into a series of functionally distinct domains separated by regions of two flexible polypeptide chains (Figure 2.6), and joined by a pair of C-terminal disulphide bonds (Alberts, et al., 2002). Fibronectin has a molecular weight of approximately 440 kDa containing Type I, II and III modules (Alberts, et al., 2002). Type I and II are stabilised by intrachain disulphide bonds and Type III modules are not, thus allowing for it to unfold due to its sensitivity to external mechanical forces (Alberts, et al., 2002; Wang, et al., 2016). Fibronectin is categorised as pFn which circulates in the blood stream and cFn which are created by fibroblasts (Kular, et al., 2014). Plasma Fn is soluble and sythesised by hepatocytes whereas, cFn is insoluble and produced by macrophages, endothelial cells and epithelial cells (Ekaidem, et al., 2011).

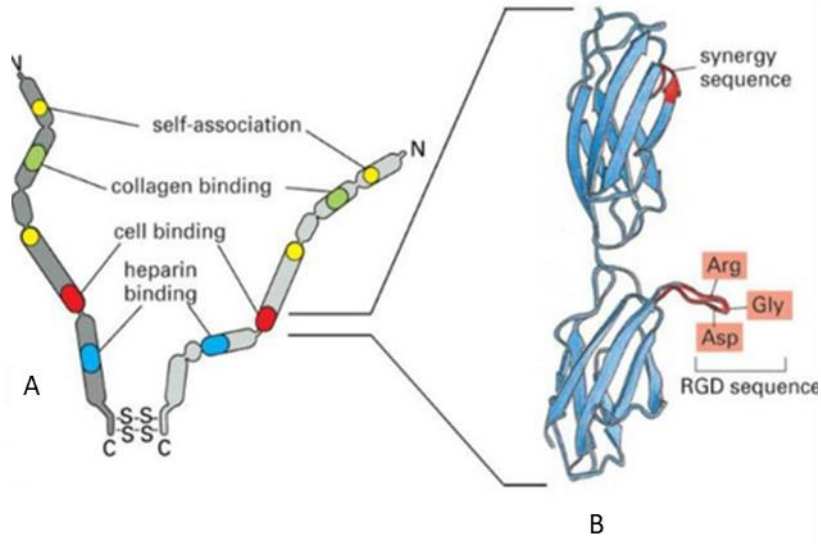

Figure 2.6: (A) represents the two polypeptide chains that resemble each other. Both chains have a length of approximately 2500 amino acids which are folded by a series of domains that are responsible for binding to a specific molecule or cell and is connected by flexible segments of polypeptides. (B) illustrates a three-dimensional structure of two Type III Fn modules. The tripeptide RGD and the synergy sequence forms part of the major cell binding site (Adapted from Alberts, et al., 2002)

2.9.2 Physiological role of fibronectin

Fibronectin is found in the BM of the ECM where it plays a role in cell adhesion and wound healing in response to injury (Kular, et al., 2014). In addition, Fn has also been known to play an important role in embryonic development (Kular, et al., 2014). Fibronectin is vital in tissue construction and reconstruction especially during physiological and pathological processes such as embryogenesis and fibrosis cancer. It is also responsible for wound healing which is made possible by the cell to cell and cell to matrix interactions of Fn (Ekaidem, et al., 2011; Kubow, et al., 2015 and Wang, et al., 2016). Wang, et al., (2016), supports this statement by further explaining that, different biological behaviors are either moderated by plasma or cellular Fn. Plasma Fn is commonly known for initiating blood clotting during early wound

healing whilst cFn is responsible for late wound healing, neovascularisation and angiogenesis (Wang, et al., 2016).

According to Ekaidem, et al., (2011), changes in Fn concentrations in body fluids and tissues have been identified in various disease conditions. Increased Fn levels in blood plasma have been reported in diseases such as diabetes mellitus, collagen vascular disease, renal and gastrointestinal cancer, rheumatoid vasculitis, chronic obstructive pulmonary disease, ischemic heart disease, acute myocardial infarction, coronary artery disease, trauma and hypertensive disorders of pregnancy such as PE (Ekaidem, et al., 2011). Although Fn has proven to be necessary during physiological remodeling processes its sometimes-abnormal accumulation and abundant presence within tissues have been associated with tumorigenesis, arthrosclerosis and fibrosis thus, explaining its possible role in certain disease states (Missirlis, et al., 2017). Fibronectin has therefore been thoroughly studied and widely used as coating for *in vitro* cell adhesion and migration research (Missirlis, et al., 2017).

2.9.3 Clinical use of circulating levels of fibronectin in pregnancy

The role of Fn has also been explored in studies comparing preeclamptic pregnancies with other diseased groups such as obesity and diabetes against control groups of normotensive pregnancies (Chavarria, et al., 2002; Ekaidem, et al., 2011). A Nigerian study evaluated pFn levels in 58 obese and 105 non-obese pregnant women as well as a control group of 60 non-pregnant women to determine a possible risk factor for PE (Ekaidem, et al., 2011). The study found the mean pFn levels to be lower in the non-obese pregnant women in trimester 1 than non-pregnant women by 24% (Ekaidem, et al., 2011). Furthermore, these levels were significantly higher in obese pregnant women compared to non-obese pregnant women in the

second and third trimesters ($p < 0.05$) and 28.57% of obese pregnant women developed PE at 18-24 weeks gestation (Ekaidem, et al., 2011).

An earlier study conducted in Mexico examined cFn levels throughout pregnancy in 26 preeclamptic pregnancies and 52 normotensive pregnancies which were matched from the same cohort that started off with 378 healthy nulliparous women (Chavarría, et al., 2002). It was reported that circulating Fn levels increased in both study groups however, the increase was significantly higher in women who had PE compared to those who did not ($p = 0.006$) with sensitivity, specificity, and positive and negative predictive values at 73%, 87%, 29%, and 98%, respectively, (OR = 16.1, 95% CI = 8.6-30.2) (Chavarría, et al., 2002).

Taking a different approach, Madazli, et al., (2005), evaluated 122 normotensive pregnant women in Turkey to assess if alterations in serum levels of Fn, PIGF and activin A could be detected in patients before they develop PE thus, determining the clinical utility of such markers (Madazli, et al., 2005). Results indicated that maternal serum levels during second trimester for Fn and activin A were significantly higher when compared to PIGF (Madazli, et al., 2005). Moreover, PIGF levels were significantly lower in women who subsequently developed PE compared to those who did not ($p = 0.001$) (Madazli, et al., 2005). The study concluded that, Fn, PIGF and activin A are all useful predictors of PE with PIGF having the highest predictive value, followed by activin A and then Fn (Madazli, et al., 2005).

Fetal Fn (fFn), a subtype of human Fn is found in the amniotic membrane and fluid and in the chorion-decidua interface where it is responsible for maintaining pregnancy in the female body

(Chen, et al., 2016). Fetal Fn testing has been adopted to predict preterm birth throughout the world. This is possible because fFn does not enter vaginal secretion towards the second and third gestational periods therefore, the determination of fFn content in vaginal secretion has been known to predict the occurrence of premature birth (Chen, et al., 2016). Abbott, et al., (2015), highlighted the benefit of fFn testing by stating that "fFn measurements using quantitative testing is a good negative predictor of spontaneous preterm birth in both symptomatic and asymptomatic high-risk women after approximately 22 weeks of pregnancy". In retrospect, a positive-negative result based on a threshold of 50ng/mL is generated (Abbott, et al., 2015).

Although fFn testing may have its benefits Esplin, et al., (2017), expressed some disadvantages of fFn testing. The sensitivity and suboptimal positive predictive value of these tests are generally low and there is a possibility of missing important risk discrimination due to the use of a single threshold for a continuous variable (Esplin, et al., 2017). In contrast, the use of these tests are based on their high negative predictive value to identify women who are unlikely to deliver preterm which is not always reliable (Liong, et al., 2014). Alternatively, the use of Enzyme Linked Immunosorbent Assay's to measure fFn has shown more reliable results. Immunoassay measurements have indicated that the risk of preterm birth is proportional to fFn concentration levels during pregnancy (Abbott, et al., 2015).

Literature proves that poor child and maternal health is directly linked to various pregnancy related disorders despite worldwide efforts to improve the quality of maternal and neonatal health and reduce child and maternal mortality (Requejo and Bhutta, 2015; Maternal and Child Health, 2017; Maternal Mortality, 2018). Contributing demographic, clinical, social and

environmental factors aggravates the situation particularly in LMICs where access to basic medical care is limited and medical resources and healthcare professionals are scarce. This is not the case in developed countries which explains the higher prevalence in child and maternal mortality and morbidity in LMICs. Concerns for the health of women and children throughout the world has led to the search for more cost effective, reliable and quick ways to prevent pregnancy related disorders and reduce pregnancy complications.

This research gave rise to the use of biomarkers as predictors for disease during early stages of pregnancy. Copeptin and Fn among various other biomarkers have been ranked as prominent markers for the prediction of pregnancy related disorders particularly PE. The use of CPP for the early detection of pregnancy related hypertensive disorders, PE in particular, has proven to be vital for risk stratification and testing therapies as a prevention mechanism (Cornelius, 2015; Karumanchi and Granger, 2015). Similarly, other studies have supported the use of Fn as a predictive marker for PE (Chavarria, et al., 2002; Ekaidem, et al., 2011).

Furthermore, these biomarkers have the added benefit of being cost effective with the ability to provide quick results however, further evaluation of these biomarkers are still required before they can be widely used in clinical settings. Scientists around the world have conducted multiple studies assessing circulating levels of these biomarkers in diseased cases of pregnancy as well as normotensive pregnancies to determine if the use of such biomarkers can be effective and reliable and their findings have shown some promising results (Table 2.1 describes these studies).

This study therefore highlights the role of Fn and CPP in conjunction with renal markers, cystatin C and LNPEP during pregnancy in an attempt to determine circulating levels throughout defined gestational intervals in HIV uninfected and HIV infected normotensive pregnancies. It also evaluates the association between BP, BMI and Hb levels against each measured biomarker.

Table 2.1: Relevant journal articles used in text

Author (year)	Biomarker	Origin	Study population and sample type	Research findings
			Copeptin	
Katan and Christ-Crain, (2010)	CPP	Switzerland	Review	Future intervention trials are needed to prove the value of CPP in improving the overall medical management of patients in different diseases.
Morgenthaler, (2010)	CPP	Germany	Review	There has been escalated progress in the use of CPP in cardiovascular and renal diseases. The study supports the use of CPP measurements in the context of therapeutic interventions.
Zulfikaroglu, et al., (2011)	CPP	Turkey	32 normotensive pregnant women, 32 mild preeclamptic pregnant women and 32 severe preeclamptic women. Plasma	Copeptin levels were 0.31±0.09 ng/mL in the normotensive pregnant group, 0.62±0.16 ng/mL in the mild pre-eclamptic group and 0.85±0.18 ng/mL in the severe pre-eclamptic group with a significant *P* value (*P* = 0.001).
Foda and Abdel-Aal, (2012)	CPP	Egypt	90 cases were included. They were divided into six groups: 1) Normal pregnancy near term, as a control group	The vaginal delivery groups had higher levels of maternal serum CPP than the elective cesarean section group (P < 0.01). Higher maternal serum CPP levels were found in cases with PE when compared to the normotensive cases.

			2) Primiparas who had vaginal delivery 3) Primiparas who had vaginal delivery and mild PE 4) Elective repeat cesarean section 5) Intrapartum cesarean section for indications other than fetal distress and 6) Intrapartum cesarean section for fetal distress. Serum	The maternal CPP levels during intrapartum cesarean section were higher than that during elective repeat cesarean section. There was a significant correlation between maternal CPP levels and the duration of the first trimester. In the presence of fetal distress, umbilical cord serum CPP levels were significantly higher than other groups.
Dobsa and Edozien, (2013)	CPP	Croatia	Review	Although CPP seems to be a promising biomarker for the prediction and diagnosis of many diseases it is still in early stages of research. It should therefore, be used as an adjunct with more specific biomarkers to increase diagnostic accuracy and prove its clinical usefulness.
Aydin, et al., (2013)	CPP	Turkey	44 pregnant women of which 15 were healthy lactating women, another 15 were lactating	-Copeptin concentrations were higher in colostrum than transitional and mature milk in healthy women and significantly higher in colostrum and transitional milk from women who had GDM.

			women with GDM and the remaining 14 were the controls, all aged between 26 and 32. Venous blood samples, colostrum and transitional and mature milk samples	
Tkachenko, et al., (2014)	CPP	USA	Review	The study concluded that, reversal of systemic vasodilation during pregnancy without subsequent activation of the RAAS may evoke a reversal of all the links in the chain of events in normal pregnancy adaptation thus leading to the risk of developing PE. A decrease of renal vasodilation decreases glomerular filtration rate.
Bolignano, et al., (2014)	CPP	Italy	Review	The study concluded that four criteria should be met in order for a biomarker to be useful in clinical practices (accuracy, simplicity, relevance and cost effectiveness).
Yeung, et al., (2014)	CPP	USA	Nulliparous pregnant women, 136 healthy pregnant women (control group), 169 preeclamptic cases, 92 cases of gestational diabetes, 101 cases of gestational hypertension, and 86 cases of preterm birth.	The median (IQR) of baseline log CPP levels among preeclamptic cases and controls measured on average at 16 weeks of gestation were 3.85 (2.57–6.30) and 3.15 (2.17–4.72) pmol/l, respectively. Baseline levels were significantly and positively associated with risk of PE before its diagnosis. Each log unit increase in baseline CPP was associated with increased risk of PE (OR = 1.61), with similar associations for preterm

			Serum	and term cases. There were no associations found for gestational diabetes and gestational hypertension.
Karumanchi and Granger, (2015)	CPP	USA	Review	The use of CPP for the early detection of pregnancy related hypertensive disorders, PE in particular, has proven to be vital for risk stratification and testing therapies as a prevention mechanism.
Santillan, et al., (2015)	CPP	USA	134 women (54 non-preeclamptic pregnant women, 50 preeclamptic pregnant women and 30 non-pregnant women). Plasma	Maternal plasma CPP was significantly higher in preeclamptic pregnancies compared to non-preeclamptic pregnancies and non-pregnant women. These concentrations still remained significant after clinical confounders such as age, BMI, chronic essential hypertension, twin pregnancies, diabetes and history of PE were controlled.
Lukaszyk and Malyzko, (2015)	CPP	Poland	Review	Copeptin has emerged as a new prognostic biomarker for various diseases.
Cornelius, (2015)	CPP	USA	Review	Copeptin is a new and promising biomarker that is specific for PE prediction and diagnosis.

Tuten, et al., (2015)	CPP	Turkey	4 groups of 20 pregnant women, 20 early onset PE, 20 late onset PE and 2 groups of 20 normotensive women as the control groups. Serum	The mean CPP levels were 0.92±0.57 ng/mL in the early onset preeclamptic group and 1.65±0.95 ng/mL in the late onset preeclamptic group. Both these values were higher compared to the control groups however, the difference was only statistically significant in the early onset PE group (P = 0.011). Copeptin levels were only associated with BP and gestational age.
Akinlade, et al., (2015)	CPP	Nigeria	3 groups of 30 pregnant women, 30 with mild PE, 30 with severe PE and 30 uncomplicated pregnancies. Serum	Copeptin levels were significantly higher in preeclamptic cases compared to controls.
Nissaisorakarn, et al., (2016)	CPP	USA	Review	The development of biomarkers to predict and diagnose PE would be extremely advantageous and important for the future.
Jadli, et al., (2017)	CPP	India	3 groups, women who subsequently developed PE (n = 33), Intra Uterine Growth Restriction (n = 81) and normal pregnancy outcome (n = 112) at 10-14 weeks of gestation. Serum and plasma	Women who developed PE presented with elevated levels of CPP compared to controls.

Joosen, et al., (2017)	CPP	Netherlands	175 women with uncomplicated pregnancies Plasma	Overall, CPP concentration levels increased during pregnancy.
Beljan, et al., (2017)	CPP	Croatia	400 nulliparous pregnant women >35 years. Serum	Only 40 women were analysed of which 7.5% developed PE with elevated levels of plasma CPP and 20% developed gestational hypertension. Copeptin was found to be statistically significant for PE prediction (p = 0.049, OR = 2.62, 95% Cl = 1.097-6.306).
			Fibronectin	
Chavarria, et al., (2002)	Fn	Mexico	26 preeclamptic pregnant women and 52 normotensive pregnant women. Plasma	Circulating Fn levels increased in both study groups however, this increase was significantly higher in women who had PE compared to those who did not (p = 0.006). Sensitivity, specificity, and positive and negative predictive values were 73%, 87%, 29%, and 98%, respectively; the odds ratio was 16.1 (95% CI = 8.6-30.2).
Madazli, et al., (2005)	Fn	Turkey	122 Normotensive pregnant women. Serum	Maternal serum levels during the second trimester for Fn and activin A were significantly higher when compared to PIGF. PIGF levels were significantly lower in women who subsequently developed PE compared to those who did not (p = 0.001).

Daley, et al., (2007)	Fn	USA	Review	There are gaps in the knowledge of cell-ECM scaffold interactions that need to be addressed by emerging technologies.
Frantz, et al., (2010)	Fn	USA	Review	An overview of the ECM discussed.
Ekaidem and Bolarin, (2011)	Fn	Nigeria	163 pregnant women who were either obese or non-obese and 60 non-pregnant women as the control group. Plasma and serum	The study found that the mean plasma Fn concentration of non-obese pregnant women was lower than non-pregnant women during first trimester but increased accordingly during the second and third trimesters. Overall Fn levels were higher in obese pregnant women than non-obese pregnant women ($p<0.05$) and 28.57% of the obese pregnant group developed PE at 18-24 weeks pregnancy. It was concluded that elevated Fn may be regarded as a risk factor for PE especially among obese women.
Liong, et al., (2014)	Fn	Australia	129 Women with singleton pregnancies admitted to the emergency department between 22 and 36 weeks of gestation presenting with symptoms of preterm labour. Cervical-Vaginal-Fluid (CVF)	The study found that fFn testing is less effective than albumin and vitamin D binding testing in predicting spontaneous preterm delivery in symptomatic women within 7 days.

Kular, et al., (2014)	Fn	UK	Review	The study has concluded that "knowledge of the age-related changes in the ECM and its implications for the wound healing process as well as potential of using the ECM as a scaffold for regenerative healing is only at the tip of the iceberg. Thus, the on-going trend of ECM research will prevail in the next few decades".
Abott, et al., (2015)	Fn	UK	1448 asymptomatic women at high risk of spontaneous preterm birth aged between 22 and 27 in their first trimester of pregnancy. CVF	Spontaneous preterm birth increased from 2.7%, 11.0%, 14.9%, 33.9%, and 47.6% with increasing concentration of fFn (less than 10, 10–49, 50–199, 200–499, and 500 ng/mL or greater, respectively). A threshold of 200 ng/mL had a positive predictive value of 37.7 (95% CI = 26.9–49.4) with specificity 96% (95% CI = 95.3–97.3). Women with a fFn concentration of less than 10 ng/mL had a very low risk of spontaneous preterm birth. Quantitative fFn discriminated risk of spontaneous preterm birth at less than 34 weeks of gestation among women with a short cervix (less than 25 mm); 9.5% delivered prematurely less than 10 ng/mL compared with 55.1% greater than 200 ng/mL ($p<.001$).
Chen, et al., (2016)	Fn	China	60 women with preterm delivery (preterm group) and 60 women with term delivery (control group).	Fetal Fn content significantly increased in cervical ripening of the preterm group while difference of fFn content in placenta was not significant. Increase of fFn content in cervical ripening was found to increase the

			Cervical ripening, placenta tissue and serum	expression of matrix metalloproteinases and inflammatory factors through the TLR4/NF pathway and SOCS3 pathway, thereby leading to premature delivery.
Theocharis, et al., (2016)	Fn	USA	Review	Fibronectin is a major component in the ECM.
Esplin, et al., (2018)	Fn	USA	9410 nulliparous women. CVF	Among the sample population, it was found that quantitative vaginal fFn and serial transvaginal ultrasound cervical length had a low predictive accuracy for spontaneous preterm birth hence, the findings did not support the use of fFn and cervical measurement tests in such study populations.

CHAPTER 3:

RESEARCH METHODOLOGY

3.1 Ethical considerations

This retrospective study made use of archived serum samples from normotensive pregnancies, which received Institutional Research Ethical Clearance (IREC) (RHDC approval: Appendix 1 and IREC 010/17: Appendix 2). This study formed part of a previously approved, larger prospective, descriptive cohort study which received both institutional (IREC 045/14: Appendix 3) and gatekeeper's permission to conduct research and access clinic records at the Cato Manor Primary Healthcare (PHC) facility (KwaZulu-Natal's department of health-HRKM 234/14: Appendix 4). Participation was strictly voluntary. All participants were informed of the study details in their preferred language without any deception or coercion. Written and verbal consents were obtained from all participants at the time of the study and all information was kept confidential. All samples and data collected are stored safely in a locked facility at the Faculty of Health Sciences, Durban University of Technology, KwaZulu-Natal, South Africa.

3.2 Study design

This was a retrospective study that used archived serum samples from normotensive pregnancies obtained from the previously approved prospective, descriptive cohort study.

3.3 Study area

The previously approved larger prospective, descriptive cohort study was conducted at the Cato Manor PHC facility in Durban, KZN. Durban is the major city of KZN and is the root of the eThekwini Metropolitan Municipality. Durban is also the central point of KZN and from it stems many towns and suburbs of which Cato Manor falls under. Cato Manor has an estimated population of 93 000 people of which approximately 51.25% are female and 48.75% are male (Cato Manor eThekwini Municipality, 2017). Cato Manor is home to some of the poorest residents of the urban poor population in KZN due to the high unemployment rate, making it a low resourced area. KwaZulu-Natal has a total of 11 health districts including the eThekwini district which is divided into 3 sub districts (eThekwini Health District, 2017). The district health services are jointly provided by the provincial department of health and the local government authority. According to the eThekwini Health District, (2017), there are 8 community health centers of which Cato Manor community falls under and 102 PHC facilities of which 43 are provincial and 59 are managed by the local authority (eThekwini Health District, 2017). Within the 102 PHC facilities there are 3 gateway clinics and 28 mobile units of which 12 are provincial and 16 are local authority (eThekwini Health District, 2017). Among all PHC facilities in the eThekwini district, there are some clinics that do not offer maternal services however, there are a total of 58 municipality PHC facilities and 41 KZN provincial administration PHC facilities that provide antenatal care services which includes the Cato Manor PHC facility (eThekwini Health District, 2017).

3.4 Study population and sampling strategy

The study population from the previously approved cohort study included 372 pregnant women from which, 41 normotensive pregnant women aged between 18-45 years were selected to be

the sample population for the current study. Convenient sampling was used to target pregnant women who reported at the Cato Manor PHC facility for their first antenatal visit.

3.4.1 Inclusion criteria

Participants were only recruited if they were pregnant and presented for their first antenatal visit to the Cato Manor PHC facility before 24 weeks of pregnancy, who agreed to participate in the study through written and verbal consent, who did not present with any disease or mental illness except for HIV, and who were within the specified age group. The chart review tool and the clinic's antenatal records were used to identify those participants that met the inclusion criteria and excluded those participants who did not.

3.4.2 Exclusion criteria

Pregnant women who did not fall under the specified age group, who presented for their first antenatal visit after 24 weeks of pregnancy, who were on any chronic medication (such as aspirin, warfarin, non-steroidal anti-inflammatory drugs) and who presented with diseases including mental illness, chorioamnionitis, chronic hypertension, eclampsia, abrubtio placentae, intra-uterine death, chronic diabetes mellitus, gestational diabetes, chronic renal disease, connective tissue disease, lipid lowering or anti-hypertensive disease, systemic lupus erythematous, sickle cell disease , anti-phospholipid antibody syndrome, thyroid disease, cardiac disease , active asthma and, pre-existing seizure disorders at initial stages were excluded. Identification of these diseases at initial stages prevented the study data from being contaminated by unrelated confounders.

3.5 Data collection and storage

Archived serum samples which were collected at three different gestational intervals (10-20 weeks, 22-30 weeks and 32-38 weeks) were utilised for this study. Samples were stored at -80ºC until analyses were done. The chart review tool (Appendix 5) was used, which captured demographic and epidemiological data such as, past and present pregnancies and diseases (including HIV), Bp, Hb levels, maternal age, birthweight, BMI and urinalysis data from demographic and epidemiological questionnaires (Appendices 6 and 7) of the previous cohort study. These factors as well as other relevant data were captured on a Microsoft Excel spread sheet in preparation for data analysis. All data collection tools including the participant questionnaires, and the chart reviews are stored in a locked facility at the Faculty of Health Sciences, Durban University of Technology.

3.6 Quantitation of serum levels of copeptin, fibronectin, leucyl/cystinyl aminopeptidase and cystatin C using Enzyme Linked Immunosorbent Assays

Archived serum samples obtained from each participant for each gestational interval was used to measure the circulating levels of CPP (Cloud-Clone Corp., 2018) and Fn (Elabscience, 2018). The circulating levels of both biomarkers were measured in accordance to the manufacturers protocol. Additionally, markers of renal function were evaluated by measuring serum levels of cystatin C (Elabscience, 2018) and LNPEP (Cloud-Clone Corp., 2018) using the commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits. These analytes were measured in order to determine the presence of any AVP degradation that may have affected CPP concentrations of the samples.

3.6.1 Principle of Enzyme Linked Immunosorbent Assays

The ELISA exploits the specificity of antibodies (Ab) and uses them to capture and quantify an analyte of interest from a given volume of sample which is done with intense levels of sensitivity (Chiswick, et al., 2015). Immunoassays are generally conducted in 96 well polystyrene microplates. Since the reactants of an ELISA are immobilised, separation of bound from unbound material is made simple thus enabling the measurement of specific analytes making ELISA's ideal for the measurement of biomarker concentrations (Overview of ELISA, 2018). There are four types of ELISA tests, direct, indirect, sandwich and competitive (Figure 3.1).

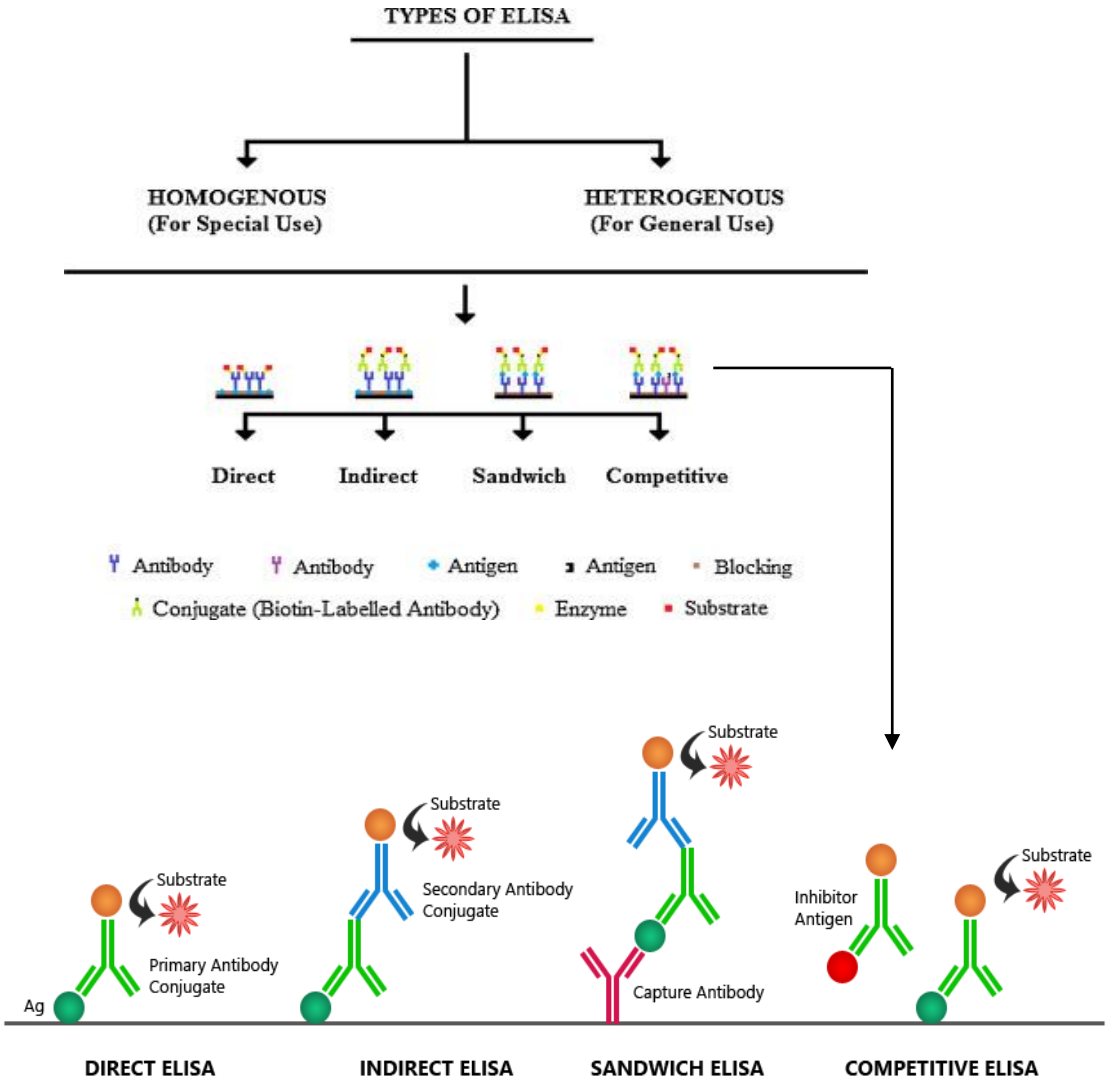

Figure 3.1: Structural comparability of ELISA formats (Adapted from ELISA fundamental principles, 2018)

Prior to conducting the immunoassays for the study, plate layouts were prepared as reference guides to ensure accurate transfer of all reagents and samples into the correct wells on the ELISA plates. Materials that were provided with each kit were stored at temperatures as specified in the user manuals upon receipt until usage. All samples and reagents for each assay were brought to room temperature prior to use as instructed by the user manuals. All immunoassays were done in duplicate, according to the manufacturers protocol and measured

using the Biochrom ELISA reader set at a wavelength of 450nm (Serial number: LJHGF-EDCA9-4KHS3-21KBQ-I0RW4, Model: SMR16.1, USCN kit Inc.).

3.6.2 The Enzyme Linked Immunosorbent Assay procedure for copeptin, fibronectin, leucyl/cystinyl aminopeptidase and cystatin C

All ELISA kits were used for the *in vitro* quantitative measurement of each marker which utilised the competitive inhibition technique (CPP) and the sandwich technique (Fn, cystatin C and LNPEP). The micro ELISA plates were first precoated with a monoclonal antibody specific to each marker. Standards and samples were pipetted into appropriate wells on the microplates according to specific plate layouts and placed into incubation at 37°C.

For CPP, a biotinylated detection antibody specific for this biomarker was added at the same point as the samples to launch a competitive inhibition reaction between biotin CPP and unlabelled CPP with the specific precoated antibody during the first incubation. In contrast, for LNPEP, cystatin C and Fn, the biotinylated detection antibodies specific for these markers were added to each of the 96 wells on the microplates after the first incubation and then incubated for a second time therefore, the ELISA technique for CPP had one less incubation period compared to the rest of the assays.

This was followed by the first wash process (×3) to remove free components using a specified wash buffer solution, thereafter followed by the addition of Avidin-Horseradish Peroxidase (HRP) conjugate to each well on the microplates and then incubated at 37°C. Following incubation, the plates were subjected to a second wash process (×5) to eliminate any remaining

free components. This was subsequently followed by the addition of the substrate solution to wells containing samples followed by the final incubation at 37°C. This was finally followed by addition of the stop solution to stop the enzyme substrate reactions, which was observed by a colour change from blue to yellow.

3.7 Measurement and calculations

The optical density (OD) for all immunoassays were measured spectrophotometrically at a wavelength of 450nm. All serum samples were diluted with phosphate buffer solution (PBS) using the following dilutions [1:2 dilution for LNPEP, cystatin C and CPP and a 1:5 dilution for Fn]. The OD values were directly proportional to the concentration values for LNPEP, cystatin C and Fn but, inversely proportional for CPP which was ascertained by the use of a standard curve. A trend line and equation were then derived which was used to calculate concentration levels of serum samples per gestational period for each marker (Examples of standard curves obtained for each marker are shown in figure 3.2). The calculated concentration levels were then multiplied by the dilution factors for each marker to obtain actual concentrations. Results of the assays are presented in pg/ml for CPP, LNPEP, cystatin C and Fn.

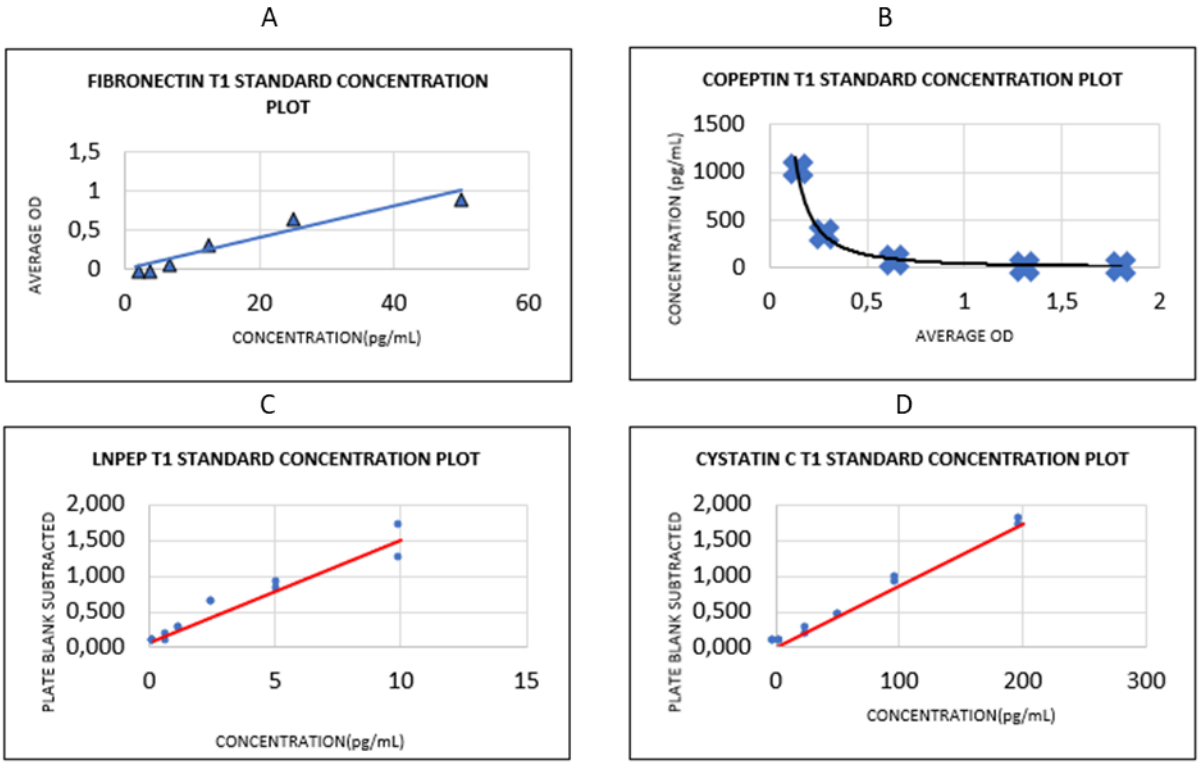

Figure 3.2: ELISA standard curves for Fibronectin (A), Copeptin (B), LNPEP (C) and Cystatin C (D) for the first gestational period

3.8 Data analysis:

All data including biomarker concentrations and demographic and clinical parameters were extracted from the chart reviews of all included participants and captured onto Microsoft Excel. Recoding and cleaning procedures were done through range and spot checking of the data. Both Excel and STATA version 15 were used for statistical analysis. Descriptive statistics were determined and represented as means, medians, standard deviations and inter-quartile ranges depending on how the data was distributed (mean and standard deviation for normally distributed data and median and inter-quartile range for non-normally distributed data). Human Immunodeficiency Virus status was identified as a dichotomous variable and thus evaluated as HIV positive and HIV negative. Body Mass Index and Hb levels were evaluated as both continuous and categorical variables. Body Mass Index was dichotomised as <24.99 kg/m^2 and

≥25 kg/m^2 and Hb levels were dichotomised as <11 g/dL and ≥11 g/dL. *P* values for the comparison of biomarker concentrations per trimester were obtained using the Kruskal Wallis test. The Wilcoxon Rank Sum test was used to compute *p* values for BMI, systolic and diastolic BP's and Hb levels stratified by HIV status. This test was also used to compute *p* values for all biomarker concentrations stratified by HIV status, BMI and Hb levels. Only *p* values <0.05 were considered statistically significant. The Spearman's Rank Correlation test was used to calculate *r* values to show the strength of correlation between biomarker concentrations and BMI, systolic and diastolic BP and Hb levels. A matrix of *p* and *r* values were obtained again using the Spearman's Rank Correlation test for the biomarker concentrations per trimester against themselves. Again, only *p* values <0.05 were considered statistically significant.

CHAPTER 4:

RESULTS

The demographic data for the specified study population is shown in Table 4.1. Forty-one (n=41) normotensive pregnant woman were recruited at three different gestational periods (10-20, 22-30 and 32-38 weeks). The mean maternal age of the study population was 26.40 years, whilst 3(7.31%) were smokers and 16(39.02%) consumed alcohol during pregnancy. Of the total sample, 26(63.42%) completed secondary education whilst 10(24.39%) proceeded to completing tertiary education however, only 21(51.22%) were employed.

Table 4.1: Demographic characteristics of maternal cohort with normotensive pregnancies (n=41)

Demographic Characteristics	Mean(SD) or n(%)
Age (years)	26.40(5.46)
Smoking	3(7.31)
Alcohol	16(39.02)
Education	
No Education	5(12.19)
Secondary School	26(63.42)
Tertiary Education	10(24.39)
Employment Status	
Employed	21(51.22)

The clinical characteristics of the study population is shown in Table 4.2. The mean gestational age at booking was 16 weeks. Maternal weight increased throughout pregnancy as expected (Table 4.2). The median BMI values increased between gestational periods 1 to 2 and thereafter remained constant until the end of gestation (Table 4.2). The median Hb levels fell between 11.00 and 11.30 g/dL during 10-38 weeks of pregnancy however, 56.00% were identified as anaemic. Anaemia was defined by haemoglobin levels <11.0 g/dL (Nandlal, et al., 2014).

Additionally, approximately 68.00% (n=28) of the total population were complicated with HIV of which approximately 64.00% (n=18) were on the Prevention of Mother to Child Transmission (PMTCT) programme. In terms of BP, both systolic and diastolic BP increased from 105.00-112.00 mmHg and 62.00-70.00 mmHg respectively, between gestational periods 1 to 2 followed by a decrease from 112.00-110.00 mmHg and 70.00-67.00 mmHg respectively between gestational periods 2 to 3. Of the total population, 39.00% were nulliparous and nulligravida.

Of the total study population, approximately 76.00% gave birth through vaginal delivery, with 90.00% of babies being born with birthweights greater than 2500g (Table 4.3). Normal APGAR scores ranging between 7-10 were noted for all newborn babies.

The circulating levels of CPP and Fn together with the concentrations of renal markers (LNPEP and cystatin C) are shown in Table 4.4. The concentrations of CPP increased between 10-20weeks (Table 4.4) and subsequently stabilised at 68.19pg/ml throughout pregnancy. In contrast, the levels of Fn increased further at 22-30 weeks (Table 4.4) and thereafter dropped to 100100pg/ml in the final weeks of gestation whereas, there was an overall reduction in both renal marker concentrations throughout pregnancy (Table 4.4).

Table 4.2: Clinical characteristics of maternal cohort with normotensive pregnancies (n=41)

Clinical Characteristics	**Median(IQR) or n(%)**
Gestational age at booking (weeks)	16(8)
Maternal Height (Mean(SD)) (m)	1.59(0.06)
Maternal Weight (kg)	
10-20 weeks	70.45(14.69)
22-30 weeks	74.15(23.15)
32-38 weeks	80.60(21.00)
BMI (kg/m^2)	
10-20 weeks	26.22(6.88)
22-30 weeks	30.64(10.68)
32-38 weeks	30.36(8.19)
Haemoglobin (g/dL)	
10-20 weeks	11.00(2.00)
22-30 weeks	11.10(1.50)
32-38 weeks	11.30(1.45)
Anaemia	23(56.10)
HIV Status	
Positive	28(68.29)
Negative	13(31.71)
On PMTCT programme	18(64.29)
Systolic Blood Pressure (mmHg)	
10-20 weeks	105.00(16.00)
22-30 weeks	112.00(21.50)
32-38 weeks	110.00(24.00)
Diastolic Blood Pressure (mmHg)	
10-20 weeks	62.50(13.00)
22-30 weeks	70.00(12.00)
32-38 weeks	67.00(16.00)
Parity	
Nulliparous	16(39.02)
Primiparous/Multiparous	25(60.98)
Gravida	
Nulligravida	16(39.02)
Primigravida/Multigravida	25(60.98)

Anaemia is defined by haemoglobin levels (<11.0 g/dL)

Table 4.3: Birth outcomes of maternal cohort with normotensive pregnancies (n=41)

Birth Outcomes	Mean(SD) or n(%)
Mode of Delivery	
Normal	31(75.61)
Caesarean Section	10(24.39)
Birthweight (g)	
<2500	4(9.76)
>2500	37(90.24)
APGAR (1min)	
7-10	41(100)
APGAR (5min)	
7-10	41(100)

Table 4.4: Serum levels of CPP, Fn, LNPEP and cystatin C Median(IQR)

	CPP(pg/mL)	Fn(pg/mL)	LNPEP(pg/mL)	Cystatin C(pg/mL)
10-20 weeks	71.22(14.12)	129510(20090)**	14.59(16.55)	419.49(110.58)**
22-30 weeks	67.95(10.72)	161900(112050)**	10.01(12.73)	381.04(123.54)**
32-38 weeks	68.19(11.14)	100100(48210)**	8.81(16.34)	247.71(66.88)**

***p<0.05 was considered statistically significant**

****p<0.01 was considered statistically significant**

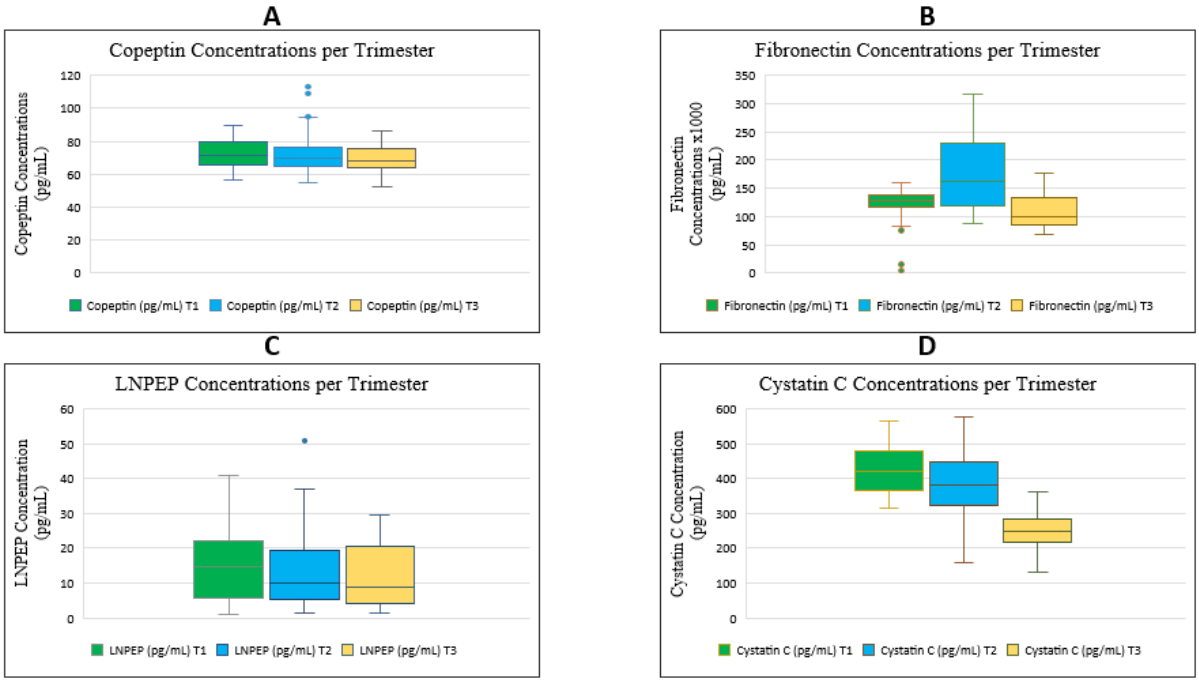

Figure 4.1: Median, quartile 1, quartile 3, IQR and outliers for biomarker concentrations per trimester: CPP(A), Fn(B), LNPEP(C) and Cystatin C(D)

Table 4.5 differentiates various clinical parameters of the study population based on HIV status, (HIV positive and HIV negative groups). The BMI measurements increased from 10 to 38 weeks gestation in both HIV positive and HIV negative women and thereafter, decreased in the third gestational period for both groups (Table 4.5). In contrast, the HIV negative group had higher systolic blood pressures during the first two gestational periods however, in the third gestational period increased values were observed among the HIV positive group (Table 4.5). Diastolic BP on the other hand, remained higher in the HIV negative population throughout pregnancy (Table 4.5). Haemoglobin levels were slightly higher in HIV positive women compared to HIV negative women throughout gestation (Table 4.5). Overall, the median

birthweights were higher amongst the HIV positive group compared to the HIV negative group (Table 4.5).

Table 4.5: Biomarker concentrations in normotensive pregnancies stratified by HIV status (n=41)

Median(IQR)

Study Characteristics	HIV -ve	HIV +ve
BMI (kg/m^2)		
10-20 weeks	25.27(6.29)	27.43(6.41)
22-30 weeks	27.19(6.57)	35.29(7.53)
32-38 weeks	26.56(4.51)	31.9(6.02)
Systolic Blood Pressure (mmHg)		
10-20 weeks	107.00(10.00)	105(19)
22-30 weeks	114.00(8.50)	105(22.5)
32-38 weeks	107.50(15.75)	110(20)
Diastolic Blood Pressure (mmHg)		
10-20 weeks	64.00(18.00)	61(10)
22-30 weeks	70.50(6.25)	68(15)
32-38 weeks	69.50(7.25)	63(14)
Haemoglobin (g/dL)		
10-20 weeks	10.80(2.13)	11(1.8)
22-30 weeks	10.65(1.13)	11.8(0.4)
32-38 weeks	10.90(0.77)	11.6(1)
Birthweight (g)		
<2500g	2390.00(one person)	2365.00(35.00)
>2500g	3171.00(470.00)	3243.00(270.00)

***p<0.05 was considered statistically significant**

****p<0.01 was considered statistically significant**

Table 4.6 illustrates the medians and IQR's of biomarker profiles stratified against HIV status and BMI. Copeptin levels were higher in the HIV positive group when compared to the HIV negative group during the first 20 weeks of gestation, however, during 22-30 weeks gestation, the HIV negative group demonstrated higher levels (Table 4.6). Thereafter, the concentrations increased in the HIV positive group during 32-38 weeks gestation (Table 4.6). Likewise, Fn levels demonstrated a similar pattern as CPP (Table 4.6). In contrast, circulating levels of LNPEP throughout gestation were higher in the HIV positive group compared to the HIV

negative group. Cystatin C levels on the other hand were higher in the HIV negative group between 10-20 weeks and 22-30 weeks gestation and declined during 32-38 weeks gestation (Table 4.6).

When biomarker profiles were stratified against BMI it showed higher CPP levels during the first 20 weeks of pregnancy amongst those with a BMI ≥25 kg/m^2 however, these CPP levels decreased as pregnancy progressed amongst those with a BMI ≥25 kg/m^2 (Table 4.6). Fibronectin levels were higher in the BMI <24.9 kg/m^2 group for the first two gestational periods but these levels increased in the BMI ≥25 kg/m^2 group in the third gestational period (Table 4.6). Both LNPEP and cystatin C concentration levels followed the same pattern as Fn throughout the three gestational periods (Table 4.6). For all biomarker profiles in gestational period 3, there were no values reported for the BMI <24.9 kg/m^2 group since all participants had an expected BMI of ≥25 kg/m^2 in their final weeks of pregnancy.

Table 4.6: Effect of HIV status and Body Mass Index on biomarker concentrations stratified by gestational periods in normotensive pregnancies (n=41)

Median(IQR)

Biomarker Profiles	HIV Status		BMI (kg/m^2)	
	HIV -ve	HIV +ve	<24.9	≥25
CPP (pg/mL)				
10-20 weeks	68.90(10.99)	71.89(14.30)	67,27(9,99)	69,87(10,72)
22-30 weeks	71.58(11.90)	66.26(9.84)	70,99(14,33)	65,93(6,59)
32-38 weeks	64.12(14.02)	68.98(11.43)	0(0)	66,31(8,80)
Fn (pg/mL)				
10-20 weeks	128220(21700)	129510(18.54)	130760(10020)	125130(22060)
22-30 weeks	183400(85300)	145700(109680)	179000(32950)	131700(50700)
32-38 weeks	100100(3660)	96460(57960)	0(0)	100520(52580)
LNPEP (pg/mL)				
10-20 weeks	6.34(10.63)*	16.84(20.24)*	12,54(4,76)	11,48(13,58)
22-30 weeks	8.54(10.72)	12.01(16.13)	6,97(4,17)	6,47(9,66)
32-38 weeks	3.60(2.47)*	14.76(17.84)*	0(0)	5,68(13,24)
Cystatin C (pg/mL)				
10-20 weeks	486.47(73.26)**	405.65(103.08)**	449,14(36,45)	437,86(122,67)
22-30 weeks	421.56(81.25)	360.88(181.95)	396,56(123,96)	378,85(137,03)
32-38 weeks	247.71(17.08)	253.39(100.31)	0(0)	245,73(66,17)

***p<0.05 was considered statistically significant**

****p<0.01 was considered statistically significant**

The circulating levels of CPP decreased as pregnancy progressed amongst those with Hb levels ≥11 g/dL, thereafter, a significant rise was observed at 32 to 38 weeks gestation but for those with Hb levels <11 g/dL, CPP levels continued to decrease throughout pregnancy (Table 4.7). In contrast, for both Hb levels <11 g/dL and ≥11 g/dL, the levels of Fn significantly increased between 10-20 weeks and 22-30 weeks gestation and then significantly decreased between 32-38 weeks gestation (Table 4.7). The circulating levels of LNPEP was higher amongst those with Hb levels <11g/dL in contrast to those with Hb levels ≥11g/dL in the first 30 weeks of pregnancy but higher in the last few weeks of pregnancy (Table 4.7). On the other hand, the levels of cystatin C decreased progressively throughout pregnancy for both Hb levels <11 g/dL and ≥11 g/dL groups (Table 4.7).

Table 4.7: Effect of Haemoglobin levels on biomarker concentrations stratified by gestational periods in normotensive pregnancies (n=41) Median(IQR)

Biomarker Profiles	Haemoglobin (g/dL)	
	<11 (g/dL)	**≥11 (g/dL)**
CPP (pg/mL)		
10-20 weeks	69.78(11.36)	73.68(10.81)
22-30 weeks	68.53(7.14)*	65.70(9.51)*
32-38 weeks	64.32(8.40)	71.04(13.37)
Fn (pg/mL)		
10-20 weeks	123640(20550)	129740(20800)
22-30 weeks	161900(104000)	183250(97500)
32-38 weeks	96470(58070)	100100(5890)
LNPEP (pg/mL)		
10-20 weeks	11.48(12.52)	11.72(14.14)
22-30 weeks	8.22(7.77)	10.02(16.35)
32-38 weeks	4.36(2.19)*	3.60(11.20)*
Cystatin C (pg/mL)		
10-20 weeks	413.44(103.84)	442.92(84.94)
22-30 weeks	361.04(179.38)	409.06(106.69)
32-38 weeks	245.73(85.60)	283.33(80.99)

***p<0.05 was considered statistically significant**

****p<0.01 was considered statistically significant**

The correlations between biomarker profiles and selected clinical characteristics are shown in Table 4.8. There was a weak inverse linear correlation between cystatin C and BMI in the second gestational period (r = -0.35). There were also significant inverse correlations between all biomarker profiles (CPP, Fn, LNPEP and cystatin C) and systolic BP at 32 to 38 weeks of pregnancy (Table 4.8) with LNPEP having the strongest correlation (r = -0.85, p = 0.0041). Fibronectin and systolic BP showed a significant but weak negative correlation at 10 to 20 weeks gestation (r = -0.36). Again, there were significant correlations between all biomarker profiles and diastolic BP in the third gestational period. In this case, correlations were inversely significant between diastolic BP and CPP, LNPEP and cystatin C and positively significant for

Fn (Table 4.8). These correlations showed a strong positive relationship for Fn (r = 0.71) and a strong inverse relationship for LNPEP (r = -0.85, p = 0.0038). Additionally, statistically significant correlations between Hb and CPP was observed during all three gestational periods with the first two periods having weak negative correlations (Table 4.8) and the third period having a fairly strong positive correlation (r = 0.68, p = 0.0397). Moreover, a significant but strong negative correlation was also identified between Hb and LNPEP at 32 to 38 weeks gestation (r = -0.88).

Table 4.9 shows correlations among biomarkers used in this study. There were no marked correlations identified but there were some relationships between certain markers. There were weak inverse correlations identified between CPP in gestational period one and Fn in gestational period 2 (r = -0.3425, p = 0.0284) as well as CPP and cystatin C (r = -0.3266, p = 0.0372). There were also weak inverse linear correlations noted between CPP in the third gestational period and Fn in the second gestational period (r = -0.5464, p = 0.0351) and a positive correlation between CPP and cystatin C in the first gestational period (r = 0.5429, p = 0,0365). Other weak positive correlations existed between Fn and cystatin C and LNPEP and cystatin C in the second gestational period (r = 0.4637 and 0.3258, p = 0.0023 and 0.0402) respectively.

Table 4.8: Spearman's Correlations between clinical characteristics and biomarker concentrations in normotensive pregnancies (n=41)

Clinical Factors	*CPP(pg/mL)*	*Fn(pg/mL)*	*LNPEP(pg/mL)*	*Cystatin C(pg/mL)*
BMI (kg/m^2)				
10-20 weeks	0.0200	-0.2000	0.04000	-0.1100
	0.9298	0.3264	0.8181	0.5592
22-30 weeks	0.0900	-0.1600	0.1700	-0.3500
	0.7752	0.5838	0.5905	0.2269
32-38 weeks	0.09	-0.26	0.09	0.20
	0.8294	0.4368	0.9801	0.4736
Systolic Blood Pressure (mmHg)				
10-20 weeks	-0.1400	-0.3600	-0.0900	0.05000
	0.4271	0.0439	0.6262	0.1802
22-30 weeks	0.0400	-0.1200	-0.1600	0.1900
	0.8968	0.6932	0.6163	0.5400
32-38 weeks	**-0.3700**	**-0.3300**	**-0.8500**	**-0.4000**
	0.3296	0.3912	0.0041**	0.2839
Diastolic Blood Pressure (mmHg)				
10-20 weeks	-0.0500	0.0800	**-0.3000**	0.2100
	0.7934	0.6676	0.0466*	0.2224
22-30 weeks	-0.0500	**-0.3000**	-0.0600	0.1700
	0.8712	0.3242	0.8542	0.5774
32-38 weeks	**-0.3700**	**0.7100**	**-0.8500**	**-0.4000**
	0.3274	0.7139	0.0038**	0.2817
Haemoglobin (g/dL)				
10-20 weeks	**-0.3100**	-0.0600	-0.1700	-0.1200
	0.0217*	0.7560	0.3391	0.4928
22-30 weeks	**-0.3100**	-0.0700	0.2300	-0.0300
	0.3516	0.8127	0.6821	0.2857
32-38 weeks	**0.6800**	-0.0400	**-0.8800**	0.0000
	0.0397*	0.9389	0.0085	0.4736

***p<0.05 was considered statistically significant, **p<0.01 was considered statistically significant**

Table 4.9: Spearman's Correlations between biomarker concentrations in normotensive pregnancies (n=4)

	CPP-1	CPP-2	CPP-3	FN-1	FN-2	FN-3	LNP-1	LNP-2	LNP-3	CYC-1	CYC-2	CYC-3
CPP-1	1 0	0.1048 0.5310	**0.5464** 0.0351*	0.1523 0.3613	**-0.3425** 0.0284*	-0.1464 0.6025	-0.1908 0.2322	-0.1610 0.3210	**-0.3929** 0.1475	-0.1860 0.2443	**-0.3266** 0.0372*	-0.0964 0.7325
CPP-2	0.1048 0.5310	1 0	0.2429 0.3831	0.2743 0.1108	-0.0977 0.5596	0.2964 0.2834	-0.0680 0.6852	-0.0421 0.8047	0.0214 0.9396	-0.1072 0.5216	0.0189 0.2318	-0.1929 0.4910
CPP-3	**0.5464** 0.0351*	0.2429 0.3831	1 0	0.2835 0.3260	**-0.5464** 0.0351*	**-0.3000** 0.2773	0.0214 0.9396	-0.2286 0.4126	-0.1464 0.6025	**0.5429** 0.0365*	**-0.3286** 0.2318	**-0.3071** 0.2655
FN-1	0.1523 0.3613	0.2743 0.1108	0.2835 0.3260	1 0	-0.2356 0.1545	**0.5780** 0.0304*	0.0986 0.5559	-0.0725 0.6696	0.1648 0.5733	-0.1927 0.2464	-0.0641 0.2012	0.1253 0.6696
FN-2	**-0.3425** 0.0284*	-0.0977 0.5596	**-0.5464** 0.0351*	-0.2356 0.1545	1 0	-0.1036 0.7134	-0.1518 0.3433	0.0118 0.9423	-0.3714 0.1728	**0.7005** 0	**0.4637** 0.0023*	-0.1857 0.5025
FN-3	-0.1464 0.6025	0.2964 0.2834	**-0.3000** 0.2773	**0.5780** 0.0304*	-0.1036 0.7134	1 0	0.0286 0.9195	0.0179 0.9496	0.2500 0.3688	0.2387 0.3977	0.3204 0.1728	0.2750 0.3212
LNP-1	-0.1908 0.2322	-0.0680 0.6852	0.0214 0.9396	0.0986 0.5559	-0.1518 0.3433	0.0286 0.9195	1 0	**0.5405** 0.0003*	**0.6786** 0.0054*	-0.2287 0.1903	0.3204 0.1728	0.2893 0.2957
LNP-2	-0.1610 0.3210	-0.0421 0.8047	-0.2286 0.4126	-0.0725 0.6696	0.0118 0.9423	0.0179 0.9496	**0.5405** 0.0003*	1 0	**0.8071** 0.0003*	-0.1069 0.5113	-0.0780 0.6280	**0.3036** 0.2714
LNP-3	**-0.3929** 0.1475	0.0214 0.9396	-0.1464 0.6025	0.1648 0.5733	-0.3714 0.1728	0.2500 0.3688	**0.6786** 0.0054*	**0.8071** 0.0003*	1 0	**-0.4929** 0.0620*	**0.3258** 0.0402*	**0.3036** 0.2714
CYC-1	-0.1860 0.2443	-0.1072 0.5216	**0.5429** 0.0365*	-0.1927 0.2464	**0.7005** 0	0.2387 0.3977	-0.2287 0.1903	-0.1069 0.5113	**-0.4929** 0.0620*	1 0	-0.1321 0.6387	0.1143 0.6841
CYC-2	**-0.3266** 0.0372*	0.0189 0.2318	**-0.3286** 0.2318	-0.0641 0.2012	**0.4637** 0.0023*	0.3204 0.1728	-0.0780 0.6280	**0.3258** 0.0402*	-0.1321 0.6387	**0.5228** 0.0005*	1 0	0.1143 0.6851
CYC-3	-0.0964 0.7325	-0.1929 0.4910	**-0.3071** 0.2655	0.1253 0.6696	-0.1857 0.5025	0.2750 0.3212	0.2893 0.2957	**0.3036** 0.2714	**0.3036** 0.2714	0.1143 0.6841	0.1143 0.6851	1 0

***p<0.05 was considered statistically significant, **p<0.01 was considered statistically significant**

CHAPTER 5:

DISCUSSION

In SA, government health services are free for children under the age of five and for pregnant and breastfeeding women however, the high death rates of new-born babies and mothers are still of great concern (UNICEF, 2017). Although the progress related to maternal and child health indicates a decline in child and maternal mortality rates, the United Nations' target for child and maternal mortality has not yet been attained in SA (Mulaudzi, et al., 2016). Based on this premise Dobsa and Edozien, (2013), has highlighted the need for cost effective and reliable biomarkers for the early detection of pregnancy related disorders. Copeptin and Fn has recently gained much interest as accurate prognostic biomarkers (Santillan, et al., 2014). It is believed that such biomarkers can provide reliable and reproducible quantitative data of practical value and measurement can be easy, rapid and inexpensive to perform (Strimbu and Tavel, 2010).

This study therefore, determined the circulating levels of CPP and Fn as well as LNPEP and cystatin C in a group of normotensive pregnant women. Our study also aimed to determine how the concentrations of each biomarker varied during pregnancy and if variations existed between the HIV positive and HIV negative groups as well as, whether maternal factors such as BMI, BP and gestational age or birthweight had any impact in their expressions. The data may assist in establishing the baseline levels of each biomarker within the selected study population. More than half of our study population tested HIV positive at their first antenatal visit. Of the 68% who were HIV positive, 64% were on the PMTCT programme (Table 4.2). These findings were similar to that of Sebitloane and Moodley who conducted a study on 1461 pregnant women of which 79% were HIV positive and 63% were on HAART (Sebitloane and Moodley, 2017). Of note, 56% of the entire population were anaemic which according to

Sebitloane and Moodley is a common expectation during pregnancy in HIV positive women (Sebitloane and Moodley, 2017). Less than half the population in our study were nulliparous and nulligravida (Table 4.2). As expected, the BMI of all women increased throughout pregnancy in our study however, no statistical significance was noted. On the other hand, a weak negative correlation between BMI and cystatin C in the second gestational period was noted (Table 4.8).

There were fluctuations in biomarker levels throughout pregnancy, with CPP, LNPEP and cystatin C showing an overall decrease between 10 and 38 weeks gestation. Our findings for CPP were consistent with that of Santillan and colleagues for the group of normotensive pregnant women assessed in their study (Santillan, et al., 2014). Our findings for LNPEP and cystatin C however, were inconsistent with Soma-Pillay and colleagues who stated that concentrations of these renal markers should increase as pregnancy progresses (Soma-Pillay, et al., 2016). Fibronectin on the other hand, increased in the second gestational period and then decreased in the third gestational period. The fluctuation in Fn concentrations reported in our study were inconsistent with Ekaidem and co-workers who reported an exponential increase in fibronectin concentrations throughout pregnancy amongst a similar study population (Ekaidem, et al., 2011). Additionally, variations in biomarker concentrations were also observed between the HIV positive group versus the HIV negative group. To our knowledge, our study is the first to measure circulating levels of CPP, Fn, LNPEP and cystatin C in normotensive pregnant women complicated with HIV. Our findings indicated slightly lower recordings for both systolic and diastolic blood pressures within the HIV positive group, except at gestational period 3, where systolic BP was higher amongst the HIV positive group which was substantiated by Macdonald-Wallis, et al., (2012). On the other hand, Hb levels were higher in the HIV positive group throughout pregnancy which was also reported by Nandlal,

et al., (2014). Overall, there were significant relationships found between CPP, Fn, LNPEP and cystatin C and BP in the third gestational period and some significant relationships with BMI and Hb levels as well.

In our study, the median CPP concentrations increased within the first gestational period of pregnancy and subsequently stabilised as pregnancy progressed. These findings were consistent with that of Santillan, et al., (2014), who measured CPP levels in normotensive and preeclamptic pregnancies and non-pregnant women. In their study Santillan and colleagues revealed that maternal plasma CPP was significantly higher in preeclamptic pregnancies compared to non-preeclamptic pregnancies and non-pregnant women (Santillan, et al., 2014). When we considered only their normotensive group the concentrations of CPP within this group stabilised from approximately 700 pg/mL in trimester 1 to about 650 pg/mL in trimester 2 (Santillan, et al., 2014). Moreover, these concentrations remained significant even after clinical confounders such as age, BMI, chronic essential hypertension, twin pregnancies, diabetes and history of PE were controlled (Santillan, et al., 2014). Despite the lack of PE cases in our study, our results corroborate their findings for the normotensive group however, the median CPP concentrations in our study were much lower than that reported by Santillan and colleagues (Santillan, et al., 2014). These findings could be attributed to the use of serum in comparison to plasma which was used by Santillan's group (Santillan, et al., 2014). It is possible that these variations may be linked to the higher blood volume in plasma as compared to serum.

We also measured the circulating levels of renal markers cystatin C and LNPEP as indicators of renal function during pregnancy. Both these markers decreased throughout pregnancy in

our study however, Soma-Pillay, et al., (2016), suggested a four-fold increase in LNPEP concentrations from mid pregnancy onwards in normal pregnancies. Similarly, Obrenovic, et al., (2011), demonstrated statistically significant increases in serum cystatin C levels during the third trimester of pregnancy (0.69mg/l in trimester 1, 0.78mg/l in trimester 2 and 1.21mg/l in trimester 3) amongst 109 normotensive pregnant women (Obrenovic, et al., 2011). These variations could be associated with changes in renal function and AVP degradation (Santillan, et al., 2014). Obrenovic and co-workers do not support the use of cystatin C as a reliable marker of kidney function in pregnancy because it is believed that elevated cystatin C levels during pregnancy can be directly linked to a combination of factors such as endotheliasis, hormonal influence and glomerular filtration rate alterations (Obrenovic, et al., 2011). Thus, the reduction in cystatin C and LNPEP concentrations throughout pregnancy in our study is inconsistent with that reported by Soma-Pillay and colleagues and Obrenovic and co-workers (Obrenovic, et al., 2011; Soma-Pillay, et al., 2016). These variations could be attributed to the high HIV status that was found in our study. Earlier studies report that HIV infection may be associated with kidney complications such as HIV associated nephropathy resulting in protein loss in the urine and reduced renal function (Lucas, et al., 2004). Although HAART slows down kidney function, it does not completely stop the loss of kidney function even with effective HIV suppression (Choi, et al., 2010).

The levels of Fn in our study increased at 22 to 30 weeks gestation followed by a significant decrease at 32 to 38 weeks' gestation, which was inconsistent with that reported by Ekaidem and co-workers (Ekaidem, et al., 2011). These investigators evaluated plasma Fn levels in obese and non-obese pregnant women and demonstrated increased Fn concentrations in both groups, with higher concentrations reported in obese pregnant women than non-obese pregnant women ($p<0.05$) (Ekaidem, et al., 2011). Likewise, Chavarria and colleagues also reported an

exponential increase in circulating plasma fibronectin levels during pregnancy (Chavarria, et al., 2002). Their study evaluated preeclamptic cases and normotensive controls and found that increased Fn levels were significantly higher in women who had PE compared to those who did not (p = 0.006, OR = 16.1 and 95% CI = 8.6-30.2) (Chavarria, et al., 2002). The variations observed in our study and that of Ekaidem, et al., (2011), and Chavarria, et al., (2002) may be associated with the use of serum versus plasma.

We also compared the median concentration levels for both CPP and Fn based on the HIV status of the study group. Our study demonstrated similar expression patterns for both CPP and Fn throughout pregnancy for both the HIV positive and HIV negative groups. The concentrations of both biomarkers were higher in the HIV positive group in the first 20 weeks of pregnancy however, their expression was higher in the HIV negative group between 22 to 30 weeks gestation. In contrast, their expression levels were higher in the HIV positive group when compared to the HIV negative group during the third gestational period. Our findings also highlight variations in the expression of circulating levels of cystatin C and LNPEP during pregnancy. Whilst median concentration levels of LNPEP was higher in the HIV positive group throughout pregnancy, the median cystatin C levels were higher in the HIV negative group for the first 30 weeks of pregnancy. To our knowledge, this is the first study that evaluated the levels of CPP, Fn, LNPEP and cystatin C in pregnancies complicated with HIV therefore, there are no substantial comparisons available.

Interestingly, 56% of our study population were anaemic, with most of the HIV positive study participants also being anaemic. Recent studies have indicated that anemia is one of the most common anomalies that HIV positive women face during pregnancy (Sebitloane and Moodley,

2017). In our population, most of the participants were on a PMTCT programme, which is associated with mitochondrial toxicity and irregular reticulocyte counts which may aggravate the risk of developing anaemia (Sebitloane and Moodley, 2017). An earlier South African study which investigated a pregnant cohort of 408 HIV positive women, also reported a 75.8% incidence of anaemia (Hb<11 g/dL) during pregnancy and postpartum (Nandlal, et al., 2014), however, most cases of anaemia during pregnancy in HIV infected women were resolved post-delivery (Nandlal, et al., 2014). This was in contrast to Sartorius, et al., (2013), who demonstrated that anaemia resolved during pregnancy before delivery in women receiving HAART as opposed to women who were not receiving HAART.

In our study, the median haemoglobin levels throughout pregnancy for the HIV positive group was ≥11 g/dL in contrast to the HIV negative group. Our findings corroborates that reported by Nandlal and coworkers (Nandlal, et al., 2014). It is possible that the higher Hb levels observed in the HIV positive group may be linked to the time of HAART implementation. It is likely that this particular group may have just started their course of HAART and the effects were thus minimal or none at all. Our findings also demonstrate a strong positive relationship between CPP and Hb levels in contrast to a strong inverse relationship between LNPEP and Hb levels between 32 and 38 weeks gestation. Copeptin is a hypothalamic stress hormone that is stimulated at increased stress levels (Katan and Christ-Crain, 2010). Since CPP and Hb levels significantly increased during the third gestational period it is likely that the increased stress levels during final stages of pregnancy may be a possible cause for the elevated circulating levels of both CPP and Hb. Moreover, elevated LNPEP levels may be in response to the 40% increase in the polypeptide hormone secretion of atrial natriuretic peptide during the third gestational period and the first week postpartum (Soma-Pillay, et al., 2016). Natriuretic peptide levels are generally higher in hypertensive pregnancies in contrast to normotensive

pregnancies, thus the inverse relationship between LNPEP and Hb levels is noteworthy since our study only included normotensive pregnancies (Soma-Pillay, et al., 2016). More recently, Ilan and colleagues reported that the expression of the haemoglobin gene HBA1 is regulated by stress, suggestive that an intracellular signal is essential for coping with stress, which is subsequently necessary for the production of haemoglobin and may be activated by haemoglobin itself (Ilan, et al., 2017).

Although HIV treatment may have a positive effect on anaemia in pregnant women, several studies have mentioned that HAART has a negative impact on fetal development causing many cases of stillbirth or low birthweight (Townsend, et al., 2007; Conroy et al., 2017). In our study, almost all babies had normal birthweights (>2500g), with median birthweights (for groups <2500g and >2500g) being slightly higher in the HIV positive group than the HIV negative group. Furthermore, all babies exhibited excellent APGAR scores at both 1 and 5 minutes. The small sample size in our study is a limitation. Since the study was conducted in a PHC facility this was to be expected because in such facilities, most women tend to access the closest regional hospital for delivery and many women recruited into this cohort were last to follow up. This limited our access to sample collection for all pregnancies that were initially recruited at the first antenatal booking. This impacted the sample number of babies evaluated at delivery making it difficult to draw a significant conclusion from such small numbers.

Our study demonstrated lower recordings for both systolic and diastolic BP's during the first 20 weeks of pregnancy, which subsequently increased towards the normal ranges during the second gestational period and thereafter dropped again in the third gestational period. This is in line with that noted by Macdonald-Wallis, et al., (2012), who reported that the BP levels in

normotensive pregnancies should decrease in the first 20 weeks of pregnancy and then increase until delivery at which point BP levels should stabilise. The variability observed in our study may be linked to our small sample size, nevertheless, our data reports a strong inverse correlation between systolic and diastolic BP's and LNPEP in the third gestational period and a strong positive correlation between diastolic BP and Fn during the same period. A significant inverse relationship was also noted between both systolic and diastolic BP's and CPP and cystatin C in the third gestational period as well however, these relationships were not as strong. Interestingly, a similar pattern was observed amongst those who were HIV negative when BP was stratified against HIV status however, in the HIV positive group, the median BP levels remained low in the first two gestational periods and thereafter increased in the third gestational period. These results were similar to that reported by Kalumba, et al. (2013), in KZN, South Africa, who highlighted slight increases in BP towards the end of gestation in normotensive pregnancies complicated by HIV infection. Limited studies exist regarding the relationship between BP and HIV status, since most studies focus on the impact of HIV on the incidence of hypertensive disorders of pregnancy such as preeclampsia (Moodley, 2013). Further investigation is therefore warranted on the direct relationship between BP and HIV status during pregnancy.

Human Immunodeficiency Virus is directly associated with a compromised immunity especially among those who are not on HAART (Moodley, 2013). A compromised immunity may result in decreased BP recordings among pregnant women thus reducing the risk of hypertensive diseases such as PE in such populations (Kalumba, et al., 2013). In our study, increased BP levels during the third gestational period may be attributed to the use of PMTCT amongst the HIV positive women, which may have strengthened their immune systems by the time they reached final stages of pregnancy. There is however, conflicting data regarding the

relationship between HIV infection and the development of hypertensive disorders such as PE (Wimalasundera, et al., 2002; Suy et al., 2006). These studies demonstrated that the risk of PE development was higher in women on HAART compared to those who were not. Overall their results suggest that HIV infected women irrespective of whether they were on HAART or not had a significantly lower risk of developing PE compared to uninfected women (Wimalasundera, et al., 2002; Suy, et al., 2006).

Study limitations include missing data and the small sample numbers which prevented us from conducting multivariate regression. Only 15 of the total participants were evaluated in the third gestational period. This could be due to late antenatal attendance or to the participants moving to different healthcare facilities closer to delivery. It is common for South Africans to provide false residential addresses to healthcare facilities for the purpose of being admitted to a more resourced facility with better treatment, care and aesthetics. It is possible that many participants of this study did the same so that they could be moved to a different facility. Having a smaller group in the third gestational period could have been the reason for many variabilities that were reported. Missing data for some variables could have contributed to uneven distributions.

CHAPTER 6:

CONCLUSION

In SA, poor child and maternal health remains a significant burden due to unequal development gains resulting in women and children in LMICs not benefitting to the same degree as those in developed countries (Maternal and Child Health, 2017; Tobar, et al., 2017). Focusing on SDG 3, the global maternal mortality ratio needs to decrease by 7.5% per annum but currently stands at only 2.4% (Tobar, et al., 2017). To help accelerate the process, the WBG has introduced 86 active projects focusing on antenatal care and delivery services to assist in improving child and maternal health on a global level which is inadequate (Tobar, et al., 2017). Based on this premise, it is believed that the investigation and implementation of new, cost effective and reliable biomarkers are needed to help alleviate this problem. This study therefore determined the baseline serum levels of copeptin and fibronectin together with leucyl/cystinyl aminopeptidase and cystatin C in normotensive pregnant women to help contribute to this field of research.

The results of our study demonstrated that 68% of the participants recruited were HIV positive, of which 64% were on HIV treatment. Our study also revealed a fluctuation in serum levels of CPP, Fn, LNPEP and cystatin C throughout pregnancy. An overall reduction was observed in the concentration values for CPP, LNPEP and cystatin C as pregnancy progressed. Fibronectin levels on the other hand, increased between 22-30 weeks gestation and decreased between 32-38 weeks gestation. In addition, the concentrations of CPP and Fn were higher in the HIV positive group during the first gestational period and then increased in the HIV negative group during the second gestational period however, during the third gestational period, the concentrations remained higher in the HIV positive group compared to the HIV negative group.

In contrast, LNPEP concentrations were higher in the HIV positive group throughout pregnancy whilst cystatin C levels were higher in the HIV negative group during the first 30 weeks of pregnancy.

Our findings also indicated an increase in BMI throughout pregnancy, whilst systolic and diastolic BP's within the HIV positive group decreased. Haemoglobin levels throughout pregnancy were also higher in the HIV positive group compared to the HIV negative group. Birthweights were normal however, there was a slight elevation in median birthweights in the HIV positive group than the HIV negative group.

The results of our study therefore, suggest that the investigated biomarker profiles fluctuate during pregnancy progression due to physiological changes that occur in pregnant women. Although this study was conducted only on normotensive pregnant women, it is expected for these baseline biomarker levels be used in comparison or as reference values with other pregnancy related studies of similar design and control groups.

6.1 Recommendations

- Since our study is one of the first studies to take initiative in evaluating CPP, Fn, LNPEP and cystatin C in HIV positive and HIV negative groups of normotensive pregnant women, it would be beneficial for future studies to further evaluate these biomarker profiles in similar study populations.

- The concentration values obtained for each biomarker in our study should also be used as reference values for verification purposes in other studies to explore their roles in various pregnancy related disorders.
- Since our study population was relatively small, the results of our study should be interpreted with caution and confirmed in larger cohort studies.
- Since research on these biomarkers are still limited, further evaluation is needed to increase their potential diagnostic accuracy and clinical use.

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APPENDICES

Appendix 1: Ethical Clearance

7 November, 2017

Student No: 21108604

Ms K Deepnarain
37 Blenford Crescent
Sunford
Phoenix
4068

Dear Ms Deepnarain

MASTERS IN HEALTH SCIENCES

I am pleased to advise that:

1. The Research and Higher Degrees Committee approved the following:

    (i) Your research proposal and dissertation title, being:

Epidemiological evaluation of circulating levels of copeptin and glycosylated fibronectin during pregnancy.

Please note: ANY PROPOSED CHANGES in the DISSERTATION TITLE require the approval of your supervisor and the Faculty Research Committee.

    (ii) Supervisor – **Dr N Govender**
    (iii) Co-Supervisor – **Professor P Reddy**

2. Your request for funding totalling **R 10 000.00** subject to any literature referred to in Section A of the PG 4a form being accessioned by this University, and any equipment purchased shall become the property of the department.

NOTE: - This funding is not paid directly to you but is controlled by the Faculty. Any proposed changes to this funding allocation needs the approval of your supervisor, and Faculty Research Committee

The University Research Committee has stipulated that:

(a) Ownership of any patent registered in respect of the results of your Master's Degree in Technology studies is retained by you as the initiator of the project;

(b) Should you make any Drift from the results of your Master's Degree in Technology studies, you will be required to repay pro rata, the **R 10 000.00** investment which the

University Research Committee has made in approving your request for funding;

(c) If the Durban University of Technology provided the equipment/materials for the creation of artefacts, this cost would be refunded to the University if such artefacts were sold and

(d) Durban University of Technology is given first refusal in respect of any possible future sale by you of any patent that may be registered in respect of your said project.

(e) All journal articles, referenced in your dissertation, are to accompany your ring-bound copies when submitting for examination purposes.

May I remind you that notwithstanding Rule LX.CM2, if a student fails to obtain the Masters Degree within two years of first registering for the fifth year, re-registration may be denied. The Academic Board may refuse to renew such registration or may impose any conditions it deems fit.

Should you experience any problems relating to your research studies, your supervisor must be informed as soon as possible. If the difficulty persists, you must then approach your Head of Department and thereafter the Dean of the Faculty.

Yours sincerely

Mr S Reddy
FACULTY RESEARCH OFFICER

12/01/2018

Student's signature in acceptance of the conditions contained herein.

Date:

Appendix 2: IREC010/17

DUT
DURBAN
UNIVERSITY OF
TECHNOLOGY

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

9 February 2017

IREC Reference Number: **REC 14/17**

Dr N Govender
Department of Basic Medical Sciences
Faculty of Health Sciences
Durban University of Technology

Dear Dr Govender

ROLE OF BIOMARKERS IN EARLY DETECTION OF PREECLAMPSIA IN UNDER RESOURCED COUNTRIES

I am pleased to inform you that Full Approval has been granted to **Phase One** of your proposal REC 14/17.

The Proposal has been allocated the following Ethical Clearance number **IREC 010/17**. Please use this number in all communication with this office.

Approval has been granted for a period of two years, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the IREC. This form must be submitted to the IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Yours Sincerely

Professor J K Adam
Chairperson: IREC

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2017 -02- 09
INSITUTUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA

Appendix 3: IREC45/14

Institutional Research Ethics Committee
Faculty of Health Sciences
Room M5 49, Hazelfield School Site
Gate 6, Ritson Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2900
Fax: 031 373 2407
Email: lenahsd@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

15 July 2014

IREC Reference Number: **REC 34/14**

Prof M N Sibiya
Department of Nursing
Faculty of Health Sciences
DUT

Dear Prof Sibiya

A multi-staged multi-disciplinary health care approach in reducing maternal morbidity and mortality rates in a selected district in KwaZulu-Natal

I am pleased to inform you that Provisional Approval subject to piloting of the data collection tools has been granted to your proposal REC 34/14.

The Proposal has been allocated the following Ethical Clearance number **IREC 045/14**. Please use this number in all communication with this office.

Approval has been granted for a period of one year, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the IREC. This form must be submitted to the IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC SOP's. In addition, you will be responsible to ensure gatekeeper permission.

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Please note that you may continue with validity testing and piloting of the data collection tools. Research on the proposed project may not proceed until IREC reviews and approves the final documents. If there are no changes to the data collection tools, kindly notify IREC in writing.

Appendix 4: HRKM234/14

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM 234/14
Enquiries : Mr X Xaba
Tel : 033 – 395 2805

Dear Prof MN Sibiya

Subject: Approval of a Research Proposal

1. The research proposal titled 'A multi-staged multi-disciplinary health care approach in reducing maternal morbidity and mortality rates in a selected district hospital in KZN' was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Cato Manor for a period of three years.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200 and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 11/02/14

Appendix 5: Chart Review Tool

CHART REVIEW

Study ID: _____

CRITERIA	
Age	
Race	
Parity	
Gravida	
HIV status	
History previous pregnancy	
• Anemia	
• Congenital abnormalities	
• Eclampsia	
• PPH	
• APH	
• Other. State……..……..	
Chronic medical conditions	
• HIV and AIDS	
• TB	
• Diabetes	
• Hypertension	
• Other, State……..……..	
Current antenatal data:	
• Last normal menstrual period	
• Estimated Delivery Date	
• Gestational age at booking (weeks)	
• Current gestational age	
• Ultrasound done during this pregnancy	
• Maternal height	
• Weight	
• BP (each visit)	
• Maternal Heart examination	
• Urinalysis test	
• Hemoglobin test	
• Rh factor test	
• Calcium supplements given	
• Folic supplements given	
• Date for 2nd Trimester Visit	
• Date for 3rd Trimester Visit	

Appendix 6: Demographic Questionnaire

STUDY ID

1. SOCIO-DEMOGRAPHIC QUESTIONNAIRE

This questionnaire covers certain aspects of your life, including work and personal details, health and illness, lifestyle and social life that is relevant to health. The answers to these questions will be kept strictly confidential and the information will not be identifiable on any reports or publications.

1. GENERAL INFORMATION

Fieldworker name: _____
Date: _____

Please answer all questions by marking the correct answer with **X,** except where otherwise indicated.

Where do you live?

.....

2. PERSONAL INFORMATION

2.1 Your role in the family

Daughter	Mother	Grandmother	Other Specify

2.2 When were you born? Year: Month: Day:

2.3 How old are you? _____ years

3. ACCOMMODATION AND FAMILY COMPOSITION

3.1 Do you live in?

Town/City	Farm	Squatter camp	Rural village	Hostel	Township	Other, specify……..

3.2 How are you currently living?

Homeless	
Living with relatives	
Living with friends	
Hostel accommodation	
Squatter home	
Rented house/flat	
Own house/flat	
Employees Properties	
Other, specify…….	

3.3 Do other people live in the house with you?

Yes	No

3.4 How many people are permanent residents living in the house with you? (Only if these people eat and sleep in this house at least 4 days a week?)

1	2	3	4	5	6	7	8	9	10	10+

3.5 How long have you been staying permanent in this house?

< 1 year	1-5 years	>5 years

3.6 In what type of house are you staying?

Brick	Clay	Wood	Tin/shack

3.7 How many rooms does your house have?

1 room	2 rooms	3 rooms	4 room	>5 rooms

3.8 Are there other houses/shacks within the same yard of the main house?

Yes	No

3.9 Do you have the following facilities/ services at home?

3.9.1 Water

Tap in the house	
Tap outside the house (in yard)	
Borehole	
Spring / river / dam water	
Fetch water from elsewhere	

3.9.2 Toilet facilities

None	
Pit latrine	
Flush / sewage	

Bucket system	
Other, specify…….	

Waste removal	Yes	No	3.9.3
Tarred road in front of house	Yes	No	3.9.4
Gravel road in front of house	Yes	No	3.9.5
Access to electricity	Yes	No	3.9.6

3.10 To what extent do you have problems with the state of your house (e.g. size, repairs, damp, etc.)?

……
……

3.11 Do you have problems with the following?

Mice/ Rats	
Cockroaches	
Ants	
Flees	
Mosquitoes	
Geckos	
Frogs	
Snakes	
Bed Bugs	

3.12. What is the floor inside your house made of?

Cement	
Tiles	
Carpet	
No floor	
Sand/mud	
Dung	
Other, please state	

4. WORK STATUS AND INCOME

4.1. Are you currently employed?

Yes	No

If YES, go to Question 4.5.

4.2. If NO, how would you describe your current status (tick one box only)?

Unemployed	Vendor	Housewife	Student	Other, specify……

4.3. Are you actively looking for paid employment at the moment?

Yes	No

4.4. How long have you been unemployed?

< 6 months	6-12 months	1-3 years	> 3 years

4.5. If YES (question 4.1) is your current job a:

Permanent position	Temporary position	Fixed term contract	Other, specify……..

4.6. Are you doing part time jobs as a second job on weekends and school vacations?

Yes	No

4.7 What is the exact title of your current job?
(Including self-employed)

4.8. What is the total income in the household per month?

< R1500	R1501-R3000	R3001-R5000	R5001-R7000	R7001-R9000	R9001-R11 000
R11 001-R13 000	R13 001-R15 000	R15 001-R17 000	R17 001-R19 000	R19 001-R21 000	> R21 000

4.9. Please specify the monthly income in the household (if willing)……..

4.10 Do you receive any of the South African Government social grants?

Child grant	Disability grant	Foster grant

4.11. How often does it happen that you do not have enough money to buy food? for you and your family?

Always	Often	Sometimes	Seldom	Never

4.12. How many people e.g. partner, relatives and others (including yourself) contributed to your household income from any source, (including wages/salary from paid employment, money from second or odd jobs income from savings investments, pension, rent or property, benefits and or maintenance etc.) in the last 12 months?

People	0	1	2	3	4	5	6	7	8	9

4.13. How often do you buy food?

Every day	Once a week	Once a month	Other, specify……

4.14. Where do you buy food?

Tuck shop	Street vendor	Wholesalers	Supermarket	Other, specify……

4.15 What type of transport do you use to get around?

Taxi	
Bus	
Train	
Own car	
Bicycle/ Motorbike	
Other Specify	

4.16 How much money is spent on food PER MONTH? (Tick only one box)

R 0 – R 500	R 501 – R 1000	R 1001 – R 1500	R 1501 – R 2000	R 2001 – R 2500	R 2501 – R 3000	> R 3000	I do not know

5 EDUCATION AND LANGUAGE

5.1. What is your highest education level?

None	Primary School	Standard 8	Standard 10	College/FET	Other post school

5.2 What language is spoken mostly in the house?

Zulu	Xhosa	English	Afrikaans	Other, specify……

5.3 Do you have any other children of your own?

Yes	No

Number: ……..

5.3 How many of your children have birth certificates?

None	1	2	3	4	5	6	7	8	All

5.4 How many of your children have completed their immunisation schedule?

None	1	2	3	4	5	6	7	8	All

5.5 Have any of your children died in the past?

Yes	No

Reason: ……..

5.6 Number of children attending school

None	1	2	3	4	5	6	7	8	All

5.7 How do the children get to school?

Walk	Bus	Taxi	Parents car	Other, specify…….

Food practices in the household

Tick one block for every question:	Father	Mother	Sibling	Grandma	Grandpa	Aunt	Uncle	Cousin	Friend	Other
5.8 Who is mainly responsible for food preparation in the house?										
5.9 Who decides on what type of food is bought for the household?										
5.10 Who is mainly responsible for feeding/serving the children?										
5.11 Who is the head of this household?										
5.12 Who decides how much is spent on food?										

5.13 How many meals do you eat per day?

0	1	2	3	> 3

5.14 Where do you eat most of your meals?

Home	Friends	Work	School	Other, specify…….

5.15 Where do your children eat most of their meals?

Home	Friends	School	Other, specify…….

6. ASSETS

6.1 Does your home have the following items and how many?

	Yes
Electrical stove	
Gas stove	
Primus or paraffin stove	
Microwave	
Hot plate	
Radio	
Television	
Refrigerator	
Freezer	
Telephone/ Cell phone	
Bed with mattress	
Mattress only	
Lounge suite	
Dining room suite	

Electrical iron	
Electrical, kettle	
Car	
Bicycle	
Motorbike	

6.2 What type of fuel do you usually use for food preparation?

Wood fire	Paraffin	Electricity	Gas	Coal/Charcoal	Other, specify……

6.3 What type/s of material are your pots made off (tick all relevant options)?

Cast iron	Aluminium	Stainless steel	Clay	Other, specify……

Thank you very much for your co-operation. We appreciate the time.

Appendix 7: Epidemiological Questionnaire

STUDY ID

2. EPIDEMIOLOGY 1ST TRIMESTER

Thank you for agreeing to be part of this study and for taking the time to fill out this questionnaire with us.

Please read this before starting.

- It's your choice whether or not to do the survey.
- Your answers will be kept **confidential**.
- Whether or not you answer the questions will **not** affect your health care or any benefits you may get.
- You can skip questions you don't want to answer.
- Please put a cross (X) next to your chosen answer.

Recruitment Assistant Name Date	
What is your marital status?	☐1 Married ☐2 Living together ☐3 Single ☐4 Divorced ☐5 Separated ☐6 Widow ☐7 Other _____
Just before I became pregnant… **(Please tick only one)**	☐1 I wanted to have a baby ☐2 I had mixed feelings about having a baby ☐3 I did not want to have a baby
When you got pregnant with your new baby, were you trying to get pregnant?	☐1 Yes ☐2 No
Was this pregnancy forced?	☐1 Yes If yes, please comment; ☐2 No

Have you/your partner at any time during the last year used the following methods to avoid becoming pregnant? ***(Fill in all that apply)***	☐$_1$ Implant ☐$_2$ Injection ☐$_3$ Pill ☐$_4$ Traditional methods ☐$_5$ Condom ☐$_6$ IUD ☐$_7$ Withdrawal ☐$_8$ Spermicides (foam, suppositories, cream) ☐$_9$ Safe period ☐$_{10}$ Withdrawal ☐$_{11}$ No such methods ☐$_{88}$ Other If other, please specify:
Did you use any home remedies to stop your pregnancy?	☐ Yes ☐ No If yes, please specify:
What were your reasons for not using a contraceptive? **Check <u>all</u> that apply.**	☐$_1$ I didn't mind if I got pregnant ☐$_2$ I thought I could not get pregnant at that time ☐$_3$ I had side effects from the birth control method I was using ☐$_4$ I had problems getting birth control when I needed it ☐$_5$ I thought my husband or partner or I was sterile (could not get pregnant at all) ☐$_6$ My husband or partner didn't want to use anything ☐ Religious purposes/beliefs ☐$_7$ Other _____
What age did you become sexually active?	Age:
What age did you first use contraception?	Age:
Have you ever heard of emergency contraception (EC) or the morning after pill (MAP) before?	☐$_1$ Yes ☐$_2$ No
Have you ever used EC/MAP before?	☐$_1$ Yes ☐$_2$ No
If Yes, how many times have you used EC/MAP in the year previous to you falling pregnant?	☐$_1$ Once ☐$_2$ 2-4 times ☐$_3$ 5-10 times ☐$_4$ over 10 times Other_____
What are the time frames to use EC?	☐$_1$ within 12 hours of sexual intercourse ☐$_2$ within 24 hours of sexual intercourse ☐$_3$ within 3 days of sexual intercourse ☐$_4$ within a week of sexual intercourse ☐$_5$ Other _____ ☐ I don't know
How many months pregnant were you when you discovered you were pregnant?	_____months
How many months pregnant were you when you had your first antenatal visit?	_____months _____I don't know
Did you receive antenatal care as soon as you found out you were pregnant?	☐$_1$ Yes – From where? ☐$_2$ No – Please comment why not?

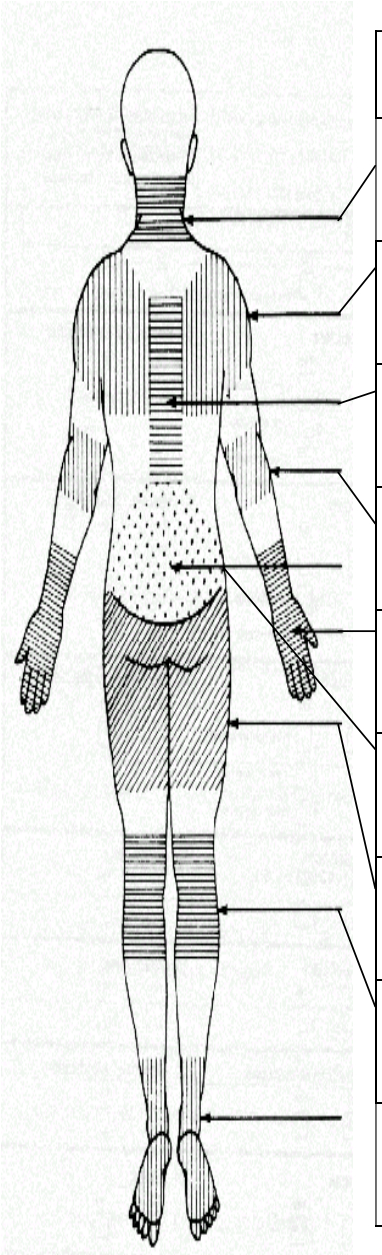
Did any of these things keep you from getting antenatal care at all or as early as you wanted? (You may choose more than one reason, if applicable)	☐1 I couldn't get an appointment when I wanted one. ☐2 I had no money for transportation to get to the clinic. ☐3 I had too many other things going on. ☐4 I was trying to hide this pregnancy from my family ☐5 I didn't know that I was pregnant ☐6 I didn't want anyone else to know I was pregnant ☐7 I didn't want antenatal care ☐8 I could not take time off from work ☐9 I had no one to take care of my other children
How many times have you been pregnant (Include all pregnancies that ended in abortion, miscarriage or stillbirth)	_____pregnancies
Did you experience any of the following with your ***previous pregnancies*** (if yes, then state number of times next to it)	☐1 Abortion Number ___ ☐2 Miscarriage Number___ ☐3 Stillbirth Number ___ ☐4 Early neonatal deaths Number___ ☐5 Ectopic Pregnancy Number___
How many live children do you currently have?	_____ Ages_____ _____
Have you had any of the following problems during ***previous pregnancies?*** *(Tick all that apply.)*	☐ Serious nausea and vomiting ☐ Hypertension ☐ Threatened abortion ☐ Diabetes during pregnancy ☐ Sugar in urine ☐ Problems with incontinence ☐ Bleeding from the vagina ☐ Other:
Do you know what a pap smear is?	☐1 Yes ☐2 No
Have you ever had a pap smear?	☐1 Yes ☐2 No
How often have you had a pap smear?	☐1 Once a year ☐2 Once in 2 years ☐3 Once in 3-5 years ☐4 Don't remember
Why is it important to have a pap smear?	_____ _____
Select all the conditions that you were treated by a doctor for **before you became pregnant?**	☐ Diabetes ☐ Heart problems ☐ High cholesterol ☐ High blood pressure ☐ Hypothyroidism ☐ Hyperthyroidism ☐ Anaemia ☐ Ulcers – if so, please state where… ☐ Cancer ☐ TB ☐ Endometriosis

	☐ Ovarian cysts ☐ Epilepsy ☐ HIV ☐ Stress – please comment… ☐ Depression/Anxiety ☐ Fainting attacks ☐ Fits ☐ Headaches ☐ Neck pain ☐ mid back pain (pain between the shoulders) ☐ Low back pain ☐ Pelvic pain (pubic or groin) ☐ Hip pain ☐ Knee pain ☐ Foot and ankle pain ☐ Shoulder pain ☐ Elbow pain ☐ Wrist and hand pain		
Are you currently taking any medication?	☐$_1$ Yes ☐$_2$ No		
If yes, what is the medication for, where did you get it from and how often are you taking it?	Medication for?	Where from?	How often?
	_____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____	_____ _____ _____ _____ _____ _____ _____ _____
Did you use medications before becoming pregnant?	☐$_1$ Yes ☐$_2$ No		
Please indicate which of these medications you have used (at a frequency of more than once) over the 12 months **prior to your pregnancy?**	☐ Antibiotics ☐ Pain Killers ☐ Immune Boosters ☐ ARV ☐ TB Drugs ☐ Isihlambezo ☐ Chronic medication ☐ Traditional Medication ☐ Other Please Specify_____		
Are you currently taking any of the medications indicated above?	☐$_1$ Yes ☐$_2$ No		

	How often? _____
Have you used any recreational drugs prior to your pregnancy?	☐$_1$ Yes ☐$_2$ No How often? _____
Do you use any of these drugs now during your pregnancy?	☐ Dagga ☐ Wonga ☐ heroine ☐ cocaine ☐ sugars ☐ other…
Have you been smoking/using snuff while pregnant?	☐ No ☐ Sometimes ☐ Daily
Do you smoke?	☐ No ☐ Yes If yes, how many _____ cigarettes per week _____ cigarettes per day
Have you ever had alcohol?	☐$_1$ Yes ☐$_2$ No
How often did you drink alcohol in the 3 months before you became pregnant?	☐ Approximately 6-7 times a week ☐ Approximately 4-5 times a week ☐ Approximately 2-3 times a week ☐ Approximately once a week ☐ Approximately 1-3 times a month ☐ Less than once a month ☐ Never
How often do you consume alcohol during this pregnancy?	☐ Approximately 6-7 time a week ☐ Approximately 4-5 times a week ☐ Approximately 2-3 times a week ☐ Approximately once a week ☐ Approximately 1-3 times a month ☐ Less than once a month ☐ Never
What type of alcohol do you usually drink? *(Fill in one or several boxes.)*	☐ Homemade/traditional beer ☐ Purchased traditional Beer ☐ Wines ☐ Ciders ☐ Spirits (*vodka, gin, whisky, liqueur)* ☐ Other specify
How many hours sleep do you currently get a night?	☐ Less than 4 hours per night ☐ 4 – 8 hours per night ☐ All night
Are you experiencing any pregnancy related cravings?	☐ Yes ☐ No Please specify? _____
Do you do any of the following activities on a daily basis?	☐ Carry water ☐ Walk long distances

	☐ Gardening work ☐ House work ☐ Manual labour/lifting ☐ Carry Children Other:_____
Do you currently have musculoskeletal (bone/muscle/joint) pain?	☐ Never ☐ Rarely ☐ Sometimes ☐ Very Often ☐ Always
Did you experience pain in a previous pregnancy?	☐ Yes ☐ No If yes, where?
Only answer the following questions if you have indicated that you have musculoskeletal pain **during pregnancy**	
Has the pain prevented you from spending time with your family and friends?	☐ Yes ☐ No
Has the pain made you feel concerned or worried about your health?	☐ Yes ☐ No
Has the pain made you feel sad or down?	☐ Yes ☐ No
Are you able to cope with the pain?	☐ Yes ☐ No
How have you treated your pain?	☐ Medication: specify_____ ☐ Just living with it ☐ Other comment:

Thank you for taking the time to complete this questionnaire.



Have you experienced MSK pain in any of the areas in the during your pregnancy:		When did the pain starts	Is your pain:	Has your pain interfered with your ability to perform your daily activities, such as gardening, house work etc.	Has the pain affected your ability to work?
Neck	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Shoulders	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Upper Back	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Elbows	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Wrist/ Hands	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Low Back	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Hips/ Thighs	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Knees	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____
Ankles/ Feet	☐ Yes ☐ No	☐ 2 weeks ago ☐ 3-8 weeks ago ☐ 3-6 months ago ☐ + 6 months	☐ Mild ☐ Moderate ☐ Severe	☐ Yes ☐ No If yes comment _____	☐ Yes ☐ No If yes comment _____