THE ROLE OF ADIPONECTIN, LEPTIN, TNF-α AND RESISTIN IN HIV ASSOCIATED PRE-ECLAMPSIA

by

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submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

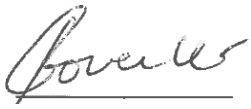
Obstetrics and Gynaecology
College of Health Sciences
University of KwaZulu-Natal
Durban

2015

PREFACE

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

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



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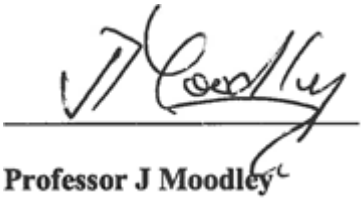

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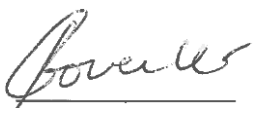
Signed:  Date: 30/03/2015

DEDICATION

To my parents, for instilling in me the desire to progress and to my brother whose faith and moral support made this achievable

To God, without whose love, grace, wisdom, knowledge and mercy none of my achievements would have been possible

Medicine is not only a science; it is also an art. It does not consist of compounding pills and plasters; it deals with the very processes of life, which must be understood before they may be guided. *- Paracelsus*

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and gratitude to:

- Professors J Moodley and T Naicker, for allowing me the opportunity to work on this thesis and be a part of the Placental Research team. The generosity of time and attention during the course of my study assisted my professional growth as an academic and researcher;
- Professor T Naicker for her support, encouragement and her invaluable supervision with regards to this thesis;
- Optics and Imaging Centre, DDMRI, College of Health Sciences, where this study was conducted;
- Professor M Christianson and Dr P Hadley (Serum Staten Institut, Copenhagen) for their invaluable support, guidance and use of their laboratory to conduct the molecular analyses of my study in a timely and efficient manner;
- Mr V Dorsamy for his invaluable time and assistance in statistical analysis;
- Miss A Ajith for her great assistance with formatting;
- RK Khan Hospital and the patients for their consent and participation in the study;
- Mrs T Esterhuizen, for her help with the statistical analysis.

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ABBREVIATIONS

≥	greater than, equal to
aa	amino acid
ACR	albumin : creatinine ratio
ACRP	adipocyte complement related protein
AdipoR	adiponectin receptor
AIDS	autoimmune deficiency syndrome
APGARs	Activity, Pulse, Grimace, Appearance, Respiration
ANOVA	analysis of variance
apM1	adipose most abundant gene transcript
ARV	anti retro viral
BMI	BODY MASS INDEX
BP	blood pressure
CD4	cluster of differentiation 4
cm	centimetre
CRH	corticotrophin releasing hormone
CT	cytotrophoblasts
CV	coefficient of variation
Cys	cysteine
DBP	diastolic blood pressure
EDTA	Ethylenediaminetetraacetic acid
ELISA	enzyme linked immunosorbent assay
EOPE	early onset pre-eclampsia
ET	endothelin
EVT	extravillous cytotrophoblast
fAd	full length adiponectin
FIZZ	found in inflammatory zone
g	gram
gAd	globular adiponectin
GA	gestational age

GH	growth hormone
h	hours
HAART	highly active anti-retroviral therapy
HALS	HIV associated lipodystrophy
hCG	human chorionic growth factor
HELLP	haemolysis, elevated liver enzymes, low platelets
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
hPL	human placental lactogen
HRP	horseradish peroxide
IGF	insulin like growth factor
IGF2	insulin like growth factor 2
IL	interleukin
IUGR	intrauterine growth gestation
JNK	Jun N-terminal kinase
kDa	kilodalton
kg/m^2	kilogram per meter squared
LEPR / OBR	leptin receptor
LGA	large for gestational age
LOPE	late onset pre-eclampsia
LSD	least significant difference
MAPK	mitogen activated protein kinase
mg	milligram
ml	millilitres
mm3	millimetre cubed
mmHg	millimetres mercury
MMP	matrix metallo-proteinases
mRNA	messenger ribonucleic acid
MUAC	mid upper arm circumference
OB	obese
OR	odds ratio
PE	pre-eclampsia

PIGF	placental growth factor
PMTCT	preventing mother to child transmission
RELM	resistin like molecule
RETN	A gene on chromosome 19p13.2 that encodes resistin
SA-PE	streptavidin phycoerythrin
SBP	systolic blood pressure
sEng	soluble endoglin
sFlt	soluble fms like tyrosine kinase receptor
SODD	Silencer of Death Domain
SPHERE	Statewide Partnership for HIV Education in Recovery Environments
SPSS	Statistical Package for the Social Sciences
ST	syncytiotrophoblasts
TACE	TNF alpha converting enzyme
TGF-β	transforming growth factor beta
TIMP	tissue inhibitor proteins
TMB	tetramethylbenzidine
TNF-α	tumour necrosis factor alpha
TNFR	tumour necrosis factor receptor
TRADD	Tumor necrosis factor receptor type 1-associated DEATH domain protein
TST	triceps skinfold thickness
Val	valine
VEGF	vascular endothelial growth factor
SANDO	South African National Department of
MRC	Medical Research Council

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ABSTRACT

Introduction and aims

Hypertensive disorders of pregnancy, in particular, pre-eclampsia, remains an enigmatic problem with global disease burden shared amongst industrialised and non-industrialised countries. It has been estimated that hypertensive disorders complicate 5 – 10% of pregnancies. The leading cause of maternal deaths in sub-saharan Africa is AIDS (43.7%). The Saving Mothers Guidelines for the tri-ennium 2005 – 2007 in South Africa found that hypertensive disorders were directly linked to maternal deaths in 15.7% of cases, of which 83% represents pre-eclampsia. Additionally South Africa now faces the challenge of obesity. These three conditions (HIV, pre-eclampsia and obesity) impact on each other causing adipokine dysregulation. The aim of the study was to examine the levels of adiponectin/leptin/TNF-α and resistin amongst non-pregnant, normotensive and pre-eclamptic pregnant cohorts in respect of their BMI and HIV status.

Methods

Following institutional ethical approval and informed consent, serum was obtained from a total of 328 women attending the RK Khan Hospital, a regional and district hospital in eThekwini, KwaZulu-Natal. Women were recruited into two groups ie., non-pregnant (n = 120; 36.58%) and pregnant group (n = 208; 63.41%). Pregnant women were further, categorised into the normotensive pregnant (n = 118; 35.97%) and the pre-eclamptic (n = 90; 27.43%) groups. The pregnant cohort was also sub-stratified in accordance with their HIV status. Clinical demographics, height, weight, body mass index (BMI), mid upper arm circumference (MUAC), triceps skin fold thickness were recorded. Indications and mode of delivery as well as associated complications, fetal ultrasound abnormalities, neonatal outcomes (APGARS),

weight, placental shape weight and appearance were noted. Serum was assessed by a double antibody sandwich ELISA technique using the DuoSet ELISA Development System for human adiponectin and TNF-α. Additionally, serum leptin and resistin was detected by the Bioplex immunoassay (Biorad). Absorbance was read spectrophotometrically at 450 nm (Systems). SPSS version 21 was used to analyse the demographic and experimental data. A p value < 0.05 was considered as statistically significant.

Results

Irrespective of the HIV status, body mass index and maternal weight (p = 0.325 vs 0.138) were not statistically significantly different between the normotensive and pre-eclamptic groups respectively. Likewise, the distribution of BMI was the same across the study groups with respect to HIV status (p = 0.124).

Mean adiponectin levels varied between 897.93±126.18, 17.19±11.56, 23.16±21.39 and 24.61±12.869 in the non-pregnant, normotensive pregnant, EOPE and LOPE groups respectively. Leptin levels varied from 4887.25±705.29, 2732.27±580.18, 955.75±527.64 and 310.23±177.43 in the non-pregnant, normotensive pregnant, EOPE and LOPE groups respectively. TNF-α was undetected in the non-pregnant group as compared to 608.52±84.89, 661.03±202.60 and 616.43±117.53 in the normotensive pregnant, EOPE and LOPE groups respectively. Resistin varied from 7497.13±1921.95 in the non-pregnant group compared to 3536.50±730.04, 1017.63±69.58 and 286.92±160.30 in normotensive pregnant, EOPE and LOPE groups accordingly.

The levels of TNF-α, leptin and resistin were significantly different within the normotensive pregnant versus pre-eclamptic groups. Except for adiponectin ($p < 0.292$); TNF-α ($p < 0.044$), leptin ($p < 0.004$) and resistin ($p < 0.006$) were statistically significantly different within the pregnant cohorts. The study demonstrated statistically significant differences in adiponectin/ leptin/ TNF-α and resistin between non-pregnant, normotensive and pre-eclamptic cohorts with respect to HIV status and BMI. There were significant differences in the levels of adiponectin/leptin/resistin and TNF-α with respect to HIV status ($p=0.00$). Additionally, a statistically significant difference in the level of adiponectin in the non-pregnant as compared to the normotensive cohorts ($p<0.00$) was noted. Furthermore,, there were statistically significant differences in the levels of TNF-α, leptin and resistin in the normotensive as compared to pre-eclamptic cohorts ($p<0.000$). This study was able to depict baseline adiponectin / leptin / resistin and TNF-α levels according to BMI in the local population.

Conclusion

This study was expedient in the fact that patients were all standardized according to ethnicity, sub-analysed according to BMI and all samples taken from the third trimester of pregnancy – one of the first such studies to be performed within South Africa as well as globally. This study reports significant differences in the BMI of the non-pregnant and pregnant groups, but no significant differences within the pregnant cohorts. In conclusion this study establishes an adipokine baseline for future reference with regards to South African Black pregnant and non-pregnant women. Albeit at term, the study shows a statistically significant difference in the levels of adiponectin/leptin/resistin and TNF-α in HIV positive patients within the non-pregnant versus pregnant population. Within the pre-eclamptic cohort there was no statistically significant difference in EOPE versus LOPE.

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

1.1 NORMAL PREGNANCY

Pregnancy is a unique condition in that a growing fetus with its own foreign genetic make-up requires nutritional support from the mother, however direct maternal leucocyte interaction with fetal cells creates a hostile milieu. This hurdle is overcome via the placenta, which plays a critical role at the materno-fetal interface.

Placental development is a highly specialized series of events critical for normal fetal growth and development. Some of the essential roles of the placenta include formation of the materno-fetal interface preventing rejection of the fetal allograft, allowing gaseous exchange, transport of nutrients/excretion of fetal waste and acting as an endocrine organ releasing peptides and hormones (Fig.1.1)(Jansson and Powell, 2000, Cunningham *et al*., 2009).

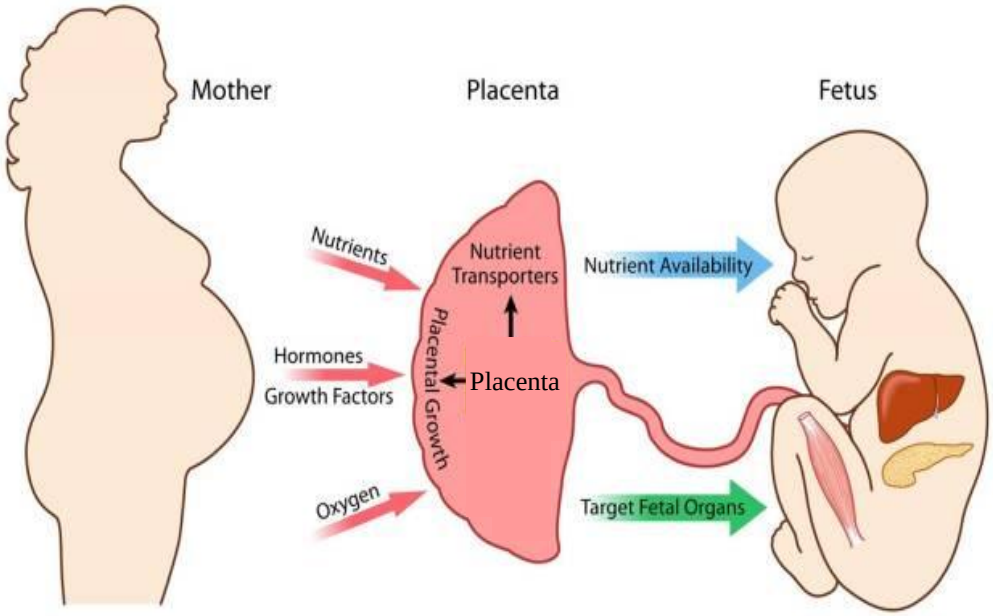

Figure 1.1: Materno-fetal interaction via the placenta as adapted from Jansson and Powell, 2000

1.2 PLACENTATION AND EARLY DEVELOPMENT

The development of the fetus and placenta is a continuum of events commencing at fertilisation. Post fertilisation, the zygote (Fig 1.2a) undergoes rapid mitosis (Fig 1.2b) to form the morula (Fig 1.2c), which on day 5, enters the uterus and forms the blastocyst. The blastocyst becomes a fluid filled cavity and allows polarisation of cells (Fig 1.2d). The formation of the human placenta begins with the trophectoderm, which is the first tissue to differentiate at the morula stage of development, giving rise to a layer of trophoblastic cells encircling the blastocyst (Cunningham *et al*., 2009; (Marieb, 2003). The outer trophoblastic layer of the blastocyst goes on to form the placenta and fetal membranes. On day 6, implantation entails movement of the blastocyst to an optimal position (usually the mid to upper anterior or posterior uterine wall), adhesion and invasion into the uterine wall (Fig 1.2e) where it has access to the glycogen nourishment (Cunningham *et al*., 2009, Marieb, 2003).

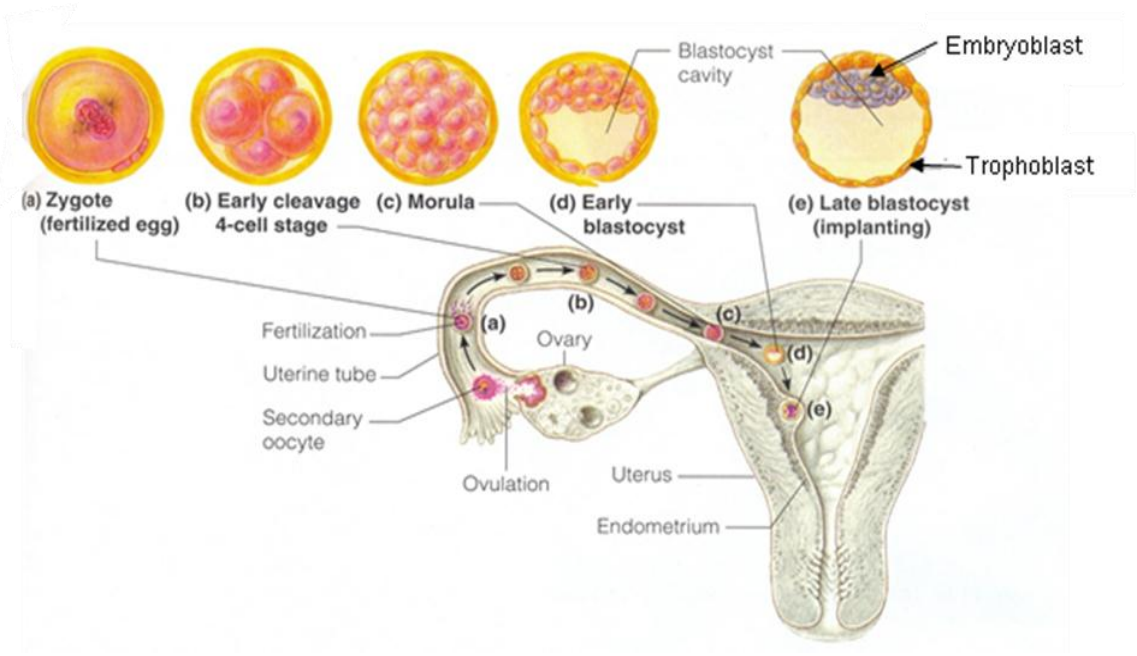

Figure 1.2: Schematic diagram illustrating stages of cleavage of the zygote, to form the blastocyst. Adapted from (Marieb, 2003).

As the trophoblast erodes deeper into the decidua, vacuoles form and become confluent to form lacunae by day 13. The lacunar space in due course becomes the intervillous space. The stem cell line of the placenta is the progenitor cytotrophoblasts (CT). Differentiation of CTs occur along one of two pathways, ie formation of the villous cytotrophoblast ultimately forming the syncytiotrophoblast (ST) or the extravillous cytotrophoblast (EVT) layer (Cunningham *et al*., 2009).

The specialised syncytiotrophoblast has several functions, including transport of gases, nutrients, and waste products and synthesis of peptide and steroid hormones that regulate placental, fetal, and maternal systems. Extravillous trophoblasts have both a proliferative and invasive cohort. There is also a migratory EVT, which is neither invasive nor proliferative. These cells populate the cell islands, septum, chorionic plate and chorion leave (Cunningham *et al*., 2009).

During the 4^{th} – 5^{th} week of pregnancy the EVT erupts into 2 columns with a proliferative aspect at the base and invasive aspect at the distal portion of the column. Invasive EVTs that invades the decidua are called interstitial EVTs, whereas those that invade and remodel the spiral arteries are called endovascular EVTs. Endovascular invasion (intramural or intra-arterial) involves replacement or displacement of vascular smooth muscle and endothelial cells with a flaccid fibrinoid type material. This leads to transformation of the narrow spiral arteries into wide bore utero-placental arteries/sinusoids. This physiological conversion of a small calibre spiral artery into a large bore flaccid conduit with a low resistance high flow supply of blood, ensures oxygen and nutrient demands of the fetus are met (Kaufmann *et al*., 2003).

1.2.1 Other functions of EVTs

Extravillous trophoblast cells express specific proteins defining the stage and role of the differentiation and invasion process. These include integrin cell-extracellular matrix antigens, matrix metallo-proteinases (MMPs), signal transduction proteins such as transforming growth factor-ß (TGF-ß), vascular endothelial growth factor (VEGF) and VEGF receptors, and insulin-like growth factor 2 (IGF2) (Kaufmann *et al*., 2003).

Invasion of the decidua and myometrium by the EVT is achieved with degradation of the extracellular matrix by means of several members of the MMP protease family. The activity of these MMPs is regulated by their tissue inhibitors (TIMPs) (Huppertz *et al*., 1998, Kaufmann *et al*., 2003, Huppertz *et al*., 2006, Xu *et al*., 2000, Lunghi *et al*., 2007). TIMP-1, an inhibitor of all MMPs, and TIMP-2 have been found in decidual cells (Charnock-Jones and Burton, 2000, Caniggia *et al*., 1999) and EVTs (Wolf *et al*., 1991, Liu *et al*., 2012, Kaufmann *et al*., 2003). Hepatocyte growth factor (HGF) stimulates trophoblast invasion via the met receptor and induction of MMP-9. The decidua prevents uninhibited EVT invasion by secreting locally acting factors (cytokines, protease inhibitors), which modulate trophoblast invasion (Huppertz *et al*., 1998, Plaisier, 2011).

Metabolic and endocrine function is a tightly controlled orderly process in healthy pregnancies. The placenta is not innervated, and hence any communication between it, the mother, and the fetus must involve humoral agents. The signalling molecules from the placenta act locally through paracrine and autocrine regulation. The hormones produced by the placenta can be split into two categories: peptide hormones (human chorionic gonadotropin [hCG], human placental lactogen [hPL], cytokines, growth hormone [GH], insulin-like growth factors [IGF's],

corticotropin releasing hormone [CRH], vascular endothelial growth factor [VEGF], placental growth factor [PIGF]) and steroid hormones (estrogens, progesterone and glucocorticoids) (Cunningham *et al*., 2009).

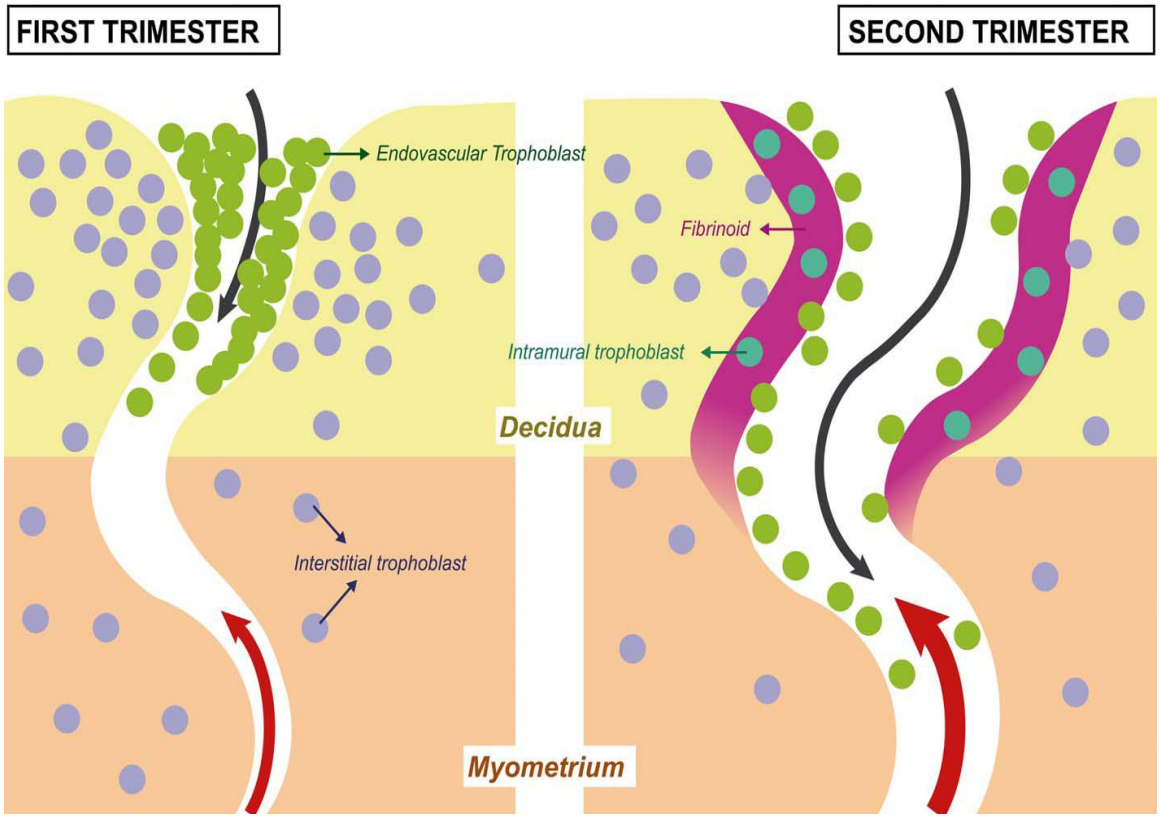

Figure 1.3: Diagram illustrating the concept of two waves of trophoblastic invasion into the spiral artery. On the left hand side one can see the endovascular trophoblast migration into the decidual segments of the spiral arteries which occurs during the first trimester. On the right hand side which is illustrating a second semester spiral artery, this endovascular migration is seen extending into the myometrial segments the of the spiral arteries. In addition, the intramural trophoblast invasion which transforms the vessel walls and alters vasoreactivity is illustrated. Red arrow: direction of blood flow; black arrow: direction of endovascular trophoblast migration. Adapted from (Pijnenborg *et al*., 2006).

1.3 HYPERTENSIVE DISORDERS OF PREGNANCY

Hypertensive disorders of pregnancy, in particular, pre-eclampsia, remain an enigmatic problem with global disease burden shared amongst high and low income countries. It has been estimated that worldwide, hypertensive disorders complicate 5 – 10% of all pregnancies (Cunningham *et al*., 2009). The recent South African Saving Mothers Guidelines for the tri-ennium 2008 –2010 demonstrates that hemorrhage and hypertensive disorders of pregnancy are the commonest direct cause of maternal deaths (2012 Saving Mothers). Additionally, their previous report for the tri-ennium 2005 – 2007, 83% of maternal deaths were attributed to pre-eclampsia (NCCEMD, 2007)**.**

1.3.1 Classification and characteristics of hypertensive disorders

Pre-eclampsia is part of a spectrum of hypertensive disorders that may complicate pregnancy. As specified by the National High Blood Pressure Education Program (NHBPEP) Working Group (National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2000), the classification is as follows:

a) Gestational hypertension

Gestational hypertension is characterised by:

- BP of 140/90 mm Hg or greater on 2 separate occasions at least 6 hours apart after 20 weeks gestation
- Proteinuria < 0.3 g/24 h
- BP returns to normal less than 6 weeks' postpartum
- The final diagnosis is only confirmed once the postpartum period is complete

b) Chronic hypertension

Chronic hypertension may be diagnosed when:

(a) BP 140/90 mm Hg or greater occurs before pregnancy or diagnosed before 20 weeks' gestation; not attributable to gestational trophoblastic disease or

(b) there is hypertension first diagnosed after 20 weeks' gestation and persistent after 12 weeks' postpartum.

Preexisting chronic hypertension may present with superimposed pre-eclampsia presenting as new-onset proteinuria after 20 weeks' gestation.

c) Pre-eclampsia/eclampsia

Pre-eclampsia/eclampsia is diagnosed with a BP of 140/90 mmHg or greater measured on 2 separate occasions at least 6 hours apart after 20 weeks' gestation in women with previously normal BP. The presence of proteinuria (≥ 0.3 g protein in 24 h urine specimen) is also a prerequisite.

Eclampsia is defined as seizures that cannot be attributable to other causes, in a woman with pre-eclampsia.

d) Superimposed pre-eclampsia

Superimposed pre-eclampsia (on chronic hypertension) is characterized by:

(a) the new onset of proteinuria (≥300 mg/24 h) in a woman with hypertension but no proteinuria before 20 weeks' gestation or;

(a) a sudden increase in proteinuria or BP, or a platelet count of less than 100 000/mm^3, in a woman with hypertension and proteinuria before 20 weeks' gestation.

1.3.2 Pre-eclampsia

Pre-eclampsia is a systemic pregnancy syndrome that is typically characterised by new onset hypertension ie., blood pressure (BP) ≥ 140/90 mmHg on two separate occasions at least six hours (h) apart) after 20 weeks gestation in the presence of proteinuria (with proteinuria defined as the urinary excretion of ≥ 300 mg protein in a 24 h urine collection) (National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2000). Blood pressure returns to normo-tension within 6 weeks' following delivery. It is a multi-organ disease, characterised by a generalised endothelial dysfunction and an exaggerated systemic inflammatory response (Redman and Sargent, 2005). Pre-eclampsia is associated with a shallow trophoblast invasion of spiral arteries (Kaufmann *et al*., 2003). A consequence of this lack of physiological conversion of the maternal spiral arteries, is that they remain small bore conduits hence there is restricted oxygen and nutrient supply to the fetus (Kaufmann *et al*., 2003). The resulting hypoxia triggers the clinical symptoms of pre-eclampsia. The only effective intervention to reverse the syndrome is delivery of the placenta.

1.3.3 Clinical parameters for pre-eclampsia diagnosis

a) Blood pressure

Blood pressure must be measured with the parturient in a seated position and the right upper arm lying at the level of the heart. The paturient should not be in a decubitus position, so that the arm is above the right atrium. The blood pressure is taken according to the Korotkoff sounds (Shennan *et al*., 1996).

The correct cuff size is dependent on the mid upper arm circumference:

- for arm circumference of up to 33 cm – standard size (13x23 cm)
- for arm circumference between 33 - 41 cm – large size (33x15 cm)
- for an arm circumference of 41 cm or more – a thigh cuff (18x36 cm)
- use a larger cuff rather than too small a cuff size, minimize errors (Milne *et al*., 2005)

Table 1.1 Correlation of Korotoff sound with auscultation of blood pressure

Korotkoff sound	**Auscultation**	**Implication**
I	Clear cut snapping tone	Systolic blood pressure
II	Succession of murmurs	
III	Disappearance of murmurs	
IV	Muffling of sounds	Diastolic BP in pregnancy

b) Urine tests

Proteinuria is defined as the presence of at least 300 mg of protein in a 24 h urine sample (Schroeder, 2002). Additionally, significant proteinuria should be diagnosed on a 24 h urine total protein collection preferably. Studies show that up to 30% of supposed gestational hypertensive patients diagnosed on the basis of trace proteinuria on random clean catch midstream urine have 300 mg of protein on a 24 h urine total protein (Andrus and Wolfson, 2010). However a finding of 1+ proteinuria on a clean-catch mid-stream urine is sufficient to make a diagnosis of significant proteinuria.

There may also be a role for the use of protein-creatinine ratio in the diagnosis of proteinuria using random urine samples. Values of 0.14 – 0.3 mg have been used to diagnose proteinuria. There is no consensus as to the best threshold for diagnosis of significant proteinuria.

However, up to 10% of pre-eclamptics and 20% of eclamptics are aproteinuric (Waugh *et al*., 2005); (Durnwald and Mercer, 2003). The HELLP syndrome may also occur in the absence of proteinuria. Hyperuricaemia may be one of the earliest manifestations of pre-eclampsia, however the sensitivity is low (0 – 55%) whilst the specificity is relatively high (77 – 95%) (Buhimschi *et al.*, 2009). Baweja *et al* (2011) suggested measuring spot urinary albumin: creatinine ratio (ACR) values (Baweja *et al*., 2011). If measured early in the second trimester, an ACR of 35.5 mg/mmol or higher may predict pre-eclampsia before clinical symptoms develop (Baweja *et al*., 2011).

1.3.4 Classification

Pre-eclampsia may be sub classified according to the stage of placentation:

a) **Early onset pre-eclampsia** (< 34 weeks; EOPE): these individuals are characterised by abnormal placentation (as interstitial and endovascular trophoblastic invasion is completed within 15 weeks of gestation).

b) **Late onset pre-eclampsia** (> 34weeks; LOPE): is more often associated with adequate placentation but excessive foetal demands.

Early onset pre-eclampsia is associated with a four-fold increased risk of stillbirth in subsequent pregnancy as compared to no elevated risk of stillbirth in women with late onset

disease (Scherer *et al*., 1995). Comparison of 456 668 singleton deliveries between early and late onset pre-eclamptics has revealed that the rate of all adverse birth outcomes, except for large for gestational age (LGA), were significantly higher amongst women with EOPE compared with women without early-onset disease (Lisonkova and Joseph, 2013). Amongst the women with EOPE, approximately 12% delivered at 34 weeks' gestation or later, and almost one half of babies (49.5%) were very low ie., birth weight < 1500 g. With the exception of neonatal death rates, all other adverse birth outcomes were significantly higher among mothers with LOPE compared with those without pre-eclampsia.

Notably the rate of fetal death is approximately 6 times higher, whilst the rate of perinatal death or serious neonatal morbidity is 16 times higher amongst women with EOPE (Lisonkova and Joseph, 2013).

Alternatively, pre-eclampsia may be sub-classified on the severity of blood pressure (National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2000):

a) Mild pre-eclampsia is generally considered to be a BP ≥ 140/90 mmHg on 2 occasions, at least 6 hours apart, but without evidence of end-organ damage, in a woman who was normotensive before 20 weeks' gestation. In a patient with pre-existing essential hypertension, superimposed pre-eclampsia is diagnosed if systolic blood pressure (SBP) has increased by 30 mmHg or if diastolic blood pressure (DBP) has increased by 15 mmHg.

b) Severe pre-eclampsia is defined as SBP of 160 mmHg or higher or DBP of 110 mmHg or higher on 2 occasions at least 6 hours apart, with or without proteinuria of more than 5 g

in a 24-h collection or more than 3+ on 2 random urine samples collected at least 4 hours apart; pulmonary edema or cyanosis; oliguria (< 400 mL in 24 hours); epi-gastric pain and/or impaired liver function; thrombocytopenia and oligohydramnios.

c) Decreased fetal growth or placental abruption Decreased fetal growth is the failure of the fetus to achieve expected growth norms as established by ethnic specific growth charts. Placental abruption is the premature separation of a normally situated placenta from the uterine wall prior to the delivery of the baby.

1.3.5 Risk factors for pre-eclampsia

1. Primiparity – a meta-analysis of 26 eligible studies found a summary odds ratio (OR) for PE in primiparous women compared to their multiparous counterparts of 2.42 (95% CI: 2.16- 2.71) with a range of 1.4 to 5.5 (Luo *et al*., 2007) .
2. Previous pre-eclampsia – a previous normal pregnancy is associated with a reduced incidence of PE in a subsequent pregnancy (Trogstad *et al*., 2001), however previous PE is a strong risk factor for PE in multiparous women. The recurrence risk is about 14%.
3. Maternal pre-pregnancy body mass index (BMI) – High BMI is consistently found to increase PE risk. The odds ratio in obese women (BMI > 30 units) is reported to vary from 3 - 5 compared to their normal weight counterparts (Duckitt and Harrington, 2005). This finding however is mainly associated with LOPE rather than EOPE.
4. Underlying medical conditions – conditions associated with endothelial damage e.g. diabetes mellitus, antiphospholipid antibodies, autoimmune or renal disease, are associated with increased risk of PE development (Chappell *et al*., 2008). Chronic

hypertension is an important risk factor for the development of superimposed PE (Conde-Agudelo *et al*., 1999).

5. Smoking in pregnancy – this is associated with a reduced risk of PE development (OR: 0.5 - 0.8) compared with non-smokers (England and Zhang, 2007, Sibai *et al*., 2000).
6. Pregnancy specific factors – multiple gestations carry a two to three time's greater risk for PE development compared with singleton gestations (Prasannan-Nair *et al*., 2006). Twin and molar pregnancies are associated with greater placental mass, the subsequent increased placental debris is believed to result in a maternal systemic inflammatory response, lowering the threshold for PE development (Wong *et al*., 2007, Mbah *et al*., 2010). Alternatively the increased paternal genetic contribution may be an explanation. Glutathione S-transferase P1-1 is a major biotransformation enzyme in placenta and decidua. The 105I Le→Val polymorphism in the glutathione S-transferase P1 gene is associated with lower enzyme detoxification capacity. This polymorhism is contributed by paternal genes and may lead to pre-eclampsia (Zusterzeel *et al*., 2002, Zusterzeel *et al*., 2001).
7. Ethnicity – Black nulliparous women carry twice the risk of developing PE compared to Whites (ACOG, 2002).

Heterogeneity of the disease has been suggested by numerous studies, however the exaggerated inflammatory response noted in pre-eclampsia, is now speculated to be due to either an excessive placental stimulus or due to an overactive maternal response to a normal placental stimulus (Figure 1.1). Focus has recently shifted to adipokines and their possible relationship to the development of pre-eclampsia.

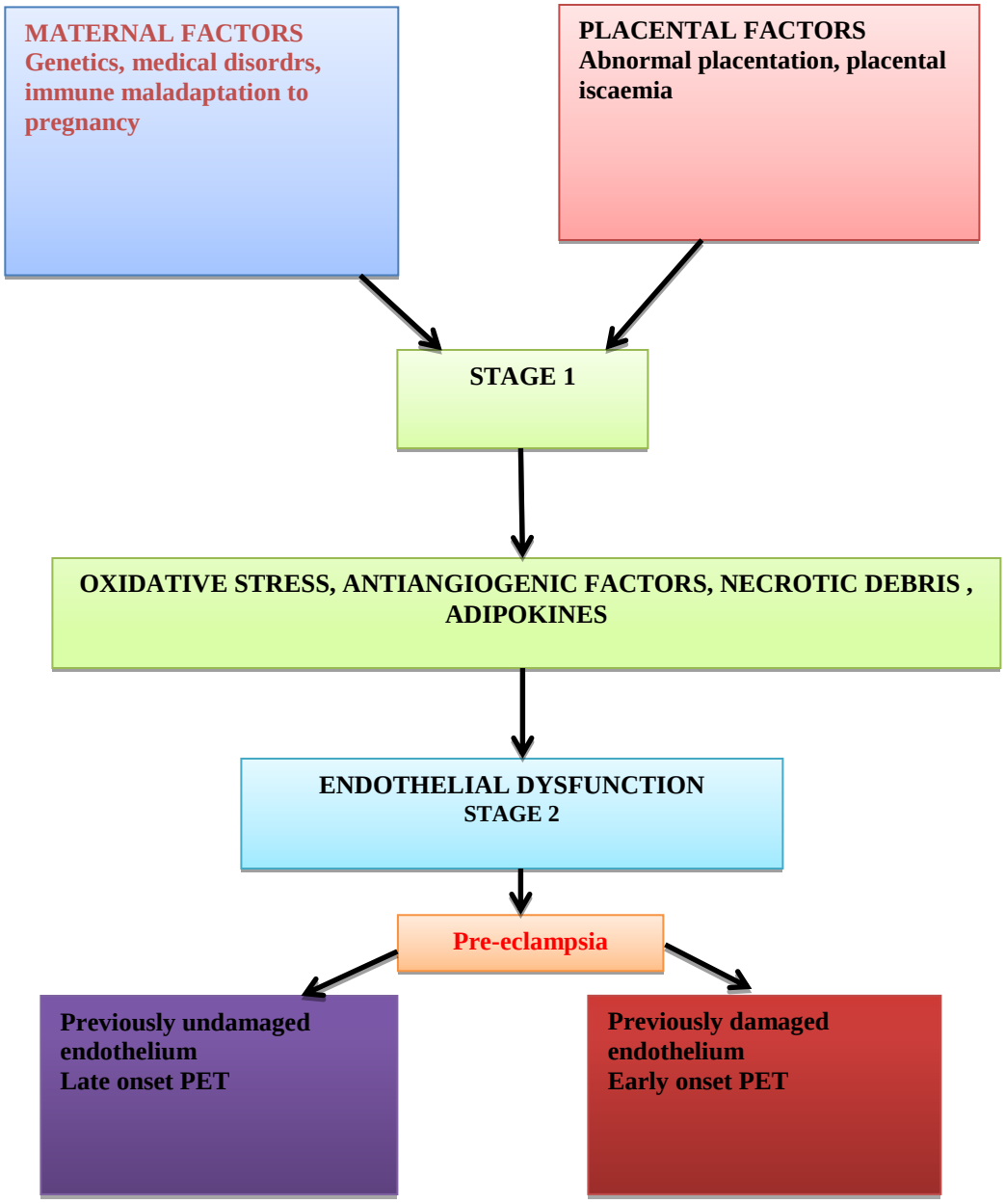

Figure 1.4: Development of pre-eclampsia: Causal model

1.3.6 Placentation in pre-eclampsia

Abnormal trophoblastic invasion of the uterine vessels is considered the main protagonist of the pre-eclampsia syndrome (Ishihara *et al*., 2002). Studies have shown an inverse correlation between the degree of spiral arteriole invasion and the severity of the pre-eclampsia (Ishihara *et al.;* 2002). Because of the placental hypo-perfusion following incomplete trophoblastic invasion as a result of the release of systemic vasoactive compounds there is an exaggerated inflammatory response, vasoconstriction, endothelial damage, capillary leak, hypercoagulability, and platelet dysfunction, all contributing to organ damage and the clinical manifestations of the disease.

The shallow placentation results from the fact that the invasion of the decidual arterioles by cytotrophoblasts is incomplete which may in itself arise from failure in alteration of the molecular expression necessary for the differentiation of the cytotrophoblasts, as required for pseudovascularization.

The invasive cytotrophoblasts fail to replace the tunica media, which means that the mostly intact arterioles capable of vasoconstriction are still present. Previous studies evaluating the histology of the placental bed demonstrates few cytotrophoblasts beyond the decidual layer.

Dramatic changes in trophoblast differentiation occur in various pathophysiological situations and may underlie pregnancy disorders, such as pre-eclampsia and fetal growth restriction (IUGR). Interstitial EVT density, however, does not differ between normal pregnancy and pre-eclampsia. Compared to the minimal EVT apoptosis seen in normal pregnancy, in pre-

eclamptics it has been found that 15 - 50% of cells are apoptotic, a finding associated with macrophages around spiral arteries (Myatt, 2002, Lyall and Myatt 2002).

In normal pregnancy, shedding of syncytiotrophoblast fragments into the maternal circulation (approximately 100 000 fragments/day) as a result of apoptosis or necrosis, does not elicit a maternal immune response. However, the rate of syncytiotrophoblast apoptosis is increased from 2 - 3% in normal pregnancy to 5 - 6% in pregnancies complicated by IUGR (Ishihara *et al*., 2002) or pre-eclampsia (Leung *et al*., 2001).

Additionally, in a recent study, the protein expression of the anti-angiogenic soluble fms-like tyrosine kinase receptor (sFlt1) and soluble endoglin (sEng) was found to be increased in contrast to the proangiogenic placental growth factor and transforming growth factor beta 1 in pre-eclamptic compared with normotensive pregnancies, irrespective of the HIV status (Govender *et al*., 2014).

1.4 ADIPOKINES

Human pregnancy is typified by endocrine and metabolic maternal adaptations including increase in weight, body fat mass, and insulin resistance (Cunningham *et al*., 2009). These changes reflect a physiological adaptation necessary to meet the energy demands of the fetus and prepare the mother for delivery and lactation. Adipose tissue is recognised as both a metabolic and endocrine organ, secreting several proteins such as adiponectin, leptin, resistin, and tumor necrosis factor alpha (TNF-α). During pregnancy, the placenta is an additional source of these proteins.

1.4.1 Adiponectin

Adiponectin is also known as adipocyte complement-related protein of 30 kDa (ACRP30), adipoQ, adipose most abundant gene transcript 1 (apM1), and gelatin-binding protein of 28 kDa (GBP28). It is an adipocyte-specific, secreted protein with roles in glucose and lipid homeostasis. Circulating adiponectin occurs in relatively high concentrations of 5-30 µg/ml, and occupies 0.01% of plasma proteins (Ukkola and Santaniemi, 2002). The hormone reflects sexual dimorphism and is inversely correlated with percentage of body fat in adults (Gable *et al*., 2006). This hormone has been found to be an insulin sensitising factor, modulating the endothelial inflammatory response with a direct anti-arthrogenic effect (Nakatsukasa *et al*., 2008).

Adiponectin itself is encoded on chromosome 3q27, a region that has been highlighted as affecting genetic susceptibility to non-insulin dependent diabetes mellitus and obesity (Mori *et al*., 2002). Adiponectin exerts its insulin-sensitizing action via its reducing hepatic glucose production and enhancing insulin activity in the liver. Its circulating levels are downgraded in insulin-resistant states such as obesity and type 2 diabetes.(Yamauchi *et al*., 2002, Kadowaki *et al*., 2007).

1.4.1.1 Adiponectin structure

Structurally, adiponectin is a 244 amino acid (aa) long polypeptide with four distinct regions,

a) a short signal sequence that targets the hormone for secretion outside the cell,

b) a short species specific aa sequence,

c) a 65 aa with similarity to other collagenous proteins and,

d) a globular domain.

Adiponectin self-associates into large structures, the protein trimers continue to self-associate to form hexamers/dodecamers (Figure 1.5). The full length (fAd) can be proteolytically processed to generate a globular (gAd) truncated 16.5 kDa protein, which represents the active form.

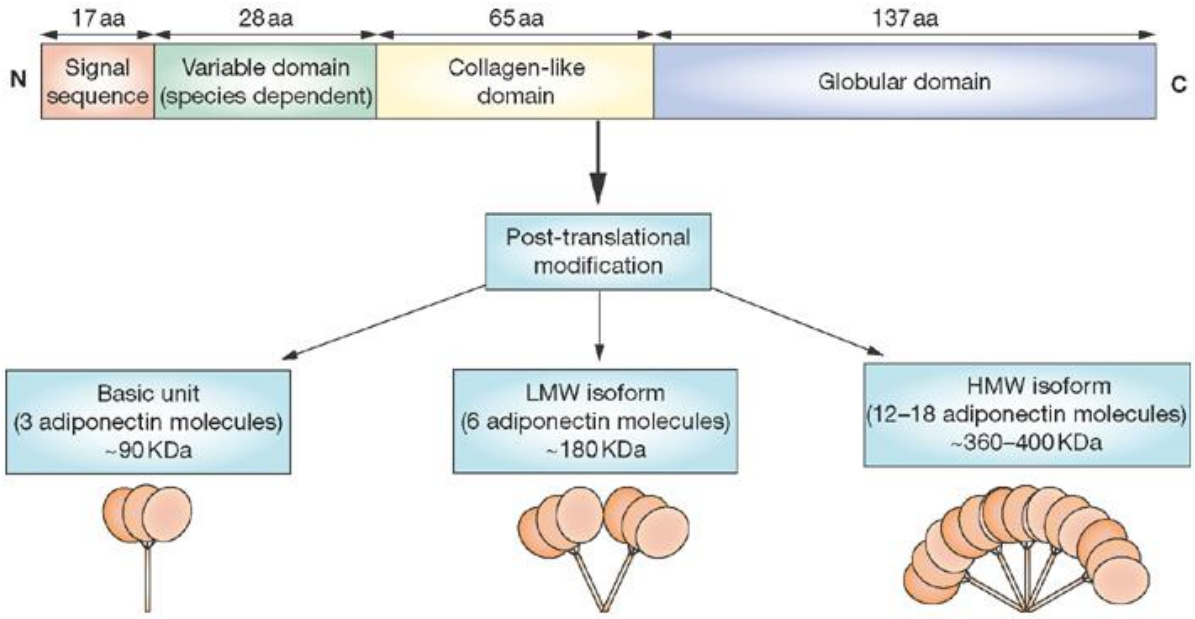

Figure 1.5: Structure of adiponectin (Goldstein *et al*., 2009)

1.4.1.2 Receptors for Adiponectin

The receptors for adiponectin, AdipoR1, and AdipoR2, have distinct expression patterns and bind to the different adiponectin isomers with different affinities. AdipoR1, expressed with the highest levels in skeletal muscle, primarily binds globular forms of adiponectin. AdipoR2, mainly expressed in the liver, binds fAd with higher affinity than gAd.

1.4.1.3 Adiponectin in pregnancy

Data on the circulating levels of adiponectin in pre-eclamptic women are contradictory. Some studies have shown that serum levels of adiponectin are higher in women with pre-eclampsia compared to normal healthy pregnant women (Ouyang *et al*., 2009); (Haugen *et al*., 2006); (Hendler *et al*., 2005a); (Kajantie *et al*., 2005); (Lu *et al*., 2006); (Naruse *et al*., 2005); (Ramsay *et al*., 2003). Conversely, low serum levels of adiponectin have also been reported in pre-eclampsia (Hendler *et al*., 2005b); (Cortelazzi *et al*., 2007); (D'Anna *et al*., 2005); (Suwaki *et al*., 2006). Others have shown no difference in circulating concentrations of adiponectin between normotensive pregnant women and pre-eclampsia (Degawa-Yamauchi *et al*., 2003). In pregnancy adiponectin levels range between 2.7– 25 µg/ml (Nien *et al*., 2007) whilst in the non-pregnant group adiponectin levels range between 3.5 – 22.4 µg/ml. They also demonstrated that no difference in adiponectin concentrations was found between non-pregnant and overweight pregnant females at all gestational ages (Nien *et al*., 2007).

Moreover, they demonstrated a significantly lower adiponectin concentration in overweight pregnant women compared to their normal weight counterparts. Notably, adiponectin levels significantly decreased with advancing gestational age (Nien *et al*., 2007). Table 1.2 - 1.3 outlines the generalised reference range of adiponectin in pregnancy (Nien *et al*., 2007) .

Table 1.2: Plasma adiponectin concentrations (μg/mL) in normal weight (BMI<25) pregnant women.

GA	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile
11–14 weeks	5.6	7.7	10.2	12.9	17.4
15–18 weeks	4.8	7.6	9.5	11.9	14.8
19–22 weeks	5.7	7.2	9.0	11.5	14.6
23–26 weeks	5.6	7.2	8.6	10.8	14.1
27–29 weeks	4.2	6.4	8.3	10.9	12.2
31–34 weeks	4.6	5.5	8.2	10.4	12.2
>37 weeks	5.0	6.7	8.6	11.3	13.4

Table 1.3: Plasma adiponectin concentrations (ug/mL) in overweight BMI>25 pregnant women

GA	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile
11–14 weeks	4.2	6.2	7.9	9.7	11.9
15–18 weeks	5.3	7.1	8.2	11.2	16.1
19–22 weeks	4.3	5.4	6.5	8.4	12.2
23–26 weeks	4.8	5.7	6.7	9.5	12.4
27–29 weeks	4.3	4.6	7.5	9.7	13.9
31–34 weeks	4.9	5.6	6.5	8.3	9.6
>37 weeks	5.1	6.0	7.2	9.1	11.3

The limitation of the latter study however, was that the samples were randomly drawn from the NIH sample bank. Results were confounded by race, diet and sub-categorisation for complicated pregnancies and their effect on adiponectin levels.

Adiponectin has been found at the feto-maternal interface during the process of spiral artery remodelling. Although preceding reports suggest it is produced and secreted by the human placenta, more contemporary studies cast doubt on these initial observations (Caminos *et al*., 2005, Chen *et al*., 2006, Corbetta *et al*., 2005, Pinar *et al*., 2008). These reports demonstrate that human first trimester trophoblasts express AdipoR1 and AdipoR2 receptors through which adiponectin exerts its function and influences placental functions (Tie Weiwei *et al*., 2009).

1.4.2 Leptin

Body weight is synchronized by a complex system, including both peripheral and central factors. One of the two hormones that plays an important role in the adaptation of food intake and body weight is leptin. Leptin is an important hormone in satiety and fatty acid oxidation and is directly correlated with the percentage of body fat (Henson and Castracane, 2006). It is secreted by adipose tissue in a pulsatile mode usually 2–3 h after meals at a frequency dependent on the adipose tissue mass. Circulating leptin levels (normal range: 1–15 ng/mL) diametrically reflects the amount of energy stored in the adipose tissue and is proportionate to the body adipose mass.

In 1994, the human obese (OB) gene and its product leptin was identified and characterized (Zhang *et al*., 1994b). The OB gene is located on chromosome 7 (7q31.3) and is composed of three exons and two introns spanning 18 kb (Masuzaki *et al*., 1995, Isse *et al*., 1995); (Gong *et*

al., 1996). It encodes a protein consisting of 166 amino acids with a putative signal sequence (Masuzaki *et al*., 1995). Only one OB mRNA species has been found in abundance in human adipose tissues (Masuzaki *et al*., 1995).

1.4.2.1 Leptin structure

Human leptin is a 167 aa protein, manufactured primarily in white adipose tissue and is directly proportional to total body fat content (Fig. 1.6). Leptin acts on the central nervous system, in particular the hypothalamus, suppressing food intake and stimulating energy expenditure (La Cava *et al*., 2004). Additionally, it is produced in the placenta, ovaries, mammary tissue and liver. It is a pro-inflammatory cytokine that belongs to the type I cytokine superfamily and has structural similarity with interleukin-6 (La Cava *et al*., 2004).

1.4.2.2 Leptin receptors

Leptin acts through the leptin receptor (LEPR or OBR). The OBR gene is located on chromosome 1 (1p31), is constituted of 18 exons and 17 introns, and encodes a protein consisting of 1162 amino acids (Chung *et al*., 1996); (Meier and Gressner, 2004). One of the splice variants of the OBR gene, the one with the longest intracellular domain (OB-Rb) and full signalling capabilities, is widely expressed in the human brain (Campfield *et al*., 1996); (Burguera *et al*., 2000); (Hegyi *et al*., 2004)). OB-Rb is highly expressed in the hypothalamus and cerebellum (Burguera *et al*., 2000); (Considine *et al*., 1996). In addition, the leptin receptor is expressed in other tissues, such as the human vasculature, stomach and placenta (Sobhani *et al*., 2000); (Henson *et al*., 1998); (Sierra-Honigmann *et al*., 1998). Both long and short leptin receptor (OBR) isoforms are present in placenta, and are co-localized with leptin to the syncytiotrophoblast at the maternal interface (Bodner *et al*., 1999).

1.4.2.3 Leptin and pregnancy

Recent studies suggest that leptin is essential to trophoblastic proliferation and survival, as well as to regulating cell proliferation, inhibiting apoptosis, stimulating protein synthesis and regulating fetal growth and development (Postovit et al., 2001, Otero, 2005). In a recent study, a direct relationship between leptin and BMI, but not between leptin and pre-eclampsia was noted (Hendler *et al*., 2005a). The correlation of leptin levels with pre-eclampsia were investigated by others as well (Kafulafula *et al*., 2002); (Arita *et al*., 1999).

In contradiction to the aforementioned studies, an increase in leptin levels in pre-eclamptic patients was noted (McCarthy *et al*., 1999); (Ning *et al*., 2004); whilst Khosrowbeygi and Ahmadvand (2013) showed a significant increase in the leptin/adiponectin ratio in pre-eclamptic Bangladeshi women (Khosrowbeygi and Ahmadvand, 2013). However, all the above studies were small in number and could not be generalised to the local population. They also failed to take into account possible effects of HIV and antiretroviral drugs.

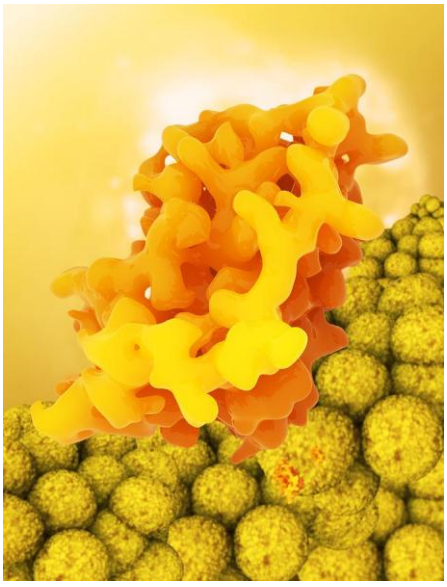

Figure 1.6: Leptin attaching to adipose tissue as depicted by (Andrade, 2014)

1.4.3 Resistin

Resistin is a cytokine that was discovered in 2001 by Steppan *et al*., (Steppan, 2001). Resistin circulates at high concentrations in diet-induced and genetic varieties of obesity and has been found to modulate insulin action on hepatic glucose (Chen *et al*., 2005). Circulating levels of resistin are proportional to adiposity. High levels of resistin have been observed in normal pregnant women at term, while this increase is less evident in women with PE (Milan *et al*., 2002).

Resistin itself is a cysteine-rich protein that is encoded by the RETN gene in humans. It is a signalling molecule expressed in monocytes, macrophages and adipocytes. In adipocytes, resistin gene expression is induced during fat cell differentiation. Resistin has been shown to increase transcriptional events, leading to an increased expression of several pro-inflammatory cytokines including (but not limited to) interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumour necrosis factor-α (TNF-α) (Silswal *et al*., 2005, Maffei *et al*., 1995).

1.4.3.1 Structure and synthesis

Human resistin, a 12.5-kDa protein, contains 108 amino acids as a propeptide. Its hydrophobic signal peptide is cleaved before its secretion. Resistin circulates in human blood as a dimeric protein consisting of two 92-amino acid polypeptides that are linked by a disulfide bridge (Aruna *et al*., 2003) at Cys-26. Holcomb *et al*. first described the gene family and its tissue-specific distribution (Holcomb *et al*., 2000). By comparison of bronchoalveolar lavages from control mice with lavages from mice subjected to experimentally induced asthma, they identified, by microsequencing, a protein that was up-regulated in the asthmatic lung. This novel protein, FIZZ1 (found in inflammatory zone 1) is also known as resistin-like molecule α

(RELMα). One of two additional homologs, FIZZ2, also known as RELMβ, was found to be localized in proliferating epithelia at the base of the crypts in the intestinal tract. FIZZ2/RELMβ is also present in rapidly dividing epithelia by demonstrating a marked increase in intestinal tumors compared with control epithelia (Steppan *et al*., 2001b). RELM is also produced in adipose tissue (Steppan *et al.;* 2001b).

The third homolog, FIZZ3, is known as resistin or adipocyte-specific secretory factor and is identical to the fat specific homolog (Rajala *et al*., 2002). Steppan *et al*., demonstrated that resistin is increased in type II diabetes and is a potential link between obesity and insulin resistance (Steppan *et al*., 2001a). Notably, injection of recombinant resistin into mice reduces glucose tolerance and insulin action, whereas neutralization with anti-resistin antibodies improves insulin action.

1.4.3.2 Functions

The potential function of resistin or its homologs requires further, study (Flier, 2001b). As fat cells (adipocytes) store more fat molecules and enlarge, they release several products that can modify the body's sensitivity to insulin. Free fatty acids and TNF-α cause insulin resistance, and leptin, which regulate energy balance is implicated in development of insulin sensitivity.

Initial studies have demonstrated that obesity induced by a high-fat diet, mutation of the leptin gene (*ob/ob* mice), or mutation in the leptin receptor gene (*db/db* mice) is associated with increased circulating resistin concentration. Resistin increases blood glucose and insulin concentration in mice. It also impairs hypoglycemic response to insulin infusion. In addition, anti-resistin antibodies decrease blood glucose and improve insulin sensitivity in obese mice (Ukkola, 2002). Resistin suppresses insulin-stimulated glucose uptake in cultured 3T3-L1

adipocytes, and this effect is prevented by anti-resistin antibodies. These data suggest that resistin provokes insulin resistance and that hyper-resistinemia contributes to impaired insulin sensitivity in obese rodents (Shuldiner *et al*., 2001); (Way *et al*., 2001b); (Moore *et al*., 2001b) and (Lay *et al*., 2001). The latter group also observed lower resistin mRNA in adipose tissue in a model of mouse obesity viz., diet-induced obesity. Likewise, they observed hyper-insulinemia, hyperglycemia, hypertriglyceridemia, and hypertension in a rat model of obesity.

The physiologic role of resistin in humans remains unclear. Given the incomplete homology between human and mouse resistin and the absence of one of the three resistin isoforms, resistin in humans may have a different physiologic role to that in mice.

There is no correlation between body weight, adiposity, insulin resistance and resistin mRNA concentration. Thus, the role of resistin and other members of the FIZZ/RELM family in humans remains to be established. These proteins may be involved in the regulation of cell proliferation and differentiation. Given the production of FIZZ1/RELMα and of resistin in inflammatory cells it is a possibility that their involvement in chronic inflammatory reactions may be associated with obesity (Gomez-Ambrosi and Fruhbeck, 2001).

1.4.3.3 Resistin in pregnancy

Resistin is significantly higher in normal pregnant compared to non-pregnant women. Pre-eclamptic women have significantly lower resistin levels than their normotensive counterparts at similar gestational ages (Cortelazzi *et al*., 2007). Elevated levels of serum resistin was noted in the third trimester of normotensive pregnancy compared to non-pregnant healthy females. In comparison, resistin levels were lower in pre-eclamptics matched for gestational age and BMI

(Chen *et al*., 2005). It is plausible to hypothesise that a possible link exists between resistin and placental mass. This study will interrogate this correlation.

Figure 1.7: Structure of resistin: An illustration of resistin assembled as a trimer, the less stable but more biologically active state of the hormone modified from Amity Tung, *Molecules of the Quarter* UCLA Department of Chemistry and Biochemistry

1.4.4 Tumour necrosis factor (TNF)

TNF is involved in systemic inflammation and is a member of a group of cytokines/adipokines that stimulate the acute phase reaction. Although it is primarily produced by macrophages, it is also produced by endothelial cells and adipocytes (Locksley *et al*., 2001).

The TNF gene is located on chromosome *6p21.3*, spans 3 kilobases and contains 4 exons. The last exon codes for more than 80% of the secreted protein (Nedwin *et al*., 1985). TNF is primarily produced as a 212-amino acid-long type II transmembrane protein arranged in stable

homotrimers (Kriegler *et al*., 1988, Tang *et al*., 1996). Emanating from this membrane-integrated form the soluble homotrimeric cytokine (sTNF) is released via a proteolytic cleavage via TNF alpha converting enzyme (TACE), also called ADAM17; (Black *et al*., 1997). The soluble 51 kDa trimeric *s*TNF tends to dissociate at concentrations below the nanomolar range, thereby losing its bioactivity (Black *et al*., 1997).

1.4.4.1 Tumour necrosis factor receptors

TNF can bind to two receptors, TNF receptor type 1 (TNF-R1) and TNF receptor type 2 (TNF-R2). TNF-R1 is expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, whereas TNF-R2 is found only in cells of the immune system, and responds to the membrane-bound form of the TNF homotrimer (Guoqing and Goeddel, 2002).

Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein SODD from the intracellular death domain. This dissociation enables the adaptor protein TRADD to bind to the death domain, serving as a platform for subsequent protein binding. Following TRADD binding, three pathways can be initiated (Guoqing and Goeddel, 2002, Wajant *et al*., 2003).

- Activation of NF-κB: NF-κB is a transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory response and anti-apoptotic factors.
- Activation of the mitogen activated protein kinase (MAPK) pathways: Of the three major MAPK cascades, TNF induces a strong activation of the stress-related Jun N-

terminal kinase (JNK) group. The JNK pathway is involved in cell differentiation, proliferation, and is generally pro-apoptotic.

- Induction of death signaling: Like all death-domain-containing members of the TNFR superfamily, TNF-R1 is involved in death signaling (Gaur and Aggarwal, 2003). However, TNF-induced cell death plays only a minor role compared to its overwhelming functions in the inflammatory process.

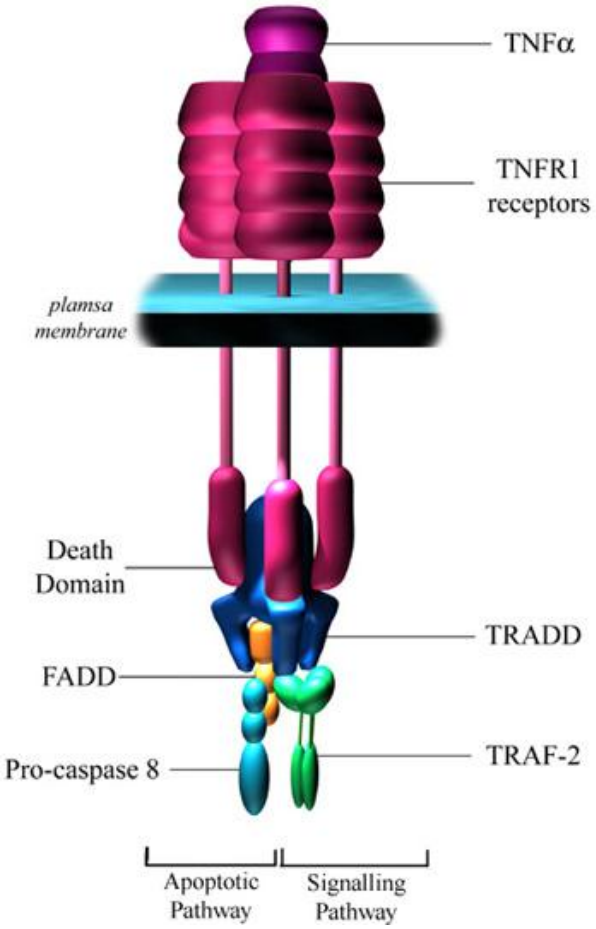

Figure 1.8: This is a model of the Tumor Necrosis Factor Receptor1 (TNFR1) for Tumor Necrosis Factor Alpha (TNF-α). TNF-α is shown bound to the extracellular ligand-binding domain of the transmembrane TNFR1 – modified from: php.med.unsw.edu.au

1.4.4.2 TNF in pregnancy

In normal pregnancy, at physiologic concentrations, TNF-α acts as a regulatory apoptotic agent that limits the invasive abilities of extravillous trophoblastic cells necessary for appropriate placental anchorage and blood flow toward the intervillous space (Fukushima *et al*., 2003). TNF-α has been shown to directly increase transcription of the vasoconstrictor peptide endothelin (ET), ET-1 (Marsden and Brenner, 1992, Roberts *et al*., 2006). Because endothelial damage is a known stimulus for ET-1 synthesis, increases in the production of ET-1 and activation of ETA receptors have been proposed to participate in the pathophysiology of hypertension during pre-eclampsia (Roberts *et al*., 1991, Taylor and Roberts, 1999). Furthermore, plasma concentrations of ET (ET-1) are increased two- to threefold in patients with pre-eclampsia compared to normal pregnant women. This increase occurs late in the disease process, suggesting that it may play a role in the progression rather than the initiation of pre-eclampsia (Roberts et al., 1991, TAYLOR et al., 1990, Taylor and Roberts, 1999, Nova et al., 1991, Wang et al., 1994).

The role of certain adipose derived proteins has been implicated in enhancing pro-inflammatory responses. In contrast to HIV infection, pre-eclampsia is associated with immune hyper-reactivity. HIV infection is also associated with muscle wasting, hence the levels of these hormones may be dysregulated and requires investigation (Matarese *et al*., 2005, Lago *et al*., 2007, Garg, 2004). Additionally, this dysregulation may serve as a predictor test for the predisposition to the development of pre-eclampsia.

1.5 HIV IN SA

Sub-Saharan Africa carries the majority of the AIDS disease burden when considering the HIV pandemic (UNAIDS, 2008, Ramjee *et al*., 2012). South Africa has more than six million people infected with HIV/AIDS (UNAIDS, 2013) Geographic variations of the epidemic in South Africa reflect a provincial difference with KwaZulu-Natal having the highest incidence. It is also considered the epicentre of this global pandemic (Ramjee *et al*., 2012).

In South Africa, the national HIV prevalence amongst women has dropped from 30.2% to 29.1% (Udjo, 2006). Notably, the epidemic has devastating consequences on women of reproductive age 15 - 24 y (Moodley and Moodley, 2005). Approximately 30% of South African parturients are co-infected with HIV (Kalumba *et al*., 2013). The latest report of the National Committee on Confidential Enquiries into Maternal Deaths in South Africa indicates that HIV/AIDS contributes to approximately 41% of all maternal deaths (Dpt of Health South Africa, 2012). As expected, KZN has a 40% infection rate in pregnant women (Kalumba *et al*., 2013).

1.5.1 Role of HIV in pre-eclampsia

The change in body fat distribution is a common finding in individuals with HIV infection, being treated with antiretrovirals. This condition has many similarities with rare, congenital and acquired lipodystrophies. This is associated with depletion of subcutaneous fat, increased triglycerides and profound insulin resistance (Addy *et al*., 2003). Recent studies have shown that these patients have marked changes in circulating levels of adipocyte secreted hormones, including leptin and adiponectin. This may contribute to the noted metabolic abnormalities (Nagy *et al*., 2003, Chaparro *et al*., 2005).

In HIV lipodystrophies, adiponectin levels are significantly lower in patients with fat redistribution and correlate inversely with serum triglycerides and insulin resistance, with levels being lowest in individuals with peripheral lipoatrophy and central lipohypertrophy (Chaparro *et al*., 2005, Verkauskiene *et al*., 2006, Khan *et al*., 2006).

A significant increase in pre-eclampsia and fetal death in HIV-infected pregnant women on highly active antiretroviral therapy (HAART) have been reported (Suy *et al*., 2006). Pregnant women attending the Hospital Clinic in Barcelona, Spain were catechised to determine an association between pre-eclampsia and/or fetal death and HAART. During January 2001 - August 2003, 8 295 women delivered babies of whom 82 (0.9%) were HIV infected. Overall, 237 (2.9%) of the HIV-uninfected women developed pre-eclampsia and 40 (0.5%) was associated with fetal death. Alarmingly, the HIV-infected women had a much higher rate of pre-eclampsia (11%) development with a significantly higher incidence of fetal death (6.1%) (Suy *et al*., 2006).

Of interest is the mirrored effect of the inflammatory adipokines on HIV susceptibility. HIV-1 seropositive women with clinical conditions of pro-inflammatory mediator production in the placenta were found to be at increased risk of HIV-1 transmission to their fetuses (Parry *et al*., 2006). Moreover, inflammatory mediators such as TNF-α produced locally by the placenta augment HIV-1 infection and replication (Vigano *et al*., 1998, Hamamoto *et al*., 1990).

1.5.2 Role of HIV in adipokine formation

The advent of highly active anti-retroviral therapy (HAART) was a turning point in the history of the acquired immune deficiency syndrome. Side effects of HAART therapy include insulin resistance, metabolic abnormalities, and changes in body shape (Carr *et al*., 1998a).

HIV associated lipodystrophy syndrome (HALS) is one the first and commonest secondary effects noted. HALS characteristically occurs as peripheral lipoatrophy of subcutaneous adipose tissue (in the face, limbs and buttocks), visceral fat accumulation, and lipomatosis, especially in the dorsocervical area ("buffalo hump") (Giralt *et al*., 2011).In obesity, secretion of peptide hormones such as leptin and adiponectin by adipose tissue is disturbed, a disruption that has been associated with insulin resistance, metabolic syndrome, and cardiovascular diseases (Kadowaki et al., 2007); Guzik *et al*., 2006).

Evidence suggests that adipokines play a role in metabolism, energy homeostasis, weight regulation, and many other biological processes (Carr *et al*., 2004).Studies on mice show that adiponectin decreases liver and muscle triglycerides by up-regulating the expression of molecules involved in fatty acid oxidation and muscle energy expenditure (Fruebis *et al*., 2001). Levels of adiponectin have been found to be lowered in obesity, diabetes, non-alcoholic liver disease and some subsets of lipodystrophy including HALS patients – Addy *et al.*, 2003; Kadowaki, 2005).

As obesity and HIV have become critical disorders of the South African population and pre-eclampsia remains one of the instrumental causes of maternal mortality it has been decided to design a study to address the impact of all three on one another.

1.6 AIMS /OBJECTIVES OF STUDY

1.6.1 Primary objectives

1. Compare serum levels of adiponectin, leptin, resistin and TNF-α (adiponectin / leptin / resistin / TNF-α) in the pregnant compared to the non-pregnant Black population in the maternal serum.
2. Compare serum levels of adiponectin / leptin / resistin / TNF-α in HIV+ve parturients as compared to HIV-ve parturients in the maternal serum.
3. Compare serum levels of adiponectin / leptin / resistin / TNF-α in patients with early onset as compared to late onset pre-eclampsia in the maternal serum.
4. Compare serum levels of adiponectin / leptin / resistin / TNF-α in patients with normotensive pregnancy compared to late onset pre-eclampsia in the maternal serum.
5. Compare serum levels of adiponectin / leptin / resistin / TNF-α in patients with pre-eclampsia and HIV as compared to patients with pre-eclampsia but without HIV in the maternal serum.

1.6.2 Secondary objectives

1. Compare the serum levels of adiponectin / leptin / resistin / TNF-α in HIV+ve pregnant women with a CD_4 count above 350 in the maternal serum.
2. Compare the levels of adiponectin / leptin / resistin / TNF-α in HIV+ve pregnant women with a CD_4 count below 350 in the maternal serum.
3. Compare the levels of adiponectin / leptin / resistin / TNF-α in pregnant HIV+ve women on ARVS and those not on ARVs in the maternal serum.

1.6.3 Hypotheses

1. The levels of adiponectin / leptin / resistin / TNF-α differ in the pregnant and non-pregnant populations.
2. The levels of adiponectin / leptin / resistin / TNF-α differ in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia.
3. The levels of adiponectin/ leptin /resistin / TNF-α in pre-eclamptics also differ according to HIV status.
4. The difference in levels of adiponectin/ leptin / resistin / TNF-α in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia are also altered by the HIV status of the patients.

CHAPTER 2

MATERIALS AND METHODS

2.1. STUDY SITE, ETHICS APPROVAL AND INFORMED CONSENT

This prospective study was approved by the Institutional Postgraduate Committee (Addendum 1) and was conducted at RK Khan Hospital in KwaZulu-Natal, South Africa. Ethical approval was obtained from the Biomedical Research Ethics Committee (BREC 256 /12 – Addendum 2). Permission to perform the study at RK Khan Hospital was obtained from the Hospital Manager (Addendum 3). The study was conducted during the period of 1st August 2012 – 30th April 2014. Written informed consent was obtained from each participant in both English and Zulu (Addendum 4).

2.2. STUDY POPULATION

Participants were recruited at the Obstetric Unit (antenatal ward) of RK Khan Hospital by the primary investigator, a qualified obstetrician/gynaecologist, during the antepartum period. International standard definitions of pre-eclampsia were employed during the selection process (National High Blood Pressure Education Program Working Group on High Blood Pressure in, 2000). All participants were managed by standard of care policies in accordance with the National Department of Health, South Africa. Non pregnant patients were recruited from the Gynaecology Unit (contraceptive clinic) at RK Khan Hospital. All non-pregnant participants (more than 6 weeks post-partum) in good clinical health and are between the ages of 18 – 45 y were eligible for participation.

Participants were recruited into one of three groups:

- non pregnant normotensive population (n = 119),
- pregnant normotensive population (n = 118) and
- pre-eclamptics (n = 91).

A schematic outline of the three study groups are shown in Figure 2.1. Each study group was further, sub-divided according to HIV status. HIV positive participants were further, stratified by their CD4 count and ARV usage.

In addition, all pre-eclamptic patients were further, classified as either early or late onset disease. Early onset pre-eclampsia (EOPE) was defined as onset of pre-eclampsia after 20 weeks of gestation, but prior to 34 weeks completed gestation. Late onset pre-eclampsia (LOPE) was defined as the onset of pre-eclampsia after 34 weeks gestation.

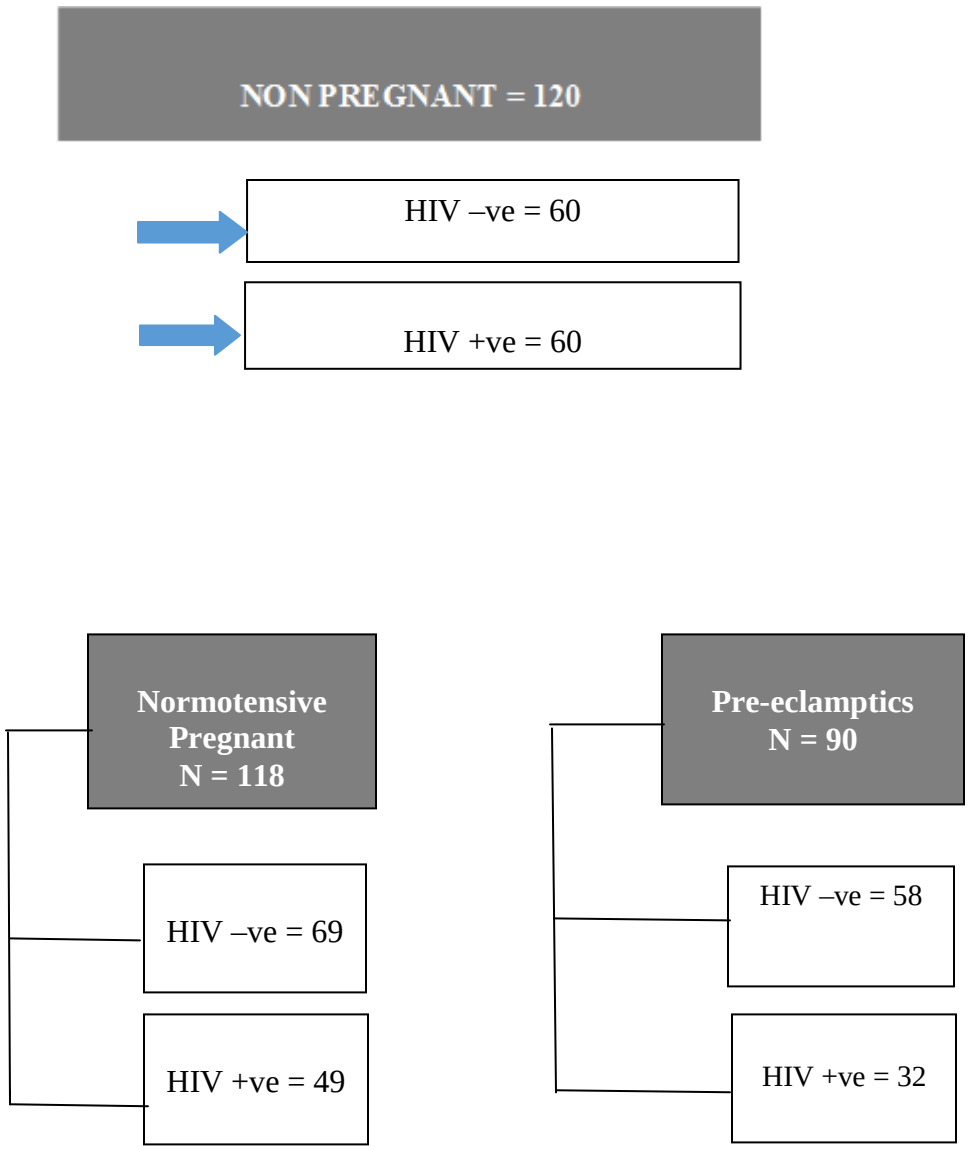

Figure 2.1: Schematic diagram of pregnant and non-pregnant cohorts. Each cohort was stratified according to HIV status

2.2.1. Inclusion Criteria

All Black South African patients over 18 years of age were eligible for participation in the study. HIV status and CD_4 count were examined prior to entry into the study. Patients were categorised into non-pregnant normotensive, pregnant normotensive and pre-eclamptic cohorts.

The pre-eclamptic group was further, sub-categorised according to gestational age, i.e., ≥ 34 weeks gestation. The pregnant population was further, compared to a non-pregnant, healthy reproductive age female population according to HIV status and CD_4 count. Non pregnant participants were eligible for entry provided they were between 18 – 45 y. Their HIV status had to be known.

2.2.2. Exclusion Criteria

Exhaustive exclusion criteria were used in an effort to maintain a homogenous study population. The following patients were excluded from the study:

a) Patient's with chronic hypertension
b) Non-Black African patients
c) Gestational diabetics
d) Chronic diabetics
e) Patients with unknown HIV status
f) Unbooked patients
g) Patients with chorioamnionitis
h) Polycystic ovarian syndrome patients
i) Patients with thyroid disorders
j) Patients with chronic renal disease
k) Patients with cardiac failure
l) Patients with connective tissue disease / antiphospholipid syndrome
m) Patients with abruption placentae

2.3. SAMPLE COLLECTION

2.3.1. Patient Demographics

Participant demographics were collated by the researcher into a pre-designed data sheet (Appendix 2.4) during the antepartum period. These included maternal age, residential area, smoking and drug habits, HIV status and CD_4 counts, antiretroviral usage (3-drug *versus* PMTCT regimens), parity, gravidity, previous reasons for miscarriage, blood pressures at confirmation of pre-eclampsia, height, weight, body mass index (BMI), mid upper arm circumference (MUAC), triceps skin fold thickness, presence and degree of oedema, urine dipstick findings at time of diagnosis of pre-eclampsia. Indications and mode of delivery as well as associated complications were noted. Fetal ultrasound abnormalities, neonatal outcomes in terms of APGARS, baby weight, placental shape and weight and appearance and umbilical cord length were recorded.

The Body Mass Index (BMI) was calculated by dividing the body weight (kg) by the height squared (m) i.e.,

$$BMI = \frac{weight}{(height)^2}$$

Obesity was defined as a BMI of at least 30 kg/m^2 (Kafulafula and Moodley, 2001). Additionally, the mid upper arm circumference was taken with a measuring tape at the midpoint between the olecranon and the acromion process of the right arm. The triceps skin fold thickness was measured with the participant standing upright with arms loosely hanging at

her sides. The subcutaneous tissue and skin of the midpoint of the posterior part of the right arm was measured with a skin fold caliper. The mid-arm muscle circumference was calculated by using the following formula:

Mid-arm muscle circumference = mid-arm circumference – [3.14 X triceps skin fold thickness] (Huang *et al*., 2001).

2.3.2. Blood collection

Peripheral venous blood was collected during the antepartum period as pre-eclampsia rapidly resolves post-partum. Four vials each containing 10 ml of blood were collected per patient in EDTA anti-coagulant tubes and plain vials, after application of a tourniquet above the ante-cubital fossa. HIV tests and CD_4 counts are offered as part of the standard of care in the ante-natal clinic. Only those patients agreeing to HIV-testing were offered placement in the study.

Each patient data analysis form had a hospital identification number. In an effort to maintain participant anonymity, each patient was allocated a study identification number. The study identification number was written onto specimen collection vials. Blood samples were then transported in a cooler box within two hours of collection to the Optics and Imaging Centre, Doris Duke Medical Research Institute at the Nelson R. Mandela School of Medicine to be centrifuged at 4000 rpms for 20 minutes. The specimens were then stored at -80^{o}C until required.

In September 2014, these specimens were subsequently transferred by an international courier at -80^{o}C to the Staten Serum Institute, Copenhagen, Denmark. Conformance to international

standards of specimen export and transportation were strictly adhered to (Addendum -2.5). The experimental procedures were performed under combined supervision of the primary investigator and collaborators at the Statens Serum Institut.

2.4. ENZYME-LINKED IMMUNOSORBENT ASSAY DETERMINATION OF SERUM ADIPONECTIN AND TNF- α

2.4.1. Principle of serum adiponectin and TNF-α detection

This technique was first described by Engvall and Perlmann in 1972 and once more in 1982 by Gaastra (Engvall and Perlmann, 1972, Gaastra, 1984). It has been described as the double antibody sandwich technique. Antibodies against the antigen to be measured are adsorbed to a solid phase support and antigen is then added to the adsorbed antibodies. Thereafter a second enzyme labelled antibody is added. The addition of an enzyme substrate allows measurement as the degree of colour is proportional to the original bound antigen-antibody (Gaastra, 1984).

The ELISA testing was performed using the DuoSet ELISA Development System and instructions followed from the human Adiponectin (Acrp30 catalogue: DY 1065)

- Capture antibody – 360 µg/ml of mouse anti-human adiponectin was reconstituted with 1.0 ml of PBS and 720 µg/ml of mouse anti-human TNF-α is reconstituted in 1.0 ml of PBS.
- Detection antibody – 360 µg/ml of biotinylated mouse anti-human adiponectin was reconstituted with 1.0 ml of reagent diluent and 90 µg/ml of biotinylated goat anti-human TNF-α is reconstituted with 1.0 ml of reagent diluent)

- Standard – 70 ng/ml of recombinant human adiponectin was reconstituted with 0.5 ml of reagent diluent. The standard was allowed to incubate for a minimum of 15 minutes with gentle agitation prior to further, dilutions and 370 ng/ml of recombinant human TNF-α is reconstituted with 0.5 ml of reagent diluent and gently agitated for a minimum of 15 minutes.
- Streptavidin-HRP – 1.0 ml of streptavidin was conjugated to horseradish –peroxidase.

The adiponectin, and TNF-α assay utilizes either a mouse anti-human adiponectin or TNFα, antibody as appropriate for immobilization on the microtiter wells. Biotinylated mouse anti-human adiponectin antibody along with streptavidin conjugated to horseradish peroxidase (HRP) is added to enable detection of the antigen through colour change. This is followed with the addition of a reagent diluent (200µl) and incubated as directed by the kit being used. Post washing a HRP substrate, tetra-methylbenzidine (TMB), was then added with the resultant development of a blue colour (Figure 2.2). Progress of colour development was stopped by the addition of Stop Solution – this changed the colour to yellow. The concentration of adiponectin and TNF-α was directly proportional to the colour intensity of the test sample. Absorbance was read spectrophotometrically at 450 nm (Systems).

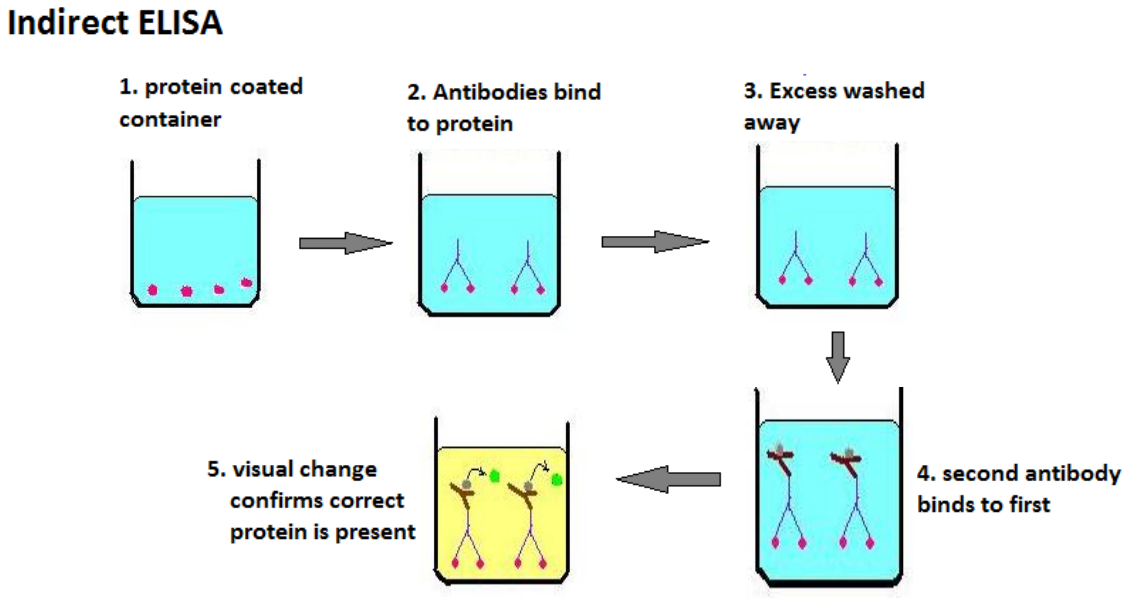

Figure 2.2: Schematic diagram illustrating ELISA procedure – adapted from (Ramawi, 2012)

2.4.2. Procedure for adiponectin and TNF-α detection by ELISA (RnD Systems)

Table 2.1 Table outlining the ELISA procedure for the determination of serum adiponectin and TNF-α

Step	Process	Method	Time
1	diluted samples	100 µl of sample in reagent diluent	2 h (1 h preparation + 1 h incubation)
2	wash wells	Wash buffer	40 min
3	detection antibody	100 µl detection Ab diluted with reagent diluent	40 min
4	wash wells	Wash buffer	x3
5	Streptavidin-HRP	100 µl of Streptavidin-HRP	40 min
6	stop solution	50 µl stop solution	20 min

2.4.3. Detection limits and co-efficient of variation (CV)

The coefficient of variation (CV) was defined as the ratio of the standard deviation σ to the mean μ:

$$C_v = \frac{\sigma}{\mu}$$

C_V shows the extent of variability in relation to mean of the population.

The detection limit for the adiponectin assay was 1.43 μg/l; coefficient of variation (C_V) within and between assays was < 4.58%. For the TNF assay the detection limit was 1.39 μg/l; C_V within and between assay was < 3.02%.

2.5. DETERMINATION OF SERUM LEPTIN AND RESISTIN BY BIOPLEX IMMUNOASSAY

2.5.1. Principle of serum leptin and resistin detection by Bioplex assays

Bioplex cytokine assays are multiplex bead assays designed to quantify multiple cytokines in diverse matrices, including serum, plasma and tissue samples. This multiplexing allows quantification of multiple cytokines in a single well. The advantage of this method is that it allows for the creation of a complete cytokine profile from limited samples and is more time efficient.

The principles behind the procedure are:

1. Fluorescently dyed beads are present to which biomolecules are bound.
2. A flow cytometer with 2 lasers and associated optics are needed to measure the biochemical reactions that occur on the surface of the beads.
3. A high speed digital signal processor is used to manage the fluorescent output.

Each colour-coded bead is conjugated to a specific reactant, which is specific for a target molecule. The assay is designed in a capture sandwich immunoassay format.
In this study a specific antibody is directed against the cytokine of interest and is covalently coupled to colour coded beads which reacts with the sample containing an unknown amount of the cytokine.

As in other multiplex assays reaction mixture is subsequently detected by the addition of Streptavidin-phycoerythrin (SA-PE). This binds to the biotinylated detection antibodies. The constituents of each well are drawn up into a flow based suspension array system which identifies and quantifies each specific reaction based on bead colour and fluorescence.

The magnitude of the reaction is then measured using fluorescently labelled reporter molecules associated with each target protein. Unknown cytokine concentrations of the sample are calculated from a standard curve that has been derived from a recombinant cytokine standard. These standard curves are provided by the multiplex assay used – in this case the Bioplex Pro assay kit was used (BIORAD, 2014).

2.5.2 Preparation and procedure:

The plate layout was planned ahead of the experiment. Approximately 30 minutes were required to bring the assay buffer, wash buffer and sample diluent to room temperature. The vacuum manifold was set to -1 to -3 mmHg for the filter plate. A single vial of standard was reconstituted in 500 µl of diluent similar to the final sample type (matrix). This was vortexed for 5 seconds and thereafter incubated on ice for 30 minutes.

A fourfold standard dilution series and blank were prepared. The standard dilutions were vortexed for 5 seconds in between liquid transfers. The serum samples were prepared in 1:4 dilution with the sample diluent. The coupled beads were vortexed for 30 seconds and were diluted to 1X assay buffer – this is light sensitive hence it was performed away from light.

The plates were subsequently washed three times with 100 µl wash buffer. The diluted 1X SA-PE was vortexed and 50 µl was added to each well and then vortexed for 5 seconds and incubated on ice for 30 minutes. The plate was then washed three times with 100 µl wash buffer. The beads were then resuspended in 125 µl assay buffer. The plate was covered and centrifuged at 850 rpm for 30 seconds. The sealing tape was then removed and the plate was read using Bioplex Manager Software (BIORAD, 2014).

A series of known concentrations of an analyte was used to construct a plot of signal intensity *vs* concentration. The plot was mathematically modelled to derive an equation that may be used to predict the concentration of the unknown samples. Both, the type of mathematical model as well as the fit of the model were considered as they have a direct effect on the accuracy of the results. Curve fitting was a critical component of immunoassay performance.

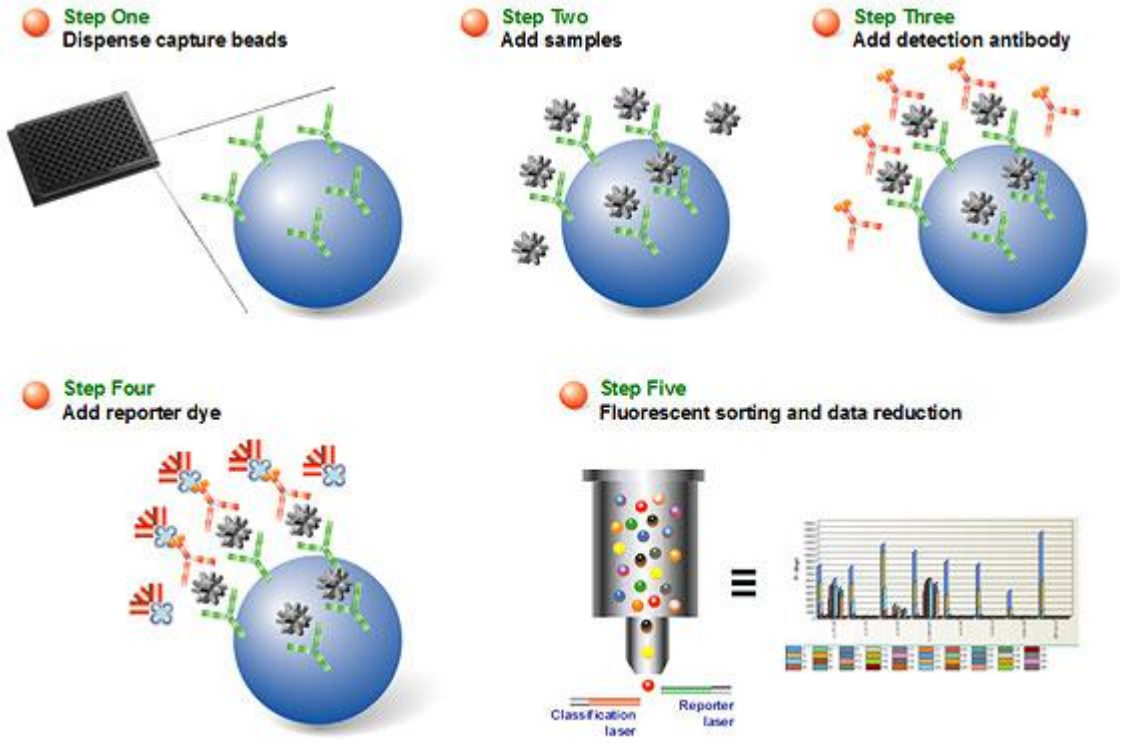

Figure 2.3: Diagram of Bioplex test procedure as adapted with permission from Biorad

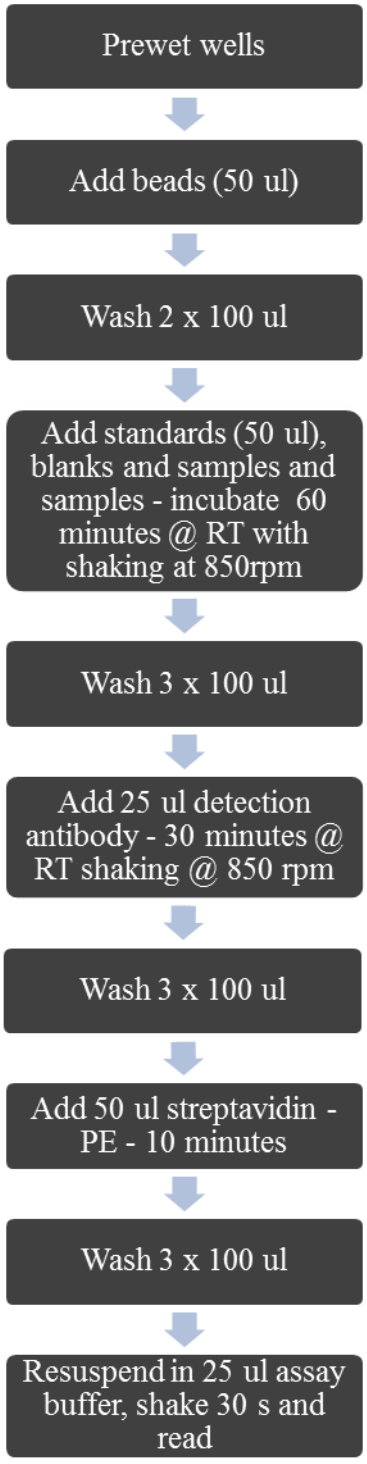

Figure 2.4: Schematic Bioplex workflow (BIORAD, 2014)

2.5.3 Linear regression

This is the simplest method for determining concentration from a standard curve. The plot was constructed on concentration *vs* response. This method has been used traditionally to quantitate results of ELISA and other immunoassays (Wild, 2005).

2.5.4 Logistic regression:

Immunoassay data may also be used in a non-linear regression routine. The log of the concentration was plotted on the X-axis *vs* the response (fluorescence intensity) plotted on the Y-axis (Wild, 2005).

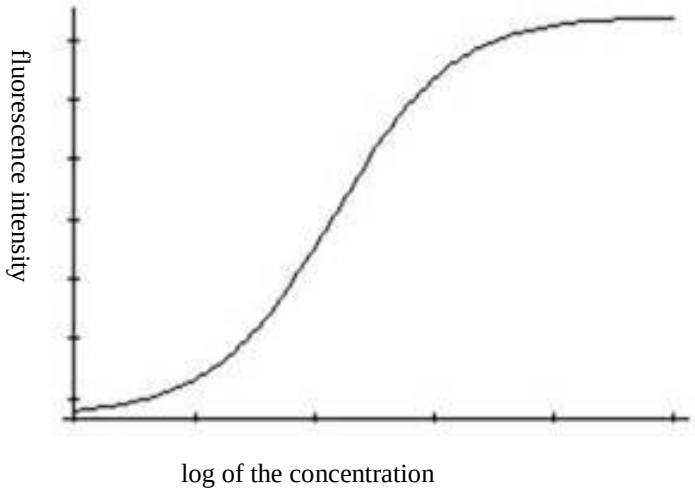

Figure 2.5: Logistic regression curve

2.6 STATISTICAL ANALYSIS

SPSS version 21 was used to analyse the data. A p value < 0.05 was considered as statistically significant. Demographic variables were summarised using mean, standard deviation and range for continuous variables, and frequency tables for categorical variables. Outcome variables were extremely non-normally distributed, therefore non-parametric tests were used to compare median levels between groups. Two independent groups were compared using Mann-Whitney U tests. Kruskal-Wallis tests were used to compare more than two independent groups.

CHAPTER 3

RESULTS

3.1 STUDY POPULATION

A total of 328 women attending RK Khan Hospital, a regional and district hospital in Chatsworth, a suburb in the eThekwini health district, KwaZulu-Natal were studied. Based on inclusion and exclusion criteria (2.2.1 and 2.2.2), women were recruited into two groups *ie*., non-pregnant (n = 120; 36.58%) and pregnant group (n = 208; 63.41%). Pregnant women were further, categorised into the normotensive pregnant (n = 118; 35.97%) and the pre-eclamptic (n = 90; 27.43%) groups (Figure 3.1).

The pregnant cohort was also sub-stratified in accordance with their HIV status. Of the 118 normotensive pregnant women, 49 (41.52%) were HIV positive whilst 69 (58.47%) were HIV negative. Likewise the pre-eclamptic women were also stratified according to their HIV status into HIV positive (n = 32; 35.55%) and HIV negative (n = 58; 64.44%; Fig 3.2).

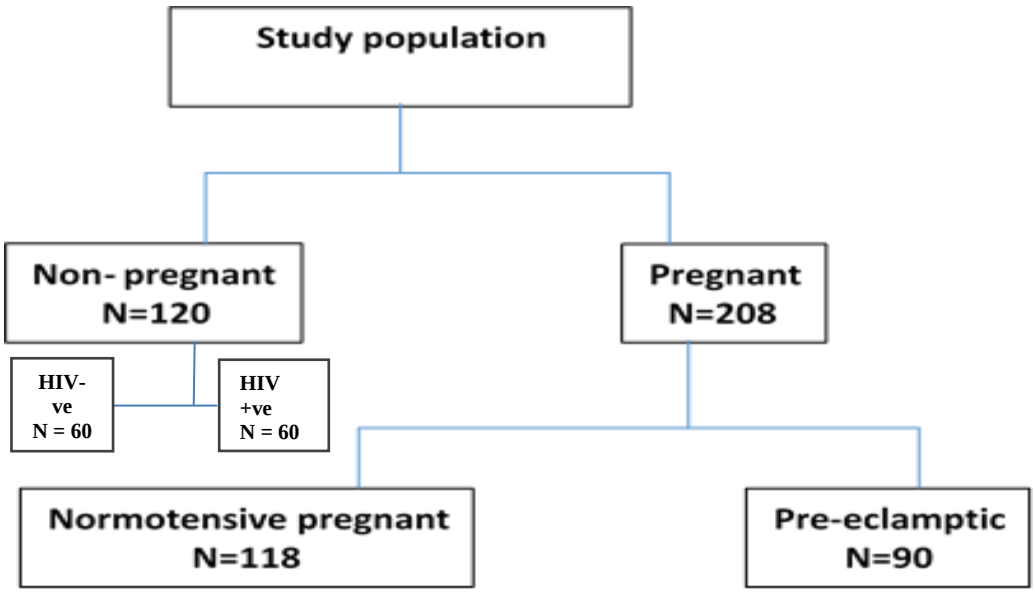

Figure 3.1: Schematic outline of study population

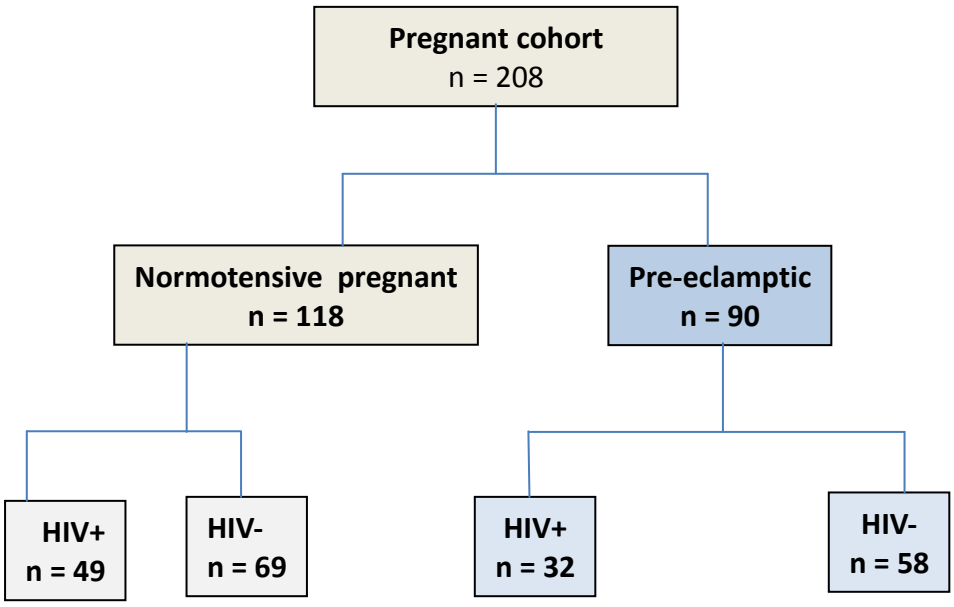

Figure 3.1: Schematic outline of subcategories within the pregnant cohort based on HIV infection

3.2 DEMOGRAPHIC AND CLINICAL DATA OF STUDY POPULATION

3.2.1 Maternal Age

Age was not normally distributed amongst the study groups. Additionally, both log and square root transformations of the age data failed to achieve normality. A Kruskal-Wallis H test was then used to analyse mean ranks of age (raw data) across the study groups.

Patient age ranged between 18 - 45 y across all the study population (Table 3.1; Figure 3.3). The mean age of study participants in the non- pregnant group was 27.75 ± 7.8 y (range: 21 – 37 y) compared to 27.30 ± 5.7 y (range: 18 - 41 y) in the normotensive group. The mean age of participants in the EOPE group was 26.38 ± 6.1 y (range: 18 - 31 y) compared to 26.62 ± 7.5 y (range: 19 – 38 y) in the LOPE group. Upon initial inspection there appeared to be no significant difference in and between study groups. However, there was a statistically significant difference in the distribution of age across the categories according to HIV status ($p < 0.00$). Patients were older in the HIV+ve group as compared to the HIV-ve group.

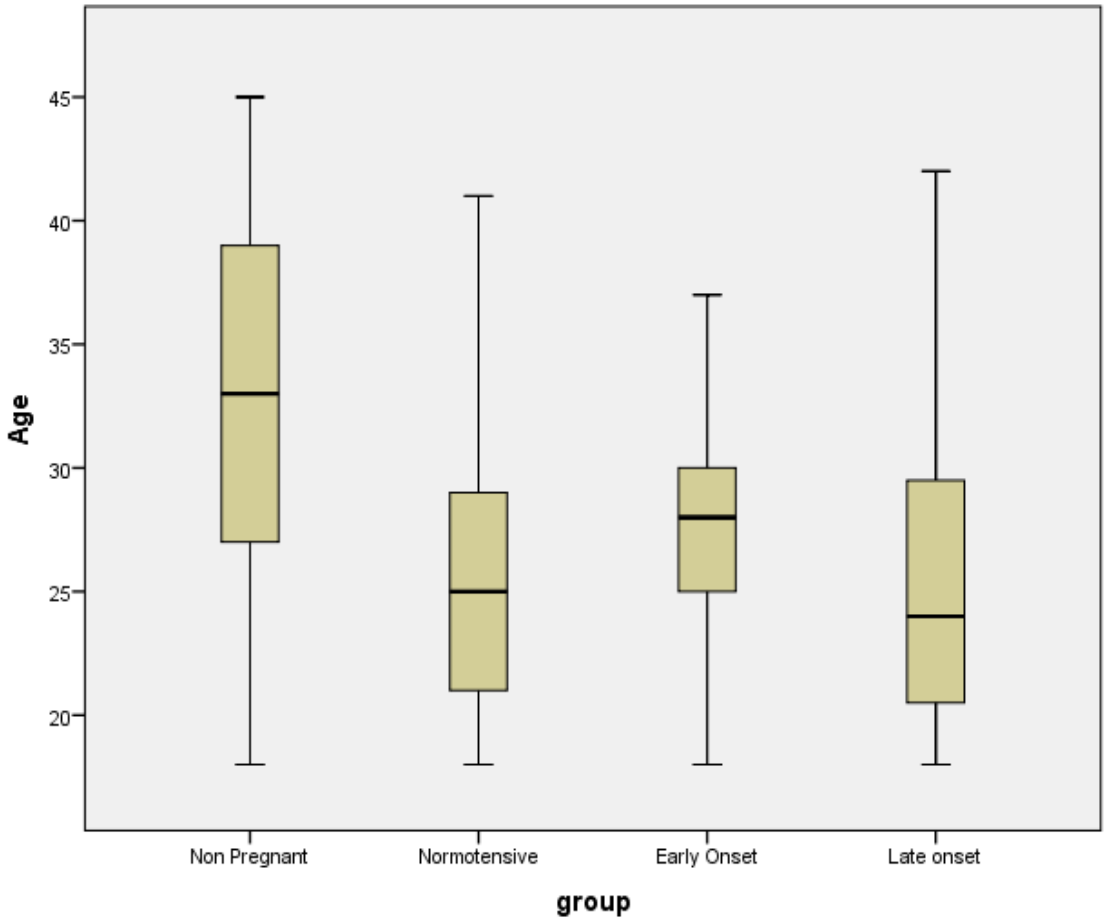

Figure 3.2: Boxplot of maternal age (y) across the study population

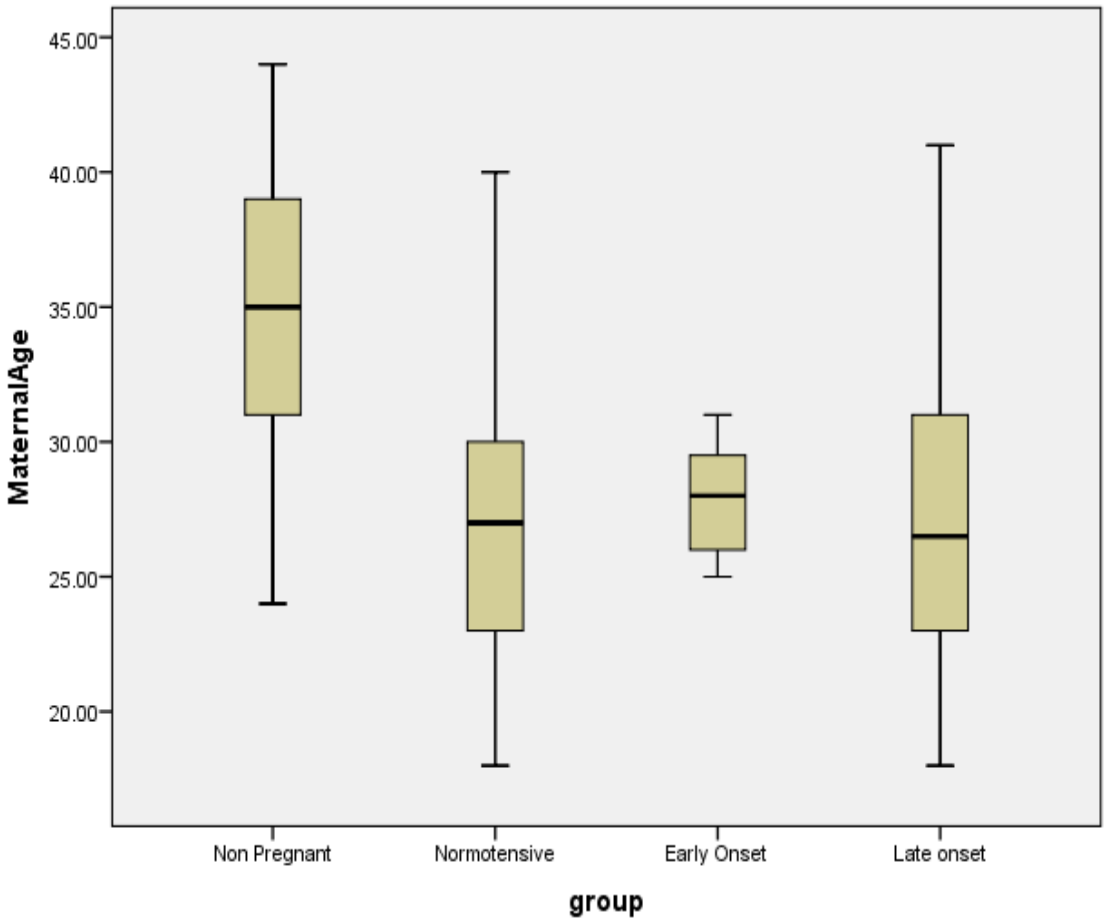

Figure 3.3: Boxplot illustrating maternal age (y) across the HIV positive groups

A Kruskal-Wallis H test showed that there was a statistically significant difference in age between the different groups, [$\chi^2(3) = 69.723$; $p < 0.000$], with a mean rank age score of 217.28 for non-pregnant, 125.27 for normotensive pregnant, 161.32 for EOPE and 121.49 for the LOPE groups. The effect size (η^2) of the group was 21.7% [calculated as $\chi^2/(n-1)$].

Pairwise Mann Whitney U comparisons were made between pairs of groups to identify which groups were different. A Bonferroni *post hoc* analyses (adjusted $p < 0.008$) revealed that there were statistically significant differences between median ages of the non-pregnant group *versus* normotensive pregnant, EOPE and LOPE groups respectively (U: 3035.5; $p < 0.000$; U:1175;

$p < 0.001$; U:1267; $p < 0.000$). There were no significant differences between normotensive pregnant *versus* EOPE and LOPE group combinations.

When comparing the HIV positive *versus* the HIV negative participants, the Levene's test of equal variances using a 2 tailed T-test showed a statistically significant difference in age between the 2 groups ($p < 0.000$).

Furthermore, when considering the effect of HIV, post Kruskal-Wallis H test and Bonferroni *post hoc* analysis, a statistically significant difference between the different groups, [($\chi^2(7) = 85.486$); $p < 0.000$], was found across the categories (Table 3.1; Figure 3.5).

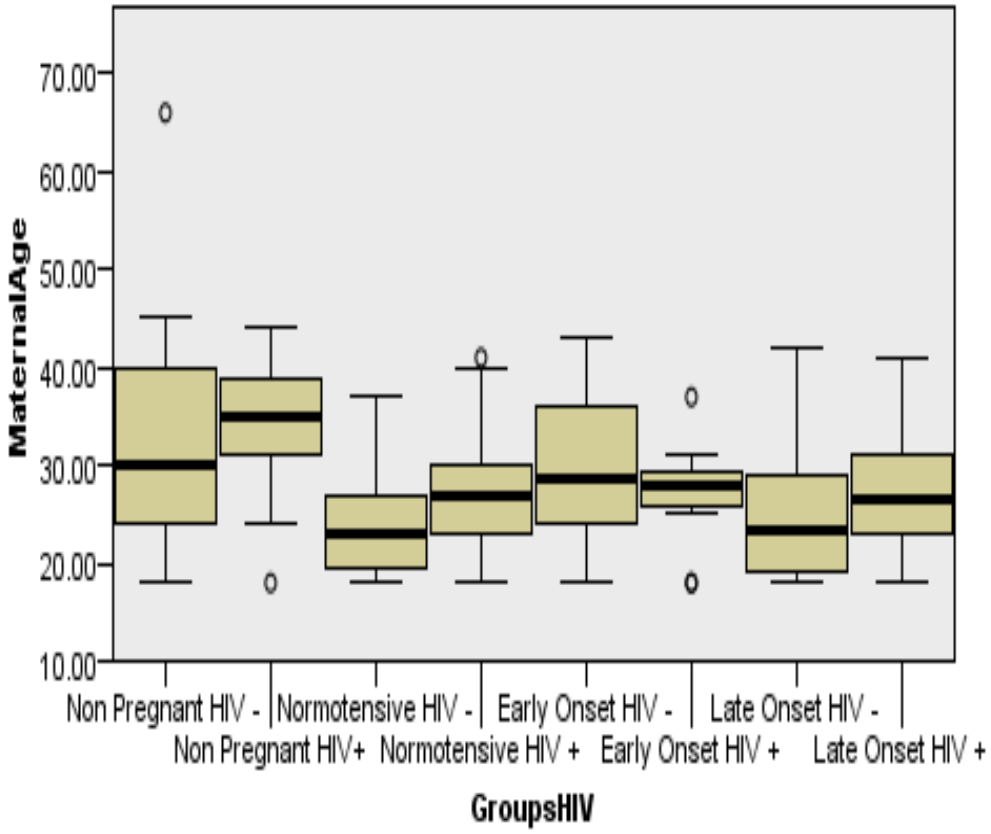

Figure 3.4: Boxplot illustrating maternal age (y) based on HIV status

3.2.2 Blood Pressure

3.2.2.1 Systolic blood pressure

The mean systolic blood pressure for non-pregnant participants was 133 ± 26.47 mmHg *versus* 117.7 ± 9.31 mmHg of the normotensive pregnant participants. The mean systolic blood pressure for EOPE was 168 8.07 mmHg compared to 161.08 ± 22.55 mmHg in the LOPE group. A detailed statistical assessment of systolic blood pressure across the study groups is outlined in Table 3.3 and Figure 3.6 respectively.

There was a statistically significant difference between groups as determined by one-way ANOVA ($F(3,322) = 308.790$, $p = 0.000$). A Fisher's least significant difference (LSD) *post-hoc* test revealed that the systolic blood pressure was statistically significantly lower in the non-pregnant group (118.903 ± 10.907 mmHg, $p = 0.000$) compared to the early onset (168.654 ± 15.552 mmHg, $p = 0.000$) and late onset pre-eclamptic groups (163.38 ± 16.787 mmHg; Figure 3.6). There were no statistically significant differences between the non-pregnant and normotensive groups ($p > 0.05$). There were no statistically significant difference of the systolic blood pressure between the EOPE and LOPE groups ($p > 0.050$).

However, of interest is the fact that when Levene's test where equal variances was not assumed and a 2 tailed T-test was then performed, a statistically significant difference of $p = 0.000$ was found between all groups.

After considering the effect of HIV, the Kruskal-Wallis H test and Bonferroni post hoc analysis, demonstrated a statistically significant difference between the systolic blood pressure across different groups,[$(\chi^2(7) = 192.040)$; $p = 0.000$], was demonstrated (Figure 3.7).

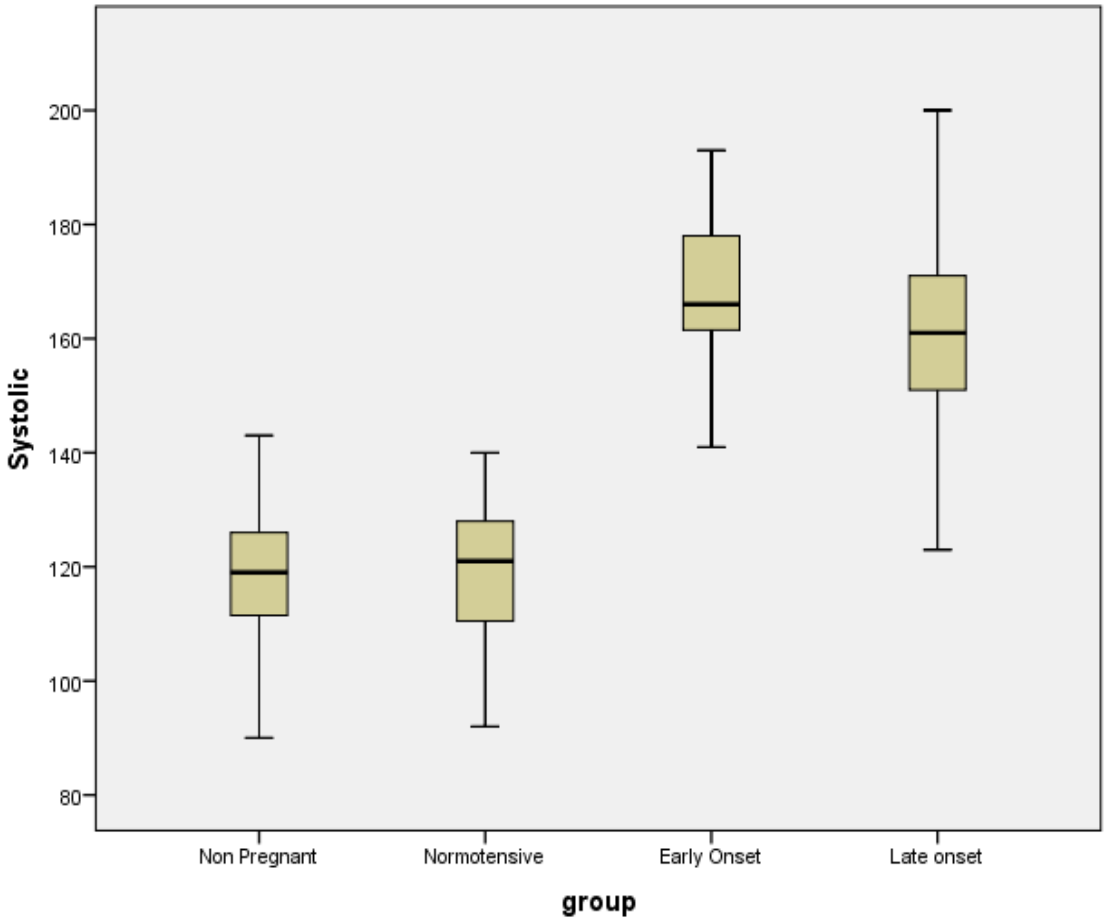

Figure 3.5: Boxplot showing the systolic blood pressure (mmHg) across groups

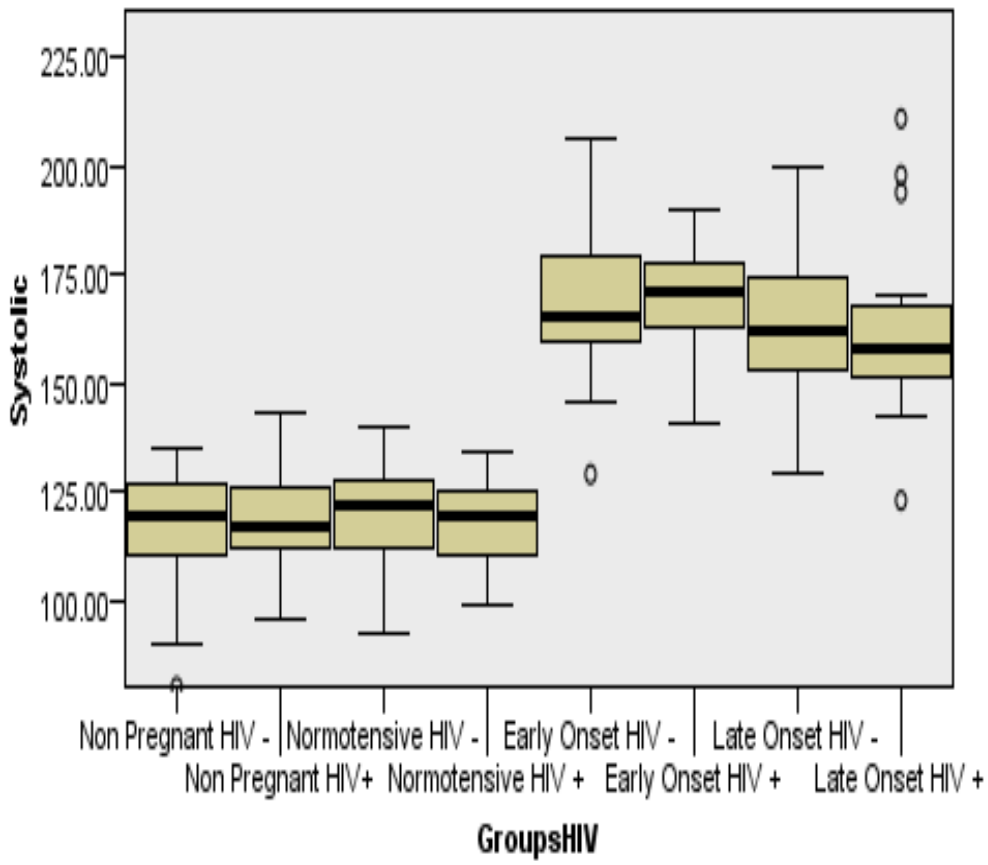

Figure 3.6: Boxplot illustrating systolic blood pressure (mmHg) based on HIV status

3.2.2.2 Diastolic blood pressure

The mean diastolic blood pressure for non-pregnant participants was 77.25 ± 15.17 mmHg ($p <$ 0.003) compared to 73.34 mmHg normotensive pregnant group. The mean diastolic blood pressure was 109.13 ± 14.567 mmHg compared to 96.54 ± 7.6 mmHg in the EOPE and LOPE groups respectively.

There was a statistically significant difference in the mean ± standard deviation of the diastolic blood pressure between groups as determined by one-way ANOVA [$F(3,322) = 146.320$, $p =$ 0.000]. A Fisher's least significant difference post-hoc test revealed that the mean diastolic blood pressure was statistically significantly lower in the non-pregnant group (72.34 ± 11.247 mmHg) compared to the EOPE (104.91 ± 14.567 mmHg,) and the LOPE groups (100.97 ± 7.655 mmHg) ($p = 0.000$; Figure 3.8). There were no statistically significant differences between the non-pregnant and normotensive groups ($p = 0.607$). Likewise, there were no statistically significant differences between the EOPE and LOPE groups ($p = 0.107$).

Only when, the Levene's test compared the pre-eclamptic group as a whole to the normotensive group (irrespective of equal variance or not), the 2 tailed T-test showed a statistical significance of p = 0.000 (Table 3.1).

When considering the effect of HIV, the Kruskal-Wallis H test and Bonferroni post hoc analysis demostrated a statistically significant difference between the different groups, [($\chi 2(7) = 170.898$); p = 0.000] (Figure 3.9).

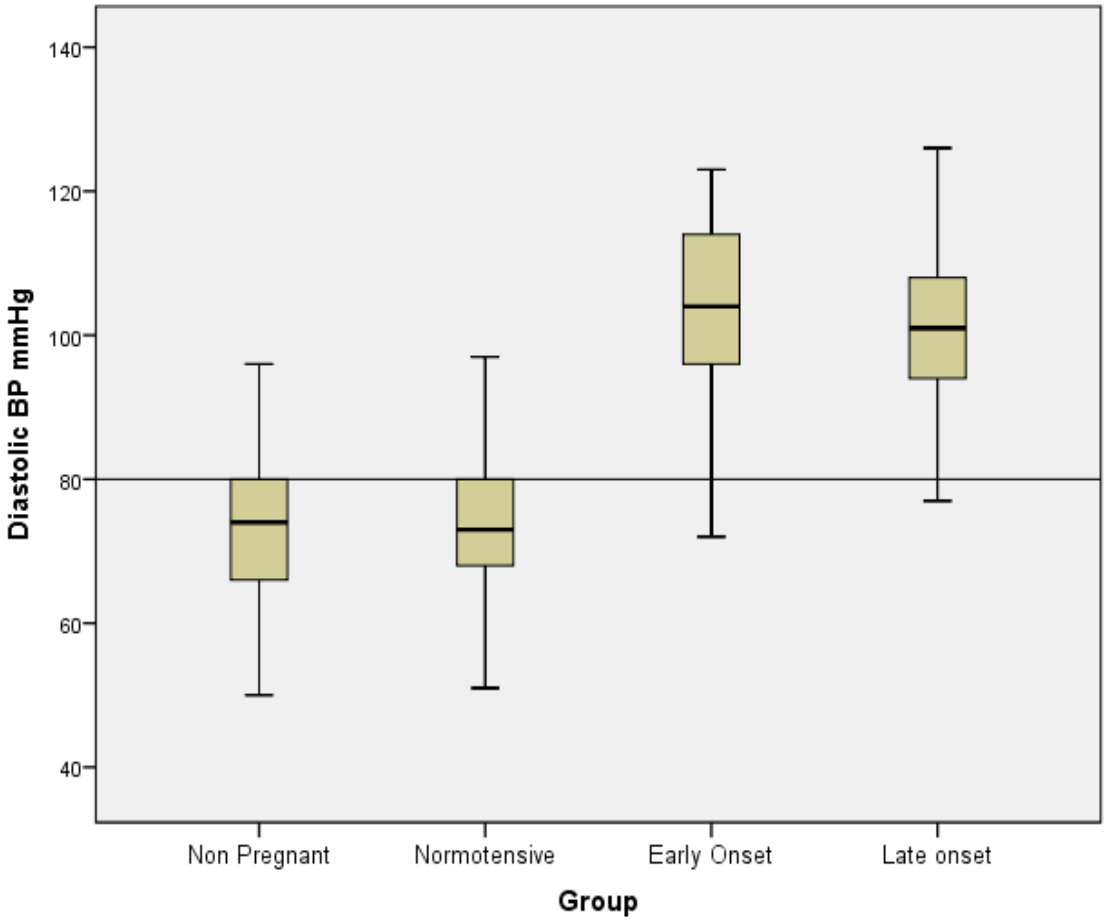

Figure 3.7: Boxplot of diastolic blood pressure (mmHg) across study groups

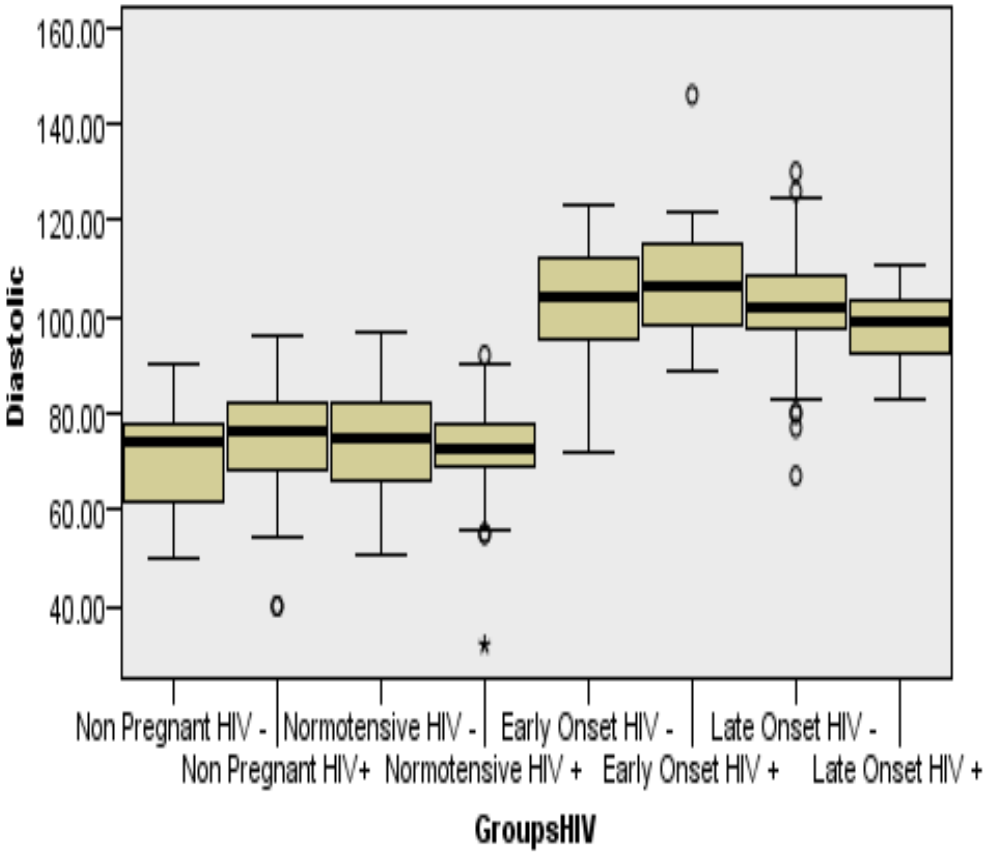

Figure 3.8: Boxplot illustrating diastolic blood pressure (mmHg) based on HIV status

3.2.3 Gestational Age

The mean gestational age of normotensive pregnant participants was 38.98 ± 1.5 weeks. The mean gestational age of participants with EOPE was 32 ± 3.8 weeks *versus* 36.92 ± 1.847 weeks in the LOPE groups.

Gestational age was not normally distributed but there was homogeneity of variance amongst groups. Therefore a Kruskal Wallis omnibus test was performed to check for a difference amongst the groups. The Kruskal-Wallis H test showed a statistically significant difference in gestational age between the different groups, [($\chi^2(2) = 83.196$); $p = 0.000$], with a mean rank gestational age score of 131.50 for normotensive, 28.98 for EOPE and 87.33 for the LOPE groups. The effect size (η^2) of the difference in mean ranks was 40.6% [calculated as $\chi^2/(n-1)$ = 83.196 divided by 206-1] demonstrating the effect of the mean rank on gestational age.

Mann Whitney U tests performed indicated that the normotensive groups was significantly higher than both the EOPE (U=190.50; $p < 0.000$) and LOPE groups (U = 1654.00; $p = 0.000$). The pregnancies EOPE groups terminated at an earlier gestation than the LOPE groups (U = 205.0; $p = 0.000$).

Furthermore, the Levene's test followed by a 2 tailed T-test compared the pre-eclamptic group as a whole (EOPE + LOPE) to the normotensive group (irrespective of equal variance or not), a statistical significance of $p = 0.000$ was demonstrated. In addition, when the Levene's test compared the EOPE and LOPE groups (irrespective of equal variances or not) and a 2 tailed T-test then performed, a statistically significant difference of $p = 0.000$ was noted (Table 3.1).

The study demonstrated there was no statistically significant difference in the distribution of gestational age across the pregnant categories according to HIV status (Figure 3.10).

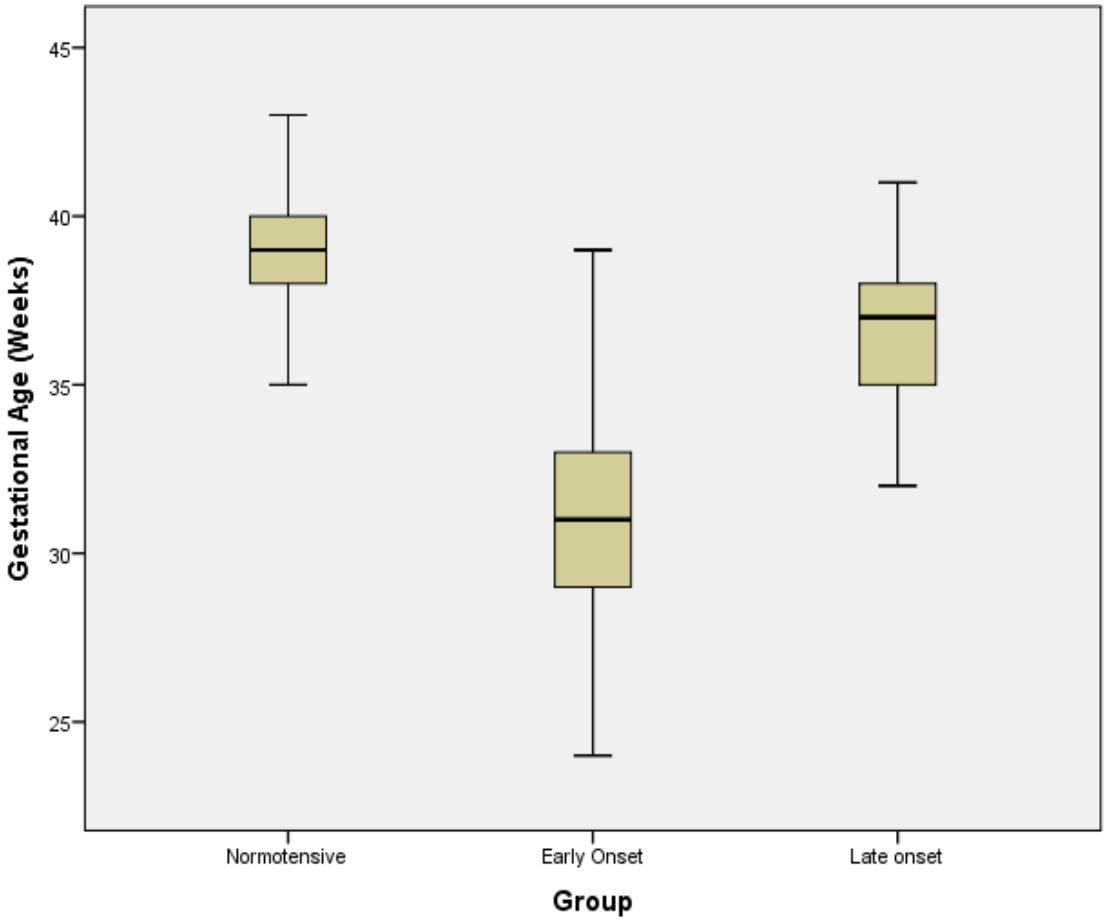

Figure 3.9: Boxplot of gestational age (weeks) across pregnant groups

3.2.4 Indications for delivery

Within the pregnant cohort (normotensive pregnant + pre-eclamptic); 65.9% of deliveries were in fetal interest as compared to maternal indications. The Pearson Chi-square test showed a statistically significant difference between the groups of $p = 0.000$. Pregnant patients (45.4%) had elective caesarean section compared to the 27.4% requiring emergency caesarean section.

Over four percent (4.6%) required induction of labour whilst 22.6% had a spontaneous normal vaginal delivery.

3.2.5 Clinical complications in the pre-eclampsia groups

Clinical complications included one case of abruption placentae, 9 cases of eclampsia, 5 cases of imminent eclampsia and 12 cases of severe pre-eclampsia. Moreover, there were 6 cases of stillbirths. In the EOPE group, there were 5 patients that developed eclampsia and one case of abruption placentae.

3.2.6 Birth weights across study groups

Babies of normotensive participants had a mean birth weight of 3.237 ± 0.40 kg. The weight of babies in the EOPE group was 1.623 ± 0.78 kg compared to 2.718 ± 0.51 kg in the LOPE group (Figure 3.11).

Baby weights were not normally distributed amongst the study groups. A Kruskal-Wallis H test showed that there was a statistically significant difference in baby weight between the different groups, [$\chi^2(2) = 76.430$; $p < 0.000$]. The effect of size (η^2) on the difference in mean ranks was 37.7%.

Pairwise Mann Whitney U tests with Bonferroni *post hoc* analyses (adjusted $p < 0.016$) showed that the normotensive pregnant baby weight differed significantly compared to EOPE (U: 165.0, $p = 0.000$) and LOPE groups (U: 1804.5 $p = 0.000$). There was a significant difference between early onset and late onset groups (U: 268.5; $p = 0.000$).

Furthermore, when the Levene's test was performed comparing the pre-eclamptic group as a whole (EOPE + LOPE) to the normotensive group (with/out equal variance), the 2 tailed T-test showed a statistical significance of p = 0.000 between babies of the pre-eclamptic mothers compared to the normotensive mothers.

Further,, when considering the effect of HIV on baby weight, the Kruskal-Wallis H test and Bonferroni *post hoc* analysis, demonstrated a statistically significant difference between the all cohorts, [($\chi^2(5) = 78.678$); $p < 0.000$; Figure 3.12].

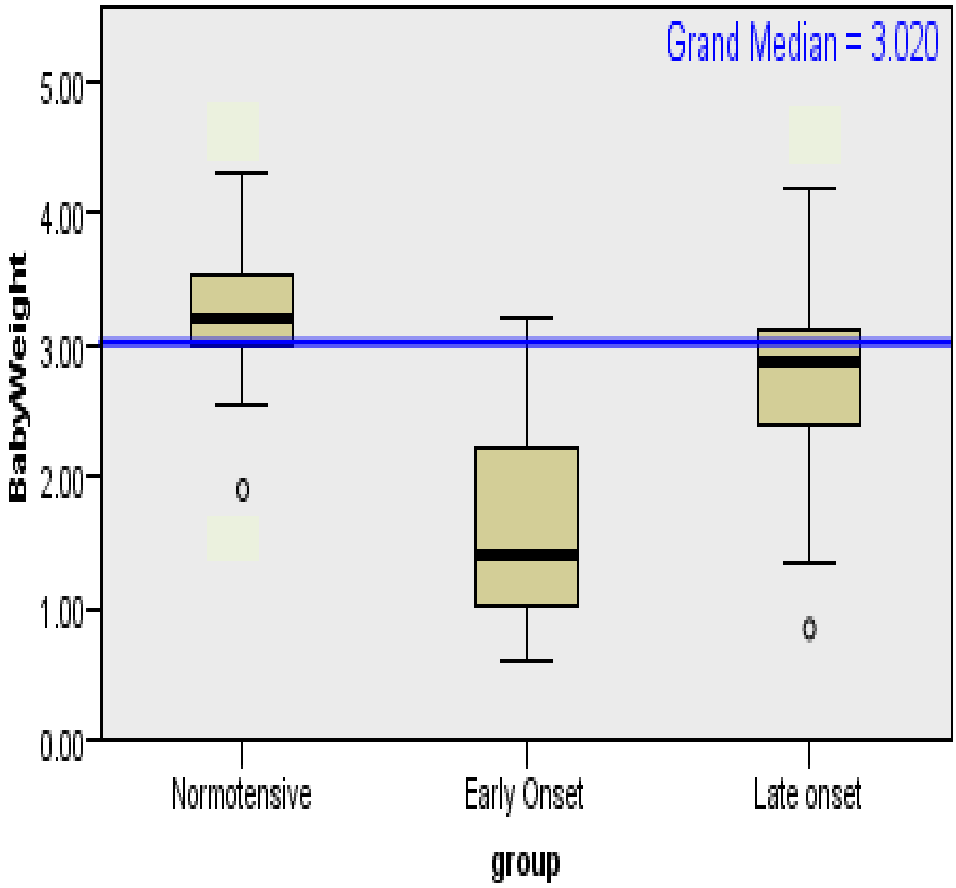

Figure 3.10: Boxplot showing birth weight (kg) across study groups

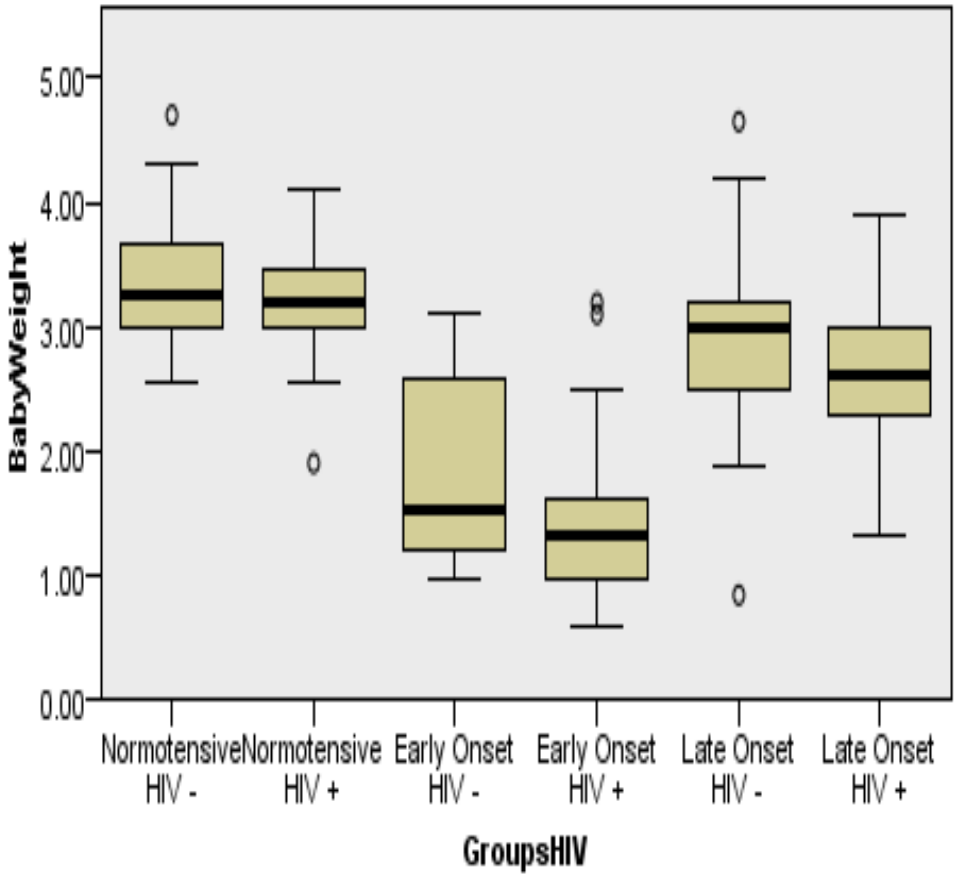

Figure 3.11: Boxplot illustrating baby weight (kg) across all groups based on HIV status

3.2.7 Placental weights across pregnant groups

In normotensive pregnant patients the mean weight ± standard deviation of the placenta was 612.45 ± 81.57 g. The mean placental weight in the EOPE group was 430.00 ± 142.23g compared to 558.46 ± 35.76 g in the LOPE group (Figure 3.13).

Placental weight was not normally distributed amongst the groups. A Kruskal-Wallis H test showed that there was a statistically significant difference in placental weight between the different groups, $\chi^2(2) = 15.674$, $p = 0.000$.

Statistically significant differences after Bonferroni *post hoc* analyses (adjusted $p < 0.008$) revealed that there were differences between placenta weight of normotensive pregnant and EOPE groups (U: 647.0; $p = 0.000$). There was no difference between normotensive and the LOPE group (p = 0.484). There was a significant difference between early onset and late onset as well (U: 223.0; $p < 0.001$).

When considering the effect of HIV on placental weight, the Kruskal-Wallis H test and Bonferroni *post hoc* analysis, showed a statistically significant difference between the different study groups, [($\chi^2(5) = 23.446$); $p < 0.000$], (Figure 3.14).

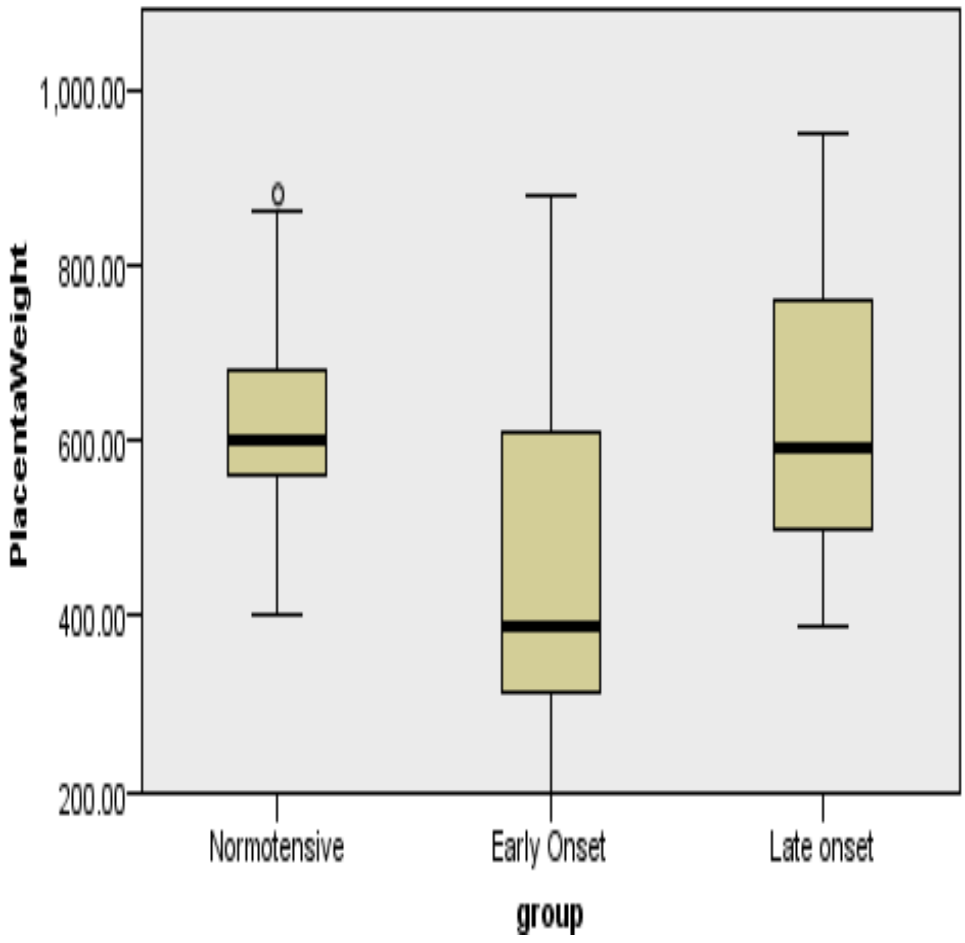

Figure 3.12: Boxplot showing placental weight (g) across pregnant groups

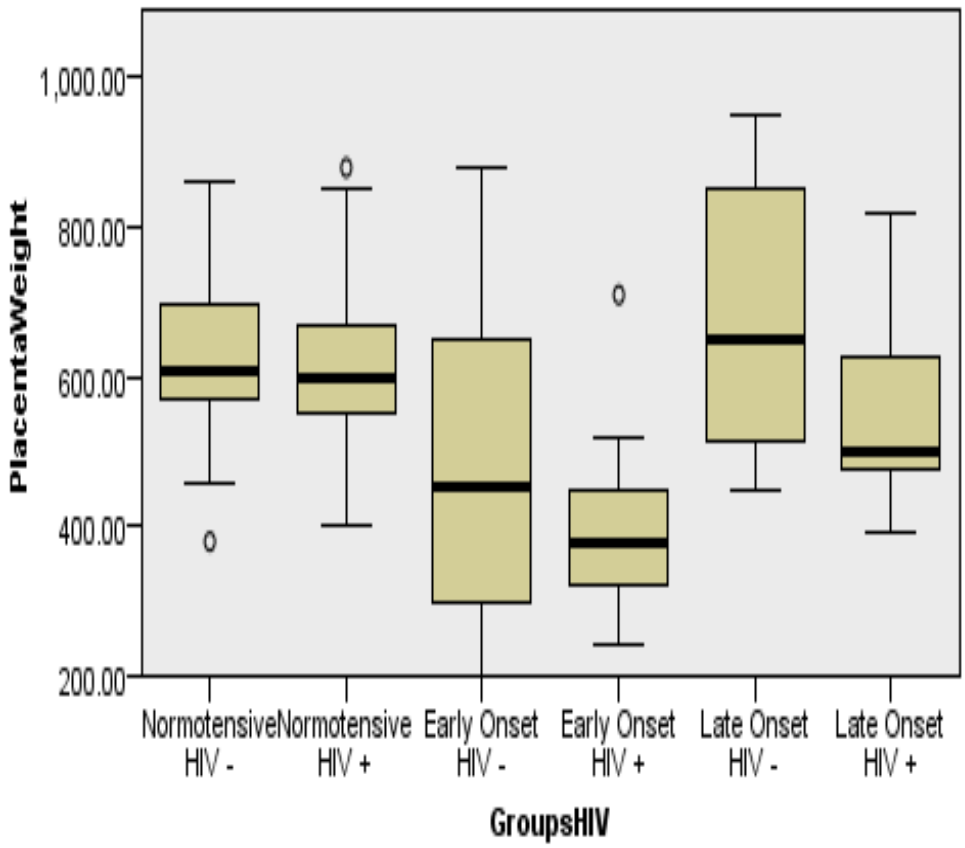

Figure 3.13: Boxplot illustrating effect of HIV status on placental weight (g)

3.2.8 HIV status

More than half of the sample cohort was HIV negative (n = 179; 54.9%). Forty five percent was HIV positive (n = 147; 45.1%).

3.2.8.1 Cluster of Differentiation 4 (CD4) count (cells/mm^3)

HIV positive non-pregnant participants had a mean CD4 count of 473 ± 188.630 cells/mm^3 ($p < 0.216$) compared to 417.91 ± 198.389 cells/mm^3 ($p < 0.629$) in the HIV positive normotensive pregnant group. HIV participants with EOPE had a mean CD4 count of 325.38 ±

166.036 cells/mm^3 (SE: 58.703; p < 0.664) compared to 388.08 ± 100.853 cells/mm^3 (p < 0.803) in the LOPE group (Figure 3.15).

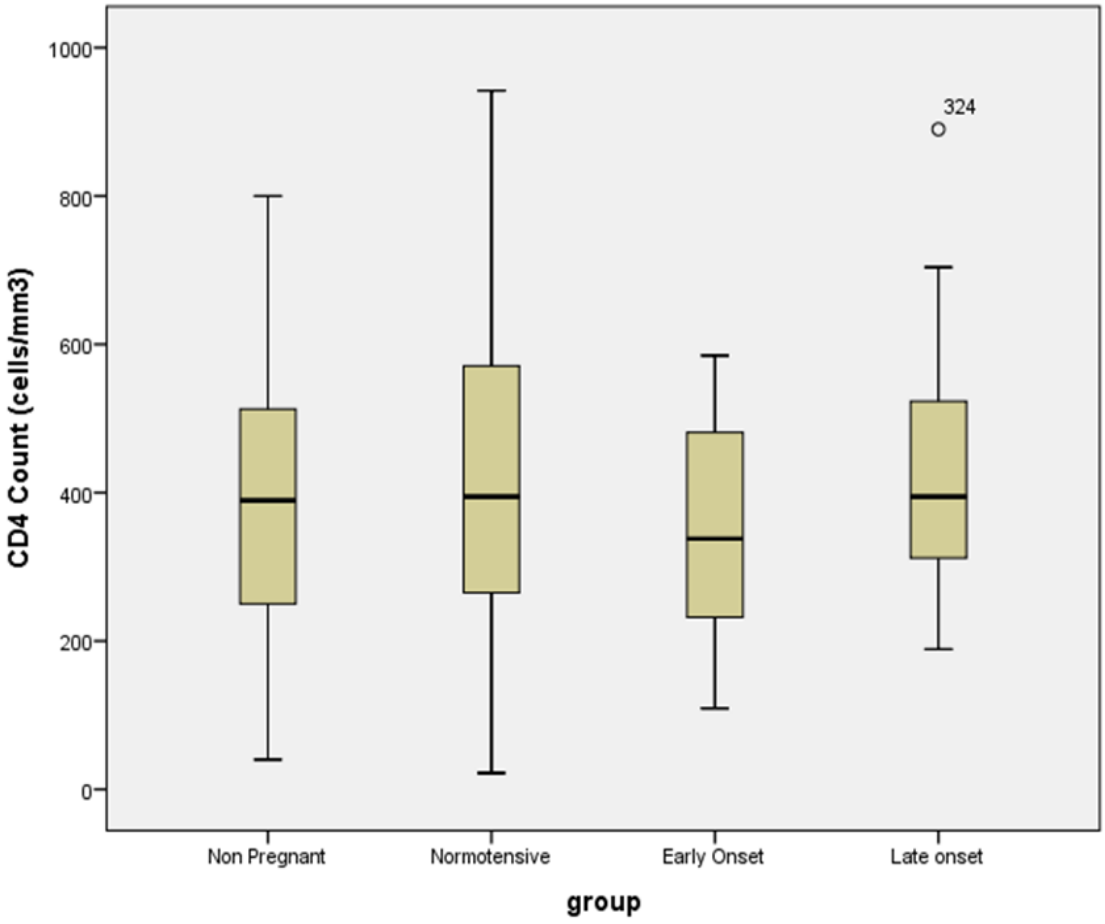

Figure 3.14: Boxplot showing CD4 count (cells/mm^3) across study groups

There were no statistically significant differences between group means as determined by one-way ANOVA [F (3,138) = 0.930; p < 0.428].

After considering the effect of HIV, the Kruskal-Wallis H test and Bonferroni post hoc analysis, demonstrated no statistically significant difference between the different groups,[(χ^2(3) = 2.627); p < 0.453], was found (Figure 3.16).

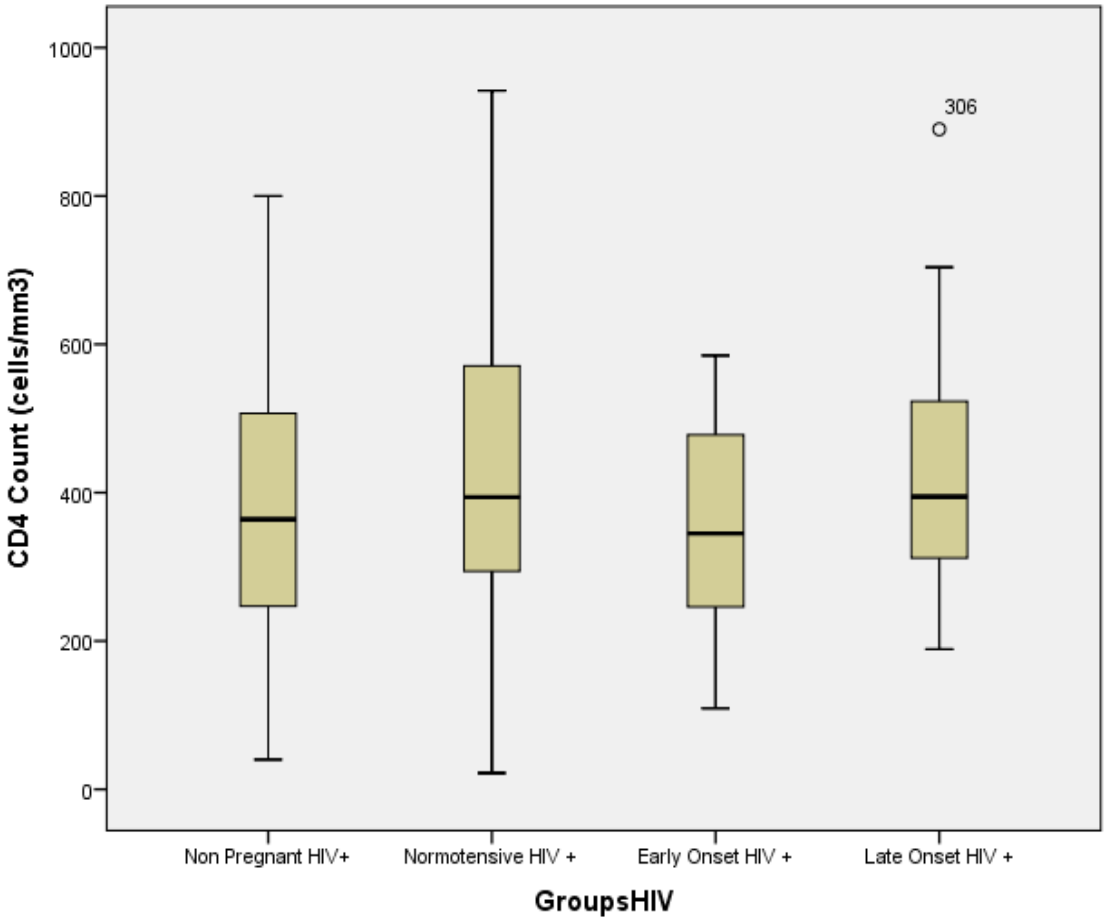

Figure 3.15: Boxplot illustrating CD4 count (cells/mm^3) in HIV positive groups

3.2.8.2 Anti – retroviral (ARV) Usage

Antiretroviral therapy was received by 36.6% of the non-pregnant participants compared to the 63.4% majority within the study population that received ARVs. Some of the pregnant participants (18.6%) did not take ARVs compared to 81.4% of pregnant participants that did take ARVs therapy. With regards to ARV usage, a Pearson Chi– square test demonstrated a statistically significant difference $p < 0.009$, between the pregnant and non-pregnant groups.

Table 3.1 Demographic data of study groups

	Non Pregnant	Normotensive pregnant	EOPE	LOPE
	N = 120	N = 118	N = 32	N = 52
Age (y)	33.01 ± 7.8	25.61 ± 5.7	27.78 ± 6.1	28.55 ± 7.5
Maternal Weight (kg)	75 ± 13	73 ± 13.8	81.75 ± 15	82 ± 19.7
Systolic (mmHg)	133 ± 26	117 ± 9.3	168 ± 8	161 ± 22
Diastolic (mmHg)	77 ± 15	73 ± 85	109 ± 16.9	96.5 ± 7.6
Height (m)	1.563 ± 0.113	1.574 ± 0.0676	1.563 ± 0.0158	1.593 ± 0.0751
CD4 cell count (cells/mm^3)	473 ± 188	417 ± 198	325 ± 166	388 ± 100
Gestational Age (wks)	0	38.98 ± 1.5	32 ± 3.8	36.92 ± 1.847
Baby weight (kg)	-	3.23 ± 0.058	1.623 ± 0.274	2.716 ± 0.1422
Placental weight (g)	-	612.45 ± 11.89	430 ± 50.28	558.46 ± 37.65

3.3 ANTHROPOMETRIC DATA ACROSS STUDY GROUPS

3.3.1 Maternal Weight

The mean weight ± standard deviation of the non-pregnant women was 75.0 ± 13.14 kg compared to 73.17 ± 13.89 kg of the normotensive pregnant women. The mean weight of EOPE women was 81.75 ± 15.06 kg compared to 82.523 ± 19.71 kg of LOPE women (Table 3.1).

When considering the effect of HIV on maternal weight, the Kruskal-Wallis H test and Bonferroni post hoc analysis, showed no statistically significant difference between the different groups,[($\chi^2(7) = 11.004$); $p < 0.138$]; (Figure 3.17).

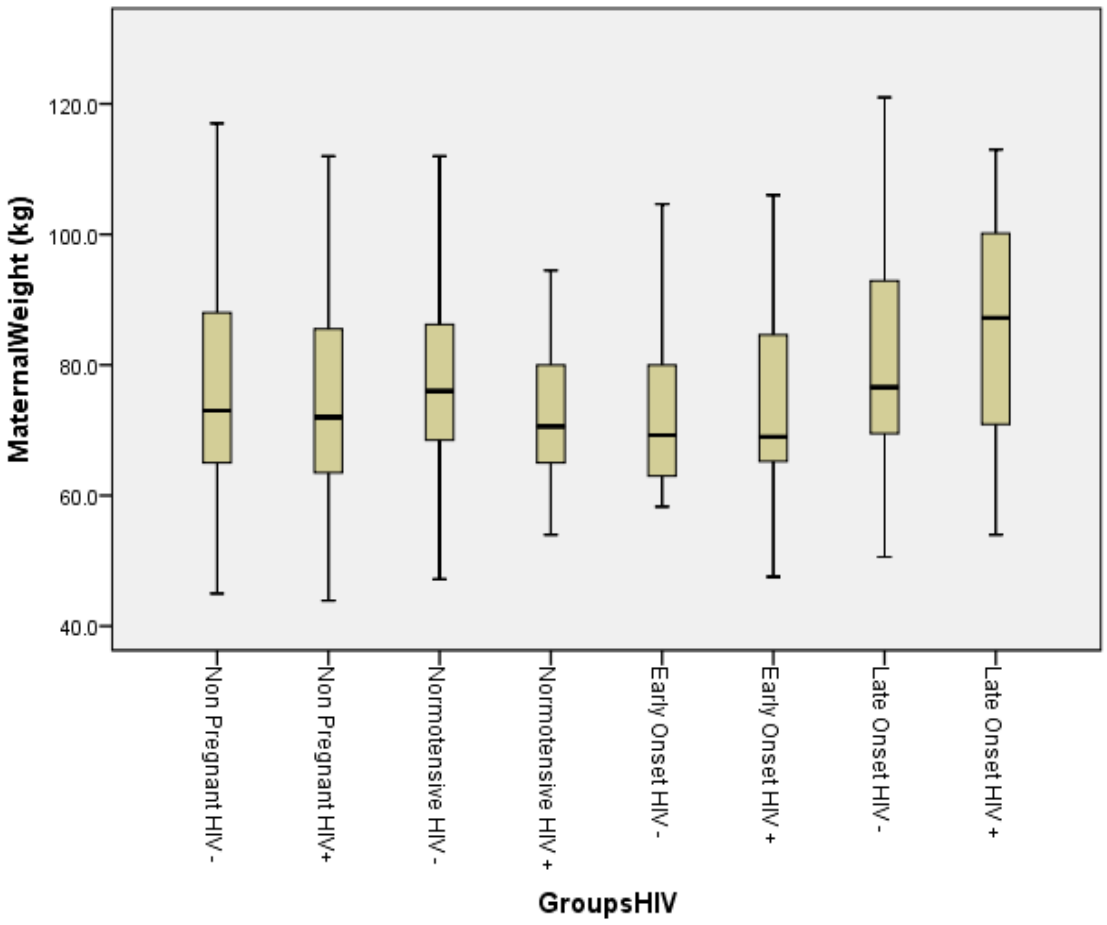

Figure 3.16: Boxplot illustrating maternal weight (kg) across HIV groups

3.3.2 Maternal Height

The mean height of non-pregnant women was 1.563 ± 0.113 m whilst that of normotensive participants was 1.574 ± 0.676 m. The mean height was 1.531± 0.090 m and 1.593 ± 0.075 m in EOPE and LOPE women, respectively (Table 3.2).

After considering the effect of HIV across the categories, the Kruskal-Wallis H test and Bonferroni post hoc analysis, demonstrated no statistically significant difference between the different groups,[($\chi^2(7) = 6.119$); $p < 0.526$].

3.3.3 Maternal Body mass index

The BMI of the non-pregnant and normotensive pregnant women was 28.55 ± 4.24 kg/m^2 and 28.89 ± 6.80 kg/m^2, respectively. The mean BMI of EOPE and LOPE women was 32.73 ± 7.65 kg/m^2 and 32.97 ± 8.54 kg/m^2 respectively (Figure 3.18). The mean BMI of the study population fell into either the overweight (BMI - 27.5 – 30 kg/m^2) or mildly obese categories (BMI- 30.5 – 35 kg/m^2).

Body mass index per group was not normally distributed. After $\log_{10}$ transformation of the data, a one way ANOVA revealed no statistically significant difference between the groups [$F(3,322) = 1.161$; $p = 0.325$].Whilst there was a significant difference between the non-pregnant and pregnant cohorts, there was no statistically significant differences between the normotensive, EOPE and LOPE groups ($p = 0.325$). Likewise, the distribution of BMI was the same across the categories with respect to HIV status ($p = 0.124$).

After considering the effect of HIV across the categories the study found that after the Kruskal-Wallis H test and Bonferroni post hoc analysis, no statistically significant difference between the different groups,[($\chi 2(7) = 9.330$); $p < 0.230$], was found (Figure 3.19).

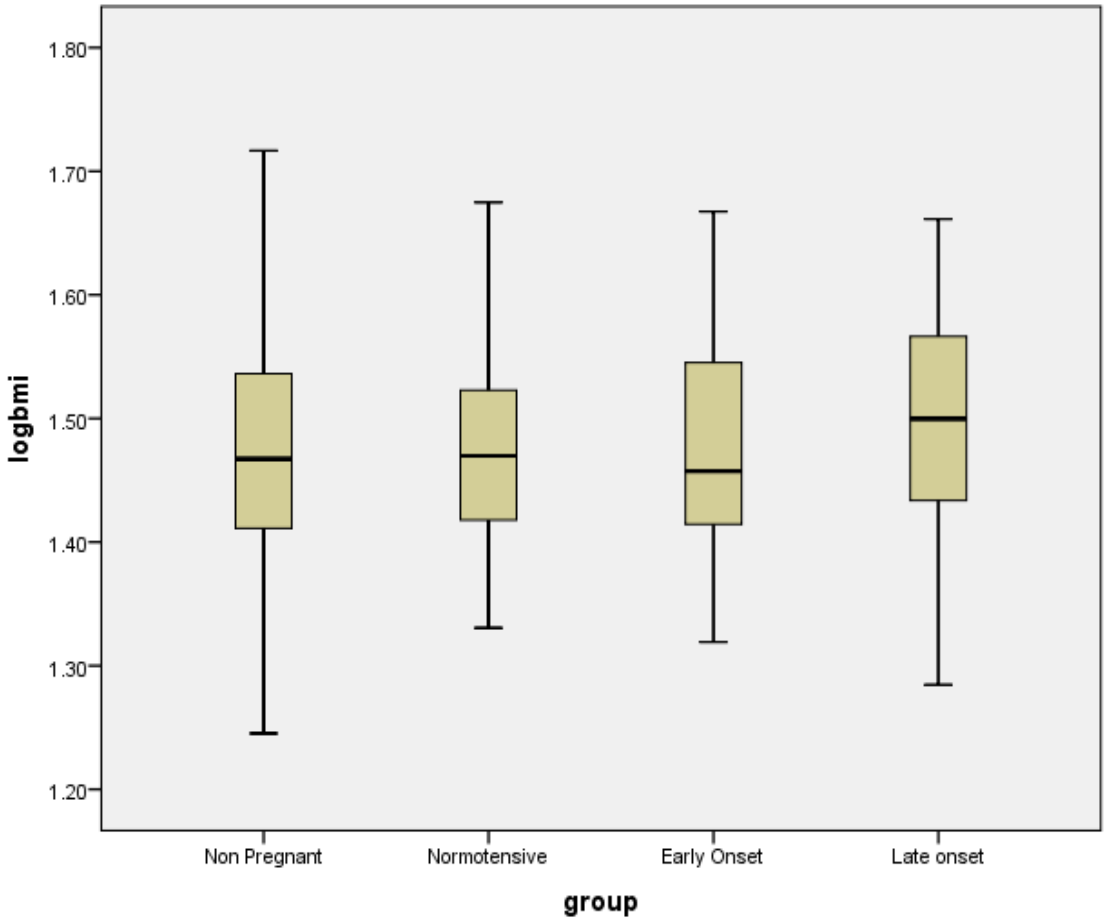

Figure 3.17: Boxplot showing BMI (kg/m^2) across study groups

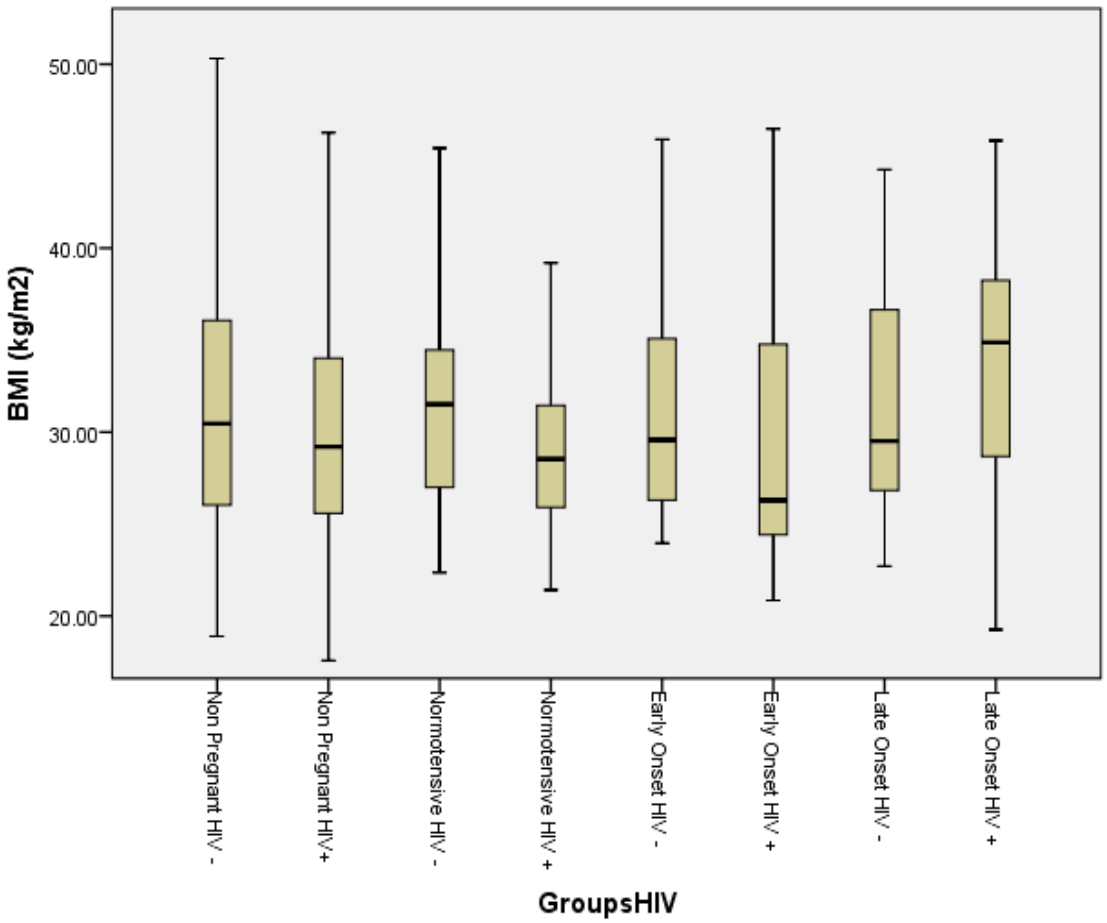

Figure 3.18: Boxplot showing BMI (kg/m^2) across subgroups based on HIV status

3.3.4 Mid upper arm circumference (MUAC)

The mid upper arm circumference was 31.53 ± 2.63 cm in non-pregnant women compared to 28.38 ± 4.59 cm in the normotensive pregnant women. EOPE participants had a MUAC of 33.38 ± 4.69 cm compared to28.23 ± 1.43 cm in the LOPE group (Figure 3.20; Table 3.2).

One way ANOVA tests of both mid-upper arm circumference [F(3.322) = 5.740; p = 0.01] and triceps skinfold thickness [F(3.322) = 5.411; p = 0.001] showed that there was significant difference between at least one of the groups analysed.

Post hoc tests showed that the mean MUAC for non-pregnant women (31.53 ± 5.845 cm) was significantly higher than normotensive pregnant women (28.73 ± 4.717 cm; $p < 0.000$) but not significantly different from EOPE and LOPE groups.

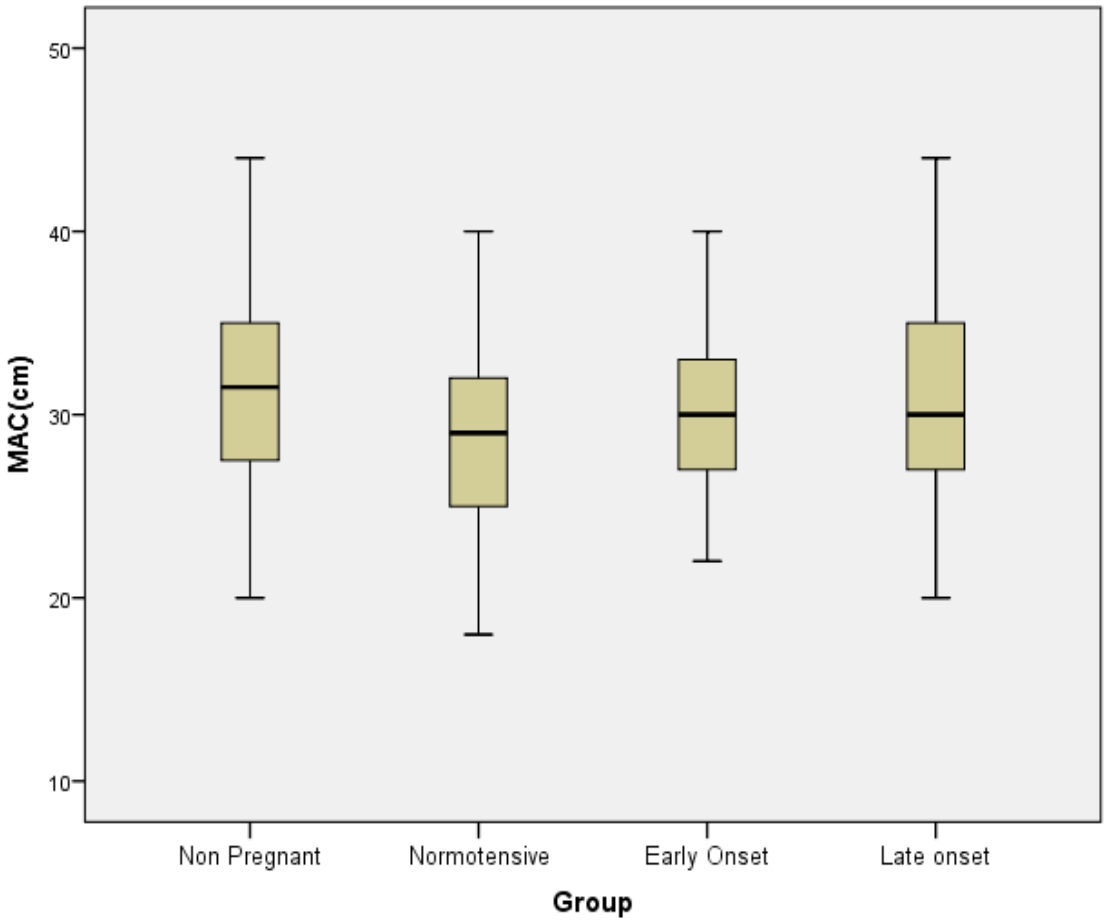

Figure 3.19: Boxplot showing mid arm circumference (cm) across study groups

When considering the effect of HIV, it was shown that after the Kruskal-Wallis H test and Bonferroni post hoc analysis, a statistically significant difference between the different groups,[($\chi^2(7) = 23.253$); $p < 0.002$], was found (Figure 3.21).

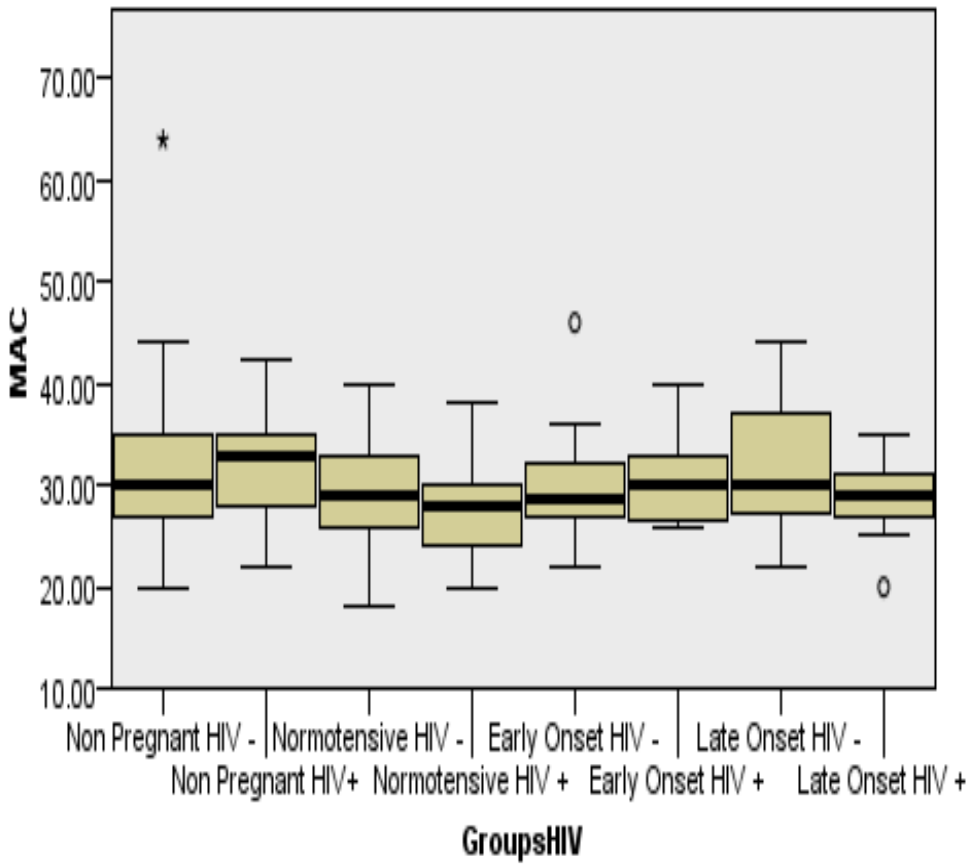

Figure 3.20: Boxplot illustrating mid arm circumference (cm) based on HIV status

3.3.5 Triceps skin fold thickness

Fisher's Least significant difference *post hoc* test revealed that triceps skin fold thickness in the normotensive pregnant group (18.14 ± 7.025 mm) was significantly lower than both non-pregnant (21.58 ± 8.812 mm; $p < 0.002$) and LOPE (22.89 ± 10.638 mm) groups but not significantly different from the EOPE group (18.94 ± 9.148 mm) (Table 3.2; Figure 3.22). The triceps skin fold thickness in the EOPE group was lower than LOPE group ($p < 0.036$).

Non-pregnant women had a mean triceps skin fold thickness of 13 ± 4.69 mm compared to 18.34 ± 7.08 mm in normotensive women. The mean triceps skin fold thickness in EOPE and

LOPE women was 23.38 ± 12.18 mm and 16.90 ± 8.47 mm respectively. There was a statistically significant difference in triceps skin fold thickness between the normotensive group and other groups ($p < 0.000$). There were no other statistically significance between non pregnant, EOPE and LOPE groups.

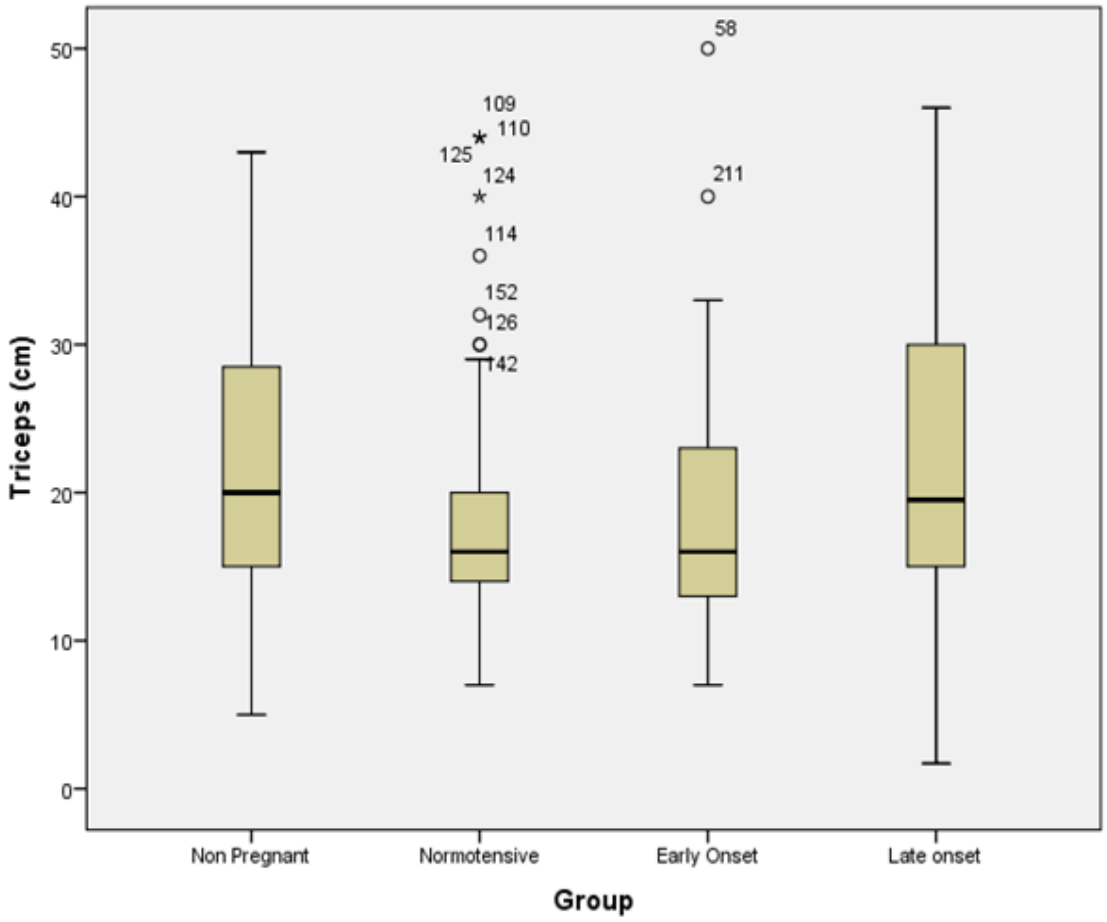

Figure 3.21: Boxplot of mean triceps circumference (mm) across study groups

After analysing the effect of HIV on triceps skinfold thickness across the groups, it was found that after the Kruskal-Wallis H test and Bonferroni post hoc analysis, a statistically significant difference between the different groups, [($\chi^2(7) = 20.457$); $p < 0.005$], was shown across the categories (Figure 3.23).

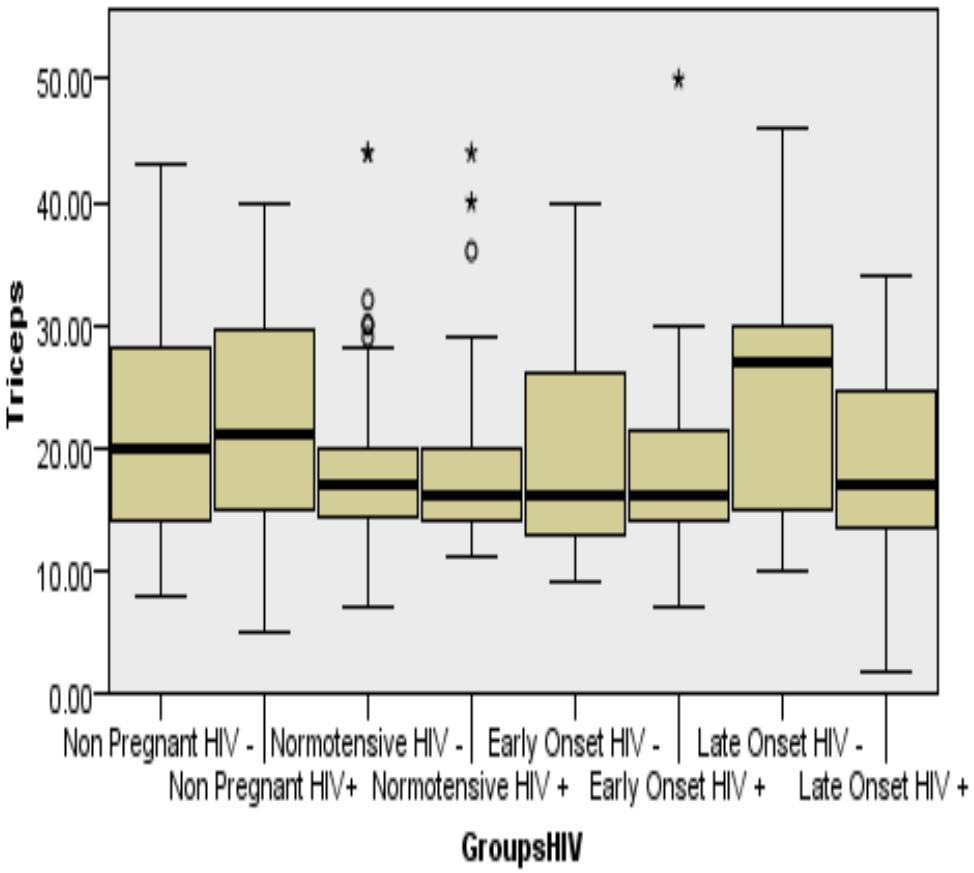

Figure 3.22: Boxplot illustrating mean triceps circumference (mm) based on HIV status

Table 3.2: Anthropometric and CD_4 data across study groups

	Non Pregnant	Normotensive Pregnant	EOPE	LOPE
	N = 120	N = 118	N = 32	N = 52
Height (m)	1.563 ± 0.113	1.574 ± 0.0676	1.563 ± 0.0158	1.593 ± 0.0751
BMI (kg/m^2)	28 ± 4.2	28.8 ± 6.8	32.7 ± 7.6	32.97 ± 8.5
MUAC (cm)	26 ± 2.6	28±4.5	33 ± 4.6	28.23 ± 5.1
Triceps skin fold (cm	21.58 ± 4.6	18.14 ± 7.07	18.94 ± 12	22.89 ± 8
CD4 cell count ($cells/mm^3$)	473 ± 188	417 ± 198	325 ± 166	388 ± 100

3.4 ASSESSMENT OF SERUM LEVELS OF ADIPONECTIN USING ELISA

The median adiponectin level was 446.55700 µg/ml and ranged between 305.27050 - 725.32175 µg/ml. The mean adiponectin level was 897.93 ($p < 0.080$); 17.19 ($p < 0.000$); 23.16 ($p < 0.017$); 24.61 ($p < 0.324$), in the non-pregnant, normotensive pregnant, EOPE and LOPE participants, respectively (Table 3.3).

The effect of HIV on adiponectin across the categories, using the Kruskal-Wallis H test and Bonferroni *post hoc* analysis, showed a statistically significant difference between the different groups, [($\chi^2(7) = 24.540$); $p < 0.001$] (Figure 3.24).

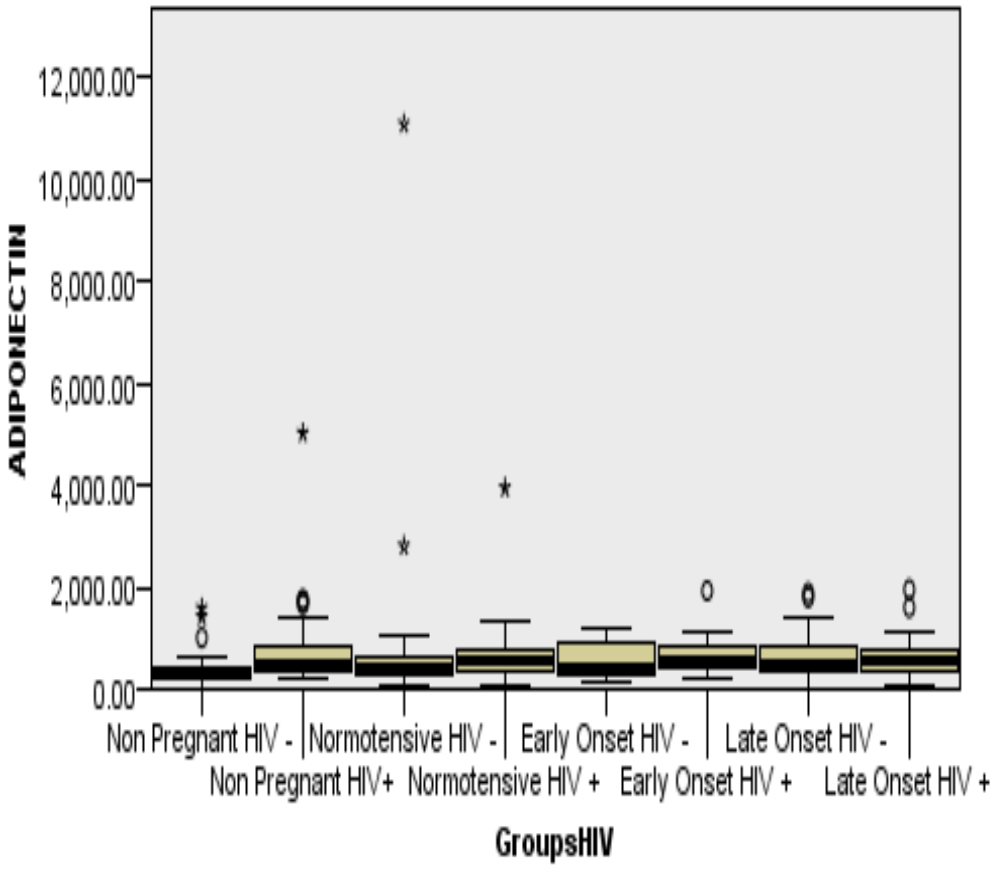

Figure 3.23: Boxplot illustrating adiponectin (µg/ml) based on HIV status

3.5 ASSESSMENT OF SERUM LEVELS OF LEPTIN USING THE LUMINEX TECHNIQUE

The median leptin concentration was 153.00 pg/ml) with an inter quartile range of 64.25 – 3028.06 pg/ml. Non-pregnant participants had mean leptin concentrations of 4887.25 pg/ml ($p < 0.125$) compared to 2732.27 ($p< 0.000$) in the normotensive pregnant groups. Participants with EOPE had a mean leptin concentration of 955.75 ($p < 0.000$) compared to 310.23 ($p < 0.000$) in the LOPE groups. When considering the effect of HIV on mean leptin level using the Kruskal-Wallis H test and Bonferroni *post hoc* analysis, a statistically significant difference between the different groups,[($\chi^2(7) = 16.926$); $p < 0.018$], across the categories was noted (Figure 3.25).

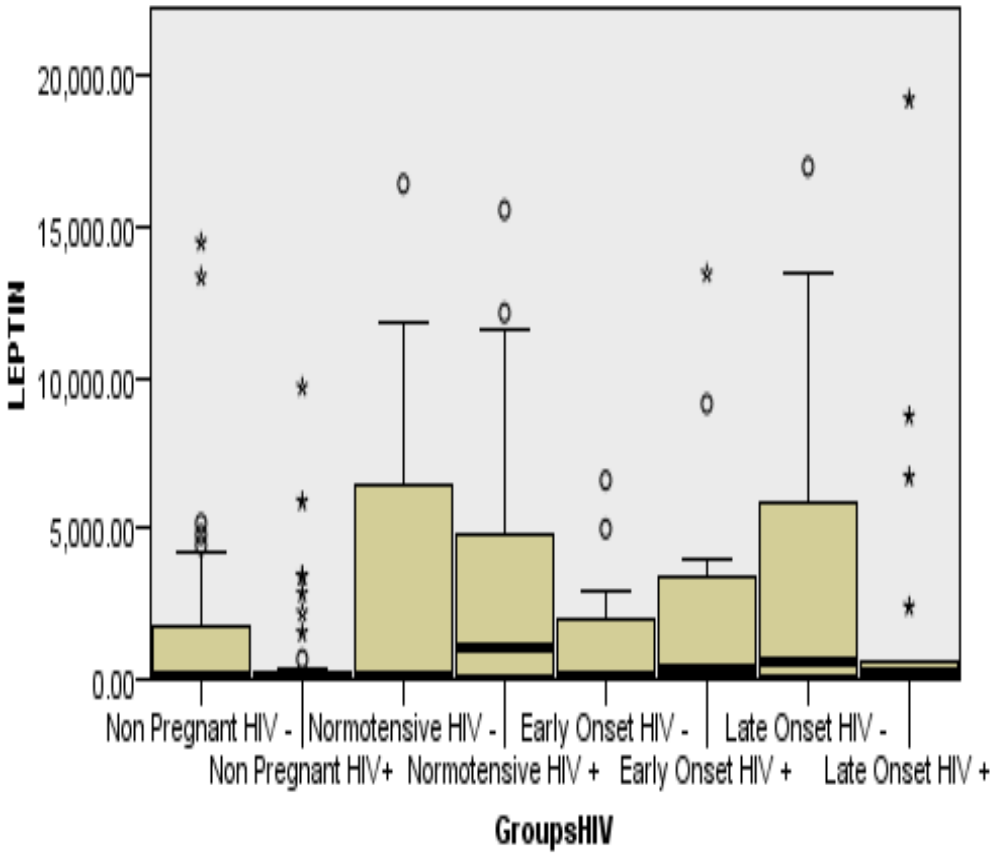

Figure 3.24: Boxplot illustrating leptin (pg/ml) based on HIV status

3.6 ASSESSMENT OF SERUM LEVELS OF TNFα USING LUMINEX TECHNIQUE

Non-pregnant participants did not have measurable levels of TNF-α. Normotensive participants had a mean TNF-α concentration of 608.521 pg/ml (p = 0.000). Participants with EOPE had a mean TNF-α concentration of 661.03 (p = 0.000) compared to 616.439 (p = 0.000) in the LOPE group (Table 3.3).

After considering the effect of HIV on TNF-α concentration, the Kruskal Wallis test and *post hoc* Bonferroni analyses revealed a statistically significant difference, [($\chi^2(7) = 14.160$); $p < 0.048$], across study groups (Figure 3.26).

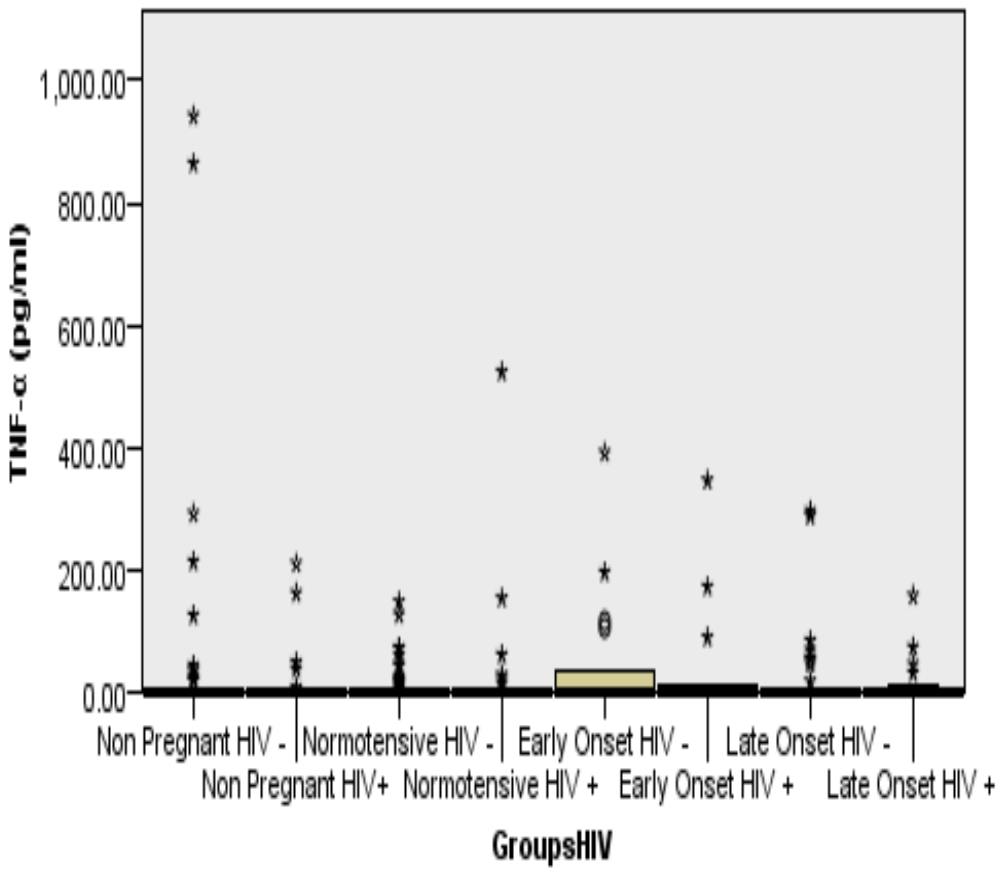

Figure 3.25: Boxplot illustrating mean TNF-α (pg/ml) based on HIV status

3.7 ASSESSMENT OF SERUM LEVELS OF RESISTIN USING THE LUMINEX TECHNIQUE

Non-pregnant participants had a mean resistin concentration of 7497.13 pg/ml compared to 3536.50 pg/ml in the normotensive pregnant groups. The mean resistin concentration was 1017.63 pg/ml in the EOPE group compared to 286.92 pg/ml ($p < 0.000$) in the LOPE group.

When considering the effect of HIV on resistin concentration, the Kruskal-Wallis H test and Bonferroni *post hoc* analysis, a statistically significant difference between the different groups, [($\chi^2(7) = 18.625$); $p < 0.009$], was noted (Figure 3.27).

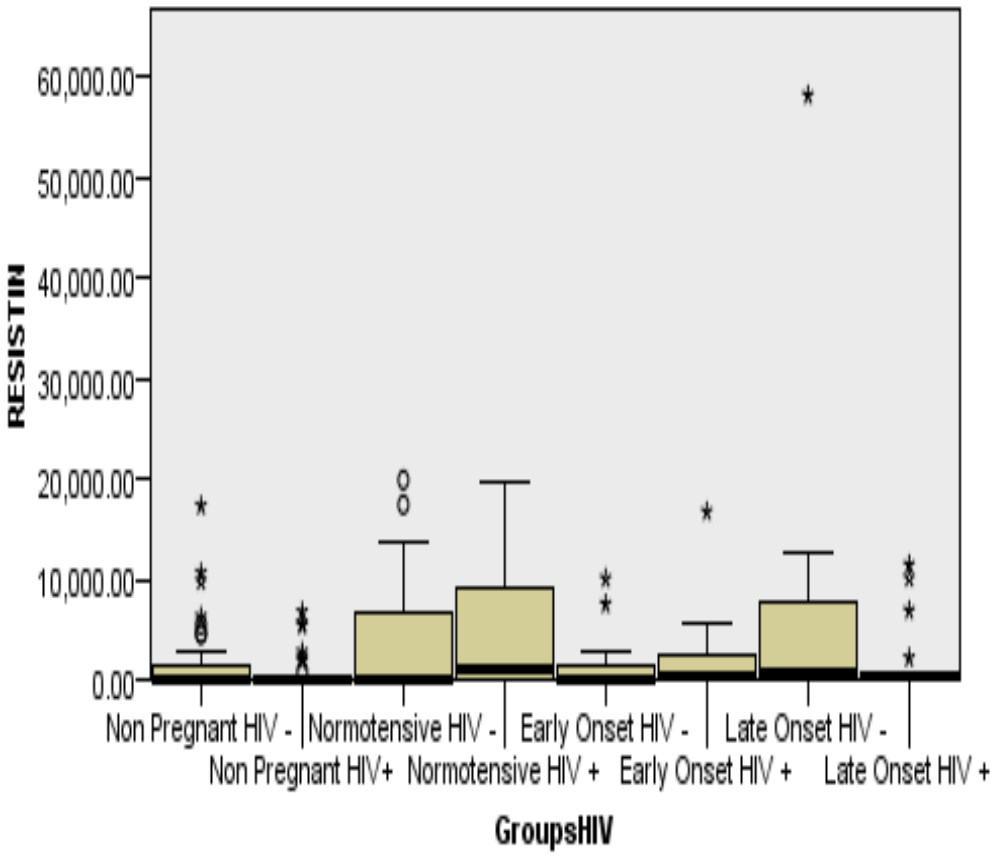

Figure 3.26: Boxplot illustrating mean resistin (pg/ml) based on HIV status

Table 3.3: Detailed outline of adipokine levels across study groups

	Non Pregnant	Normotensive Pregnant	EOPE	LOPE
	N = 120	**N = 118**	**N = 32**	**N = 52**
ADIPONECTIN µg/l	897.93 ± 126.18	17.19 ± 11.568	23.16 ± 21.392	24.61 ± 12.869
LEPTIN pg/ml	4887.25 ± 705.294	2732.27 ± 580.183	955.75 ± 527.642	310.23 ± 177.438
TNF-α pg/ml	0	608.52 ± 84.899	661.036 ± 202.604	616.439 ± 117.53
RESISTIN pg/ml	7497.13 ± 1921.956	3536.50 ± 730.044	1017.63 ± 691.585	286.92 ± 160.308

Table 3.4: Adipokine levels in relation to BMI groups

	5	10	25	50	75	90	95
BMI	22.75	23.94	26.19	30.14	34.95	40.42	44.22
ADIPONECTIN µg/ml	142.06	192.78	305.27	446.56	725.32	1061.39	1371.04
LEPTIN pg/ml	50.00	51.00	64.25	153.00	3028.06	7827.25	11235.38
RESISTIN pg/ml	50.00	56.50	71.00	168.00	3085.13	9849.75	12921.25
TNF pg/ml	.00	.00	.00	.00	.00	51.50	152.56

3.8 ANALYSIS OF RESULTS WITH RESPECT TO THE PRIMARY NULL HYPOTHESES OF THE STUDY

3.8.1 Null hypothesis: The levels of adiponectin/leptin/resistin/TNF-α does not differ amongst the pregnant and non-pregnant cohorts.

The levels of TNFα/adiponectin/leptin and resistin were all statistically significantly different between the pregnant and non-pregnant cohorts (Table 3.5). The mean concentration of TNF-α was 22.181 ± 90.24 pg/ml ($p = 0.000$) in the pregnant cohort. In this pregnant cohort, the mean concentration of adiponectin, leptin and resistin was 614.980 µg/l ± 780.84 ($p = 0.000$); 2266.537 pg/l ± 3747.85 ($p = 0.000$) and 2790.471 pg/l ± 5363.09 ($p = 0.000$), respectively.

The levels of TNF-α, leptin and resistin were significantly different within the normotensive pregnant *versus* pre-eclamptic groups. Using the Mann-Whitney test it was demonstrated that TNF- α was statistically significant within the pregnancy groups ($p < 0.044$). Likewise, leptin ($p < 0.004$) and resistin ($p < 0.006$) were statistically significant between the pregnancy groups. Only Adiponectin, as mentioned, failed to reach statistical significance within the pregnancy subgroups ($p < 0.292$).

The null hypothesis was therefore rejected.

Table 3.5: Detailed outline of adipokine levels between pregnant vs non-pregnant study groups

	PREGNANT			NON PREGNANT			
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	**p-values**
TNF-α pg/ml	0	0	524	0	0	942	0.00
Adiponectin µg/l	476.7	27	11099	414.6	66	5030	0.00
Leptin pg/ml	189.0	50	19239	88.0	50	14507	0.00
Resistin pg/ml	230.0	50	58241	101.0	50	17338	0.00

3.8.2 Null hypothesis: The levels of adiponectin/leptin/resistin/TNF-α does not differ in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia

The differences in TNF-α, adiponectin, leptin and resistin concentration failed to reach statistically significant *p*-values between EOPE *versus* LOPE using the Mann-Whitney Test. Therefore the null hypothesis was accepted (Table 3.6).

Table 3.6: Detailed comparison of adipokine levels between EOPE and LOPE study groups

	LOPE			EOPE			
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	**p-values**
TNF-α	0	0	297	0	0	392	<0.098
Adiponectin	554.3	27	1957	446.2	168	1931	<0.913
Leptin	196	50	58241	182	50	13467	<0.538
Resistin	230	50	58241	230.5	50	16763	<0.669

3.8.3 Null hypothesis: The levels of adiponectin/leptin /resistin /TNF-α in pre-eclamptics does not differ according to HIV status

There were no statistically significant differences in the levels of TNF-α, adiponectin, leptin and resistin in the pre-eclamptic HIV+ve *versus* the HIV-ve groupings. In this instance the null hypothesis was retained (Table 3.7).

Table 3.7: Detailed comparison of adipokine levels between HIV negative and positive study groups

	HIV-ve			HIV+ve			
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	**p-values**
TNF-α pg/ml	0	0	392	0	0	346	<0.696
Adiponectin µg/l	453.6	167	1889	559.5	60	1957	<0.499
Leptin pg/ml	185	50	13495	196	55	19239	<0.800
Resistin pg/ml	230.5	50	58241	191	50	16763	<0.671

3.8.4 Null hypothesis: The difference in levels of adiponectin/leptin/resistin/TNFα in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia are not altered by the HIV status of the patients.

The study results from use of the Mann-Whitney Test demonstrated that there were no statistically significant differences in the levels of adiponectin, leptin, TNF-α and resistin within the pregnancy groups with respect to HIV status. The levels for TNF-α was $p < 0.665$; adiponectin was $p < 0.197$; leptin was $p < 0.493$ and resistin was $p < 0.653$.

3.9 FURTHER, COMPARISONS

3.9.1 CD4 Count

There was no statistically significant findings in the levels of TNF-α, adiponectin, leptin and resistin within the HIV+ve pregnant population based on CD4 >350 compared to CD4 <350 (Table 3.8).

Table 3.8: Detailed comparison of adipokine levels based on CD4 levels.

	CD4 < 350			**CD4 ≥ 350**			
	Median	**Minimum**	**Maximum**	**Median**	**Minimum**	**Maximum**	**p-values**
TNF pg/ml	0	0	346.446	0	0	523.552	0.386
Adiponectin ug/l	601.51	117	3954	505.1	59.7	1252	0.122
Leptin pg/ml	277	53	13467	279	50	19239	0.848
Resistin pg/ml	267	50	19598	285	50	13901	0.837

3.9.2 ARVs

There were no statistically significant differences in the levels of adiponectin /leptin /resistin /TNF-α in pregnant HIV+ve women based on receipt of HIV therapy. TNF-α achieved a *p* value of 1 whilst adiponectin ($p < 0.735$), leptin ($p < 0.215$) and resistin ($p < 0.326$) across groups.

3.9.3 BMI

There were no statistically significant differences in levels of TNF-α, Leptin, and Resistin when compared across subdivisions of BMI in the non-pregnant population (Table 3.4). TNF-α achieved a $p < 0.933$ vs leptin that achieved a $p < 0.234$ vs resistin that achieved a $p < 0.108$. However adiponectin levels did reach statistical significance amongst the BMI subdivisions in the non-pregnant population; $p < 0.002$.

There were no statistically significant differences in the levels of TNF-α, adiponectin, leptin and resistin in the pregnant population across the subdivisions of BMI. TNF-α achieved a $p < 0.466$. Adiponectin achieved a $p < 0.091$ whilst leptin achieved a $p < 0.275$ and resistin achieved a $p < 0.214$. The study was able to establish adipokine levels according to BMI (Table 3.9).

Table 3.9: Adipokine levels stratified according to BMI

	BMI			
	<27	**27 – 30**	**30-35**	**>35**
TNF α pg/ml	0.00±105.066	0.00±134.489	0.00±31.083	0.00±60.639
Adiponectin ug/ml	593.469±617.586	431.988±572.660	396.307±1254.792	403.714±347.278
Leptin pg/ml	321.00±3479.098	114.00±3057.903	131.00±3836.619	104.00±4427.365
Resistin pg/ml	320.00±4868.865	130.50±3795.870	128.00±7392.624	109.00±4356.158

Using the data collected it was possible to depict the relationship between TNFα / Adiponectin / leptin and resistin and their medians with respect to change in Body mass index (Table 3.9).

It was possible to further, stratify the median TNFα / Adiponectin / Leptin and Resistin levels with respect to pregnancy status and HIV status. This has not been attempted in the local population previously (Table 3.10).

Table 3.10: Adipokine levels according to Pregnancy and Non pregnancy state in relation to HIV status

	Non pregnant		Pregnant	
	HIV -ve	**HIV+ve**	**HIV-ve**	**HIV+ve**
TNFα pg/ml	0.00±189.028	0.00±39.403	0.00±58.609	0.00±72.710
Adiponectin ug/ml	349.272±300.190	517.943±758.705	452.95±1032.234	557.574±511.288
Leptin pg/ml	90.00±3029.725	74.50±1791.436	153.00±4128.513	321.00±4037.204
Resistin pg/ml	101.00±3375.880	90.00±1527.309	175.00±6849.363	283.50±4763.811

CHAPTER 4

DISCUSSION

4.1 INTRODUCTION - OBESITY, HIV AND PRE-ECLAMPSIA

Maternal morbidity and mortality in South Africa is weighed down in a quagmire of old and new challenges. Currently, pre-eclampsia and HIV infection remain two of the top five causes of maternal mortality in South Africa (NCCEMD, 2007). Following on the heels of the HIV infection, South Africa now faces a new pandemic, that of obesity.

In 2011, the overall HIV prevalence amongst antenatal women was 29.5% (95%CI: 28.7- 30.2) (Health, 2011a SANDO). Geographical variations of the epidemic in South Africa reflects a provincial distribution, with KwaZulu-Natal having the highest HIV prevalence followed by Mpumalanga, Free State and North-West, all with prevalence rates greater than 30.0% (Health, 2011bSANDO).

Concurrently, it has been estimated that 1.3 billion people are overweight or obese (Obesity Task Force, 2005). Not only is obesity affecting high income countries but, it is becoming more evident and an increasing problem in low-middle income countries (Puoane *et al*., 2002, Filozof *et al*., 2001, Rivera *et al*., 2002).

South Africa has the highest overweight and obesity rate in sub-Saharan Africa: seven out of 10 women have significantly more body fat than what is considered healthy. Of the 70% of overweight South African women, 42% are obese (Ng *et al*., 2014). In neighboring sub-Saharan African countries such as Namibia (19.8%), Lesotho (24.1%) and Zimbabwe (33.5%)

women are less obese than South Africa. Eritrea only has 4.7% obese women and Ethiopia 1.8% – respectively, a 10-20 fold lower prevalence of obese women than South Africa (Ng *et al*., 2014).

A progressive increase in the prevalence of obesity in South Africa, particularly among women and young girls has been reported in many studies (Labadarios *et al*., 2005, Senekal *et al*., 2003, Walker, 1995) A report published by the South African Medical Research Council (MRC) in 2007, cited that "56% of adult women and 29% of adult men were overweight/obese, while 17% of children under the age of 9 years were overweight" (Walker, 1995, Stein, 2007). They also report that "approximately 60 people die from obesity-related disease every day in South Africa". The primary reason for this high mortality amongst Black women, perhaps emanates from a strong belief that increased body weight is considered an indicator of wealth, good health and success, whilst being thin is associated with being HIV-infected (Mvo *et al*., 1999, Ndlovo and Roos, 1999).

Globally, obesity remains a key risk factor for hypertension and cardiovascular disease (WHO, 2003). Pregnancy, obesity and overweight all lead to increased insulin resistance. Insulin resistance is a physiological process in pregnancy, but obesity and overweight leads to pathological changes *ie*., inflammatory processes and sub-clinical inflammation (Challis *et al*., 2009, Wang and Nakayama, 2010, Stupin and Arabin, 2014).

TNF-α, leptin, resistin and adiponectin and many other adipokines are released into the maternal circulation with increasing obesity. They altogether contribute to the development of obesity specific morbidities such as hypertension, diabetes and cardiovascular disorders (Ronti

et al., 2006, Fischer-Posovszky *et al*., 2007). Adipose tissue produces TNF-α, which increases with an increasing degree of obesity (Hotamisligil, 1993). This elevation of TNF-α contributes to insulin resistance. In 1994, leptin was identified as a secretory product of adipose tissue (Zhang *et a*l., 1994a). Leptin up-regulation contributes to inflammation and changes in metabolic activity. In obesity, adipokines and receptors are up-regulated in visceral adipose tissue (VAT) (Ronti *et al*., 2006, Hu *et al*., 1996, Milan *et al*., 2002).

Since two-thirds of HIV infected women of reproductive age reside in sub-Saharan Africa, this region is experiencing a major obstetric dilemma, (Coovadia and Coutsoudis, 2000). Also, in view of the fact that KZN province is considered the epicenter of the global HIV pandemic, women of reproductive age are vulnerable. This is further, exacerbated by the high frequency of pre-eclampsia, the commonest direct cause of maternal deaths in South Africa. Hence, it is imperative that one adequately clarifies the interaction of the two diseases.

Pregnancy in itself reflects a modest maternal inflammatory response, whilst pre-eclampsia is identified as an excessive inflammatory response (Redman and Sargent, 2005, Redman *et al*., 1999). Thus, when combined with the immune insufficiency stimulated by HIV infection, it is possible that the immune hyper-reactivity is perhaps prevented, thereby inhibiting pre-eclampsia development.

A lower incidence of pre-eclampsia in HIV positive women has been reported (Boyajian *et al*., 2012); (Wimalasundera *et al*., 2002, Haeri *et al*., 2009, Mattar *et al*., 2004). Similarly, consistent evidence has been described in South Africa where HIV positive women were found to be at a lower risk of developing pre-eclampsia (Kalumba *et al*., 2013). In the pre-HAART

era, pre-eclampsia was an uncommon complication of pregnancy in HIV-infected women, the occurrence being even less frequent than in the general population (Stratton *et al*., 1999). Another study has demonstrated that with the routine use of HAART, the incidence of pre-eclampsia in HIV-infected pregnant women increases to a level similar to that of HIV-uninfected women (Wimalasundera *et al*., 2002). Additionally, the incidence of pre-eclampsia and fetal deaths increases in the HIV infected pregnant women (Suy *et al*., 2006). The latter study attributes this risk with the exposure to HAART prior to pregnancy and that insulin resistance and endothelial inflammation may have been the potential underlying conditions.

In light of the pervasive HIV infection, high pre-eclampsia prevalence and the very high levels of obesity in South Africa, this novel study attempts to reconnoiter the relationship amongst this deadly troika. Given the role of this trio in immune responses, this study explores the role of adipose related proteins (adipokines) in HIV associated pre-eclampsia in KwaZulu-Natal.

4.2 PATIENT DEMOGRAPHICS

4.2.1 Maternal age

In this study, the mean age of pregnant participants was 27.3 *vs* 26.3 *vs* 26.6 years in the normotensive pregnant, EOPE and LOPE cohorts, respectively. These maternal ages appear to be largely in keeping with global trends of women delaying their first pregnancy. However, one should take into consideration that due to ethical considerations, maternal age of under 18 years was an exclusion criteria in the sampling strategy of this study.

The younger participants in the EOPE cohort is supported by classical risk factors such as nulliparity (Luo *et al*., 2007), Black ethnicity and primipaternity (Zhang *et al*., 1997, ACOG,

2002), all of which are risk factors for pre-eclampsia development. The age of the normotensive pregnant cohort in this study is similar to that of previous studies (Aksornphusitaphong and Phupong, 2013).

The distribution of HIV according to age demonstrates that the HIV positive participants tended to be older than the HIV negative participants. This attests to the fact that local incidence of the HIV disease seems to be stabilizing, as supported by local statistics (Lehola, 2014).

4.2.2 Blood pressure

Currently both the International Society for the Study of Hypertension in Pregnancy and the Working Group of the National High Blood Pressure Education Program in the United States both define pre-eclampsia as either systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, with associated proteinuria after 20 weeks' gestation (Brown *et al*., 2001, 2000 and 183(1):S1-S22). As expected there were statistically significant higher systolic and diastolic blood pressure readings in this studys' pre-eclamptic cohort as compared to the normotensive non-pregnant and normotensive pregnant population. Furhermore, it was found that there were statistically significant differences with respect to HIV status and blood pressure across the non-pregnant, pre-eclamptic and normotensive cohorts (p=0.000).

A posturing inspection of BP in this study revealed a trend towards a lower systolic and diastolic blood pressure in HIV naive individuals. This trend is in keeping with the development of insulin resistance, obesity and hypertension in HIV infection (Gazzaruso *et al*., 2003). Furthermore,, it is well documented that the use of HAART induced complications of

metabolic syndromes such as impaired glucose tolerance, diabetes, hypertriglyceridemia, and reduced levels of high-density lipoprotein (HDL) are risk factors for cardiovascular disease development, including blood pressure elevation (Hadigan *et al*., 2001) (Samaras *et al*., 2007, Wolz *et al*., 2000); (Carr *et al*., 1998a, Samaras *et al*., 2007, Carr *et al*., 1998c, Carr *et al*., 1998b).

4.2.3 Gestational age, birth weights and placental weights

This study demonstrates significant differences in the gestational age at delivery when comparing the normotensive pregnant, EOPE, and LOPE cohorts *ie*., 38.98 *vs* 32 *vs* 36.92 weeks gestation. Similarly, and as expected there were significant differences in the birth weight of the pregnant cohort (p=0.000). Babies in the EOPE cohort were significantly smaller than both, the normotensive pregnant or LOPE groups (p=0.000) whilst there was no difference in birth weight between the normotensive pregnant and LOPE cohorts. This finding is in keeping with other studies, where babies from the EOPE cohort fell into the very low birth weight category (Kucukgoz Gulec *et al*., 2013, Lisonkova and Joseph, 2013). Both type of pregnancy (EOPE) and HIV status were risk factors for low birth weight in this study.

A large scale Tanzanian study in 2001 found that socio economic status, vitamin deficiency, parasitic infection, HIV positivity and stage of disease as risk factors for lower birth weight. When examining the effect of HIV status on baby weight in this study, lower birth weight babies in the EOPE HIV positive participants compared to the LOPE HIV negative/normotensive pregnant HIV positive and negative cohorts are found, respectively. In this study birth weight was lower in the EOPE HIV negative participants when compared to the LOPE HIV negative and normotensive pregnant HIV negative/positive participants

respectively. The LOPE HIV negative group also had lower birth weight babies than the normotensive HIV negative group. LOPE HIV positive participants had lower birth weight when compared to the normotensive pregnant HIV positive/negative participants.

Although earlier studies by Minkoff *et al.,* (1990) found no association between HIV infection and risk for low birth weight, these were small studies. Notably they did not consider the stage of HIV disease (Minkoff *et al*., 1990). Likewise, later studies also did not show a correlation (Castetbon *et al*., 1999). In contrast, studies by Stratton *et al*., (1999) support the correlation of low birth weight with HIV infection (Stratton *et al*., 1999).

Infant birth weight does not differ among women in the earlier stages of HIV disease, but has been shown to decrease significantly in stage III than in stage I infection (Dreyfuss *et al*., 2001). This may be attributed to the fact that with increased HIV infection, the immune system is further, down regulated eventuating in a deterioration of nutritional reserves.

Similarly, this study reports statistically significant smaller placentas in the EOPE cohort compared to the other pregnancy cohorts. There was however, no difference between the normotensive and LOPE pregnant cohorts. This outcome is indicative of the difference in pathophysiology between the EOPE and LOPE cohorts. Early onset pre-eclampsia is characterized by abnormal placentation and resultant hypoxia. It is also is consistent with the <34 weeks gestational age in the EOPE group. Moreover, poor fetal/neonatal outcomes particularly, lower birth weight has been previously reported in the EOPE group (Lisonkova and Joseph, 2013). The shallow placentation with resultant decreased blood flow and ensuing lower placental weight in the EOPE group expounds placental inefficiency.

Indications for delivery and complications

In this study, the indications for delivery were in fetal interest, with the commonest complication being one case of abruption placentae (3%), nine cases of eclampsia (27%), five cases of imminent eclampsia (15%) and twelve cases of severe pre-eclampsia (36%). Moreover, there were six cases (18%) of stillbirths.

In the study by Minire *et al*., (2013), maternal complications in pre-eclampsia were examined precisely and showed that liver damage occurred in 4.9% and 12.3% had renal impairment. Detachment of the placenta (abruption) was encountered in 7% of cases and 0.7% of patients had an epileptic attack in the study by Minire *et al*., (Minire et al., 2013). HELLP syndrome was present only in 4.2%, pulmonary edema occurred in 5.6% of subjects, DIC was found in 2.8% of cases and only one patient (0.2%) was registered with encephalopathy (Minire *et al*., 2013).

As the researcher was unable to obtain liver and renal function test results in all participants it is not possible at this time to speculate on the true incidence of liver and renal dysfunction in the local setting. This would have been an interesting area of investigation, as there may be contributory disease to these organs with use of various antiretroviral drugs.

4.2.4 HIV status / CD4 / ARV usage

The estimated overall HIV prevalence rate is approximately 10,2% of the total South African population (Lehola, 2014). The total number of people living with HIV is estimated to be approximately 5.51 million in 2014. For adults, aged 15–49 years, an estimated 16.8% of the

population is HIV positive (Lehola, 2014). As per study design and inclusion criteria, forty five percent (45.1%) of the overall study population was HIV infected. It must be noted that the study was conducted at a large referral hospital, there is a higher number of HIV positive individuals in such a setting. Further, in the Obstetric ward, a younger population tends to be the norm and this is a high risk group for the development of sexually transmitted infections. This study specifically included participants in the 18-45yr age group, an age group that carries the highest incidence of HIV nationwide (Lehola, 2014). As participants were of a similar age throughout the study groups their time of exposure to HIV infection may be similar.

As the national government had instituted policy at the time of the study, that all pregnant women with a CD4 count less than 350 cells/mm^3 be commenced on an ARV triple regimen and those with a CD4 >350 receive PMTC this may explain the significant difference in ARV usage between the non-pregnant and pregnant populations.

4.3 ANTHROPOMETRIC MEASUREMENTS

4.3.1 BMI

In this study, significant differences in the BMI between the non-pregnant and pregnant groups, but no significant differences between the pregnant cohorts were noted. BMI was calculated on individuals in their third trimester of pregnancy. To date there is no standardised way to calculate BMI in pregnancy. BMI is based on population and ethnicity specific charts, however these charts are unavailable as yet for the SA region (to our knowledge). There have however been comparisons made within the North American adolescent population (Lynch *et al.*, 2007) and as well as a WHO expert consultation on obesity in Asian populations (WHO Expert consultation, 2004) .

The WHO Expert Consultation concluded that the proportion of Asian people with a high risk of type 2 diabetes and cardiovascular disease was still substantial at BMI's lower than the existing WHO cut-off point for overweight (= 25 kg/m^2) (WHO Expert consultation 2004) . However, the cut-off point for observed risk had varied from 22 kg/m^2 to 25 kg/m^2 amongst the different Asian populations and for the high risk cohorts, it varied from 26 kg/m^2 to 31 kg/m^2 (WHO Expert consultation, 2004). The Consultation, therefore, recommended that the current WHO BMI cut-off points should be retained as the international classification.

The cut-off points for BMI of 23, 27.5, 32.5 and 37.5 kg/m^2 were recommended for use as points for public health action. This same consultation recommended that countries should use all categories (i.e. 18.5, 23, 25, 27.5, 30, 32.5 kg/m^2 , and in many populations, 35, 37.5, and 40 kg/m^2) for reporting purposes, with a view to facilitating international comparisons (WHO Expert consultation, 2004). Study analysis showed that the BMI of the non-pregnant and normotensive pregnant women was 28.55 ± 4.24 kg/m^2 and 28.89 ± 6.80 kg/m^2, respectively, although there was no statistically significant difference.

The relationship between BMI and hypertension in subpopulations of Ethiopian, Vietnamese and Indonesian population has been investigated (Tesfaye *et al*., 2006).

Table 4.1: The International Classification of adult as underweight, overweight and obesity according to BMI

Classification	**BMI(kg/m^2)**	
	Principal cut-off points	**Additional cut-off points**
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Source: Adapted from WHO, 1995, WHO, 2000 and WHO 2004.

The comparison between the pregnant cohorts allows for a more uniform comparison, however the majority of the participants fell into the overweight, pre-obese and grade 1 obesity category. Duckitt and Harrington found that raised BMI is a risk factor for pre-eclampsia development. It is only plausible to link the finding of similar BMI amongst the normotensive and pre-eclamptic cohorts in this study to a high pre-pregnancy weight. Pre-pregnancy weight was not

available for the participants. A consideration of high pre-pregnancy weight is conceivable since 70 % of South African women are considered overweight.

4.3.2 Mid upper arm circumference (MUAC)

In this study, the non-pregnant cohort (31.53 ± 2.63 cm) had a significantly higher mid upper arm circumference in comparison to the normotensive pregnant cohort (28.38 ± 4.59 cm). However, there was no difference between the pre-eclamptic cohorts. The EOPE group had a mid upper arm circumference of 33.38 ± 4.69 cm compared to 28.23 ± 1.43 cm for the LOPE group. According to the SPHERE (Statewide Partnership for HIV Education in Recovery Environments, 2011) Guidelines, mid upper arm circumference may be used as a screening tool for pregnancy weight, e.g., as a criterion for entry into a feeding programme. The guidelines recommends a cut-off point for risk with a range from 21 - 23 cm.

Furthermore,, mid upper arm circumference is considered a good indicator of the protein reserve of a body, and a thinner arm reflects wasted lean mass, *ie*., malnutrition (Cogill, 2003). The WHO Collaborative Study of 1995 implicates mid upper arm circumference values of <21-23 cm (OR 1.9; 95%; CI: 1.7-2.1) as having significant risk for low birth weight babies (WHO 1997, Kelly *et al*., 1996, WHO 1995).

The findings are in keeping with a South African survey of 2002 which depicts an increasing mid upper arm circumference amongst Black South African women (Puoane *et al*., 2002). Obesity is a risk factor for hypertension and cardiovascular development. It is also in keeping with the recent report showing high levels of overweight and obesity in adult women (Ng *et al*., 2014).

When comparing the effect of HIV status on the mid upper arm circumference, it was demonstrated that normotensive HIV positive participants had a lower MUAC when compared to the non-pregnant HIV negative or the LOPE HIV negative participants. This finding is congruent with the Womens' Inter-Agency study that found the incidence of peripheral and central lipo-atrophy amongst HIV-infected women to be double that amongst HIV-uninfected women, after adjustment for age and race (Tien *et al*., 2003). A drawback of the latter study was that it did not include pregnant women. Obviously due to pregnancy it was not possible to assess central lipo-atrophy in this study.

Supportive evidence for this local study findings has been put forward by Villamor *et al*., (2002). They examined lipodystrophy amongst pregnant participants of a low socio-economic class, HIV-positive mothers had an adjusted 34% excess prevalence of wasting, compared with HIV- negative women (Villamor *et al*., 2002).

4.3.3 Triceps skin fold thickness (TST)

This study demonstrated that the triceps skin fold thickness was significantly lower in the normotensive pregnant women (18.14 ± 7.025 mm) compared to both non-pregnant (21.58 ± 8.812 mm; $p < 0.002$) and LOPE (22.89 ± 10.638 mm) but was not significantly different from the EOPE group (18.94 ± 9.148 mm).

It had long been established that "at nearly all sites, skinfold thicknesses increases up to about 30 weeks of pregnancy" (Taggart *et al*., 1967). Increases were shown to be greater at central and least at peripheral sites, and were not proportional to the initial skinfold thickness. From 30

to 38 weeks of pregnancy, the distribution pattern of skin fold thickness had been found to be variable: the mid-thigh skinfold increased whilst at other sites there was little change or a decrease was noted. All sites decreased between 38 weeks of pregnancy and the end of the first post-partum week. Evidence at that time suggested that this change, was not related to the presence or absence of oedema, and occurred about the time of parturition (Taggart *et al*., 1967).

A later study by Sidebottom *et al.* revealed that the subcutaneous body fat stores of healthy pregnant women remained stable ($p>0.13$) during the first 6 weeks after conception, and increased from 6 to 35 weeks by 1.5 mm at the triceps, 4.2 mm at the subscapular, and 7.3 mm ($p<0.01$) at the thigh areas (Sidebottom *et al*., 2001).

In this study, the normotensive pregnant participants delivered at ±38.9 weeks whilst the EOPE participants delivered at ± 32 weeks of gestation. Superficially it would seem reasonable to assume that since EOPE participants delivered at an earlier gestation this would account for the seemingly higher TST levels in this cohort. True significance would only be established if the participants TST had been established pre-pregnancy, through the different trimesters of pregnancy and into the postpartum period.

When considering the effect of HIV status on the triceps skinfold thickness, the normotensive HIV positive participants had a significantly lower TST when compared to the LOPE HIV negative cohort. The study results are congruent with peripheral muscle wasting observed in HIV positive patients (Dannhauser *et al*., 1999). This is an area requiring supplementary research.

4.4 ADIPOKINE LEVELS

4.4.1 Adiponectin

In this study, a mean adiponectin level during the third trimester (all bloods were obtained just prior to delivery) of pregnancy was found to be 897.93 µg/ml *vs* 17.19 µg/ml *vs* 23.16 µg/ml *vs* 24.61 µg/ml in the non-pregnant, normotensive pregnant, EOPE and LOPE cohorts, was found respectively.

Ukkola *et al.*, (2002*)* demonstrated an inverse relationship between adiponectin levels and the degree of adiposity present. It would seem logical to assume that adiponectin levels would be lower in a pregnant state due to increased levels of adipose tissue – as is the trend in this local study. However, Nien *et al.*, (2007b) found that there was no significant difference in adiponectin levels in the non-pregnant and pregnant state.

An Egyptian study in 2011 found that "serum adiponectin levels in pre-eclamptic women were significantly higher than in normal pregnant women and the increase was more marked in cases of severe pre-eclampsia"(Abd-Alaleem *et al*., 2011). Nien *et al*., (2007a) in a study on severe pre-eclampsia had similar findings. This study showed a trend of higher adiponectin levels in the preeclamptic cohort as a whole as compared to the normotensive cohort but this failed to reach statistical significance (p= 0.292).

It could of course be that this is actually a true reflection that there is no significant increase in adiponectin levels in preeclapsia (O'Sullivan *et al*., 2006, Odden *et al*., 2006, Dalamaga *et al*., 2011). This is possible as the study reflects a high risk population of the same ethinicity at a single time frame in pregnancy. Although insulin resistance is thought to play a pivotal role in

the development of pre-eclampsia, the rise may be mitigated by the trimester at which testing was performed.

4.4.2 Leptin

The reference range of leptin is 3600 – 72400 pg/mL in adult females (Biorad diabetic assay). In this study it was found that non-pregnant participants had a mean leptin concentrations of 4887.25 compared to 2732.27 pg/ml in the normotensive pregnant groups. Participants with EOPE had a mean leptin concentration of 955.75 compared to 310.23 pg/ml in the LOPE groups (p=0.000). These were statistically significant findings.

The findings in the current study of higher leptin levels in the EOPE cohort as compared to the LOPE cohort seems in keeping with many other studies, however the overall finding of lower levels of leptin in the pre-eclamptics as a group compared to the normotensive cohort requires further, investigation ((Anato *et al*., 2000, Martinez-Abundis *et al*., 2000, El Shahat *et al*., 2013). It may be possible that the rise in leptin was ameliorated by the use of anti-hypertensives. The relative difference in leptin levels within the pre-eclamptic cohort may point to leptin being a marker for the degree of severity of the pre-eclampsia. Thus far debate remains ongoing regarding the role of leptin in pre-eclampsia.

4.4.3 TNF-α

TNF-α has a reference range of 42 – 203 pg/ml (Biorad Diabetes Assay kit). The current study showed non-pregnant participants had no measurable TNF-α levels. This may be attributed to the rapid breakdown of TNF-α once drawn from the participants. Normotensive participants

had a mean TNF-α concentration of 608.521 pg/ml. Participants with EOPE had a mean TNF-α concentration of 661.03 compared to 616.439 pg/ml in the LOPE group (p=0.000).

Walsh *et al.,* (2013) have shown that insulin resistance, even at levels below those diagnostic of gestational diabetes, is associated with maternal and fetal inflammatory response. Hence an elevated TNFα level is expected as compared to a healthy non pregnant population. Further, Vitoratos *et al.,* (2010) found that the levels of TNF-α were significantly elevated in preeclamptic as compared to normotensive pregnant participants. Similar findings in were shown in this study and this was found to be statistically significant (p=0.000).

4.4.4 Resistin

The reference range of resistin is 6390 – 26400 pg/ml (Biorad Diabetic assay). This local study found that non-pregnant participants had a mean resistin concentration of 7497.13 pg/ml compared to 3536.50 in the normotensive pregnant groups. The mean resistin concentration was 1017.63 pg/ml in the EOPE group compared to 286.92 pg/ml in the LOPE group respectively.

4.5 ADIPONECTIN, LEPTIN, RESISTIN AND TNF-α DIFFERENCES IN NON PREGNANT VERSUS PREGNANT POPULATIONS

The information garnered from this local study enables us to establish a novel range of adipokine levels according to BMI for non-pregnant and pregnant Black South African women. This information is further, stratified according to type of pregnancy into normotensive pregnant, EOPE and LOPE pregnancies. Moreover full stratification according to HIV status is for the first time recorded for Black South Africans.

In this local study, significantly lower levels of adiponectin, leptin and resistin in the pregnant cohort as compared to the non-pregnant cohort were found. TNF-α was only detected in the pregnant cohort. It is well recognised that pregnancy is a unique physiological state characterised by a temporary reversible insulin resistance and a modest level of immune elevation (Catalano *et al*., 1991, Buchanan *et al*., 1990, Ryan *et al*., 1985). Despite this, there is a dearth of literature regarding the adiponectin, leptin, resistin and TNF-α levels in healthy pregnant women. Correlation of these levels with HIV infection and regarding variation according to the trimester of pregnancy is lacking.

4.5.1 Adiponectin

To our knowledge there has been thus far, only one prior study that attempts to establish the change in adiponectin level according to the trimester of pregnancy (Mazaki-Tovi *et al*., 2007). Limitations of this study was its small sample size (n = 80). Although BMI was standardised in their study, they did not have a homogenous sample according to ethnicity. In this local study standardisation for BMI was considered. Moreover, this study was expedient in the fact that patients were all standardized according to ethnicity, sub-analysed according to BMI and all samples taken from the third trimester of pregnancy – one of the first such studies to be performed within South Africa as well as globally. This enables an establishment of a reference range for adipokines according to pregnancy type as well as HIV status in Black South African women in the third trimester pregnancy.

In pregnancy the adiponectin levels range between 2.7 – 25 µg/ml compared to 3.5 – 22.4 µg/ml in the non-pregnant (Nien *et al*., 2007). There was no difference demonstrated in the

adiponectin concentrations of non-pregnant and overweight pregnant females at any gestational age (Nien *et al*., 2007). However there was significantly lower adiponectin concentrations in overweight pregnant women compared to their normal weight counterparts. There was a trend of adiponectin decrease with advancing gestational age (Nien *et al*., 2007).

The problem with the latter study however, was that the samples were randomly drawn from the NIH sample bank hence there was a heterogenous sample population. Influence of race as well as sub-categorization for complicated pregnancies on adiponectin levels are serious drawbacks to the study.

In contrast to the above study, the current study demonstrates a decrease in adiponectin levels between all pregnant cohorts. This may indicate that the relative insulin resistance of pregnancy has not been overcome. Raised serum adiponectin concentrations are associated with increased insulin sensitivity and glucose tolerance (Goldfine and Kahn, 2003). Supporting this concept is the fact that hypo-adiponectinaemia in pregnancy is a negative predictor of insulin resistance, beta cell dysfunction and increased risk of diabetes mellitus (Retnakaran *et al*., 2010). As explained above, other studies have demonstrated the negative correlation between adiponectin levels and insulin resistance.

Another possible explanation for the difference in this study findings from other studies may be due to ethnic variation – the majority of the above mentioned studies were conducted on Caucasian participants. A study by Retnakaran *et al*., (2010) on South Asian women in pregnancy showed women of South Asian descent exhibited significantly reduced plasma

concentrations of adiponectin in pregnancy compared with their Caucasian and Asian counterparts, thus suggesting a greater risk for diabetes.

4.5.2 Leptin

Leptin is a satiety hormone hence it inhibits hunger and is secreted in proportion to adipose mass. In fact, the most important variable that determines circulating leptin concentrations in the body is fat mass (Speakman *et al*., 2002). Many studies have indicated that maternal peripheral leptin levels are enhanced during pregnancy (Helland *et al*., 1998, Jaquet *et al*., 1998, Hardie *et al*., 1997, Highman *et al*., 1998, Butte *et al*., 1997, Schubring *et al*., 1997, Schubring *et al*., 1998, Sivan *et al*., 1998).

A decline of leptin level after the second trimester and closer to parturition has been demonstrated (Henson and Castracane, 2006). These findings are consistent with a point out of time sampling in third trimester of pregnancy.

Castellano *et al.,* (2013) found an increase in leptin in the non-overweight (BMI $\leq 25 kg/m^2$) as compared to the overweight pregnant participants (Castellano Filho *et al*., 2013). Whilst most of this studys' pregnant participants fell into the overweight/obese category the implication of low serum leptin in pregnant participants, could be attributed to maternal hunger in an attempt to meet the increased nutritional demands of pregnancy in the presence of a low adipose mass. The relatively high leptin levels in the non-regnant cohorts would be indicative of insulin sensitivity. The lack of difference in leptin concentration within the pregnant cohorts may be due to the obesity of this cohort.

4.5.3 TNF-α

A 45% increase in TNF-α levels in late pregnancy has been reported (Kirwan *et al*., 2002). Further, other studies have shown that TNF-α is a predictor of insulin resistance in pregnancy (Clapp and Kiess, 2000, Beckmann *et al*., 1997). This is similar to and consistent with the low adiponectin levels demonstrated in this study, effectively showing that insulin resistance of pregnancy had not been overcome.

4.5.4 Resistin

Enlarged adipocytes release several products that can modify the body's sensitivity to insulin. Free fatty acids and TNF-α cause insulin resistance, and leptin, which regulates energy balance, probably causes insulin sensitivity. Yet there is a paucity of information on the potential function of resistin or its homologs in pregnancy (Flier, 2001a).

Further, in animal models, lower resistin mRNA in adipose tissue in different models of mouse obesity, such as diet-induced obesity, and in rat models characterized by hyperinsulinemia, hyperglycemia, hypertriglyceridemia, and hypertension have been demonstrated (Moore *et al*., 2001a, Lay *et al*., 2001). This further, substantiates current study findings of a failure to overcome insulin resistance in the pregnant cohort.

The levels of TNF-α, Leptin and Resistin were statistically significant different within the normotensive versus pre-eclamptic groups. Only Adiponectin failed to show statistically significant differences within the pregnancy subgroups

Both leptin and resistin were statistically lower and TNF-α higher in the preeclamptic cohort when compared to the normotensive cohort. During pregnancy, leptin is produced by both maternal and fetal adipose tissues, as well as by the placental trophoblast. Leptin induces human chorionic gonadotrophin production in trophoblast cells, regulates placental growth, enhances mitogenesis and stimulates amino acid uptake (Hauguel-de Mouzon *et al*., 2006). Most studies have found higher levels of leptin in pre-eclampsia (Hendler *et al*., 2005a).

Placental leptin mRNA production is upregulated by tumour necrosis factor (TNF) -α and interleukin (IL)-6 (Nuamah *et al*., 2004). It is thought that TNF-α is raised in pre-eclampsia in order to stimulate leptin production and increase nutrient supply to an underperfused placenta (Moore *et al*., 2003).

It would be expected that leptin levels in this preeclamptic cohort ought to be increased as well. The above studies however did not consider the confounding factor of HIV – a study by Azzoni *et al* (2010) showed a negative correlation between leptin and viral load in HIV positive individuals (Azzoni *et al*., 2010). As this study did not account for viral loads, this may account for the seemingly unique findings of lowered leptin levels amongst the pre-eclamptic cohort in this study.

The combination of a high viral load, together with the chronic inflammatory state induced by TNF may together account for patients with pre-eclampsia with low leptin levels. Studies on resistin levels in pre-eclampsia are few and conflicting – Hendler *et al* (2005) found no correlation between resistin and pre-eclampsia (Hendler *et al*., 2005a), whilst Cortelazzi *et al* (2007) hypothesized that lower levels of resistin in PE might be related to a reduction in

placental production of the adipokines because of the smaller size of the placenta (Cortelazzi *et al*., 2007).

However, statistically significant difference in placental weight between this studys' EOPE and other pregnant cohorts were found. Deductive reasoning would suggest that there should also have a difference in resistin levels in the EOPE versus other pregnancy cohorts. Other reasons for low resistin levels in pregnancy would be the onset of gestational diabetes. Studies evaluating the links between insulin resistance, obesity and diabetes have reported low levels of resistin (Heilbronn *et al*., 2004, Savage *et al*., 2001, Way *et al*., 2001a).

However the presence of diabetes was an exclusion criterion in this study. The answer may lie in the raised TNF-α levels – Fasshauer *et al.,* (2001) found that TNF-α was a negative regulator of resistin and a 70-90% decrease in resistin mRNA and protein secretion was noted after TNF-α administration (Fasshauer *et al*., 2001).

The levels of adiponectin / leptin / resistin / TNF-α does not differ in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia. There was no statistically significant difference depicted in the levels of adiponectin/leptin/tnf or resistin between the EOPE vs LOPE cohorts. This may indicate that there is a similar disease process occurring, but more likely the impact of HIV has a confounding effect. Further, since only one time point in pregnancy was assessed firm conclusions cannot be drawn.

4.6 ADIPONECTIN, LEPTIN, RESISTIN AND TNF-α LEVELS IN NON PREGNANT HIV PATIENTS VS PREGNANT HIV PATIENTS ACCORDING TO CD4 COUNTS

This study generated the following findings:

- non pregnant HIV negative participants had lower adiponectin levels than the normotensive HIV positive / and non-pregnant HIV positive participants respectively
- The non-pregnant HIV positive participants had lower leptin levels than the normotensive HIV positive participants
- The non-pregnant HIV positive participants had lower resistin levels than the normotensive HIV positive participants.
- The mean maternal age is lower in normotensive HIV negative participants than that of non-pregnant HIV negative and non-pregnant HIV positive participants.

The levels of adiponectin in HIV-positive ARV naive patients were lower compared to the healthy subjects (Li *et al*., 2011). In ARV treated patients, the levels of adiponectin is lower than in HIV- negative patients. Both protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs) alter adipokine secretion from human subcutaneous adipocytes (Lagathu *et al*., 2007). Further,, an interesting study by Sankale *et al*., (2006) showed that human subcutaneous adipocytes exposed to HIV-1 dramatically increased the secretion of adiponectin (Sankale *et al*., 2006).

In this study, a statistically significant difference in the levels of adiponectin/leptin/resistin and TNF-α in HIV positive patients within the non-pregnant *versus* pregnant population matched for CD4 count, was found. Within the pre-eclamptic cohort there was no statistically significant difference in EOPE versus LOPE when matched to CD4 count. In a similar vein the study by

Arama *et al*., (2012) also showed no difference in adipokine, leptin or resistin levels when comparing healthy treatment naive HIV positive patients compared to treated HIV positive patients (Arama *et al*., 2012). However, the latter study reported significantly higher levels of TNF-α in participants with persistent viral load. It is not possible to comment on this aspect as viral load assessment was not assessed in this local study as it is not a standard of care practice. Research with a larger number of patients and prospective study designs may be required to draw statistically significant and relevant conclusions regarding the association between adipokines and HIV replication.

The difference in levels of adiponectin/ leptin / resistin / TNFα in women who develop early onset pre-eclampsia as compared to women who develop late onset pre-eclampsia are not altered by the HIV status of the patients

This study did not show any statistical significance in the levels of adiponectin/leptin/TNF-α/or resistin among the pregnant cohorts irrespective of CD4 counts and ARV usage. The lipodystrophy syndrome of HIV patients has been well documented in the literature. It is characterised by fat redistribution (lipodystrophy or lipoatrophy), fat loss from the face, buttocks and extremities (lipoatrophy), and mixed fat disturbances (lipodystrophy and lipoatrophy (Carr *et al*., 1998a, Carr *et al*., 1998c, Saint-Marc *et al*., 1999, Behrens *et al*., 2000, Hakeem *et al*., 2008). As mentioned earlier, there was no statistically significant differences in mid upper arm circumference or triceps skinfold thickness between the EOPE and LOPE cohorts. This may account for the similar levels of adiponectin/leptin/resistin and TNF-α in both cohorts.

4.7 ADIPONECTIN, LEPTIN, RESISTIN AND TNF-α LEVELS ACCORDING TO BMI

There were no statistically significant differences in levels of TNF-α, Leptin, and Resistin when compared across subdivisions of BMI in the non-pregnant population. Adiponectin did show statistically significant differences across BMI subdivisions in the non-pregnant population. Further, there were statistically significant differences in BMI between the non- pregnant and pregnant cohorts.

There was no statistically significant differences in the levels of TNF-α, Adiponectin, Leptin and Resistin in the pregnant population across the subdivisions of BMI. Adiponectin levels have been shown to be paradoxically lower in obese patients when compared to non obese patients (Arita *et al*., 1999; Hu *et al*., 1996). Further,, it has been shown that weight reduction in obese individuals is accompanied by an increase in plasma adiponectin concentrations (Esposito *et al*., 2003). This a difficult area of interpretation as there are no standardised BMI charts for pregnancy globally.

4.8 LIMITATIONS OF THE STUDY

Blood samples were only drawn for adipokine assessment at one time point in the study ie., in the third trimester. Preferably patients should be recruited in the pre-pregnancy state and followed up through all three trimesters. However, in the current setting, patients only present to a referral hospital late in pregnancy. An assessment of viral load in the pre-pregnancy and through each trimester would have strengthened the study. This however, this was not possible as it is not standard of care practice in South Africa. These are limitations imposed by the Department of Health. It must be acknowledged that the findings of this study are correlative

as there are mechanistic experiments included in this thesis to outline the role of the tested factors in pre-eclampsia with or without HIV.

4.9 STRENGTHS OF THE STUDY

The strengths of this study include its strict inclusion and exclusion criteria thus ensuring as homogenous a population as possible. The study is innovative because adipokine levels are compared across non-pregnant, normotensive-pregnant and pre-eclamptic women associated with HIV infection. It is also novel in that it correlates adipokine levels with HIV status. This pioneering study establishes a baseline for adipokines across the non-pregnant, normotensive-pregnant and pre-eclamptic women associated with and without HIV infection in Black South African women.

4.10 CLINICAL IMPLICATIONS OF THE STUDY

To our knowledge it is the first such study to address the three aspects of obesity, HIV and pre-eclampsia and their inter-relationship. It is a relatively large study and establishes the marked difference in adiponectin/leptin/resistin and TNF-α levels between normotensive pregnancy and pre-eclampsia. It is plausible to implicate the deficient placentation with adipokine dysregulation as the pathological process of EOPE development compared to that of LOPE.

4.11 FUTURE RESEARCH

Future research should assess adipokines from pre-pregnancy across all 3 trimesters of pregnancy. In addition to the maternal serum levels, cord blood should also be assessed for these adipokines. Viral load, and ARV usage should be specifically included irrespective if it is

not a standard of care practice. Moreover, placental tissue and umbilical cord should be assessed by immunohistochemistry for the paracrine secretion of these adipokines.

4.12 CONCLUSION

This study was expedient in the fact that patients were all standardized according to ethnicity, sub-analysed according to BMI and all samples taken from the third trimester of pregnancy – one of the first such studies to be performed within South Africa as well as globally. The study reports significant differences in the BMI of the non-pregnant and pregnant groups, but no significant differences within the pregnant cohorts. In conclusion this study establishes an adipokine baseline for future reference with regards to South African Black pregnant and non-pregnant women. Albeit at term, this study shows a statistically significant difference in the levels of adiponectin/leptin/resistin and TNF-α in HIV positive patients within the non-pregnant versus pregnant population. Within the pre-eclamptic cohort there was no statistically significant difference in EOPE versus LOPE. The ubiquitous HIV infection, high pre-eclampsia and obesity prevalence in South Africa, warrants this novel study that reconnoiters the relationship amongst this deadly troika.

CHAPTER 5

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CHAPTER 6

ADDENDUM

ADDENDUM 1 POSTGRADUATE APPROVAL

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

24 August 2012

Prof T Naicker
School of Laboratory Medicine & Medical Sciences
Dept of Optics & Imaging

Dear Prof Naicker

PHD PROTOCOL: "The role of Adiponectin, Leptin, TNFα and Resistin in HIV associated Pre-eclampsia"
Student: Dr V. Govender, student number: 993210275 (Obs & Gynae)

I am pleased to inform you that the abovementioned study has been approved.

Please note:

- The Academic Leader: Research must review any changes made to this study.
- The study may not begin without the approval of the Biomedical Research Ethics Committee.

May I take this opportunity to wish the student every success with the study.

Yours sincerely

for Professor JK Burns
Academic Leader School Research
School of Clinical Medicine

C Dr. V Govender
PROF J. MOODLEY

Biomedical Research Ethics Committee
Westville Campus

Postgraduate, Higher Degrees & Research
School of Clinical Medicine, NRMSM Campus
Postal Address: P/Bag X3, Congella, Durban, 4013, South Africa
Telephone: +27 (0) 31 260 4745 **Facsimile:** +27 (0) 31 260 4723 **Email:** jantjies@ukzn.ac.za **Website:** www.ukzn.ac.za

ADDENDUM 2 INSTITUTIONAL ETHICS APPROVAL

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

13 February 2013

Dr. V Govender
Department of Obstetrics and Gynaecology
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Govender

PROTOCOL: The role of Alponectin, Leptin, TNF and Resistin in HIV associated Pre-eclamptics.
REF: BE256/12.

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 05 September 2012.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 05 February 2013 to queries raised on 21 January 2013 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 13 February 2013.

This approval is valid for one year from **13 February 2013.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **12 March 2013.**

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

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Professor S Collings (Chair)
Humanities & Social Sc Research Ethics Committee
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban, 4000, South Africa
Telephone: +27 (0)31 260 3587/8350 **Facsimile:** +27 (0)31 260 4609 **Email:** ximbap@ukzn.ac.za / snymanm@ukzn.ac.za
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

ADDENDUM 3 PERMISSION TO CONDUCT RESEARCH

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager/s for signature.

For **King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH)** studies please submit the document together with the following:

1. Research proposal and protocol.
2. Letter giving provisional ethical approval.
3. Details of other research presently being performed by yourself if in the employ of KEH, (individually or as a collaborator).
4. Declaration of all funding applications / grants, please supply substantiating documentation.
5. Complete the attached KEH Form - *"Research Details"*

Once the document has been signed it should be returned to **Mrs Patricia Ngwenya:** Biomedical Research Ethics Administrator, Room N40, Govan Mbeki Building, Westville Campus, University of KwaZulu-Natal.

To: Chief Medical Superintendent / Hospital Manager

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address: RK Khan Hospital

Investigator/s:
Principal: Dr V Garender
Co-investigator: Prof. T Naicker
Co-investigator: Prof. J Moodley

Signature of Chief Medical Superintendent/Hospital Manager:

Date: 28/1/2013

HOSPITAL C.E.O.
R.K. KHAN HOSPITAL
28 -01- 2013
PRIVATE BAG X004
CHATSWORTH 4030

Site 2 address:

Investigator/s
Principal:
Co-investigator:
Co-investigator:

Signature of Chief Medical Superintendent / Hospital Manager:

Date:

NB: Medical Superintendent/s / Hospital Manager/s to send a copy of this document to Natalia

ADDENDUM 4 CONSENT DOCUMENT

Title of Study: The Role of adiponectin, TNF, leptin and resistin in HIV associated pre-eclamptics

Good day. Miss/ Mrs _____. My name is _____

Thank you for giving me the time to speak to you.

My colleagues and I are currently doing research on high blood pressure and pregnancy. Research is just the process to learn the answer to a question. In this study, we want to learn what causes this high blood pressure in pregnant women and what factors may be involved in causing this to happen.

When you are pregnant, the baby growing inside of you actually receives nutrition from a piece of tissue that is attached to your womb called the placenta or after birth. We think that certain substances/hormones made in the fat affect the way that this placenta develops and leads to high blood pressure.

We are inviting you to participate and require your permission to be included in this research study**.** This is what your role will be in this study. We need you to donate a blood sample to perform some laboratory tests on it. This will not harm you or your baby in any way. Please be aware that an experienced doctor will be available to draw out the blood. The taking of the blood will be done at the same time as the normal or routine blood tests are done to avoid many venipunctures. The taking of blood may cause minor discomfort.

All these samples are being collected here in this hospital from South African woman and will be studied at the Nelson Mandela Medical School. You will not directly benefit from this research in this pregnancy but other women or you in the future may benefit. The taking of the bloods will not interfere with the treatment you will be getting.

Please note that this research is being done for a higher degree or university qualification. The results of this will benefit medical science.

Please remember that you can refuse to enter the study and if you do you will not be disadvantaged in any way and all our discussions are private and confidential.

Thank you for your time and assistance in this study.

Islwoko sokufundwayo:

Ngiyabingelela Nkosazane / Nkosikazi. Igama lami ngingu _____

Ngiyabonga ukuthola ithuba lokukhuluma nawe

Mina nozakwethu senza ucwaningo ngomfutho wegazi nokukhulelwa. Ucwaningo ngomfutho nokuphendulwa kwemibuzo. Kulolucwaningo sifuna ukathola ukuthi ubangwa yini umfutho ophezulu wegazi komama abakhulelwe nemiphumela yokuthi loko kwenzeke.

Uma ukhulelwe ingane ekhula ngaphakathi ithola ukudla ngesicubu senyama esinamathele esibelethweni esibizwa ngomzanyana. Sicabangela ukuthi ukulakheka kwamafutha kuphazamisa indlela umzanyana akhula ngayo lokho okwenza ukwenyuka komfutho wegazi. Sifisa ukukumema sisebenzisane nawe kulolucwa-ningo. Yilokhu ozosenzela khona. Sicela usinikelele ngegazi lokwenza lokuwaningo. Lokhu akuzukukulimaza wena noma umntwana nanoma ngayiphi indlela. Sifisa wazi ukuthi udokotela oqeqeshekile ozobakhona ukuthatha igazi. Ukuthathwa kwegazi kokwenziwa kanyekanye nalokhe okujwayelekile ukuvimbela ukujovwa kaningi. Ukuthathwa kwegazi kungenza uzizwe ungemnandi kahle.

Lezizibonisо eziqoqwe kwabesifazane base mzansi Africa kuzofundwa nguzo eskoleni sobudokotela iNelson Mandela. Akukho wena ozokuthola ngqo ngalolucwaning kadwa abanye besifazane noma wena ngokuzayo. Ukuthathwa kwegazi akuzulwba namthelela kwimithi oyithdayo.

Lolucwaningo lwenzelwe ezemfundo ephakeme. Imiphumda izosiza kwezobudokotela.

Khumbula ukuthi ungakwazi ukunqaba ukungenela lolucwaningo futhi ngalokho akukho okokulahlekela.

Siyabonga isikhethi sakho nokusiza ngalesesisifundo.

Information for non pregnant women:

Title of Study: The Role of adiponectin, TNF, leptin and resistin in HIV associated pre-eclamptics

Good day. Miss/ Mrs _____. My name is _____

Thank you for giving me the time to speak to you.

My colleagues and I are currently doing research on high blood pressure and pregnancy and HIV. Research is just the process to learn the answer to a question. In this study, we want to learn what causes this high blood pressure in pregnant women and what factors may be involved in causing this to happen. We also want to know how HIV may also lead to problems with high blood pressure.

When you are pregnant, the baby growing inside of you actually receives nutrition from a piece of tissue that is attached to your womb called the placenta or after birth. We think that certain substances/hormones made in the fat affect the way that this placenta develops and leads to high blood pressure. We also think that HIV on its own can increase these substances that leads to high blood pressure when you are not pregnant.

We are inviting you to participate and require your permission to be included in this research study. This is what your role will be in this study. We need you to donate a blood sample to perform some laboratory tests on it. This will not harm you. Please be aware that an experienced doctor will be available to draw out the blood. The taking of the blood will be done at the same time as the normal or routine blood tests are done to avoid many venipunctures. The taking of blood may cause minor discomfort.

All these samples are being collected here in this hospital from South African woman and will be studied at the Nelson Mandela Medical School. You will not directly benefit from this research but other women or you in the future may benefit. The taking of the bloods will not interfere with the treatment you will be getting.

Please note that this research is being done for a higher degree or university qualification .The results of this will benefit medical science.

Please remember that you can refuse to enter the study and if you do you will not be disadvantaged in any way and all our discussions are private and confidential.

Thank you for your time and assistance in this study

Ulwazi kwabesifazane abangakhulelwe

Ngiyabingelela Nkosazane / nkosikazi_____

Igama lami ngingu_____. Ngibonga ithuba ongipha lona lokuxoxa nawe.

Mina nozakwethu senza ucwaningo ngonifutho ophezulu wegazi, ukukhulelwa nesandulele ngculazi. Ubwaningo indlela yokufunda ukuphendula imibuzo. Kulokufunda sifuna ukuthola izimbangela zomfutho ophezulu wegazi kwabesifazane abakhulelwe.

Uma ukhulelwe, ingane ekhula ngaphakathi ithola ukudla ngesicubu senyama esinamathele kwisibeletho esbizwa ngomzanyana. Sicabagela ukuthi ukwakheka kwamafutha ikhona nokuthi isandilela ngculazi ngokwaso singabangela ukwenyaka komfutho wegazi noma ungakhulelwe.

Siyakumena ngokwemvume yakho ukuba ube yingxenye yalokcwaningo. Yilokhu ozosisiza ngakho kulesisifundo. Sifisa unikele ngegazi elizosetshenzi swa ukuyohlolwa. Lokhu akunabungozi kuwe. Qaphela ukuthi udokotela oqeqehekile ozobekhona ukuthatha igazi. Ukuththwa kwegazi kuzokwenziwa ngasikhathi sinye namanye amagazi ukubalekela ukujovwa kiningi. Ukuthathwa kwegazi kungenza uzizwe ungemnandi kahle.

Leziziboniso eziqoqwe kwabesifazane baseMzansi Afrika kuzofundwa ngazo esikoleni sobudokotela iNelson Mandela. Akukho lutho wena ozokuthola ngqo kulolucwaningo kodwa abanye besifazane noma wena ngokuzayo. Ukuthathwa kwegazi akuzukuba namthelela kwimithi oyitholayo.

Lolucwaningo lwenzelwe ezenfundo ephakeme. Imiphumela iyosiza kwezobudoktela.

Khumbula ungakwazi ukunqaba ukungenela lolcwaningo futhi ngalokho akukho okokulahlekela lolucwaningo futhi ngalokho akukho okokulahlekela. Okoxoxiwe kuzogcinwa kuyimfihlo kuvikelekile.

Sibonga ukubambisana kulesisifundo.

ADDENDUM 5 PATIENT DATA FORM

THE ROLE OF ADIPONECTIN / RESISTIN / LEPTIN / TNFα IN HIV-ASSOCIATED

PRE-ECLAMPSIA

Study no: ☐

IP number ☐

Category (tick): (more than 1 catergory may require a tick)

1. Pre-eclamptic HIV +ve CD4 < 350: ☐
2. Pre-eclamptic HIV +ve CD4 > 350: ☐
3. Pre-eclamptic HIV -ve CD4 : ☐
4. Pregnant Normotensive HIV +ve CD4 < 350: ☐
5. Pregnant Normotensive HIV +ve CD4 > 350: ☐
6. Pregnant Normotensive HIV -ve: ☐
7. Onset pre-eclampsia before 34 wks ☐
8. Onset of pre-eclampsia at or after 34wks ☐
9. Normotensive , Non Pregnant ☐

☺**No exclusion criteria present** (check against list) ☐

Please place hospital sticker here

THE FOLLOWING BLOODS MUST BE TAKEN FOR THE PURPOSES OF THIS STUDY. PLEASE TICK IF THE BLOODS TAKEN

1. **ADIPONECTIN** ☐
2. **LEPTIN** ☐
3. **RESISTIN** ☐
4. **TNFα** ☐

General hospital information

Admission date		RKK no.	

Demographics

Age			
Area of Residence (tick)	Rural	Urban	
Smoke (y/n)		No. of cigarettes/day	

HIV Status	+ve	-ve
CD4 counts		
Anti Retroviral therapy	Yes	no
HAART		
PMCTC		

Reason for attending clinic if Non pregnant, Normotensive patient_____
_____

MATERNAL TREATMENT

Type of Treatment	**Yes**	**No**
Magnesium sulphate		
Aldomet		
Monoohydralazine		
Nifedipine		
Dihydralazine (nepresol)		
Labetalol		

Others		

Clinical Data

Parity	P: G:	Weeks gestation on admission	
Reason for previous pregnancy loss (If any)			
Highest BP	Systolic:	Diastolic:	
Maternal weight		Maternal height	
Midarm circumference		Triceps skin fold thickness	

Oedema (tick)	ankle	Up to knee	Up to groin	Generalised (facial)

Lab results (or attach copy of results)	proteinuria	Dipstick			
		Lab 24hr protein			
		Creatinine clearance			
	Full blood count	Red cell count		White cell count	
		Haemoglobin		Neutrophils	
		Haematocrit		Lymphocytes	
		Mean cell volume		Monocytes	
		Mean cell Hb		Eosinophils	
		Platelets		Basophils	
	Urea and electrolyte	Sodium		Urea	
		Potassium		Creatinine	
		Chloride		Anion gap	
		CO_2			
	Liver function tests	Total protein		Alkaline phos	
		Albumin		AST	
		Globulin		ALT	
		Alb : Glob		LDH	
		Total bilirubin			

Antenatal Fetal Investigations

Type (tick)	Note any abnormalities
Sonar	
Doppler	
Electronic fetal HR	

Birth details

Weeks of gestation at time of birth	

Date of birth		**Time of birth**	
Indication for delivery (tick one)	Maternal interest	Fetal Distress	Combination of Maternal and fetal interest.
		CTG abnormal	
		MSL	
		IUGR	
	Explain above if relevant	Explain above if Relevant	Explain above if relevant
	Diagnosis: Eclampsia, severe abruptio infection		

Method of Delivery (tick one)	Normal vaginal		Caesarean	
	Spontaneous		Elective	
	Induced		Emergency	

Complications in labour.	Eclampsia – related (tick)	Severe pre-eclampsia	Imminent eclampsia
	Abruptio-placentae		
	Other (explain)		

Baby details at birth

APGAR	1 min		5 min	
Baby (tick)	Live		Stillborn	
	Perinatal death (1st 7days)		Neonatal death (up to 28 days)	
Baby weight (kgs)				

Placental details

Shape	Normal		abnormal	
Weight (grams)				
Diameter (cm)				
Thickness (cm)	Less than 2cm	2-3cm	More than 4	
Colour	Maternal surface		Dark Maroon	Pale
	Fetal surface		Dark	Pale

Infarcts (maternal surface)	Amount of infarcted tissue	clear	mild	severe
	Colour of infarcts (if present)	Pale grey	Very dark	Both
Clots (maternal surface) tick	None	few	many	
Umbilical cord	Point of attachment	central	peripheral	
	Length	Less than 30 cm	30-90 cm	Greater than 90 cm
	No of vessels	3	2	
	Oedema	present	absent	

FOLLOW UP DATA PRIOR TO DISCHARGE FROM HOSPITAL

Date:_____ Inpatient / Outpatient visit: _____

Oedema (tick)	ankle	Up to knee	Up to groin	Generalised (facial)

Any other observations/clinical data/information of relevance for mother:
(Maternal complications / morbidity)

_____
_____
_____
_____
_____

Baby weight: _____ Maternal BP: _____

Feeding choice	formula	Breast	flash heating	not fed	TPN
Cranial scan					

Morbidities in early NN period

Resp Distress	HMD, TTN, Pneum ?Mas, other	
CNS	Asphyxia, meningitis	
Metabolic	hypoglycaemia, electrolyte imbalance	Other
hypothermia,		
Infections	Minor	Skin,
		eye,
		umbilicus,

		Suspected sepsis
		normal WCC +CRP
	Major	Pneumonia,
		Septicaemia (positive BC),
		meningitis (positive culture
		NEC,
		susp sepsis + low wcc and raised CRP (negative culture)

All positive cultures = severe infections. CPAP and ventilalion = severe illness

Any other observations/clinical data/information of relevance for child:
(Neonatal complications / morbidity)

_____
_____
_____
_____
_____

FOLLOW UP DATA AFTER DISCHARGE FROM HOSPITAL

Date:_____ Inpatient / Outpatient visit: _____

Oedema (tick)	Ankle	Up to knee	Up to groin	Generalised (facial)

Baby weight: _____ Maternal BP: _____

Any other observations/clinical data/information of relevance for mother or child:

_____
_____
_____
_____
_____

HIV status of baby 6 weeks post delivery	HIV +ve (PCR)	HIV –ve
CD4 count		
Baby NVP and AZT Bactrim yes/no		(7 days or 28 days)

Late Morbidities

Neurological impairment	
BPD	
ROP	
Nutritional	

Outcomes

Alive well	
Alive ill - record morbidities as above	Minor infections, HIV related infections,ROP and Audiology if small babies (<34 weeks), feeding choices
ENND	
LNN	

ADDENDUM 6 COLLABORATION WITH STATENS SERUM INSTITUT

STATENS SERUM INSTITUT

Prof Anita Naicker
Optics & Imaging Centre
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
University of KwaZulu-Natal
Private Bag X7
Durban
4013

Clinical Biochemistry, Immunology and Genetics
Our ref: SSI/UKZN_2013_01

15 April 2013

Re: Confirm interest in Collaboration

Dear Professor Naicker,

This letter is to confirm my interest in collaborating on the research proposed in Dr Vineshree Govender's PhD proposal entitled "The role of Adiponectin, Leptin, TNFα and Resistin in HIV associated Pre-eclampsia".

The Statens Serum Institut is a Danish health institute which participates in research collaborations and provides diagnostic services world-wide. At the Section of Molecular Medicine we have long history with feto-maternal medicine and a keen interest in understanding pregnancy conditions; as part of our work in this area have developed many of the techniques and technical skills Dr Govender has proposed to apply in her study.

The research proposed here would help us better understand the role adipocytokines play in pre-eclampsia with a particular emphasis on HIV positive women. Thus, I would be glad to provide academic support for this project in addition to training and technical support in the ELISA and real-time PCR portion of Dr Govender's study. Participants from Statens Serum Institut will be Dr Michael Christiansen and me, administrative support for all visits to Copenhagen will be provided by Mevis Walter (email: mewa@ssi.dk; tel: +45 3268 8636).

Yours sincerely,

Paula Hedley, Ms
Section of Molecular Medicine

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Fax: +45 3268 3860
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ADDENDUM 7 EXPORT PERMIT- SA TO COPENHAGEN, DENMARK

health

Department:
Health
REPUBLIC OF SOUTH AFRICA

Private Bag X828, PRETORIA, 0001. 27th Floor, Room 2710, Civitas. Cnr Thabo Sehume & Struben Street, PRETORIA, 0001
Tel: +27 (0) 12 395 8000. Fax: +27 (0) 12 395 8422

Reference : J1/2/4/2 No 1/14
Enquiry : Ms L Motopi
Tel : (012) 395 8366/8197
Fax : (086) 632 6815/8506

EXPORT PERMIT

In terms of Section 68 of the National Health Act 2003 (Act No. 61 of 2003) –

Prof T Naicker
Doris Duke Medical Research Institute
Nelson R Mandela School of Medicine
University of Kwazulu Natal
Congella
4013 South Africa
Tel. No.: (031) 260 4435 Fax. No.: (031) 260 4311

is hereby authorised to export from the Republic of South Africa –

2 ml x 350 samples Blood – serum

to –

Dr Paula Hedley
States Serum Institut
Department of Clinical Biochemistry
Immunology and Genetics
Statens Serum Institut
Orestads Boulevard 5
2300S, Copenhagen
DENMARK
Tel: + 0045 3268 8192 Fax: 0045 3268 3878

For – Analysis.

This export permit is subject to the following conditions:

1. The substance shall be imported into the country specified above, within the legal requirements of that country.
2. The substance shall be exported from South Africa and handled in accordance with the provisions of the National Health Act 2003 (Act No. 61 of 2003), and the regulations made in terms of the Act.
3. The export permit shall not be used for any trade or advertising purposes.
4. **This export permit shall expire on 30 June 2015.**

DIRECTOR-GENERAL: HEALTH
Date: 12/06/2014
Ms P Netshidzivhani