EVALUATION OF HAEMATOLOGICAL PARAMETERS AND IMMUNE MARKERS IN HIV-INFECTED AND NON-INFECTED PRE-ECLAMPTIC BLACK WOMEN

BY

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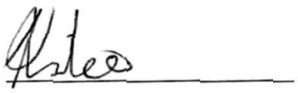
ABSTRACT

This study focuses on women with both pre-eclampsia and Human Immunodeficiency Virus (HIV). Pre-eclampsia is a pregnancy-specific syndrome that occurs after 20 weeks gestation. Thrombocytopenia is the most common haematological abnormality in pre-eclampsia. Further, studies suggest that the immunological mechanism plays some role in the aetiology of pre-eclampsia. The immunological hallmark of HIV infection is a progressive decline in the number of CD_4 T lymphocytes and significant haematological abnormalities are also common in HIV-infected individuals i.e. anaemia, thrombocytopenia and leukopenia. The study population comprised of two groups i.e., pre-eclamptic HIV-positive African women and pre-eclamptic HIV-negative African women as the control group. Samples were analysed for haematological parameters (full blood count) and immunological markers (flow cytometry). There was no statistical significance in the following parameters: RBC, Hb, haematocrit, MCV, MCH, MCHC, platelets, MPV, WBC, lymphocytes, neutrophils, eosinophils, monocytes, basophils and $CD_{8.}$ There was a statistical difference in the CD_3 and CD_4 counts between both the groups. However, the CD_3 and CD_4 counts were within the normal range in the HIV-negative pre-eclamptic group and even though CD_3 decreased, it was still within the normal range in the HIV-positive pre-eclamptic group, with CD_4 decreasing below the normal range in the HIV-positive pre-eclamptic group. This suggests that immune mechanisms involving CD estimations do not play a role in pre-eclampsia since the decrease in the counts can be solely attributed to HIV infection. Results obtained in this study do not show any severe haematological or immunological abnormalities when women have both pre-eclampsia and HIV infection.

AUTHORS DECLARATION

The experimental work presented in this thesis represents the original work by the author and has not been submitted in any form to any other university. Where use was made of the work of others, it was duly acknowledged in the text.

The research described in this study was carried out under the supervision of Prof. J. Moodley in the Department of Obstetrics and Gynaecology, Nelson R. Mandela School of Medicine, University of Kwa-Zulu Natal during the period January 2004 – May 2007.



Miss K. Naidoo

DEDICATION

✸ *Dedicated to my parents,*

Mr. and Mrs. P.S. Naidoo ✸

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LIST OF ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired Immune Deficiency Syndrome
AMA	Antimitochondrial antibody
ANA	Antinuclear Antibodies
ANG	Angiotensin
ARV	Antiretroviral
AZT	Zidovudine
BP	Blood Pressure
CD	Clusters of Differentiation
CS	Caesarean Section
DBP	Diastolic Blood Pressure
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ET-1	Endothelin-1
FBC	Full Blood Count
FITC	Fluorescein Isothiocyanate
Hb	Haemoglobin
HELLP	Haemolysis, Elevated Liver Enzymes and Low Platelet Count
HIV	Human Immunodeficiency Virus
ICAM-1	Intercellular Adhesion Molecule-1
Ig	Immunoglobulin
IL-1	Inteleukin-1
ITP	Immune Thrombocytopenic Purpura
IUGR	Intrauterine Growth Restriction
KEH	King Edward Hospital
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume

mmHg	Millimetres of Mercury
MPV	Mean Platelet Volume
NHBPEP	National High Blood Pressure Education Program Working Group
NK	Natural Killer
NNRTI’s	Non-Nucleoside Reverse Transcriptase Inhibitors
NO	Nitric Oxide
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NVD	Normal Vaginal Delivery
PCAM-1	Platelet Endothelial Cell Adhesion Molecule–1
PE	Phycoerythrin
PerCP	Peridinin Chlorophyll Protein
PG-2	Prostaglandin-2
RBC	Red Blood Cell
RDW	Red Blood Cell Distribution Width
RNA	Ribonucleic Acid
SA	South Africa
SBP	Systolic Blood Pressure
SGA	Small for Gestational Age
SIV	Simian Immunodeficiency Virus
STD	Sexually Transmitted Disease
TBX	Thromboxane
TCL	T-cell lines
TM	Transmembrane
TNF	Tumour Necrosis Factor
VCAM-1	Vascular Adhesion Molecule-1
WBC	White Blood Cell
WHO	World Health Organisation

CHAPTER 1

LITERATURE REVIEW

Physiologically women have an enhanced vulnerability or predisposition to disease. This vulnerability is increased if a woman is poor as this reduces her opportunity to access the required medical interventions. Taken together, the health hazards associated with being female are widely underestimated. Economic and cultural factors may limit women's access to clinics and healthcare workers. Further to this, the World Health Organisation (WHO) reports that less is spent on healthcare for women and girls worldwide. The World Health Report showed that, globally, the leading causes of death among women are Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS), malaria, complications of pregnancy (hypertension, ectopic pregnancies, pre-eclampsia) and childbirth and tuberculosis (Bellamy, 2004).

1.1 HYPERTENSION

Hypertension is one of the most common medical disorders in pregnancy and is associated with approximately 10% - 16% of all pregnancies. It is the leading cause of maternal, foetal and neonatal morbidity and mortality worldwide (Allen *et al*., 2004). The limited understanding of the pathogenesis of these diseases is compounded by the lack of early diagnostic tests available to identify women at an increased risk for pregnancy-induced hypertension (Wolf *et al*., 2002 (a)).

The normal blood pressure (BP) of a healthy adult is ≤ 120/80 millimeters of mercury (mmHg). Hypertension in pregnancy is defined by a systolic blood pressure (SBP) of 140mmHg or above and a diastolic blood pressure (DBP) of 90mmHg or above, on two occasions 6 hours apart (Sibai, 2001). Patient anxiety, physical stress, apprehension and excitement can cause transient elevations in BP, therefore it is necessary to confirm elevated readings on two consecutive occasions (Helewa *et al*., 1997).

During healthy pregnancy, the DBP falls by 7mmHg – 10mmHg during the first 10 weeks and decreases further until around the 20^{th} week where it is at its lowest level about 55mmHg (Reif, 2003).. The decease in SBP is smaller (Figure 1.1). This trend of decreasing BP is not found in pregnancies complicated by hypertension and therefore its absence may indicate subtle renal disease or early stages of hypertension (Reif, 2003).

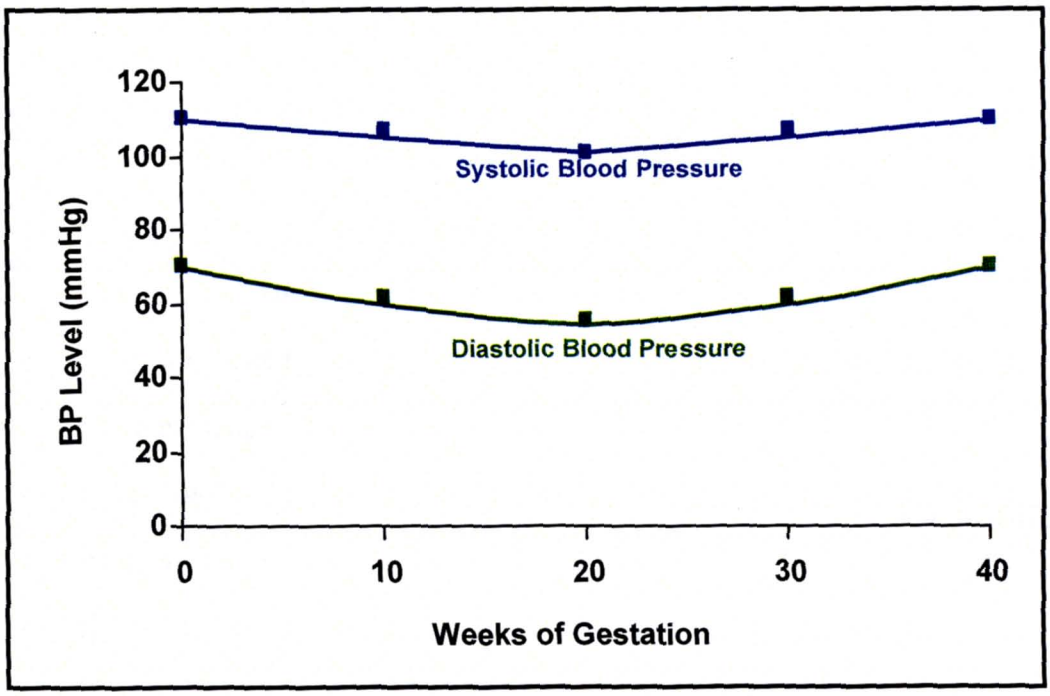

Figure 1.1: Blood pressure changes during normotensive pregnancy

Pregnant women with hypertension, either newly diagnosed or pre-existing, are predisposed to several complications such as:

- abruptio placentae,
- disseminated intravascular coagulation,
- cerebral haemorrhage,
- hepatic failure and
- acute renal failure

(Helewa *et al.,* 1997; National High Blood Pressure Education Program Working Group (NHBPEP), 2000).

Women with pregnancy-induced hypertensive disorders are at a significantly increased risk of having a small for gestational age (SGA) infant or a stillbirth as compared to women with normotensive pregnancies (Allen *et al.,* 2004). Pregnancy-induced hypertensive disorders are classified into four major categories as shown in Table 1.1. Pre-eclampsia is the second most common hypertensive disorder diagnosed amongst pregnant women (23%) (Figure 1.2).

Table 1.1: Classifications of pregnancy-induced hypertensive disorders

Diagnosis	Definition
1. Chronic Hypertension	a) Essential Hypertension: Occurring before pregnancy or within the first 20 weeks of gestation, does not resolve within three months postpartum b) Secondary Hypertension: Secondary to conditions such as renal disease, pheochromocytoma and Cushing syndrome
2. Gestational Hypertension	Hypertension after 20 weeks gestation, without the appearance of proteinuria or any other signs/symptoms of pre-eclampsia, returning to normal within three months postpartum.
3. Pre-eclampsia/Eclampsia	Mild to severe hypertension diagnosed after 20 weeks gestation, returning to normal within three months postpartum, with one or more of the following: 1. Proteinuria ($\geq$ 0.3g/day or a dipstick result $\geq$ 1+), 2. Increase in serum creatinine levels (> 1.2 mg/dL), 3. Platelet count < 100 000 cells/mm^3, 4. Evidence of microangiopathic haemolytic anaemia, 5. Elevated hepatic enzymes, 6. Headaches, 7. Blurred vision or abdominal pain, 8. Intrauterine growth retardation (IUGR) Eclampsia: Defined by seizures/convulsions with no known cause
4. Pre-eclampsia Superimposed on Chronic Hypertension	Pre-eclampsia in women who already have pre-existing chronic hypertension

Modified from National High Blood Pressure Education Program Working Group, 2000

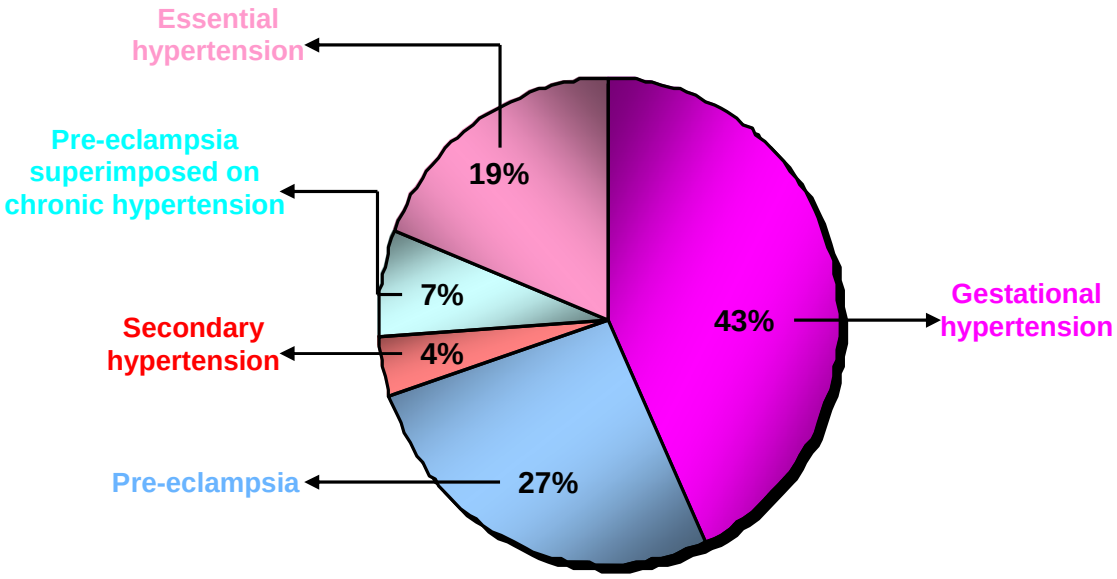

Figure 1.2: Diagnosis of hypertensive disorders (Reif, 2003)

1.2 PRE-ECLAMPSIA

Eclampsia was first recognised almost 2000 years ago as seizures occurring during pregnancy that abated postpartum. In the late 1800's, the presence of proteinuria and increased BP was associated with eclampsia and its occurrence before the onset of eclampsia was noted. This led to the term pre-eclampsia, which meant that even in the absence of seizures, maternal and foetal risk was increased due to the presence of proteinuria and increased BP. For nearly a hundred years, research focused on increased BP despite the knowledge that it did not pose a major risk to both mother and child but rather was a marker of a multisystemic syndrome (Roberts *et al.,* 2003 (b)). In recent years, the understanding on the pathophysiology of pre-eclampsia has increased but its cause remains unknown.

Pre-eclampsia is a pregnancy-specific syndrome that occurs after 20 weeks gestation or earlier in instances of trophoblastic disease such as hydatidiform mole (NHBPEP, 2000), a condition in which genetically abnormal tissue proliferates in the absence of a foetus (DiFederico *et al.,* 1999). It is defined by an increased BP of SBP $\geq$ 140mmHg and DBP $\geq$ 90mmHg and proteinuria of $\geq$ 0.3g per 24-hour urine sample or $\geq$ 1+ on dipstick (NHBPEP, 2000).

Most of the pathophysiological changes occur before clinical symptoms are present and since multiple organs are affected, the clinical presentation can appear in a variety of ways. Therefore, there is no standard test or group of tests that is able to successfully detect pre-eclampsia at an early stage. Laboratory tests are used instead to differentiate between pre-eclampsia and chronic or gestational hypertension and also to ascertain the severity of pre-eclampsia (Peters & Flack, 2004). Efforts to identify an ideal screening or predictive test for pre-eclampsia have been unsuccessful thusfar (NHBPEP, 2000).

The 24-hour urine collection (once the bladder is emptied, all urine that is passed over a 24 hour period is collected) gives the most accurate diagnosis and is therefore recommended to quantitate proteinuria. Dipstick measurement should be used when a rapid determination is required (Sibai, 2001). In the absence of proteinuria, pre-eclampsia is suspected when increased BP is present together with headaches, blurred vision, abdominal pain, thrombocytopenia (low platelet count) and abnormal liver enzymes (NHBPEP, 2000).

Eclampsia occurs when pre-eclampsia progresses to a life-threatening convulsive phase that usually occurs after mid-pregnancy or during delivery. Convulsions occurring during the first 48 hours postpartum have also been observed (Roberts *et al.*, 2003).

1.2.1 Incidence and Risk Factors

The incidence rate of pre-eclampsia in healthy nulliparous women is between 2% - 7% with increased rates in women with twin gestation (14%) and those with previous pre-eclampsia (18%) (Sibai, 2003). Geographical and racial differences in incidence have been reported but this may be attributed to socio-economic status and poor medical care (Sibai, 2001).

Risk factors for pre-eclampsia include:

- Age > 40 years (Sibai, 2003; Peters & Flack, 2004),
- Nulliparity (Sibai, 2003; Peters & Flack, 2004) ,
- Obesity (Sibai, 2003; Peters & Flack, 2004),
- African ethnicity (Sibai, 2003; Peters & Flack, 2004),
- Pre-existing chronic hypertension or renal disease (Sibai, 2003; Peters & Flack, 2004),
- Diabetes mellitus (Sibai, 2003; Peters & Flack, 2004),
- Multi-foetal gestation (Sibai, 2003; Peters & Flack, 2004),
- Family history of pregnancy-induced hypertension (Sibai, 2003; Peters & Flack, 2004),
- Pre-eclampsia in previous pregnancy (Sibai, 2003; Peters & Flack, 2004),
- Thrombophilias (Sibai, 2003; Peters & Flack, 2004),
- Abnormal uterine Doppler studies at 18 and 24 weeks (Sibai, 2003; Peters & Flack, 2004).

Vatten and Skjaerven, (2003) showed that changing partners between the first two births increases the risk of infant mortality, preterm birth and low birth weight for the second birth, which are foetal manifestations associated with pre-eclampsia. This may be due to the long intervals between both births and therefore the increase in maternal age, a risk factor of pre-eclampsia. Poor pregnancy outcomes related to change of partner have focused on the effects attributed to change of paternal antigens or genes. There is also the "selection hypothesis" where women who change partners are a selected group with lifestyle and behavioural characteristics that increase their risk to develop pre-eclampsia.

Trogstad *et al*., (2001) showed a reduced risk of pre-eclampsia in the second pregnancy in women with a change in paternity after controlling the time interval between the two births. However, increased risk of pre-eclampsia was observed in women with no previous pre-eclampsia and long pregnancy intervals. Since increasing maternal age was accounted for, the increased risk may have been due to environmental influences such as infections. A long interval between deliveries increases a mother's risk to acquire infections that may re-activate during pregnancy. Therefore, it was hypothesised that some women may develop pre-eclampsia as a consequence of a coinciding infectious stimulus adding to the inflammatory burden of a normal pregnancy.

1.2.2 Aetiology and Pathophysiology

Pre-eclampsia is a syndrome that affects almost all maternal organ systems. The pathophysiology is characterised by activation of the coagulation system and perturbations in many humoral and autacoid systems related to volume and BP control. The pathological changes are ischemic in nature and affect the placenta, kidney, liver and brain (NHBPEP, 2000).

The placenta is the key component in pre-eclampsia (Figure 1.3) (NHBPEP, 2000). Pre-eclampsia only occurs in the presence of placenta and resolution begins with delivery of the placenta. In cases of extra-uterine pregnancy, persistent pre-eclamptic signs and symptoms are observed following delivery of the foetus without the placenta (Roberts *et al.,* 2003). Pre-eclampsia can occur without uterine distension or a foetus (Roberts *et al.*, 2003).

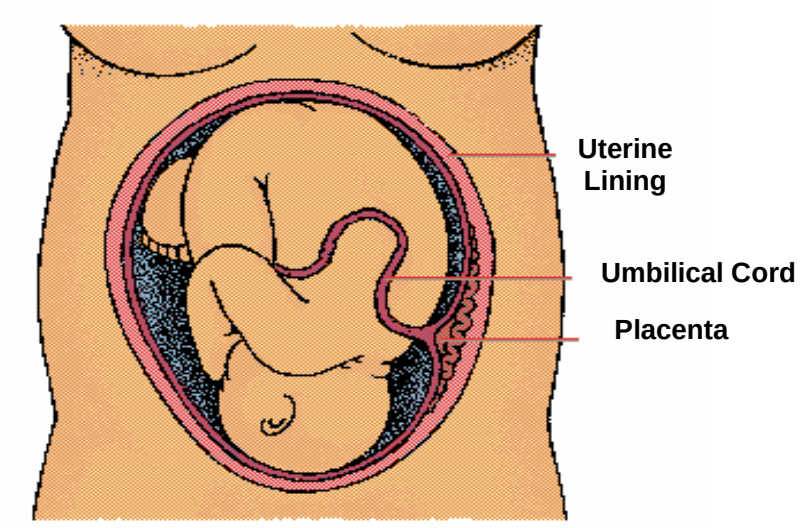

Figure 1.3: **The placenta unites the foetus to the maternal uterus (Modified from Encarta, 2006)**

The pathophysiology of pre-eclampsia can be divided into two stages. The first stage (placental ischemia) is reduced placental perfusion that is secondary to abnormal implantation and development of the placental vasculature (Stage 1). The second stage (maternal syndrome) is the maternal response to the reduced placental perfusion characterised by inflammation and maternal endothelial dysfunction (Stage 2) (Society for Paediatric Pathology, 2003).

Decreased placental perfusion is thought to result in oxidative stress at a placental level. With subsequent release of trophoblastic debris, apoptotic cells and a variety of substances which lead to maternal endothelial dysfunction and a sterile inflammatory response. The link between the two stages, however, is still being investigated since placental factors are not exclusively responsible for the maternal syndrome of pre-eclampsia. Furthermore, it is uncertain as to how immune mechanisms link into this hypothesis. Intrauterine growth restriction and pre-term births are associated with abnormalities in Stage 1, without the occurrence of the maternal syndrome (Society for Paediatric Pathology, 2003).

1.2.2.1 Stage 1: Placental Ischemia

The formation of the placenta is the apposition of foetal and maternal tissues to allow physiological exchange. Foetal and maternal tissues come into intimate contact at several sites such as the syncytiotrophoblast intervillous space, chorion laeve decidua parietalis and the placental bed (Society for Paediatric Pathology, 2003). The resulting area of mixed origin between the placenta and decidua is called the maternofoetal junctional zone (Figure 1.4). The basal plate forms the bottom of the intervillous space and attaches to the placenta after delivery. The placental bed attached to the uterine wall and remains in the uterus after delivery (Kaufman *et al.*, 2003). The decidua and myometrium make up the placental bed, which contains the origins of the spiral arteries (Society for Paediatric Pathology, 2003).

During early human pregnancy, extravillous trophoblast derived initially from the primitive cytotrophoblast shell and the anchoring villi (columns) infiltrate into the decidua and superficial myometrium in the placental bed. These cytotrophoblasts that invade the uterine interstitium comprise the interstitial trophoblast (Society for Paediatric Pathology, 2003).

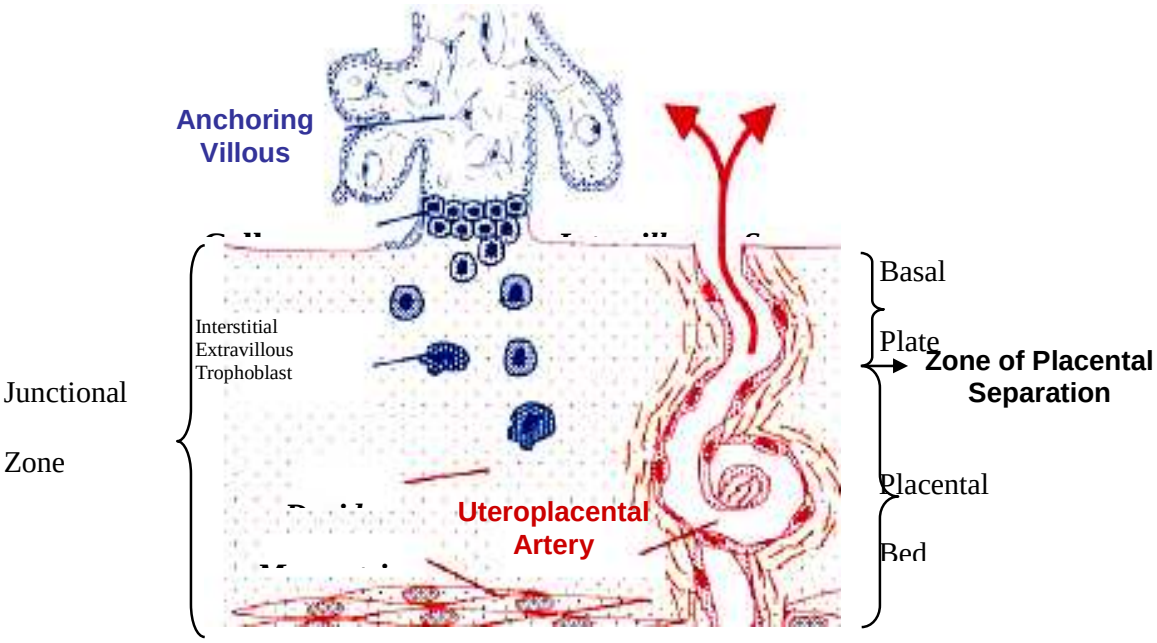

Figure 1.4: Interstitial and endovascular trophoblast invasion in < 6 weeks normal gestation. Blue = foetal tissues, red = maternal tissues (Modified from Society for Paediatric Pathology, 2003)

Cytotrophoblast cells (endovascular cytotrophoblast) invade the uterine spiral arteries (terminal branches of the uterine artery) replacing the endothelial layers of the vessels resulting in the destruction of the medial elastic, muscular and neural tissues. By the end of the second trimester of pregnancy, the uterine spiral arteries are lined entirely by cytotrophoblast and endothelial cells are absent in the endometrial or superficial myometrial regions (Figure 1.5). These changes result in the formation of a low resistance arteriolar system, which increases the blood supply to the foetus (Mushambi *et al.*, 1996; Granger *et al.*, 2001). The remodelling of the uterine spiral arteries is important for normal growth and development of the foetus (Kaufman *et al.*, 2003).

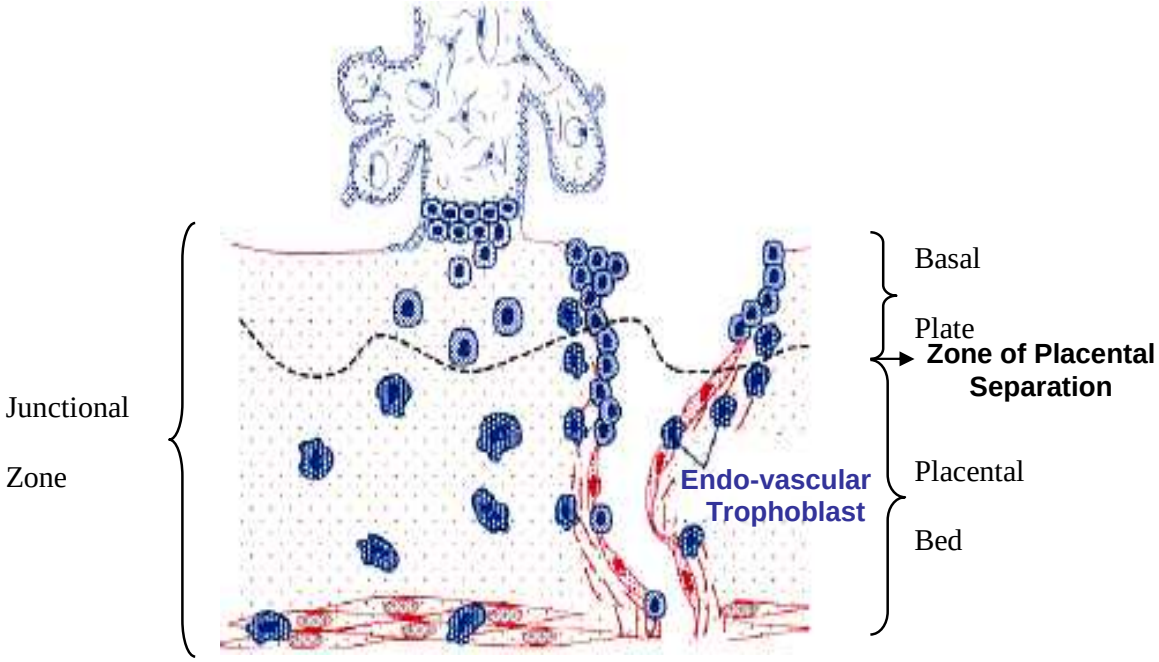

Figure 1.5: Interstitial and endovascular trophoblast invasion in > 20 weeks normal gestation. Blue = foetal tissues, red = maternal tissues (Modified from Society for Paediatric Pathology, 2003)

In pre-eclampsia and IUGR trophoblastic invasion is incomplete (Figure 1.6). Invasion of the uterine spiral arteries is limited to the proximal decidua with 30% - 50% of the spiral arteries of the placental bed escaping endovascular trophoblast remodelling (Mushambi *et al.,* 1996; Granger *et al.,* 2001). The cause of this may be failure of cytotrophoblast cells to express the adhesion molecules necessary for normal remodelling of the maternal spiral arteries (NHBPEP, 2000). These cells enable the trophoblast to adhere to the extracellular matrix, form colonies and target cells in the vessel wall (Kaufman *et al.,* 2003). However, well-known maternal risk factors for pre-eclampsia and IUGR (renal disease, diabetes, obesity and stress) render it unlikely that trophoblastic failure is the sole pathogenic mechanism (Kaufman *et al.,* 2003).

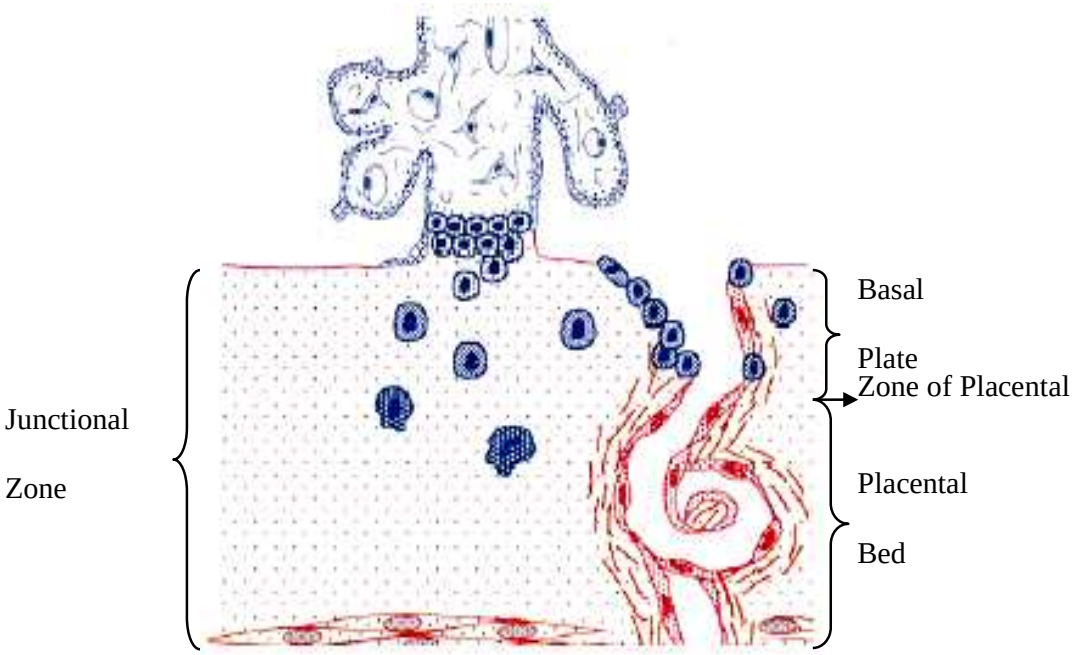

Figure 1.6: Interstitial and endovascular trophoblast invasion in >20 weeks pre-eclampsia/IUGR. Blue = foetal tissues, red = maternal tissues (Modified from Society for Paediatric Pathology, 2003)

Medical conditions associated with microvascular disease, such as hypertension, diabetes and collagen vascular disease increases the risk of pre-eclampsia, further supporting the theory that impaired placental perfusion is an important event leading to pre-eclampsia (Roberts *et al.,* 2003). Women with pre-eclampsia have a distinctive lesion, acute atherosis, found on the spiral arteries, which results in the obstruction of the decidual vessels as well as increased frequency of placental necrosis, both of which can lead to decreased placental perfusion and placental hypoxia (Granger *et al.,* 2001; Peters & Flack, 2003). In addition, placental hypoxia in pre-eclampsia is supported by reports of decreased clearance rates of various radioactive compounds and steroids by the pre-eclamptic placenta (Granger *et al.,* 2001).

1.2.2.2 Stage 2: Endothelial Dysfunction

The vascular endothelium has important functions including maintenance of fluid compartments, prevention of intravascular coagulation, modification of contractile function in smooth muscle wall and maintenance of immune and inflammatory response (Mushambi *et al.,* 1996). It has been suggested that the release of factors from the abnormal placenta, in response to ischemia, results in endothelial dysfunction of the maternal circulation (Figure 1.7) (Granger *et al.,* 2001). Evidence supporting this hypothesis has been demonstrated when sera from women destined to develop or manifesting pre-eclampsia have shown to be cytotoxic to endothelial cells in culture (Laragh & Brenner, 1995).

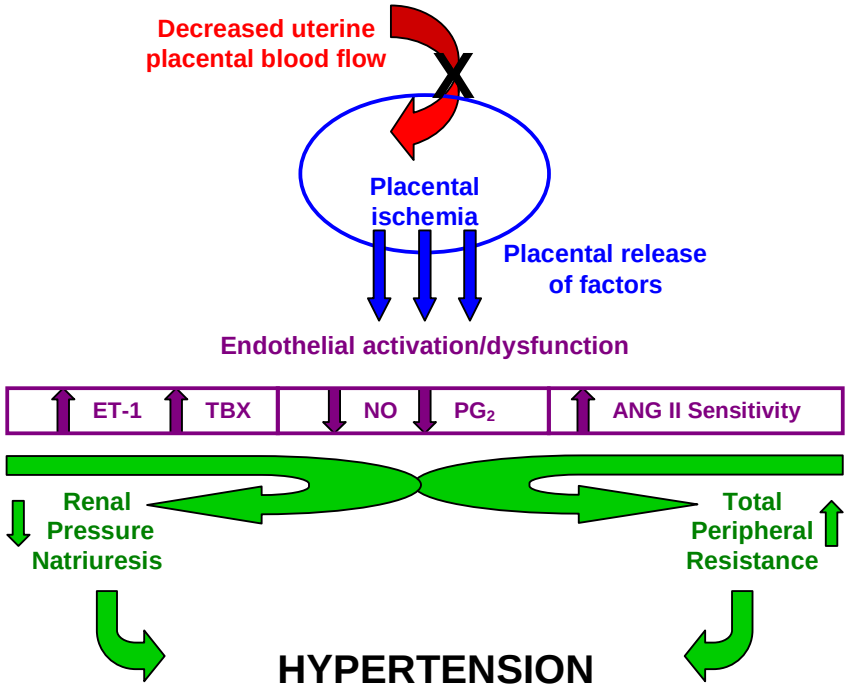

Figure 1.7: Potential mechanisms whereby reduced placental perfusion may lead to hypertension (Modified from Granger *et al.*, 2001)

High levels of circulating substances known to be markers of endothelial damage have been reported in women who develop pre-eclampsia, further supporting the theory that pre-eclampsia is an endothelial cell disorder (Granger *et al.,* 2001). An imbalance of anticoagulation and procoagulation forces are found in pre-eclampsia whilst increases in proteins of the coagulation cascade have been reported in women with pre-eclampsia. Circulating levels of fibronectin and plasma thrombomodulin , an anticoagulation factor, are significantly increased in women with pre-eclampsia as early as 20 weeks and 24 weeks of pregnancy respectively. These levels increase relative to the severity of the disease and therefore can act as biomarkers reflecting the severity of the disorder. Von Willerbrand factor, another coagulation cascade factor, is also elevated in women with pre-eclampsia (Granger *et al., 2001*).

Enhanced platelet activation and increased levels of platelet endothelial cell adhesion molecule–1 (PCAM-1) also occurs in women who develop pre-eclampsia. Other adhesion molecules, vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM-1) and E-selectin, are also significantly elevated in women who develop pre-eclampsia. Plasma levels of ICAM-1 and VCAM-1 have shown to be elevated at 3 to 15 weeks before the onset of clinical manifestations (Granger *et al.,* 2001).

Evidence of endothelial dysfunction as an early event in pre-eclampsia suggests that it is a possible cause and not a result of the pregnancy-specific disorder and many markers may function as predictors of the syndrome in women who develop pre-eclampsia (Granger *et al.,* 2001).

1.2.3 Maternal Manifestations

The maternal risks associated with pre-eclampsia are convulsions, cerebral haemorrhage, abruptio placentae with disseminated intravascular coagulopathy, pulmonary oedema, renal failure, liver haemorrhage and death (Sibai, 1996).

Hypertension is common among women with pre-eclampsia during the second half of pregnancy but vasoconstrictive influences may be present earlier. Numerous surveys suggest that women who eventually develop pre-eclampsia have BP's falling slightly higher in the normal ranges as early as the second trimester (NHBPEP, 2000). Blood pressure in pre-eclampsia is characterised by a reversal of the vasodilatory characteristics of uncomplicated pregnancy which results in a marked increase in peripheral resistance (Peters & Flack, 2004). Normally the vasculature of the normotensive woman manifests a decreased pressor response to several vasoactive peptides and amines, especially to angiotensin II. However, the vessels of women with pre-eclampsia become hyper-responsive to these hormones (NHBPEP, 2000) and the abnormal pressor response to angiotensin II has shown to be increased significantly by 22 weeks gestation in women who later develop pre-eclampsia (Mushambi *et al.*, 1996).

Blood pressure also behaves differently in women with pre-eclampsia, who can have highly labile pressures and could experience a flattening or reversal of normal circadian BP rhythms, with their highest values recorded at night (Peters & Flack, 2004). Blood pressure returns to normal postpartum, often within the first few days or two to four weeks in severe cases. The mechanisms underlying vasoconstriction and altered vascular reactivity in pre-eclampsia remain obscure. Research has focused on the renin-angiotensin

system, catecholamines, prostaglandins and nitric oxide and endothelial cell products and other circulating factors which may influence volume or vascular reactivity and permeability (NHBPEP, 2000).

1.2.4 Foetal Manifestations

The foetal risks associated with pre-eclampsia are severe growth restriction, low birth weight, hypoxemia, acidosis, preterm birth and perinatal death (Sibai, 1996). Preterm birth is associated with increased mortality and morbidity rates and long-range neurological disability. Fifteen percent of all preterm births are a consequence of early deliveries during pre-eclampsia to prevent further deterioration of the mother and foetus (Roberts *et al.*, 2003).

The conditions of foetal growth are controlled mainly by genetics during the first trimester of pregnancy and are affected by the natural environment in the second and third trimesters of pregnancy (Yamada *et al.,* 2004). Impairment of placental perfusion caused by placental disease and vasospasm has been suggested to be one of the major reasons for the high incidence of foetal death, IUGR, SGA infants and perinatal mortality. In addition, the state of the mother (severe pre-eclampsia or the possibility of progression to eclampsia) frequently requires early termination of the pregnancy (Mushambi *et al.*, 1996).

Intrauterine growth restriction is the primary cause of low birth weight and perinatal morbidity. It is associated with an increased risk of cerebral palsy, short stature and sub-normal intellectual and psychological performance during later childhood (Yamada *et al.*, 2004). Odegard *et al*. (2000) showed significant IUGR in severe and early-onset pre-eclampsia and an increased risk in women with recurrent pre-eclampsia of having an SGA infant. Allen *et al.* (2004) also demonstrated an increased risk among women with hypertensive disorders of having an SGA infant with the risk higher in hypertensive women with a twin pregnancy. Hypertensive women were shown to be at a significantly higher risk of stillbirth compared with women having normotensive pregnancies.

Positive correlations between pregnancy oestrogens and foetal size have been reported and decreases in serum oestradiol levels have been found in women with foetal growth restriction. Oestrogens are known to stimulate cell proliferation and placenta growth and therefore supports the link between abnormal placental development and foetal growth (Yamada *et al.*, 2004).

1.2.5 Management

Management of pre-eclampsia requires early diagnosis and close medical supervision with delivery being the best cure. Management is based on the initial evaluation of maternal and foetal well-being, the severity of the disease and the length of gestation (Sibai, 1996).

Women with mild pre-eclampsia require close observation in the hospital. Outpatient management should be considered if compliance is expected to be good, hypertension is mild and the foetus is normal. The management should include close monitoring of the mother's BP, weight, proteinuria and platelet count. Bed rest is recommended with the proposed results being reduction of oedema, improved foetal growth, prevention of progression to severe pre-eclampsia and improved outcomes of pregnancy (Sibai, 1996).

In women with severe pre-eclampsia, hospitalisation and delivery is strongly recommended when there is evidence of impending eclampsia, multi-organ dysfunction, and foetal distress or when severe pre-eclampsia develops after three weeks. During hospitalisation, anti-hypertensive drug therapy is administered if required with the aim being to keep systolic BP between 140 – 155 mmHg and diastolic BP between 90 – 105 mmHg (Sibai, 2003). Short-acting antihypertensive agents, hydralazine, labetol and nifedipine are commonly used to control very high BP in women with severe hypertension during pregnancy (Magee *et al*., 2003).

1.2.6 Pre-eclampsia in South Africa

Pre-eclampsia tends to be more severe and life-threatening in developing countries since little or no antenatal care is available to low-to-middle income people. Pre-eclampsia therefore remains undiagnosed until the condition has reached an advanced or dangerous stage. In addition, these women are more likely to have more underlying health and social problems which increases their risk of developing pre-eclampsia. These problems include chronic hypertension, malnutrition, anaemia, chronic malaria and a tendency to have many babies, starting at a young age (Medical Research Council, 2002).

Hypertensive disorders are the most common direct cause of maternal deaths in South Africa (SA). The recent Saving Mothers report (2002 – 2004) indicated that hypertensive disorders contributed 19.1% (n=628) of all deaths and pre-eclampsia/eclampsia syndrome accounted for 518 (82.5%) of deaths in SA (Table 1.2). However, the magnitude of the problem resulting from hypertensive disorders of pregnancy might be under reported due to the poor reporting in some provinces (Saving Mothers, 2006).

Table 1.2: Primary obstetric causes of death associated with hypertensive disorders in pregnancy

Types of Disorders	N	%
Eclampsia	347	55.3%
Proteinuric Hypertension	171	27.2%
Chronic Hypertension	37	9.2%
Haemolysis, Elevated Liver Enzymes, Low Platelets (HELLP syndrome)	70	11.1%
Rupture of Liver	3	0.5%

Adapted from Saving Mothers, 2006

1.3 HUMAN IMMUNODEFICIENCY VIRUS

Human Immunodeficiency Virus belongs to a group of viruses called retroviruses (Figure 1.8). Two distinct viruses, HIV types 1 and 2 (HIV-1, HIV-2), cause AIDS. Human Immunodeficiency Virus - 1 is responsible for the great majority of infections globally, with HIV-2 being very rare outside West Africa (Grant & De Cock, 2001).

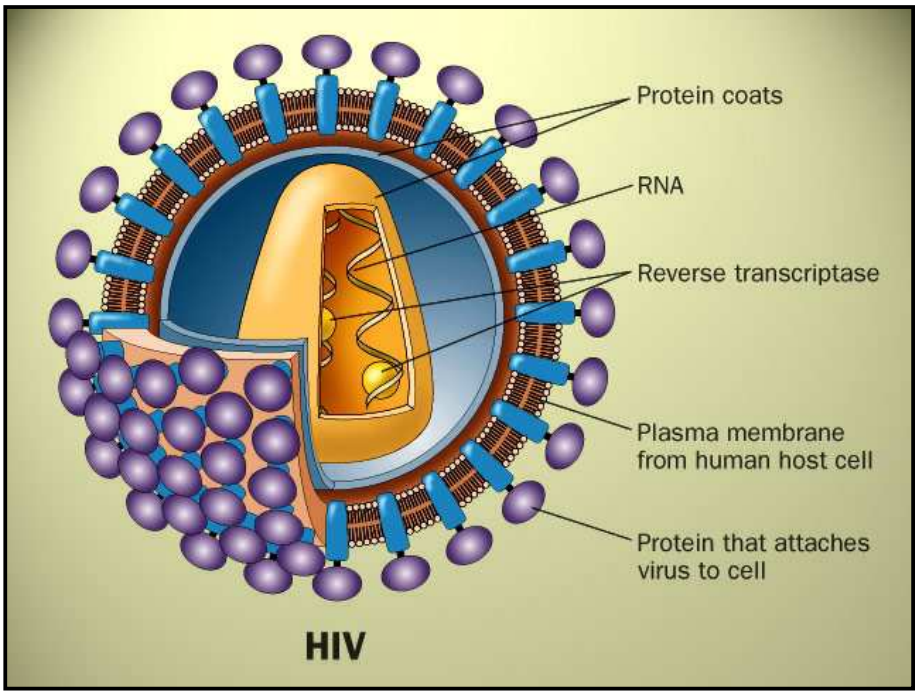

Figure 1.8: Anatomy of Human Immunodeficiency Virus (Chemistry Pictures, 2006)

Human Immunodeficiency Virus is present in the blood, semen and vaginal fluids of infected people. Although sophisticated laboratory techniques are able to isolate the virus from other body fluids of infected people (such as saliva or tears), the level of the virus in these fluids is too low to be infectious. HIV is transmitted:

- through unprotected sexual intercourse; mainly heterosexual in Sub-Saharan Africa,
- through blood to blood contact: by sharing injecting equipment among drug users, by blood transfusion from infected people and needle-stick injuries amongst health workers
- vertical transmission: from HIV positive women to her baby during the course of pregnancy, birth and breastfeeding (AIDSmap Treatment and Care, 2005).

The chances of a woman being infected by one act of sexual intercourse with an HIV positive man are about 1 in 100. The chances of a man being infected by one act of sexual intercourse with an HIV positive woman are about 1 in 1000 but this probability may increase considerably if one of them has a sexually transmitted disease (STD). The STDs act as co-factors in HIV transmission by increasing susceptibility to HIV (Singhal & Rogers, 2003).

Human Immunodeficiency Virus is a lentivirus. In common with all retroviruses, lentiviruses have three major genetic loci (*gag*, *pol*, *env*) that encode the core proteins, the reverse transcriptase and integrase and the envelope proteins respectively. The lentiviruses are distinguished from other retroviruses by several characteristics:

- They possess 6 unique accessory genes that encode non-structural proteins;
- The viral attachment protein (the surface envelope protein, SU) binds to the clusters of differentiation$_4$ (CD_4) molecule that is found on the CD_4 subset of T lymphocytes and on monocytoid cells and this determines their cellular host range;
- They are capable of replicating in non-dividing cells as well as dividing cells;
- They cause lifelong infections that are associated with a number of chronic diseases including AIDS but they do not encode oncogenes;
- They are strictly exogenous viruses and host genomes do not include copies of their sequences (Nathanson, 2002).

1.3.1 History

The transmission of an animal virus to a human host (zoonotic transmission) enables the rapid spread of a virus beyond the geographic range of its original animal host. It is believed that the most serious viral epidemic resulting from zoonotic transmission is caused by HIV. Exactly when and how the Simian Immunodeficiency Virus (SIV) was transmitted from a non-human primate to humans and began to diversify, resulting in the emergence of HIV's is still under investigation (Hillis, 2000).

The HIV-1 virus was first identified in 1983 at the National Cancer Institute in Bethesda, Maryland by Dr Robert Gallo and other medical scientists. At about the same time, Dr Luc Montagnier of the Pasteur Institute in Paris isolated HIV-2 from AIDS patients (Singhal & Rogers, 2003).

Scientists have tried to trace the origins of the epidemic. A dozen AIDS cases were retrospectively identified in 1978 – 1979. Scattered cases of HIV infection or AIDS were identified in the United States and Haiti from 1972 – 1976. The earliest known case of HIV-1 has been identified in one of 1213 stored blood samples that were gathered in 1959 in Africa. The individual, designated "L70", was an adult male living in Kinshasa, Democratic Republic of Congo. Using sophisticated mathematical models and computer tools and knowing that L70's infection probably had ancestors in the B, D and F subtypes of HIV-1, biologists estimate that the first HIV case occurred in Africa in the 1930's (Singhal & Rogers, 2003).

When AIDS first emerged as a clinical problem in the 1980's, many patients died early from acute illnesses such as pneumonia and tuberculosis. Cumulative experience and increased awareness have led to the use of prophylaxis, early diagnosis and more effective treatment and management of HIV infection and AIDS (Wood *et al.*, 1997).

1.3.2 Virus-Cell Interactions

The entry of HIV into permissive host cells is a multi-step process that involves a primary receptor and a co-receptor (Figure 1.9). The primary receptor is CD_4, an immunoglobulin superfamily molecule, expressed on two major cell types, the CD_4 subset of T lymphocytes and the cells of the monocyte lineage. The co-receptor is one of several members of the chemokine family of molecules, particularly CCR5 expressed on T lymphocyte and T-cell lines (TCL). Initially, the viral attachment protein (SU protein) binds to CD_4, which triggers a conformational change in the SU protein that leads to binding to the co-receptor. Binding to the co-receptor triggers a second change in the transmembrane (TM) envelope proteins resulting in the approximation of the N-terminal domain of the TM protein to the plasma membrane of the cell. A hydrophobic fusion peptide at the N-terminal domain of the TM protein inserts into the plasma membrane, leading to fusion between the viral envelope and cellular membrane (Nathanson *et al.*, 2002).

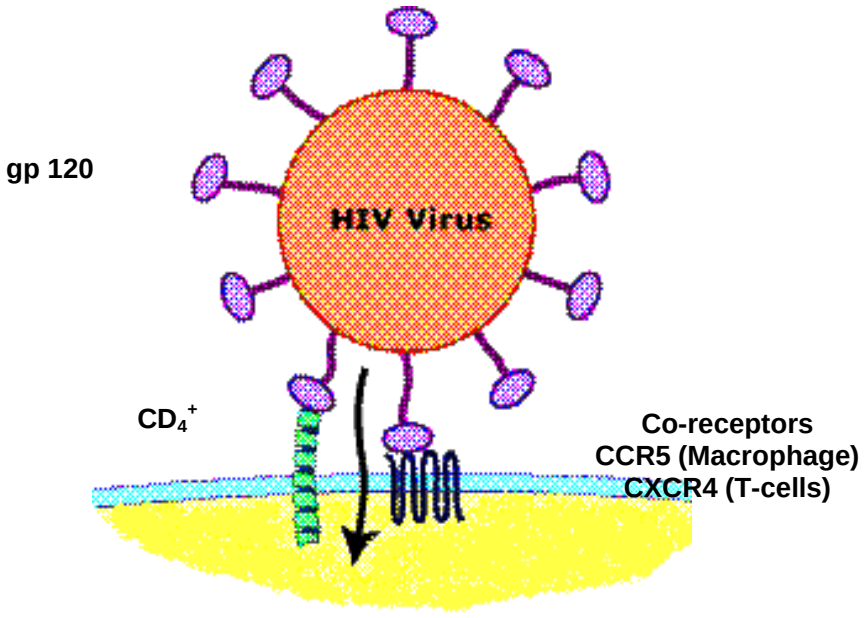

Figure 1.9: Entry of HIV into Host Cells (The Body, 2003)

Human Immunodeficiency Virus commandeers host cells, turning them into factories churning out virus particles. When HIV infects a cell, reverse transcriptase copies the ribonucleic acid (RNA) into double-stranded deoxyribonucleic acid (DNA), which is then spliced into the host DNA. This new integrated DNA (the HIV provirus) manufactures viral proteins and RNA copies of the virus genome. Proteins and RNA are brought together to form new HIV particles, which bud from the cell and go onto infect other host cells (Figure 1.10) (Welcome Trust, 2000).

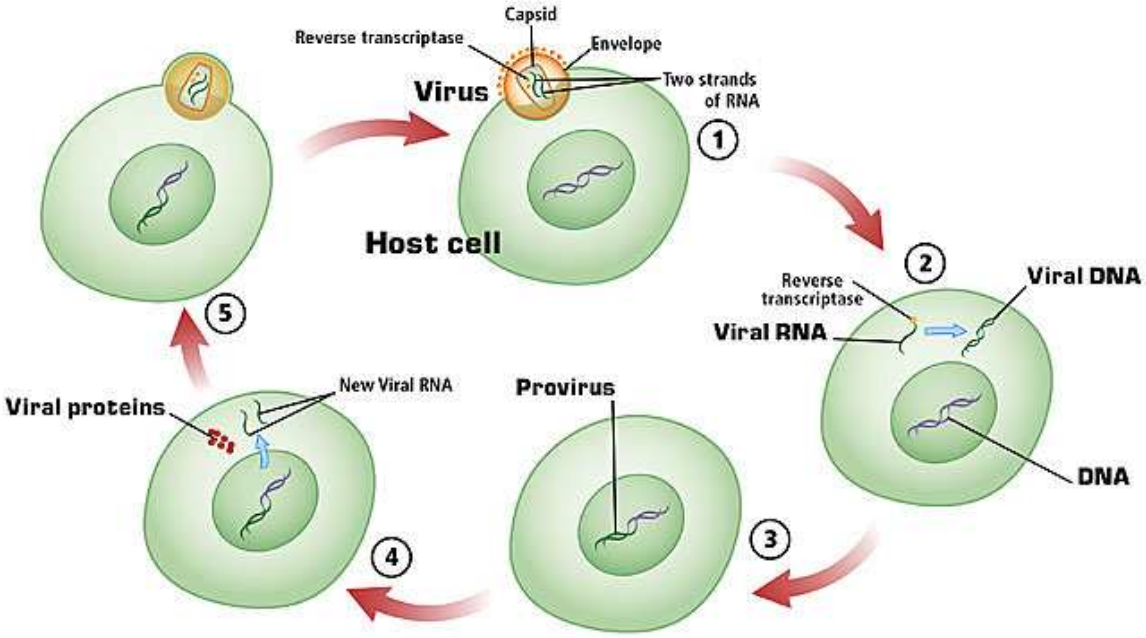

Figure 1.10: The replication cycle of HIV (Rediscovering Biology, 2006)

Human Immunodeficiency Virus replicates slowly in permissive cells. If the infected lymphocyte is actively dividing, then viral replication proceeds at a maximal rate. If the infected T cell is resting, the provirus can enter the nucleus and integrate but remains latent until the T cell begins to divide, when HIV replication proceeds (Nathanson *et al.,* 2002).

The hallmark of HIV infection is a progressive decline in the number of CD_4 T lymphocytes over many years. CD_4 T lymphocytes act as "helper cells" for the induction of both cellular (CD_8 cytolytic T cells) and humoral (antibody) immune response. Therefore HIV infected patients are impaired in their ability to develop immune responses and are particularly susceptible to new infections against which they have no immunity (Nathanson *et al.,* 2002).

1.3.3 Clinical Features

- **Group I: Primary HIV Infection**

Primary HIV infection is also called the seroconversion illness or acute HIV infection. It represents the stage of infection after the acquisition of the virus when antibodies are developing. Some people have been found to present with symptoms at the time of seroconversion which includes fever, sore throat, night sweats, malaise, lymphadenopathy (abnormal enlargement of the lymph nodes), diarrhoea and relative and absolute lymphocytosis in the peripherial blood. These severe illnesses are rare (Timbury, 1994; Mindel & Tenant-Flowers, 2001). Primary HIV infection is characterised by rapid viral replication and a dramatic drop in the T helper cell count (Welcome Trust, 2000).

- **Group II: Asymptomatic Infection**

After Primary HIV Infection, HIV antibodies continue to be detectable in the blood. The amount of virus in the blood and lymphoid tissues falls to very low levels and the rate of HIV replication is slow but does not cease. CD_4 lymphocyte counts are within normal limits or generally above 350 x 10^6 cells/l. This phase may persist for 10 years or more (Mindel & Tenant-Flowers, 2001).

- **Group III: Persistent Generalised Lymphadenopathy**

Persistent generalised lymphadenopathy may be a presenting feature of HIV infection in a person who is otherwise well. HIV related lymphadenopathy persists for at least 3 months (Mindel & Tenant-Flowers, 2001).

- **Group IV: Symptomatic HIV Infection**

The progression of HIV infection is a result of a decline in immune competence that occurs due to increased replication of HIV from sites where it has been latent. As the disease progresses, infected persons suffer from constitutional symptoms, skin and mouth problems and haematological disorders (Mindel & Tenant-Flowers, 2001). Progression to AIDS correlates with a severe decrease in the CD_4 count (Timbury, 1994).

1.3.4 Treatment

The course of HIV infection is variable and unpredictable, with a wide range of potential complications, rates of progression and survival. Some patients remain free of serious symptoms and complications until they have reached an advanced stage of immunosuppresion, while others suffer debilitating malaise and fatigue or frequent non-life threatening complications throughout their infection. Medical management of people with HIV is a balance between acute treatment and attempting to control chronic symptoms and conditions (Wood *et al.,* 1997).

Antiretroviral (ARV) therapy has shifted the model of care delivery from one of terminal care to one of chronic disease management (Levi & Kates, 2000). Antiretroviral drugs inhibit the replication of HIV. When antiretroviral drugs are given in combination, HIV replication and immune deterioration can be delayed and survival and quality of life improved (World Health Organisation, 2006).

The common classes of drugs currently available are the nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT), lamivudine (3TC), abacavir, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine and efavirenz2 and the protease inhibitors such as indinavir, ritonavir and lopinavir (Family Health International, 2006). Selection of ARV treatment regimens for programmes and individual patients should consist of frequency of dosage, side effects, maintenance of future treatment options, need for storage, concurrent conditions, viral strains and cost and access (World Health Organisation, 2006).

Several guidelines for ARV therapy concur that treatment is indicated for patients who are symptomatic (e.g., have thrush, fevers, thrombocytopenia or wasting), have AIDS (CD_4 cell counts of less than 200 cells/mm^3 or AIDS defining conditions), who are pregnant (to prevent perinatal transmission). The controversy among the guidelines revolves around the appropriate CD_4 cell count or HIV RNA levels at which to initiate therapy in asymptomatic pre-AIDS patients (Khalsa, 2006). The WHO recommends that in ARV therapy programmes in resource-limited settings, HIV infected adolescents and adults should start ARV therapy when they have clinical AIDS, regardless of the CD_4 count. When total lymphocyte count can be assessed, in addition, people with WHO stage II or III HIV disease should be offered treatment. When CD_4 counts are available, all HIV infected people with less than 200 CD_4 cells/mm^3 should be offered treatment (WHO, 2006).

1.3.5 HIV/AIDS in South Africa

South Africa has one of the fastest growing HIV epidemics in the world. In SA, the epidemic is mainly concentrated among the African population, which makes up to 90% of the total population of 43 million (Singhal & Rogers, 2003).

The human impact of the HIV epidemic in South Africa has been devastating and the need to tackle this pandemic on all fronts with new vaccines, medical interventions and campaigns to reduce the rate of transmission grows more urgent (Welcome Trust, 2000). Contributing factors to these epidemiological trends may include barriers faced by women in accessing care as well as financial constraints resulting in women depending on the public sector for the financing and delivery of the care (Levi & Kates, 2000). Refusal of condom use by male partners is compounded by women playing the socially submissive role in most relationships also contributes daily to this pandemic (Pettifor *et al.*, 2004)

Surveys amongst women attending antenatal clinics in the public sector have been undertaken by the South African Department of Health annually. These surveys provide the best available estimates of HIV infection among the South African population. These show that SA has experienced a very rapid spread of HIV during the last decade. In 1990, the first year of the survey, the prevalence was less than one percent (Dorrington *et al*., 2001).

The findings of the 2005 survey indicate that HIV prevalence in SA among pregnant women is 30.2%, compared to 29.5% observed in 2004. HIV prevalence continues to vary by province as shown in Table 1.3. KwaZulu-Natal has the highest prevalence in South Africa of 39.1% in 2005. Further to this, the Department of Health estimates that over 90, 000 babies became infected with HIV through the mother-to-child transmission route (Department of Health, South Africa, 2005).

Table 1.3: HIV prevalence by province among antenatal clinic attendees, South Africa: 2003 – 2005

Province	HIV Prevalence 2003 (CI 95%)	HIV Prevalence 2004 (CI 95%)	HIV Prevalence 2005 (CI 95%)
KwaZulu-Natal	37.5 (35.2 – 39.8)	40.7 (38.8 – 42.7)	39.1 (36.8 – 41.4)
Mpumalanga	32.6 (28.5 – 36.6)	30.8 (27.4 – 34.2)	34.8 (31.0 – 38.5)
Gauteng	29.6 (27.8 – 31.5)	33.1 (31.0 – 35.3)	32.4 (30.6 – 34.3)
North West	29.9 (26.8 – 33.1)	26.7 (23.9 – 29.6)	31.8 (28.4 – 35.2)
Free State	30.1 (26.9 – 33.3)	29.5 (26.1 – 32.9)	30.3 (26.9 – 33.6)
Eastern Cape	27.1 (24.6 – 29.7)	28.0 (25.0 – 31.0)	29.5 (26.4 – 32.5)
Limpopo	17.5 (14.9 – 20.0)	19.3 (16.8 – 21.9)	21.5 (18.5 – 24.6)
Northern Cape	16.7 (11.9 – 21.5)	17.6 (13.0 – 22.2)	18.5 (14.6 – 22.4)
Western Cape	13.1 (8.5 – 17.7)	15.4 (12.5 – 18.2)	15.7 (11.3 – 20.1)
South Africa	27.9 (26.8 – 28.9)	29.5 (28.5 – 30.5)	30.2 (29.1 – 31.2)

(Adapted from the Department of Health, 2005)

N.B. The true value is estimated to fall within the two confidence limits, thus the Confidence Interval (CI) is important to refer to when interpreting data

Human Immunodeficiency Virus prevalence is different among the different age groups suggesting different patterns of risk (Table 1.4). Nearly 40% of women attending antenatal clinics aged between 25-29 years are HIV positive. Women in the early twenties and late thirties show lower rates at about 30% prevalence. Older women and teenagers have prevalence rates below 20% (Department of Health, South Africa, 2005).

Table 1.4: HIV prevalence by age group among antenatal clinic attendees, South Africa: 2003 – 2005

Age Group (Years)	HIV Prevalence 2003 (CI 95%)	HIV Prevalence 2004 (CI 95%)	HIV Prevalence 2005 (CI 95%)
<20	15.8 (14.3 – 17.2)	16.1 (14.7 – 17.5)	15.9 (14.6 – 17.2)
20 – 24	30.3 (28.8 – 31.8)	30.8 (29.3 – 32.3)	30.6 (29.0 – 32.2)
25 – 29	35.4 (33.6 – 37.2)	38.5 (36.8 – 40.3)	39.5 (37.7 – 41.3)
30 – 34	30.9 (28.9 – 32.9)	34.4 (32.2 – 36.6)	36.4 (34.3 – 38.5)
35 – 39	23.4 (20.9 – 25.9)	24.5 (21.9 – 27.2)	28.0 (25.2 – 30.8)
40+	15.8 (12.3 – 19.3)	17.5 (14.0 – 21.0)	19.8 (16.1 – 23.6)

(Adapted from the Department of Health, 2005)

N.B. The true value is estimated to fall within the two confidence limits, thus the Confidence Interval (CI) is important to refer to when interpreting data

Gender power inequities, economic vulnerabilities and inconsistent condom use play a key role in the HIV epidemic. In SA, multiple partnerships are condoned and even encouraged for men, while women are expected to be monogamous and unquestioning of their partner's behaviour. Sexual refusal or negotiation by women may result in suspicions of infidelity and carry the risk of violent outcomes. In the context of poverty, partnerships with men who can provide financially are essential, transactional relationships in which sex is exchanged for material goods or money (Pettifor *et al.*, 2004).

1.4 MANIFESTATIONS OF PRE-ECLAMPSIA AND HUMAN IMMUNODEFICIENCY VIRUS

1.4.1 Haematological

Thrombocytopenia is the most common haematological abnormality in pre-eclampsia occurring in one-third of all patients. Platelet counts < 100 000 cells/mm^3 indicate severe disease, which occurs in only 15% of women (Mushambi *et al.,* 1996). Levels may continue to fall if delivery is delayed and therefore increases the risk of bleeding. The cause of thrombocytopenia is unclear. Enhanced platelet aggregation and destruction may be due to endothelial damage and increments in circulating fibronectin, laminin and preprocollagen type II (markers of endothelial cell injury) have been described which may correlate with increases in products of platelet granules, the increments consistent with activation (Laragh & Brenner, 1995).

Thrombocytopenia is linked to DIC which is characterised by the widespread activation of coagulation, which results in the intravascular formation of fibrin and ultimately thrombotic obstruction of small and midsize vessels (NHBPEP, 2000). There are many reports documenting increased consumption of various clotting components as well as decreases in inhibitors of coagulation.

Thrombocytopenia is a common finding in HIV infection and increases as the disease progresses. It is an immune mediated destruction resulting in a severe decrease in platelets and abundance of megakaryocytes in the bone marrow. Suggested mechanisms include circulating platelets being damaged IgG, immune complexes or antibodies to specific platelet surface antigens (McPhedran, 1999).

During pregnancy, the foetal demand for iron increases maternal daily iron requirements from =/- 1 to 2.5 mg/day in early pregnancy and 6.5 mg/day in the third trimester. This demand by the developing foetus may result in the mother to develop iron deficiency anaemia (Steer, 2000). Iron deficiency is considered the most important cause of low haemoglobin concentration and may have independent effects on other maternal, pregnancy and infant outcomes such as immunity, pre-eclampsia, maternal mortality, malformation, foetal loss, prematurity and IUGR (Friis *et al.,* 2001).

Anaemia is the most common finding in HIV infection, particularly in people with advanced stages of HIV and can be defined as a reduction below normal of the haemoglobin concentration and red blood cell mass (Kline, 2005). Usually normocytic normochromic anaemia manifests. A persistently low haemoglobin concentration is predictive of a poor outcome. The possible causes of anaemia include:

- Drug-induced anaemia
- Opportunistic infections such as tuberculosis
- Anaemia caused by bone marrow infections
- Decreases in vitamin B12, folate and iron (Northfelt, 1998).

During normal pregnancy upregulation of the inflammatory cascade and increase in white cell count occurs (Sattar & Greer, 2002). Leukopenia (decrease in white blood cells) is frequently associated with HIV infection which results from the parallel decline in the three most numerous kinds of leukocytes: neutrophils, lymphocytes and monocytes. The main causes of leukopenia are not known, although HIV invasion of myeloid precursors or of cells producing granulocyte-stimulating cytokines have been suggested. Leukopenia tends to worsen gradually with progression of HIV infection with sudden plunges in the white cell count if a drug suppresses the marrow (McPhedran, 1999).

1.4.2 Immunological

The vascular endothelium plays a role in the maintenance of immune response therefore endothelial dysfunction during pre-eclampsia results in immunological alterations. Further to this, various studies suggests that the immunological mechanism plays some role in the aetiology of pre-eclampsia. Studies have found a decrease in the functional activities of the natural killer (NK) cells and an increase in the serum levels of immunoglobulin (Ig)E, antinuclear antibodies (ANA) and antimitochondrial antibodies (AMA) (Cetiner *et al*., 1998).

Cetiner *et al*., (1998) also reported conflicting results from other studies with regards to CD_4/CD_8. Studies have demonstrated that the rates of CD_4 in pre-eclamptic women begin to decrease in the early stages of pregnancy before the occurrence of pre-eclampsia and normal levels are only reached several weeks after delivery. In another study, it was found that the rates of CD_4/CD_8 in pre-eclamptic women were lower when compared to normal pregnant women.

There are also immune hypotheses to explain abnormal placentation in pre-eclampsia. It is suggested that a breakdown of the usual adaptations in the immune system required to sustain a normal gestation, the placenta and the uteroplacental circulation. This is supported by observations that pre-eclampsia occurs primarily in nulliparous women, whose subsequent gestations tend to be uncomplicated thereafter, unless there is a change in paternity; some evidence that the foetus of pre-eclamptic mothers may be more genetically compatible with their mothers than foetuses in normal pregnancies and that certain pathologic changes in the placental vasculature resemble those of allograft rejection (Laragh & Brenner, 1995).

Further evidence of immunological phenomena has been reported. Beginning early in the second trimester, women in whom pre-eclampsia develops later have a significantly lower proportion of T-helper cells , antibodies against endothelial cells have been found in 50% of women with pre-eclampsia versus 15% of control women, deposition of IgM, complement (C3) and fibrin have been noted in the walls of pre-eclamptic spiral arteries (Dekker & Sibai, 1998).

In the early stages of HIV infection, where few symptoms are seen, there may be a slight depression in CD_4 T cells which is temporary at this stage. Immune abnormalities appear during the second stage, when the virus begins to replicate at a high level which results in a decrease in the percentage and total numbers of CD_4 T cells. The decrease in the CD_4 T cells remain the most widely used parameter to assess disease progression. This decrease may be due to several factors, including fusion of uninfected T cells with virally infected cells, the development of pores in the cell membrane as HIV buds from the cell surface and the killing of cells expressing gp120 by cytotoxic lymphocytes programmed to recognise this vial protein (Stevens, 1996).

Another immune phenomenon is an upset in the balance of cytokines. In response to the presence of HIV, macrophages produce tumour necrosis factor – α (TNF-α) and interleukin-1 (IL-1), thereby activating CD_4 helper T cells. Activated helper T cells, in turn, produce an array of lymphokines (plays a role cell mediated immunity) which can induce viral replication in both infected T cells and macrophages. As HIV infection progresses, the CD_4 T-cell population continues to drop, resulting in immunosuppression. This results in decreasing signals to multiple limbs of the immune response, so that the AIDS patient becomes susceptible to a host of opportunistic infections (Stevens, 1996).

Further, Wimalasundera *et al.,* (2002) proposed that effective ARV treatment may restore a HIV infected pregnant woman's immune response to foetal antigens, and therefore could reinstate the pathological processes that result in pre-eclampsia. Pre-eclampsia may therefore be another manifestation on immune restoration, similar to the exacerbation of other immune-mediated diseases that follows effective ARV. The clinical presentation of pre-eclampsia and effects of ARV could overlap and complicate diagnosis and management in these women. Conflicting results by Matter *et al.*, (2004), demonstrate that pre-eclampsia development is inhibited in HIV seropositive women, and there is a possibility that HIV infection inhibits or blocks other factors that may play a role in the development of pre-eclampsia.

1.5 OBJECTIVE

Current literature suggests that there exists an overlap of haematological abnormalities in HIV infected individuals and pre-eclamptic women. Further to this, it is known that an individual's immune status is debilitated during HIV infection. The immune system is also altered during pregnancy and exacerbated in pre-eclampsia.

The focus of this research endeavour is to compare, contrast and evaluate to help better understand the haematological and immune manifestations in women with both pre-eclampsia and HIV. This study endeavours to assist in creation of a more comprehensive tool to evaluate, diagnose and manage women affected with both these diseases and improve prognosis and care.

Therefore the objectives are:

1) Evaluation of haematological parameters in HIV negative pre-eclamptic women.

2) Evaluation of immune markers in HIV negative pre-eclamptic women.

3) Evaluation of haematological parameters in HIV positive pre-eclamptic women.

4) Evaluation of immune markers in HIV positive pre-eclamptic women.

CHAPTER 2

METHODOLOGY

2.1 STUDY DESIGN

2.2.1 Study Population

Ethical approval for this research study was granted by the University of KwaZulu-Natal Ethics Committee on 09 November 2004 (Reference Number H136/04). Women were recruited for this cross-sectional study from King Edward VIII Hospital (KEH) in Kwa-Zulu Natal, Durban. The study population comprised of two groups i.e., pre-eclamptic HIV-positive African women and pre-eclamptic HIV-negative African women as the control group. HIV-negative and HIV-positive pre-eclamptic women diagnosed with a BP $\geq$ 140/90 mmHg (BP measured with Welsh and Allyn Vital Monitors) and proteinuria $\geq$ 1+ at > 24 weeks gestation were screened pre-delivery. Those that were interested in participating voluntarily signed the informed consent which included permission to access HIV results. Women underwent routine HIV screening (Abbott Determine® HIV rapid test, USA) at KEH including pre- and post-test counseling. The HIV status of each woman was accessed from the participant's hospital records.

Pre-eclampsia is a pregnancy-specific syndrome that occurs after 20 weeks gestation. Pre-eclampsia is a clinical diagnosis: only women with a BP of $\geq$ 140/90 mmHg and proteinuria $\geq$ 1+ were included. A full medical history was taken into account to record any additional medical conditions which may affect the parameters being tested. This did not affect recruitment.

2.2.2 Statistical Analysis

Sample size was determined as 18 per group. Group sample sizes of 18 and 18 achieve 80% power to detect a difference of 440.0 between the null hypothesis that both group means are 820.0 and the alternative hypothesis that the mean of group 2 is 380.0 with estimated group standard deviations of 600.0 and 200.0, with a significance level (alpha) of 0.05000.

SPSS version 13 (SPSS Inc., Chicago, Illinois) was used to analyse the data. Bivariate analysis was undertaken to assess associations between HIV status and various outcomes. Categorical outcomes were assessed using Pearson's Chi Square tests or Fisher's Exact tests where appropriate. Quantitative outcomes were tested for normality and found to be significantly skewed. Thus non-parametric Mann - Whitney tests were used to compare median values between HIV negative and positive groups. Distribution of the data in each group was graphically presented with box and whisker plots and tabulated showing medians, interquartile ranges and percentiles. A p value of < 0.05 was considered statistically significant.

2.2.3 Collection of Blood Samples

Pre-eclampsia occurs after 20 weeks gestation, returning to normal within three months postpartum. Any dilution effects from intravenous fluids or blood loss may have affected post delivery samples. Therefore, venous blood samples were collected pre-delivery by trained clinical staff into two tubes containing ethylenediaminetetraacetic acid (EDTA), which acts as chelating agent and anticoagulant. Both samples were labelled accordingly and transported within an hour of collection to the respective laboratories – Department of Haematology, KEH (routine testing) and Department of Optics and Imaging/Department of Physiology, University of Kwa-Zulu Natal where samples were processed by trained laboratory staff.

2.2 EVALUATION OF THE HAEMATOLOGICAL PARAMETERS IN HIV-INFECTED AND NON-INFECTED PRE-ECLAMPTIC BLACK WOMEN - FULL BLOOD COUNT

Haematology is the study of blood cells and the factors that affect their functioning. The cells that circulate in the blood stream are divided into three types:

- white blood cells (WBC's) (leukocytes),
- red blood cells (RBC's) (erythrocytes) and
- platelets (thrombocytes).

The full blood count (FBC) is an automated count of these cells and provides information on the number, type, size, shape and some of the physical characteristics of the cell.

The FBC was carried out on the Beckman Coulter^{R} HmX Haematology Analyser (Beckman Coulter, Florida, USA) (Figure 2.1). All reagents were purchased from Beckman Coulter (Florida, USA).

Calibration procedure was performed using S-CAL calibrator. Coulter Latron^{TM} primer and control was analysed once at the beginning of each day. The Cycling Coulter 5C-cell control was used to monitor the performance of instruments.

Figure 2.1: Beckman Coulter HmX Haematology Analyser (Beckman Coulter, 2007)

Table 2.1: Haematological parameters measured

Parameter	Normal Reference Range
Red Blood Cells	3.5 - 5.0 x10^12/l
Haemoglobin (Hb)	11.5 - 13.5g/dL
Haematocrit	37.0 - 52.0%
Mean Cell Volume (MCV)	78.0 - 99.0 fl
Mean Cell Haemoglobin (MCH)	27.0 - 32.0 pg
Mean Cell Haemoglobin Concentration (MCHC)	30.0 - 35.0g/dL
Red Blood Cell Distribution Width (RDW)	11.5 - 14.5%
Platelets	150 - 450 x10^9/L
Mean Platelet Volume (MPV)	7.4 - 10.4 fl
White Blood Cells (WBC)	4.0 - 11.0 X10^9/L
Neutrophil (%)	40 - 75%
Lymphocyte (%)	20 - 45%
Monocyte (%)	2 - 10%
Eosinophil (%)	1 - 6%
Basophil (%)	0 - 1%
Neutrophil (absolute)	2.0 - 7.5 x10^9/L
Lymphocyte (absolute)	1.5 - 4.0 x10^9/L
Monocyte (absolute)	0.5 - 1.5 x10^9/L
Eosinophil (absolute)	0.04 - 0.4 x10^9/L
Basophil (absolute)	0.0 - 0.1 x10^9/L

2.3 EVALUATION OF THE IMMUNE MARKERS IN HIV-INFECTED AND NON-INFECTED PRE-ECLAMPTIC BLACK WOMEN – FLOW CYTOMETRY

The immune system depends upon the recognition of exogenous materials as being foreign to the body, known as an antigen. This results in the activation of the immune system with the purpose of neutralising or destroying the antigen. Lymphocytes play a key role in immune response and T lymphocytes are divided into functional subsets as identified by the presence of different surface markers:

- **T helper cells**

T helper cells assist other lymphocytes to perform their effector functions by secreting a variety of locally acting mediators known as cytokines. T cells are characterised by the surface marker CD_4 as well as the pan-T cell markers CD_2 and CD_3, which are present on all mature T cells.

- **Cytotoxic T cells**

These lymphocytes function in the killing of virus-infected and malignant cells. Most carry the surface marker CD_8 as well as the pan-T cell markers.

- **Suppressor T cells**

The exact role of suppressor cells *in vivo* is unclear but it is thought to be responsible for "switching off" the immune response once the initiating stimulus is removed and possibly for suppressing immune responsiveness to self-antigens. Suppressor cells also carry the CD_8 marker (Young & Heath, 2000).

Flow cytometry is often used to determine the types of markers and receptors on the surface of a cell. It is commonly used in HIV infection to assay the CD_3 values, CD_4/CD_8 ratio, to evaluate disease progression and monitor therapy (Rose *et al.*, 1992).

A normal immune response requires a balance between the regulatory activities of several interacting cell types – primarily T-helper and T-suppressor cells. By using highly specific antibodies, flow cytometry can analyse several lymphocyte markers including the following:

- CD_3 – To measure mature T cells when the immune system malfunctions
- CD_4 – To identify and characterise the proportion of T-helper cells in autoimmune or immunoregulatory disorders
  To detect immunodeficiency disorders such as AIDS
  To differentiate T-cell acute lymphoblastic leukaemia from T-cell lymphomas and other lymphoproliferative disorders
- CD_8 – To identify and characterise the proportion of T-suppressor cells in auto – immune and immunoregulatory disorders (McCann, 2002).

Flow cytometry is used in a variety of different fields including immunology, pathology and medicine. It is a powerful technique to simultaneously analyse multiple characteristics of thousands of individual cells in a relatively short period of time. Flow cytometry makes these multi-parametic measurements on single cells as opposed to population measurements (National Institute of Environmental Health Sciences, 2006). High precision and sensitivity, combined with the large numbers of cells that can be examined, allow resolution of even minor subpopulations from complex mixtures with high levels of statistical validity. The ability to separate physically these populations by flow sorting allows further functional and morphologic correlations (Preffer, 1988).

2.3.1 Principles and instrumentation

A suspension of cells stained with two monoclonal antibodies conjugated with either a green or orange emitting fluorescent dye is introduced into the laminar flow of a confining sheath fluid within the flow cell of a cytometer. The rapidly moving sheath separates cells and ensures individual and uniform interrogation by the precisely focused laser light (Figure 2.2) (Preffer, 1988).

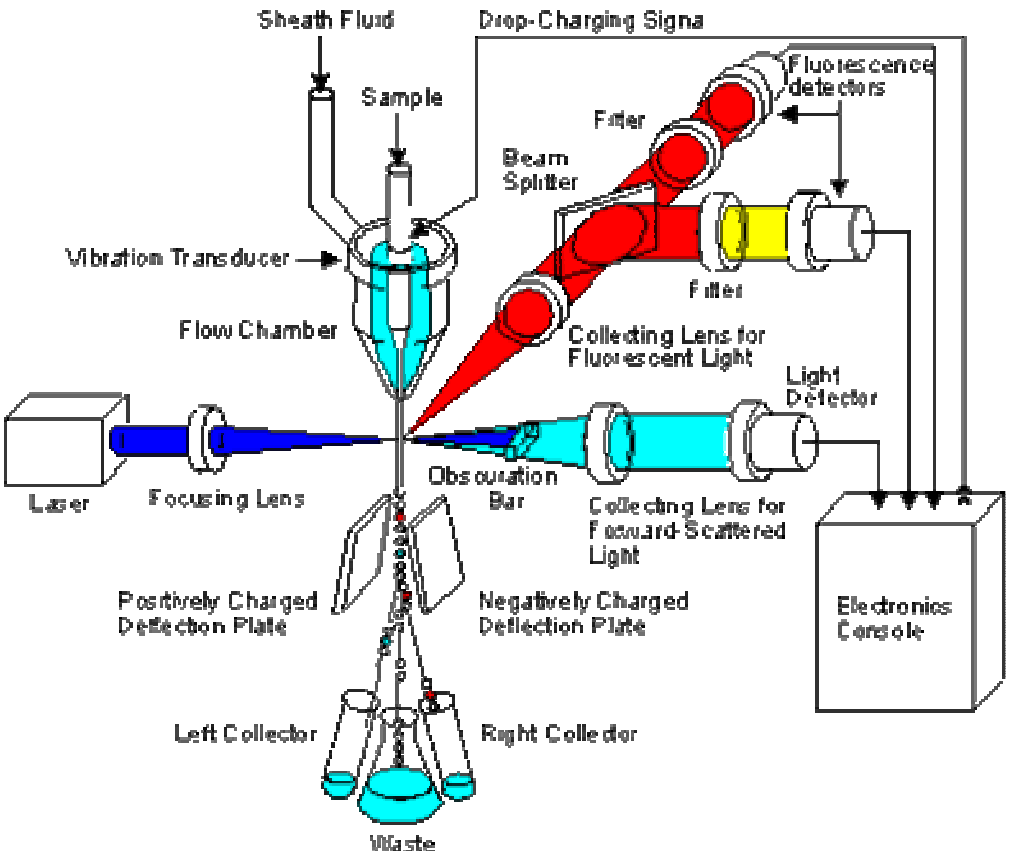

Figure 2.2: Schematic representation of the instrumentation of a flow cytometer

(Decker, 2006)

The presence of a cell in the path of the laser scatters light in all directions and excites the fluorescent dyes conjugated to the monoclonal antibodies bound to the cell. This results in either reflected or refracted light reaching the detector. The pattern of light scattering is dependent on cell size and shape, giving relative measures of theses cellular characteristics as cells flow through the beam (Simmer, 2003).

Fluorescence based detection depends on the absorption of the light by the cell and the subsequent re-emission of this light at a different frequency. Flow cytometers make use of this technology by employing filters to block the original light source from reaching the detector, while the fluorescence emission is allowed through for detection, which allows a very low background of stray light to reach the detector. Various fluorescent dyes are commercially available with the most common dyes used being fluorescein isothiocyanate (FITC) and phycoerythrin (PE). The amount a cell scatters or fluoresces light is measured is collected by a light sensitive diode and converted into electronic signals (Simmer, 2003).

2.3.2 Display and interpretation of data

Dot plot displays use two parameters to graph the data generated by flow analysis, with each dot representing the passage of one cell through the detector. The X- and Y-axes measure the different emissions, displaying a dot for each of the cells that show that particular emission. Plots are usually divided into four areas. A cell of a particular population type will show up as a dot in the quadrant of the dot plot designated for that population (Simmer, 2003).

2.3.3 Flow Cytometry

Due to operational circumstances, samples were processed on the Coulter Epics MCL Flow Cytometer (Beckman Coulter, Florida, USA) (Figure 2.3) and the FACSCalibur Flow Cytometer (Becton Dickinson, California, USA) (Figure 2.4). Both these instruments can be used interchangeably provided that they are both aligned and standardised correctly.

Figure 2.3: Beckman Coulter Epics MCL Flow Cytometer (Beckman Coulter, 2007)

Figure 2.4: Becton Dickinson FACSCalibur Flow Cytometer (BD Biosciences, 2007)

2.3.3.1 Beckman Coulter Epics MCL Flow Cytometer

All reagents were purchased from Beckman Coulter, Florida, USA. FLOW-CHECK and Epics IMMUNO-BRITE beads were used to align and determine linearity/fluorescence intensity respectively. Zones were gated to analyse maximum cells for each sample which were expressed as percentage values. These were converted to absolute counts using the FBC parameters.

- 100ul of sample was aliquoted into 75 x 12 mm polyurethane tubes
- CYTO-STAT Monoclonal antibodies were added:
    - IgG1 RDI / FITC – isotypic control to exclude non-specific staining cells
    - T4 RDI / T8 FITC – specific for CD_4 and CD_8
    - T3 RDI – specific for CD_3
- The samples were vortexed and allowed to incubate in the dark at room temperature for 30 minutes to allow antigen/antibody binding
- Following this, the samples were then passed through a Coulter Q-Prep Workstation (Beckman Coulter, Florida, USA) which automatically dispensed:
    - Reagent A – 600ul formic acid (lysing)
    - Reagent B – 265ul sodium chloride, sodium sulphate and sodium carbonate (stabilising)
    - Reagent C – 100ul paraformaldehyde (fixing)
- After preparation on the Coulter Q-Prep Workstation the samples were analysed on the flow cytometer

2.3.3.2 Becton Dickinson FACSCalibur Flow Cytometer

All reagents and materials were purchased from Becton Dickinson, California, USA. CaliBRITE beads and FACSComp were used for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation and checking the instrument sensitivity. A control was run to optimise instrument settings and perform quality control.

A three-colour direct immunoflourescence reagent, TriTEST CD_4 FITC / CD_8 PE / CD_3 peridinin chlorophyll protein (PerCP) was used to determine absolute counts of CD_4, CD_8 and CD_3 together with TruCOUNT Tubes. TruCOUNT tubes contain a lyphilised pellet that dissolves during sample preparation, releasing a known number of fluorescent beads.

By gating the bead population during analysis, MultiSET Software automatically calculated subset absolute counts. MultiSET provided automatic gating using an Expert Gate to exclude unwanted cells and debris.

- 20ul of TriTEST CD_4 / CD_8 / CD_3 reagent was aliquoted into the TruCOUNT Tube
- 50ul of well-mixed whole blood was thereafter aliquoted into the TruCOUNT Tube
- Samples were vortexed and incubated for 15 minutes in the dark at room temperature
- 450ul FACS Lysing Solution was aliquoted into the tube
- Samples were vortexed and incubated for 15 minutes in the dark at room temperature
- Samples were then analysed on the flow cytometer

CHAPTER 3

RESULTS

3.1 SAMPLE SIZE

Sixty blood samples were obtained with only 46 being processed (Table 3.1). Of the 46 samples, 45 were processed for haematological parameters and 33 were processed for immune markers. This was due to operational circumstances leading to samples not being processed timeously. Sample integrity was maintained, the laboratory equipment was validated for the 33 samples. The remaining specimens produced results that could not be validated.

Table 3.1: Case processing summary of the sample size for each parameter

Parameters	Included	HIV (-)	HIV (+)	*Total*
Red Blood Cells	45	25	20	46
Haemoglobin	45	25	20	46
Haematocrit	45	25	20	46
Mean Cell Volume	45	25	20	46
Mean Cell Haemoglobin	45	25	20	46
Mean Cell Haemoglobin Conc.	45	25	20	46
Red Blood Cell Distribution Width	45	25	20	46
Platelets	45	25	20	46
Mean Platelet Volume	45	25	20	46
White Blood Cells	45	25	20	46
Neutrophils (%)	45	25	20	46
Lymphocytes (%)	45	25	20	46
Monocytes (%)	45	25	20	46
Eosinophils (%)	45	25	20	46
Basophils (%)	45	25	20	46
Neutrophils (absolute)	44	25	19	46
Lymphocytes (absolute)	45	25	20	46
Monocytes (absolute)	44	25	19	46
Eosinophils (absolute)	45	25	20	46
Basophils (absolute)	45	25	20	46
CD3 (absolute)	33	18	15	46
CD4 (absolute)	33	18	15	46
CD8 (absolute)	33	18	15	46

HIV (-) – Human Immunodeficiency Virus negative
HIV (+) – Human Immunodeficiency Virus positive
Total samples = 100%

3.2 CLINICAL DATA

There was no statistical significance ($p < 0.05$) between both groups, in respect of the maternal clinical data (Table 3.2). Among the HIV-negative pre-eclamptic group, 48.0% were ≤ 24 years and 52.0% were > 24 years. Among the HIV-positive pre-eclamptic group, 28.6% were ≤ 24 years and 71.4% were > 24 years (p=0.179). Among the HIV-negative pre-eclamptic group, 52.0% were nulliparous and 48.0% had ≥ 1 child previously. Among the HIV-positive pre-eclamptic group, 23.8% were nulliparous and 76.2% had ≥ 1 child previously (p=0.051). There was a trend towards statistical significance in respect to parity.

Among the HIV-negative pre-eclamptic group, 52.0%, 40.0% and 8.0% had proteinuria of 2+, 3+ and 4+ respectively. Among the HIV-positive pre-eclamptic group, 47.6%, 52.4% and 0.0% had proteinuria of 2+, 3+ and 4+ respectively (p=0.349). Among the HIV-negative pre-eclamptic group, 60.0% were at a gestational age between 24 – 36 weeks and 40.0% were >36 weeks. Among the HIV-positive pre-eclamptic group, 61.9% were at a gestational age between 24 – 36 weeks and 38.1% were >36 weeks (p=0.895).

Among the HIV-negative pre-eclamptic group, 68.0% had a caesarean section (CS) and 32.0% had normal vaginal delivery (NVD) as compared to the HIV-positive pre-eclamptic group where 52.4% had a CS and 47.6% had NVD (p=0.280). Among the HIV-negative pre-eclamptic group, 40.0% had a BP of <160/110 mmHg and 60.0% had a BP of >160/110 mmHg. Among the HIV-positive pre-eclamptic group, 38.1% had a BP of <160/110 mmHg and 61.9% had a BP of >160/110 mmHg (p=0.895).

Table 3.2: HIV status versus maternal clinical data

Clinical Data		HIV Negative	HIV Positive	P value
Age Group (years)	**≤ 24**	n = 12 (48.0%)	n = 6 (28.6%)	0.179 (NS)
	> 24	n = 13 (52.0%)	n = 15 (71.4%)	
Parity	**Nulliparous**	n = 13 (52.0%)	n = 5 (23.8%)	0.051 (NS)
	≥ 1	n = 12 (48.0%)	n = 16 (76.2%)	
Proteinuria	**2+**	n = 13 (52.0%)	n = 10 (47.6%)	0.349 (NS)
	3+	n = 10 (40.0%)	n = 11 (52.4%)	
	4+	n = 2 (8.0%)	n = 0 (0.0%)	
Gestational age (weeks)	**24 – 36**	n = 15 (60.0%)	n = 13 (61.9%)	0.895 (NS)
	> 36	n = 10 (38.1%)	n = 8 (38.1%)	
Mode of Delivery	**CS**	n = 17 (68.0%)	n = 11 (52.4%)	0.280 (NS)
	NVD	n = 8 (32.0%)	n = 10 (47.6%)	
Blood Pressure (mmHg)	**< 160/110**	n = 10 (40.0%)	n = 8 (38.1%)	0.895 (NS)
	> 160/110	n = 15 (60.0%)	n = 13 (61.9%)	

CS – caesarean section
NVD – normal vaginal delivery
HIV – Human Immunodeficiency Virus
NS – Not significant

There was no statistical significance ($p < 0.05$) between the groups in respect of foetal clinical data (Table 3.3). Among the HIV-negative pre-eclamptic group, 68.0% gave birth to babies with a weight of ≤2500 grams and 32.0% to >2500 grams. Among the HIV-positive pre-eclamptic group, 61.9% gave birth to babies with a weight of ≤2500 grams and 38.1% to >2500 grams ($p=0.665$). Among the HIV-negative pre-eclamptic group, 88.0% and 12.0% had baby outcomes of alive and deceased respectively. Among the HIV-positive pre-eclamptic group, 85.7% and 14.3% had baby outcomes of alive and deceased respectively ($p=0.819$).

Table 3.3: HIV status versus foetal clinical data

Clinical Data		HIV Negative	HIV Positive	P value
Weight of Baby (grams)	**≤ 2500**	n = 17 (68.0%)	n = 13 (61.9%)	0.665 (NS)
	> 2500	n = 8 (32.0%)	n = 8 (38.1%)	
Baby Outcome	**Alive**	n = 22 (88.0%)	n = 18 (85.7%)	0.819 (NS)
	Deceased	n = 3 (12.0%)	n = 3 (14.3%)	

HIV – Human Immunodeficiency Virus
NS – Not significant
HIV Negative deceased = Early neo-natal death (n=2), macerated stillbirth (n=1)
HIV Positive deceased = Macerated stillbirth (n=2), fresh stillbirth (n=1)

3.3 HAEMATOLOGICAL PARAMETERS

There was no statistical significance ($p < 0.05$) in haematological parameters between both the groups (Table 3.4). Red blood cells are within the normal reference values of 3.5 – 5.0 x 10^12/l in both groups, HIV-negative = 3.91 x 10^12/l and HIV-positive = 3.79 x 10^12/l (p=0.522). Haemoglobin was within the normal reference values of 11.5 – 13.5 g/dL in the HIV-negative group, 11.5 g/dL and slightly lower than normal in the HIV-positive group, 11.1 g/dL but cannot be considered clinically significant (p=0.576).

Haematocrit was slightly lower in both groups as compared to the normal reference values of 37.0 – 52.0 % but not clinical significant. In the HIV-negative group, haematocrit was 34.1% and in the HIV-positive group, haematocrit was 33.2% (p=0.545). Mean cell volume was within the normal reference values of 78.0 – 99.0 fl in both groups, HIV-negative = 87.4 fl and HIV-positive = 87.5 fl (p=0.664). Mean cell haemoglobin was within the normal reference values of 27.0 – 32.0 pg in both groups, HIV-negative = 29.3 pg and HIV-positive = 30.3 pg (p=0.320). Mean cell haemoglobin concentration was also within the normal reference values of 30.0 – 35.0 g/dL in both groups, HIV-negative = 34.1 g/dL and HIV-positive = 34.3 g/dL (p=0.873). Red cell distribution width was within the normal reference values of 11.5 – 15.5 % in both groups, HIV-negative = 14.4 % and HIV-positive = 14.5 % (p=0.936).

Platelets were within the normal reference values of 150 – 450 x 10^9/L in both groups, HIV-negative = 205 x 10^9/L and HIV-positive = 225 X 10^9/L (p=0.749). Mean platelet volume was within the normal reference values of 7.4 – 10.4 fl in both groups, HIV-negative = 9.5 fl and HIV-positive = 9.6 fl (p=0.373).

Table 3.4: Red blood cell and platelet parameters among HIV-negative and HIV-positive pre-eclamptic women

Parameters		HIV Negative	HIV Positive	P value
Red Blood Cells (3.5 - 5.0 x10^12/l)	**Minimum**	3.09	2.43	0.522 (NS)
	Median	3.91	3.79	
	Maximum	4.78	4.53	
Haemoglobin (11.5 - 13.5g/dL)	**Minimum**	8.30	7.60	0.576 (NS)
	Median	11.50	11.10	
	Maximum	14.50	13.70	
Haematocrit (37.0 - 52.0%)	**Minimum**	25.80	21.40	0.545 (NS)
	Median	34.10	33.20	
	Maximum	42.10	38.80	
Mean Cell Volume (78.0 - 99.0 fl)	**Minimum**	70.30	67.50	0.664 (NS)
	Median	87.40	87.50	
	Maximum	95.10	96.90	
Mean Cell Haemoglobin (27.0 - 32.0 pg)	**Minimum**	21.40	21.70	0.320 (NS)
	Median	29.30	30.30	
	Maximum	35.20	32.50	
Mean Cell Hb Concentration (30.0 - 35.0g/dL)	**Minimum**	30.40	30.50	0.873 (NS)
	Median	34.10	34.30	
	Maximum	37.10	35.90	
RDW (11.5 - 14.5%)	**Minimum**	12.50	13.00	0.936 (NS)
	Median	14.40	14.55	
	Maximum	24.60	21.10	
Platelets	**Minimum**	123.00	95.00	0.749

(150 - 450 x10^9/L)	**Median**	205.00	225.00	(NS)
	Maximum	458.00	431.00	
Mean Platelet Volume (7.4 - 10.4 fl)	**Minimum**	7.30	7.40	0.373 (NS)
	Median	9.50	9.65	
	Maximum	12.30	14.50	

HIV – Human Immunodeficiency Virus
NS – Not significant
Hb – Haemoglobin
RDW – Red Blood Cell Distribution Width

White blood cells were within the normal reference values of 4.0-11.0 x 10^9/L in both groups, HIV-negative = 10.8 x 10^9/L and HIV-positive = 9.0 x 10^9/L (p=0.201) (Table 3.5). Neutrophils (%) were slightly higher in the HIV-negative group, 76.7% as compared to the normal reference values of 40 – 75%, indicative of slight neutrophilia. In the HIV-positive group, neutrophils (%) were within the normal reference values, 71.2% (p=0.249).

Lymphocytes (%) were slightly lower (lymphopenia) in the HIV-negative group, 17.0% as compared to the normal reference values of 20 – 45%. In the HIV-positive group, lymphocytes (%) were within the normal reference values (p=0.226). Monocytes (%) were within the normal reference values of 2.0 – 10.0 % in both groups, HIV-negative = 4.0% and HIV-positive = 4.2% (p=0.304).

Eosinophils (%) were slightly lower in both groups as compared to the normal reference values of 1 – 6 %, indicative of eosinopenia. In the HIV-negative group, eosinophils (%) were 0.7% and in the HIV-positive group, eosinophils (%) were 0.9% (p=0.162). Basophils (%) were within the normal reference values of 0 – 1.0 % in both groups, HIV-negative = 0.3% and HIV-positive = 0.32% (p=0.564).

Neutrophils (absolute) were slightly higher, neutrophilia, in the HIV-negative group, 7.8 x 10^9/L, as compared to the normal reference values of 2.0-7.5 x 10^9/L. In the HIV-positive group, neutrophils (absolute) were 6.2 x 10^9/L, within the normal reference values (p=0.145). Lymphocytes (absolute) were within the normal reference values of 1.5-4.0 x 10^9/L in both groups, HIV-negative and HIV-positive = 1.6 x 10^9/L (p=0.918).

Monocytes (absolute) were slightly lower in both groups as compared to the normal reference values of 0.5-1.5 x 10^9/L suggestive of possible monocytopenia. In the HIV-negative and HIV-postive group, monocytes (absolute) 0.4 x 10^9/L (p=0.607). Eosinophils (absolute) were within the normal reference values of 0.04-0.4 x10^9/L in both groups, HIV-negative and HIV-positive = 0.1 x 10^9/L (p=0.623). Basophil (absolute) was constant (count = 0.00) when HIV status was positive. It has been omitted.

Table 3.5: White blood cell parameters among HIV-negative and HIV-positive pre-eclamptic women

Parameters		HIV Negative	HIV Positive	P value
White Blood Cells (4.0 - 11.0 X10^9/L)	**Minimum**	5.00	4.60	0.201 (NS)
	Median	10.80	9.05	
	Maximum	22.70	17.00	
Neutrophils (40 - 75%)	**Minimum**	49.20	36.00	0.249 (NS)
	Median	76.70	71.25	
	Maximum	91.50	88.60	
Lymphocytes (20 - 45%)	**Minimum**	6.40	6.50	0.226 (NS)
	Median	17.00	21.15	
	Maximum	41.20	47.00	
Monocytes (2 - 10%)	**Minimum**	0.60	1.10	0.304 (NS)
	Median	4.00	4.25	
	Maximum	17.00	14.00	
Eosinophils (1 - 6%)	**Minimum**	0.10	0.10	0.162 (NS)
	Median	0.70	0.90	
	Maximum	2.40	4.60	
Basophils (0 - 1%)	**Minimum**	0.00	0.10	0.564 (NS)
	Median	0.30	0.20	
	Maximum	0.50	0.80	
Neutrophils (absolute) (2.0 - 7.5 x10^9/L)	**Minimum**	2.50	2.20	0.145 (NS)
	Median	7.80	6.20	
	Maximum	20.70	14.20	

Lymphocytes (absolute) (1.5 - 4.0 x10^9/L)	**Minimum**	1.00	0.90	0.918 (NS)
	Median	1.60	1.60	
	Maximum	2.70	3.10	
Monocytes (absolute) (0.5 - 1.5 x10^9/L)	**Minimum**	0.10	0.10	0.607 (NS)
	Median	0.40	0.40	
	Maximum	1.30	1.00	
Eosinophils (absolute) (0.04 - 0.4 x10^9/L)	**Minimum**	0.00	0.00	0.623 (NS)
	Median	0.10	0.10	
	Maximum	0.20	0.50	

HIV – Human Immunodeficiency Virus
NS – Not significant

3.4 IMMUNOLOGICAL MARKERS

There was a statistical significance as well as a clinical significance in CD_3 (p=0.015) and CD_4 (p=0.002) between both the groups. In the HIV-negative group and HIV-positive group, the CD_3 count was 1423 and 978 respectively (normal reference values = 800 – 2800 cells/ul) (Table 3.6, Figure 3.1) and CD_4 count was 925 and 274 respectively (normal reference values = 550 – 1955 cells/ul) (Table 3.6, Figure 3.2). There was no statistical significance in CD_8 between both the groups (p=0.842), HIV-negative group = 618.5 cells/ul and HIV-positive group = 633.0 cells/ul (normal reference values = 250 – 1200 cells/ul) (Table 3.6).

Table 3.6: Immune markers among HIV-negative and HIV-positive pre-eclamptic women

Parameters		HIV Negative	HIV Positive	P value
CD_3 (absolute) (800 – 2800 cells/ul)	**Minimum**	674.00	338.00	0.015 (S)
	Median	1423.00	978.00	
	Maximum	2712.00	2284.00	
CD_4 (absolute) (550 – 1955 cells/ul)	**Minimum**	176.00	96.00	0.002 (S)
	Median	925.00	274.00	
	Maximum	1716.00	782.00	
CD_8 (absolute) (250 – 1200 cells/ul)	**Minimum**	114.00	110.00	0.842(NS)
	Median	618.50	633.00	
	Maximum	921.00	1333.00	

HIV – Human Immunodeficiency Virus
S – Significant

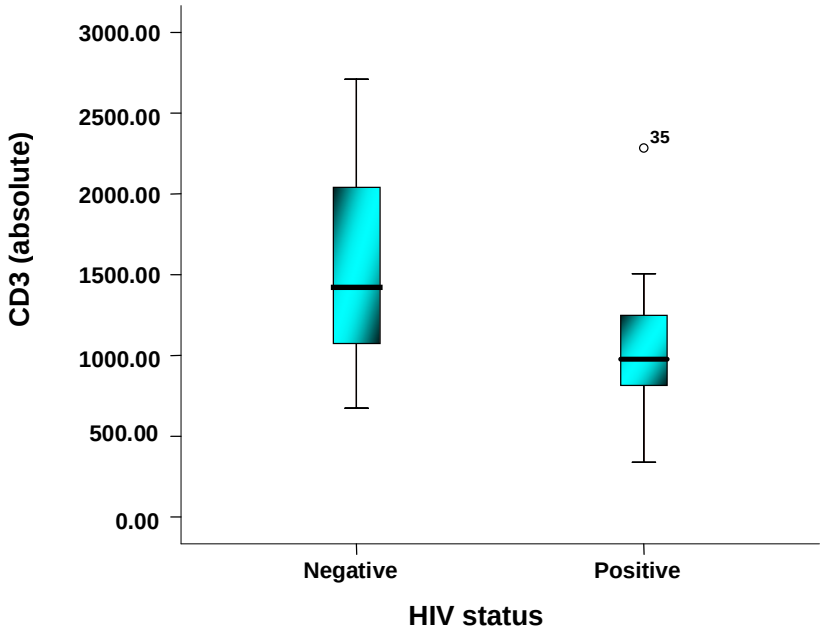

Figure 3.1: Graphically representation of CD_3 (absolute) distribution among HIV-negative and HIV-positive pre-eclamptic women

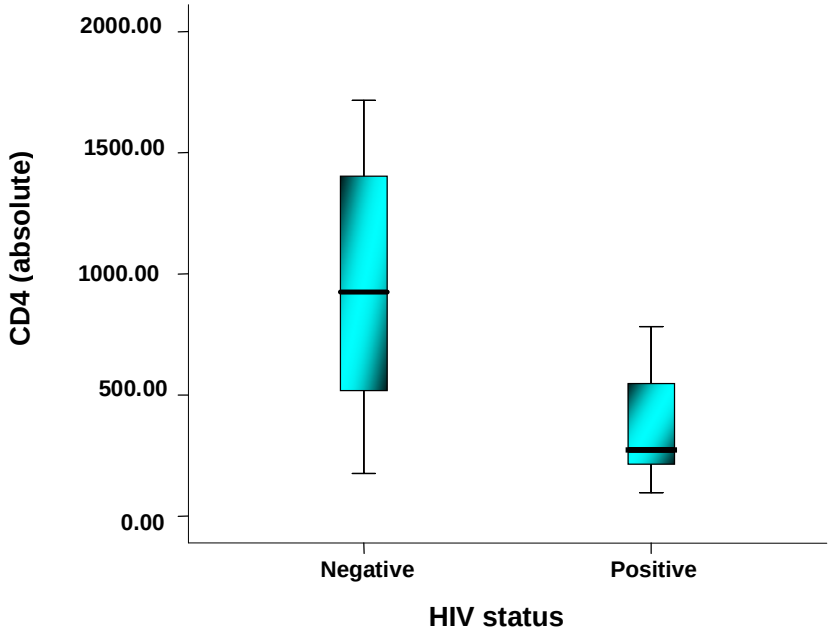

Figure 3.2: Graphically representation of CD_4 (absolute) distribution among HIV-negative and HIV-positive pre-eclamptic women

CHAPTER 4

DISCUSSION

❖ **No Difference in Maternal Demography**

There was no statistical significance in age among the two groups (Table 3.2). However, the difference in > 24 years old among the HIV-positive group from 28.6% to 71.4% is in keeping with statistics reported by the South African Department of Health where the highest prevalence among antenatal clinic attendees were reported amongst the 25 – 29 and 30 – 34 year age groups (South African Department of Health, 2005).

There was no statistical significance in parity among both the groups (Table 3.2). It has been suggested that the pathophysiology of pre-eclampsia differs according to parity. Barden *et al.* (1999), however, reported lack of differences in birthweight and biochemical and endothelial markers in pre-eclamptic primigravida and multigravida groups which contradicts other studies. Among the HIV-positive pre-eclamptic group, 23.8% were nulliparous and 76.6% had a parity of ≥ 1. Women with previous pregnancies may suggest inconsistent condom use which puts them at a greater risk for HIV infection.

- **No Difference in Pregnancy Outcomes**

It is known that elective CS is the best option in HIV-positive women to reduce the risk of mother to child transmission (Mohlala *et al.*, 2005). There was no statistical difference in mode of delivery between both the groups (Table 3.2). It is not standard practice in the public sector hospitals in Durban to perform routine elective CS for HIV positive pregnant women at the time of the study and this probably accounted for the lack of difference between the groups. The possible mechanisms for vertical transmission during the peripartum (before, during or after giving birth) include transplacental microtransfusions of maternal blood into the foetal circulation during contractions, labour and separation of the placenta before clamping of the umbilical cord, ascending infection from the vagina through the cervix after rupture of the amniotic fluid and absorption of the virus through the infants immature digestive tract (Mohlala *et al.*, 2005).

There was no statistical significance in gestational age among both the groups (Table 3.2). Pre-eclampsia occurs > 20 weeks gestation, but is not specific to any particular gestational period after 20 weeks gestation. Pre-eclampsia did not present itself earlier during the gestational period due to HIV infection.

- **No Difference in Clinical Parameters**

Women with BP of > 160/110 mmHg were slightly higher between the two groups as compared to < 160/110 mmHg but this was not statistical significant (Table 3.2). Increased BP in pre-eclampsia is mainly due to a reversal of the vasodilation characteristics of normal pregnancy replaced by marked increases in peripheral vascular resistance (NHBPEP, 2000). Studies have shown no association of HIV infection with hypertension (Jung *et al.,* 2004).

There was no statistical significance in proteinuria (Table 3.2) among both the groups. As mentioned previously, proteinuria is key feature in pre-eclampsia. Proteinuria can also be linked to HIV infection among patients with HIV-associated nephropathy and renal failure which could be the reason as to why there was no difference in proteinuria between both the groups (Szczech *et al.,* 2002).

- **No Difference in Birth Outcomes**

There was no statistical significance in the weight of the baby (Table 3.3) and baby outcome (alive or deceased) (Table 3.3) between both the groups. Low birth weight due to placental insufficiency has been reported frequently among women with pre-eclampsia, although there was no evidence of low birth weight in the results obtained.

❖ **No Difference in Haematological Parameters**

Leukopenia (low WBC) in usually noticed during late stages of HIV infection due to a parallel decline in the most numerous WBC's, neutrophils, lymphocytes and monocytes. It is suggested that lymphopenia and monocytopenia (HIV-1 can be recovered from monocytes) results from a direct attack on the cells by HIV through CD_4 sites (McPhedran, 1999). There was no statistical difference in the lymphocyte, monocyte and neutrophil counts shown in Table 3.5, however lymphocytes (%) and neutrophils (absolute and %) in the HIV-negative group were observed to be slightly lower than the normal reference values. Further, lower monocytes (absolute) were observed in both groups. No severe decline among the HIV-positive group may have been due to it being early stages of infection.

The slightly higher neutrophils (absolute and %) in the HIV-negative group could support the theory that neutrophils are activated in both the placental bed and maternal circulation of women with pre-eclampsia. Neutrophil activation is associated with a free radical release that can either affect endothelial function directly or contribute indirectly through production of lipid peroxides. It is not known whether a circulating factor is responsible for the neutrophil activation and any subsequent lipid peroxidation. It may also be due to exposure to hypoxic conditions or local inflammatory agents encountered during passage of neutrophils through the placental circulation. An alternative explanation is that neutrophils obtained from pre-eclamptic women are sensitised to circulating serum factors, whereas neutrohils obtained in the non-pregnant state are not (Barden *et al*., 2001).

There was no statistical difference in eosinophils and basophils (absolute and %) between both the groups (Table 3.5) suggesting that these parameters are not affected in pre-eclampsia.

There was no statistical significance in platelets and MPV between the two groups (Table 3.4). In pre-eclampsia, thrombocytopenia is the most common haematological abnormality. Platelet counts < 100 000 cells/mm^3 are indicative of serious disease. The cause is unclear but it has variously been ascribed to platelet deposition at sites of endothelial damage and to an immunological process (Laragh & Brenner, 1995).

Thrombocytopenia is also evident in HIV infection. Possible aetiologies include immune-mediated destruction, thrombotic thrombocytopenic purpura, impaired haematopoiesis (formation of blood cells) and toxic effects of medication. In many instances, thrombocytopenia is associated with a normal or higher number of megakaryocytes (source of blood platelets) in the bone marrow and elevated levels of platelet associated immunoglobulin. These patients have the clinical syndrome known as immune thrombocytopenic purpura (ITP) (Northfelt, 1998). The possible reason as to why pre-eclampsia and/or HIV infection showed no effect on platelets may be due to it being early stages of HIV infection or mild (not severe) severe pre-eclampsia.

There was no statistical significance in the RBC's between both the groups (Table 3.4). Red blood cells contain Hb and reduction in these parameters could result in anaemia. Anaemia is common in pregnancy due to increased foetal demand (Chotnopparatpattara *et al.*, 2003) and in HIV infection where a persistently low Hb is predictive of a poor outcome. Haemoglobin was slightly lower among the HIV-positive pre-eclamptic group but not indicative of severe anaemia (Table 3.4). Anaemia is the most common finding in people with advanced stages of HIV and can be defined as a reduction below normal of the haemoglobin concentration and red blood cell mass (Kline, 2005).

In pre-eclampsia, higher Hb concentrations have been reported. Failure of the plasma volume to expand and subsequently, the Hb concentration to drop is associated with ≤ 3-fold increase in the incidence of pre-eclampsia in pregnancy (Steer, 2000). However, the results in this study showed that pre-eclampsia and HIV infection had no effect on Hb, again, suggesting early stages of HIV infection and/or mild pre-eclampsia.

One of the reported features in pre-eclampsia is impaired blood rheology (deformation and flow) due to altered RBC aggregation and deformability. However, in a study by Pepple *et al.* (2001), no statistical difference in RBC's among control groups and pre-eclamptics was found suggesting that RBC aggregation and deformability are not altered in pre-eclampsia in keeping with the results obtained in this study. Further investigation is recommended.

Haematocrit was slightly lower than the normal reference value between both groups but was not statistically significant (Table 3.4). Haematocrit is the percentage of the volume of whole blood that is composed of RBC's. Haematocrit values have been reported to be low in HIV infection, especially late during the course of infection, related to compromised nutritional and antioxidant status (Bogden *et al.*, 2000). Anim-Nyame *et al*. (2000) reported no difference in haematocrit values in normal pregnant and pre-eclamptic women.

Pre-eclampsia and HIV infection showed no effect on MCV, MCH, MCHC and RDW (Table 3.4) which could be attributed to the normal values observed for RBC's, haematocrit and Hb since MCV, MCH, MCHC and RDW are calculated automatically from RBC's, haematocrit and Hb.

- **Significant Difference in Immunological Parameters**

The vascular endothelium plays a role in the maintenance of immune response therefore endothelial dysfunction during pre-eclampsia results in immunological alterations to some degree (Cetiner *et al*., 1998). Further to this, the immune responsiveness of women is altered during pregnancy in order to retain protective properties against disease and also allow tolerance of the foetus. Diseases such as pre-eclampsia have been suggested to arise as a result of maladaptations in theses immune alterations (Mahmoud *et al*., 2003).

There was a statistical significance in CD_3 and CD_4 between both the groups (Table 3.6, Figure 3.1, Figure 3.2). However, the CD_3 and CD_4 counts were within the normal reference values in the HIV-negative pre-eclamptic group and even though CD_3 was lower, it was still within the normal reference values in the HIV-positive pre-eclamptic group, with CD_4 below the normal reference values in the HIV-positive pre-eclamptic group. This suggests that immune mechanisms involving CD estimations do not play a role in pre-eclampsia since the decline in the counts can be solely attributed to HIV infection. There was no statistical difference in CD_8 among both the groups. This is in keeping with results reported by Cetiner *et al*. (1998), where no statistical significance in CD_4 and CD_8 was found in pre-eclampsia when compared to normal pregnant controls.

Further, their study found decreased levels of NK cells in pre-eclamptic women than in normal pregnant women which may be involved in the pathomechanism of pre-eclampsia. However, contradicting results by Mahmoud *et al*. (2003) showed a statistical significance in the number of T lymphocytes: CD_2, CD_3, CD_4, CD_8 and CD_{19}. These findings suggested systemic alteration in maternal immunity associated with the pre-eclamptic state. The difference in results could be due to the small sample size used by Mahmoud *et al*, (2003), of the 54 women with pregnancy-induced hypertension, only 14 were protein uric and therefore pre-eclamptic. In this study, 45 samples were evaluated in each pre-eclamptic HIV-negative and HIV-positive group.

The results show HIV infection lowered CD_3 and CD_4 during pregnancy. Ibrahim *et al.* (2004), showed no deterioration in the CD_4 count in any of the trimesters among HIV infected pregnant women, and seemed to only increase post delivery. The reasoning for this, suggested by Ibrahim *et al.* (2004), may have been due to the cross sectional nature of the study. Further, with the exception of the third trimester CD_8 counts, among all the trimesters the CD_4 and CD_8 counts showed significant differences between the HIV-infected and non-infected pregnant groups. These lower CD_4 counts in the HIV-infected group might reflect a longer period of infection prior to parturition.

The hallmark of HIV infection is declining CD cell numbers. The rate of CD_4 depletion differs among individuals and occurs in four stages:

- A rapid decline for 12 to 18 months at the time of seroconversion
- A gradual decline over several years during the asymptomatic period
- A more rapid decline which can last at least several months just before AIDS develops
- Continued CD_4 decline until death.

CD_8 numbers are usually elevated in HIV infection. This occurs during the first few months of infection but remains fairly stable thereafter (Rose *et al.*, 1992). This may be reason as to why the CD_8 count is within the normal range in the HIV – positive group. Abnormally low CD_3 counts herald the final stages of disease progression and since it may have been early stages of HIV infection, this was not observed.

Also of note, are the low CD_4 counts but normal WBC (Table 3.5) and lymphocyte counts in the HIV-positive group suggesting early HIV infection. Throughout the period of HIV infection, until AIDS develops, total WBC, lymphocyte (absolute and percentage) counts remain relatively stable and are comparable to levels in healthy normal controls. Only late in HIV infection, a decrease is noted in these parameters (Rose *et al.,* 1992).

CHAPTER 5

CONCLUSION

Results obtained in this study do not show any severe haematological or immunological abnormalities when women have both pre-eclampsia and HIV infection. Haematological manifestations i.e. anaemia, thrombocytopenia and leukopenia, key features in either pre-eclampsia or HIV infection were not observed. The decrease in CD_3 and CD_4 counts can be attributed solely to HIV infection. No other immunological alterations were noted suggesting that the immune mechanisms do not play a role in the development of pre-eclampsia.

Therefore, these results suggest that a more specific management is not required when women have both pre-eclampsia and HIV infection. Medical care should be administered on the basis of grade of pre-eclampsia (mild or severe) and stage of HIV infection taking into account maternal and foetal well-being.

Results obtained from this study contradict outcomes of other studies conducted. Further investigation is warranted in these areas. Limitations to this study may include small sample size and neglected ARV treatment history amongst the HIV-positive group, which may have played a role in the stability of the haematological parameters. Also, tracking the women over all three trimesters may have produced more significant results. Due to the small sample size, this study may serve to determine the study size required for a larger study to evaluate the hematological parameters and immune markers among HIV-infected and non-infected pre-eclamptic women in South Africa.

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