

**TO IDENTIFY THE CHANGES IN THE HAEMODYNAMICS
IN PATIENTS WITH PRE-ECLAMPSIA
USING BRAIN NATRIURETIC PEPTIDE
AND DOPPLER STUDIES**

**A dissertation submitted to the
Faculty of Health Sciences
University of Kwa Zulu-Natal**

**In partial fulfilment of the requirements
for the Master of Medical Science degree**

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***This study is dedicated to my
Lord and Saviour Jesus Christ and to my family.
Thank you for believing in me.
Your love and support has carried me through.***

STATEMENT OF DECLARATION

I declare that this dissertation is my own unaided work. It is submitted in partial fulfilment for the Master of Medical Science Degree at the University of Kwa Zulu-Natal. It has not been submitted before for any degree or examination at any other educational institution.

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Contents

Contents	5
ABBREVIATIONS	7
CHAPTER 1.....	11
INTRODUCTION	11
Epidemiology	11
Haemodynamics and cardiac changes in PE.....	15
Investigative evaluation of maternal haemodynamics	16
The evaluation of ventricular function	18
Timing of testing	19
Ultrasound and doppler studies	20
Types of doppler studies	23
Correlating central haemodynamics with uteroplacental circulation	26
Effect of treatment	27
Rationale for this study.....	29
CHAPTER 2.....	31
STUDY DESIGN AND METHODS	31
2.1. Aim and objectives	31
2.2 Study Design	31
2.3. Inclusion and Exclusion Criteria.....	32
2.4. Methodology	32
CHAPTER 3.....	39
RESULTS.....	39
3.1 Demographic Data	39
HIV Status	39
Body Mass Index (BMI).....	39
3.2. Laboratory Investigations	40
3.3. Fetal ultrasound at initial assessment.....	41
3.4. Echocardiography findings	42

3.5. B-TYPE Natriuretic Peptide Levels	43
3.6. Correlation Studies (Table 7)	44
3.6. Pregnancy Outcomes.....	45
CHAPTER 4.....	50
DISCUSSION	50
CHAPTER 5.....	54
CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS.....	54
5.1. Conclusion	54
5.2. Recommendations.....	54
5.3. Limitations	54
5.4. Disclosure of Interest.....	55
REFERENCES	56
APPENDIX	69
a) INFORMED CONSENT ENGLISH.....	69
b) INFORMED CONSENT (ISIZULU) ISIVUMELWANO SOKUBA INGXENYE YOCWANINGO.....	70

ABBREVIATIONS

AEDF	Absent End Diastolic Flow
AIDS	Acquired Immunodeficiency Syndrome
ANP	Atrial Natriuretic Peptide
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
CPD	Cephalopelvic Disproportion
DIC	Disseminated Intravascular Coagulation
DOH	Department of Health
ECHO	Echocardiography
EDTA	Ethylenediaminetetraacetic acid
E/ Ea ratio	Mitral valve Doppler inflow velocity (E) / Mitral annulus n Tissue Doppler (Ea)
EFW	Estimated Fetal weight
ENND	Early Neonatal Death
FBC	Full Blood Count
FIG	Figure
GA	Gestational Age
HELLP	Syndrome: <u>H</u> aemolysis/ <u>E</u> levated <u>L</u> iver enzymes/ <u>L</u> ow <u>P</u> latelets
HIV	Human Immunodeficiency Virus
IE	Imminent Eclampsia
IOL	Induction of Labour

IUGR	Intra Uterine Growth Restriction
LA	Left Atrium
LV	Left ventricle
MSL	Meconium Stained Liquor
NICU	Neonatal Intensive Care Unit
PE	Pre-eclampsia
PI	Pulsatility Index
PROM	Prolonged Rupture of Membranes
REDF	Reversed End-Diastolic Flow
ROM	Rupture of Membranes
SA	South Africa
SB	Stillbirth
SD	Standard Deviation
TDI	Tissue Doppler Index
U/S	Ultrasound
UNK	Unknown

TO IDENTIFY THE CHANGES IN THE HAEMODYNAMICS IN PATIENTS WITH PRE-ECLAMPSIA USING BRAIN NATRIURETIC PEPTIDE AND DOPPLER STUDIES

ABSTRACT

AIM:

To determine the haemodynamic changes in pre-eclampsia by using echocardiography and cardiovascular markers i.e. Brain Natriuretic Peptide (BNP); and relating these changes to perinatal outcomes.

MATERIAL AND METHODS:

A prospective study was conducted at a large regional hospital in Durban, KwaZulu Natal. One hundred and fifteen primiparous patients were studied; 63 were normotensive pregnancies and 52 were pre-eclamptics. Patients were matched for age and gestational age. Study participants were examined during pregnancy, labour and within the puerperium. Transthoracic echocardiography, tissue Doppler imaging, umbilical artery Doppler, and laboratory investigations were performed. The ratio of the mitral inflow (E) to the early diastolic tissue Doppler velocity (Ea) was measured as a marker of the left ventricular filling pressure.

RESULTS:

BNP levels were significantly increased in the antepartum (23.8 (2 - 184.1) vs 6.0 (0.5 - 45.2) pmol/L; $p < 0.001$) and during labour (15 (1.8-206.4) vs 8.7 (1.9 -24.8) pmol/L; $p = 0.01$) in the pre-eclamptic group when compared to the normotensive pregnancies. In the postpartum period, median BNP levels were 4.2 (1.7-51.4) and 5.95 (2.2-38.7) pmol/L in the pre-eclamptic and normotensive groups, respectively. There was no statistically significant difference in the tissue Doppler E/Ea between the pre-eclamptic when compared to the normotensive group of patients (11.02 ± 5.6 vs 9.16 ± 2.6 ; $p = 0.058$). The caesarean section rate was 47% in the pre-eclamptic group and 32% in the normotensive group. Eight subjects were lost to follow-up, therefore no inference could be made from this data. There were 2 stillbirths in the pre-eclamptic group, none in the normotensive group.

CONCLUSION:

In pregnancies complicated by pre-eclampsia, changes in the haemodynamic state are accompanied by raised blood levels of BNP in comparison to normotensive pregnancies. These revert to baseline values in the puerperium.

CHAPTER 1

INTRODUCTION

EPIDEMIOLOGY

HYPERTENSIVE DISORDERS OF PREGNANCY

Hypertensive disorders of pregnancy complicates approximately 10-16% of pregnancies and is one of the leading cause of maternal, fetal and neonatal morbidity and mortality worldwide (1, 2). A recent population-based study estimates that hypertensive disorders of pregnancy affect 12% of pregnant women in Durban, South Africa (SA) (3). Hypertensive disorders of pregnancy accounts for about 9% of maternal deaths in Africa and Asia, and about 25% of maternal deaths in Latin America and the Caribbean. The maternal deaths are mainly due to pre-eclampsia and eclampsia (4). According to the recent National Confidential Enquiry into Maternal Deaths (NCCEMD) report in SA, which assessed avoidable factors and missed opportunities in deaths that occurred at health facilities from 1 January 2005 until 31 December 2007, approximately 15% of all deaths were due to hypertension and its complications. Hypertensive disorders of pregnancy were the commonest direct cause of maternal deaths in SA. It was found that over one half (52.7%) of all hypertensive-related deaths were avoidable. The highest number of deaths (21.2%) occurred in the province of Kwa-Zulu-Natal. Furthermore cardiac disease accounted for 2.2% of all deaths (5).

Hypertensive disorders of pregnancy have been classified by The National High Blood Pressure Education Program of the National Health Lung and blood institution (NHLBI) into the following categories: gestational hypertension, chronic hypertension, PE and superimposed PE (6). Gestational hypertension is a working definition used when an elevated blood pressure (BP) (systolic BP \geq 140mmHg and diastolic \geq 90mmHg) is first detected after the twentieth week of pregnancy (in the absence of proteinuria) and returns to normal in the puerperium. Women diagnosed with gestational hypertension may eventually fulfill diagnostic criteria for PE if proteinuria subsequently develops. In the absence of proteinuria, chronic hypertension is diagnosed when elevated blood pressures are present before 20 weeks gestation and remain elevated postpartum. Pre-eclampsia is defined as the presence of hypertension, and proteinuria exceeding 0.3 g/day after the twentieth week of pregnancy in a previously normotensive woman. PE occurs in 2-5% of

pregnancies worldwide but it complicates up to 10% of pregnancies in developing countries where emergency care is often inadequate or lacking (6).

PRE-ECLAMPSIA

The cause of PE remains unclear. Pre-eclampsia is characterised by the new onset of hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mmHg respectively on two occasions at least 6 hours apart) with proteinuria (protein excretion of ≥ 300 mg in a 24 hour urine collection, or a dipstick of $\geq 2+$) that develops after 20 weeks gestation in a previously normotensive woman (7, 8). The cure is delivery of placenta. Known maternal characteristics such as obesity, ethnicity (black race), diabetes, collagen vascular disease, multiple pregnancies, previous episodes of PE, thrombophilias, molar pregnancy and extremes of age (<20 or >40 years) increase the risk for PE. Women with chronic hypertension have a 15–25% increased risk of developing superimposed PE (9). The prognosis for mum and baby depends on the severity of the pre-eclampsia. In mild disease, there is a good prognosis. In severe disease, complications include abruption placenta, eclampsia, renal and liver disease, disturbances of haemostasis and the HELLP syndrome. A higher incidence of pre-eclampsia has been found among women who conceive with assisted reproduction techniques, nulliparous women, and in women with autoimmune conditions demonstrating the probable influence of an inexperienced maternal immune system (10, 11). Women with pre-existing metabolic, vascular or renal disease are at increased risk for superimposed PE (12, 13). This is possibly due to their increased sensitivity to the physiological changes of pregnancy.

Identifying “at-risk” women is important because modern obstetric care places emphasis upon the primary care setting for expectant women. A marker which identifies high-risk women would allow for closer supervision in secondary care. Such a marker would also facilitate recruitment for trials of potential therapeutic agents, for accurate diagnosis, and for timely intervention whenever problems develop.

PATHOPHYSIOLOGY OF PRE-ECLAMPSIA

The placenta is considered central in the pathogenesis of PE (14). This is known to be the case because PE occurs only in pregnancy and resolves after delivery of the placenta. Furthermore, it can occur in the absence of a viable fetus for example, in molar pregnancies (15).

The pathophysiology of PE it is believed to be multifactorial. The teaching has been that PE develops due to an immune maladaptation between the mother and the fetus. Poor placentation is considered the first step in the two-stage theory (16). Trophoblastic invasion of the maternal spiral arterioles occurs early in pregnancy and results in the formation of wide-bore vessels carrying maternal blood to the developing fetus. Defective trophoblastic invasion, possibly due to genetic or immune mechanisms, results in the spiral arterioles remaining as narrow conduits. The result is tissue anoxia due to hypoperfusion (17). The consequence of the tissue anoxia is the release of apoptotic cells, trophoblastic debris and angiogenic factors that causes widespread vascular endothelial damage (18, 19). This leads to the second step of the disorder which is the development of maternal signs and symptoms affecting multiple systems of the body (20).

BIOCHEMICAL MARKERS OF PRE-ECLAMPSIA

The ideal marker would be non-invasive, cheap, with a high sensitivity and specificity and suitable for resource limited settings. Based on the etiology of this life-threatening pregnancy disorder, a major focus of research has recently been maternally expressed proteins and the identification of placental factors showing abnormal expression in pre-eclamptics placentas (21, 22). Their potential use for non-invasive early prediction or early detection would be invaluable. The availability of such markers could potentially improve surveillance of high risk patients and result in earlier referrals and better outcomes. The impact and cost of treating the complications of PE (e.g prolonged ICU admissions, etc) for both the family and the health system could potentially be reduced.

Misdiagnosis and failure to recognise warning symptoms is still an issue in our health care system owing partly to the multiple clinical symptoms associated with the syndrome (23). The availability a reliable biochemical indicator might thus help in making the clinical diagnosis. Biochemical markers would allow the classification of pre-eclamptic patients into categories based on their severity and this would direct the clinical management and improve the pregnancy outcomes (24).

IDENTIFICATION OF NOVEL BIOMARKERS

Although a number of promising biomarkers already exists, a lot of effort is being made to find novel candidates that bear a greater potential to identify women at risk for PE, in order to provide the best possible care for these mothers and children (25, 26). Several approaches have been made (27, 28, 29). Microarrays screen the placental transcriptome for up- and down-regulated transcripts in pre-eclamptic samples and compare these to healthy controls. Comparative transcription analyses

have been performed by numerous groups (30, 31, 32). These groups were however, studying the molecular mechanisms of PE rather than potential biomarkers. The microarray based screening for RNA molecules in maternal circulation that are transcribed in placenta has been reported as a potential source for pregnancy related biomarkers (33, 34), including PE (35, 36). Gene experiments are being performed frequently due to the rapid evolution of the microarray technology.

Another direction of research in the field of complex diseases is metabolomics. It involves the analysis of endogenous and secreted metabolites in a biological system. The reports investigate the placental metabolome using varying oxygen tensions in the plasma of pre-eclamptic patients. It is suggested that this technology may bring new insights into placental function (37, 38).

THE NATRIURETIC PEPTIDE SYSTEM

Most of the molecular markers that have been described above relate to the pathogenesis of the PE process. The early sequelae in PE involve well documented haemodynamic changes which have been investigated and corroborated by invasive and non-invasive techniques. The natriuretic peptide system, first discovered in the early 1980s to regulate salt and body fluid balance, has been shown to closely reflect fluid dynamics in the circulation (39).

Human cardiocytes manufacture a family of structurally related peptide hormones that include atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP), and their metabolically associated peptides (40). Release of the natriuretic peptides is stimulated by haemodynamic stress, such as wall stretch, ventricular dilation, and increased pressures resulting from fluid overload. The natriuretic peptides have powerful natriuretic, diuretic, antimitotic, and vascular smooth muscle relaxing actions. The natriuretic peptides are antagonists for the sympathetic nervous system and the renin-angiotensin-aldosterone axis (41).

BNP has emerged as a superior biomarker to ANP for clinical applications involving heart failure and left-ventricular dysfunction (42). In a study by Cowie and colleagues (43), BNP showed a greater predictive power as an indicator of heart failure when compared with either ANP or its metabolites. Contributing factors are BNP's longer half-life, activation at the gene level and greater quantity in left ventricular tissue. Also, BNP has a 2- to 3-fold more powerful natriuretic and blood pressure lowering effect compared to ANP (44). Thus in vitro diagnostics for BNP and associated metabolites have been the focus for clinical applications (45).

BIOSYNTHESIS AND SECRETION OF BNP AND NT-proBNP

Human BNP is derived from 134 amino-acid precursor termed pre-proBNP1-134. Haemodynamic stress activates cleavage of a 26-aa signal peptide sequence from the N-terminus of pre-proBNP1-134. The remaining proBNP1-108 prohormone is then cleaved by corin; the N-terminal pro-BNP1-76 (NT-proBNP) fragment and active 32-peptide, C-terminal BNP77-108 (BNP) hormone are then released into the circulation. BNP and the metabolically inert NT-proBNP are released in a 1:1 molar ratio.

The release and metabolism of BNP and NT-proBNP is a far more complicated process. The intact prohormone proBNP1-108 may be released into circulation and may cross-react with immunoassays for BNP and NT-proBNP. Also, N-terminal proBNP1-76 in blood may be comprised of numerous fragments and not a single 77 amino-acid molecular entity. Furthermore, BNP appears to undergo metabolism following release into the circulation. Truncation of the 32-amino acid peptide at either the C-terminal or N-terminal arms to an inactive form of BNP has been reported (46).

MECHANISM OF ACTION OF NATRIURETIC PEPTIDES

The primary function of ANP and BNP is to regulate blood volume and pressure. Under high blood volume or pressure, ANP and BNP are released into the circulation. In target organs such as the kidneys and peripheral blood vessels, the peptides activate their receptor, natriuretic peptide receptor –A (NPR-A), and increase intracellular cGMP production. This leads to natriuresis, diuresis, and vasodilation resulting in the lowering of blood volume and pressure. ANP and BNP also suppress the renin-angiotensin release, which is an additional mechanism to regulate vascular tone (47). The blood levels of BNP and NT-proBNP, reflect haemodynamic stress, and as such, may serve as a marker of early haemodynamic changes that accompany PE.

HAEMODYNAMICS AND CARDIAC CHANGES IN PE

During normal pregnancy, plasma volume increases steadily throughout the first two trimesters and reach a plateau at approximately 32 weeks gestation. In a singleton pregnancy at term, plasma volume is nearly 50% more than that seen in a non-pregnant woman. Total peripheral vascular resistance decreases in normal

pregnancy, cardiac output increases, reaching a peak of 30-50% of the pre-pregnancy level by the end of the 2nd trimester. There is usually no further increase (48). In patients with PE, there is a further rise in cardiac output as well as an increase in total peripheral resistance (49).

There is evidence that cardiac function is altered in PE. Significant changes in the volume status and systemic resistance have been documented in patients with pre-eclampsia. Left ventricular mass (and index) in PE patients is higher than normotensive patients and is associated with increased left ventricular wall thickness. There appears to be no difference in left ventricular diastolic filling rates between PE and normotensive patients (49). A further significant increase in left ventricular end-systolic and end-diastolic volumes and reduction in contractile function has been found in PE women. Under these conditions, changes in volume loading may affect ventricular function. There are limited data available on the changes in cardiac structure and function during PE. Most studies focus on changes in normotensive patients (50). A study found that there was reduced cardiac function in pregnancies complicated by pre-eclamptic IUGR when compared to normotensive IUGR pregnancies (51). This was due to reduced intrinsic contractility and reduced diastolic filling. Some markers of the changes in diastolic filling would help to understand the haemodynamic changes of PE. Left ventricular diastolic filling may precede changes in systolic function. Therefore, assessment of diastolic function may provide an early marker of PE.

INVESTIGATIVE EVALUATION OF MATERNAL HAEMODYNAMICS

Several diagnostic investigations have been used to evaluate the cardiac changes that occur in PE, but because of the physiological changes seen even in normal pregnancies (Table 1), interpretation of these findings is often difficult.

Table 1: Chest X Ray and Electrographic Changes in normal Pregnancy

Chest X-ray	Electrocardiogram
Increased cardiothoracic ratio	ST segment and T wave changes
Straightened left heart border	Atrial and ventricular ectopics
Increased pulmonary vascular markings	Leftward shift of QRS axis
Small pleural effusions (early post partum period)	Small Q wave, inverted T wave in lead III

TISSUE DOPPLER ECHOCARDIOGRAPHY

What is required is a sensitive measure of the cardiac changes that occur during PE, which may easily be measured non-invasively. Until recently most of the non-invasive studies of the hemodynamic changes of PE have employed echocardiography with the focus being on cardiac dimensions and systolic function. Tissue Doppler imaging of the heart is a technique that has enabled the evaluation of changes in ventricular filling at a much earlier stage than can be detected by conventional echocardiography. This technique measures the frequency of ultrasound returning from the moving myocardium to estimate the velocity of the myocardial wall. Isaaq et al (1989) were the first to describe the clinical applications of tissue Doppler in cardiac disease, demonstrating that low myocardial velocities at the posterior mitral annulus correlated with abnormal posterior wall motion on left ventricular angiography (52). Gulati et al (1996) demonstrated that tissue Doppler systolic mitral annular velocities correlated with global left ventricular ejection fraction as assessed by radionuclide ventriculography (53). Diastolic tissue Doppler velocities reflect myocardial relaxation, and in combination with conventional Doppler measurements, ratios (transmitral early diastolic velocity / mitral annular early diastolic velocity (E/Ea) have developed to non-invasively estimate left ventricular filling pressure. It has been shown that Tissue Doppler derived Ea correlated with the invasively measured left ventricular time constant of relation (Tau), establishing Ea as a relatively load independent measure of myocardial relaxation in patients with cardiac disease (54).

Echocardiography is a good diagnostic tool as it allows for the structural as well as functional assessment of the heart. Furthermore the cardiovascular changes that have been documented appear to coincide with increases in plasma BNP and have

been observed to be linearly related to the left ventricular structural and functional changes observed in patients with PE (49).

THE EVALUATION OF VENTRICULAR FUNCTION

Our study was designed to evaluate BNP changes in PE as a measure of ventricular function. As previously mentioned, BNP and ANP are polypeptide neurohormones synthesized by cardiac atrial and ventricular myocytes in response to stretch (55). They have a regulatory and modulatory role in the cardiovascular system. They are secreted as prohormones, proANP and proBNP. They are then split into equimolar amounts of active ANP and BNP, and inactive amino-terminal fragments NT-proANP and NT-proBNP (56). BNP levels reflect long-term intravascular volume status. It shows less affinity for clearance receptors and therefore has a longer half life than ANP. ANP is more dependent on atrial filling volume than filling pressure and is rarely measured in clinical practise. BNP and NT-proBNP are more commonly measured, often interchangeable, based on clinician's preference and laboratory availability (57). There is evidence however, that NT-proBNP measurement is superior in detecting mild systolic or diastolic heart failure or asymptomatic left ventricular dysfunction (LVD) (58). There is fast synthesis and release into the blood by myocardial stretch which is increased in LVD. Left ventricular stretch activates neuronal and hormonal pathways that results in BNP being secreted directly into the blood during ventricular dilatation. Increased plasma levels of BNP have been found in patients with LVD from various causes. BNP has a high sensitivity, specificity and predictive value for LVD and is an approved marker for the diagnosis of congestive heart failure in patients with dyspnoea in an acute care setting (59). During normal pregnancies, haemodynamic changes do not place significant stress on the heart. However, increased ANP and BNP levels of statistical significance were found in patients with PE as compared to normotensive pregnant patients. A study which evaluated maternal, umbilical plasma and amniotic fluid BNP and ANP levels in nineteen normotensive pregnant women and thirty five pre-eclamptic patients suggested that increased ANP and BNP levels were a sequel of PE pathophysiology and did not play an etiological role in PE (60). Another study looked at the trends in BNP levels during pregnancy. BNP blood levels were taken during the 1st, 2nd and 3rd trimester in normotensive as well as in patients with PE. In normotensive pregnancies, median BNP levels are < 20pg/ml and stable throughout gestation. An increase in BNP levels in pregnancies complicated by mild PE and an even higher increase in severe pre-eclampsia was found. This may reflect ventricular strain and/or subclinical cardiac dysfunction associated with pre-eclampsia (61). In a study

investigating the role of BNP in the circulation of patients with pregnancy induced hypertension, they found that BNP and ANP participate in maintaining homeostasis of the maternal circulation. This cross-sectional study looked at thirty six normal pregnancies and compared levels of BNP and ANP to seventeen women with pregnancy induced hypertension. They found statistically significant increased levels of BNP and ANP in patients with pregnancy induced hypertension. In patients with severe pregnancy induced hypertension, an eight-fold increase in BNP levels were found. There was a positive correlation between the plasma BNP levels and the mean arterial blood pressure ($r=0.62$, $P<0.001$) (62). Numerous studies have confirmed the definite increase in BNP levels in patients with PE (63, 64). In a study looking at the natriuretic peptides and PE, it was found that the high BNP concentrations found in pre-eclampsia reflect cardiac strain caused by high afterload (64). In a review of the charts of fifteen obstetric patients who presented with acute dyspnoea, seven of them had PE with elevated BNP levels. This correlated with acute ventricular overload and they responded well to volume management and diuresis. In two patients, markedly elevated serum BNP levels and significant left ventricular dysfunction was found that was not apparent by standard clinical evaluation. It was thus concluded that serum BNP levels provided valuable information that could be used in the management of obstetric patients with acute dyspnoea and may assist in identifying patients with cardiac dysfunction that might have been otherwise missed on clinical evaluation (65).

TIMING OF TESTING

So, if serum BNP is activated during pregnancies complicated by PE, the crucial question is “when is the optimal time to test these levels”; and “when do they return to normal?” In a study comparing proBNP levels in pre-eclamptic versus normotensive obstetric patients antenatally, and then later in the puerperium, the proBNP level in the pre-eclamptic group showed a seven-fold increase to that of the control group ($p > 0.05$). Of note is that in the puerperium, these levels normalised (66). In the non-pregnant situation, a study by Bayes-Genis et al., found that there was a good association between the New York Heart Association functional class and NT pro-BNP concentrations; NT pro-BNP concentrations in the emergency rooms did not significantly differ from those after 24 hours; NT pro-BNP concentrations on day 7 did not differ from that at 6 or 12 months and could be considered as the basal stable level for that particular patient (67).

We know that there are dramatic changes in circulating volume in the perinatal period which would influence BNP levels in relation to labour and uterine contractions. Not much is known about changes in BNP on an hour to hour basis. Vasoactive hormones are said to play an important role in the pathogenesis of pre-eclampsia thus linking placental hypoperfusion with hypertension, systemic disease and proteinuria. In a study evaluating the diurnal patterns of vasoactive hormones in PE and comparing these to a normotensive comparable control group, it was found that in the control group there was diurnal variations (blood samples were taken every 2 hours over a 25 hr period) in serum BNP levels amongst others. The BNP concentrations in the PE group tended to be higher throughout the 25 hours but had blunting of the normal diurnal variation (68). Although this study did show certain trends, in clinical practise it is difficult for serum sampling to be done at exactly the same time in our patients.

ULTRASOUND AND DOPPLER STUDIES

Ultrasound allows for a detailed, painless and safe analysis of the placenta and fetus. Numerous investigative modalities have been used by researcher's e.g X-rays by Panigel demonstrating placental maternal blood jet lags, Magnetic Resonance Imaging, 2-dimensional grey-scale ultrasound, colour imaging and now 3-dimensional ultrasound imaging (69). Several medical disorders of the pregnant woman or her fetus begin or end in the placenta. Examples of these include PE, IUGR, triploidy, etc. Ultrasound is the optimal investigation method because of the benefits mentioned above as well as the fact that it allows for placental structural and functional assessment. Three dimensional ultrasound permits evaluation of the placenta in several planes, more precise depiction of internal vasculature and a more accurate assessment of volume.

Pre-eclamptic vascular changes are seen relatively early in pregnancy. At the level of the uterine circulation, significant haemodynamic changes have been documented. Harrington et al confirmed that the development of PE was associated with failure to modify the uterine circulation in early pregnancy as shown by abnormal Doppler values done between 12-16 weeks gestation (70). In another study investigating the vascular mechanical properties and endothelial function in PE with special reference to bilateral uterine artery notch (bilateral uterine artery notch is associated with ischaemic pathology), it was found that a significant number of placentas in the group with PE did not show notable ischaemic or other morphological changes that could explain the role of the placenta in the development of PE. Women with PE

showed signs of endothelial dysfunction significantly more pronounced in women with the bilateral uterine artery notch (71).

Inadequate placental perfusion has led to the use of Doppler ultrasonography to assess the velocity of the blood flow in the uterine arteries. A persistence of an early diastolic notch after 24 weeks of gestation or abnormal flow velocity ratios has been associated with inadequate trophoblast invasion. Pregnancies associated with an abnormal uterine Doppler after 24 weeks of gestation (high pulsatility index and/or presence of an early diastolic notch) are associated with a more than six fold increase in the rate of pre-eclampsia (72).

Among high-risk patients with a previous PE, Doppler ultrasound of the uterine arteries has an excellent negative predictive value, thus it is an important tool in patient management and care which is of paramount benefit for patients with PE in a previous pregnancy. A systematic review evaluated the use of Doppler ultrasonography in PE. A total of 74 studies were included. Of these studies, 69 were cohorts, 3 were randomized controlled trials and 2 were case-control studies. These studies involved a total of 79547 patients, 2498 of whom developed PE. The authors showed that Doppler ultrasonography of the uterine arteries were more accurate in the second trimester than in the first trimester. The most predictive measurement of the Doppler ultrasonography to predict PE was shown to be the pulsatility index (alone or in combination with a persistent notching after 24 weeks of pregnancy) (73).

A study showed that the combined measurement of uterine perfusion in the second trimester and analysis of angiogenic markers have a high detection rate for pre-eclampsia (74). However, data does not support the use of Doppler ultrasonography for routine screening of low risk patients for PE. A multicentric study involving nine hundred and sixteen low risk pregnancies were followed up with end points being PIH, low birth weight for gestational age, small for gestational age with abnormal outcome and PIH requiring delivery. The study was blinded. Umbilical and uterine artery Dopplers were performed nineteen to twenty four weeks gestation and at twenty six to thirty one weeks gestation. This study found the positive predictive value to be low. It did, however, identify the severe forms of PIH and small for gestational age fetus' in mid pregnancy but could not be used as a screening tool in a low risk population where the prevalence of the disease is low (75). Doppler velocimetry of the umbilical cord has been found to be a more specific and sensitive method of fetal surveillance than cardiotography. It is a fast and convenient method for monitoring fetal well being in high risk pregnancies (76).

Ultrasound and Umbilical Artery Dopplers have also been well studied and their importance in predicting fetal outcomes is well established (77, 78). The use of Doppler studies even in mild PE has been shown to be essential and prompt response to abnormalities validated as shown in the following case report. A pregnant woman with mild PE and severe growth restriction, with reverse end-diastolic flow velocity detected on uterine arteries by colour Doppler was managed expectantly. Follow up Doppler studies of the internal iliac and umbilical arteries also showed reverse flow. This fetus died two weeks after initial abnormality detected (79).

Another study estimated systolic/diastolic ratios in a low risk population to see if this could be used as a screening tool. It was found not to be useful as the positive predictive value was calculated to be between zero and five percent. It was also found to be of no clinical use in predicting IUGR in this population group. One thousand six hundred and sixty five Doppler examinations were performed on five hundred and sixty five women in this study. Dopplers were performed at 27-31, 32-36 and 37-42 weeks gestation. Low dose aspirin (60mg) was also used in this double blinded prevention trial from twenty four weeks gestation. Aspirin did not affect the relation between the Doppler indices and outcomes using the logistic regression model (80). In our study, we looked at the Resistance Index (RI) in patients in the third trimester.

“Reverse Flow” as a sonographic finding, indicates the appearance of retrograde blood flow in the diastolic part of the cardiac cycle and is an indicator of deterioration of the fetal condition (81). Reversed end-diastolic flow velocity is well known to be an ominous finding. A study conducted on thirty patients during the third trimester with reversed end diastolic flow found a perinatal mortality rate of fifty percent. All newborns were admitted to the NICU. Statistically significant complications included caesarean sections, fetal acidosis and small for gestational age babies (82). It is thus imperative that intensive and frequent surveillance be carried out and prompt aggressive management plans be implemented in an attempt to alleviate these devastating outcomes when reverse end-diastolic flow is identified.

Abnormal Doppler umbilical artery waveform is a strong predictor of perinatal mortality and is associated with a poor perinatal outcome. The study, conducted over 2 year period, included 43 patients with severe PE. Of these, twenty-two (52%) had abnormal Doppler umbilical pulsatility index. The abnormalities included: positive end diastolic velocities with elevated pulsatility index values (13 patients/ 59%), absent end diastolic velocometries (7 patients/ 32%) and reversed flow (2 patients/

9%). There were 6 perinatal deaths in this group with abnormal umbilical artery Dopplers, two in the reversed flow (100%), two in the absent flow (28%) and 2 in the positive end diastolic flow (15%). There were no perinatal deaths in the group with normal umbilical Doppler waveform. Of note is that neonates with abnormal pulsatility index had lower birth weights, lower APGAR score at 5 minutes, higher admission rates to the neonatal intensive care units and significant neonatal morbidity as compared to those with normal velocimetry ($p < 0.05$) (83).

Interestingly, in a study looking at the independent contribution of absent or reversed end-diastolic umbilical artery Doppler flow in the prediction of adverse neonatal outcomes, only an increased frequency of hypoglycaemia (OR 1.7) and polycythemia (OR 1.7) were found whereas other negative outcomes could be explained by prematurity and IUGR. Two hundred and seventy pregnancies complicated by PE with either absent or reversed flow were followed up. Other outcomes included low APGAR scores, thrombocytopenia, jaundice, neonatal mortalities, etc. Gestational age, oligohydramnios and IUGR were controlled for (84). An abnormal Doppler result usually precedes the appearance of an abnormal biophysical profile. Fetal well being by weekly assessing umbilical artery Dopplers and biophysical profiles were investigated in patients with pre-eclampsia in their third trimester. It was found that thirty eight percent of patients had an abnormal Doppler on the last evaluation before delivery. In this group, significantly higher blood pressures and serum uric acid were recorded, lower platelet counts, higher incidence of IUGR, lower APGAR score at five minutes, a higher incidence of perinatal deaths and higher operative delivery rates. Abnormal Doppler results clearly help to identify fetus at risk requiring intensive surveillance (85).

TYPES OF DOPPLER STUDIES

So which Doppler examination is the best in evaluating underperfusion and should be used in the management of patients with PE? Numerous sites have been evaluated with often contradicting results. Intraplacental villous artery Doppler measurements have been found to be of no value in the clinical management of patients with severe pre-eclampsia (86). A study looking at forty three patients; twenty normotensive and twenty three with severe PE found that intraplacental villous flow were within normal limits even in patients with abnormally high umbilical artery resistance indices. There was no difference in the intraplacental villous artery resistance indices between the two groups. Failure to detect villous artery Doppler flow signals was associated with fetal compromise (86).

Whether uterine artery Doppler, umbilical artery Doppler or both should be examined in high risk pregnancies has been a topic of much debate with conflicting results. A study examined two hundred and eighty women with singleton pregnancies in the third trimester. While blood flow in the cerebral and umbilical arteries remained unchanged, Doppler indices from the main uterine branches showed increased resistance. This study concluded that increased uterine artery resistance is an early indicator of increased risk to the fetus when compared with umbilical and fetal cerebral artery Dopplers (87). Another study involving one hundred and forty two patients with intra-uterine growth restriction (birth weight <10th percentile) and/ or PE/ HELLP Syndrome, was conducted where they examined the umbilical artery Doppler and bilateral uterine artery Dopplers. Patients were divided into six groups based on Doppler findings i.e. uterine artery unilaterally/ bilaterally normal/ pathological and umbilical artery normal/ pathologic. The median birth weight, gestational age at delivery and frequency of occurrence of complications were calculated. The birth weight and gestational age at delivery were significantly lower where there was a pathologic result in all three vessels. PE/ HELLP Syndrome were found in only six percent of patients who had pathological findings in the umbilical artery Doppler. However, ninety percent of patients who had pathologic findings on uterine artery Doppler developed PE/ HELLP syndrome. The umbilical artery Doppler was normal in twenty eight percent of this high risk group. Without performing the uterine artery Dopplers, the impaired placental haemodynamics would have been missed. The authors advised examination of both uterine and umbilical artery Doppler as an important tool in assessing placental performance and high risk groups (88). In another study confirming the importance of both uterine and umbilical artery Doppler examinations in predicting adverse outcomes in pregnancies complicated by PE, a correlation between maternal blood pressure, proteinuria and placental vascular resistance was sought. One hundred and eighty pre-eclamptic patients were included in the study. They found that the uterine artery velocimetry was not related to the maternal blood pressure or the degree of proteinuria. However, abnormal arterial blood velocity waveforms on both sides of the placenta were significantly related to increased caesarean section rates, younger gestation at delivery, lower birth weight and increased frequency of admissions to the neonatal intensive care unit (NICU). Bilateral rather than unilateral uterine artery notches were predictive of poor perinatal outcome. This study concluded that the best predictor of adverse outcomes was a combination of umbilical and uterine artery Doppler waveform results. They could also be used to predict the need for operative interventions during labour and delivery (89).

To support the value of performing both uterine and umbilical artery Dopplers, a retrospective study looked at the frequency of increased uterine artery vascular impedance in the third trimester, its relationship to abnormal umbilical artery Doppler and adverse pregnancy outcomes. Five hundred and seventy pregnancies complicated by PE were analysed. Increased umbilical artery vascular impedance was seen in 59 cases (10.4%) whereas increased uterine artery pulsatility index or notching, or both were seen in 207 patients (36.3%). Signs of increased uteroplacental vascular impedance were more common in severe than in mild PE (57.4% vs. 31.4%). This study showed that only one-third of pre-eclamptic cases analysed showed signs of increased uterine artery vascular impedance in the third trimester. Increased vascular resistance was more frequent in the uterine than in the umbilical artery and was strongly related to adverse outcomes of pregnancy (90). Uterine artery Dopplers are technically difficult to perform in the third trimester and are discomforting for the patient especially when the presenting head has engaged into the pelvis.

The use of uterine artery Doppler waveform in the third trimester in growth restricted infants delivered at ≥ 34 weeks gestation was looked at. This study was conducted over four years and all consecutive euploid singleton fetuses with accurate dating diagnosed with IUGR were included into the study. After PE was controlled for, it was found that in growth restricted infants delivered at \geq thirty four weeks gestation, the presence of abnormal Doppler waveforms at the uterine arteries at diagnosis is associated with a four-fold increased risk of adverse neonatal outcomes (these events included increased rates of admission to NICU and low birth weights) (91).

However, another study found that an abnormal umbilical artery waveform was a strong and independent predictor of adverse perinatal outcomes in patients with PE (92). This study looked at seventy two consecutive patients admitted with severe PE. Doppler velocimetry was performed within seven days of delivery and perinatal outcomes noted. Adverse perinatal included necrotising enterocolitis, APGAR score $<$ seven at five minutes, perinatal death, acute renal failure and significant neonatal morbidity. After confounding variables were adjusted for, it was found that an abnormal umbilical artery waveform was a significant independent predictor of adverse perinatal outcomes (OR 14.2; $p < 0.005$) (92). Another study supporting the use of umbilical artery flow velocimetry alone included one hundred and seventy one women with hypertensive pregnancies at thirty six to forty two weeks gestation. The Doppler ultrasonography profile included umbilical artery Doppler flow velocimetry waveforms, amniotic fluid volume, fetal movements, placental grading

and fetal growth pattern. Doppler ultrasonography profiles were correlated with the neonatal outcome (one minute APGAR score or admission to the NICU as the main outcomes). Only four patients had PE. The majority of patients (one hundred and forty five) had gestational hypertension, while twenty two had chronic hypertension. Doppler ultrasonography was found to have a high predictive value in assessing fetal wellbeing in hypertensive pregnancies. They also concluded that the umbilical artery Doppler flow velocimetry alone is a significant variable in the multiple logistic regression analysis (93).

CORRELATING CENTRAL HAEMODYNAMICS WITH UTEROPLACENTAL CIRCULATION

A study using Doppler velocimetry measurements was designed to correlate the relationship between the uteroplacental circulation, central haemodynamics and perinatal outcomes in pregnancies complicated by severe PE. Patients enrolled into the study had no other medical conditions. Doppler investigations were performed on the maternal central haemodynamics as well as umbilical and uterine arteries on admission. Patients were divided into three groups based on their systemic vascular resistance levels. They were then followed up and perinatal outcomes analysed. An inverse relationship was noted between the left ventricular function and cardiac index, and vascular resistance. As the vascular resistance increased, the left ventricular and cardiac index decreased. Small for gestational age was found to be statistically significant in the group with high resistance and was postulated to be due to reduced blood flow to the uteroplacental circulation. The authors concluded that in order to maintain adequate uteroplacental perfusion in pregnancies with high uterine artery resistance, a high cardiac output in low systemic-vascular-resistance might compensate (94).

Two studies have shown a correlation between intra-uterine weight and maternal ventricular function. The first study was performed to compare the maternal cardiac function in patients with pregnancies complicated by intra-uterine growth restriction and those with small for gestational age babies. This study showed that in the intra-uterine growth restriction group, maternal cardiac output was lower while the total vascular resistance was higher when compared to the mums in the small for gestational age group. A model using total vascular resistance, left atrial diameter, fetal middle cerebral artery pulsatility index and gestational age yielded a sensitivity of 96.2% and a specificity of 84.6% for the detection of intra-uterine growth restriction. The authors concluded that maternal echocardiography provides a

sensitive tool for identifying pregnancies complicated by intra-uterine growth restriction (95).

A second study showed that certain haemodynamic changes in pregnancies complicated by intra-uterine growth restriction preceded the clinical manifestation of the intra-uterine growth restriction. While physiological changes in blood pressure, heart rate and stroke output were seen in the small for gestational age group, in the IUGR group, the total vascular resistance (TVR) was increased. A significant inverse linear correlation was observed between TVR and weight ($r=0.83$; $p<0.001$) (96).

EFFECT OF TREATMENT

Methyldopa is the most common antihypertensive drug used in pregnancy in our setting. A study looking at its effects on umbilical artery blood flow found that it decreases umbilical artery vascular resistance in mild PE and chronic hypertensive pregnant women. The study involved ninety patients who were subclassified as either treated or untreated chronic hypertensives, pre-eclamptics or normotensive patients. They were commenced onto treatment with methyldopa twenty four hours after admission. The pulsatility index was measured on two occasions i.e. day zero and day seven. This study showed a statistically significant decrease in the BP recordings in the chronic hypertensive patients only ($p <0.05$). The PI in the umbilical artery decreased in the treated pre-eclamptic and chronic hypertensive group, while there was no difference in the normotensive and untreated pre-eclamptics and chronic hypertensives. After treatment, there was no difference in the umbilical artery PI in all five groups and birth weights were similar (97).

There has been contradicting results from studies looking at the effects of plasma volume expansion and drugs on Doppler velocimetry (98, 99, 100). For example, plasma volume expansion was not shown to influence the pulsatility indices of the umbilical and middle cerebral arteries in a study involving two hundred and sixteen patients with severe pre-eclampsia and other hypertension related complications. Patients were infused with 250mL hydroxyethyl starch 6% twice daily in 4 hours and NaCl 0.9% between these doses and with intravenous medication while the control group did not receive any plasma expanders. Measurements of the umbilical and middle cerebral pulsatility indices were done on 4 occasions and not found to be any different between the groups. The only difference was a significant decrease in the haemoglobin concentration as would be expected in the treatment group (98). A randomised trial of plasma volume expansion in hypertensive disorders of pregnancy

looked at the changes on the pulsatility indices of the fetal umbilical artery and middle cerebral artery after treatment was initiated. It was found that there was a significant decrease in the umbilical artery pulsatility index after both plasma volume expansion and dihydrallazine administration (99). The response to acute versus chronic antihypertensive drugs has also been evaluated. In patients with pregnancy-induced hypertension, acute and chronic blood pressure reduction did not change the systolic / diastolic ratios in the umbilical or maternal brachial artery. This study did however show a transient rise in the uteroplacental systolic/diastolic ratio (100). Another study looked at the effect of a single oral dose of nifedipine compared to that of an infusion of hydralazine on central haemodynamics in pregnancies complicated by severe pre-eclampsia. There was a similar reduction in blood pressure and systemic vascular resistance and a similar rise in heart rate and cardiac output. There was a greater decrease in pulmonary capillary wedge pressures with dihydralazine (101). Magnesium sulphate and qingxintong administration have been found to increase the time average velocity and volume of blood flow ($p < 0.05$) through the uterine and umbilical arteries in patients with pregnancy induced hypertension. This study compared thirty one patients with PIH and seventy one normotensive patients. Initially the TAV and Q values were low ($p < 0.05$) but subsequently improved after treatment. Interestingly, this study also showed elevated levels of serum estriol and human placental lactogen levels (102). As discussed earlier, colour flow Doppler imaging is clearly a valuable predictive index of fetal and placental function (88). Another study mentioned above looked at the role of low dose aspirin (60mg) in the prevention of pre-eclampsia in a low risk population. Healthy nulliparous patients with a singleton pregnancy were enrolled into the study at twenty four weeks gestation. They were followed up throughout pregnancy with regular Doppler examinations of the umbilical arteries and adverse pregnancy outcomes noted. Logistic Regression model was used and treatment with aspirin was not found to affect the relation between Doppler indices and adverse pregnancy outcomes (80).

ADVERSE MATERNAL AND FETAL OUTCOMES IN PRE-ECLAMPSIA

In the mother, PE can manifest either as a mild syndrome with proteinuria and hypertension late in pregnancy or a severe disease with widespread endothelial dysfunction and end organ damage. It is a multisystem condition. Endothelial dysfunction is believed to be due to an imbalance in circulating vasoactive substances. This results in 'leaky' capillary beds which produces clinical edema. This may be generalised and patients are prone to pulmonary oedema. When cerebral

oedema is present, patients present with headaches, visual changes (e.g. blurring of vision) and seizures. The hallmark renal lesion of PE is termed glomerular endotheliosis. It is associated with reductions in glomerular filtration rate (GFR) and proteinuria. Thrombotic microangiopathy, also known as The HELLP syndrome is characterised by Haemolysis, Elevated Liver enzymes and Low Platelets and develops in 10–20% of pregnancies complicated by severe PE (103).

Women who previously experienced PE remain at increased long-term risk for cardiovascular and cerebrovascular events, relative to women with normotensive pregnancies (104). In a retrospective study of more than 1 million Canadian women, those who previously suffered PE, gestational hypertension, placental abruption, or placental infarction were twice as likely to develop premature cardiovascular disease at a median follow-up of 8.7 years, compared with those without a maternal placental syndrome (105).

Furthermore infants of affected mothers are at risk for the complications of premature delivery, intrauterine growth restriction, oligohydramnios and still births (106).

RATIONALE FOR THIS STUDY

Non-invasive methods for cardiac monitoring have limitations; therefore there is a need for reliable biomarkers of cardiac function and risk assessment. However the available biomarkers to date have not consistently been able to discriminate among the different clinical phenotypes in hypertensive disorders of pregnancy. Hence there is a need for exploration of other biomarkers of PE. Given that the vasoconstriction and volume retention that characterises hypertensive disorders of pregnancy, it is possible that the biomarkers of cardiac stress may be useful in diagnosing women with these disorders. BNP is a protein released by the ventricles in the presence of myocytic stretch, and has been correlated to left ventricular filling pressures and, independently, to other cardiac morphological abnormalities. In addition, BNP is significantly affected by age, sex, renal function and obesity. Given its correlation with multiple cardiac variables, BNP has high sensitivity, but low specificity, for the detection of elevated left ventricular filling pressures. The clinical use of BNP to diagnose patients with heart failure is now well established but little is known about the clinical use of this marker in hypertensive disorders of pregnancy. There is evidence that cardiac function is altered in PE. Significant changes in the volume status and systemic resistance have been documented in patients with PE. Given that BNP is a protein released from the cardiac ventricles in response to

myocytic stretch, elevated left ventricular diastolic pressures cause elevation of plasma BNP. We performed Doppler studies of the heart and measured plasma BNP levels in pre-eclamptic and normotensive patients and correlate these findings with perinatal outcomes.

CHAPTER 2

STUDY DESIGN AND METHODS

2.1. AIM AND OBJECTIVES

AIM:

The aim of this study is to identify haemodynamic changes in patients with pre-eclampsia by evaluating serum BNP levels and performing Doppler studies of the heart and umbilical cord.

OBJECTIVES:

- 1) To evaluate the changes in volume status in PE using BNP and echocardiographic changes
- 2) To evaluate the role of BNP in the management of PE
- 3) To relate changes in BNP to fetal outcome as assessed by the APGAR score and fetal condition.

2.2 STUDY DESIGN

- Study population:
Consenting primigravidae in the third trimester of pregnancy
- Sampling strategy:
Convenient sampling
- Statistical planning (variables/ confounders):
52 pre-eclamptic and 63 normotensive pregnant patients
- Sample size:
115 patients

2.3. INCLUSION AND EXCLUSION CRITERIA

- Inclusion criteria:
 - 1) Women in their first pregnancy who had a blood pressure of at least 140mmHg systolic and 90 mmHg diastolic pressure on 2 occasions for the first time after the twentieth week of pregnancy. All women satisfying this criteria, had to have at least one plus of proteinuria on urinary dipstick.
 - 2) Patients in whom informed consent can be obtainable
- Exclusion criteria:
 - 1) Multiple pregnancy
 - 2) Multigravidae
 - 3) History or clinical evidence of pre-existing hypertension/ cardiac or renal disease

2.4. METHODOLOGY

2.4.1 Data Collection Methods And Tools

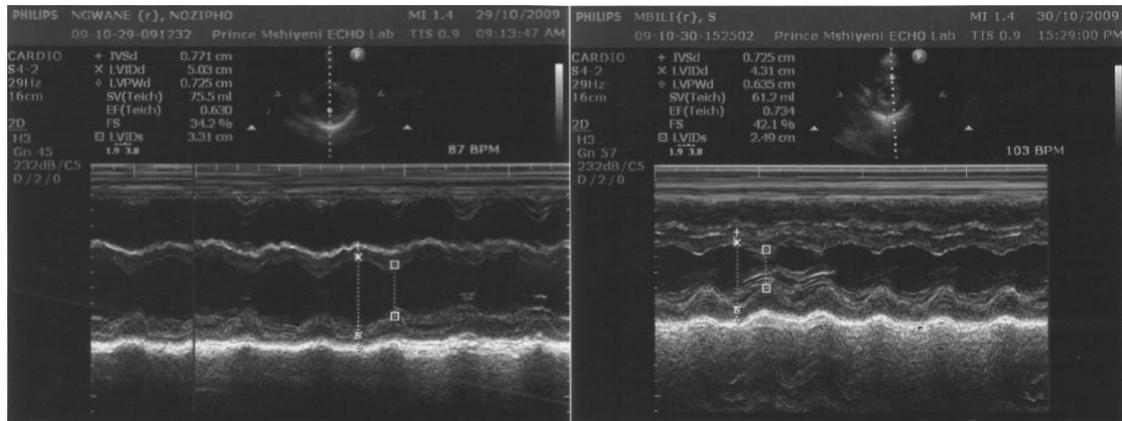
Selection of suitable participants was undertaken after discussion and obtaining informed consent (see appendix 1) from antenatal patients attending Prince Mshiyeni Memorial Hospital antenatal clinic in Durban. A full history and clinical examination was performed. Baseline blood investigations included a full blood count, urea and creatinine, urates and BNP levels. Proteinuria was tested for by the dipstick method (Ames). Echocardiography (including a Tissue Doppler Imaging) was performed by an experienced sonographer at recruitment. An obstetric ultrasound was performed on all patients. Fetal wellbeing was assessed at ultrasound in terms of appropriate growth for gestation and placental sufficiency with the aid of placental Doppler flow measurements. Pregnancies were followed and timing and mode of delivery noted. Plasma BNP levels were repeated during labour (or within 24 hours post delivery). The baby at birth was assessed in respect of APGAR scores, admission to neonatal intensive care and condition on Day 7 following delivery. Maternal serum BNP was collected within the puerperium.

2.4.2. Data Analysis Techniques

a) Echocardiography and Tissue Doppler

All patients had standard M-mode 2-dimensional Doppler echocardiograms performed by the same highly skilled examiner who was unaware of the patient's blood pressure values. Doppler echocardiography was performed using a HD imaging system (Philips) with phased array transducer and an emission frequency of 3.0 megaHertz with the patient in the left decubitus position. Parameters were measured e.g. the LV end-systolic and end-diastolic dimensions, LV wall thickness, and left atrial (LA) dimensions were measured according to American Society of Echocardiography guidelines using the leading edge method (107). The left atrial volume was estimated using the biplane ellipsoid formula. The LV end-systolic and end-diastolic volumes and the ejection fraction were measured from the apical four-chamber view using the Modified Simpson's method (108). Tissue Doppler Imaging (TDI) was performed with transducer frequencies of 1.8–3.6 MHz with minimum optimal gain as possible to obtain the best signal to noise ratio. The mitral inflow pattern was used to measure the peak early (E) and late (A) filling velocities. End expiratory tissue Doppler views were obtained of the inferoseptal side of the mitral valve in pulsed mode. The angle between the Doppler beam and the longitudinal motion of the annulus was kept to a minimum in order to obtain the highest tissue velocities with the spectral pulse wave Doppler velocity adjusted to an appropriate range. The diastolic tissue velocity pattern at the annulus was used to measure the peak early (Ea) and late (Aa) tissue velocities. The ratio of the peak early mitral inflow velocity to the peak early tissue velocity (E/Ea) was calculated as an estimate of the left ventricular filling pressure. The following figures are shown of 2 randomly selected study patients.

Figure 1a: M-Mode recording in a normotensive patient (A) and in a pre-eclamptic patient (B).



Patient A (Normotensive)

Patient B (Pre-eclamptic)

Figure 1b: Doppler recordings in a normotensive patient (A) and in a pre-eclamptic patient (B) across the mitral valve.

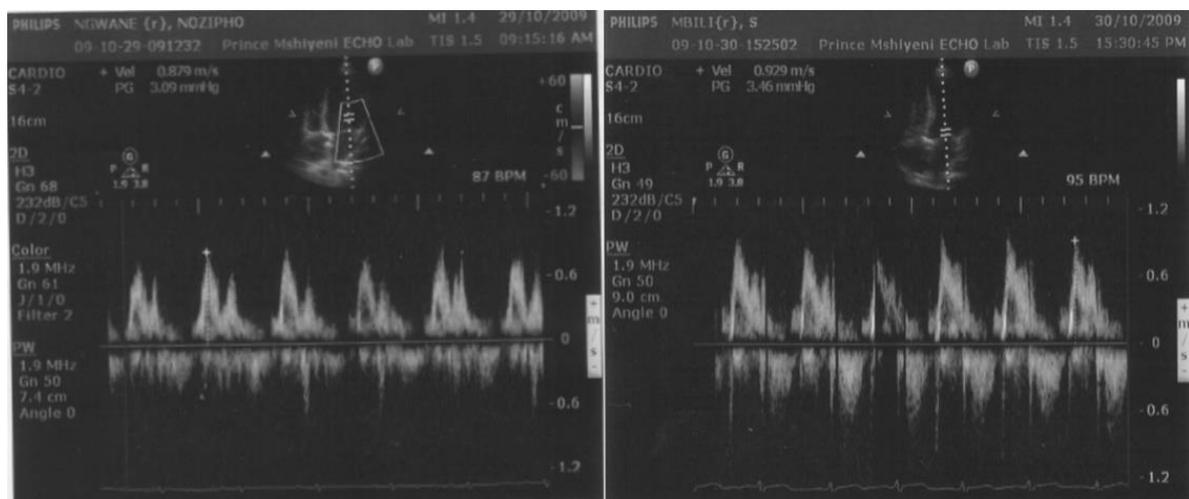
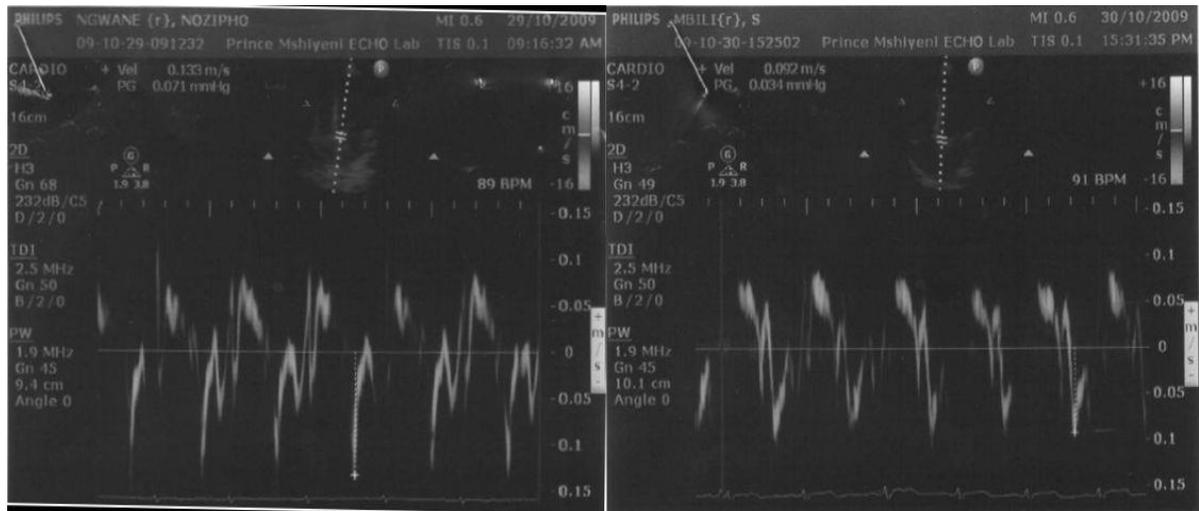


Figure 1c: Tissue Doppler recordings in a normotensive patient (A) and in a pre-eclamptic patient (B) across the lateral wall of the mitral valve annulus.



Patient A (Normotensive)

Patient B (Pre-eclamptic)

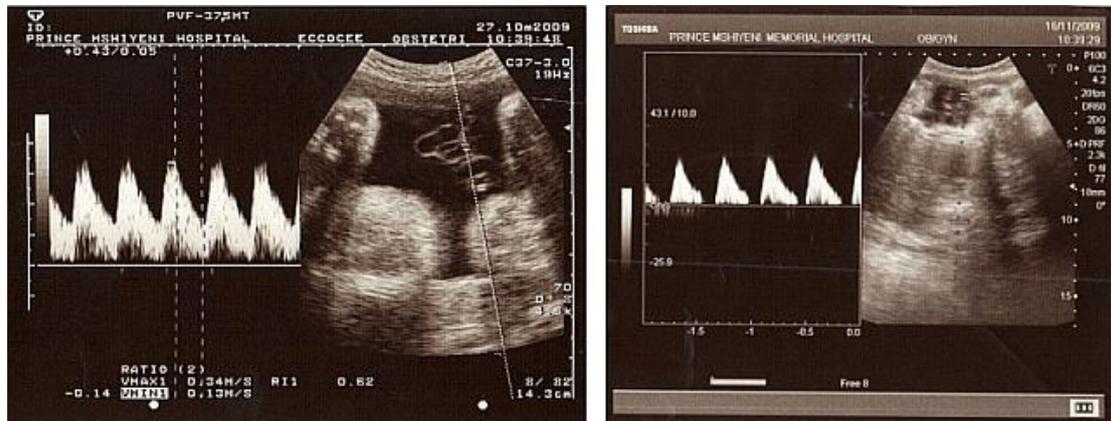
b) Brain Natriuretic Peptide

Three venous blood samples were taken in plastic specimen tubes containing ethylenediaminetetraacetic acid (EDTA). Three specimens were collected from participants i.e. at the time of recruitment, intra-partum and the last specimen was collected from day 7 post delivery until the end of the puerperium. The samples were transported on ice to the laboratory where they were centrifuged. Standard handling of specimens was practised (109). The plasma was stored at -20°C and NT-proBNP was assayed in batches by standard electrochemiluminescence immunoassay (“ECLIA”) using the Modular Analytics E170 (ELECTYS module) and Elecsys 1010/2010 analyzer (Roche diagnostics,). Samples from cases and controls were stored for the same duration and were handled together, identically. According to the National Committee for Clinical Laboratory Standards (NCCLS) (109), the resting BNP values considered normal for this methodology lie below 100 pg/mL. The within-assay and total precision coefficients of variation for NT-proBNP mean 208 pmol/l is 0.8% and 4.5% respectively. The reading sensitivity is $<2.0\text{-}5000\text{pg/ml}$ (0.58-1445pmol/L)

c) Fetal Ultrasound and Umbilical Artery Doppler

Routine biometrical ultrasound was performed on patients using a Toshiba (Nemio) scanner in B-Mode. In B-Mode, the image shows all the tissue that is transversed by the ultrasound probe in a 2-dimensional view. It is also known as B-Mode sections. If multiple B-Mode images are watched in rapid sequence, they become 'real time' images (110). A low frequency (3.75 MegaHertz) curvilinear probe was utilised with patients lying supine. The sonographer was blinded to the study. Standard principles of measurements were used (111). The estimated fetal weight (EFW) was automatically calculated after measurement of the abdominal circumference (AC), femur length (FL) and biparietal diameter (BPD). BPD was measured as the distance between the parietal eminences on either side of the skull. The projection of the femur in a transverse section was identified. It was scanned at 90 degrees to contain a longitudinal section. Measurement was made from one end to the other end. The abdominal circumference was measured in a cross sectional view (as round as possible), at the level of the fetal liver. Antero-posterior and transverse diameter from the outer edge of the fetal abdomen on one side to the outer edge on the other side were used. The abdominal circumference was measured by multiplying the sum of the two dimensions by 1.57 i.e. $AC = (AP \text{ diameter} + T \text{ diameter}) / 1.57$. Umbilical artery Doppler studies were then performed on all patients at the time of the fetal ultrasound. Pulsed wave Doppler was used to measure the speed of flow in the umbilical artery. The Resistance Index (RI) was computed by measuring the peak of systole and then dividing it by the sum of measurements at peak systole and diastole. $RI = \text{systole} / (\text{systole} + \text{diastole})$ as an average over three cardiac cycles (111).

Figure 2: Transabdominal ultrasound showing (a) normal umbilical artery Doppler in a normotensive pregnant patient and (b) absent end diastolic flow in the umbilical artery Doppler in a pre-eclamptic pregnant patient.



Patient A

Patient B

2.4.3. Statistical Analysis

SPSS version 11.5 (SPSS Inc. Chicago ILL, USA) was used for statistical analysis. Outcome measures were BNP levels, echocardiographic findings and pregnancy outcomes in both groups. Chi square statistics or Fisher's exact tests were used where appropriate to examine associations between categorical exposures and outcomes. Independent two sample t-tests were used to compare mean BNP levels between two categories. A p value of <0.05 was considered as statistically significant. Results are presented as variables and standard deviation (SD), and for BNP and U/S as median values with ranges in brackets. BNP values were not normally distributed and were log transformed for analysis. Spearman's test was used for correlation studies between BNP, TDI and RI.

2.4.4. Funding

Funding was obtained from The Department of Cardiology Research fund.

2.4.5. Ethical Approval

The University of KwaZulu/ Natal Postgraduate committee approved the study (PG 032/08). Ethics approval was obtained from The University of Kwa Zulu-Natal

Biomedical Research Ethics Committee (Reference number: E335/05). Informed consent was obtained from all patients involved before inclusion into the study (Appendix 1). KwaZulu/Natal Department of Health approval was obtained in August 2009.

CHAPTER 3

RESULTS

3.1 DEMOGRAPHIC DATA

One hundred and fifteen primiparous pregnant patients gave consent to participate in the study. Fifty two patients were pre-eclamptic with a mean age of 21.5 ± 4.7 years and sixty three patients were normotensive pregnancies with a mean age of 20.4 ± 3.7 years ($p=0.063$). The systolic and diastolic blood pressure of the pre-eclamptic patients were significantly higher when compared to the normotensive pregnancies ($p<0.001$). There was no difference in gestational age at entry and in mode of delivery in both groups. Demographic data of the normotensive pregnancies and the pre-eclamptic patients are shown in Table 2. By design there were no differences in gestational age at enrolment. Gestational age at delivery was similar in both groups.

HIV STATUS

Seventeen patients in both groups were HIV positive of which five had CD4 cells count less than 200 cells/mm.

BODY MASS INDEX (BMI)

Both groups were considered overweight. There was no statistical significance in the body mass index between the pre-eclamptic group when compared to the normotensive pregnancies (29.4 ± 7.9 vs 27.9 ± 5.5 ; $p=0.129$).

Table 2: Demographic data in normotensive and pre-eclamptic patients (mean \pm SD)

Parameter	Normotensive (n=63)	Pre-eclamptic (n=52)	p value
Age (years)	20.4 \pm 3.7	21.5 \pm 4.7	0.063
BMI (weight / height ²)	27.9 \pm 5.5	29.4 \pm 7.9	0.129
Gestational age at entry (wks)	34.5 \pm 2.7	34.3 \pm 2.7	0.567
*Gestational age at delivery (wks)	37.8 \pm 1.7	35.7 \pm 2.8	<0.001
Edema	0 (0-1+)	2+ (1+-4+)	<0.001
HIV Infected	17 (27%)	17 (32%)	
Blood Pressure (mmHg)			
SBP	127.03 \pm 12.9	163.2 \pm 17.6	<0.0001
DBP	78.32 \pm 8.6	104.4 \pm 11.9	<0.0001
Pulse (bpm)	84.7 (72 - 96)	86.0 (72-106)	0.271

SBP= systolic blood pressure; DBP= diastolic blood pressure; CVS=cardiovascular; bpm= beats per minute; SD= standard deviation

*Gestational age at delivery for 7 normotensives and 1 pre-eclamptic could not be determined as they did not return for follow-up (n=56 and 51 respectively).

3.2. LABORATORY INVESTIGATIONS

The detailed laboratory analysis of serum and urine is shown in Table 3. There was no difference in hemoglobin levels between the two groups. Platelet count and haematocrit were lower in the pre-eclamptic group. Ten patients in the pre-eclamptic group developed thrombocytopenia and one in the normotensive pregnancies. Serum urea, creatinine and uric acid levels were significantly elevated in the pre-eclamptic group. The mean level of proteinuria as assessed by dipstick was 2+ in the pre-eclamptic group and 0 in the normotensive pregnancies.

Table 3: Laboratory variables (mean and SD given)

Parameter	Normotensive (n=63)	Pre-eclamptic (n=52)	p Value
Full blood count			
Hemoglobin (g/dl)	10.9 ± 1.3	11.3 ± 1.4	0.190
Platelets (x10 ⁹ /L)	251.8 ± 82.8	215.5 ± 82.6	0.021
Haematocrit (%)	33.1 ± 3.1	34.2 ± 3.6	0.073
Blood chemistry			
Urea (mmol/L)	2.5 ± 0.7	3.08 ± 1.18	0.002
Creatinine (µmol/L)	54.5 ± 8.9	62.4 ± 12.7	<0.001
Uric acid (mmol/L)	0.27 ± 0.06	0.33 ± 0.07	<0.001
Urine Dipstick			
Proteinuria	0 (0 to 1+)	2+ (1+ to 3+)	<0.001

3.3. FETAL ULTRASOUND AT INITIAL ASSESSMENT

There was no difference in the GA and EFW between the groups. The liquor volume was adequate in 60 patients with the normotensive pregnancies and forty two in the pre-eclamptic group. In the normotensive group there was one patient with polyhydraminos and in one patient, the liquor volume was decreased. In the pre-eclamptic group there were six patients with polyhydraminos and in three patients the liquor volume was decreased. The RI was significantly higher in the pre-eclamptic group compared to the normotensive group (0.68 ± 0.06 vs 0.63 ± 0.05; p<0.001). Results expressed as mean (+/-SD). Details are shown in Table 4.

Table 4: Fetal ultrasound at initial assessment

Parameter	Normotensive pregnancies (n=63)	Pre-eclamptic (n=52)	p Value
Resistance index	0.63 (±0.05)	0.68 (±0.06)	p<0.001
Gestational age (weeks)	34.5 (±2.711)	34.2 (±2.678)	0.567
Estimated fetal weight (kilograms)	2.58 (±0.66)	2.54 (±0.721)	0.749

Parameter	Normotensive pregnancies (n=63)	Pre-eclamptic (n=52)	p Value
Liquor volume	Adequate (n=60)	Adequate (n=42)	

3.4. ECHOCARDIOGRAPHY FINDINGS

All the parameters between the two groups were within normal limits. There were no differences in chamber dimensions between the groups. The relevant data are shown in Table 5.

Table 5: Echocardiography findings (median and ranges given)

Parameter	Normal range	Normotensive (n=41)	Pre-eclamptic (n=36)	p Value
LV Diastole (mm)	35-55	49 (37-56)	50 (39-60)	0.501
LV Systole (mm)	23-34	33 (23-40)	34 (24-41)	0.565
Fractional shortening (%)	27-35	32 (27-42)	32 (26-38)	0.296
Ejection fraction (%)	50-75	63.52 (54 -62)	63.44 (56-66)	0.250
LV posterior wall (mm)	6-10	7 (5-9)	7 (5-10)	0.099
Septal thickness (mm)	6-10	7 (5-9)	7 (4-10)	0.063
Left atrium (mm)	19-39	35 (25-41)	38 (32-55)	0.081
Aortic root (mm)	20-37	23 (21-29)	24 (19-30)	0.061
Tissue Doppler	<8	9.2 (4.5-12.25)	11.0 (7.3-15.4)	0.058

LV= Left Ventricular

Ejection Fraction

There was no difference in ejection fraction between the two groups. The details are shown in Table 5.

Tissue Doppler Imaging

There were no significant differences in the tissue Doppler S wave or the Tissue Doppler E/Ea ratio in the pre-eclamptic group when compared to the normotensive pregnancies (11.02 ± 5.6 vs 9.16 ± 2.6 ; $p=0.058$).

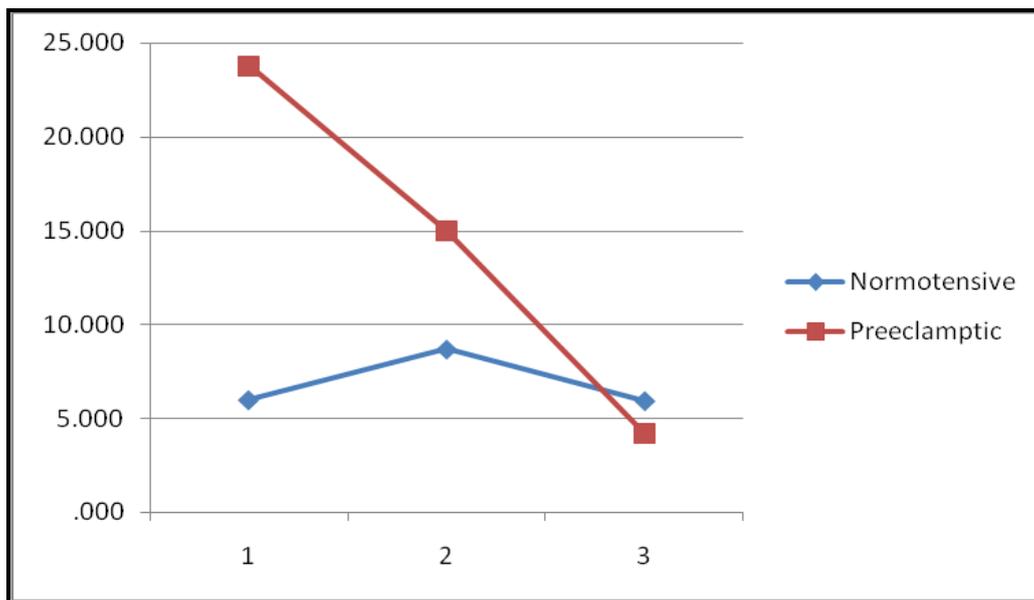
3.5. B-TYPE NATRIURETIC PEPTIDE LEVELS

BNP type natriuretic levels were significantly increased in the antepartum (23.8 (2 - 184.1) vs 6.0 (0.5 - 45.2) pmol/L; $p<0.001$), during labour (15.0 (1.8-206.4) vs 8.7 (1.9 - 24.8) pmol/L; $p=0.017$) in the pre-eclamptic group compared to the normotensive pregnancies. BNP levels decreased in the postpartum period 4.2 (1.7-51.4) vs 5.95 (2.2-38.7) pmol/L; $p=0.140$ in the pre-eclamptic and normotensive groups respectively to levels that were not significantly different. Median values in both groups are shown below (Table 6, Fig 3)

Table 6: BNP levels (median values plus range) in normotensive and pre-eclamptic pregnancies.

Parameter	N	Normotensive	N	Pre-eclamptic	p Value
BNP (p/mol)					
Antepartum (1)	59	6.0 (0.5 - 45.2)	49	23.8 (0.2-184.1)	<0.001
Labour (2)	32	8.7 (1.9 -24.8)	34	15.0 (1.8-206.4)	0.017
Postpartum (3)	28	5.95 (2.2-38.7)	21	4.2 (1.7-51.4)	0.140

Figure 3: Median BNP at different timepoints in pregnancy



Median BNP by PE and time point (Key: 1= antenatal, 2= intrapartum, 3= postpartum)

3.6. CORRELATION STUDIES (TABLE 7)

BNP1 and E/Ea and RI

There was a small correlation between baseline BNP and E/Ea ($r=0.22$; $p=0.051$) at study enrolment. This was however not statistically significant. Statistical significance was found when BNP1 and umbilical artery Doppler RI at study enrolment were compared ($r=0.321$; $p=0.001$). It is important to note that the correlation was small and significant, possibly influenced by the sample size.

BNP3 and E/Ea and RI

There was no correlation between BNP after delivery and the predelivery E/Ea and RI parameters.

Table 7: Correlation of BNP, E/Ea and RI in the pre-eclamptic group

			E/Ea	RI	BNP1	BNP3
Spearman's rho	E/Ea	Correlation Coefficient	1.000	.180	.222	-.293
		Sig. (2-tailed)	.	.110	.051	.060
		N	80	80	78	42
	RI	Correlation Coefficient	.180	1.000	.321**	-.143
		Sig. (2-tailed)	.110	.	.001	.326
		N	80	113	106	49

** Correlation is significant at the 0.01 level (2-tailed). BNP values were logarithmically transformed to get an appropriately distributed normal distribution.

3.6. PREGNANCY OUTCOMES

3.6.1. Maternal

The number of maternal complications was higher in the pre-eclamptic group compared to the normotensive group. Details of the complications are shown in Table 8.

Table 8: Maternal Complications

Parameter	Normotensive pregnancies (n=56)	Pre-eclamptic (n=51)
Thrombocytopenia	1	10
Pulmonary edema	0	2
Eclampsia	0	11
HELLP syndrome	0	1

Thrombocytopenia = < 150 x10⁹/L

ANTIHYPERTENSIVES TREATMENT USED IN THE PRE-ECLAMPTIC GROUP

Thirty three pre-eclampsics required rapid acting antihypertensive for blood pressure control. The antihypertensive of the three patients are shown in Table 9. Echocardiography and BNP at enrolment were performed prior to the administration of antihypertensive therapy.

Table 9 Antihypertensive regimen of pre-eclampsics (n=51)

Antihypertensive regimen	No
Aldomet	18
Adalat	14
Adalat + MgSO ₄	9
Adalat + Amlodipine	2
Adalat + Hydrallazine	1
Adalat + Hydrallazine +MgSO ₄	5
Adalat+ Labetalol	1
Adalat+ Labetalol + MgSO ₄	1

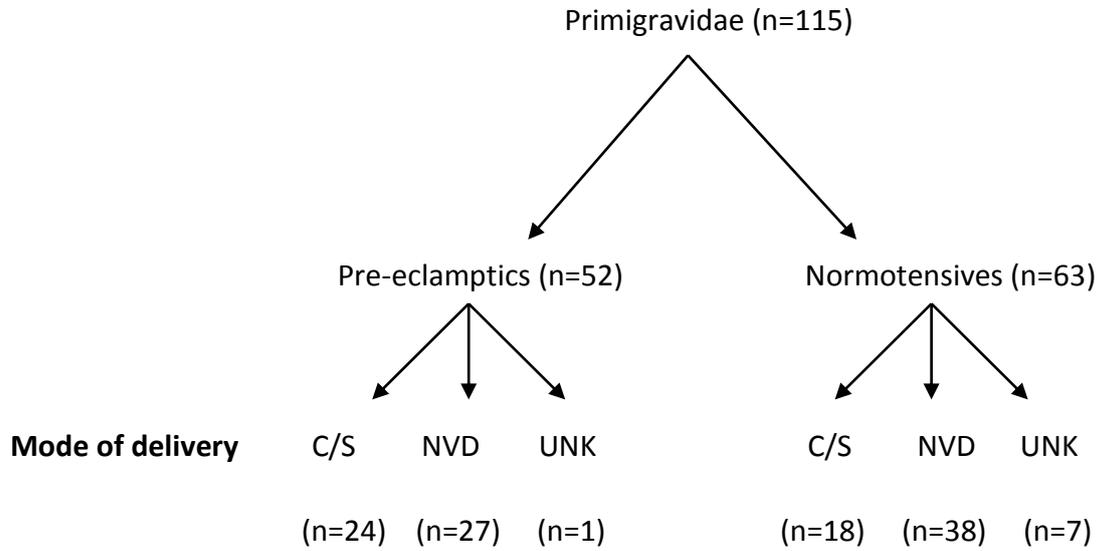
EMERGENCY POST DELIVERY CARE

Seven pre eclampsics required emergency care after delivery; two were admitted to intensive care unit and five to high care. There were no normotensive patients admitted to high care or the intensive care unit.

3.6.2 Mode Of Delivery

The mode of delivery of 8 patients (7 patients in the normotensive group and 1 in the pre-eclamptic) could not be established as they did not return for follow up. The caesarean section rate was 47% in the pre-eclamptic group and 32% in the normotensive group (Fig 4). Because eight subjects were lost to follow-up, no inference could be made from this data. The mode of delivery and indication for cesarean section is shown in Table 10.

Figure 4: Flow diagram showing mode of delivery



- UNK = unknown

Table 10: Indications for Caesarean section

INDICATION FOR C/S	PE (n=51)	Normotensive (n=56)
Severe PE	7	0
Eclampsia	4	0
Fetal distress	6	5
CPD	0	3
Big baby	0	2
Failed IOL	2	1
Poor progress	1	3
Breech presentation	1	1
PROM	1	2
MSL 3	0	1
An/oligohydramnious	2	0

3.6.3. Perinatal Outcome

There was no difference in the number of live births between the 2 groups. The birthweight was significantly lower in the pre-eclamptic pregnancies compared to the normotensives (2.6 ± 0.8 kg vs 3.14 ± 0.42 kg; $p < 0.0001$). One neonate in the pre-eclamptic group was born with Down's syndrome.

Table 11: Perinatal outcome in the pre-eclamptic and normotensive pregnancies. Results are expressed as mean \pm SD and for APGAR mean (range).

Variable	Pre-eclampsia	Normotensive pregnancies
Birth weight (kg)	2.64 ± 0.8	3.14 ± 0.42
Apgar scores		
1 min	7 (0-10)	8 (3-10)
5 min	8 (0-10)	9 (0-10)
Perinatal outcome	PE (51)	Normotensive (56)
Live births	47	55
Still births	2	0
ENND	1	1

Early Neonatal Deaths

- 1) Patient 1, a 25yr old POG1, HIV infected patient, presented at 28 weeks of gestation with blood pressure of 224/134 mmHg, pulse 106 beats per minute, proteinuria 2+, edema 1+. Tissue Doppler E/Ea was markedly elevated at 42, as was the RI (0.74), uric acid levels (0.43) and she had a thrombocytopenia ($122 \times 10^9/L$). She was in pulmonary edema. She was given rapid acting blood pressure lowering drugs (nifedipine, hydralazine, labetalol, methyldopa and $MgSO_4$) and intravenous diuretics. She had an induction of labor at 30 weeks and delivered 1kg baby with poor Apgar scores who died an hour later.
- 2) Patient 2, a 24 year old POG1 HIV negative patient, presented at 40 weeks of gestation with BP of 126/82 mmHg. Tissue Doppler E/Ea was 15.4, RI 0.67,

ejection fraction 68%. She delivered a 3.4 kg baby with low APGAR scores that died two days later from acute respiratory distress.

Stillbirths

- 1) A 23 yr old booked patient presented with severe lower abdominal pain and decreased fetal movements. She was receiving highly active antiretroviral therapy with a CD3 count of 173 cells/ uL. She was pre-eclamptic with baseline BP 154/94 and 1+ proteinuria. She was diagnosed with an abruptio placentae Grade 3B. She delivered a 3.0 kg macerated stillborn. Tissue Doppler E/Ea was 13.24 and BNP 20.8pmol/L. Post-delivery the BNP fell to 2.7pmol/L.
- 2) 29 year old patient presented with imminent eclampsia. She was HIV infected with a CD4 count of 200 cells/uL. Her BP was 175/ 110. Ultrasound confirmed the diagnosis of an intra-uterine death and no visible pools of liquor (she gave no history of ROM). She subsequently delivered a 740 gram macerated stillborn.

CHAPTER 4

DISCUSSION

Pre-eclampsia is associated with vasoconstriction and increased left ventricular afterload accompanied by a reduced cardiac output, hypovolemia and low to normal or slightly increased cardiac filling pressures (112). This study shows that the haemodynamic changes that accompany PE are associated with significant changes in LV filling as reflected by increased BNP levels.

Hypertensive disorders of pregnancy contributed to 15.7% of maternal deaths in South Africa. According to the NCCEMD 2005-2007 report, this is second only to AIDS-related deaths. Of note is that 53% of these deaths were clearly avoidable. It is vital that all health care providers are aware of the severity of this condition, the availability of markers, and the correct treatment of this common obstetric condition. It is important to study primigravidae, as so called “true PE” usually occurs in first pregnancies after the the 34th week of pregnancy. Thus, our patients could be considered a homogenous group of patients. Furthermore, all our patients were Black South Africans who were Zulu speaking. Most other studies included heterogenous population groups (113, 114).

In women with PE, there may be modification of left ventricular structure and function due to the increase in systemic blood pressure. Furthermore, there is a statistically significant increase in left ventricular mass. Increased left ventricular end-systolic and end-diastolic volumes have also been demonstrated while significant reductions in left ventricular ejection fraction and percentage of fractional shortening have been found (112). The ejection fraction between the two groups was insignificant in our study possibly due to the fact that even in heart failure, up to 40-50% of patients may still have normal ejection fractions (115).

An increasingly popular marker of increased left ventricular filling pressure is assessment by tissue Doppler imaging. Our study showed no difference in the tissue Doppler E/Ea ratio between the pre-eclamptic and normotensive groups (11.02 ± 5.6 vs 9.16 ± 2.6 $p=0.058$) or in any of the other measured parameters.

BNP is a recognised marker of patients with LV dysfunction. In patients with PE, BNP has been observed to be linearly related to the left ventricular structural and functional changes seen (116) and even in non pregnant patients with a wide range

of hemodynamic changes, circulating levels of BNP were shown to be independently associated with cardiac stress and risk of progressive heart failure and death (117).

It has been established that BNP is synthesised by cardiac atrial and ventricular myocytes and released into blood in response to stretch. Evidence shows that NT-proBNP measurement is superior to ANP in the detection of mild systolic heart failure, diastolic heart failure, and asymptomatic left ventricular dysfunction because of its longer half - life (41, 118, 119).

Numerous studies have looked at the relationship between BNP and PE. In a recent study, Tihtonen et al. (2007) found that concentrations of natriuretic peptides (NT-pro-BNP and NT-pro-ANP) were significantly higher in PE than in the normotensive` and those with chronic hypertension (120). Frantz et al, in 2009 and Kale et al, in 2005 have confirmed that BNP concentrations are elevated in PE and other hypertensive disorders of pregnancy than in normal pregnancy (121, 122). Furthermore, the long term risk of death from cardiovascular disease is higher in women who have had pre-eclampsia (123).

Women with PE have increased sensitivity to angiotensin II resulting in increased peripheral vasoconstriction and volume retention (124). BNP is associated with a derangement in the renin-angiotensin system. It suppresses renin release (125). BNP also suppresses endothelin release which is an additional mechanism to regulate vascular tone. Factors such as pro inflammatory cytokines and endothelin-1 are also known to stimulate natriuretic peptide release. Furthermore, the intense vasoconstriction observed in PE, may lead to myocardial remodelling with subclinical ventricular dysfunction and activation of BNP (126). It is now known that circulating plasma BNP levels are increased even in healthy pregnancies but the increased serum BNP levels in PE are greater than in normotensive pregnancies (127). This suggests that BNP activation in PE is a response to the pathophysiological changes in the utero-placental circulation.

We have shown that plasma BNP levels were significantly increased in the pre-eclamptic compared to the normotensive patients in the antepartum period; and decreased in the post partum period. While it appears that the increase in circulating plasma BNP is most likely explained by the rising cardiac filling pressure, it should be remembered that PE is documented to be a relatively hypovolaemic state with peripheral vasoconstriction. Our findings suggest that BNP may be involved in the pathophysiology of PE. This hypothesis is confirmed by the decrease in BNP levels in the puerperium when the placenta has been removed.

The correlation between elevated blood pressures and clinical indices were clearly demonstrated in this study. Significant changes were seen in the pre-eclamptic group with regard to blood pressure, pedal oedema, proteinuria, uric acid and serum creatinine levels, highlighting the need for intervention. These are but a few of the well documented indicators of worsening disease progression of PE and the need for prompt intervention highlighted. The relationship between proteinuria and the heart need further evaluation as well as its relationship to HIV infection.

Our study showed a statistically significant increase in the RI on umbilical Doppler velocimetry. Doppler velocimetry of the umbilical cord has been found to be a more specific and sensitive method of fetal surveillance than cardiotography. It is a fast and convenient method for monitoring fetal well being. Its importance in high risk pregnancies is well documented (128-131). A study looking at the perinatal outcomes in babies born to mums with severe PE showed that an abnormal Doppler umbilical artery waveform is a strong predictor of perinatal mortality and is associated with a poor perinatal outcome. Of note is that neonates with abnormal pulsatility index had lower birth weights, lower APGAR score at 5 minutes, higher admission rates to the neonatal intensive care units and significant neonatal morbidity as compared to those with normal velocimetry ($p < 0.05$) (83). The umbilical artery circulation is normally a low impedance circulation with an increase in the amount of end-diastolic flow with advancing gestation (132). Umbilical artery Doppler waveforms reflect the status of the placental circulation. With increasing gestation, there is an increase in the end-diastolic flow as a result of an increase in the number of tertiary stem villi that occurs with placental maturation (133, 134). Another study showed that when the fetal growth velocity was reduced, changes in fetal circulation preceded this. Ratios of the fetal Doppler parameters provides clear evidence of deterioration in the fetal condition which can aid in the diagnosis and management of the intra-uterine growth-restricted fetus (135)

In our study, 7 pre-eclamptic patients required emergency care post delivery in keeping with the association between PE and adverse pregnancy events. An increased rate of caesarean sections, lower APGAR scores, lower birthweight and an increased admission rate to NICU has been described in PE (136, 137). Our study showed an increase in the number of abdominal deliveries in the pre-eclamptic group when compared to the normotensive control group. The caesarean section rate was 47% in the pre-eclamptic group and 32% in the normotensive group. Eight subjects were lost to follow-up, therefore no inference could be made from this data. The two highest indications were fetal distress ($n=6$) and hypertensivers disorders of pregnancy namely PE and eclampsia ($n=11$) in the PE group. In the

normotensive control group, the greatest indications for caesarean sections were fetal distress (n=5) followed by cephalopelvic disproportion (CPD) (n=5). The lower birthweight in the pre-eclamptic group is most likely due to placental insufficiency. Of the 7 pre-eclamptics who required emergency care, 2 required ICU admission.

CHAPTER 5

CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

5.1. CONCLUSION

PE is a leading cause of maternal and fetal/neonatal mortality and morbidity (1). Therefore the early identification of patients with an increased risk for PE is important. The availability of highly sensitive and specific biochemical markers would allow not only for early detection of patients at risk but also permit close surveillance. This study showed that there are significant differences in BNP during PE and this reflects the haemodynamic changes in this condition. Serum BNP levels were significantly elevated in pregnancies complicated by PE, particularly in those with more severe disease. There was no statistical difference in the Tissue Doppler (E/Ea ratio) between the two groups. No inference could be made to the post delivery findings as eight patients were lost to follow-up.

5.2. RECOMMENDATIONS

BNP levels in asymptomatic pregnant patients were found to have little clinical value as they were not markedly elevated. However, there is clear evidence of its activation in PE and as in the non-pregnant patient; it could be used to identify left ventricular dysfunction. It could be particularly beneficial in symptomatic patients. Further research is needed to evaluate whether or not NT-pro-BNP can be used as an early marker to predict negative outcomes in patients with PE of varying grades of severity.

5.3. LIMITATIONS

As mentioned, most of the changes in BNP occurred within normal ranges for our laboratory. The presence and extent of any structural changes may indeed be disease-specific, occurring in some conditions but not in others (138) and also likely that BNP activation is more pronounced in severe PE.

In vitro stability of BNP and NT-proBNP after patient collection is also an important consideration, particularly when samples are referred to an outside laboratory for

testing. Reports indicate that BNP is stable in either EDTA whole blood or plasma for up to 24 hours if the specimen is refrigerated. Frozen plasma specimens for BNP measurement are reportedly stable for approximately 3 months. NT-proBNP is stable for up to 3 days when plasma or serum is stored refrigerated; NT-proBNP appears to be stable for years when frozen at -70° C. Overall, available data indicate that in vitro measurements of NT-proBNP are more stable compared to those of BNP. We addressed the stability issue by transporting all our specimens on ice.

The study was conducted on patients in the 3rd trimester. Earlier recruitment might have shown whether or not BNP could be used as an earlier marker of PE. One of the difficulties in this study was that patient compliance and follow up was not ideal, due to numerous reasons, mainly socio-economic. Most subjects wished to be discharged immediately after delivery, making follow up evaluation difficult.

Our study included 115 primigravidae with a mean age of 20 years in the normotensive and 22 years in the pre-eclamptic groups respectively. The mean BMI in the normotensive group was 27.852 while in the pre-eclamptic group it was 29.342 ($p=0.129$). Extremes of age, nulliparity and an increased body mass index are well documented risk factors for pre-eclampsia. Obesity has been found to increase the risk of pre-eclampsia three-fold and this risk increases linearly with increasing body mass index. Besides increasing the rates of pre-eclampsia, escalating obesity also increases perinatal morbidity (139).

Lastly, repeating this study would call for substantial funding since BNP is an expensive laboratory investigation. Follow up studies may be limited.

5.4. DISCLOSURE OF INTEREST

No conflict of interest is disclosed by the author.

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APPENDIX

a) INFORMED CONSENT ENGLISH

Consent to participate in the study

Study title: To identify the changes in the haemodynamics in patients with Pre-Eclampsia using BNP and Doppler studies of the heart and uterine circulation.

You have been asked to participate in a research study.

You have been informed about the study by Dr S Fayers.

You may contact Dr S Fayers at any time if you have questions at (031) 2604250.

You may contact the Medical Research Office at the Nelson Mandela School of Medicine at (031) 2604604 if you have any questions about your rights as a research subject.

Your participation in this research is voluntary, and you will not be penalized or lose any treatment benefits if you refuse to participate or decide to discontinue participation.

Your blood samples will be discarded after the study and will not be used for any further tests.

If you agree to participate, you will be given a signed copy of this document and the participant information sheet with the summary of the research.

The research study including the above information has been described to me verbally. I understand what my involvement in the study means and I voluntarily agree to participate.

Signature of participant

Date

Signature of witness

Date

***b) INFORMED CONSENT (ISIZULU) ISIVUMELWANO
SOKUBA INGXEENYE YOCWANINGO***

Isihloko sesifundo

Ukusetshenziswa kohlobo u-B lwe Natriuretic peptide emazingeni okukhalima isifo esenyusa umfutho wegazi ezinyangeni ezintathu zokugcina zokukhulelwa.

Ucelwe ukuba ube ingxenye esifundweni socwaningo.

Utsheliwe ngesifundo nguDokotela S Fayers.

Ungamthinta uDokotela S Fayers nganoma isiphi isikhathi uma unemibuzo ku 0795047462.

Ungathintana nehhovisi Lezocwaningo Lwezempilo Esikoleni Sezifundo Zokwelashwa i Nelson Mandela ku (031)2604604 uma unemibuzo ngamalungelo onawo njengomuntu obambe iqhaza ocwaningweni.

Ukubamba kwakho iqhaza kulolu cwaningo akuphoqiwe, futhi uma ukhetha ukungabi ingxenye yalo angeke ujeziswe noma ulahlekelwe inzuzo yakho yokwelashwa uma wenqaba noma uthatha isinqumo sokuyeka.

Igazi lakho lizolahlwa emva kocwaningo, futhi ngeke lisetshenziselwe okunye ukuhlolwa.

Uma uvuma ukuba ukubamba iqhaza kulolu cwaningo, uzonikezwa isifaniso salo mqulu esisayiniwe, neminingwano yobambe iqhaza ebeka kafuphi ngocwaningo.

Isifundo socwaningo kubandakanya nolwazi olubhalwe ngenhla kuchazwe kimina ngomlomo. Nginyaqonda ukuthi kuchazani ukuba ingxenye kulolu cwaningo futhi ngiyavuma ngokuzikethela ukubamba iqhazao.

Isignesha yobambe iqhaza

Usuku

Isignesha kafakazi

Usuku