Spot urine protein to creatinine ratio testing: new techniques for detecting proteinuria in pre-eclampsia

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DECLARATION

I, Rajesh Gangaram, hereby declare that this is my original work and has not previously been submitted to this or any other university.

…… ……..
Dr. R. Gangaram Date

DEDICATION

To my wife Prebashini and my precious daughter Nandini who make every moment of my life a joyful one.

ACKNOWLEDGEMENTS

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PUBLICATIONS OR PRESENTATIONS

1. R Gangaram, M. Naicker, J Moodley, T Padayachi. Evaluation of the Clinitek® 50 microalbumin:creatinine ratio urinalyser and visual urinary dipsticks in normotensive and hypertensive pregnant women.

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Abstract accepted for poster presentation at SASOG 2008

2. R Gangaram, M. Naicker, J Moodley. Accuracy of the spot urinary microalbumin:creatinine ratio and visual dipsticks in hypertensive pregnant women.

To be submitted for publication to Eur J Obstet Gynaecol

Abstract accepted for poster presentation at SASOG 2008.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

BMI	body mass index
CC	creatinine clearance
CI	confidence interval
FSB	fresh stillbirth
Eng	Endoglin
ENND	early neonatal death
ERPF	effective renal plasma flow
GFR	glomerular filtration rate
GH	gestational hypertensives
HDP	hypertensive disorders of pregnancy
HELLP	haemolysis, elevated liver enzymes, low platelets
HLA	human lymphocyte antigens
ISSHP	International Society for the study of Hypertension in pregnancy
KIRs	Killer immunoglobulin like receptors
LR+	likelihood ratio for a positive result
LR-	likelihood ratio for a negative result
MA	microalbuminuria
MSB	macerated stillbirth
NK	natural killer cells
NPV	negative predictive value
PE	pre-eclampsia
PlGF	placental growth factor
PPV	positive predictive value
PRECOG	pre-eclampsia community guideline
sflt	soluble film- like tyrosine kinase
sVEGFR	soluble vascular endothelial growth factor receptor
TPE	total protein excretion
UAE	urinary albumin excretion
UAC	urinary microalbumin to creatinine ratio

VE-cadherin	vascular endothelial – cadherin
VEGF	vascular endothelial growth factor
WHO	World Health Organization

ABSTRACT

Background:

The most commonly employed screening method for proteinuria is a semi-quantitative dipstick urinalysis, but it has been shown to be inaccurate in pregnancy. New developments in the assessment of proteinuria have included the use of urinary albumin measurements. The Clinitek® Microalbumin Reagent Strip (Bayer Healthcare LLC, USA) is a semi-quantitative dipstick test. It is used to measure the spot urinary microalbumin to creatinine ratio that is read using the Clinitek 50 portable urine chemistry analyzer.

Aims

We embarked on a pilot study to validate the Clinitek 50 system by determining the accuracy of spot urinary microalbumin to creatinine ratio dipsticks and conventional visual dipsticks (Makromed®) compared to the laboratory urinary microalbumin to creatinine ratio quantification to detect significant proteinuria in normotensive and hypertensive antenatal attendees. The accuracy of spot urinary microalbumin to creatinine ratio dipsticks and conventional visual dipsticks were then compared to a 24 hour urinary protein (gold standard) to detect significant proteinuria in hypertensive disorders of pregnancy. We then determined the role of proteinuria as assessed by the diagnostic accuracy of both the 24 hour urinary protein (gold standard) and the spot urinary microalbumin to creatinine ratio dipstick, in pregnancy outcomes of these participants.

Methods

This was a prospective study conducted at hospitals serving the Durban Metropolitan region in South Africa. To validate the urinary microalbumin to creatinine ratio dipstick, fifteen normotensive healthy pregnant women and 11 women with new onset

hypertension in pregnancy were recruited .Each women had a spot midstream urine, which was assessed for proteinuria using a semi-quantitative visual dipstick (Makromed®) and analysed using the semi-quantitative urinary microalbumin to creatinine ratio dipsticks (Clinitek® Microalbumin) read on the Clinitek® 50 urine chemistry analyser. A result of ≥1 + on visual dipsticks and a spot urinary microalbumin to creatinine ratio UAC of > 300mg/g (33.9mg/mmol) was considered as positive for significant proteinuria. The results were compared to the laboratory quantitative measurement of the urinary microalbumin to creatinine ratio.

The study group comprised 163 women presenting with newly diagnosed hypertension during pregnancy after 20 weeks of gestation, being recruited from antenatal clinics. Each participant had a spot urine sample that was tested by trained midwives for proteinuria using a semi-quantitative visual dipstick (Makromed®). Participants were admitted to the ward where a spot midstream urine sample was collected and analysed using the semi-quantitative urinary microalbumin to creatinine ratio dipsticks. A 24 hour quantitative urinary protein analysis was completed. The results of the urinary microalbumin to creatinine ratio dipsticks and conventional visual dipsticks were compared to the 24 hour urinary protein (gold standard) to detect significant proteinuria. A urinary microalbumin to creatinine ratio of < 300mg/g (nil and trace on visual urine dipsticks) was considered to be a negative result. A urinary microalbumin to creatinine ratio ≥ 300 mg/g (1+ to 4+ on visual urine dipsticks) was considered to be a positive result. Urinary protein ≥ 0.3 g/24 hours was considered significant proteinuria. The outcomes of pregnancy in 2 sub-categories viz. those with and without significant proteinuria were compared using the 24 hr urinary protein measurement. A secondary analysis of outcomes of pregnancy was performed by subcategorizing the participants according to the diagnostic accuracy of the urinary microalbumin to creatinine ratio dipsticks.

Results

In the 26 patients enrolled in the initial study , the visual dipstick had a sensitivity of 25% (95% CI [0.04-0.64]) and specificity of 89% (95% CI [0.64 -0.98]).The urinary microalbumin to creatinine ratio dipsticks had a sensitivity of 88% (95% CI [0.47-0.99]), specificity of 89% (95% CI [0.64-0.98]), negative predictive value (NPV) of 94% (95% CI [0.69-1.00]) and positive predictive value (PPV) of 78% (95% CI [0.40-0.96]).

In the 163 patients subsequently enrolled the visual dipstick had a sensitivity of 51 % (95% CI [0.41-0.61]) and specificity of 91% (95% CI [0.81-0.96]) .The PPV and NPV was 89 %(95% CI [0.77-0.95]) and 58% (95% CI [0.48-0.67]) respectively. The urinary microalbumin to creatinine ratio dipsticks had a sensitivity of 63% (95% CI [0.52-0.72]) and specificity of 81 % (95% CI [0.70-0.89]). The PPV was 82% (95% CI [0.71-0.90]) and NPV was 62% (95% CI [0.51-0.71]).

Our results show that in hypertensive pregnant women, significant proteinuria determined by the quantitative 24 hour urinary protein is associated with delivery at an earlier gestational age, increased induction of labour and lower birthweights compared to the non-proteinuric hypertensives (gestational hypertension). There is also a trend towards an increased maternal morbidity and perinatal mortality. When the groups were classified into pre-eclampsia and gestational hypertension using the diagnostic accuracy of the urinary microalbumin to creatinine ratio dipsticks, there were no differences in the clinical outcomes between the false negatives and true negatives except a trend towards a higher caesarean section rate in the false negatives.

Conclusion

The urinary microalbumin to creatinine ratio dipstick read on the Clinitek 50 system provides a semi – quantitative result of the urinary microalbumin to creatinine ratio that has good sensitivity and specificity. Furthermore, the urinary microalbumin to creatinine ratio dipstick has a good negative predictive value and a result of < 300mg/g rules out significant proteinuria and avoids unnecessary investigations in pregnancy.

Both the visual dipstick (Makromed®) and the urinary microalbumin to creatinine ratio dipstick read on the Clinitek 50 system are not accurate when compared to the total 24 hour urinary protein. Differences between the urinary microalbumin to creatinine ratio and 24 hour total urinary protein may be due to the variation in the albumin fraction of the total urinary protein of pre-eclampsia, technical problems with imprecision of the assay technique and clinical causes of false positives and negatives. The improved sensitivity of the automated urinary microalbumin to creatinine ratio dipstick over the visual dipstick suggests it may be a suitable substitute for the visual dipstick in clinical practice

Hypertension in pregnancy associated with significant proteinuria is associated with greater adverse maternal and fetal outcome. Outcome of pregnancy is similar when a classification of gestational hypertension is made based either on the 24 hour urinary protein or the urinary microalbumin to creatinine ratio dipstick read on the Clinitek 50 system. The urinary microalbumin to creatinine ratio dipstick is a good screening test to rule out significant proteinuria. It has the potential to improve accuracy of screening for proteinuria and enhancing safety by preventing incorrect diagnosis and unnecessary investigation. Further research is required to determine its full impact and cost effectiveness in the clinical setting.

CHAPTER 1- LITERATURE REVIEW

1. INTRODUCTION

Hypertensive disorders of pregnancy are a major cause of maternal and perinatal mortality worldwide. In well resourced countries such as the UK, hypertensive disorders of pregnancy (HDP) were second only to thromboembolism as the main cause of maternal death (Lewis, 2004). In the United States, HDP represented almost 15% of pregnancy related deaths (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). In South Africa, it has been the commonest cause of direct primary obstetric related deaths since 1998, and accounted for 19.1% of maternal deaths in the triennial report 2002-2004 (Saving Mothers, 2006).The Confidential Enquiry into Stillbirths and Deaths in Infancy report in the UK cited one in six stillbirths as occurring in pregnancies complicated by maternal hypertension (Maternal and Child Health Research Consortium, 1998). Long term healthcare implications for women who develop HDP include an increased risk of developing cardiovascular complications later in life (Haukkamaa et al., 2004; Ramsay et al., 2003; Wilson et al., 2003) and their offspring have an increased risk of hypertension, heart disease and diabetes (Smith and Kenny, 2006).

POSSIBLE AETIOLOGY

Of the HDP, pre-eclampsia remains the leading cause of maternal and perinatal mortality (Smith et al., 2006).While the aetiology of pre-eclampsia remains unknown, there has been extensive research trying to elucidate the key steps in this multisystem disease process. Pre-ecalmpsia is primarily thought to be caused by a maternal response to abnormal placentation while other theories have included ischaemia leading to oxidative stress within the placenta and maternal circulation, and immune maladaptation (Sibai et al., 2005).

Key steps proposed in the disease process include deficient remodelling of the spiral arterioles in early pregnancy leading to placental ischaemia and an increase in placental oxidative stress that results in the release of circulating factors that target maternal vascular endothelium and this in turn causes maternal and fetal effects (Smith et al., 2006).

SCREENING

Many screening tests for predicting pre-eclampsia based on dysfunction of placental perfusion, vascular resistance, endocrinology and the fetoplacental units have been described. A systematic review conducted by the World Health Organization (WHO) to evaluate these screening methods found the majority of them to have low predictive potential and thus not suitable for use in routine clinical practice (Conde-Agudelo et al., 2004). Antenatal screening therefore consists predominantly of detection of a raised blood pressure and proteinuria. Once detected, treatment of pre-eclampsia has remained delivery of the fetus and placenta for the last century.

BLOOD PRESSURE MEASUREMENTS

Advancements in blood pressure measurement have involved the development of automated blood pressure measuring equipment. Unlike the mercury sphygmomanometer that relies on detecting Korotkoff sounds, these devices evaluate oscillometric signals obtained from a cuff during deflation. They have an advantage of removing observer error and facilitating repeated measurements and patient self monitoring. However, a number of studies have demonstrated that oscillometric measurement is inaccurate in hypertensive pregnancies (Villar et al., 2004). Individual machines may produce large errors and it is recommended that each device be validated for accuracy using protocols published by the Advancement of Medical Instrumentation and the British Hypertension Society (O'Brien et al., 1993).

MEASUREMENT OF PROTEINURIA

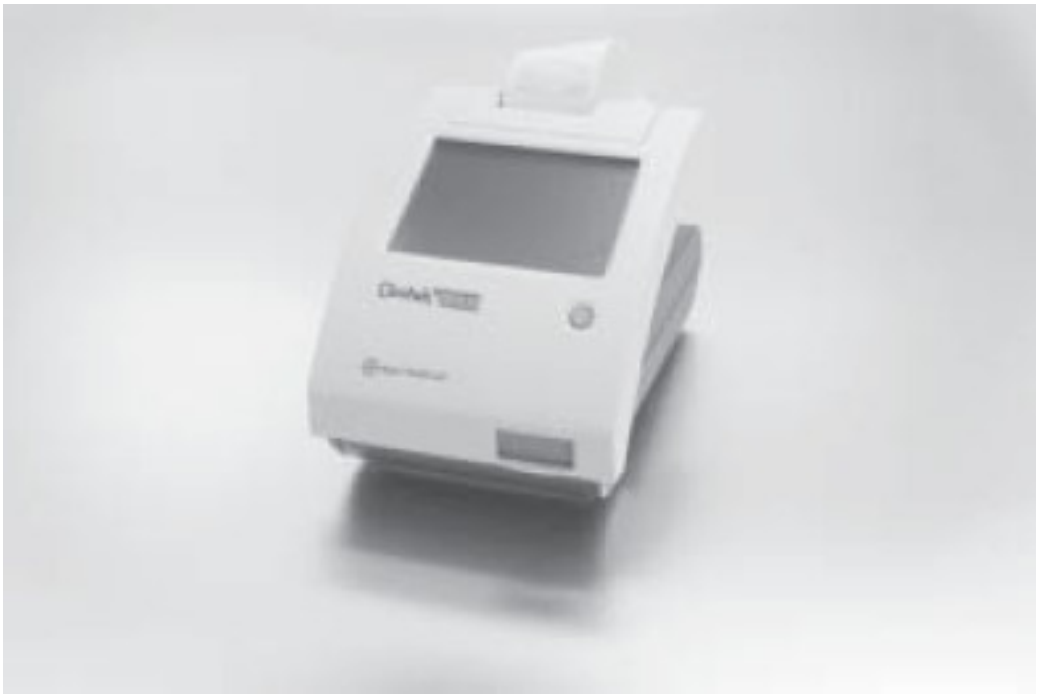
There have been several advancements in the detection of proteinuria since the nineteenth century. It was in 1827 that Bright boiled a teaspoon of urine and discovered "albuminous urine" in patients with oedema and related this to severe and protracted disease of the kidneys (Bright et al., 1827). In 1843, the obstetrician John Lever separated the proteinuria of pregnant women in whom hypertension was developing from that of "Morbus Brightii" (Bright's disease). Since this discovery, proteinuria has been used to define pre-eclampsia and classify disease severity. Patterns of proteinuria have also been investigated to distinguish pre-eclampsia from other proteinuric diseases (Karumanchi et al., 2007).

Traditionally a quantitative 24 hour total urinary protein excretion has been used to quantify proteinuria. New developments in proteinuria assessment have included the use of urinary albumin measurements. While small amounts of albumin can be detected in the urine of a healthy population, the term microalbuminuria (MA) has been used to refer to a range of urinary albumin excretion that is above the reference ranges but below amounts referred to as significant proteinuria. In the non pregnant population MA has been extensively studied. It has been used as a marker to predict an increase risk of cardiovascular and renal disease in the general population, in type 1 and 2 diabetics and in patients with essential hypertension (Hillege et al., 2002; Gerstein et al., 2001).

In the pregnant population, there is limited literature regarding MA. It has been used as a clinical tool to predict pre-eclampsia and as an early predictor of hypertensive complications and perinatal outcome (Das et al., 1996; Bar et al., 1996; Nakamura et al., 1992; Chhabra et al., 2002).Waugh et al., (2005a) have suggested that MA may correlate better with other clinical measurements of disease severity as it may more accurately reflect the glomerular dysfunction associated with the glomerular endotheliosis of pre-eclampsia.

Microalbuminuria dipsticks compared to the traditional visual urinary dipsticks have also been shown to be a better screening test for clinically significant proteinuria (Higby et al., 1995; Waugh et al., 2005a). Various semi quantitative dipstick tests have been used for detection of MA. The Clinitek Microalbumin Reagent Strip (Bayer Corporation, Elkhart, IN) is an example of semi- quantitative dipstick test for MA that is read using the Clintek 50 portable urine chemistry analyzer (Figure 1). This type of automated point of care urine analyzers are able to provide rapid results, avoiding inter observer error and provide automated documentation of results. However, unlike automated blood pressure measuring devices, no established protocols exist for assessing the accuracy of these devices.

Figure 1: Picture of Clinitek 50 portable urine chemistry analyzer



DEFINITION OF PRE-ECLAMPSIA

Hypertensive disorders of pregnancy include chronic hypertension, gestational hypertension, pre-eclampsia and chronic hypertension with superimposed pre-eclampsia (Table 1) (Davey et al., 1988). Pre-eclampsia is a multisystem disorder of unknown aetiology that is unique to human pregnancy. The International Society for the study of Hypertension in pregnancy (ISSHP) defines pre-eclampsia as the occurrence of hypertension in combination with proteinuria developing after 20 weeks gestation in a previously normotensive, non-proteinuric patient (Davey et al., 1988). Hypertension is defined as a blood pressure of at least 140mmHg (systolic) or at least 90 mmHg (diastolic) on two occasions 4- 6 hours apart. Proteinuria is defined as the excretion of 300mg or more of protein every 24 hours. If 24 hour urine samples are not available, proteinuria is defined as a protein concentration of 300mg /l or more (≥1 + on dipstick) in at least two random urine samples taken at least 4-6 hours apart or a spot protein to creatinine ratio of 30 mg/mmol or more (Sibai et al., 2005). In order to detect pre-eclampsia, blood pressure measurement and dipstick analysis of urine for protein have become a part of routine antenatal screening.

Table 1:

Classification of Hypertensive disorders of pregnancy
Pre-eclampsia / Eclampsia
Gestational Hypertension
Chronic Hypertension
Chronic Hypertension with superimposed pre-eclampsia

1.6 RISK FACTORS

A systematic review of controlled trials by Duckitt et al., (2005) found the unadjusted relative risks (RR) for developing pre-eclampsia were antiphospholipid antibodies (RR 9.72 , 95% confidence interval (CI) [4.34-21.75]), history of pre-eclampsia (RR 7.19, 95% CI [5.85-8.83]), pre-existing diabetes (RR 3.56, 95% CI [2.54-4.99]), multiple pregnancy (RR 2.93, 95% CI [2.04-4.21]), nulliparity (RR 2.91, 95% CI [1.28-6.61]), family history of pre-eclampsia (RR 2.90, 95% CI [1.70-4.93]), nulliparous women aged ≥ 40 (RR 1.68, 95% CI [1.23-2.29]), multiparous women aged ≥ 40 (RR 1.96, 95% CI [1.34-2.87]), and a raised body mass index (BMI) at booking (RR 1.55, 95% CI [1.28-1.88]) (Table 2).The risk of pre-eclampsia is also increased with pre-existing hypertension and renal disease, a preganacy interval of ≥ 10 years and a raised diastolic blood pressure at booking, and confirmed proteinuria (Duckitt et al.,2005 ; Milne et al.,2005).These evidence based risk factors have been recommended for use by the NICE guidelines (2003) and the pre-eclampsia community guideline (PRECOG) (Milne F et al., 2005) to screen for likelihood of the development of pre-eclampsia .

Table 2:

Risk factors for Pre-eclampsia		
	RR	**95%CI**
antiphospholipid antibodies	9.72	4.34-21.75
history of pre-eclampsia	7.19	5.85-8.83
pre-existing diabetes	3.56	2.54-4.99
multiple pregnancy	2.93	2.04-4.21
nulliparity	2.91	1.28-6.61
family history of pre-eclampsia	2.90	1.70-4.93
nulliparous women aged ≥ 40	1.68	1.23-2.29
multiparous women aged ≥ 40	1.96	1.34-2.87
raised body mass index (BMI)	1.55	1.28-1.88

1.7 PATHOPHYSIOLOGY OF PRE-ECLAMPSIA

1.7.1 'DISEASE OF THEORIES'

Pre-eclampsia has often been claimed to be the 'disease of theories'. While the exact aetiology of pre-eclampsia is unknown, there is substantial evidence that the placenta, in particular the trophoblast is necessary for the development of this disorder (Shah et al., 2007; Redman et al., 2000 and 2005; Roberts et al., 2000 and 2001). The spectrum of the clinical syndrome varies and is thought to be due to maternal endothelial dysfunction.

The endothelial dysfunction is thought to arise from placental hypoxia with oxidative stress or from the interaction between a normal placenta and conditions that make the mother susceptible to microvascular disease like chronic hypertension, diabetes mellitus and renal disease (Redman et al., 2005). Often it is due to a combination of the above, resulting in the wide spectrum of disease presentation.

There are currently two main theories regarding the primary precipitating factor in the disease process. In the two stage model it is suggested that a relative reduction in placental blood flow secondary to either defective placentation or maternal microvascular disease leads to release of circulating factors that cause endothelial dysfunction and that this gives rise to the clinical features seen (Roberts et al.,2002; Smith et al.,2006) (Figure 2).

In the continuum theory it is suggested that pre-eclampsia is predisposed to by factors that increase the maternal systemic inflammatory response to pregnancy. It is suggested that this is in response to a relative increase in trophoblastic debris that may be caused by a large placenta, an abnormal stimulus from a small placenta, or an excessive maternal sensitivity to such stimuli (Redman et al., 2000) (Figure 3).

Figure 2: Two Stage Model. Adapted from Roberts et al., (2002)

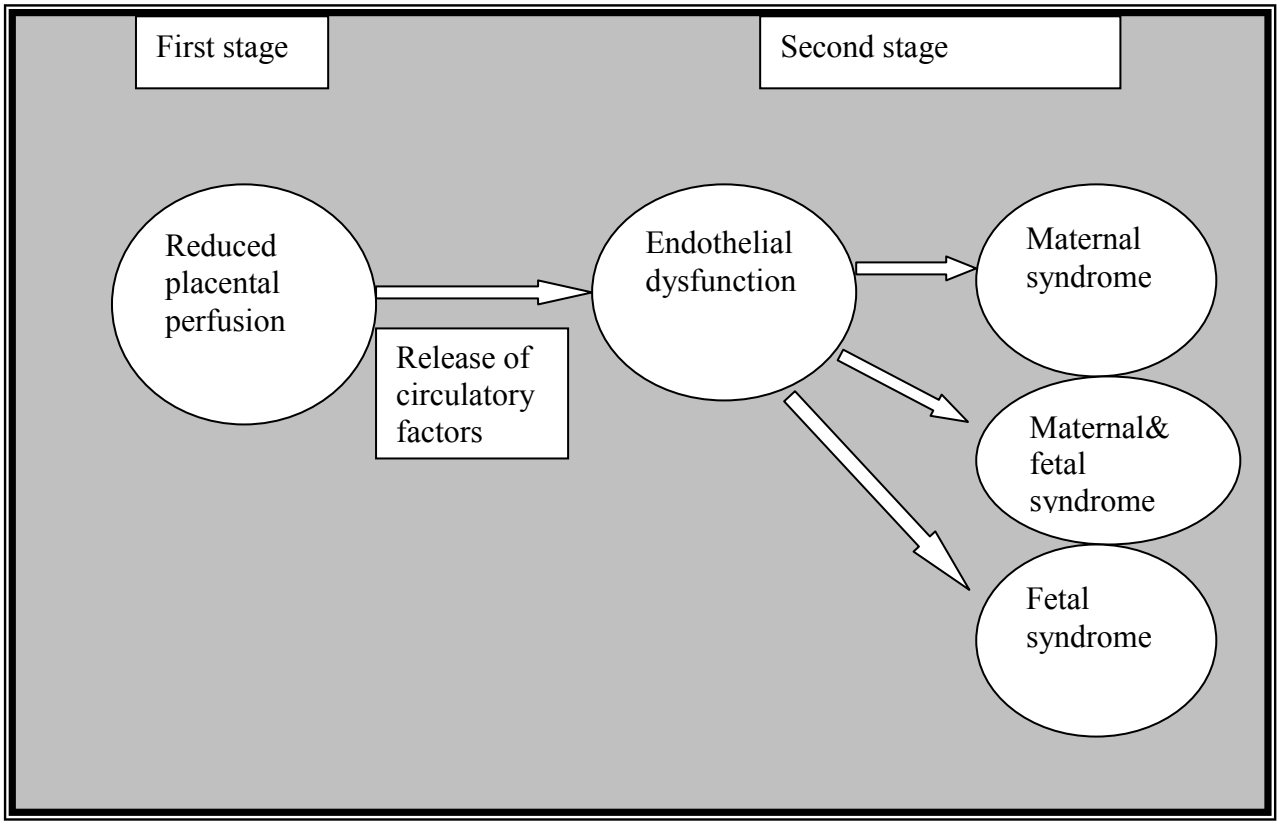
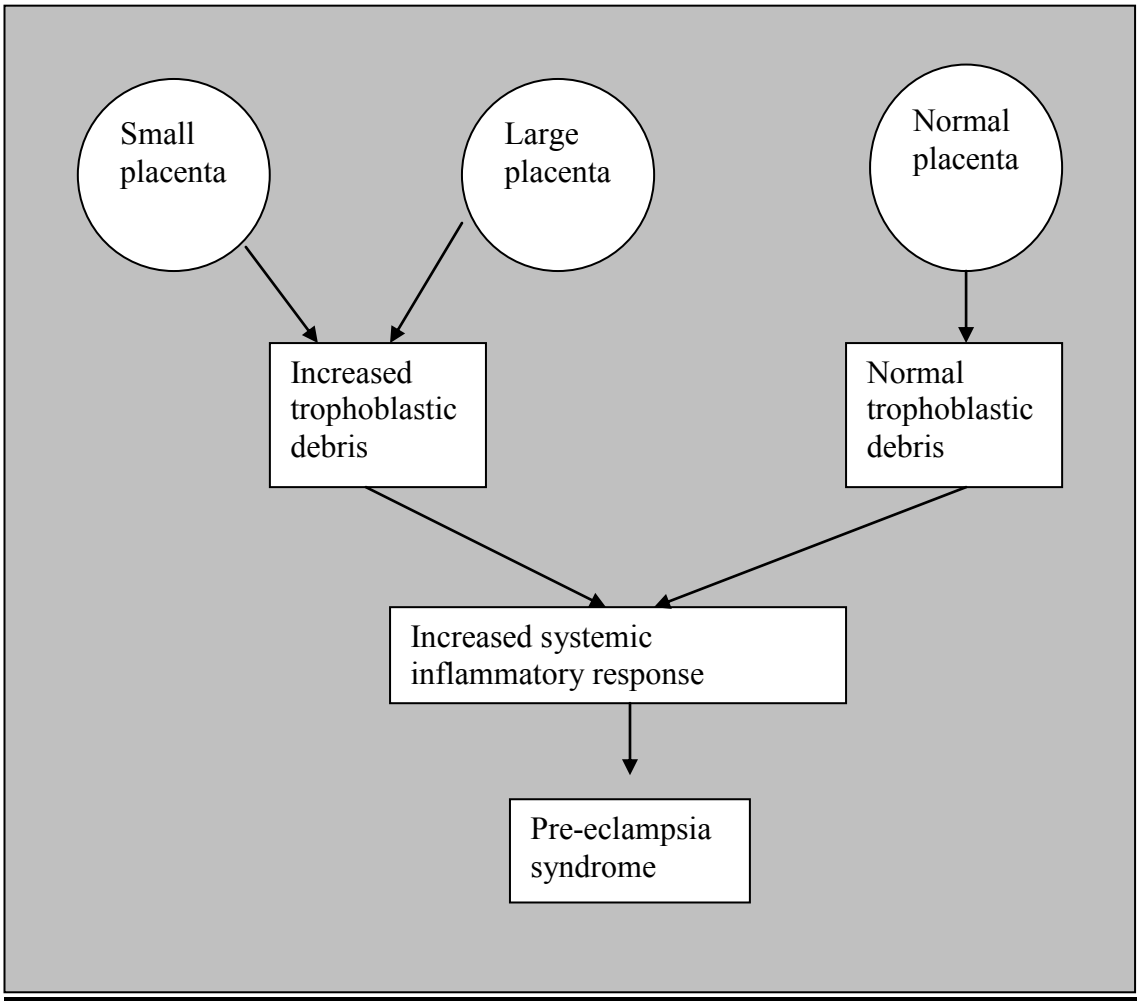


Figure 3: Continuum Theory. Adapted from Redman et al., (2000)



1.7.2 'UTEROPLACENTAL' MODELLING IN NORMAL PREGNANCY

In pregnancy the spiral arteries undergo various physiological changes to be transformed into 'uteroplacental' vessels. Trophoblastic invasion occurs in two phases with the first occurring between 6 to 12 weeks of gestation and the second between 14 to 18 weeks of gestation.

This invasion of maternal tissue at the implantation site consists of vascular and interstitial invasion (Shah, 2007). Zhou et al., (1997) found that cytotrophoblast cells transform from an epithelial phenotype to an endothelial phenotype as they invade the myometrium. Integrins (receptors that bind extracellular matrix ligands including fibronectin, lamnin and collagen), cadherins (molecules that mediate cell to cell adhesion) and immunoglobulin superfamily adhesion receptors (VCAM-1 AND PECAM-1) play an important role in invasion and acquisition of an endothelial phenotype (Zhou et al., 1997; Roberts et al., 2002).

This transformation is brought about by upregulating the expression of adhesion molecules such as vascular endothelial-cadherin (VE-cadherin) and $\alpha V\beta_3$ integrin and downregulation of molecules that restrain invasion ($\alpha_6\beta_4$, E-cadherin) and is thought to be required for successful endovascular invasion and normal placentation. These changes secondary to trophoblastic invasion transform the spiral arterioles into a high flow; low pressure system in order to support the pregnancy. Interstitial invasion consists of cytotrophoblasts invading the decidual tissue between blood vessels by extravillous trophoblasts from the anchoring villi.

1.7.3 'UTEROPLACENTAL' MODELLING IN PRE-ECLAMPSIA

Impaired placentation in pre-eclampsia has been attributed to failure of trophoblasts to adopt an endothelial phenotype and endovascular invasion failing to proceed beyond the superficial portions of the spiral arteries in early pregnancy (Zhou et al., 1997; Smith et al., 2006).

Disordered expression of several adhesion molecules that are normally involved in the conversion to an endothelial phenotype are thought to be responsible for the shallow implantation with limited vascular invasion (Zhou et al., 1997).Pijnenborg et al., (1996) found impaired trophoblast attachment on fibronectin and vitronectin in pre-eclamptic pregnancies, which may reflect differences in expression of matrix receptors.

Immunological factors have also been thought to contribute to defective trophoblastic invasion. Interaction between the Natural Killer (NK) cells and trophoblasts are important in placentation and may be defective in pre-eclampsia. Extravillous trophoblasts express a combination of human lymphocyte antigens (HLA) class 1 molecules, namely HLA-C, HLA-E and HLA-G, which are all ligands for NK cell receptors. From these, HLA-C is the only polymorphic antigen that signals paternal antigens (Redman et al., 2005; Parham et al.,2005).Killer immunoglobulin like receptors (KIRs), are NK receptors that recognize polymorphisms of HLA-C. Pregnant women who are homozygous for group A KIR haplotypes and carry a fetus that expresses HLA-C2 are at highest risk of pre-eclampsia (Redman et al., 2005; Parham et al.,2005). Group A KIRs haplotypes inhibit NK cells and it is thought that in pre-eclampsia overly inhibited NK cells may lead to understimulation of trophoblasts (Parham et al., 2005).

In pre-eclampsia spiral arteries that would normally achieve a mean diameter of 500 microns have reduced distensibility and only achieve a mean diameter of 200 microns (Shah, 2007). This altered vascular modelling results in reduced placental perfusion

and relative ischaemia occurs.

1.7.4 ENDOTHELIAL DYSFUNCTION IN PRE-ECLAMPSIA

Maternal endothelial dysfunction is responsible for the clinical features of the disease. Hypertension results from enhanced vascular sensitivity to angiotensin II and norepinephrine with subsequent vasoconstriction. This is further aggravated by a decrease in production and activity of prostaglandins like prostacyclin and nitric

oxide. Increase endothelial cell permeability results in leakage of protein from the intravascular space giving rise to oedema and proteinuria. Platelet aggregation results due to an imbalance of aggregatory and anti-aggregatory factors. These include prostacyclin, thromboxane and serotonin (Sibai et al., 2005).

According to the two stage model, the hypoxic placenta releases factors into the maternal circulation that results in an exaggerated systemic inflammatory response of which endothelial dysfunction is a key component. Recently there has been much interest in factors that are thought to be responsible. Vascular endothelial growth factor (VEGF) is an angiogenic factor that is expressed by the placenta (Shah, 2007). It plays an important role in blood pressure regulation and in maintaining the integrity of the glomerular filtration barrier (Maynard et al., 2003).

Soluble film- like tyrosine kinase (sflt), also known as soluble vascular endothelial growth factor receptor (sVEGFR) is elevated in pre-eclampsia and is associated with decreased free VEGF and placental growth factor (PlGF) (Maynard et al.,2003; Shibata et al.,2005; Mutter et al.,2007). Over expression of sflt1 in rats leads to hypertension, proteinuria and glomerular endotheliosis , which are characteristic of the clinical manifestations of pre-eclampsia suggesting that this factor may have a causal role in pre-eclampsia (Maynard et al.,2003). However, sflt1 is not raised in every women affected, suggesting that other factors may play a role.Endoglin (Eng), a placental derived soluble transforming growth factor β co-receptor, an anti – angiogenic factor, is elevated in patients with pre-eclampsia (Venkatesha et al.,2006).

Administration of sflt1 together with Eng in pregnant rats results in a pre-eclampsia like syndrome including features of HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome suggesting that these two factors may work in concert (Venkatesha et al.,2006).

1.8 RENAL CHANGES IN NORMAL PREGNANCY

1.8.1 THE GLOMERULAR FILTRATION BARRIER

The kidney is composed of many glomeruli. Each glomerulus serves as a filtering unit that allows the passage of water and small molecules while serving as a barrier to larger molecules like albumin. The glomerular filtration barrier is composed of three layers which include an endothelial layer, the glomerular basement membrane and the epithelial cell layer composed of cells called podocytes.

The glomerular filtration rate (GFR) refers to the ultrafiltrate of plasma across the above mentioned layers (Hladunewich, 2005). Glomerular filtration rate depends upon effective renal plasma flow (ERPF), the ultrafiltration coefficient 'K_f' (the product of the available surface area for ultrafiltration and the porosity of that surface) and Starling forces acting across the glomerular wall (the net oncotic pressure and the transglomerular hydrostatic pressure difference) (Moran et al., 1999).

Passage of solutes through the glomeruli is determined by their size and charge selectivity. The podocyte layer that lines the Bowman's capsule is thought to be the primary filtration barrier to the passage of large molecular weight proteins (Hladunewich, 2005).

1.8.2 PROTEINURIA AND GLOMERULAR FILTRATION RATE IN NORMAL PREGNANCY

The methods of screening for proteinuria and the level of significant proteinuria in HDP have remained controversial. In the non-pregnant women daily urine protein excretion averages 20-80 mg/day with an upper limit of protein excretion of two standard deviations above the mean or 150 mg /day. This consists of 40% albumin, 15-20 % immunoglobulin (IgG 5-10%, IgA 3% and light chains 5-10 %) and the remainder is Tamm-Horsfall glycoprotein derived from the tubules and the lower urinary tract (Kumar et al., 1990).

In normal pregnancy, GFR and ERPF increase by approximately 50%. Clinically, GFR is determined by measuring creatinine clearance (CC). Creatinine clearance reliably correlates with GFR provided that a complete urine collection is obtained during an accurately timed period. Creatinine clearance is significantly increased by 4 weeks gestation, peaks at 9-11 weeks gestation and is then sustained until the 36th week of gestation. In the last four weeks of pregnancy CC reduces by 15-20 % (Davison et al., 1980). In pregnancy CC may be increased to values of 150-200ml/min. These renal haemodynamic changes result in greater quantities of colloids and solute passing by the glomerular barrier per unit time.

In addition there are changes in glomerular permeability and altered tubular reabsorption of filtered proteins that may result in increased excretion of protein. Thus it is normal in pregnant women for total protein excretion (TPE) and urinary albumin excretion (UAE) to be significantly elevated after the 20th week of gestation (Maybury and Waugh, 2004).Currently the accepted upper limits of normal for protein excretion in pregnancy are 300mg/24 hours for TPE and 30mg/24 hours for UAE (Higby et al.,1994). Kuo et al (1992) have suggested that a threshold for pregnancy should be lowered to 200mg /24 hours for TPE but it is the 300mg threshold that remains in clinical use.

1.9 RENAL CHANGES IN PRE-ECLAMPSIA

In pre-eclampsia there is a reduction of both GFR and ERPF by 30-40% compared with normal pregnancy (Lafayette et al., 1998; Moran et al., 2003).It is postulated that the basis for the hypofiltration is largely secondary to structural changes in the glomerulus as opposed to renal vasoconstriction with a depression in renal plasma flow (Hladunewich, 2005). Rarely, prolonged renal hypoperfusion with resulting acute tubular necrosis can occur in severe pre-eclampsia.

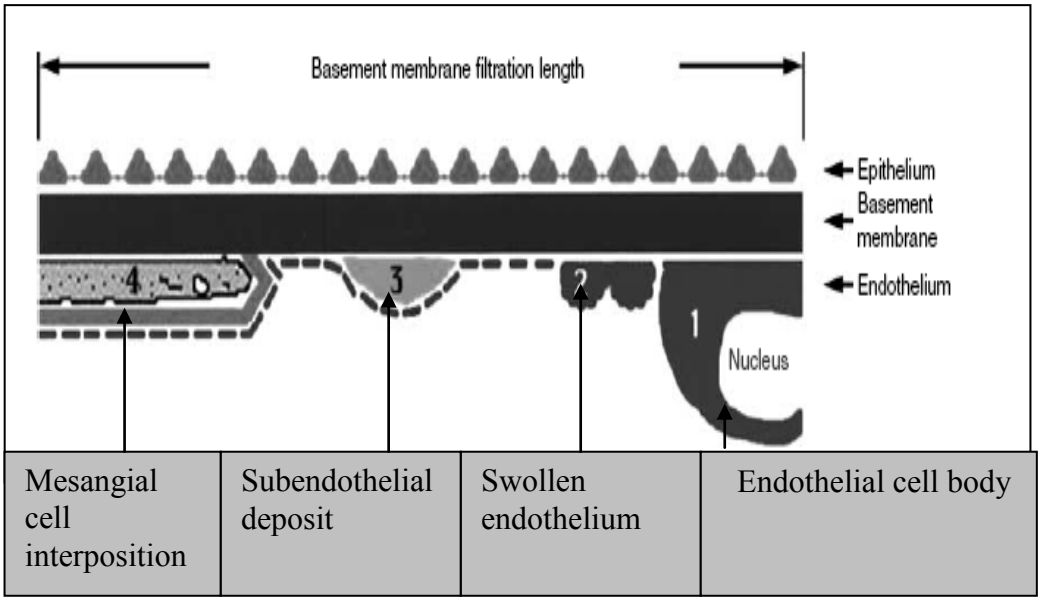
Proteinuria may rarely precede hypertension but usually accompanies or follows it. After pregnancy is terminated, proteinuria commonly disappears within 3 to 8 weeks,

but occasionally persists for months. Pre-eclampsia is the leading cause of nephrotic syndrome during pregnancy. Protein excretion may vary from less than a gram to 8 to 10 g per day. The urinary sediment is usually bland with red blood cells and cellular casts being rare (Karumanchi et al., 2005).

In pre-eclampsia, glomeruli undergo structural changes with endothelial vacuolization and hypertrophy of the cytoplasmic organelles defined as glomerular endotheliosis (Spargo et al., 1959). Loss of both size and charge selectivity of the glomerular barrier contribute to the development of albuminuria (Moran et al., 2003).The proteinuria of pre-eclampsia is thus considered to be non-selective. Figure 4 provides a schematic representation of the glomerular filtration barrier with the characteristic changes accompanying pre-eclampsia**.**

Recently, Garovic et al., (2007) demonstrated the presence of 4 podocyte markers (podocin, podocalyxin, synaptopodin, and nephrin) in patients with pre-eclampsia at the time of delivery. Podocyturia (i.e., urinary excretion of podocytes) may contribute to proteinuria in pre-eclamptics and may indicate loss of podocytes from the glomerulus leading to disruption of the glomerular filtration barrier and subsequent proteinuria.

Figure 4: Schematic representation of the glomerular filtration barrier with characteristic changes accompanying pre-eclampsia. Adapted from Hladunewich (2005).



1.10 PROTEINURIA AND PREGNANCY OUTCOME

There are several key questions that need to be answered when one looks at the relationship between proteinuria and pregnancy outcome. These include:

1. How has the threshold for significant proteinuria been determined and does it have any correlation to clinical outcome?
2. Is proteinuric hypertension associated with greater adverse maternal and fetal outcome?
3. Is the severity of adverse maternal and fetal outcomes related to the degree of proteinuria?

1. How has the threshold for significant proteinuria been determined and does it have any correlation to clinical outcome?

Proteinuria above a threshold of ≥ 300mg/24 hours has been used as a criterion to differentiate pre-eclampsia from gestational hypertension in the classification system for hypertensive disorders of pregnancy (Davey and MacGillivray, 1998).It is used both as a marker of severity and disease progression. The threshold for significant proteinuria is based on reference ranges from a normal pregnant population using the upper centile (95^{th} to 99^{th}) to define significance. Higby et al., (1994) published the first adequate study establishing normal urinary protein and albumin excretion in pregnancy. They reported figures of 260mg per 24 hour for urinary protein and 29 mg per 24 hour for albumin as the upper limit of normal excretion during pregnancy and also found that there was a statistically significant increase in protein excretion after 20 weeks of gestation. Waugh et al., (2005a) in a prospective study of 197 women found that whilst 300mg/24 hours may be above the 95^{th} centile for an obstetric population, it is a threshold of 500mg/24 hours that is more predictive of adverse outcome.

2. Is proteinuric hypertension associated with greater adverse maternal and fetal outcome?

Proteinuria associated with hypertension in pregnancy is associated with greater adverse maternal and fetal outcome. In pregnant women with mild chronic hypertension but no proteinuria, the outcome of pregnancy is similar to non - hypertensive pregnant women (Sibai et al., 1983) whilst hypertension together with proteinuria is associated with poor fetal outcome, an increased rate for small for gestational age pregnancies, increased perinatal mortality and maternal morbidity (Chan et al., 2005; Brown et al., 1996; Ferranzani et al., 1990; Chua and Redman, 1992; Lin et al., 1982).

3. Is the severity of adverse maternal and fetal outcomes related to the degree of proteinuria?

There is conflicting evidence in the literature regarding the degree of proteinuria and adverse maternal and fetal outcome. Chan et al., (2005) found that in women with pre-eclampsia, the probability of adverse maternal outcomes increased with both increasing maternal age and increasing spot urine protein to creatinine ratios. They found that a spot protein to creatinine ratio of 900mg/mmol or more for all ages, was associated with an increase in risk of developing adverse maternal outcomes (likelihood ratio for a positive result of 7 or more).The probability of adverse fetal outcome was also increased with an increasing spot urine protein to creatinine ratio and was significantly greater when gestation at initial presentation was less than 34 weeks. Chan et al., (2005) however could not determine a specific spot protein to creatinine ratio that could be used as a definitive screening test for adverse outcomes. Other studies have suggested that the severity of proteinuria is not proportionally linked to increasing adverse maternal and fetal outcomes. Chua et al., (1992) found that delivery was necessary within 2 weeks of the onset of heavy proteinuria (> 5g/24 hours) in 88.1% of cases and that in a subset of more preterm pregnancies, pregnancies could be safely prolonged for up to 4 weeks with intensive monitoring.

Schiff et al., (1996) concluded in a retrospective study that the amount of proteinuria and the rate of increase in proteinuria during conservative management were not important predictors of maternal and perinatal outcome. A prospective study by Hall et al., (2001) similarly found that significant increases in proteinuria or heavy proteinuria was not associated with poorer maternal and fetal outcomes compared to smaller increases of proteinuria of < 5g/24 hours. Newman et al., (2003) found in a retrospective review that the magnitude of proteinuria, even when massive (> 10g/24 hours), did not correlate with increased maternal or neonatal morbidity in pre-ecampsia. They concluded that neonatal morbidity appeared to be more a function of prematurity rather than massive proteinuria itself.

1.11 DIPSTICK URINALYSIS

Proteinuria is assessed most appropriately by the biochemical quantitative measurement of total protein excretion over a 24-hour period. This is an impractical screening test. The most commonly employed screening method for proteinuria antenatally is a semi-quantitative dipstick urinalysis (Halligan et al., 1999).

Several studies have questioned the value of dipstick urinalysis. Kuo et al., (1992) compared the dipstick diagnosis of significant proteinuria in 24-hour urine collections with dipstick urinalysis in 68 hypertensive pregnant women admitted to hospital with 0.3g/l proteinuria on urinalysis. They found a wide range of total protein values at a urine score of 1+ on dipstick urinalysis.

Meyer et al., (1994) retrospectively reviewed case records of 300 hypertensive women. They reported that 60 % of women with a negative or trace dipstick result had significant proteinuria defined as ≥ 0.3 g protein/24 hours and a significant false positive rate of 26 % with a 1+ dipstick result.

Brown et al., (1995) compared ward urinalysis for protein obtained on a midstream sample before and after a 24-hour urine collection and compared this with the 24-hour urine protein excretion. Urinalysis was also performed on a mixed aliquot of each 24-hour urine sample. The positive predictive value for urinalysis ranged from 38 % (pre-collection) to 60% (for test on 24 hour aliquot). Negative predictive values ranged from 86 to 88% respectively.

Waugh et al., (2001) found that the amount of protein assessed quantitatively was dependent on the biochemical assay employed. The positive and negative predictive values for urine dipstick analysis were dependent on the type of assay used as the gold standard. They however found that dipstick urinalysis has a significant false negative rate regardless of the type of assessment.

Gangaram et al., (2005) evaluated the accuracy of dipstick urinalysis in a single voided urine sample and in an aliquot of a 24-hour urine collection in the assessment of proteinuria in 198 hypertensive pregnant women, using the 24-hour urine protein excretion as the gold standard. The positive predictive value (PPV) for dipstick urinalysis ranged from 64.9% (single voided urine sample) to 94.2% (24 hour urine aliquot). The negative predictive value (NPV) ranged from 75.2% (single voided urine sample) to 84.2% (24 hour urine aliquot).

These studies show that random semi-quantitative dipstick analysis in the diagnosis of proteinuria in pregnancy is imprecise and its value is questionable. False positive results may subject patients to the inconvenience of over investigation and unnecessary interventions, while false negative results may jeopardise the health of the woman and her fetus.

1.12 INACCURACY OF DIPSTICK URINALYSIS- THE ROLE OF AUTOMATION

There are several factors that may contribute to the inaccuracy of urine dipstick analysis. These include observer error (Bell et al., 1999) and factors that may influence concentration of protein in an individual urine specimen such as contamination, exercise, posture, osmolality and urinary pH (Halligan et al., 1999).

In order to overcome inter observer errors, automated technology has been introduced to read the urine dipstick. Saudan et al., (1997) found that an automated urinalysis device (Clinitek 100 Ames) improved the positive predictive value of urinalysis from 24% to 47% at the 1+ (0.3 g/l) concentration and from 53% to 83% at the 2+ (1g/l) concentration, without significantly altering the false negative rate. Although the automated device eliminated inter- and intra-observer variability, there were still

persistently high rates of false positives encountered at the 1+ level, though only to half the extent of the visual urinalysis.

Waugh et al., (2005b) performed a study comparing visual dipstick testing with automated methods. They found automated dipstick testing has a significantly better positive (PPV 78% vs. 64%) and negative predictive value (NPV 84% vs. 65%) for detecting 300mg/24 hours protein excretion.

1.13 LABOROTARY MEASUREMENT OF PROTEINURIA – THE 'GOLD STANDARD'

There is a lack of a clear 'gold standard' for quantitative measurement of proteinuria. There are currently more then ten different assays, none of which has gained universal acceptance. This makes it difficult to compare the outcomes of various studies with similar methodology where there are differences in the assay used as the laboratory gold standard.

In pregnancy, Waugh et al., (2001) compared urine dipstick analysis to the Benzethonium chloride assay and the Bradford assay. They found a variation between dipstick urinalysis and the two different assays and attributed this to protein assay specificity and the observed protein compositions of the samples on electrophoretic analysis. Thus the measurement of proteinuria is dependent on the type of assay used in the laboratory.

1.14 PROTEIN:CREATININE RATIOS IN SPOT URINE SAMPLES

The gold standard for determining protein excretion is the 24 hour urine collection. The need for a 24 hour collection is due to the variation in protein excretion during the day. Factors that may contribute to this variation include variation in water intake and excretion, rate of diuresis, exercise, recumbency and diet (Price et al., 2005).

The major problem with the 24 hour protein collection is that it is often impractical in the outpatient setting with problems of incomplete collection. In order to overcome this, the spot protein to creatinine ratio has been proposed. During the day urinary protein and creatinine excretion rates are fairly constant provided the glomerular filtration rate is constant. Thus a ratio of the concentrations of urinary protein and creatinine in a single voided urine sample would reflect the cumulative excretion during the day since the ratio of two stable rates would cancel out the time factor (Ginsberg et al., 1983).

Recent studies have suggested a strong correlation between the protein/ creatinine ratio and 24 hour urine protein level in women with pre-eclampsia. Jaschevatzky et al., (1990) measured the protein:creatinine ratio in 35 pre-eclamptic patients and 70 healthy pregnant women. They found a close correlation between the protein/ creatinine ratio in random urine samples and the 24 hour protein excretion ($r = 0.927$; $p < 0.001$) and the 24-hour protein:creatinine ratio ($r = 0.920$; $p < 0.001$) in the pre-eclamptic patients.

Besides showing a significant correlation between the 24 hour urine protein and the protein:creatinine ratio ($r = 0.93$; $p < 0.001$), Neithardt et al., (2002) in addition found that the protein:creatinine ratio appears to predict trends in protein excretion over time.

Other studies have found contradictory results. Durnwald et al., (2003) found a poor correlation ($r^2 = 0.41$) between the protein:creatinine ratio in 220 women with suspected pre- eclampsia. Al et al., (2004) similarly found a poor correlation ($r = 0.56$,

$p < 0.01$) in patients with new onset mild hypertension in late pregnancy. A systematic review by Price et al., (2005) concluded that there was sufficient data to demonstrate a strong correlation between the protein:creatinine ratio in a random urine sample and 24 hour protein excretion. They also found that protein:creatinine ratio in a random urine sample might be used to rule out significant proteinuria as defined by a 24 hour urine excretion measurement.

To increase the applicability of the use of the protein:creatinine ratio in clinical practice semi-quantitative protein:creatinine ratio dipsticks have been developed. Roy et al., (2003) described the first assessment of a protein:creatinine ratio dipstick (Autistics Pro: Bayer Diagnostics).They tested a midstream urine of 171 hypertensive pregnant women with protein:creatinine ratio dipsticks on a validated Urinanalyser (Clinitek 50: Bayer diagnostics) and compared this with the use of visual and automated dipstick analysis using the 24 hour total protein measurement as the gold

standard. They found the sensitivity (94.5%) and specificity (95.7%) of the protein:creatinine ratio dipsticks to be superior to visual dipstick urinalysis for the prediction of 300mg protein / 24 hours at the 1+ threshold.

1.15 MICROALBUMINURIA

1.15.1 HISTORY OF MICROALBUMINURA

The term microalbuminuria (MA) was first used by Viberti et al., (1981) to describe urinary excretion rates of albumin that could not be detected by standard urinary dipsticks, but only by more sensitive assays for albumin, to predict development of overt proteinuria in diabetic patients. While the clinical utility of MA has been largely studied and applied in diabetic populations to screen and monitor for incipient nephropathy, more recently it has been found to be an independent predictor of cardiovascular disease in patients with diabetes, hypertension and in the general population (Busby et al., 2005; Verdecchia et al., 2004; Heerspink et al., 2006;

Ruggenenti et al., 2006). It has also been suggested as a marker of endothelial dysfunction (Verdecchia et al., 2004). These findings have led to an increased interest in the role of microalbuminuria in hypertensive disorders of pregnancy.

1.15.2 DEFINITION OF MICROALBUMINURIA

Microalbuminuria is defined as a urinary excretion rate of albumin between 20µg and 200µg/min or between 30 mg/day and 300mg/day. Traditionally this has been measured using a 24 hour urine collection. The definition has been expanded to include spot urinary microalbumin to creatinine ratio (UAC) of 30 to 300mg/g.

When using the UAC, various factors affecting albumin and creatinine excretion need to be taken into account. Factors affecting albumin excretion include blood pressure, time of day, fasting, salt intake and volume status (Khosla et al., 2006).

During the 1990s, the most sensitive strips for detection of albuminuria had thresholds for detection of 20 mg/l. Thus a lower limit of 30 mg/day was chosen for the definition of microalbuminuria as the average daily urine output of 1.5 l was multiplied by 20mg/ l. The upper limit of 300 mg / day was chosen as the sensitivity of the older dipsticks for albumin was 100 to 300 mg/l (Heerspink et al., 2006).

Recent studies have demonstrated that subjects with even slight increases in urinary albumin excretion in the normal range have an increase risk for development of cardiovascular morbidity and mortality (Gerstein et al., 2001) .This suggests that the limits chosen for microalbuminuria are arbitrary and the best cut offs still need to be identified (Heerspink et al., 2006).

1.15.3 PATHOPHYSIOLOGY OF MICROALBUMINURIA

Microalbuminuria appears to be more a marker of vascular disease than a pathogenic factor. Factors known to influence the development of MA include an increased body mass index (BMI), hypertension, endothelial dysfunction, a decrease in high density

lipoprotein levels, insulin resistance, smoking, salt sensitivity, increasing age and a DD ACE- genotype (Khosla et al., 2006; Verdecchia et al., 2004)

Patients with MA have an elevated transcapillary escape rate of albumin, and usually the presence of one or more of the above risk factors. The mechanism of vascular injury differs among diabetic and hypertensive populations. In hypertensive patients with MA, increases in microvascular pressure results in endothelial damage, leading to generalized vascular leakiness. Excess protein is deposited in the extracellular matrix, resulting in the capillary basement membrane becoming sclerosed.This response is mediated through various stimuli such as, complement activation, macrophages, neutrophils, and endothelial stimulation from other inflammatory insults (Khosla et al., 2006; Verdecchia et al., 2004).

In diabetic patients, the glycated state of albumin transforms it into an antigenic like molecule that is associated with generation of free oxygen radicals that causes direct injury to the glomerular membrane. This impairs glomerular filtration of proteins resulting in increased albumin excretion (Stehouwer et al., 1997; Khosla et al., 2006).

The link between diabetic and non-diabetic MA may be impaired insulin resistance, leading to an increased amount of glycated albumin.

1.15.4 MICROALBUMINURIA IN PREGNANCY

Recently there has been interest in the measurement of microalbumin in the urine of pregnant women. Microalbuminuria is defined as urinary excretion of albumin that is persistently above normal, although below the sensitivity of conventional semi-quantitative test strips (Maybury and Waugh, 2004).

Proteinuria in pregnancy is due to selective glomerular filtration and non-selective (proximal tubule) reabsorption. In non-pregnant women there is an albumin filtration of 500mg -600mg/day (Maybury and Waugh, 2004). During pregnancy proteinuria

gradually increases with levels of 5mg/100ml in the first and second trimesters and 10mg/100ml in the third trimester. Levels in the third trimester may reach 300mg/ml in normal pregnancy (Davison, 1985). The majority of additional albumin excretion in pregnancy is from nocturnal excretion (Douma et al., 1995). Gestation specific reference ranges for urinary microalbuminuria, creatinine concentration and microalbumin to creatinine ratio have been described for uncomplicated pregnancies (Waugh et al., 2003b).

1.15.5 MICROALBUMINURIA AND HYPERTENSIVE DISORDERS OF PREGNANCY

It has been suggested that a phase of micoalbuminuria may precede overt proteinuria in pre-eclampsia (Bar et al., 1996). There has been mixed results in the literature on the usefulness of MA as an early predictor of pre-eclampsia.

Lopez-Espinoza et al., (1986) found no evidence that gross proteinuria detected in patients with pre-eclampsia was preceded by a gradual increase in microalbuminuria, and Konstantin-Hansen et al., (1992) concluded that MA could not be used to predict pre-eclampsia in low risk pregnant women.

Nakamura et al., (1992) in a study of 199 normotensive pregnant women at 20 and 30 weeks of gestation, found the fasting urinary albumin to creatinine ratio to be significantly higher in women destined to develop pregnancy induced hypertension. Using a cut off value of more than 16mg/g as a positive test result, the negative predictive value was 94% for 20 weeks and 96% for 30 weeks gestation and thus they concluded that this was a useful screening tool for predicting pregnancy induced hypertension.

Das et al., (1996) concluded that microalbuminuria was a significant risk factor for prediction of pre-eclampsia. Using ≥ 20 μg/ml of urinary albumin as a positive test, they found a sensitivity of 64, 42% and specificity of 91, 84%.

Microalbuminuria dipsticks have also been used to detect clinically significant proteinuria (Higby et al., 1995; Waugh et al., 2005a). Higby et al., (1995) compared two screening tests for MA, namely the Micro-bumintest and Multistix 10SG with a 24 hour quantitative urinary protein measurement. They found the Micro-bumintest to have good sensitivity (87%), specificity (99%), PPV (81%) and NPV (99%) compared to the Multistix 10SG which had a lower sensitivity (36%), specificity (97%), PPV (68%) and NPV (88%).

Besides being used as a predictor of pre-eclampsia it has also been suggested that MA may correlate better with other clinical measurements of disease severity as it

may more accurately reflect the glomerular dysfunction associated with the glomerular endotheliosis of pre-eclampsia (Waugh et al., 2005a).

1.15.6 POINT OF CARE INSTRUMENTS

Microalbuminuria dipsticks compared to the traditional visual urinary dipsticks have also been shown to be a better screening test for clinically significant proteinuria (Higby et al., 1995; Waugh et al., 2005a). Various semi-quantitative dipstick tests have been used for detection of MA. In order to allow routine testing of the antenatal population for microalbuminuria, quantitative and semi-quantitative point of care urinanalysers have been developed.

The DCA 2000 (Bayer Corp., Elkhart, IN) is a point of care instrument that provides a quantitative result of the UAC. Waugh et al., (2003a) found that the DCA 2000 is accurate for the measurement of albumin to creatinine ratios in the uncomplicated population. In the hypertensive pregnant population they found the DCA 2000 remained accurate though when the albumin concentration was greater than 40mg/l the 95 % limits of agreement are broader. They found the DCA 2000 to have a

sensitivity of 94% [95% CI (0.85-0, 98)] and specificity of 98% [95%CI (0.85-0.98)] to detect significant proteinuria (Waugh et al., 2005).

The Clinitek Microalbumin Reagent Strip (Bayer Corporation, Elkhart, IN) is a semi-quantitative dipstick test for MA that is read using the Clintek 50 portable urine chemistry analyzer. Waugh et al., (2005a) found that the Clinitek 50 system had a sensitivity of 58% [95% CI (0.47-0.70)] and specificity of 83% [95% CI (0.74-0.90)] for detecting significant proteinuria. The same study found the semi-quantitative visual Multistix 8SG used to detect proteinuria to have a lower sensitivity of 51% [95% CI (0.39-0.62)] and specificity of 78% [95% CI (0.68-0.86)] for detecting significant proteinuria.

This type of automated point of care urine analyzers are able to provide rapid results, avoiding inter observer error and provide automated documentation of results. Based on the potential benefits of such instruments, we decided to test the clinical utility of the Clinitek 50 system in our setting.

CHAPTER 2

2.1 AIMS

The use of semi-quantitative UAC dipstick analysis using point of care urinanalysers may offer significant advantages. These include decreasing the need for timed 24 hour urine collections, a reduced need for hospital admission and rapid availability of results with improved accuracy over other forms of dipstick urinalysis for proteinuria. We therefore decided to embark on study using the Clinitek® Microalbumin reagent strip (Bayer Healthcare LLC, USA) that is analysed on the Clinitek® urinalyser (Bayer Healthcare LLC, USA) with the following aims:

- Determine the accuracy of spot UAC dipsticks and conventional visual dipsticks compared to laboratory UAC quantification to detect significant proteinuria in a normotensive and hypertensive population attending antenatal clinic.

- Determine the accuracy of spot UAC dipsticks and conventional visual dipsticks compared to a 24 hour urine protein collection (gold standard) to detect significant proteinuria in HDP.

- Determine the role of proteinuria as determined by the 24 hour urinary protein and the UAC dipstick read on the Clinitek 50 system on pregnancy outcome in HDP.

2.2 ETHICS AND STUDY LOCATION

This was a prospective study conducted at hospitals serving the Durban Metropolitan region in South Africa, viz. King Edward VIII, R.K. Khan and Inkosi Albert Luthuli Central Hospitals. Institutional ethical approval was obtained (no.E042/05) and all participants gave consent. Recruitment began in January 2006 and ended in September 2007.

CHAPTER 3 – DIPSTICK VALIDATION

3.1 INTRODUCTION

As previously mentioned, the use of point of care analysers to determine the UAC ratio in pregnancy, may offer significant advantages over conventional visual dipsticks in the assessment of significant proteinuria in pregnancy. We therefore embarked on a pilot study to validate the Clinitek 50 system by determining the accuracy of spot UAC dipsticks and conventional visual dipsticks compared to laboratory UAC quantification to detect significant proteinuria in normotensive and hypertensive antenatal attendees.

3.2 METHOD

In order to validate the UAC dipstick we required participants with and without significant proteinuria. We thus chose one population group with a low prevalence of proteinuria (normotensives) and the other with a high prevalence (hypertensives).

A series of 15 normotensive pregnant women and 11 women with new onset hypertension in pregnancy after 20 weeks of gestation were recruited at the antenatal clinic. Exclusion criteria included women with chronic renal disease, eclampsia and urinary tract infections.

Each woman had a spot midstream urine (morning specimen) collected. A small amount of this specimen was tested for proteinuria using a semi-quantitative visual dipsticks (Makromed®, Makro Medical, RSA) by trained midwifes at the antenatal clinic; another small amount of the urine specimen was then analysed using the semi-quantitative microalbumin:creatinine ratio dipsticks (Clinitek® Microalbumin, Bayer Healthcare LLC, USA) read on the Clinitek® 50 urine chemistry analyser. This test was carried out by two doctors who were specifically trained to use the instrument as a side room investigation.

The results from the printout of the test and the semi-quantitative tests were recorded in a structured data sheet (Appendix 1). The remainder of the sample was then sent to the laboratory for quantitative measurement of the UAC. The midwives and the laboratory personnel were blinded to the results.

The results of the semi-quantitative measurement of visual dipsticks (Makromed®) for proteinuria done routinely at the antenatal clinic and the UAC dipsticks were compared to the quantitative measurement of the UAC in the laboratory. A result of ≥1 + on visual dipsticks was considered positive for proteinuria. A spot urinary microalbumin to creatinine ratio of > 300mg/g (33.9 mg/mmol) was considered as positive for significant proteinuria. Figure 5 illustrates a flow diagram of the methodology.

3.3 TEST METHODS

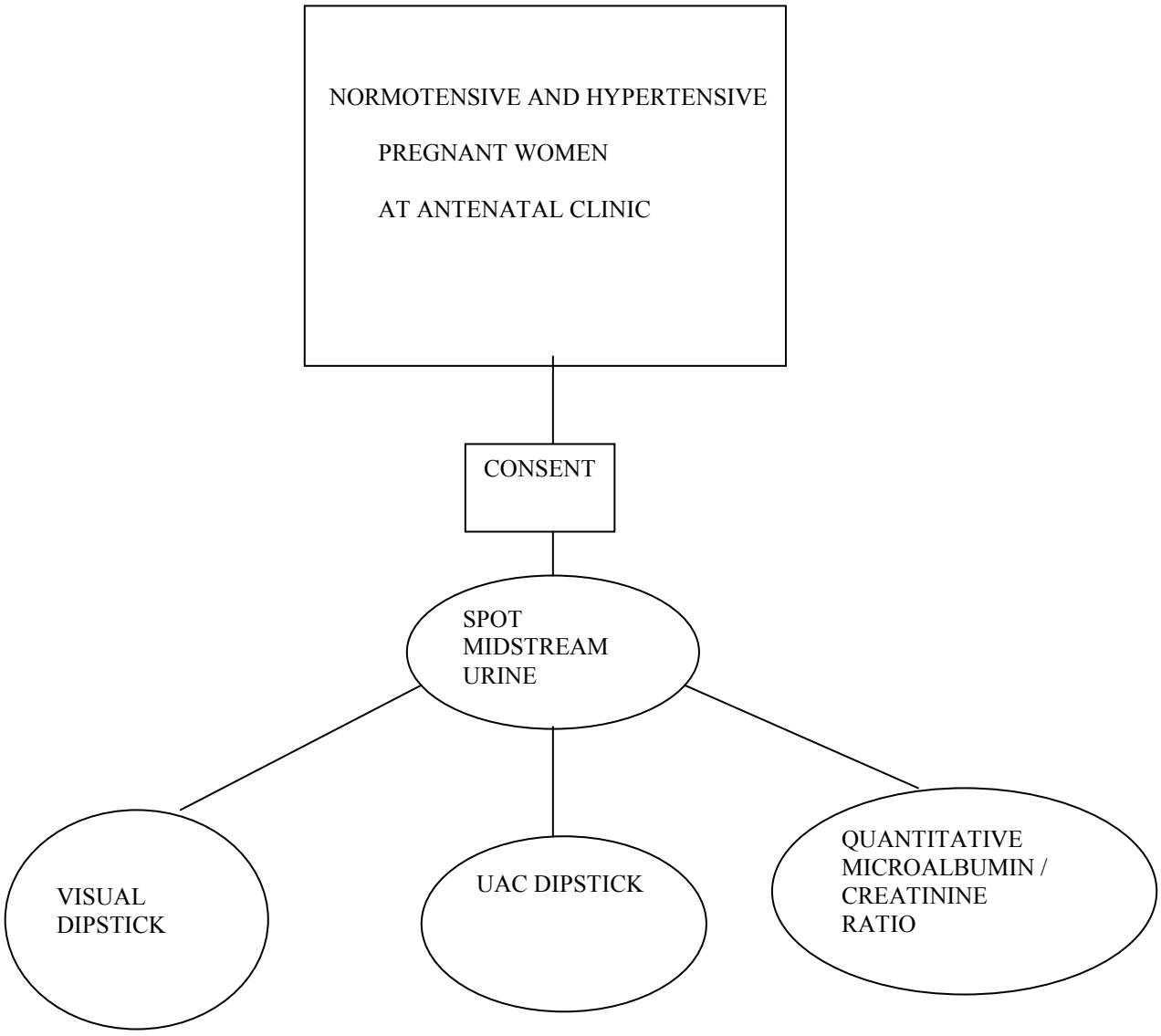
Semi-quantitative visual dipsticks (Makromed®, Makro Medical, RSA) were used to test for proteinuria. This visual dipstick is sensitive for albumin (15-20 mg/dl albumin) with its active agent being tetrabromophenol blue.

The dipstick test used to detect MA was the Clinitek® Microalbumin reagent strip (Bayer HealthCare LLC, USA). This test is based on albumin binding to sulphonephthalein dye and creatinine forming a copper creatinine complex that catalyzes the reaction of di-isopropyl-benzene dihydroperoxide and 3,3', 5,5'-

tetramethylbenzidine. Both these reactions produce colours that are read reflectometrically in the Clinitek 50 portable urine chemistry analyser. Albumin concentrations are reported as 10, 30, 80 and 150 mg/l, creatinine concentrations as 10, 50,100,200 and 300 mg/l and UAC as < 30 mg/g, 30-300 mg/g and > 300mg/g.

The Multigent Microalbumin assay was used for the quantitative measurement of albumin on the spot urines on the Architect c6000® System. This assay is an immuno-turbidimetric assay that uses polyclonal antibodies against human albumin. The coefficient of variation (CV) of this assay was ≤ 5%. Creatinine was measured quantitatively using the Jaffe´ reaction on the same analyser.

Figure 5: FLOW DIAGRAM OF METHODOLOGY (1)



3.4 STATISTICAL ANALYSIS

With the use of the laboratory UAC as the standard, sensitivity, specificity, and positive and negative predictive values for the visual urinary dipstick and the UAC dipstick read on the Clinitek® 50 system was determined using the SPSS package (version 2006) for analysis.

3.5 RESULTS

A total of 26 pregnant patients were recruited of whom 15 were normotensive and 11 were patients with hypertension. Table 3 shows the characteristics of the study population.

Table 4 shows the comparison of the UAC dipsticks and conventional visual dipsticks to the laboratory UAC quantification. The visual dipstick had a sensitivity of 25% (95% CI [0.04-0.64]) and specificity of 89% (95% CI [0.64-0.98]).The UAC dipsticks had a sensitivity of 88% (95% CI [0.47-0.99]) , specificity of 89% (95% CI [0.64-0.98]), negative predictive value of 94 % (95% CI [0.69-1.00]) and positive predictive value of 78% (95% CI [0.40 -0.96]).

The hypertensive and normotensive pregnant women were sub analysed and a comparison of UAC dipsticks to laboratory UAC quantification made in both groups (Table 5). In the normotensive group the sensitivity and specificity of the UAC dipstick was 67% (95% CI [0.13-0.98]) and 92 % (95% CI [0.60-1.00]) and in the hypertensive group it was 100% (95% CI [0.46-0.98]) and 83% (95% CI [0.36-0,99]) respectively.

Table 3. Clinical characteristics of participants

Number of participants	26
Number of normotensives	15
Number of hypertensives	11
Age (mean)	27 years
Parity	
P_0	8
P_{1-4}	16
$P_{>4}$	2

Table 4: Comparison of UAC dipsticks and conventional visual dipsticks to laboratory UAC quantification

	SENSITIVITY	SPECIFICITY	PPV	NPV
VISUAL DIPSTICKS	25% [0.04-0.64]	89% [0.64-0.98]	50% [0.09-0.91]	73% [0.50-0.88]
**UAC DIPSTICKS	88% [0.47-0.99]	89% [0.64-0.98]	78% [0.40-0.96]	94% [0.69-1.00]

**A UAC of < 300mg/g (nil and trace on urine dipsticks) was considered to be a negative result. A UAC ≥ 300 mg/g (1+ to 4+ on urine dipsticks) was considered to be a positive result.

NPV = Negative Predictive Value

PPV = Positive Predictive Value

[ ] = 95 % Confidence Interval

Table 5 Comparison of UAC dipsticks to laboratory UAC quantification in normotensive and hypertensive pregnant women

	n	SENSITIVITY	SPECIFICITY	PPV	NPV
NORMOTENSIVES	15	67% [0.13-0.98]	92% [0.60-1.00]	67% [0.13-0.98]	92% [0.60-1.00]
HYPERTENSIVES	11	100% [0.46-0.98]	83% [0.36-0.99]	83% [0.36-0.99]	100% [0.46-0.98]

** A UAC of <300mg/g (nil and trace on urine dipsticks) was considered to be a negative result. A UAC ≥300 mg/g (1+ to 4+ on urine dipsticks) was considered to be a positive result.

NPV = Negative Predictive Value

PPV = Positive Predictive Value

[ ] = 95 % Confidence Interval

n = number of patients

3.6 DISCUSSION:

Visual dipsticks are used routinely to screen for proteinuria in pregnancy. Detection of 1+ or more of proteinuria on visual dipsticks in the hypertensive pregnant patient is followed by the measurement of total urinary protein over a 24 hour period which is used as the gold standard in the diagnosis of pre-eclampsia.

Although the ISSHP has proposed the use of spot urinary protein to creatinine ratio as an alternative to the 24 hour urine collection it does not appear to be widely used in practice (Cōté et al., 2008) .The majority of our patients have a 24 hour urine collection as inpatients in order to overcome difficulties with collection and transportation problems. This contributes to an increase in the number of admissions and financial costs to the hospital services. At the same time pre-eclampsia is a major cause of maternal mortality and both the early and correct diagnosis together with the institution of appropriate management is critical in preventing both maternal and perinatal mortality. We have thus looked at newer methods to screen for proteinuria.

The measurement of albumin excretion has been suggested as an alternative to total protein measurement as it may provide a more reliable methodology with improved sensitivity (Newman et al., 1995). The results of this study show the Clinitek system to have improved sensitivity and predictive values compared to the visual dipstick.

In pregnancy, the Clinitek 50 system has been evaluated in hypertensive women. Waugh et al., (2005a) in a study comparing various methods for detecting significant proteinuria have shown a sensitivity of 58% [95% CI (0.47, 0.70)] and a specificity of 83% [95% CI (0.74, 0.90)] compared to the 24 hour urinary protein. In our study, we chose to initially compare the semi quantitative UAC dipstick to the quantitative laboratory UAC measurement, in order to validate the UAC dipstick method prior to comparing it to the 24 hour urinary protein.

The Clinitek system has mainly been evaluated in the non pregnant diabetic population in screening for microalbuminuria. Le Floch et al., (2001) screened 302 diabetic outpatients for microalbuminuria using the Clinitek system and compared it to the reference method of the biological laboratory. Using a positive result as UAC ratio of $\geq$ 30mg/g they found a sensitivity of 79% , specificity 81%, PPV 46% , NPV 95% and likelihood ratio of 4.2.They concluded that due to the excellent NPV, the Clinitek system was a good screening test for microalbuminuria and that positive results should be confirmed using a reference assay.

Parsons et al., (1999) evaluated the performance of the Clinitek system and compared it to a lateral flow device for the semi-quantitation of albumin (Micral 11 Roche Diagnostics, Lewes, UK) and also a laboratory based procedure. The imprecision of the Clinitek device was assessed by observing the discrepancy between duplicates in 144 urine samples from patients with diabetes and or renal disease. Discrepancies in the albumin estimation were 6.9% and creatinine estimation was 12.5%. Using a UAC ratio with a cut off of < 30mg/g they found the Clinitek system to have a sensitivity of 76.3%, specificity of 89.1% and a PPV of 89%.

In a study of 127 urine samples from paediatric patients with various disorders, Osta et al., (2003) compared the Clinitek 50 system and the DCA 2000 analyser against usual reference laboratory methods. Using an albumin cut off of 30 mg/l , they found the Clinitek 50 system had a sensitivity of 91.7 % , specificity 86%, positive predictive value 55% and negative predictive value of 98%. They concluded the Clinitek system is a semi-quantitative method that is easy to use, low in cost and useful for screening. Table 6 shows a summary of the findings of the above studies.

This is a pilot study and is limited by the small number of participants. It is however to the best of our knowledge, the first study to compare the semi- quantitative UAC dipstick read on the Clinitek 50 system to a quantitative laboratory method of UAC

measurement in a normotensive and hypertensive pregnant population. Although a sub analysis of the two groups was done (Table 5), the study was not powered to

detect differences between the normotensive and hypertensive participants.

The results of the visual dipsticks were in keeping with those noted in a previous study (Gangaram et al.,2005).The visual urinary dipsticks (Makromed®) were not accurate with low sensitivity and negative predictive values (Table 4). There are several reasons for the inaccuracy of visual dipsticks. These include inter-observer error and that dipstick urinalysis on random antenatal urine specimens yields a measure of protein concentration at a given time. The use of the UAC obviates the latter as the day urinary protein and creatinine excretion rates are fairly constant provided GFR is constant thus cancelling the time factor. Use of a point of care instrument for reading the UAC dipsticks eliminates inter-observer error.

Table 6: Summary of previous studies reporting performance of the Clinitek 50 system measuring UAC compared to a laboratory based procedure

Reference	n	Cut off	Sensitivity	Specificity	PPV	NPV
Le Floch et al.,(2001)	302	30mg/g	79%	81%	46%	95%
Parsons et al.,(1991)	144	30mg/g	76.3%	89.1%	89.7%	Not calculated
Osta et al.,(2003)	127	30 mg/l albumin	91.7%	86%	55%	98%

NPV = Negative Predictive Value

PPV = Positive Predictive Value

n = number of patients

3.7 CONCLUSION

The UAC dipstick read on the Clinitek 50 system provides a semi-quantitative result of the UAC that has a good sensitivity and specificity. Due to its good NPV, a result of < 300mg/g would rule out significant proteinuria and avoid unnecessary investigations in the pregnant population. The Clinitek 50 system is a point of care instrument that is user friendly, provides rapid results in the form of a print out, and avoids interobserver error. It thus provides a better alternative to visual dipsticks for screening for proteinuria. Further research is required to see how it compares to the quantitative 24 hour urine protein measurement (gold standard) in a hypertensive pregnant population.

CHAPTER 4 - COMPARISON TO 24 HR URINARY PROTEIN

4.1 INTRODUCTION

The 24 hour urinary protein collection has been used as the gold standard for quantification of proteinuria in HDP. However, the procedure is time consuming, cumbersome and prone to collection errors. Visual dipsticks are used routinely to screen for proteinuria but have been shown to have poor sensitivity and specificity (Gangaram et al., 2005). In Chapter 3, we have shown that the UAC dipstick read on the Clinitek 50 system provides a semi-quantitative result of the UAC that has good sensitivity and specificity when compared to the laboratory UAC. Use of this point of care system may therefore reduce the need to perform 24 hour urine collections. We therefore embarked on a study to determine the accuracy of spot UAC dipsticks and conventional visual dipsticks compared to a 24 hour urinary protein (gold standard) to detect significant proteinuria in HDP.

4.2 METHOD

Women presenting to the antenatal clinic with hypertension during pregnancy after 20 weeks of gestation were recruited. Hypertension was defined as a blood pressure of at least 140mmHg (systolic) or at least 90 mmHg (diastolic) on two occasions 4- 6 hours apart. Women with eclampsia, diabetes, chronic renal disease and urinary tract infection were excluded from the study.

In our setting hypertensives in pregnancy are routinely admitted for investigations and planning of clinical management. Routine investigations include assessment of the haematological and renal systems. On admission each participant has a spot urine sample that is tested by trained midwives for proteinuria using a semi-quantitative visual dipstick (Makromed®).

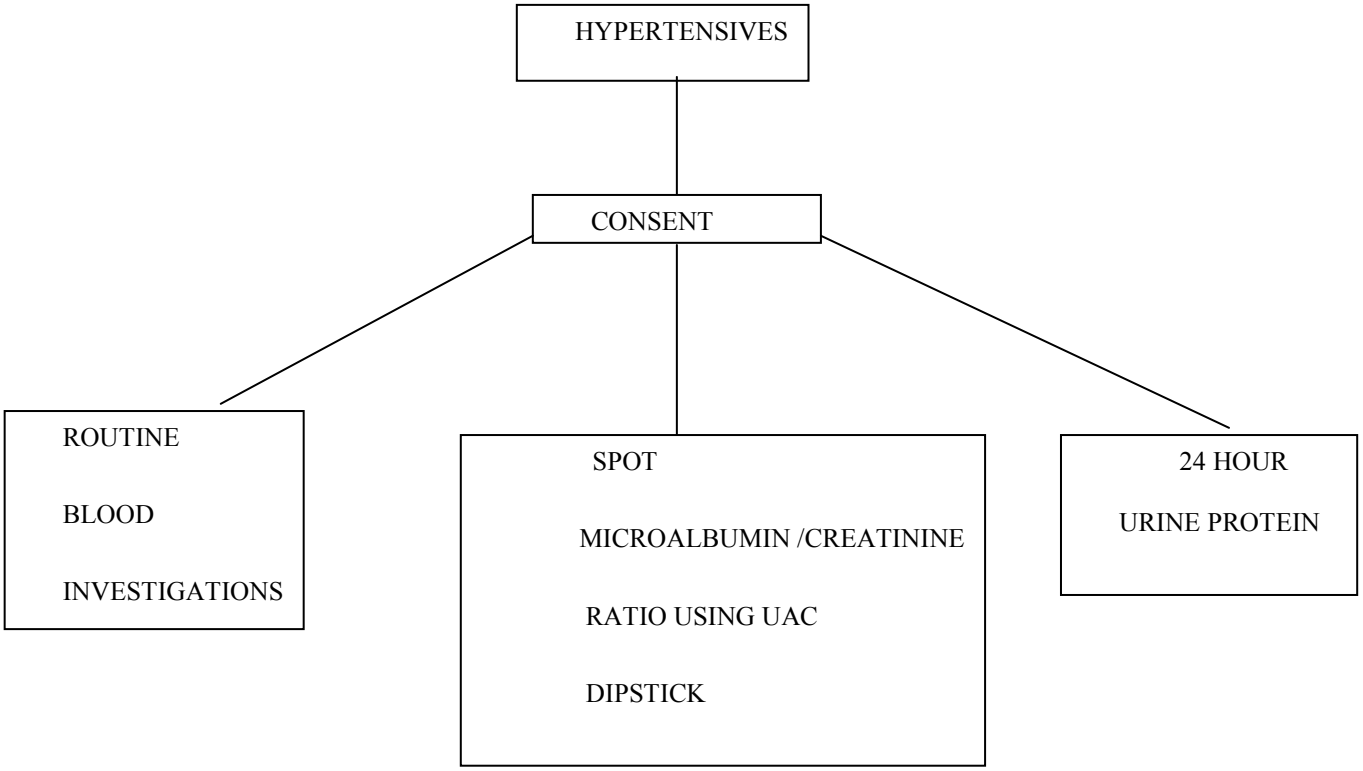
In addition, a spot midstream urine sample was collected and analysed using the semi-quantitative UAC dipsticks (Clinitek® Microalbumin, Bayer Healthcare LLC, USA) read on the Clinitek® 50 urine chemistry analyser. This test was carried out by two doctors who were specifically trained to use the instrument as a side room investigation.

A 24 hour urine collection was then commenced and a quantitative measurement of protein in the urine was estimated in the laboratory (figure 6). Both the clinician managing the patient and laboratory technician measuring the 24 hour urine protein were blinded to the results of the UAC dipsticks, thus not interfering with standard patient management. Results were recorded in a structured data sheet (Appendix 2).

4.3 TEST METHODS

The test methods utilized was the semi - quantitative visual dipsticks (Makromed®, Makro Medical, RSA) and the Clinitek 50 system as described in chapter 3.The quantitative 24 hour total urinary protein was measured by the Biuret method on a Beckman Synchron LX20 multichannel analyzer.

Figure 6: FLOW DIAGRAM OF METHODOLOGY(2)



4.4 STATISTICAL ANALYSIS

With the use of the quantitative 24 hour urinary protein as the gold standard, sensitivity, specificity, and positive and negative predictive values for the visual urinary dipstick and the UAC dipstick read on the Clinitek® 50 system was determined using the SPSS package for statistical analysis. A negative result was considered to be a UAC of < 300mg/g (nil and trace on urine dipsticks). A positive result was a UAC ≥ 300 mg/g (1+ to 4+ on urine dipsticks).Urinary protein ≥ 0.3 g/24 hrs was considered significant proteinuria.

4.5 RESULTS

A total of 163 participants were recruited. Their mean age was 28 years and there were 20 primigravida and 143 multiparous patients. Table 7 shows the characteristics of the study population.

Table 8 shows the comparison of the semi-quantitative UAC dipsticks and conventional visual dipsticks to the quantitative 24 hr urinary protein measurement.

The visual dipstick had a sensitivity of 51 % (95% CI [0.41-0.61]) and specificity of 91% (95% CI [0.81-0.96]) .The PPV and NPV was 89 % (95% CI [0.77-0.95]) and 58% (95% CI [0.48-0.67]) respectively.

The UAC dipsticks had a sensitivity of 63% (95% CI [0.52-0.72]) and specificity of 81 % (95% CI [0.70-0.89]). The PPV was 82% (95% CI [0.71-0.90]) and NPV was 62% (95% CI [0.51-0.71]).

Table 7: Characteristics of the study population.

Number of participants	163
Age (mean)	28 years
Parity	
P_0	20
P_{1-4}	141
$P_{>4}$	2

Table 8: Comparison of semi- quantitative UAC dipsticks and visual dipsticks to the quantitative 24 hr total urinary protein measurement in the study population

	Sensitivity	Specificity	PPV	NPV
Visual dipsticks	51% [0.41-0.61]	91% [0.81-0.96]	89% [0.77-0.95]	58% [0.48-0.67]
UAC dipsticks**	63% [0.52-0.72]	81% [0.70-0.89]	82% [0.71-0.90]	62% [0.51-0.71]

**A UAC of <300mg/g (nil and trace on urine dipsticks) was considered to be a negative result. A UAC ≥300 mg/g (1+ to 4+ on urine dipsticks) was considered to be a positive result. Urinary protein ≥ 0.3 g/24 hrs was considered significant proteinuria.

NPV = Negative Predictive Value

PPV = Positive Predictive Value

[ ] = 95 % Confidence Interval

4.6 DISCUSSION

The results show that both the visual dipstick and the UAC dipstick, read on the Clinitek 50 system are not accurate when compared to the total 24 hour urinary protein. Whilst the Clinitek 50 system had a better sensitivity than the visual dipstick, overall they both showed low sensitivity and poor negative predictive values.

In a previous study (Gangaram et al., 2005) we have shown the visual dipstick done on a spot urinary sample to be inaccurate. The two main reasons postulated for this include inter observer error and the fact that various factors may influence urinary protein concentration at a given point in time. By using an automated device like the Clinitek 50 system, it was thought that inter observer error would be omitted. The slightly improved sensitivity of the Clinitek 50 system as compared to the visual dipstick probably reflects this to a certain extent, however it suggests that it probably isn't a major contributor to inaccuracy of the visual dipstick in an environment where there is trained staff routinely conducting the test.

A 24 hour urinary collection is done to account for the variation in protein excretion that occurs during the day. By doing a protein to creatinine ratio on the spot urinary sample, the time factor is cancelled and thus the ratio reflects the cumulative excretion during the day. We measured the urinary albumin as opposed to the total urinary protein and this may explain the differences in results. As albumin only accounts for about 10% of total protein excretion in pregnancy (Higby et al.,1995) and the non selective proteinuria of pre-eclampsia contains a range of molecular weight proteins (Waugh et al.,2005a) , this may explain why UAC ratio did not closely predict total protein excretion. Waugh et al., (2005a) have suggested that microalbuminuria may correlate better with other clinical parameters of disease severity as it may more accurately reflect the glomerular pathology associated with the glomerular endotheliosis of pre-eclampsia.

Another factor that may have influenced the results is the lack of clear guidelines in the literature of what should be the gold standard for measurement of total urinary protein in the laboratory. There are many assays available and total protein

measurement may vary according to the assay used (Waugh et al., 2001). This may have been a further confounding variable in the study.

In a study of 171 hypertensive pregnant women Waugh et al., (2005a) compared the visual dipstick (Multistix 8SG), automated Multistix 8SG and UAC dipstick read on the Clinitek 50 system to the 24 hour urinary protein excretion (300mg/24hrs). They found that the use of an automated visual dipstick (Multistix 8SG) improved sensitivity from 51% [95% CI (0.39-0.62)] to 82% [95% CI (0.71-0.90)].They also found the automated UAC dipstick, using a threshold of 3.4 mg albumin / mmol creatinine to have a sensitivity of 58% [95% CI (0. 45-0.70)] , specificity of 83% [95% CI (0. 74-0.90]) , likelihood ratio for a positive result (LR+) 3.43 [95% CI (2.12 -5.57)] and likelihood ratio for a negative result (LR-) of 0,50 [95% CI (0.38-0.66)].

In the same study the DCA 2000, which is a point of care device that gives a quantitative measurement of microalbumin performed significantly better for the detection of 'significant proteinuria' in hypertensive pregnancies. Using a cut off value of 2mg albumin/mmol creatinine they found a sensitivity of 94% [95% CI (0.85-0.98)], specificity 94% [95% CI (0.85-0.98), LR+ of 14.6 [95% CI (6.74-31.8)] and LR- of 0.069 [95% CI (0.030-0.16)]. They hypothesized that the improvement seen with the DCA 2000 was partly due to the use of appropriate pregnancy specific thresholds which are not reflected by conventional dipsticks.

Bar et al., (1996) suggested that a phase of microalbuminuria may precede pre-eclampsia. Our study was limited by the fact that we evaluated the UAC to detect significant proteinuria only. It is possible that some of the clinical causes of our false positives may have been attributed to participants who would have gone on to develop pre-eclampsia at a later stage. In their study of the DCA 2000, Waugh et al., (2005a) noted that all their false positive results went on to develop significant proteinuria.

Technical considerations in terms of assay technique of the Clinitek 50 system may have also contributed to some of the false positives and false negatives. Parsons et al., (1999) investigated the imprecision of the device and found discrepancies in the

albumin estimation was 6.9% and creatinine estimation was 12.5%. They found a decrease in sensitivity, specificity and positive predictive value for the UAC compared with the albumin value alone for the Clinitek system and attributed this to the poorer precision of the creatinine assay.

4.7 CONCLUSION

Both the visual dipstick (Makromed®) and the UAC dipstick read on the Clinitek 50 system are not accurate when compared to the total 24 hour urinary protein. Reasons for inaccuracy of the visual dipstick include inter-observer error and factors that affect urine concentration. Differences between the UAC and 24 hour urinary protein may be due to the variation in the albumin fraction of the total urinary protein of pre-eclampsia, technical problems with imprecision of the assay technique and clinical causes of false positives and negatives. The improved sensitivity of the automated UAC dipstick over the visual dipstick suggests it may be a suitable substitute for the visual dipstick in clinical practice. It is a point of care instrument that is easy to operate, provides rapid results in a print out form and avoids inter-observer error. Whether the UAC correlates better to other clinical measurements of disease severity or clinical outcomes needs to be further investigated.

CHAPTER 5 – PREGNANCY OUTCOMES

5.1 INTRODUCTION

In chapter 4, the study results showed that the UAC dipstick read on the Clinitek 50 system is not accurate when compared to total 24 hour urinary protein estimation. It has been suggested that MA may correlate better with other clinical measurements of disease severity as it may more accurately reflect glomerular dysfunction associated with the glomerular endotheliosis of pre-eclampsia (Waugh et al., 2005a). We therefore determined the role of proteinuria as determined by the 24 hour urinary protein and the UAC dipstick read on the Clinitek 50 system on pregnancy outcomes in HDP.

5.2 METHOD

Women recruited to the study described in chapter 4 were followed up. The outcomes of pregnancy in 2 sub-categories viz. those with and without proteinuria were compared. Significant proteinuria (≥ 0.3g/24 hours) was measured by the gold standard 24 hour urinary protein and the UAC dipstick read on the Clinitek 50 system. The pregnancy outcomes were compared utilizing the following variables:

Maternal outcomes:

1. Gestational age at delivery.
2. Caesarean section – Primary elective / emergency
3. Induction of labour
4. Complications – abruptio placentae , eclampsia, high care admission

Perinatal outcomes:

1. Stillbirths / early neonatal deaths
2. Birth weight
3. Apgar scores – 1 minute / 5 minute

4. Perinatal mortality

5.3 STATISTICAL ANALYSIS

Outcome of pregnancy was compared between those with pre-eclampsia (proteinuria ≥0.3g/24 hours as measured by the quantitative 24 hour urine collection) and those with gestational hypertension. The SPSS package was used for statistical analysis. Analyses testing of the frequency of adverse events within the groups were done using the Chi-square test. Birthweights were compared using Student-t test, whilst apgar scores and biochemical indices were compared using the Mann -Whitney U test.

A secondary analysis of outcomes of pregnancy was performed by sub-categorizing the participants according to the diagnostic accuracy of the UAC dipsticks. A UAC of < 300mg/g was considered to be a negative result and UAC ≥ 300 mg/g a positive result. Analyses testing of the frequency of adverse events within groups was done using the Chi-square test with Bonferroni correction. ANOVA was used to calculate mean birthweights. Birth apgars and biochemical indices were compared using the Kruskal-Wallis test.

5.4 RESULTS

Outcome of pregnancy in hypertensives as classified according to the 24 hr urinary protein

The outcomes of pregnancy in 163 hypertensives were compared between those with pre-eclampsia (proteinuria ≥ 0.3g/24 hours as measured by the quantitative 24 hour urine collection) and those with gestational hypertension. Complete data was only

available for 155 patients. There was incomplete data available for 8 patients. Reasons for this included patients delivering at other healthcare facilities or delivery prior to coming to hospital due to lack of accessibility to transport. There were 69 gestational hypertensives (GH) and 94 patients with pre-eclampsia (PE).

Table 9 shows the baseline characteristics at entry in the two groups. Table 10 shows the laboratory parameters of the two groups at entry. Baseline platelet count was significantly lower in the PE group (222.5 vs. 245 x 10^9/ l, p =0.045). Urea, creatinine and urates were significantly higher in the PE group. These results are in keeping with the endothelial and renal dysfunction associated with pre-eclampsia.

Table 11 shows the maternal outcomes in the groups. Mean gestational age of delivery was significantly lower in the PE group, 36 weeks compared to 38 weeks in the GH (p =0.003).There was a trend towards more caesarean sections in the PE group (69.1% vs. 49.2%) with the majority being emergency caesarean sections (69.1% vs. 50%).

Of the vaginal deliveries there were significantly more inductions in the PE group (62.9% vs. 21.2%). There was no maternal mortality in either group and there was a trend towards increased maternal morbidity in the PE group (8.8% vs. 4.6%). We could not demonstrate significance in this result most likely due to the limited sample size.

Table 12 shows the perinatal outcomes in the groups. The mean birthweight was significantly lower in the PE group (2351g vs. 2854g, p < 0.001). This may have been as a result of intrauterine growth impairment or preterm delivery. There was no significant difference in the apgar scores in the two groups. There was a trend towards an increase in perinatal mortality in the PE group (10% vs. 3.1%, p =0.053).

Outcome of pregnancy in hypertensives as classified according to the UAC ratio

We assessed whether the UAC ratio correlates better to clinical measurements of disease severity and clinical outcomes. While false positives for PE may lead to over investigation, false negatives may lead to less close surveillance and result in serious maternal and perinatal consequences. Outcomes of pregnancy were re-analyzed by sub-categorizing the participants according to the diagnostic accuracy of the UAC ratio dipsticks.

Table 13 shows the baseline laboratory parameters. There were no significant differences between the true negatives and false negatives in the GH group except for the higher median creatinine value in the false negatives.
There were also no significant differences in terms of gestational age of delivery, maternal morbidity (Table 14) and the perinatal outcomes (Table 15) between the true negatives and false negatives in the GH group. The only difference noted was in the mode of delivery with a trend to a higher caesarian section rate in the false negatives compared to the true negatives.

Table 9: Baseline characteristics at entry in the study groups

At entry	GH	PE
Number(n)	69	94
Mean Gestational age (weeks)	31.6	31.2
Parity		

P_0	6	14
P_{1-4}	62	79
$P_{>4}$	1	1

GH = Gestational Hypertension

PE = Pre- eclampsia

n = Number of patients

Table 10: Baseline Laboratory parameters in the study groups

(median)	GH	PE	p-value
Platelets ($x10^9$ /l)	245	222.5	0.04
Urea (mmol/l)	2.1	2.5	0.001
Creatinine (µmol/l)	53	63.5	0.001
Urates (mmol/l)	0.25	0.3	0.004

GH = Gestational Hypertension

PE = Pre- eclampsia

Table 11: Maternal outcomes in the study groups

	GH (n=65)	PE(n=90)	p- value
Mean gestational age at delivery(weeks)	38	36	0.003
Mode of delivery			
Caesarean section (c/s)	32 (49.2%)	55 (61.1%)	0.077

Emergency c/s	16 (50%)	38 (69.1%)	
Elective c/s	16 (50%)	17 (30.9%)	
Vaginal delivery	33 (50.8%)	35 (38.9%)	0.001
Spontaneous	26 (78.8%)	13 (37.1%)	
Induced	7 (21.2%)	22 (62.9%)	
Morbidity	3 (4.6%)	8 (8.8%)	0.19
Abruptio Placentae	2	1	
Eclampsia	0	1	
High care admission	1	6	

GH = Gestational Hypertension

PE = Pre- eclampsia

n = Number of patients

Table 12: Perinatal outcomes in the study groups

	GH(n=65)	PE(n=90)	p- value
Mean Birthweight (grams)	2854	2351	0.001
Apgars (median**)**			
1 minute	8	8	0.074
5 minute	9	9	0.058

Perinatal Deaths	2 (3.1%)	9 (10%)	0.053
MSB (n)	1	6	
FSB (n)	0	0	
ENND (n)	1	3	

GH = Gestational Hypertension

PE = Pre- eclampsia

n = Number of patients

MSB = macerated stillbirth

FSB = fresh stillbirth

ENND = early neonatal death

Table 13: Baseline Laboratory parameters in the study groups as classified by the UAC dipsticks

	PE		GH		
(mean)	True positives	False positives	True negatives	False negatives	p-value
Platelets ($x10^9$ /l)	212	238	239	248	0.016
Urea (mmol/l)	2.6	2.2	2.2	2.1	0.001
Creatinine (µmol/l)	100	125	108	126	0.001
Urates (mmol/l)	0.32	0.21	0.26	0.25	0.001

GH = Gestational Hypertension

PE = Pre- eclampsia

Table 14: Maternal outcomes in the study groups as classified by the UAC dipsticks

	PE		GH		
	True positives	False positives	False negatives	True negatives	p-value
n	55	13	35	52	
Mean gestational age at delivery(weeks)	34	37	38	38	0.001

Mode of delivery					
Caesarean section (c/s)	33 (60%)	7(53.8%)	22(62.9%)	25(48.1%)	0.180
Emergency c/s	23(69.7%)	5(71.4%)	15(68.2%)	11(44%)	
Elective c/s	10(30.3%)	2(28.6%)	7(31.8%)	14(56%)	
Vaginal delivery	22(40%)	6(46.2%)	13(37.1%)	27(51.9%)	0.02
Spontaneous	6(27.3%)	5(83.3%)	7(53.8%)	21(77.8%)	
Induced	16(72.2%)	1(16.7%)	6(46.2%	6(22.2%)	
Morbidity	6(10.9%)	0	2(5.7%)	3(5.8%)	0.025
Abruptio Placentae	0	0	1	2	
Eclampsia	0	0	1	0	
High care admission	6	0	0	1	

GH = Gestational Hypertension

PE = Pre- eclampsia

n = Number of patients

Table 15: Perinatal outcomes in the study groups as classified by the UAC dipsticks

	PE		GH		
	True positives	False positives	True negatives	False negatives	p-value
Birthweight (grams)	2092	2884	2760	2847	0.001
Apgars					
1 minute (mean)	8	8	8	8	0.197
5 minute (mean)	9	9	9	9	0.142

Perinatal Deaths	8 (14.5%)	1 (7.7%)	1 (2.9%)	1 (1.9%)	0.053
MSB (n)	5	0	1	1	
FSB (n)	0	0	0	0	
ENND (n)	3	1	0	0	

GH = Gestational Hypertension

PE = Pre- eclampsia

MSB = macerated stillbirth

FSB = fresh stillbirth

ENND = early neonatal death

n = number

5.5 DISCUSSION

Our results show that in hypertensive pregnant women, significant proteinuria determined by the quantitative 24 hour urinary protein is associated with delivery at an earlier gestational age, increased induction of labour and lower birthweights compared to the non – proteinuric hypertension (gestational hypertension). There is also a trend towards an increased maternal morbidity and perinatal mortality. These findings are similar to those of previous studies which have demonstrated that proteinuria associated with hypertension in pregnancy is associated with greater adverse maternal and fetal outcomes (Chan et al., 2005; Brown et al., 1996; Ferranzani et al., 1990; Chua and Redman, 1992; Lin et al., 1982).

When the groups were classified into PE and GH using the UAC dipsticks, there were no differences in the clinical outcomes between the false negatives and true negatives except a trend towards a higher caesarean section rate in the false negatives. The results suggest that classification as GH based either on a quantitative 24 hour urinary protein collection or a negative UAC dipstick test is associated with a similar clinical outcome. Thus there appears to be a good correlation between the UAC and measurements of disease severity and clinical outcomes in hypertensive pregnant women.

There are several possible explanations for this. The clinical syndrome of PE is thought to be due to maternal endothelial dysfunction .This is thought to result from placental hypoxia with oxidative stress or from the interaction between a normal placenta and conditions that make the mother susceptible to microvascular disease (Redman et al., 2005) .The net result of endothelial dysfunction is an increase in vascular permeability systemically and at glomerular level. Microalbuminuria occurs as a result of this endothelial dysfunction (Verdecchia et al., 2004).

Furthermore, urinary albumin has been shown to be a sensitive marker to early changes in glomerular permeability (Newman et al., 1995) and thus the UAC may detect structural renal changes associated with pre-eclampsia earlier.

The findings of this study is important in the clinical context as it demonstrates that the UAC dipstick is a good screening test to rule out significant proteinuria. The visual dipstick test which is widely used is a poor screening test (Gangaram et al.,2005) while the gold standard 24 hour urinary protein is far from perfect. It is a test which is cumbersome, time consuming, prone to collection error and leads to delay in diagnosis. Lack of a gold standard assay for measurement of protein may also contribute to its inaccuracy (Waugh et al., 2005b). Use of the UAC may lead to a decrease in the number of 24 hour urinary protein specimens required and a decrease

in hospital admissions. A positive UAC dipstick test still mandates a 24 hour urinary protein collection to accurately quantify proteinuria and confirm the diagnosis.

Although the ISSHP and the Australasian Society for the Study of Hypertension in pregnancy have proposed the use of the spot urinary protein to creatinine ratio as an alternative to the 24 hour urinary collection (Brown et al.,2001; Brown et al., 2000) , it doesn't appear to be widely used (Cōté et al.,2008).A recent systematic review (Cōté et al.,2008) found the spot protein to creatinine ratio to be a reasonable 'rule-out' test for detecting significant proteinuria in hypertensive pregnancy , however , the review found information on the use of the UAC to be limited. Our results suggest that the UAC dipstick read on the Clinitek 50 system would be a reasonable rule out test. In addition, it would be more amenable to widespread use as it is a point of care system that can be easily used by midwives and doctors and provides rapid results in the form of a printout.

Limitations of the study include that participant numbers were not large enough to demonstrate the expected finding of a significant difference in perinatal mortality and maternal morbidity between the two groups. We also did not analyse perinatal morbidity in detail.

Although we did not do a detailed cost analysis, approximate cost per test of the visual dipstick is R10, UAC dipstick R15 and 24 hour urinary protein R45 (admission costs not included). A reduction in the number of unnecessary 24 hour urinary protein tests done may justify the added cost of the UAC dipstick compared to that of the visual dipstick.

While false positives in screening may lead to over diagnosis and an increase in surveillance and cost, it may be justified in a condition like PE where one is trying to prevent adverse maternal and fetal outcome. The UAC dipstick read on the Clinitek 50 system is a new technique that has potential to improve accuracy of screening for proteinuria and enhance safety by preventing incorrect diagnosis and unnecessary investigations.

5.6 CONCLUSION

Hypertensive pregnancy associated with significant proteinuria is associated with greater adverse maternal and fetal outcome. Outcome of pregnancy is similar when a classification of GH is made based either on the 24 hour urinary protein collection or the UAC dipstick read on the Clinitek 50 system. The UAC dipstick is a good screening test to rule out significant proteinuria. It has potential to improve accuracy of screening for proteinuria and enhancing safety by preventing incorrect diagnosis. Further research is required to determine its full impact in the clinical setting.

CHAPTER 6

6. SUMMARY OF FINDINGS

Antenatal screening for pre-eclampsia consists predominantly of detection of a raised blood pressure and proteinuria. Traditionally screening for proteinuria has consisted of performing a semi-quantitative visual dipstick test. If positive for significant proteinuria this is then followed by a quantitative 24 hour urinary protein.

The visual dipstick has been shown to be inaccurate (Gangaram et al., 2005), while the 24 hour urinary protein collection is cumbersome and fraught with collection errors. New developments in proteinuria assessment have included the use of urinary albumin measurements. This study has investigated the role of the UAC dipstick read on the Clinitek 50 system in detection of proteinuria in the HDP.

The initial investigation was to validate the UAC dipstick in both a normotensive and hypertensive pregnant population. It showed that the UAC dipstick read on the Clinitek 50 system provides a semi-quantitative result of the UAC that has a good sensitivity 0.88 (95% CI [0.47-0.99]) and specificity 0.89 (95% CI [0.64-0.98]). Due to its good NPV 0.94 (95% CI [0.69-1.00]), a result of < 300mg/g would rule out significant proteinuria and avoid unnecessary investigations in the pregnant population. It thus provides a better alternative to visual dipsticks for screening of proteinuria.

A comparison was then made between the visual dipstick and the UAC dipstick read on Clinitek 50 system and the 24 hour urinary protein which is the current 'gold standard'. Both the visual dipstick and the UAC dipstick were found to be inaccurate. Reasons for inaccuracy of the visual dipstick include inter-observer errors and factors that affect urine concentration. Differences between the UAC and 24 hour total urinary protein may be due to the variation in the albumin fraction of the total urinary protein of pre-eclampsia, technical problems with imprecision of the assay technique and clinical causes of false positives and negatives.

We then set out to determine if the UAC correlates better to other clinical measurements of disease severity or clinical outcomes. The findings were that hypertensive pregnancies associated with significant proteinuria as determined by the 24 hour urinary protein is associated with greater adverse maternal and fetal outcome.

Pre-eclampsia was associated with delivery at an earlier gestational age, increased induction of labour and lower birthweights compared to GH. There was also a trend towards an increased maternal morbidity and perinatal mortality in the PE group. We found that the outcome of pregnancy is similar when a classification of GH is made based either on the 24 hour urinary protein or the UAC dipstick read on the Clinitek 50 system. A possible reason for the good clinical correlation of the UAC may be related to the fact that microalbuminuria is a marker of endothelial dysfunction which is responsible for many of the clinical manifestations of pre-eclampsia. Furthermore the UAC may reflect structural renal changes associated with pre-eclampsia earlier.

The UAC dipstick is a good screening test to rule out significant proteinuria. A positive result would still require quantification of proteinuria using the 24 hour urinary collection. It has potential to improve accuracy of screening for proteinuria and enhancing safety by preventing incorrect diagnosis. It is also more likely to be used widely in the clinical setting since it is a point of care system that is easy to use and provides rapid results in the form of a printout. Further research is required to determine its full impact and cost effectiveness in the clinical setting.

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APPENDIX 1

DATA SHEET: UAC dipstick validation

Demographics

1. Study number:

2. Hospital:

3. Age:

4 Parity:

5. Gestational age (entry):

6. Normotensive / Hypertensive

Biochemical data

7. Visual dipstick

Nil	
Trace	
1+	
2+	
3+	
4+	

8. UAC dipstick
   a. Microalbumin:
   b. Creatinine
   c. UAC:

9. Laboratory Quantification
   a. microalbumin
   b. Creatinine
   c. UAC

APPENDIX 2

DATA SHEET–UAC dipstick vs. 24 hr urinary protein and Pregnancy outcomes

Demographics

1. Study number:

2. Hospital:

3. Age:

4 Parity:

5. Gestational age (entry):

Biochemical data

7. Visual dipstick

Nil	
Trace	
1+	
2+	
3+	
4+	

8. UAC dipstick

a. Microalbumin:
b. Creatinine
c. UAC:

9. 24 hr urinary collection
    a. Total protein
    b. Creatinine clearance

Maternal (Entry)

10. Platelets:

11. Urea:

12. Creatinine:

13. Urates:

Outcomes

Maternal (Delivery)

14. Platelets:

15. Urea:

16.Creatinine:

17. Urates:

18. Gestational age at delivery:

19. Delivery

a. Induction
b. Spontaneous
c. Elective C/S
d. Emergency C/S

20 Morbidity

a. Abruptio
b. Eclampsia
c. HELLP syndrome
d. High care
e. ICU

Neonate

21. Apgar score (Birth):

22. Apgar score (5min):

23. Mortality

    a. FSB
    b. MSB
    c. ENND
    d. None

24. Birth weight: