ACCURACY OF URINE MICROALBUMIN AND SERUM URIC ACID IN THE DIAGNOSIS OF PREECLAMPSIA

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DECLARATION

The work described in this thesis was carried out at the Department of Physiology, KNUST. This work has not been submitted for any other degree.

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ABSTRACT

Preeclampsia (PE), a multisystemic disorder of pregnancy characterized by proteinuria and hypertension after 20 weeks of gestation, is one of the leading causes of maternal morbidity and mortality worldwide. Its aetiology is not fully understood though several studies attribute it to a widespread endothelial dysfunction originating from the placenta. The increasing prevalence of PE coupled with the need to identify and institutionalize more sensitive diagnostic tools has necessitated this study. This study sought to evaluate the diagnostic accuracy of urine microalbumin and serum uric acid as early markers of preeclampsia among Ghanaian women attending antenatal care at the Komfo Anokye Teaching Hospital (KATH).

This case-control study was conducted among pregnant women at the Obstetrics and Gynaecology (O&G) department of the KATH, Kumasi-Ghana from October 2011 to May 2012. A total of 146 participants were recruited for this study. Written informed consent was obtained from the participants, and information on socio-demographic characteristics, medical history and previous obstetric history were obtained through medical records of the eligible participants. Blood pressure and anthropometrics were measured according to standard procedure; urine samples were collected for estimation of spot urine protein and microalbuminuria; and venous blood sample was taken for biochemical analysis and platelet count. The percentage of married participants with preeclampsia was significantly lower than the controls (p=0.004). More control participants had formal education compared to the participants with preelampsia (p=0.008), however, more preelamptics had informal education compared to the controls (p=0.004). The prevalence of abortion and blood pressure were higher in the preelamptics than the controls. Serum uric acid and hepatic enzymes (ALT and AST)] and urine microalbumin (p=0.005) were significantly elevated in the preeclamptic participants compared to the controls. The mean platelets count and serum albumin levels were however lower in the preelamptics than the controls (p>0.001). A significant positive linear correlation was observed between spot urine protein and urine microalbumin (r=0.324, p=0.006). A negative linear correlation was observed between uric acid and spot urine micro albumin (r=0.033, p=0.786). A urinary micro albumin value of 75.45 mg/g was identified as the best threshold to

detect a spot urine protein of > +2 with a sensitivity of 92.7% and a specificity of 80.0%, PPV of 81.03% and NPV of 33.3%. Area under the curve = 0.835; asymptomatic p-value of 0.0001 at 95% CI (0.678-0.991). In contrast, serum uric acid level of 263.5 mg/g was identified as the best cut-off point to detect a spot urine protein of > +2 with sensitivity and specificity of 89.1% and 33.3% respectively (PPV of 77.2% and NPV) of 20.8%. Area under the curve = 0.552; asymptotic p-value of 0.538 at 95% CI (0.364-0.740).

Urine levels of microalbumin, as a measure of proteinuria are elevated in preeclamptics and can be used in place of spot macro protein estimation to diagnose preeclampsia especially in the early stages.

DEDICATION

I dedicate this work to my loving husband Kobina and our children, Papa Kojo, Naa Shika and Efua Effraimu.

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ABBREVIATION

ALT	Alanine aminotransferase
ALB	Albumin
ALP	Alkaline phosphatase
ACOG	American College of Obstetricians and Gynecologists
AST	Aspartate aminotransferase
BMI	Body mass index
BMJ	British Medical Journal
BCG	Bromocresol green
CI	Confidence interval
CRT	Creatinine
DBP	Diastolic blood pressure
K_2 EDTA	Dipotassium ethylenediaminetetraacetic acid
DIH	Disseminated intravascular haemolysis
HELLP Syndrome	Hemolysis, elevated liver enzyme, low platelet count
HDP	Hypertensive disorders of pregnancy
KATH	Komfo Anokye Teaching Hospital
MA	Microalbumin
NKF/KDOQI	National Kidney Foundation/Kidney Diseases Outcomes Quality Initiatives
NPV	Negative predicted value
O&G	Obstetrics and Gynaecology
PE	Preeclampsia
PLT	Platelet concentration
PPV	Positive predicted value
ROC	Receiver operating characteristic
SMS/KNUST	School of Medical Sciences/Kwame Nkrumah University of Science and Technology
SBP	Systolic blood pressure
UA	Uric acid
VDRL	Venereal Disease Research Laboratory test

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Hypertensive disorders of pregnancy are a leading cause of maternal and perinatal mortality and morbidity across the globe and for that matter in Ghana (Roberts *et al*., 2003).

The classification of hypertensive disorders of pregnancy is based on the two most common manifestations of preeclampsia: hypertension and proteinuria (Sibai *et al*., 2012). Blood Pressure (BP) can be measured using a mercury sphygmomanometer, aneroid device, or automated (usually oscillometric) BP device. In our setting mercury sphygmomanometer, which is the gold standard, is mostly used.

Most testing for urinary protein is performed to screen for preeclampsia in hypertensive pregnant women or those at increased risk of preeclampsia, although urinary protein screening is used in early pregnancy to detect pre-existing renal disease. The current recommendations have been revised to reflect the critical fact that proteinuria is but one diagnostic criterion for preeclampsia. The end-organ complications of preeclampsia may occur in the absence of proteinuria; for example, 20% of women who develop eclampsia would have had only hypertension in the week preceding their seizure, 10% would have had only proteinuria, and 10% would have had neither. There is also the need for both efficiency and economy in clinical care. There are many options for diagnosis of

proteinuria, including urinary dipstick testing, urinary protein: creatinine ratio, and various timed urine collections (most commonly, 24-hour). The method that best identifies women at increased risk of maternal and/or perinatal complications still remains unknown. However, in a retrospective study, increasing number of pluses of urinary dipstick proteinuria was associated with increasing risk of adverse maternal outcomes (von Dadelszen *et al.*, 2004). Most studies have focused on methods that best match the quantification of urinary protein by 24-hour urine collection, considered to be the gold standard. A 24-hour urine collection is timeconsuming, inconvenient, and often not completed (Cote *et al.*, 2008a). For diagnosis of proteinuria, these logistical considerations have prompted the National Kidney Foundation/Kidney Diseases Outcomes Quality Initiatives (NKF/KDOQI) of the United States of America, for example, to abandon timed collections in favour of the spot urine samples. The upper limit of normal 24-hour urine protein excretion is 0.3 g/day and is based on a 95% CI for urinary protein in pregnancy. It is used by convention; however, a urinary protein measurement of ≥0.5g/day may be a better predictor of adverse clinical outcome (Waugh *et al.*, 2004). The urinary protein: creatinine ratio has been accepted for diagnosis by the International and Australasian pregnancy hypertension societies. Ideally, this test should be performed in the morning but not on the first voided urine; however, timing may not be critical in pregnancy (Valerio *et al.*, 2005). The reported cut-off varies from 17 to 57 mg/mmol (median 26 mg/mmol) in 10 studies (1079

hypertensive women) (Neithardt *et al.*, 2002; Durnwald and Mercer, 2003). For a cut-off of 30 mg/mmol urinary protein-creatinine ratio (as recommended by the ASSHP), and among women with hypertensive disorders of pregnancy (HDP) specifically, the sensitivities and specificities were 0.85 (95% CI 0.78– 0.91) and 0.76 (0.73–0.78), respectively (Waugh *et al.*, 2004; Cote *et al.*, 2008a). Efforts are underway to improve the standardization of urinary protein and serum creatinine measurement across laboratories (Myers *et al.*, 2006). Urinary dipstick testing is inexpensive, easy, and widely used. Its usefulness is uncertain for screening either women with hypertension or those who are at increased risk of preeclampsia. A negative or trace value should not be ignored in a woman with new hypertension or symptoms or signs suggestive of preeclampsia; 12% of negative/trace results will be false negatives as assessed against 24-hour proteinuria of 0.3 g/day (Brown and Buddle, 1995) and, regardless, these women may have preeclampsia without proteinuria. More information on the determination of proteinuria using other measures of proteinuria like microalbuminuria is needed before clinical use of the urinary microalbumin can be recommended. Therefore, this study seeks to establish the diagnostic accuracy of urine microalbumin and uric acid estimations in diagnosing preeclampsia and the correlation that exists between these markers and identified risk factors.

1.2 STATEMENT OF PROBLEM

Maternal mortality, the fifth millennium development goal, is on the ascendency in the country due to several factors including hypertension and complications that arise out of hypertensive disorders of pregnancy. According to Komfo Anokye Teaching Hospital (KATH) Annual Reports (KATH, 2009; KATH, 2013a), Preeclampsia/eclampsia topped the first ten (10) causes of the admissions in the Directorate of Obstetrics and Gynaecology. In addition, preeclampsia/eclampsia was the major cause of maternal mortality spanning the period 2009 to 2013 (KATH, 2013b). In the quest to achieve this goal there is the need to reduce maternal mortality caused by hypertension in pregnancy and for that matter preeclampsia. However, the two major diagnostic components of this condition (blood pressure and proteinuria) are often beset with numerous problems in terms of the equipment and the techniques to use and the various methods for estimating proteinuria in pregnancy. In the light of the afore mentioned, this study sought to determine the diagnostic accuracy of urine microalbumin and serum uric acid in patients with preeclampsia

1.3 JUSTIFICATION

The increasing prevalence of hypertensive disorders of pregnancy coupled with the need to identify and institutionalize more sensitive diagnostic tools has necessitated this study. The findings of this study will help in creating more

awareness on this condition, and identifying novel and more sensitive markers that will help in early diagnosis of preeclampsia.

1.4 AIMS AND OBJECTIVES

The objectives of this study are:

1. To test the effectiveness of urine microalbumin as a diagnostic tool for proteinuria in women with preeclampsia.
2. To measure the effectiveness of serum uric acid as an early marker of preeclampsia.

Chapter 2

LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF PREECLAMPSIA

Pre-eclampsia typified by the development of elevated blood pressure (usually after the first trimester of pregnancy) is prevalent in many developing countries but less prevalent in the developed world where it accounts for 20% of perinatal deaths and it remains the principal cause of maternal death (Sibai, 2012). The hypertensive disorders of pregnancy are mainly due to gestational hypertension and preeclampsia (70%) and the other (30%) can be attributed to preexisting and/ or undiagnosed hypertension or kidney disease (Sibai, 2012). Preeclampsia is defined as a multisystem ailment of unknown etiology depicted by the development of elevated blood pressure to the level of 140/90 mmHg or more with proteinuria induced by pregnancy after 20 weeks of pregnancy in a previously normotensive and non-proteinuria pregnant woman (ACOG, 2002; Sibai, 2012). Preeclampsia refers to the syndrome of hypertension with proteinuria or oedema or both during pregnancy or within two days after delivery (Turner, 2010; Sibai, 2012). The American College of Obstetricians and Gynaecologists in the classification of preeclampsia defined it as a triumvirate of hypertension, proteinuria and oedema which occurs after 20 weeks of pregnancy in a hitherto normotensive woman (ACOG, 2002b; Sibai, 2012). Even though many significant

factors have been identified in epidemiological studies that may reduce a woman's risk of developing preeclampsia, the real cause remains a mystery (Sibai, 2012).

2.2 PLACENTAL FACTOR

The placenta is mainly considered to be the basic cause of the hypertensive disorders of pregnancy in that after delivery the condition regresses. Preeclampsia is a disease of the placenta as it has been described in complete molar pregnancies (Lim *et al.*, 1997).

Studies have suggested that the basic event for the development of preeclampsia is the inability of the second wave of trophoblastic invasion responsible for the destruction of the muscularis layer of the spiral arterioles and remodeling of the vasculature of the placenta (Cross *et al.*, 1994; Meekins *et al.*, 1994). With the progression of pregnancy and the increased metabolic requirements of the foetoplacental units, the spiral arterioles are unable to contain the necessary increase in blood flow. Consequently, placental dysfunction develops which clinically manifests in pregnant women as preeclampsia which results in extensive vasospasm and endothelial injury (Lim *et al.*, 1997).

Reduced placental perfusion, which originates from abnormal implantation, is noted in conditions associated with microvascular diseases such as diabetes, hypertension or thrombophilia. Additionally, it may occur where there is large placental mass such as in multiple pregnancy or hydatiform mole (gestational trophoblastic disease). The risk of preeclampsia is high in women with these

conditions (Rao *et al.*, 2006). A twin pregnancy significantly elevates the risk of incidence of preeclampsia and the early onset of preeclampsia. This is confirmed by the fourfold increase in the risk of preeclampsia found in patients with twin pregnancies (Lim *et al.*, 1997).

2.3 RISK FACTORS OF PREECLAMPSIA

2.3.1 History of preeclampsia in previous pregnancies

A woman who develops preeclampsia early in her pregnancy is likely to have a recurrent episode in later pregnancies and she also stands a greater risk of developing chronic hypertension (Lim *et al.*, 1997).

2.3.2 Parity

Preeclampsia is more likely to be reported in women during their first pregnancy (Rao *et al.*, 2006).

2.3.3 Pre-existing medical conditions:

Personal history of chronic conditions like diabetes, hypertension, vascular or kidney diseases is associated with preeclampsia. Women with a greater blood pressure in pregnancy have an increased risk of preeclampsia. Furthermore, obesity, insulin resistance and glucose intolerance are strongly linked with nonpregnant hypertension. Peripheral insulin resistance in hypertension may show that insulin function is ineffective. This allows other vasoconstrictors to mediate the effect of insulin. A strong correlation has been reported between

obesity and hypertension and this is depicted by the increased blood volume and consequently increased cardiac output to meet the high metabolic demands. Type 1 diabetes mellitus is linked with a high incidence of preeclampsia in diabetes complicated by vascular changes (Lim *et al.*, 1997).

2.3.4 Hereditary or familial tendency

Follow up studies over a period of three generations indicates that there may be a single recessive trait in preeclampsia giving an expected frequency of 20% for daughters and about 40% for sibling sisters. More so women with a family record of hypertension are two times at risk of developing preeclampsia (Sibai *et al.*, 1998; Packer, 2005; Skjaerven *et al.*, 2005).

2.3.5 Age

The risk of preeclampsia is higher in women between the ages of 20 to 35 years. The percentage of women delaying conception till after 30 years is gradually increasing and this has resulted in an increased risk of chronic hypertension in pregnancy (Sibai *et al.*, 1998; Duckitt and Harrington, 2005). The risk may be greater in younger women because they may be primiparous. However, for older women there is consistent evidence for the risk of preeclampsia as has been observed in older women with an increased risk of hypertension. Studies conducted in the United States have reported a 30% increase in the risk of hypertension in pregnancy for every additional year of age past 34 years (Sibai *et al.*, 1998).

2.3.6 Low Socio-economic status

Some mothers are unable to attend ante natal clinics regularly due to poverty. Also, financial constraints result in some mothers having reduced quantities of protein and calcium in their diet which ultimately increase their risk of preeclampsia (Sibai *et al.*, 1998). Among the essential functions of proteins include promotion of cellular growth, maintenance of normal serum osmotic pressure and transportation and conversion of fats to lipoproteins. Adequate quantities of calcium decrease vascular sensitivity to angiotensin by stimulating prostacyclin synthesis and thus calcium deficiency during pregnancy has the possibility of triggering preeclampsia. Magnesium causes vasodilation but its capacity to prevent preeclampsia remains unknown. However, it is reported that altered metabolism interferes with the synthesis of prostacyclins and results in an increased sensitivity to angiotensin and aggregation of platelets; altered metabolism also explains, partially why people of low socio economic status are at a higher risk of preeclampsia (Wang *et al.*, 1991).

2.3.7 Stress

The risk of preeclampsia is greater in women engaged in high and low risk jobs compared to women who are unemployed (Saftlas *et al.*, 2004).

Antenatal care when sought early and regularly especially in high risk pregnancy helps to identify the early signals of preeclampsia (Sibai, 2012). Women with high risk of preeclampsia are taken through the following regimen when they seek antenatal attention:

1. Regular monitoring of blood pressure and body mass index to determine excess weight gain (Sibai *et al.*, 1998).
2. Laboratory tests to determine the haemoglobin level, ABO and rhesus blood group, blood glucose (fasting or random), venereal disease reference laboratory test (VDRL) and the analysis of urine especially for protein.
3. Pregnant women at increased risk of preeclampsia should get adequate rest and sleep specifically 8 to 12 hours per night and enough rest during the daytime. This serves to return oedematous fluid back into the intravascular space (Sibai *et al.*, 1998).
4. Furthermore, high risk women should sleep in the lateral position to reduce the effect of vena caval compression and increase perfusion of the placental bed (Rao *et al.*, 2006).
5. Restriction of household activities: The amount of household work high risk women engage in should be reduced to allow for the conservation of blood which would otherwise be circulated to skeletal muscles to be channelled to the placenta and other vital organs of the mother (Saftlas *et al.*, 2004).
6. Nutrition: A well balanced diet containing all the essential nutrients should be regularly taken by the pregnant woman (Sibai, 2012).
7. The incidence of preeclampsia is decreased in women who are engaged in some form of occupational and leisure time physical activity (Weissgerber *et al.*, 2004).

2.4 CARDINAL SIGNS AND SYMPTOMS AND COMPLICATIONS OF PREECLAMPSIA

The key signs and symptoms of preeclampsia include oedema, high blood pressure and albuminuria. In a case of preeclampsia, any or all of them could be present. Any woman at risk of preeclampsia should be instructed on the symptoms to watch out for, so she can alert her ante-natal attendants (Sibai *et al.*, 1998).

2.4.1 High blood pressure

There is generalized vasoconstriction due to the increased manner in which the arterial system responds to the pressor substances. This could mainly be attributed to the increased peripheral vascular resistance, which results in the elevation of blood pressure. Vasoconstriction leads to a reduction in uteroplacental blood flow. In preeclampsia, there is a noticeable increase in how vasopressin responds to norepinephrine and to angiotensin. The development of hypertension follows the increased response to angiotensin (Rao *et al.*, 2006; Siddiqui *et al.*, 2010).

2.4.2 Oedema:

Expansion of extravascular blood volume due to reduced colloid osmotic pressure leads to fluid from the intravascular space accumulating in the interstitial tissue. This is the type of oedema seen in preeclampsia (Rao *et al.*, 2006). If this pitting oedema persists even over a period of twelve hours of bed rest, or if there is visible signs of oedema on the face, hands, abdominal wall or the labia an association with preeclampsia should be considered (ACOG, 2002b; Sibai, 2012).

Approximately 80% of women with preeclampsia are affected with oedema. When the preeclampsia is severe the oedema conspicuously appears as puffiness on the woman's face and hands (ACOG, 2002b; Sibai, 2012).

2.4.3 Weight gain

When a pregnant woman puts on more than 0.5 to 1 kg of weight per week then she needs to be watched carefully. The weight gain is attributed to fluid retention in the body tissues. An important procedure that helps in the early diagnosis of preeclampsia is the routine checking of the weight of women during the ante natal period (ACOG, 2002b; Sibai, 2012).

2.4.4 Proteinuria

Plasma proteins predominantly albumin, filters into urine as a result of glomerular endothelial damage producing proteinuria (Sibai, 2012). It appears in urine mostly after hypertension and oedema have become apparent in most cases (Sibai *et al*., 2012).

Vasoconstriction of the vascular bed of the liver leads to hypoxia and oedema of the hepatocytes (Sibai, 2012). Endothelial change and deposition of fibrin in the liver leads to impaired function and consequently haemorrhagic necrosis indicated by the tenderness of the right upper quadrant or epigastric pain, nausea or vomiting. This leads to the elevation of liver enzymes. Brain oedema and heamorrhage results in severe headache. Furthermore, blurred or double vision occurs because of spasm of the arteries and retinal oedema (Sibai, 2012). High fetal

and maternal mortality accompanies preeclampsia and to a greater extent preeclampsia is responsible for delivery before term (Roberts and Cooper, 2001; ACOG, 2002b).

2.4.5 Effects on the mother

The effects on the mother include; eclamptic seizures, which is an intense vasospasm and cerebral oedema that results in spasm of cerebral vessels, which decreases the oxygen supply. The cerebral hypoxia results in cerebral irritation and subsequently convulsions arise (Sibai, 2005). This disorder naturally originates ante natally but it resolves quickly after delivery. However, eclampsia may also develop during the post-natal period (Sibai, 2005; Sibai, 2012). Other complications include postpartum hemorrhage, postpartum shock, recurrent preeclampsia and coma.

2.4.6 Hemolysis, elevated liver enzyme, low platelet count (HELLP Syndrome)

Women at risk develop this condition, which becomes apparent after 32-34 weeks of pregnancy and in 3 out of 10 patients it occurs during the postpartum period (Wolf, 1996; ACOG, 2002b; Sibai, 2005). Hypertensive disorders of pregnancy can lead to hepatic failure, disseminated intravascular haemolysis (DIH), acute renal failure, placental abruption, placenta and cerebral haemorrhage (Stalker, 1976).

The cardiac system is overwhelmed due to the hypertension because the heart is overworked and this results in the reduction in the amount of blood to vital organs

such as the liver, kidney, pancreas and brain (Germain *et al.*, 2007). Furthermore, there is vasospasm of the afferent arterioles as a result of the hypertension which leads to reduction in the renal blood flow, which causes hypoxia and oedema of the endothelial cells of the capillaries of the glomerulus. As the condition worsens, the preeclampsia becomes severe and kidney damage results leading to the development of oliguria. Thrombocytopenia results due to increased breakdown of platelets and this could account for the disseminated intravascular coagulation (DIC) (Sibai, 2005; Sibai, 2012).

2.4.7 Effects on the foetus

Pre-term deliveries is a common outcome of preeclampsia due to spontaneous onset of labour caused by pre-term induction of labour (Bryan and Hindmarsh, 2006). The increased vasoconstriction due to preeclampsia results in reduced perfusion of the uteroplacental bed leading to intrauterine growth restriction of the foetus, decrease in the volume of the amniotic fluid and loss of tolerance for inutero conditions (Bryan and Hindmarsh, 2006).

Preeclampsia decreases flow of maternal blood through the placenta resulting in reduced availability of nutrients and oxygen to the foetus. This leads to foetal hypoxia and subsequently foetal distress (Ounsted and Ounsted, 1966; Bryan and Hindmarsh, 2006). The placental weight is thus decreased in pregnancy induced hypertension (PIH). Placental weight less than 250g can only be found in PIH. The

placental weight decreases as the preeclampsia becomes severe (Bryan and Hindmarsh, 2006). Hypovolemia due to reduced fluid volume within the blood vessels leads to reduced blood flow to the placenta and maternal organs (Ounsted and Ounsted, 1966). The complications are best controlled by regular ante natal attendance.

Preeclampsia is often diagnosed based on elevated blood pressure levels and other symptoms that may help in the easy diagnosis of preeclampsia like assessment of oedema, regular checking of weight and testing of urine for protein (Sibai, 2005).

Increase in blood pressure is the main diagnostic feature of preeclampsia (Sibai, 2005). Pregnant women at high risk should visit the laboratory early for tests to be conducted to provide baseline data and should receive regular pre-natal attention for the assessment of the progress of both mother and foetus (Sibai, 2005; Rao *et al.*, 2006).There is elevated serum uric acid due to the impaired function of the distal tubule of the kidneys, thus uric acid can be used as an early marker to predict preeclampsia (Sibai, 2005).

2.5 VARIOUS LABORATORY TESTS USED IN THE DIAGNOSIS OF PREECLAMPSIA

Laboratory diagnosis of a woman suspected of having preeclampsia (hypertension with proteinuria) primarily involves the assessment of proteinuria. This could be microalbumin, dipstick proteinuria or urine albumin-creatinine ratio.

2.5.1 Proteinuria

Higby and colleagues (Higby *et al.*, 1994) showed that an average 115 mg/day of protein is excreted in a 24 hour period in the urine of a normal pregnant woman. These results have served as the basis on which the Japanese Society of Obstetrics and Gynecology and host of the world's scientific organizations adopted 300 mg/day or more protein excreted in the 24 hours urine as a criterion for pathological proteinuria in pregnancy (Brown and Buddle, 1995; Brown *et al.*, 2000b). Since the estimation of 24 hour urine protein is not an easy procedure, most outpatient clinics screen women on prenatal visits using urine dipstick tests which detects protein levels semi-quantitatively (i.e. negative, or positive 1+, 2+, 3+) in spot urine samples. Compared with the quantity of protein detected in 24 hour urine, studies have reported that a positive result (1+ or 2+) of a dipstick proteinuria test in spot urine provides a high degree of false positive according to the criterion set for clinical proteinuria (Brown and Buddle, 1995; Brown *et al.*, 2000a). The significance of other methods for detecting clinical proteinuria in pregnancy like the spot urine protein/creatinine ratio has been mentioned in recent studies (Rodriguez-Thompson and Lieberman, 2001; Neithardt *et al.*, 2002).

Guidelines proposed by the International Society for the Study of Hypertension in Pregnancy pegged pathological proteinuria as 0.265 mg/mg creatinine or more in the spot urine (Cote *et al.*, 2008a). This method though cannot be used for every ante natal visit because of the cost involved.

Other proposals from organizations such as the Australasian Society for the Study of Hypertension in Pregnancy and the International Society for the Study of Hypertension in Pregnancy have stated that spot urinary protein: creatinine ratio can be used in place of the 24 hour urine collection (Ginsberg *et al.*, 1983). However, this method has not been widely used for the diagnosis of preeclampsia for several reasons, which have not been well clarified. In a study published in the British Medical Journal (BMJ), Cote *et al.*,(2008a) indicated that the spot urine protein: creatinine ratio acts as a reasonable test to rule out significant proteinuria of 0.3 mg/g or more in pregnancy.

Excretion of urinary albumin when persistent and above normal but below the sensitivity of the traditional semi quantitative test strip is termed microalbuminuria. Among non-pregnant women it serves as a marker of kidney damage of glomerular or tubular origin and is considered when all other possible causes of kidney pathology have been excluded (Cote *et al.*, 2008b).

The lack of harmony as to which is the most appropriate method of urine collection, the quantitative expression of albuminuria (Marshall, 1991), considerable changes as a result of posture and time of day (Winocour *et al.*, 1992) has affected the classification of microalbuminuria. An early morning urine specimen with a microalbumin or albumin creatinine ratio of >3 mg/mmol can predict up to 300 ug/min overnight excretion. If the ratio exceeds 1 mg/mmol it becomes mandatory for the laboratory to collect a 24 hour urine sample to estimate albuminuria

(Mogensen, 1984; Eshoj *et al.*, 1987). Thus, it appears that urinary albumin excretion can increase 10 to 20 fold higher than normal without the conventional dipstick method being able to detect it.

Only one study, at present, has been unable to establish that the albumin: creatinine ratio is predictive of total protein excretion. In a cohort of 22 participants Lindow and Davey (1992) were unable to diagnose proteinuria in women with heavy proteinuria.

2.5.2 Hyperuricaemia

This is a major characteristic finding in preeclampsia. Clinically, measurement of uric acid has been considered as a part of a panel of tests in women with preeclampsia to observe the severity of the disease to help with the management of the patients. The association between elevated serum uric acid and preeclamptic pregnancy has dated back over 95 years (Siemons and Bogert, 1917; Lam *et al.*, 2005). The reasons behind these elevated levels include decreased clearance of serum uric acid due to reduced glomerular filtration rate, increased reabsorption and decreased secretion of serum uric acid (Fadel *et al.*, 1969; Fadel *et al.*, 1976; Liedholm *et al.*, 1984).

Reports from several studies have identified a positive correlation between high maternal serum uric levels and harmful outcomes for the mother and foetus (Redman *et al.*, 1976; Liedholm *et al.*, 1984). However, because these basic diagnostic

tests were not conducted on large sample sizes, the accuracy of the findings are doubtful and so cannot be generalized and so optimal cut offs need to be established in predicting fetal and maternal complications (Thangaratinam *et al.*, 2009).

In the diagnosis and prediction of preeclampsia and its complications, different sensitivity and specificity of hyperuricaemia has been presented; from being the most sensitive marker of preeclampsia present (Lim *et al.*, 1998) and being equal in importance to proteinuria in identifying fetal risk in preeclamptic women (Roberts *et al.*, 2005) to being of little or no value in diagnosing and predicting preeclampsia (Weerasekera and Peiris, 2003a).

Chapter 3

MATERIALS AND METHODS

3.1 STUDY SITE/STUDY DESIGN

This non-randomized case-control study was conducted at the Obstetrics and Gynaecology (O&G) department of the Komfo Anokye Teaching Hospital (KATH) the major specialist and referral centre for the northern parts of the country from October 2011 to May 2012.

3.2 RECRUITMENT OF PARTICIPANTS

Seventy six (76) pregnant women with preeclampsia were selected using consecutive sampling from the O&G department of KATH for this study. Another 57 normotensive pregnant women were enrolled as controls. Of the 133 participants, 10 comprising 6 cases and 3 controls were dropped as they neither gave consent nor provided samples. Hence, 123 participants comprising 70 cases and 53 controls took part in the study.

3.2.1 Inclusion criteria

Participants twenty or more weeks pregnant with hypertension and proteinuria were considered eligible for recruitment into the study.

3.2.2 Exclusion criteria

Women less than twenty weeks pregnant, or carrying more than one fetus and

having preeclampsia superimposed upon chronic hypertension, those with a previous history of the condition or those with other chronic metabolic disorders were excluded.

3.2.3 Ethical clearance and consent

The participation of the respondents who are all indigenes of Ghana was voluntary and written informed consent was obtained from each of them. The study was approved by the Committees on Human Research Publication and Ethics, School of Medical Sciences / KNUST and the Research Directorate of KATH.

3.3 MEASUREMENT OF ANTHROPOMETRIC VARIABLES

Anthropometric measurements included height to the nearest centimeter without shoes and weight to the nearest 0.1 kg in light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall-mounted ruler. Body mass index (BMI) was calculated by dividing weight (kg) by height in metre squared (m^2). Waist circumference (to the nearest centimeter) was measured with a Gulick II springloaded measuring tape (Gay Mills, WI) midway between the inferior angle of the ribs and the suprailiac crest.

3.3.1 Blood Pressure (using Korotkoff 1 and 5)

Blood pressure was measured by trained personnel using a mercury sphygmomanometer and a stethoscope. Measurements were taken from the left

upper arm after subjects had been sitting for >5 min in accordance with the recommendations of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 minute rest interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

3.4 LABORATORY MEASUREMENTS

3.4.1 Urine collection and Urinalysis

Early morning urine was collected into plastic containers from the respondents and urine protein was determined using the dip-stick qualitative method (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea).

The urine sample was well mixed and transferred into a well labelled centrifuge tube. A urine reagent strip (dipstick) was then dipped into the urine sample (once) after which extra urine on the strip was allowed to drain back into the tube by holding the dipstick on the tip of the tube. The results from the procedure were read according to the time specified by the manufacturer by comparing the colour on the reagent strip to the colour on the container. The various parameters determined on the dipstick were based on the chemical principles stated below:

3.4.1.1 Protein

The test is based on the protein error of indicators principle. When pH is held constant by a buffer indicator, dyes release hydrogen ions because of the protein present and change colour from yellow to blue-green.

3.4.1.2 Specific gravity

Cations in the urine induce the release of protons from a complexing agent in the patch. This results in a non pH dependent colour change in the bromothymol blue indicator over the range of yellow through green to blue. Reagents: Bromothymol blue indicator and buffer.

3.5 BLOOD SAMPLE COLLECTION AND PREPARATION

Venous blood samples was collected after an overnight fast (8 – 12 hours). About 7 mls of venous blood were collected and, 5 ml dispensed into vacutainer® plain tubes. After clotting, it was then centrifuged at 500 g for 15 min. The serum was stored at - 80°C until assayed. The remaining 2 ml were dispensed into tubes containing 2.5 µg of dipotassium ethylenediaminetetraacetic acid (K_2 EDTA) as an anticoagulant.

3.5.1 Biochemical assays

Serum biochemistry was performed on the ATAC 8000 Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined included: ALK PHOS, ALT, AST and albumin (ALB); others were serum creatinine (CRT), serum uric acid. The serum for the estimation of the selected analytes were put into sample cups and placed in the auto analyzer. When analyze was

selected on the computer screen connected to the analyzer the automated instrument picked specified volumes of samples and reagents, mixed, incubated and had the absorbances read by the automated instrument.

The methods adopted by the automated instrument for the determination of the above parameters are as follows and all reagents were from JAS™ diagnostics, Inc. (JAS Diagnostics, Inc. Miami Florida, USA).

3.5.2 Albumin (BCG)

At a controlled pH, bromocresol green (BCG) forms a coloured complex with albumin. The intensity of colour at 630 nm is directly proportional to albumin content. The instantaneous initial absorbance is obtained as suggested by Webster (1977). The method used by the JAS™ albumin reagent is based on that of Doumas *et al.,*(1971).

3.5.3 Creatinine

Creatinine measurements are used in the assessment of renal dysfunction. Elevated creatinine levels are found in renal diseases and insufficiency with decreased glomerular filtration (uremia or azotemia if severe), urinary tract obstruction, reduced renal blood flow including congestive heart failure, shock and dehydration.

This method is based on a modification of the kinetic procedure which is fast, simple and avoids interferences (Fabiny and Ertingshausen, 1971), incorporating a surfactant and other ingredients to minimize protein and carbohydrate

interferences. Creatinine reacts with picric acid in alkaline conditions to form a colour complex (yellow-orange), which absorbs at 510 nm. The rate of formation of colour is proportional to the creatinine in the sample.

3.5.4 Serum Uric Acid

Serum uric acid measurements are most commonly used in the diagnosis of gout. Increased levels (hyperuricaemia) may be observed in leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, and conditions associated with decreased renal function.

The JAS™ procedure uses uricase, peroxidase and the chromogen TBHB to yield a colorimetric end product. Uric acid is oxidized by uricase to allantoin and hydrogen peroxide. TBHB + 4-aminoantipyrine + hydrogen peroxide, in the presence of peroxidase, produce a quinoneimine dye that is measured at 520 nm. The colour intensity at 520 nm is proportional to the concentration of Uric Acid in the sample.

3.5.5 Aspartate aminotransferase (AST)

AST is widely distributed with high concentration in the heart, liver, skeletal muscle, kidney and erythrocytes. Damage or disease to any of these tissues such as myocardial infarction, hepatitis, liver necrosis, cirrhosis and muscular dystrophy may result in raised serum levels of AST.

The present method is based on IFCC recommendations. AST catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of

NADH to NAD^+ in the malate dehydrogenase (MDH) catalyzes reaction. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate, which is normally present in serum.

3.5.6 Alanine aminotransferase (ALT)

ALT is widely distributed in tissues with highest concentrations found in the liver and kidney. Even so, ALT is considered more liver-specific than AST. Elevated levels of ALT are often only observed in liver diseases such as cirrhosis, hepatitis, or metastatic carcinoma. However, there can be elevated levels of ALT with infectious mononucleosis, muscular dystrophy, and dermatomyositis.

The procedure described herein is based on the method of Bergmeyer and Hørder, (1980; 1986). Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from L-alanine to 2-oxoglutarate to yield pyruvate and L-glutamate. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate and simultaneous oxidation of NADH to NAD^+. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the ALT activity. Endogenous sample pyruvate is rapidly and completely reduced by LDH during the initial incubation period so that it does not interfere with the assay.

3.5.7 Alkaline phosphatase

Alkaline phosphatase is a hydrolytic enzyme found in serum in many distinct forms which originate mainly from bone and liver. Pathological increases are

largely associated with hepatobiliary and bone diseases. Elevated activities are also observed in infectious hepatitis, bone disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. p-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita, (1939). Bessey, Lon and Brock published an endpoint procedure (Bessey *et al.*, 1946) while Bowers and McComb, (1966) reported a kinetic procedure. The JAS™ method is based on the kinetic photometric test, according to the IFCC. Absorbance is read at 405 nm.

3.5.8 Platelet Count

Platelet concentration (PLT) was determined by an automated blood analyzer CELL-DYN 1700®, version 1.08, (Abbott Diagnostics, Abbott Park, Illinois, USA). Measured parameters were determined by a direct analysis or count, and calculated parameters were determined by a mathematical manipulation of measured parameters or scientific constants (CELL-DYN analyzers manual).

3.5.9 Estimation of Urine Microalbumin

Low and medium molecular weight proteins are reabsorbed in the renal tubules such that the normal urine contains less than 150 mg of protein per day. Elevated levels of urine protein (proteinuria) usually more than 150 mg per 24 hours urine, almost always indicates disease. Glomerular proteinuria is due to increased

nephrotic syndrome (glomerular permeability) and may be seen in nephritis and secondary to other diseases like diabetic nephropathy. Tubular proteinuria is due to renal tubular damage leading to a slight increase in low molecular weight proteins if glomerular permeability is normal. Proteinuria observed when the renal system is functioning normally may be as a result of physiological increases in protein excretion or production of abnormally large amounts of low molecular weight proteins. Increased protein excretion could be seen during normal pregnancy, after strenuous exercise, or following prolonged maintenance of an upright posture. Increase in low molecular weight proteins may be as a result of production of Bence-Jones proteins, haemoglobinuria, due to severe haemolysis and myoglobinuria due to severe muscular damage.

When pyrogallol red molybdate complex binds to basic amino acid groups of protein molecules, there is a shift in reagent absorbance. The increase in absorbance at 578 nm is directly proportional to the concentration of protein in the urine.

Procedure

Well mixed urine samples were pipetted into test tubes labeled Blank (B) Standard (S), and (T) and the volume of the various reagents for the estimation added as shown in the table below:

Table 3.1: Estimation of urine microalbumin

Reagent	Blank (µl)	Standard (µl)	Test (µl)
Microalbumin reagent	1000	1000	1000

Microalbumin standard		20	
Sample			20
Water	20		

The mixture was well mixed and incubated for 3 minutes at 37 ºC. The absorbance of the colour developed at the end of the reaction (for standard and test) was read against the reagent blank at 578 nm within 20 minutes.

Calculations

Microalbumin concentration (mg/dl) =Absorbance of test/absorbance of standard *100

3.6 STATISTICAL ANALYSIS

Continuous variables were expressed as their mean ± SD, while categorical variables were expressed as proportion. Comparisons of the women with PE against the control group were performed using unpaired t tests, Chi-square ($\chi 2$) tests, or Fisher exact tests where appropriate. GraphPad Prism version 5.00 for windows was used for these statistical analyses (GraphPad software, San Diego California USA, www.graphpad.com). Analysis was statistically significant at $p<0.001$.

Association between spot urine protein and microalbumin and between uric acid and spot urine protein was by Pearson Correlation Coefficient. Sensitivity and specificity of microalbuminuria and uric acid as diagnostic markers was performed using receiver operating characteristics (ROC) curve. Statistical package for social science software version 16.0 (SPSS Inc. Chicago). $P < 0.05$ was interpreted as statistically significant.

Chapter 4

RESULTS

4.1 GENERAL CHARACTERISTICS OF STUDY PARTICIPANTS

Table 4-1: Demographic, clinical and biochemical data of study participants

Parameter	Control N=53	Cases N=70	P value
Education			
No	2(3.7)	10(14.3)	0.067
Basic	29(54.7)	40(57.1)	0.855
Secondary	10(18.8)	13(18.5)	0.967
Tertiary	11(20.7)	7(10)	0.123
Married	50(94.3)	48(68.5)	0.001
Occupation			
Formal	14(26.4)	6(8.6)	0.008
Informal	38(71.7)	64(91.4)	0.004
Ethnicity			
Akan	41(77.3)	52(74.3)	0.693
Ga-Adangbe	0(0)	2(2.8)	0.215
Ewe	1(1.9)	1(1.4)	0.842
Mole-Dagbani	11(20.7)	15(21.4)	0.971
Gestational age			
First trimester	3(5.6)	-	-
Second trimester	19(35.8)	37(52.8)	0.073
Third trimester	21(39.6)	33(47.1)	0.465
Abortion	6(11.3)	19(27.1)	0.041
Parity			
Nulliparous	19(35.8)	20(28.5)	0.433
Primiparous	13(24.5)	27(38.6)	0.122
Multiparous	21(39.6)	23(32.8)	0.454
Miscarriage	13(24.5)	9(12.8)	0.103
Contraceptive	17(32.1)	25(35.7)	0.705
History of hypertension	6(11.3)	6(8.5)	0.761

N=frequency

The demographic and obstetric characteristics of the studied participants are shown in Table 4-1. The percentage of married participants with preeclampsia was significantly lower than that of the controls (p=0.001). More control participants had formal education than the preelamptics (p=0.008), however, more preelamptics had informal education than the controls (p=0.004). The prevalence of abortion was higher in the preelamptics compared to the controls (p=0.041).

Table

4-2: Demographic, clinical and biochemical data of studied participants

Parameter	Preeclampsia N=70	Controls N=53	P value
Age (yrs)	30.51±0.75	30.52±0.65	0.996
BMI (kg/m^2)	25.09±0.48	25.98±0.68	0.276
SBP (mmHg)	161.60±2.22	107.20±1.36	0.001
DBP (mmHg)	101.90±2.83	66.63±1.33	0.001
UA (umol/L)	360.70±18.28	165.80±7.38	0.001
ALB (g/l)	32.50±0.58	34.24±0.69	0.058
CRT (umol/L)	73.07±4.94	82.70±2.88	0.128
AST (IU)	42.10±5.42	22.39±1.03	0.003
ALT (IU)	51.65±12.74	9.316±0.56	0.005
PLT (10^6)	242.20±9.90	264.90±22.04	0.307
MA (mg)	154.70±14.75	85.60±8.60	0.001

BMI=body mass index, SBP=systolic blood pressure, DBP= diastolic blood pressure, ALB=Albumin, CRT=creatinine, UA=uric acid AST=aspartate aminotransferase, ALT=alanine aminotransferase, PLT= platelets, MA=microalbumin

Table 4-2 shows the demographic, clinical and biochemical characteristics of the study participants. The preeclamptics had higher blood pressure (SBP and DBP) compared to the controls ($p<0.001$). Furthermore, biochemical parameters [(uric acid and hepatic enzymes (ALT and AST)] were significantly elevated compared to the controls. The mean urine microalbumin concentration was higher in the

preelamptics than the controls (p=0.005). The mean platelets count and serum albumin levels were however lower in the preelamptics than in the controls (though not significant).

Table 4-3: Demographic, clinical and biochemical data of studied participants stratified by concentration of urine microalbumin

Parameter	MA <30 (mg/g)	MA 30-300 (mg/g)	P value
Age (yrs)	31.50 ± 2.36	31.18 ± 0.82	0.919
BMI (kg/m^2)	25.68 ± 1.53	25.06 ± 0.45	0.458
SBP (mmHg)	162.5 ± 7.50	160.7 ± 2.55	0.856
DBP (mmHg)	105.0 ± 2.89	106.0 ± 3.00	0.817
UA (umol/L)	317.5 ± 72.46	357.8 ± 18.94	0.588
ALB (g/l)	33.85 ± 1.99	32.49 ± 0.69	0.611
CRT (umol/l)	55.20 ± 5.31	77.18 ± 5.88	0.331
AST (U/L)	36.35 ± 11.60	44.06 ± 6.57	0.760
ALT (U/L)	68.00 ± 1.155	55.41 ± 15.56	0.832
PLT (10^6/L)	251.50 ± 28.60	236.4 ± 9.50	0.683

MA =microalbumin

The demographic, clinical and biochemical characteristics of study participants stratified by urine microalbumin concentration is shown in Table 4-3. The preeclamptics with microalbuminuria >30-300 mg/g had insignificantly elevated DBP (p=0.817), uric acid (p=0.588) Creatinine (p=0.331) and transaminases (p=0.760; p=0.832). Platelets count (p=0.683) and serum albumin (p=0.611), SBP (p=0.856)

Table
stratified by
were reduced in the preeclamptics with urine microalbumin levels <30 mg/g though the differences were not significant.

4-4: Demographic, clinical and biochemical data of studied participants serum uric acid concentration

Parameter	UA< 360 umol/l	UA > 360 umol/l	P value
Age (yrs)	30.50 ± 0.94	30.55 ± 1.30	0.978
BMI (kg/m^2)	25.08 ± 0.61	25.11 ± 0.73	0.981
SBP (mmHg)	162.50 ± 2.64	159.50 ± 4.18	0.541
DBP (mmHg)	103.20 ± 3.40	99.18 ± 5.14	0.512
ALB (g/l)	33.33 ± 0.79	30.69 ± 0.56	0.036
CRT (umol/l)	72.13 ± 6.41	75.13 ± 7.35	0.780
AST (U/L)	39.81 ± 4.14	47.08 ± 14.89	0.538
ALT (U/L)	34.88 ± 8.15	88.24 ± 35.79	0.051
PLT (10^6/l)	231.70 ± 10.70	265.20 ± 20.68	0.116
MA (mg)	136.80 ± 14.68	193.60 ± 33.42	0.074

UA=Uric acid

Table 4.4 represents the demographic, clinical and biochemical characteristics of studied participants stratified by serum uric acid concentrations. Preeclamptics with uric acid levels <360 umol/L had significantly elevated serum albumin levels compared to those with uric acid levels >360 umol/L. However, blood pressure [SBP (p=0.542) and DBP(p=0.512)] was reduced in preeclamptics with uric acid

Table
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<360 umol/L. Conversely, serum creatinine (p=0.780), transaminases [AST (p=0.538) and ALT (p=0.051)], platelets (p=0.116) and urine microalbumin (p= 0.073) levels were elevated in the preeclamptics with >360 umol/L

4-5: Demographic, clinical and biochemical data of studied participants spot urine albumin concentration

Parameter	Spot urine Albumin < 2+ dipstick	Spot urine Albumin ≥2+ dipstick	P-value
Age (yrs)	34.20 ± 1.45	29.51 ± 0.84	**0.012**
BMI (kg/m^2)	24.36 ± 0.90	25.29 ± 0.56	0.431
SBP (mmHg)	158.70 ± 5.42	162.40 ± 2.46	0.500
DBP (mmHg)	100.10 ± 7.19	102.40 ± 3.05	0.741
UA (umol/l)	355.30 ± 48.79	362.10 ± 19.36	0.880
ALB (g/l)	32.29 ± 1.06	32.56 ± 0.69	0.852
CRT (umol/l)	59.97 ± 11.76	76.64 ± 5.37	0.168
AST (IU)	33.31 ± 5.63	44.50 ± 6.71	0.401
ALT (IU)	29.30 ± 6.37	57.75 ± 16.06	0.363
PLT (10^6/l)	238.00 ± 21.20	243.30 ± 11.28	0.826
MA (mg)	91.31 ± 32.73	172.00 ± 15.88	0.024

Table
stratified by

Table 4.5 shows the demographic, clinical and biochemical characteristics of preeclamptic participants stratified by spot urine albumin concentration. The preeclamptic participants with spot urine albumin concentration ≥2+ were younger (p=0.010) and had higher urine microalbumin levels (p=0.024) than those with spot urine albumin<2+. Transaminases (ALT, AST), creatinine, albumin, blood pressure (DBP and SBP), uric acid and platelets were elevated in the preeclamptic with spot urine albumin ≥2+ though not significant (p>0.05).

4-6: Demographic, clinical and biochemical data of studied participants gestational age (second trimester (T2)

Parameter	Control	Case	p-Value
	T2	T2	
Age (yrs)	31.21 ± 0.90	30.63 ± 1.28	0.722
BMI (kg/m^2)	24.24 ± 0.91	23.54 ± 0.58	
SBP (mmHg)	105.50 ± 2.28	168.80 ± 5.15	0.651
DBP (mmHg)	65.71 ± 2.13	107.50 ± 5.26	< 0.001
UA (umol/L)	163.60 ± 9.37	323.80 ± 26.83	
ALB (g/l)	35.25 ± 0.93	33.13 ± 2.34	< 0.001
CRT (umol/l)	82.85 ± 4.60	69.19 ± 6.01	< 0.001
AST (IU)	23.31 ± 1.64	24.10 ± 2.75	
ALT (IU)	9.92 ± 0.92	14.76 ± 1.09	
PLT (10^6/L)	247.20 ± 34.06	235.60 ± 24.05	0.317
MA (mg)	-	167.60 ± 44.10	0.105
			0.799
			0.006

Table
stratified by

0.835
-

The demographic, clinical and biochemical characteristics of preeclamptic participants stratified by gestational age is shown in Table 4.6. The preeclamptics in their second trimester had significantly elevated blood pressure (SBP and DBP), uric acid, hepatic transaminases (ALT) compared to the controls. However, though platelet count, albumin and creatinine were elevated in the controls compared to the preeclamptics the difference was of no statistical significance.

4-7: **Demographic, clinical and biochemical data of studied participants gestational age (third trimester)**

Table
stratified by

Parameter	Controls T3	Cases T3	P-Value
Age (yrs)	28.46 ± 0.83	30.50 ± 0.84	0.164
SBP (mmHg)	108.30 ± 1.96	160.60 ± 2.41	< 0.001
DBP (mmHg)	67.71 ± 2.17	106.50 ± 1.79	< 0.001
UA (umol/L)	174.10 ± 12.33	365.40 ± 20.31	< 0.001
ALB (g/l)	34.01 ± 1.02	32.42 ± 0.60	0.175
CRT (umol/l)	83.11 ± 2.69	73.57 ± 5.53	0.306
AST (IU)	20.47 ± 1.12	44.42 ± 6.05	0.019
ALT (IU)	9.52 ± 0.79	56.41 ± 14.30	0.049
PLT (10^6/L)	295.70 ± 39.42	243.00 ± 10.80	0.080
MA (mg)	-	-	

T3=Third trimester

The demographic, clinical and biochemical characteristics of studied participants stratified by gestational age (in trimesters) is shown in Table 4.7. The preeclamptics in their third trimester had significantly elevated blood pressure (SBP and DBP), uric acid, hepatic transaminases compared to the controls. However, though platelet count, albumin and creatinine were elevated in the controls compared to the preeclamptics the difference was of no statistical significance.

Table
stratified by

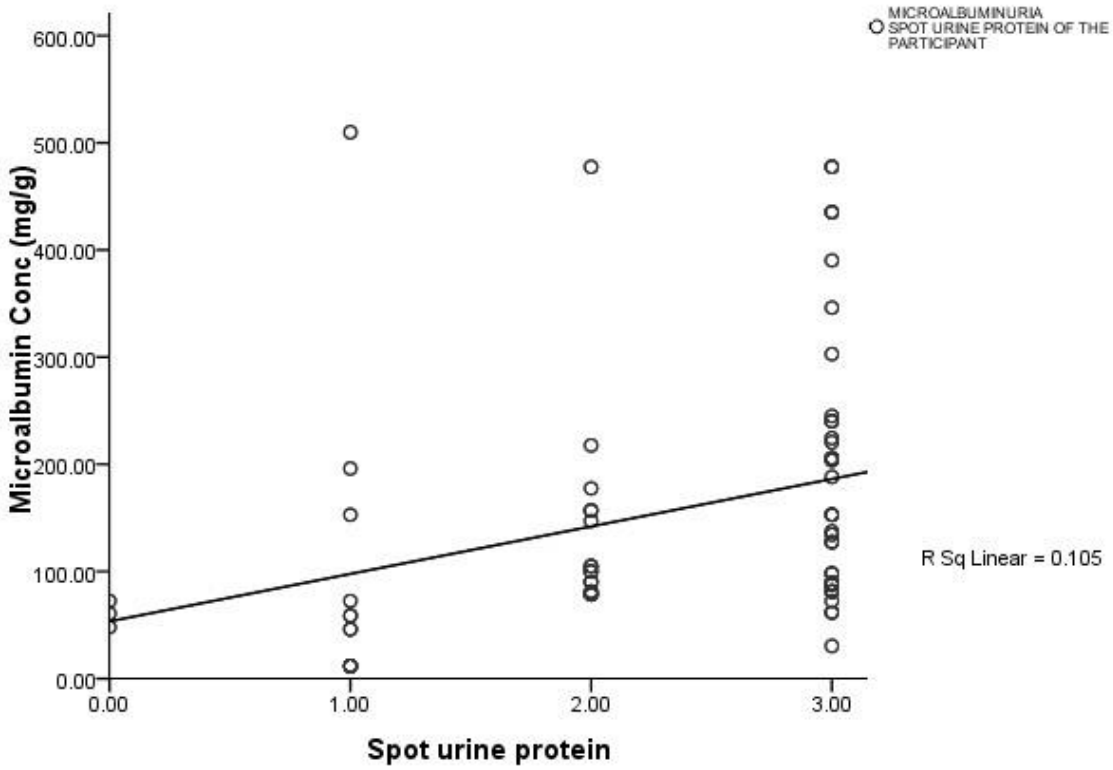

Figure 4-1: Correlation between spot urine protein and urine microalbumin concentration of preeclamptic patients.

The relationship between spot urine protein and urine microalbumin is shown in Figure 4.1. A significant positive linear correlation was observed between spot urine protein and urine microalbumin (r=0.324, p=0.006).

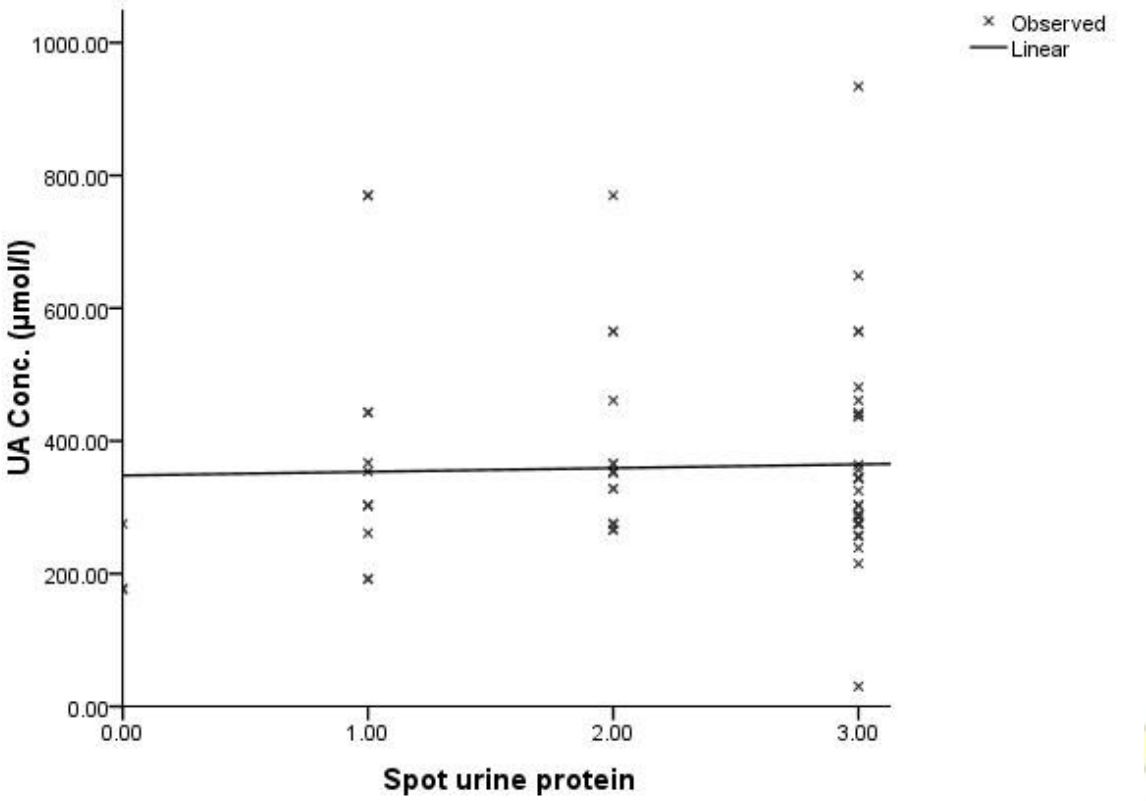

Figure 4-2: Correlation between spot urine albumin and uric acid levels of preeclamptic patients

Figure 4.2 shows the relationship between uric acid and urine microalbumin. A negative linear correlation was observed between uric acid and spot urine microalbumin (r=0.033, p=0.786).

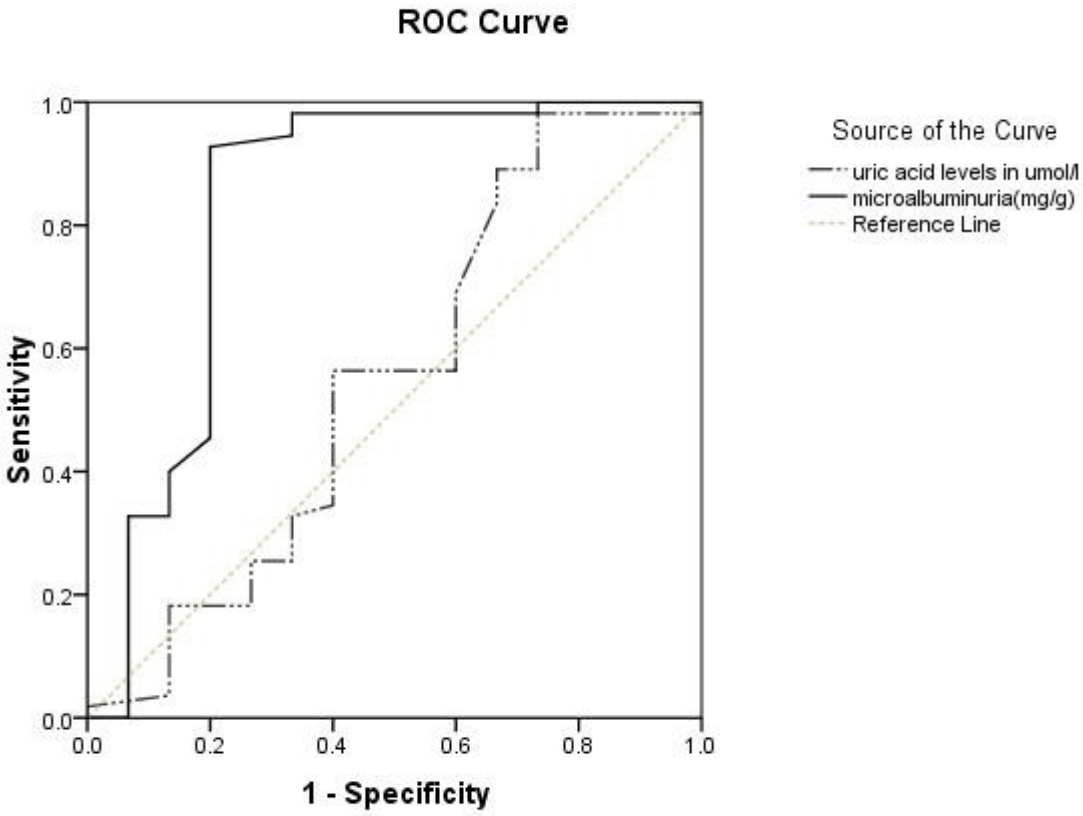

Figure 4-3: **Receiver operating characteristic curve analyses in women with preeclampsia.**

Figure 4.3 shows the receiver operating characteristic curve analysis in women with preecalmpsia. A urinary micro albumin value of 75.45 mg/g was identified as the best threshold to detect a spot urine protein of > +2 with a sensitivity of 92.7% and a specificity of 80.0%, PPV of 81.03% and NPV of 33.3%. Area under the curve = 0.835; asymptotic p-value of 0.000 at 95% CI (0.678-0.991). In contrast, serum uric acid level of 263.5 mg/g was identified as the best cut-off point to detect a spot urine

protein of > +2 with sensitivity and specificity of 89.1% and 33.3% respectively PPV of 77.2% and NPV of 20.8%).

Area under the curve = 0.552; asymptotic p-value of 0.538 at 95% CI (0.364-0.740).

Chapter 5

DISCUSSION

Preeclampsia is a multisystemic disorder peculiar to pregnancy after 20 weeks of gestation. It is characterized by widespread endothelial dysfunction throughout the maternal circulation resulting in hypertension attributable to vasoconstriction and significant proteinuria attributable to glomerular damage (Davey and Mac Gillivray, 1988; ACOG, 2002a; Jasovic-Siveska and Jasovic, 2011). The aim of this study was to evaluate the accuracy of urine microalbumin and serum uric acid as diagnostic parameters of preeclampsia.

Jasovic-Sivescka and Jasovic (2011) in a study conducted in Macedonia observed that women of low education status have an increased risk of preeclampsia. In this study however, though preeclamptics with basic education recorded the highest prevalence (57.1%) compared to controls the difference was of no statistical significance (p= 0.966). Even though the reason for this is unclear, the sample size and the cross sectional nature of the Macedonian study could account for the difference.

High rates of preeclampsia have been reported among women engaged in some form of socio-physical activity at work (Klebanoff *et al.*, 1990; Landsbergis and Hatch, 1996; Mozurkewich *et al.*, 2000). In this study, we observed that a significant percentage of the employed women had preeclampsia in agreement with earlier reports. Most of these women were employed in the informal sector, which

involves some substantial amount of physical activity. Though the percentage rate (91.4%) of preeclamptic women was higher compared to the percentage (71.4%) of healthy pregnant women, the difference was statistically significant.

Several observations support the biological plausibility of a link between job strain or strenuous physical activity and preeclampsia. Among these links is oxidative stress and uteroplacental blood flow. Preeclampsia is characterized by oxidative stress (Wang and Walsh, 1998). Prolonged or strenuous physical activity has been suggested to be detrimental to health through the induction of oxidative stress (Radak *et al.*, 1999; Tozzi-Ciancarelli *et al.*, 2002). Physical exertion promotes the accumulation of secondary products of lipid peroxidation (Radak *et al.*, 1999) and the formation of free radicals and other reactive oxygen species (Wang and Walsh, 1998). A recent study compared surgery room nurses, whose work involves prolonged standing without moving, with outpatient department nurses, who can walk during their worktime (Flore *et al.*, 2004). Interestingly, the surgery room nurses had significantly higher mean levels of reactive-oxygen-species after work. Therefore, if causal, the harmful effect of long periods of standing without walking on preeclampsia may be mediated by an elevated oxidative stress. Preeclampsia is also characterized by decreased uteroplacental blood flow (Flore *et al.*, 2004) and increased peripheral vascular resistance, which is in part mediated by over active sympathetic system (Redman and Sargent, 2001). Doppler studies indicate an increase in uteroplacental vascular resistance with physical exertion (Paisley *et al.*, 2003). Both stress and strenuous physical exertion activate the sympathetic

nervous system and therefore lead to the release of catecholamines and vasoconstriction, which, in turn, may have a detrimental effect on uteroplacental blood flow (Jackson *et al.*, 1995; Clapp, 2006). Our findings that both physically demanding and stressful work conditions increase the risk of preeclampsia are consistent with Hogue's hypothesis that hard physical work represents a set of acute physical stressors stimulating biophysical responses, which are similar to emotional stress (Roberts *et al.*, 1989).

Ethnicity did not significantly influence preeclampsia in this study. However, the Akans recorded the highest prevalence (74.3%) followed by the Mole-Dagbani (21.4%) and then the Ewe (1.4%). The rise in percentage rate of preeclamptic patients in the Akans can be attributed to their high population in Kumasi where the study site is located in Ghana (Ghana demographic profile, 2012) as well as the study site. Studies in China and the United States have linked ethnicity with preeclampsia even though the reasons are not clear (Gong *et al.*, 2012; Xiao *et al.*, 2014). For example, Rao et al. examined perinatal outcomes among Asian American/Pacific Islander women and observed that Filipino women had the highest risk of preeclampsia while East Asian women such as Chinese, Japanese, and Korean women had lowest risk (Rao *et al.*, 2006).

Gestational age showed no significance in this study (Table 4.1). However, the highest prevalence (52.8%) of preeclampsia among participants was recorded in the second trimester of pregnancy consistent with earlier findings (Sibai *et al.*, 1991). The third trimester recorded the second higher prevalence (47.1%).

Abortion has been identified as a risk factor for preeclampsia in previous studies (Sibai *et al.*, 1995; Sibai *et al.*, 1997; Saftlas *et al.*, 2003) and a protective factor in nulliparous women (Sibai *et al.*, 1997; Eras *et al.*, 2000). A significant number of preeclamptic participants enrolled in this study had had an abortion. The type of abortion determines whether a women is protected against preeclampsia or stands at an increased risk of developing the condition (Saftlas *et al.*, 2003). Though the mechanism behind how abortion affects preeclampsia is ill understood, studies have hypothesized that abortion protects against preeclampsia through an immunologic mechanism, whereby exposure to fetal antigens through the aborted pregnancy enhances development of maternal-fetal immunologic tolerance (i.e., causing "desensitization") in a subsequent pregnancy with the same father. This theory is supported by the recent finding that fetal cell microchimerism results even following first trimester pregnancy terminations (Bianchi *et al.*, 2001; Saftlas *et al.*, 2003).

Twenty seven preeclamptic women in the primiparity status recorded the highest percentage rate (38.6%) preeclampsia. This corresponds with evidence of increased primiparity being a risk factor for preeclampsia (Sibai *et al.*, 2005). Meanwhile percentage difference in parity of preeclamptic women compared to healthy pregnant women was of no statistical significance ($p>0.05$).

An association has been reported between family history of hypertension (Sanchez *et al.*, 2003), history of miscarriage (Sanchez *et al.*, 2003), contraceptive use (Bracken and Srisuphan, 1982), and prevalence of preeclampsia. However, observations

made in this study contradict these earlier reports. The reasons for these contradictions remain unclear. However, the type of contraceptive used in this study was not clarified.

There was no significant difference in the age of the preeclamptics compared to controls. This finding is consistent with observations made by several studies (Bianco *et al.*, 1996; Kashanian *et al.*, 2011) who observed no significant difference between the mean age of the preeclamptics and the healthy pregnant women. The reality of these findings can be explained by the fact that most participants were in the same age group and so could attribute to the closeness in mean age.

Increased BMI has been associated with increased development of preeclampsia (O'Brien *et al.*, 2003; Frederick *et al.*, 2006). In this study, however, the mean BMI of the preeclamptics were lower compared to the controls though the difference was not significant. This observation, though, is consistent with the findings of Fatema *et al.*, (2011) and Anderson *et al.*,(2012) but disagrees with O'Brien *et al.*,(2003) and Frederick *et al.*,(2006) who reported significantly higher mean BMI values in preeclamptics compared to healthy pregnant controls. The reasons for these discrepancies are that the previous studies worked more on the severe forms of the preeclamptic condition and that may have recruited large number of obese participants whose weighted average fetched an elevated BMI. Conversely, our study worked with a randomized sampling technique where most of the cases recruited were averagely overweight (Table 4.2). Obesity and overweight measured by BMI might act through its association with dyslipidaemia which

results in an atherosclerotic-like lesion of spiral arterioles of the placenta, characterized by foam cell invasion and intravascular fibrin deposition leading to endothelial cell dysfunction, which is a characteristic of preeclampsia (Sibai *et al.*, 1991; Levine *et al.*, 2006). Elevated mean BMI level in preeclamptics can also be due to retention of fluid in the body tissues.

The mean blood pressure (SBP and DBP) were higher in cases compared to controls. These observations agree with several findings (Levine *et al.*, 2006; Akhtar *et al.*, 2011). Increased blood pressure in preeclamptics may be best explained by its association with hypertension. The increased responsiveness of the arterial system to pressor substances causes a generalized vasoconstriction. Increase in peripheral vascular resistance appears to be the main cause for blood pressure elevation.

Vasoconstriction results in reduced uteroplacental blood flow. In preeclampsia, there is a marked increase in the response to vasopressin, to norepinephrine and to angiotensin (Levine *et al.*, 2006).

AST and ALT are intracellular enzymes involved in amino acid and carbohydrate metabolism; their elevated levels show damage to organs with cells rich in these enzymes, probably the liver. The mean AST and ALT levels were significantly higher in preeclamptics compared to normal healthy controls (Table 4.2). Elevated levels of aminotransferase in preeclampsia are consistent with several studies (Catanzarite *et al.*, 1995; Martin, 2013). In this study, ALT levels were significantly higher than AST levels in the preeclamptics compared to the controls. This

however is inconsistent with the findings of Malvino *et al.,* (2005) who observed an elevated AST than ALT among preeclamptics. These deviations can be explained that Melvino and colleagues used only severe preeclamptics as their study population. Elevated transaminases levels in preeclampsia may be explained by vasoconstriction of the hepatic vascular bed resulting in hypoxia and oedema of the liver cells (Rahman and Wendon, 2002). Another mechanism is the changes in the endothelium and deposition of fibrin in the liver leading to impaired function. Fibrin deposition in the liver can result in haemorrhagic necrosis, indicated by epigastric pain or right upper quadrant tenderness, nausea and vomiting. Necrotic liver tissue can result in elevated liver enzymes (Knox and Olans, 1996).

Significantly high levels of aminotransferase may be associated with hemolysis, elevated liver enzymes, low platelet count (HELLP syndrome) which is mostly common in multiparous women (Lee and Brady, 2009). HELLP syndrome, which is characterized by an elevated liver enzyme with AST exceeding ALT levels is inconsistent with this study (Table 4.2) and thus makes its association with preeclampsia unlikely.

Consistent with previous reports (Many *et al.*, 1996; Koike *et al.*, 1997), mean serum uric acid levels are significantly higher (p=0.001) in participants with preeclampsia than in normal pregnant women in the present study. There are several proposed mechanisms for hyperuricaemia in preeclampsia such as abnormal renal clearance, increased tissue breakdown, acidosis, a rise in the activity of the xanthine oxidase/dehydrogenase enzyme as well as the pathophysiological response to

hypovolaemia and increased oxidative stress, caused mainly by the ischemic placenta (Bainbridge and Roberts, 2008). Another mechanism involves the reduction in the clearance of uric acid due to the decrease in the GFR that occurs in many, but not all cases of preeclampsia, increased absorption and decreased renal tubular excretion in proximal tubules of the kidney (Thangaratinam *et al.*, 2006; Martin and Brown, 2011). Uric acid is known to possess antioxidant properties, and contributes about 60% of free radical scavenging activity in human serum (Waring *et al.*, 2006). The hyperuricaemia recorded in this study could be a protective response, capable of opposing harmful effects of free radical activity and oxidative stress. Furthermore, between the fetus and the placenta provides substrate for maternal xanthine dehydrogenase /oxidase, which increases the production of reactive oxygen species (ROS) and UA. Thus, the increased circulating levels of uric acid, a valuable marker of preeclampsia, may also be a marker of free radical generation in this condition (Many *et al.*, 1996).

Vasospasm in preeclampsia leads to decreased renal perfusion and subsequent decreased GFR. In normal pregnancy the GFR is increased up to 50% above prepregnancy levels. Because of this, serum creatinine levels in preeclamptic patients rarely increase above normal pregnancy levels (Weerasekera and Peiris, 2003b). In the present study the mean serum creatinine levels were elevated in healthy pregnant controls compared to preeclamptic patients though the difference was of no statistical significance (p=0.128). This finding is in agreement with Sibai *et al.*,(1997) who observed lower creatinine levels in preeclamptic patients.

Decreased serum creatinine levels may not only be attributed to renal failure but also associated with hemoconcentration due to dehydration (Sibai *et al.,* 1997). Previous studies (Burrows and Kelton, 1993; Fallahian and Nabaie, 2005; Ceyhan *et al.*, 2006) have suggested that the storage of platelet in areas with endothelial damage and peripheral consumption as the cause of thrombocytopenia in preeclampsia. In addition, the life span of platelets in preeclamptic women is reduced to 3 to 5 days and the alteration of platelet membrane accelerates its aggregation and destruction (Fallahian and Nabaie, 2005; Juan *et al.*, 2011). This confirms the low platelet levels observed in preeclamptics in this study though the difference was of no statistical significance. The decrease in platelet levels can be attributed to HELLP syndrome.

The preeclamptic patients excreted higher amount of urinary protein ($p= 0.003$) compared to healthy controls. In the present study microalbuminuria concentration for preeclamptics was markedly elevated, consistent with previous research (Rodriguez *et al.*, 1988; Poon *et al.*, 2008). Excretion of urinary protein in preeclampsia results from glomerular endotheliosis. The pathogenesis for microalbuminuria in preeclampsia may be due to damage of the glomerular filtration capacity of the kidney and a resulting decrease in tubular reabsorption of protein (Jensen *et al.*, 2010). Glomerular filtration rate ("GFR") is a quantifiable way of measuring the overall functioning of the kidneys. When the rate of blood flow is changed, it changes the blood flow to the kidneys as well. As a result, metabolic

waste is not filtered out of the kidneys quickly enough and can lead to proteinuria due to injury to the glomeruli from low oxygen levels (Martini, 2006).

Increased urinary protein excretion reflects the decrease in serum albumin concentration among preeclamptic women compared to the healthy pregnant women though the difference was of no statistical significance (p=0.058). This finding is in accordance with reports of several studies (Gojnic *et al.*, 2004; Salari and Eftekhari, 2005) which concluded that preeclamptic patients have lower serum albumin concentration than normotensive controls. A loss of albumin in the urine may be caused by renal dysfunction (nephrotic syndrome).

The mean serum albumin levels among the healthy pregnant women were higher than those presenting with preeclampsia. However, differences in their means were of no statistical significance (Table 4.2). This observation deviates from the findings of Salako *et al.*,(2003) who reported that the difference in the mean serum albumin levels was significantly higher in the preeclamptics compared to controls. The discrepancies in results attest to the fact that our study employed a case control format and a sample size of 121 compared to the large number of participants recruited by Salako and colleagues which also comprised normotensive (69.6%) and preeclamptics (21.7%). Hypoalbuminemia in preeclampsia can be explained by the reduced hepatic blood flow which is secondary to hypovolaemia created by higher filtration pressure in the capillaries (Gojnic *et al.*, 2004).

Preeclamptics with microalbuminuria, in this study had elevated mean DBP compared to normoalbuminuric group though the difference was not significant.

Increase DBP signifies the intensity of hypertension in the condition. Also, subjects with microalbuminuria recorded the highest uric acid level comparatively to the normoalbuminuric group. This finding although not similar, agrees partially with Lee *et al.*,(2006) who reported that prehypertensive subjects with microalbuminuria had higher uric acid level than those with normoalbuminuria. The findings of Lee *et al.*,(2006) in relation to uric acid were significant in contrast to observations made in this study. Moreover, transaminases levels though elevated among microalbuminurics showed no significance ($p>0.005$) when compared with subjects presenting normoalbuminuria. Reason for elevated aminotransferase in microalbuminuric group though not clear could be due to damaged liver cells. The mean platelet count and serum albumin level among the two groups was not statistically significant though the means were higher in the normoalbuminuric group (Table 4.3). No specific reasons could be assigned for this difference. However, the fact that the normotensive group was also above twenty weeks gestation age could account for the slight variation.

As previously reported the increase in uric acid occurs as a result of the reduction of glomerular filtration and hypovolemia in preeclampsia. An elevation in serum uric acid has been associated with an increased risk for the development of hypertension. Even mild hyperuricemia causes hypertension and renal injury with stimulation of the renin-angiotensin system and inhibition of neuronal nitric oxide synthase (Bainbridge and Roberts, 2008). In this study we observed that the mean

SBP and DBP were low among preeclamptics with uric acid levels >360µmol/l compared with the group who had <360µmol/l. This can be attributed to the fact that elevated level of uric acid in preeclamptic women is not simply a marker of disease severity but rather contributes directly to the pathogenesis of the disorder (Kang *et al.*, 2004). Meanwhile there was a significant elevation in serum albumin concentration among preeclamptics with uric acid levels <360µmol/l.

This study observed microalbuminuria to be directly proportional to ≥2+ spot urine albumin and inversely proportional to <2+ spot urine albumin. Microalbuminuria concentration was significantly higher among preeclamptics in the ≥2+ spot urine albumin group than in <2+ spot urine albumin group (Table 4.5). Elevated levels of microalbuminuria concentration in the ≥2+ spot urine albumin group can be explained by: firstly, the large number of preeclamptics present in this group due to the recruitment criteria for the condition under study and secondly, damage to the glomerular filtration capacity of the kidney and a resulting decrease in tubular reabsorption of protein. Also, the ≥2+ spot urine albumin group was significantly (p=0.010) older compared to the other group. This explains age as a risk factor for preeclamptics who diagnostically present ≥2+ urine protein. Women aged ≥ 40 had approaching twice the risk of developing preeclampsia, whether they were primiparous or multiparous (Bianco *et al.*, 1996). Nationwide US data suggest that the risk of preeclampsia increases by 30% for every additional year of age past 34 (Saftlas *et al.*, 2003). Young maternal age did not seem to affect the risk of developing preeclampsia, whichever cut off age was used.

There were no significant correlation between spot urine protein and uric acid levels (Figure 4.2.) Although the data in this study suggest a statistically significant difference between the mean uric acid value of the preeclamptics and healthy pregnant control groups (Table 4.2), its diagnostic ability for preeclampsia is questionable. Our study could not identify an obvious cut off point on the receiver operator curve (ROC) for uric acid level that was sufficiently sensitive and specific to distinguish preeclampsia. The best sensitivity (89.1%) and specificity (66.7%) was related to cut off point of 263.50 μmol/l. These figures are close to the values of some other studies, and the findings are consistent with those studies that did not find a clinical utility for serum uric acid in the prediction of preeclampsia (Cnossen *et al.*, 2006; Taefi *et al.*, 2008) and in contrast to some other studies (Roberts *et al.*, 2005). This might be because most of the studies that have reported a strong correlation between elevated serum uric acid and the severity of preeclampsia, have examined pregnant women with the most severe form of the disease (Acien *et al.*, 1990).

In severe preeclampsia, levels of serum uric acid might correlate with certain measures of outcome; however, in the more prevalent milder forms of the disease, the correlation of serum uric acid levels and outcomes is weak, as reported by Paternoster *et al.,* (1999) who concluded that the rate of protein excretion, the oldest laboratory test used for diagnosis, appeared to be more useful in the prediction of preeclampsia than serum uric acid. In this study 89.1% of all participants with

preeclampsia were correctly identified as such and 66.7% of all healthy pregnant controls were incorrectly identified as positive (i.e. using uric acid as diagnostic predictor of preeclampsia). This shows that a single estimate of uric acid is of little or no value in the prediction of preeclampsia. This is further confirmed by the insignificant (p=0.538) results showed by our ROC analysis (Figure 4.3).

A significant positive correlation (r=0.324, p=0.006) was established between spot urine protein (macroalbumin) and microalbuminuria. This finding partly agrees with the observations made in other studies where 24 hour urine was used in the detection of macroalbuminuria (Durnwald and Mercer, 2003; Al *et al.*, 2004). Significant correlation between proteinuria and microalbuminuria in preeclamptics suggest a strong relationship between microalbuminuria and risk of preeclampsia because microalbuminuria is a more sensitive measure of proteinuria and thus increased levels serves as an early indicator of the risk of preeclampsia. According to the ROC curve analysis, sensitivity (92.7%) and specificity (80.0%) of microalbuminuria were best identified at a threshold point of 75.45 mg/g with PPV of 81.03% and NPV of 33.3%. In comparison with uric acid accuracy, urinary microalbumin can be used as an alternative diagnostic marker to spot urine protein for predicting early onset of preeclampsia. The area (0.835) under this curve was of relevant significance (Figure 4.3). Shaarawy and Salem,(2001) reported that sensitivity of predicting preeclampsia by measuring microalbumin in early pregnancy varied between 50 to 68%, the specificity varied between 58 to 97%, PPV varied between 26 to 61% and the NPV varied between 87 to 94 %. The limitations

of this study was that it could not generalize the prevalence and incidence to the whole Ghanaian population in Kumasi, since it was a hospital-based study.

Chapter 6

CONCLUSIONS

In the diagnosis of preeclampsia, the use of microalbumin as a biomarker is more accurate than serum uric acid. Urine levels of microalbumin, as a measure of proteinuria are elevated in preeclamptics and can be used in place of spot macro protein estimation to diagnose preeclampsia especially in the early stages. Conversely, single determinations of serum uric acid estimations cannot be used to predict preeclampsia. This study further established a correlation between spot urine protein and microalbuminuria but not microalbuminuria and serum uric acid.

6.1 RECOMMENDATION

As a result of the low platelet count observed among PE in this study, coagulopathy and coexisting malaria, infections among PE need further investigation since these conditions play a role in endothelial dysfunction of the maternal and placental circulation.

Paternal, immunologic, oxidative stress and genetical influences might play a role in the aetiology of PE in Ghanaian women and thus further research into immunologic and/or molecular mechanisms involved in genetic and partner-specific tolerance is necessary to be investigated.

Since this study was hospital based, a Ghanaian population-based study is needed for investigation to identify the monthly incidence and prevalence of PE in Ghana.

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APPENDIX

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
SCHOOL OF MEDICAL SCIENCES
DEPT. OF PHYSIOLOGY

QUESTIONNAIRE

EVALUATION OF DIAGNOSTIC PARAMETERS OF PREECLAMPSIA/ ECLAMPSIA

CODE:..... **DATE:** ...../...../ 20.....

Consent has been read to participant and obtained	**Yes** ☐	**No** ☐

1.0	**Demographic Information (Please tick or write where appropriate)**	
1.1 Age	**Please write here**.....	
1.2 Ethnic group	**Please write here**.....	
1.3 Education (Highest level attained)	**Never attended School** ☐	**Junior Sec School** ☐
	Primary School ☐	**Senior Sec School** ☐
	Middle School ☐	**University / Tertiary** ☐
	Other (please write).....	
1.4 Occupation	**Please write the job you do**.....	
1.5 Marital status	**Single** ☐	**Divorced** ☐
	Married ☐	**Widowed** ☐
	Cohabiting ☐	**Other** ☐

2.0	**Anthropometric Data (Please write in the spaces provided)**

Parameter	Entry
Weight	
Height	
WHR	
SBP	
DBP	

	Obstetric History	
3.1	How old is this pregnancy	Please write.....
3.2	Is this your first pregnancy	Yes ☐ No ☐
3.3	If no, how many have you had?	Please write.....
3.4	Have you had any abortion	Yes ☐ No ☐
3.5	If you answered yes to question 3.4, please Indicate how many?	Please write.....
3.6	How many children do you have?	Please write.....
3.7	Have you had any miscarriage?	Yes ☐ No ☐
3.8	If you answered yes to Question 3.7 above, please indicate how many miscarriages.	Please write.....
	Clinical History (Please tick and/ or write where appropriate)	
3.9	Have you used any contraceptive method before?	Yes ☐ No ☐
4.0	If you answered yes to question 3.9 above, Indicate which type of contraceptive method you used.	Please tick all that apply to you Injectables ☐ Pills ☐ Condom ☐ Implants ☐ IUD ☐
4.1	How long did you use the contraceptive Method before pregnancy?	Please write.....
4.2	Have you ever been told prior to this Pregnancy that you had high blood pressure?	Yes ☐ No ☐
4.3	Do you have a sister or mother who has Had high blood pressure during pregnancy?	Yes ☐ No ☐
4.4	Did you have high blood pressure during any of your previous pregnancies?	Yes ☐ No ☐
4.5	If you answered yes in question 4.4, please Indicate which pregnancy?	Please write.....
4.6	Did you have any Kidney disease prior to this Pregnancy?	Yes ☐ No ☐

Appendix

4.7	Did you have Diabetes Mellitus prior to this Pregnancy?	Yes ☐	No ☐
	Bleeding History (Please tick and write where appropriate)		
4.8	Do you have any history of spontaneous bleeding?	Yes ☐	No ☐
	If you answered question 4.8 above, please indicate the following in the spaces provided		
4.9a	From which site (s) was the bleeding?	.....	
4.9b	Did you use to have heavy menstrual flow?	Yes ☐	No ☐
4.9c	How long does menstruation last?	Yes ☐	No ☐
4.9d	Do you have dark sports on your skin?	Yes ☐	No ☐
4.9e	Does it take long for bleeding to stop?	Yes ☐	No ☐
4.9f	Are you on any anticoagulant therapy?	Yes ☐	No ☐