A PILOT STUDY ON SENSITIVITY AND SPECIFICITY OF CYSTATIN C IN DETECTING RENAL IMPAIRMENT IN HYPERTENSIVE PREGNANCIES

By

DR FAUZIAH BINTI JUMMAAT

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE (OBSTETRICS AND GYNECOLOGY)

UNIVERSITI SAINS MALAYSIA
MAY 2011

Acknowledgements

"In the name of Allah, most Gracious, most Compassionate".

Firstly, to my most beloved husband Dr. Azreen Syazril Bin Adnan, thousands of thanks and appreciation for your valuable time day and night, endless support, never ending help, love, understanding and sharing the hardships with me in completing this study. Thank you for being my inspiration. I pray to Allah, He would give all the best thing into your life.

To our princesses Aida Farhana, Anis Fatihah and Alya Faridah, who always give me strength and always cheer up my life. May Allah be with you all the time.

To my parents Encik Jummaat Kahman and Puan Sapiah Abdullah, may Allah bless you for your sacrifices in replacing me to feed my children, send and pick them up from schools, shower them with care and love, during my limited time in this Master programme. Thank you for your continuous support and strong motivation.

In memory of my late parents in law Encik Adnan Omar and Puan Fatimah Jaman, may Allah bless you, Al-Fatihah..

To my supervisors, Assoc. Prof. Dr. Nor Aliza Abdul Ghaffar, thank you for your full support, encouragement and continuous motivation.

To Dr Julia Omar, thanks for your guidance and understanding.

Last but not least, to Prof. Dr. Syed Hatim Noor, thank you for the guidance and time.

Fauziah Jummaat

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AKI	Acute Kidney Injury
ATN	Acute Tubular Necrosis
CKD staging	Chronic Kidney Disease staging
cm	Centimeter
Cr Cl	Creatinine Clearance
CT	Computerized Tomography
DBP	Diastolic Blood Pressure
dl	Desiliter
DTPA	Diethylene Triamine Penta-acetic acid
DMSA	Dimercaptosuccinic acid
ELISA	Enzyme-linked immunosorbent assay
g	Gram
GFR	Glomerular Filtration Rate
HUSM	Hospital Universiti Sains Malaysia
i.e	id est
IL-18	Interleukin-18
IUD	Intrauterine death
KIM-1	Kidney Injury Molecule-1
KUB	Kidney Ureter Bladder
kDa	Kilo Dalton
LSCS	Lower Segment Cesarean Section
mg	Miligram
min	Minute

ml	Mililiter
NGAL	Neutrophil Gelatinase-associated Lipocalin
PENIA	Particle-enhanced nephelometric immunoassay
ROC	Receiver Operating Characteristics
SPSS	Statistical package for social science
SD	Standard Deviation
SVD	Spontaneous Vaginal Delivery
µmol	Micromol

ABSTRACT

Objectives : This study is to compare between Cystatin C and 24 hour urine creatinine clearance for detection of renal impairment in hypertensive pregnancies population.

Study Methods: 64 patients enrolled in this cross sectional study and each patient was required to collect 24 hour urine and 15 mls of blood. Blood taking was taken once in second and third trimester. Urine collection was carried out during the first visit. Creatinine clearance below 90 mls/min is taken as renal impairment and compared to the serum Cystatin C level. The Receiver Operating Characteristic Curve is drawn to obtain the sensitivity and specificity of Cystatin C.

Results : The results have shown that when compared to 24 hour urine creatinine clearance, in second trimester, Cystatin C is 84.6 % sensitive and 86.7% specific for detection of renal impairment at Cystatin C level of 0.574-0.898 (p value < 0.012) , area under curve: 0.736, positive predictive value is 0.92 and negative predictive value is 0.76. While in the third trimester, the sensitivity and specificity is 76.9% and 60% at the Cystatin C level of 0.657-1.00 (p value < 0.006), area under curve 0.838, the positive predictive value is 0.71 and negative predictive value is 0.67.

Conclusion: Our study suggests that Cystatin C is a useful diagnostic kit for diagnosis of renal impairment in hypertensive pregnancies population.

ABSTRAK

Objektif: Kegagalan fungsi buah pinggang wanita hamil yang mempunyai tekanan darah tinggi dapat dikenalpasti dengan ujian Cystatin C. Ia dibandingkan dengan ujian air kencing 'creatinine clearance' 24 jam.

Metodologi: Seramai 64 pesakit terlibat didalam kajian ini. Sampel air kencing dikumpulkan selama 24 jam dan pengambilan darah 15 ml diperlukan. Darah pesakit diambil pada trimester kedua dan ketiga. Manakala air kencing 24 jam diambil pada lawatan pertama. Nilai 'Creatinine clearance' kurang daripada 90 ml/min menunjukkan kegagalan fungsi buah pinggang dan ini dibandingkan dengan paras Cystatin C. Graf 'Receiver Operating Characteristic Curve' untuk mendapatkan sensitiviti dan spesifisiti Cystatin C dilakarkan.

Keputusan: Keputusan dibandingkan dengan ujian air kencing 'creatinine clearance' 24 jam. Trimester kedua menunjukkan Cystatin C adalah 84.6% sensitif dan 86.7% spesifik, dalam mengesan kegagalan fungsi buah pinggang. Paras Cystatin C adalah 0.574-0.898 (nilai $p< 0.012$) ,'area under curve' adalah 0.736. Manakala 'nilai prediktif positif' ialah 0.92 dan 'nilai prediktif negatif' ialah 0.76. Pada trimester ketiga, sensitiviti dan spesifisiti adalah 76.9% dan 60% masing-masing. Paras Cystatin C 0.657-1.000 (nilai $p<0.006$), 'area under the curve' adalah 0.838, 'nilai prediktif positif' ialah 0.71 dan 'nilai prediktif negatif' ialah 0.67.

Kesimpulan: Kajian ini menunjukkan Cystatin C berguna dalam mendiagnosa kegagalan fungsi buah pinggang kepada wanita hamil yang mempunyai tekanan darah tinggi.

INTRODUCTION

CHAPTER 1
INTRODUCTION

1.1 Renal impairment in hypertensive pregnancies – morbidities and mortalities

Kidney is an important organ in maintaining physiology of pregnancy. Pregnancy related acute renal failure predisposes the pregnancy to multiple medical risks, thus increased morbidity and mortality (Brown *et al.*, 2001). Preeclampsia and uncontrolled hypertension are recognized factors causing renal impairment. In third world countries pregnancy causes 25% of acute kidney injury and maternal mortality rate ranges from 9-55% (Prakash *et al.*, 1995). Preeclampsia increases maternal morbidity and mortality. Renal impairment is associated in hypertensive pregnancies (Chee, 1988) . Early detection of renal impairment in hypertensive pregnancies enable immediate referral and multidisciplinary approach. Consequently further deterioration of kidney function is preventable. Diagnostic tools with high sensitivity and acceptable specificity is important for renal function assessment (Hoek *et al.*, 2003).

Preeclampsia and eclampsia constitutes 75% as the cause of acute kidney injury in pregnancy, while sepsis 11% and followed by hemorrhage 7.2%. Fetal death was reported at 5.5 % while maternal death at 9.1% (Silva *et al.*, 2009). Another study conducted in India, discovered maternal mortality due to pregnancy related acute renal failure was 24.3% (Misra *et al.*, 2003).

Perinatal mortality rate is significantly higher with Glomerular Filtration Rate (GFR) of 70 ml/min. A consistent observation showing that serum creatinine >2.5 mg/dl (220 µmol/l) is associated with more preterm deliveries and lower birth weights than women with a lower serum

creatinine. 73% of such women were delivered before 37 weeks and 57% had intra-uterine growth retardation (Knutzen and Davey, 1977) .

1.2. Current available measures in detection of chronic kidney disease

Several investigations available in diagnosing kidney impairment. Investigations are divided to urine, blood, radiological investigation and renal biopsy. Urine analysis include urine for microalbumin, protein and hematuria. Radiological investigations include, ultrasonography of the kidneys, X-ray of the kidney, ureter and bladder, radionuclide imaging Diethylene Triamine Penta-acetic acid (DTPA), Dimercaptosuccinic acid (DMSA) scan. Renal biopsy is carried out in exclusive conditions, and general indications include assessment of causes of proteinuria and for identifying the cause of acute glomerulonephritis. Blood investigations include serum creatinine and urea level. 24 hour urine creatinine clearance is another method requiring patient to collect 24 hours urine sampling and blood for serum creatinine taken simultaneously.

1.2.1. Urine Analysis

Urine analysis is performed as initial assessment in any patients presented with renal failure. Indications include confirmation and diagnosis of proteinuria, urinary tract infection, and establishing the cause of acute renal failure. There is no contraindication for the test and it can be performed routinely by the bedside. The test is cheap and readily available. Urine phase contrast is another tool available in urinalysis to detect glomerular injury. However this test can only be performed in centers with the facility and trained staff. These investigations depend on the quality of the sample taken, uncooperative patient is a disadvantage (Hou and Peano, 1998).

Sensitivity and specificity of the test depends upon the laboratory and quality of the sample collected.

1.2.2. 24 hour urine creatinine clearance

24 hour endogenous creatinine clearance is employed as it is widely available in any laboratory facilities. However the reliability of this method is diminished by patient's accuracy in collecting timed urine sample and tubular variability creatinine secretion. This method requires patient to be present once to the clinic for sample collection and blood taking. Inadequate urine volume may result in underestimation of kidney function (Johnson, 2005). Other factors that may affect its accuracy include prolonged urine storage may cause deranged urine creatinine levels. High temperature and acidified urine will cause more conversion of creatine to creatinine. Multiplication of urinary bacterial colony especially contributes to the changes of urine pH. Drugs like cimetidine inhibits tubular secretion of creatinine, therefore improves its accuracy . Unfortunately, variability in individual response to this method has been observed. In cases of oliguric or anuric acute kidney injury this method may not be suitable. This method is taken as the 'gold standard' and under appropriate instruction to the patient will reduce the chances of inaccuracy. Inulin clearance, even though is the most accurate method of assessing creatinine clearance , is not applicable in day-to-day clinical practice (Brown *et al.*, 2001) .

1.2.3. Blood investigations

There are numerous blood investigations available in assessment of renal failure. Conventionally serum creatinine and blood urea are widely and readily available throughout the country. Indications of these tests mainly to establish the diagnosis of renal failure. The sensitivity and

specificity of these tests depends upon the clinical situation. Serum creatinine is very useful in determining the GFR. However certain drugs such as cimetidine, an H_2 antagonists inhibits tubular secretion of creatinine causing it to be falsely high in blood. Serum creatinine value is reproducible for Chronic Kidney Disease Staging (CKD staging). It is particularly useful in risks stratification of patients with renal failure in specific clinical situation. For example in pregnancy related acute renal failure, decisions to sustain or terminate the pregnancy will depend on the clinical staging of the renal impairment. CKD staging is classified to 5 clinical stages. Stage I is creatinine clearance above 90 mls/min, Stage II between 60 to 89 mls/min, Stage III between 30 to 59 mls/min, Stage IV between 15 to 29 mls/min and Stage V below 15 mls/min (K/DOQI, 2004).

1.2.4. Radiological Investigation

Radiological investigations are performed to identify aetiology of the renal impairment or supporting the diagnosis. Investigations are divided to invasive and non-invasive. Invasive investigations include computerized tomography (CT) angiogram, magnetic resonance angiography and intravenous pyelography. These investigations include the use of contrast media and carries risks of contrast induced nephropathy and allergic reactions. Magnetic resonance angiography exposes patient to nephrogenic systemic fibrosis. Non-invasive investigations include ultrasonography and Kidney Ureter Bladder (KUB) x-ray. However, non-radioopaque will not be visible in this investigation. Indications for CT scan include in renal vein thrombosis, renal artery stenosis and renal infarction. CT urography is indicated in cases of obstructive uropathy to identify the level of obstruction. Contraindications for these investigations would include hypersensitivity to contrast media.

1.2.5. Renal Biopsy

Renal biopsy is performed under local anesthesia, via posterior approach. The procedure is invasive and ultrasound guided. This procedure is indicated in unexplained state of acute or chronic kidney disease with normal renal size kidneys. The histopathological finding will help to assist the management of these patients. Information regarding the disease activity can also be obtained from this tissue diagnosis. Contraindications for this procedure include small size kidneys, multiple cysts, suspected renal tumor, hydronephrosis, urinary tract infection, bleeding tendencies and uncooperative patients. Renal biopsy is technically difficult and carries complications in pregnant ladies. Schewitz *et al.,* (1965) describes gross hematuria in 16.7% of 77 pregnant women, 4.4 % developed perirenal hematoma and one death.

Contraindication for renal biopsy include small kidney size, bipolar length less than 9cm, single kidney, coagulopathy, uncooperative patient and ongoing urinary tract infection.

1.2.6. Protein Creatinine Index Ratio And Albumin Creatinine Ratio

Normally, daily protein excretion does not exceed 150 mg, most filtered protein is reabsorbed. Proteinuria is a marker of nephropathy, gross proteinuria as in nephritic range suggest overt nephropathy. However proteinuria slightly increased in healthy pregnancy, levels above 260mg/24 hours considered abnormal. Conventionally 24 hour urine collection is used to quantify total 24 hour urine excretion, however this method requires patient to present twice to the clinic and to follow the instructions. It is bound to inaccuracy. Alternatively protein creatinine ratio can be used, it has good correlation with 24 hour urine protein collection.

1.2.7 Gold Standard Test For Diagnosis Of Renal Impairment

Inulin clearance has been recognized as the gold standard for assessment of renal function. The test which determines GFR is not feasible to be carried out in the clinical setting. Even though the investigation has high accuracy unfortunately it is tedious and requires continuous infusion of inulin and serial blood taking from the patients. The investigation is mainly been carried out in experimental laboratories (Locatelli *et al.*, 2002).

1.3. Pathophysiology of renal impairment in hypertensive pregnancies

Renal impairment in pregnancies is multifactorial and maybe preexisted or contributory to the pregnancy. Physiological renal adaptation is a natural process and important in sustaining the pregnancy and ensuring the maternal and fetal outcome. Failure of this natural mechanism resulted in homeostasis imbalance and fetal or maternal loss. There are numerous other causes of renal impairment in pregnancy but hypertension is common and early detection and appropriate management may improve the outcome. Hypertensive disorders are major cause of maternal mortality, accounting 20% of maternal death (Bosio *et al.*, 1999). Most deaths occur in women with eclampsia and severe hypertension and are due to intracerebral hemorrhage (Atlas of Kidney Disease, 2007).

The pathophysiology of hypertension in pregnancy is postulated from preeclampsia which later resulted in renal hypoperfusion and prerenal failure. The major mechanism is widespread vascular endothelial dysfunction resulting in vasospasm, intravascular coagulation, hypertension, renal, hepatic and central nervous system abnormalities (Hladunewich *et al.*, 2007).

Abnormalities in uteroplacental circulation and release of toxic substances affecting the maternal endothelial cells. Endothelial cell dysfunction results in increased platelet aggregation leading to widespread systemic vasospasm, intravascular coagulation and decreased organ flow.

In normal pregnancy, the placenta involves transformation of the branches of maternal uterine arteries, the spiral arteries from thick walled muscular arteries into saclike flaccid vessels that permit delivery of greater volume of blood to the uteroplacental unit (Dunlop, 1981).

In preeclampsia, the process is incomplete, resulting in narrowed spiral arteries and decreased perfusion of the placenta (Figure 1.1).

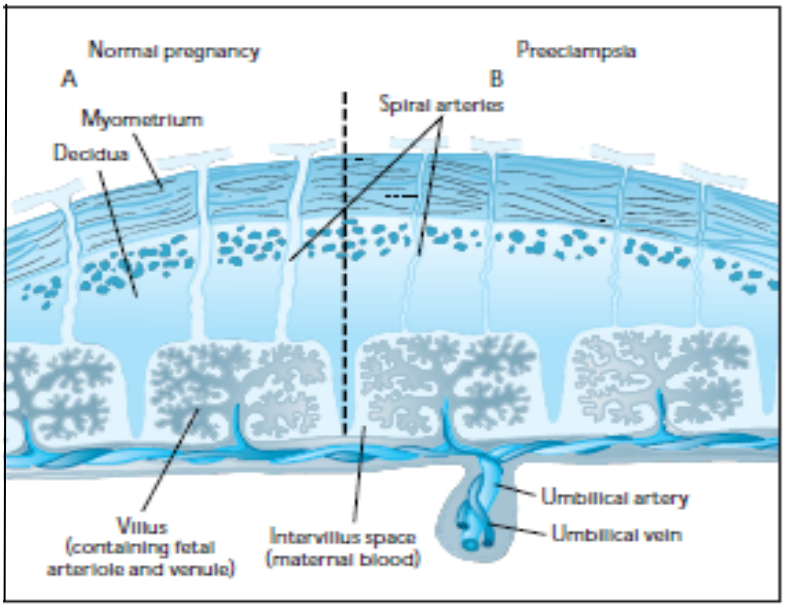

Figure 1.1 : Uteroplacental perfusion in normal pregnancy compares to preeclampsia

(Adapted from Atlas Of Kidney Disease, 2007)

Placenta ischemia leads to release of toxic substance that initiates widespread endothelial cell dysfunction and resulted in increased platelet aggregation. This sequential steps resulted in tissue and organ hypoperfusion, and kidney injury (Figure 1.2).

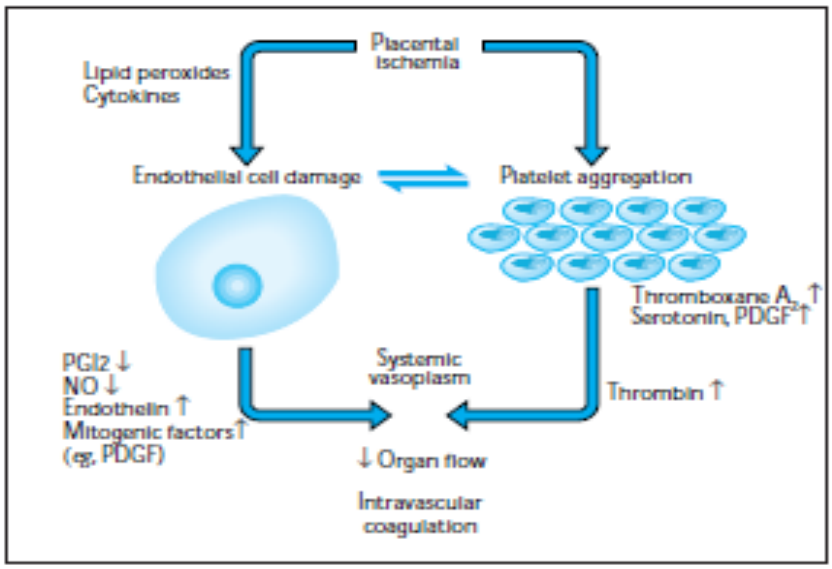

Figure 1.2 : Pathophysiology of kidney injury

(Adapted from Atlas Of Kidney Disease, 2007)

1.4. New biomarkers in detecting renal impairment

1.4.1. Cystatin C

Cystatin C is a cysteine protease inhibitor produced and released at constant rate by nucleated cells. It is freely filtered by the glomerulus and not influenced by race and muscle mass. Cystatin C was demonstrated to predict Acute Kidney Injury 1-2 days earlier than the conventional methods (Herget-Rosenthal *et al.*, 2000). Unlike creatinine, Cystatin C serum levels are virtually unaffected by age (>1 year), muscle mass, gender and race. Cystatin C was described to be an early predictive biomarker of AKI, higher accuracy compared to serum

creatinine in emergency department setting (Misra *et al.*, 2003). Patient plasma is required for analysis via immunoassays. Urinary Cystatin C assay has also been used for earlier detection of kidney injury and need for renal replacement therapy. The mean serum Cystatin C levels reflect changes in the GFR in normal pregnancy population and also in the postnatal period (Strevens *et al.*, 2002). It has also been shown that serum Cystatin C levels are independent of age, height, weight, or blood sugar level (Babay *et al.*, 2005).

1.4.2. Neutrophil Gelatinase-associated Lipocalin (NGAL)

Human NGAL is a 25 kDa protein that is expressed by neutrophils and various epithelial cells, including cells of the proximal convoluted tubule. It is upregulated after ischemic injury. Urinary NGAL is a promising biomarker for AKI because it is upregulated within 2 hours of acute renal cellular injury. NGAL is normally expressed in low levels in human tissues, including the kidney, lung, stomach and colon, the expression is marked in the presence of renal tubular cells injury. In preclinical studies, NGAL has been shown to be easily detected in blood and urine in early AKI. It has also been used as kidney biomarkers in renal transplant patients (Liangos *et al.*, 2009).

1.4.3. Kidney Injury Molecule – 1 (KIM-1)

Kidney injury molecule 1 (KIM-1) is a transmembrane protein overexpressed in proximal tubule cells of the kidney in response to ischemic or nephrotoxic injury to human and animal models. One study was prospectively evaluated the expression of KIM-1 levels in 103 patients

undergoing cardiopulmonary bypass surgery. AKI defined as 0.3 mg/dl increase of creatinine more common in patients with elevated KIM-1 levels compared with other biomarkers (Liangos *et al.*, 2009). Other studies also demonstrated urinary KIM-1 able to differentiate between pre-renal and renal causes of kidney injury. The sensitivity of specificity of KIM-1 are 99% sensitive and 95% specific, however the results obtained is not in pregnant ladies with hypertension.

1.4.4. Interleukin – 18 (IL-18)

IL-18 is a proinflammatory cytokine activated within the proximal tubule and excreted in urine after ischemic kidney injury. One study was demonstrated to compare urinary IL-18 concentration in patients with diagnosis of Acute Tubular Necrosis (ATN) with healthy controls. Patients with ATN had markedly increased IL-18 and elevated ratios of urinary IL-18 to creatinine compared with other group (Parikh *et al.*, 2006) .

1.4.5. The need for rapid and reliable diagnostic test in detecting kidney disease in hypertensive pregnancies

Rapid diagnosis of acute kidney injury in pregnancy will prevent potential adverse outcomes to the mother and fetus. Earlier intervention can be initiated to reverse the potential ongoing insult. In one study, described mortality between 20-30% in pregnant ladies with acute kidney injury (Hou and Peano, 1998). It is important of earlier initiation of dialysis for better control of

azotemia and improving the overall outcomes (Gammill and Jeyabalan, 2005). However the role of prophylactic dialysis in pregnant ladies with acute kidney injury is debatable.

HYPOTHESIS

CHAPTER 2

HYPOTHESIS

We hypothesized that Cystatin C is a reliable test for detection of renal impairment in hypertensive pregnancies that carries acceptable sensitivity and specificity.

OBJECTIVES

CHAPTER 3

OBJECTIVES

Objectives :

1. To determine sensitivity and specificity of Cystatin C in comparison to 24 hour urine Creatinine clearance in hypertensive pregnancies.
2. To compare Cystatin C level in hypertensive pregnant lady between 2^{nd} and 3^{rd} trimester.

METHODOLOGY

CHAPTER 4

METHODOLOGY

This section describes the materials and general methods used in this work. Specific methods are described in the respective chapters.

4.1. Materials

4.1.1. Subjects

This study was approved by the Research Ethics Committee (Human) on 21st November 2010 and funded by short term grant (304/PPSP/6139008) Hospital Universiti Sains Malaysia (HUSM). This pilot prospective cross sectional study was conducted in HUSM between January 2009 and January 2010. Flow chart of the study is shown in Figure 4.1. All pregnant patients beginning with 2nd trimester at the age of 16 years old to 55 years old and hypertensives including gestational hypertension, chronic hypertension, chronic hypertension with superimposed preeclampsia, preeclampsia and unclassified hypertension were included. Verbally informed consent was obtained prior to the involvement of the patients into the study followed by written consent. Recruitment was done on patients that meet the inclusion and exclusion criteria and consented to participate.

Patients included in this cross-sectional study whom had satisfied the inclusion criteria, were informed regarding their involvement in the study and explained regarding the need to collect 5 mls of blood sample exclusive of other routine blood investigations. They were also required to provide 24 hour urine collection sample for the analysis. Blood investigations was taken in serial

during the 2nd and 3rd trimester. 64 patients were selected and enrolled in this cross sectional study.

The inclusion and exclusion criteria for the patients were as follows:

4.1.1.1 *Inclusion criteria:*

- Consented for the study
- Patients beginning from 2nd trimester of pregnancy whom are hypertensives including gestational hypertension, chronic hypertension, chronic hypertension with superimposed preeclampsia, preeclampsia and unclassified hypertension.
- Age : 16-55 years

4.1.1.2 *Exclusion criteria:*

- Hyper/Hypothyroidism
- Smokers
- Receiving corticosteroids
- Suffering from any forms of malignancy

4.1.2. Reagents/Kits

The reagents were used for the analysis of the blood sample as listed in the product inlet. The laboratory analysis was performed and prepared as recommended in the product inlet. The 24 hour urine creatinine clearance and the reagents are listed in Table 4.1. The Cystatin C reagents are as listed in Table 4.2.

Table 4.1 : 24 hour urine creatinine clearance and reagents

	Reagents	Working solutions
R1	Sodium hydroxide	0.20 mol/L
R2	Picric acid	25mmol/L

Table 4.2 : Cystatin C and control reagents

Materials	Compositions
N Cystatin C Reagents	A suspension of polystyrene particles coated with approximately 0.03 g/L anti-human cystatin C from rabbits.
N Cystatin C Supplementary Reagents A	Contains rabbit immunoglobulin (14 g/L) in buffered solution.
N Cystatin C Supplementary Reagents B	Consists of an aqueous solution of polyethylene glycolsorbitan monolaureate (85 g/L) and polyethylene glycol ether (27 g/L).
N Cystatin C Control Level 1 and N Cystatin C Control Level 2	Lyophilized polygeline with urine proteins of human origin. The concentration of Cystatin C was standardized with reference to purified Cystatin C. Once reconstituted, the controls contain the Cystatin C concentration as indicated on the respective vial label.

4.2. Methods

This study was carried out in HUSM, Kubang Kerian Kelantan. It was a prospective cross sectional study which involved 64 patients and suspected clinically as renal failure related to the hypertension in pregnancy.

Patients were seen in antenatal clinic and antenatal wards. Selected patients whom satisfied the inclusion criterias were given explanations regarding the study and consent taken. Patients who agreed to participate were required to provide blood samples about 5 mls and 24 hour urine collection. The blood samples were taken to the Department of Chemical Pathology Laboratory, School Of Medical Sciences for analysis. Serum Cystatin C was measured with N Latex Cystatin C kit, a particle-enhanced nephelometric immunoassay (PENIA) method, on a fully automated BN II nephelometer (Dade Behring Diagnostics). Serum and urine creatinine will be determined by a kinetic colorimetric assay on a Hitachi 912 analyser.

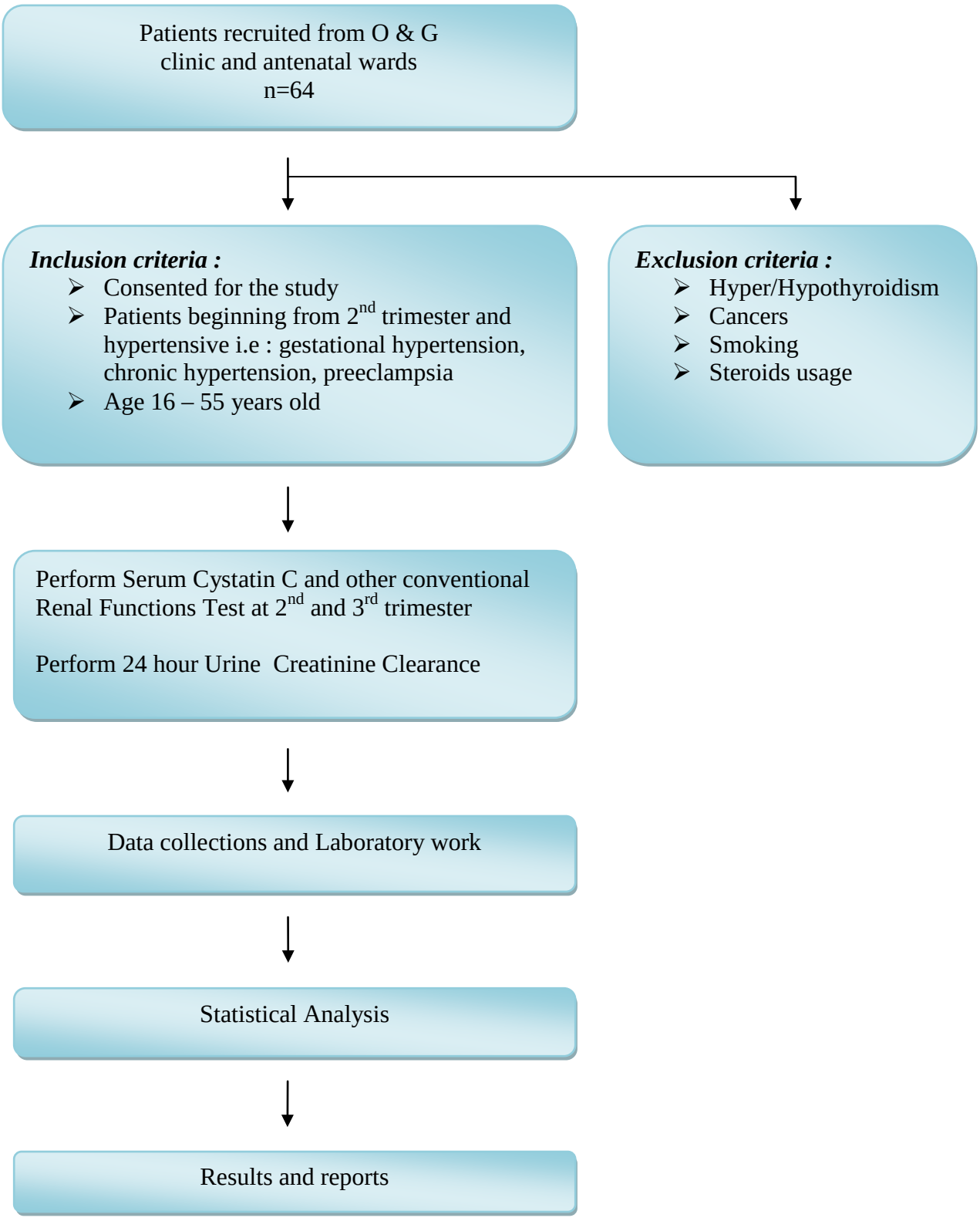

Figure 4.1: Flow chart on the methodology of study

4.2.1.Cystatin C

Patients who were selected for the study were requested for blood sample. About 5mls of blood were taken during antenatal clinic or inpatients. They were required to give the blood each in 2nd and 3rd trimester. The blood was sent to the Chemical Pathology Laboratory, HUSM. In the laboratory, the blood was run in the Eppendorf Centrifuge 5810 machine to obtain the serum and supernatant.

The serum was manually transferred to the sample cups which is small in size.

Figure 4.2 : Sample cups

The serum were kept in the freezer in temperature below -17°C before being analyzed .

The serum sample collection was equilibrated in room temperature with reagent N Latex Cystatin C and automatically diluted 1:100 with N Diluent.

Figure 4.3 : N latex Cystatin C reagent

Analyzing process started by using Dade–Behring Nephelometer Systems and results were automatically evaluated .

Figure 4.4 : Dade Behring analysis machine

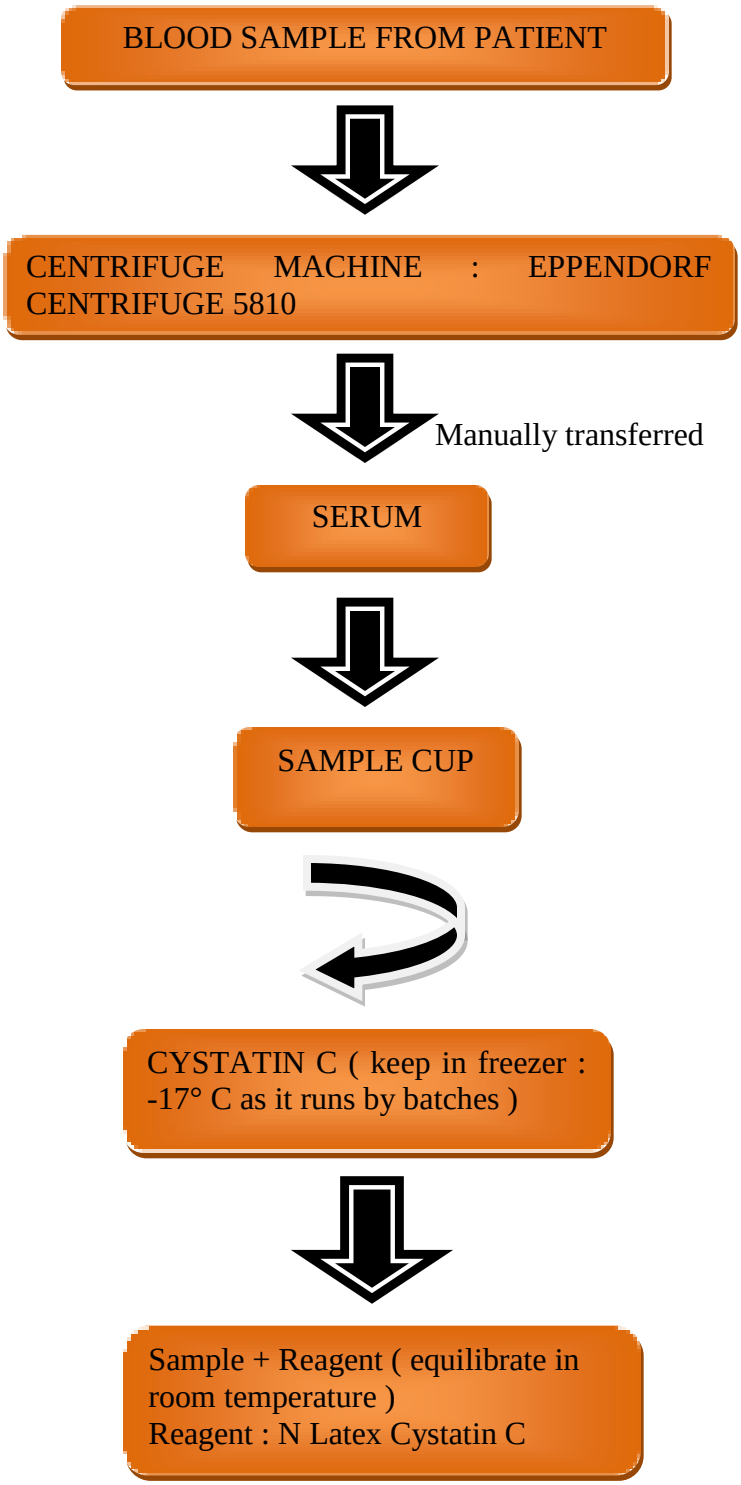

Figure 4.5 : Cystatin C process

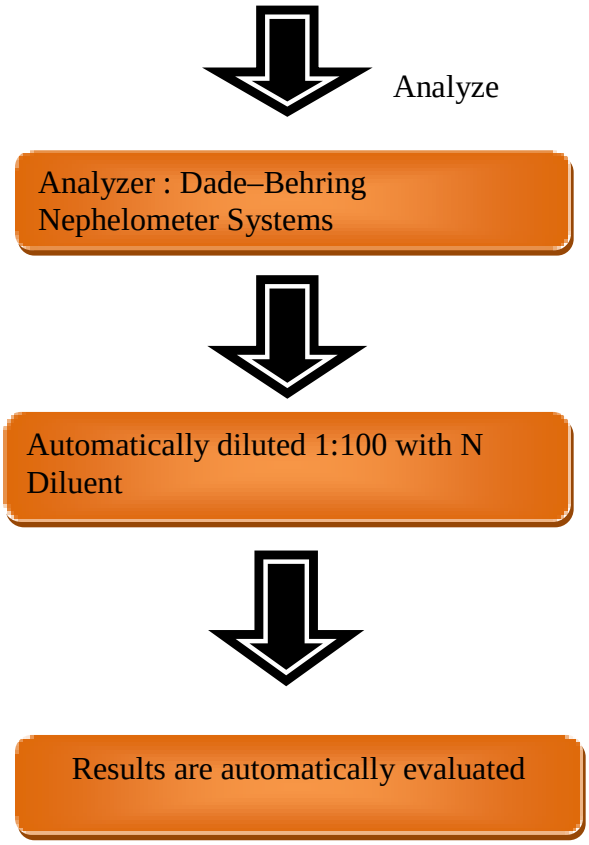

Figure 4.5 : (continue) Cystatin C process