

**MICROALBUMIN: THE POTENTIAL SCREENING TEST FOR
DETECTION OF EARLY KIDNEY DYSFUNCTION**



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Entitled

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DETECTION OF EARLY KIDNEY DYSFUNCTION**

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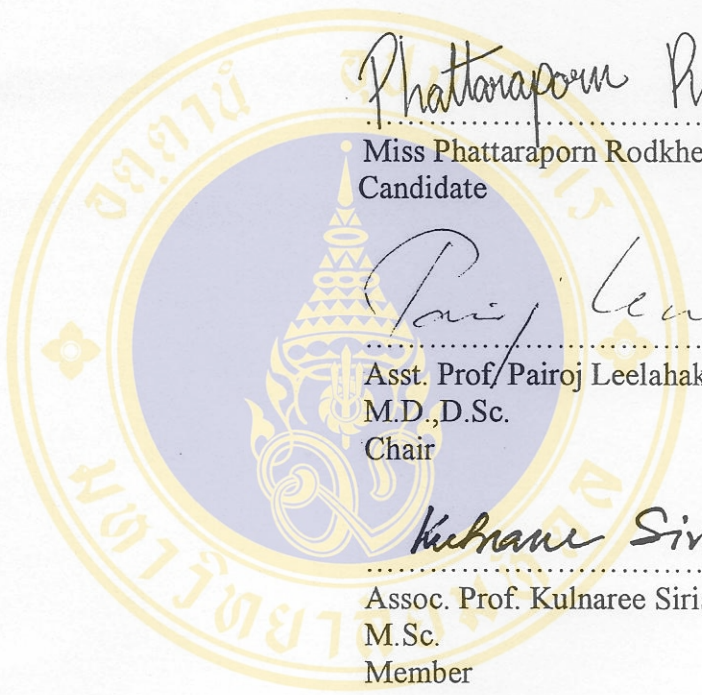
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ABSTRACT

Chronic kidney disease is a major public health problem world-wide. The number of new patients entering kidney transplantation has been increasing constantly in the past 20 years. Laboratory markers are very important for detecting impaired kidney function. Measurement of Glomerular Filtration Rate (GFR) is the gold standard for detection of kidney dysfunction. However, it is not suitable for screening kidney function. The current tests for creatinine, uric acid and Blood Urea Nitrogen (BUN) are not sensitive enough for early detection.

Testing for microalbumin shows potential as an alternative in diabetics. The object of this study was to check whether microalbumin can be detected, in subjects who have passed the standard tests. 995 subjects were tested for standard laboratory markers. All subjects tested negative for urine strip test. Then cross-sectional analysis on morning urine was performed. Microalbumin was measured by immunoturbidimetric assay. Within-assay and between-assay imprecisions were analyzed with two levels of control specimen, nominated as low and high levels. The percentage coefficient variance of within-assay and between-assay imprecision of control specimen were 2.1%, 3.5%, 4.4% and 5.9%; respectively. A detection limit of 0.9 mg/L was established. However, 66%, 24.4% and 9.6% of these populations had normal microalbumin (≤ 15 mg/L), high normal microalbumin (16-29 mg/L) and high microalbumin (≥ 30 mg/L), respectively.

It was also found that there were no significant differences [$P \geq 0.05$] between these three groups for plasma creatinine, BUN and uric acid tests. Estimate regression revealed that even before creatinine clearance problems become evident, microalbumin showed high levels in the urine [30-70mg/L; $r^2=0.07$ $P \leq 0.05$, ≥ 70 mg/L; $r^2=1$ $P \leq 0.001$]. The microalbumin test might be a useful indicator for kidney dysfunction in screening groups rather than using the conventional screening test. To find out whether quantitative urine microalbumin test could be an additional test for early detection of kidney dysfunction, a longitudinal design would need to be carried out in future research.

KEY WORDS : MICROALBUMINURIA/ CHRONIC KIDNEY DISEASE

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ไมโครอัลบูมิน: การทดสอบที่มีศักยภาพในการคัดกรองความผิดปกติในการทำงานของไตในระยะแรกเริ่ม (MICROALBUMIN: THE POTENTIAL SCREENING TEST FOR DETECTION OF EARLY KIDNEY DYSFUNCTION)

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บทคัดย่อ

ภาวะไตวายเรื้อรังเป็นปัญหาสุขภาพที่สำคัญของประชากรทั่วโลกซึ่งเห็นได้จากจำนวนของผู้ป่วยใหม่ที่ต้องการการเปลี่ยนถ่ายไตได้เพิ่มขึ้นอย่างคงที่และมีแนวโน้มที่จะเพิ่มขึ้นอย่างต่อเนื่อง การทดสอบทางห้องปฏิบัติการจึงมีความสำคัญเป็นอย่างยิ่งในการตรวจวัดประสิทธิภาพการทำงานของไตที่ลดลง วิธีการตรวจทางห้องปฏิบัติการซึ่งถือว่าเป็นวิธีมาตรฐานในการวัดความสามารถในการทำงานของไตที่ลดลงคือการวัดอัตราการกรองของไต อย่างไรก็ตามวิธีการตรวจวัดอัตราการกรองของไตไม่เหมาะที่จะนำมาใช้เป็นวิธีการตรวจคัดกรองทางห้องปฏิบัติการ วิธีการตรวจคัดกรองทางห้องปฏิบัติการที่ใช้โดยทั่วไปคือการวัดระดับของ ครีเอตินิน, ยูเรียไนโตรเจน และครีเอตินินในเลือดซึ่งยังไม่เพียงพอในการวัดประสิทธิภาพการทำงานของไตที่ลดลงในระยะแรกเริ่ม

การศึกษากาดัดขวางของการวิเคราะห์ระดับไมโครอัลบูมินในตัวอย่างปัสสาวะที่เก็บในคอนเซ็ปต์โดยการวัดความขุ่นที่เกิดจากปฏิกิริยาทางอิมมูน ค่าเฉลี่ยของความแปรปรวนในการวัดความเข้มข้นของไมโครอัลบูมินที่ระดับความเข้มข้นต่ำและสูงคือ ร้อยละ 2.1 และร้อยละ 3.5 ตามลำดับและค่าเฉลี่ยความแปรปรวนในระหว่างการวัดที่ระดับความเข้มข้นต่ำและสูงคือ ร้อยละ 4.4 และ ร้อยละ 5.9 ตามลำดับ และมีความสามารถในการวัดระดับความแตกต่างของความเข้มข้นอยู่ที่ 0.9 มิลลิกรัมต่อลิตร การวิเคราะห์ความเข้มข้นของไมโครอัลบูมินในปัสสาวะที่ตรวจพบผลปกติด้วยแถบปัสสาวะจำนวน 995 ตัวอย่างพบว่า 656 ตัวอย่างหรือร้อยละ 66 เป็นกลุ่มที่มีความเข้มข้นของไมโครอัลบูมินที่ระดับปกติ (≤ 15 มิลลิกรัมต่อลิตร) 243 ตัวอย่างหรือ ร้อยละ 24.4 เป็นกลุ่มที่มีความเข้มข้นของไมโครอัลบูมินสูงที่ปกติ (16-29 มิลลิกรัมต่อลิตร) และ 96 ตัวอย่าง หรือ ร้อยละ 9.6 เป็นกลุ่มที่ระดับความเข้มข้นของไมโครอัลบูมินที่ระดับสูงกว่าปกติ (≥ 30 มิลลิกรัมต่อลิตร)

ส่วนระดับครีเอตินิน, ยูเรียไนโตรเจน และครีเอตินินในซีรัมของทั้ง 3 กลุ่มไม่มีความแตกต่างอย่างมีนัยสำคัญ ($P \geq 0.05$) นอกจากนี้การตรวจสอบโดยความสัมพันธ์เชิงเส้นก็ังพบว่าระดับของไมโครอัลบูมินสูงเกินกว่าค่าปกติก่อนการลดลงของอัตราการกรองครีเอตินินของไต ($30-70$ มิลลิกรัมต่อลิตร; $r^2 = 0.07$ $P \leq 0.05$, ≥ 70 มิลลิกรัมต่อลิตร; $r^2 = 1$ $P \leq 0.001$) การวัดระดับไมโครอัลบูมินในปัสสาวะจึงมีประโยชน์ในการบ่งชี้การทำงานที่ผิดปกติของไตในกลุ่มคนที่ตรวจคัดกรองด้วยวิธีที่ใช้ในปัจจุบันแล้วให้ผลปกติ การตรวจไมโครอัลบูมินทางห้องปฏิบัติการในการคัดกรองการทำงานของไตที่ลดลงในระยะแรกเริ่มจึงเป็นทางเลือกอีกทางหนึ่งและควรจะมีการศึกษาต่อเพื่อติดตามผลการทดลองในครั้งนี้

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LIST OF ABBREVIATION

ACR	=	Albumin : Creatinine Ratio
AST	=	Aspartate aminotransferase
ALT	=	Alkaline aminotransferase
ALP	=	Alkaline phosphatase
ADD	=	American Diabetes Association
BMI	=	Body Mass Index
BP	=	Blood Pressure
BUN	=	Blood Urea Nitrogen
CCr	=	Creatinine Clearance
⁵¹ Cr-EDTA	=	Edthylene Diamine Tetra Acetic, labelled with chromium isotope 51
⁵¹ Cr-inulin	=	Inulin, labelled with chromium isotope 51
¹²⁵ I-Hypaque	=	Hypaque, labelled with iodine isotope 125
⁵⁷ Co-Vitamin-B ₁₂	=	Vitamin-B ₁₂ , labelled with cobolt 57
ESRD	=	End Stage Renal Disease
CKD	=	Chronic Kidney Disease
GFR	=	Glomerular Filtration Rate
GSC	=	The Glomerular Sieving Coefficient
GSM	=	Glomerular Filtration Rate
HOPE	=	The Heart Outcome Prevention Evaluation
IT	=	Immunoturbidimetric
JNC	=	Joint National Comitten on Prevention, Detection, and Treatment of High Blood Pressure
K/DQQI	=	Kidney Disease Outcome Quality Initiative
TG	=	Triglyceride
TP	=	Total Protein
NIDDK	=	The National Institute of Diabetes and Digestive and Kidney Disease

LIST OF ABBREVIATION (CONT.)

NKF	=	The Nation Kidney Foundation
nm	=	nanometer
m ²	=	meter square
mg/day	=	milligram per day
mg/dL	=	milligram per deciliter
mg/L	=	miligram per liter
RIA	=	Radioimmunoassay
UKEQAS	=	United Kingdom External Quality Assessment Scheme

CHAPTER 1

INTRODUCTION

Chronic Kidney Disease (CKD) is a major public health problem in world-wide. Research by Health Care has shown that the prevalence of kidney failure has risen steadily for over 30 years. More than 2 million people die each year because of CKD and millions more are at an increased risk. While there are many different types of kidney disease, kidneys are incredibly resilient in functioning sufficiently well to keep the body alive even after great or continuing damage. However, the onset of kidney disease often ends in kidney failure. When someone develops kidney failure, they will require treatment with dialysis or a kidney transplant. The number of patients requiring kidney transplant therapy in 1997 was 304,083 in the United States, 175,988 in Japan, and 197,721 in Europe (1). With so many patients involved, the cost of treatment will become extremely high and prohibitive (i.e. some people may not be able to afford the cost of treatment), not only in developing countries such as Thailand, but also developed countries where there may not be free health care treatment.

The major health effects of CKD, regardless of cause, include progression to kidney failure and complications of decreased kidney function. Increasing evidence indicates that some of these adverse effects can be prevented or delayed by early detection and treatment. Recent studies clearly indicate that kidney failure can be delayed or even prevented if kidney disease is diagnosed and managed early (2-4). Thus, early intervention may reduce its progression to kidney failure and reduce the complications associated with decreased kidney function. Timely intervention in these patients may play a role in reducing the cost of CKD treatment including the cost for dialysis. Kidney disease can be diagnosed without knowledge of its cause. Kidney damage is usually diagnosed by markers rather than by kidney biopsy. Unfortunately, CKD is underdiagnosed and undertreated, resulting in lost opportunities for prevention, in part because of a lack of uniform application of simple tests for detection and evaluation.

In the modern era people tend to go for health checks more frequently than the past. Consequently, the laboratory screening kidney function tests will detect more patients with treatable early kidney dysfunction thereby initiating immediate treatment. However using the present screening kidney function test the number of new patients entering kidney transplant programs has still increased constantly in the past 20 years and is forecast to continue to increase (5). One of the possible reasons may be screening process itself. A new improved screening process will be needed.

The laboratory markers are very important for detecting impaired kidney function. Kidney function tests look at whether the kidney is failing by measuring the waste products passed out in the urine and those left behind in the blood, as well as the general mineral levels in the blood which are controlled in part, by the kidneys. There are many metabolic functions and chemical interactions to be evaluated through laboratory tests of kidney function (6). In clinical practice, physicians use test results to characterize the degree of functional abnormalities in individual patients. The decision to use a particular test depends on the aspects of the test, the characteristics of the subject to be tested, and the setting in which the test is to be used (7).

Direct determination of the glomerular filtration rate (GFR) is generally accepted as the best index of kidney function. Accurate methods based on measurement of e.g. inulin clearance, radiolabelled substances or iohexol, are however, too complex and laborious for routine clinical use. In clinical practice; the routine laboratory test for assessing the glomerular function as kidney function are the plasma creatinine, Blood Urea Nitrogen (BUN) and uric acid (6,7). The Glomerular Filtration Rate (GFR) by clearance test will be requested if the plasma creatinine, BUN and uric acid have substantially risen or if the patient already has kidney failure. Unfortunately the plasma creatinine, BUN and uric acid have poor sensitivity as clinical markers in routine laboratory tests for kidney function (8,9). There is a need for new markers to test kidney function that can be more sensitive and precise in identifying kidney function. Most of the markers investigated had failed to detect early kidney dysfunction. Many laboratories are searching for new markers that can detect for impaired kidney function at an early stage (10-13).

Since normal urine contains small amounts of protein the term proteinuria describes the excretion of abnormal amounts of protein. If proteinuria is shown by

strip testing it usually means there has been an increase filtration through the glomerulus. In terms of pathophysiology the proteinuria can classify dysfunction, either due to glomerular kidney disease or due to non-glomerular kidney disease. Clinical proteinuria refers to an excess of protein excretion sufficient enough to be detected by reagent strip testing such as the Albustix strip test. This is however, a relatively insensitive test, which can usually only identify proteinuria of the order of 300 mg daily. Sub-clinical proteinuria refers to the excretion of abnormal amounts of particular proteins, which means that albumin cannot be detected by strip testing or by conventional measurement of total protein excretion.

In 1836 Richard Bright was the first physician to show clearly that albuminuria was manifestation of kidney disease (14). At the beginning of 1980s Viberti and Mogensen showed that even a slightly elevated urine albumin concentration, not detectable with a dipstick, was predictive of clinical nephropathy in diabetics (15,16). In fact, patients with normal or near normal albumin excretion have only a small risk of clinical overt nephropathy, but during a 20 year follow-up period, suggested that the level of albumin in urine is an important indicator of the potential for developing end stage renal disease.

Routine screening for microalbuminuria has been recommended in the past by the American Diabetes Association as a marker for the early detection of diabetic nephropathy (17). In the present study microalbumin was measured from a sample of the Thai population, who come for check-up for health and was correlated with all of the kidney function tests. Recommendations can then be made to the physician for the urine microalbumin test is alternative for routine clinical use in Thailand

The objectives of this study are:

1. To show that there is the prevalence of microalbumin in urine sample taken from a sample of people who have health checks in the Thai population.
2. To propose that the microalbumin test be considered the effective alternative biomarker for the early detection of kidney dysfunction in the Thai population.
3. To provide data to support and encourage the implementation of alternative practice for routine kidney function.

CHAPTER 2

LITERATURE REVIEWS

1. Chronic Kidney Disease (CKD)

There are two kidneys, each about four to five inches long and about 300g in weight. They lie in the abdomen underneath the liver on the right and the spleen on the left. Each kidney contains about one million tiny filtering units, known as glomeruli, which remove waste material and excess water from the blood to form urine. With all these intricacies of the kidney, it's no wonder the kidney is susceptible to many disorders, diseases, and conditions that can lead to the progressive destruction of the kidneys such as diabetes, infections, blood vessel diseases, high (or low) blood pressure, diseases of the blood, cancer and immune diseases (such as lupus, trauma, etc). The kidneys can also initiate diseases of their own such as infections, structural abnormalities from birth that bring about abnormal function, cancer, and high blood pressure.

Kidney damage can happen quickly, but most often develops slowly and silently, often taking years or decades for the injury to become apparent. The usual end result is damage to the kidney's filtering units, glomeruli. These filtering units normally remove toxic waste from the blood, but as the kidney's functions decline, this waste can build up to harmful levels. Gradual loss of kidney function is called chronic kidney disease. In 2002, the National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (K/DQOI) Advisory Board approved development of clinical practice to define chronic kidney disease and to classify stages in the progression of chronic kidney disease (18). The work group defined "Chronic Kidney Disease" to include conditions that affect the kidney, with the potential to cause progressive loss of kidney function or complications resulting from decreased kidney function. CKD was thus defined as the presence of kidney damage or decreased level of kidney function for three months or more, irrespective of diagnosis.

CKD is a world-wide public health, with an increasing incidence and prevalence, poor outcomes, and high cost. Outcomes of CKD include not only kidney failure but also complication of decreased kidney function and cardiovascular disease. There is ominous growth in chronic kidney disease among the world's population. Research by health Care has shown that the prevalence of kidney failure has risen steadily for over 30 years. Most patients with end-stage kidney disease will end up on dialysis because the supply of kidneys for transplants is limited (1). The human toll is substantial not only in terms of lives lost, but also in quality of life lived.

The National Institute of Diabetes and Digestive and Kidney Disease (NIDDK), National Institutes of Health (NIH), has initiated a National Kidney Disease Education program (NKDEP). The major goals are to slow the progression of kidney disease and its complications in people with early kidney impairment and prevent or slow the development of the disease in those at risk. Issues that drive the need for this education program include recognition of kidney disease as a major national health problem by healthy people 2010 (19).

- 1.1 Striking racial disparities in the prevalence, morbidity, and mortality of kidney disease and the provision of optimal care.
- 1.2 A steady, alarming growth in incidence of kidney disease, especially kidney failure, which has been doubling every 10 years. A rapidly growing numbers of people at risk.
- 1.3 Cost to the Nation of this disease.
- 1.4 Existence of science-based guidelines to slow progression of kidney disease.
- 1.5 Consensus that some early stage indicators are available.

The key to prevent end-stage kidney disease is early detection. When kidney disease is detected early, treatment such as dieting and taking medication can often help to prevent or slow the progression towards kidney failure (3,4). The laboratory markers are very important for detecting impaired kidney function. Many laboratories are searching for new markers that can detect for impaired kidney function in the early stages (10-12).

2. Laboratory evaluation of Glomerular Function

There are many metabolic functions and chemical interactions to be evaluated through laboratory tests of kidney function (18). The usefulness of any diagnostic test is based on its accuracy (by compared it to a standard), precision (related inversely to the variability of measurements), and convenience. In clinical practice, physicians use test results to characterize the degree of functional abnormalities in individual patients. The decision to use a particular test depends on the features of the test, the features of the subject to be tested, and the setting in which the test is used. An accurate estimation of prognosis is important in many respects. First, prognostic information can be used to inform patients about likely outcome of their disease. Second, the prognosis of an individual patient can be used as a guide for selecting therapeutic options. An ability to predict those at “high risk” would aid the physician in selecting the patients most likely to benefit from therapies. Third, prognostic assessments are useful in designing and evaluating randomised trials (20).

The most widely used indices of excretory function are the plasma creatinine and urea both are handled largely by glomerular filtration and their blood levels are thus substantially dependent on GFR, yet because of their different physiological properties each has a special meaning (20,21).

2.1 Plasma Blood Urea Nitrogen (BUN) as an index of Glomerular function

Urea is the end product of protein catabolism and is synthesised primarily by the liver. About a quarter of synthesised urea is metabolised in the intestine to carbon dioxide and ammonia. The ammonia thus generated returns to the liver where it is reconverted to urea. Urea is usually measured as urea nitrogen, and for historical reasons, the serum level is commonly referred to as the blood urea nitrogen (BUN). Urea is not only filtered by the glomeruli but also partially reabsorbed by tubules. The reference value of BUN is 8-12 mg/dl (21).

Deterioration of glomerular function leads to a predictable increase in BUN. When 50% of kidney function is reduced it results in doubling the BUN in the steady state. It has been reported that a patient with a controlled BUN of 10 mg/dl, has

approximately half the normal glomerular function remaining when the BUN has risen to 20 mg/dl, 25% of normal function remaining when the BUN is 40 mg/dl and 12% remaining at a BUN of 80 mg/dl. Most important is the well-recognised relationship of kidney function, the BUN level, and the clinical features of uremia. It is important to know that a BUN level greater than 100 mg/dl is associated with a higher risk of complication in both acute kidney disease and CKD and may indicate the need to initiate dialysis. However, if urea generation is reduced, the BUN may remain below this level even if GFR declines to very low levels and uremic symptoms begin arise (21-23).

BUN concentration, though widely used to assess glomerular filtration, is a less satisfactory measurement than serum creatinine chiefly because of two factors. The first is that urea clearance varies with the rate of urine flow and, secondly reason is that the plasma concentration of urea is importantly dependent on nitrogen metabolism as well as renal function (6,7). On the other hand, BUN is a better index of symptoms: patients with a high value are likely to have uremic symptoms, and those with a moderate elevation in BUN are likely to be symptom-free even if glomerular function is quite poor (23).

2.2 Plasma creatinine as an index of Glomerular function

As we know creatinine is a protein produced by muscles in the body and released into the blood. The amount produced is relatively stable in a given person. The creatinine level in the serum is therefore determined by the rate that is being removed, which is roughly a measure of kidney function. Creatinine is the waste product of creatinine phosphate, a compound found in skeletal muscle tissue. Production of creatinine rate is excreted only through the kidneys.

The utility of serum creatinine as an index of glomerular function stems from the fact that any reduction in glomerular filtration imposes a limitation on creatinine excretion (7-9). There is a continued constant release of creatinine from muscle even with this impedance to excretion which, leads to an accumulation of creatinine throughout the total body water and thus to a rise in its serum concentration. Accumulation continues until a new steady state is achieved in which the daily quantity of filtered (and therefore excreted) creatinine matches the amount released

in metabolism each day. The hypothetical changes in creatinine production following a 50% decrement in GFR (22). A new steady state is reached when serum creatinine concentration rises sufficiently so that the filtered load of creatinine, and hence its urinary excretion, again equals its generation. In the new steady state, the reduced level of GFR is reflected by a reciprocal increase in the steady-state plasma concentration. It follows that the serum creatinine concentration should be interpreted as an index of glomerular function only in the patient who is in a steady state (8,22). A patient who has been anuric for less than 24 hours, for example, and is found to have a serum creatinine of 1.4 mg/dL obviously does not have only slight impairment of kidney function, but instead has a total cessation of renal function. If anuria persists, of course, the serum creatinine will reach markedly elevated levels. Estimation of the extent impairment of glomerular filtration from the concentration of creatinine has as its basis the predictable relationship that exists between the given level of GFR and the creatinine concentration reached in the steady state. Thus, for every 50% reduction in GFR, the serum creatinine concentration doubles. A patient with a normal serum creatinine concentration of 1 mg/dL, for example, has approximately 50% of normal glomerular function remaining. Also when the serum creatinine has increased to 2 mg/dL, 25% of normal function remaining when the serum level is 4 mg and 12% at a serum creatinine of 8 mg (8,22,25,26).

Another feature about the relationship between the serum creatinine and GFR that is worth emphasising is when a small increase in creatinine concentration above the normal level implies a much larger percent change in glomerular function than the same absolute increase in serum creatinine when renal function is already moderately impaired. For example, an increase in serum creatinine from 1.0 to 2.0 mg/dl implies a 50% decrease in function; an increase from 7.0 to 8.0 mg/dl indicates only a 2 to 3% further loss of controlled GFR

2.2.1 Reference values for serum creatinine (26,27):

Adult males: 0.6-1.5 mg/dl: values are slightly higher in males due to larger muscle mass

Adult females: 0.6-1.1 mg/dl: creatinine clearance increased in pregnancy, resulting in lower serum levels

Children: 0.2-1.0 mg/dl: slight increases with age because values are proportional to body mass

When renal dysfunction occurs, the kidneys are impaired in their ability to excrete creatinine and the serum creatinine rises but that means the kidney's nephrons are destroyed by at least 50%. Here is the relationship of creatinine levels to the estimated amount of nephron loss (27,28):

Normal creatinine 0.6 – 1.5 mg

Creatinine level over 1.5 mg : over 50% nephron loss

Creatinine level 4.8 mg or more: up to 75% nephron loss

Creatinine level over 10 mg: 90% nephron loss (end-stage kidney disease)

Serum creatinine is roughly, inversely related to clearance test (22,26). If the clearance drops to one half of the old level, the serum creatinine doubles (in the steady state). So for an adult, serum creatinine of 2 is roughly a creatinine clearance of 60 ml/min; creatinine of 3 is roughly a clearance of 30; creatinine of 4 is roughly a clearance of 15, etc So why didn't the creatinine rise to only 2 when a kidney was removed. The answer is that the remaining kidney "hyperfilters" and seems to work harder, therefore kidney function is not quite halved.

The laboratory marker that has long served as the mainstay for detecting impaired kidney function is serum creatinine. Serum creatinine is an important test of kidney function, as the only pathological condition that causes a significant increase in the serum creatinine level is damage to a large number of nephrons. Unlike the BUN, the serum creatinine level is not affected by protein metabolism and by the patient's hydration status (27). Tests to measure serum creatinine used only evaluate kidney function and only kidney function changes the results. Unfortunately, the serum creatinine is an insensitive marker of kidney damage. Serum creatinine does not rise until at least half of the kidney's nephrons are destroyed or damaged. Also serum creatinine is dependent not only on GFR, but dependent upon many factors, which include muscle mass, diet, sex, certain drugs, and age. Creatinine production decreases with age, and therefore creatinine value that is within the "normal range" in an elderly patient may represent a significant decline in GFR but it may go unnoticed. Presumably, estimates of GFR based on serum creatinine alone are

incapable of detecting a glomerular injury until overwhelming impairment of the glomerular capillary wall function has occurred (8,9,26,27).

3. Laboratory evaluation of Glomerular Filtration Rate (GFR)

The standard test used to measure the filtering of the glomeruli is the clearance test. This measures the rate at which kidneys are able to remove (clear) a filterable substance from the blood. Glomerular filtration Rate (GFR) is a gold standard for measuring kidney function (28,29). In clinical practice, GFR is measured by determining the urinary excretion of a marker substance that must fulfil the key requirement that the amount filtered per minute is equal to the amount excreted in the urine per minute. This requirement is met if this substance is neither absorbed nor secreted by the renal tubules, freely filterable across the glomerular membranes and not metabolized or produced by the kidneys

GFR cannot be measured directly; it is assessed from renal clearance. The renal clearance of a substance x is given by the equation (29).

$$C_x = U_x V / P_x$$

C_x is the clearance of x

U_x is the urinary concentration of x

V is the urine flow rate

P_x is the average plasma concentration of x during the period of urine collection

3.1 Measuring of GFR using exogenous filtration marker

It has been almost half a century since inulin, a polymer of fructose with a mean molecular weight of about 5000 was first introduced as a marker for measuring the rate of glomerular ultrafiltration. In 1975 Chang RLS et al confirmed that the glomerular capillary wall is freely permeable to inulin whereas the tubule wall is completely impermeable (30). These attributes, together with its long record of safety in clinical use, have led to the adoption of inulin clearance as the method of choice when precise determination of the GFR is required. Inulin is infused intravenously to produce an appropriate constant plasma level, which is maintained

while completely timed urine sample is collected. The test takes 2-4 hours, and is too tedious and demanding for use outside the research laboratory for three reasons that were found in a study conducted in 1956 by Heyrovsky (31) and in 1970 by Robertson et al (32). The reasons suggested were that inulin estimation is not easy, patients do not readily pass urine to order when attached to an infusion pump and lastly the chores of preparing inulin, running an infusion, and taking multiple plasma samples are expensive in staff time.

Attempts have been made to overcome all these problems. Polyfructosan is a purer mixture of fructose polymers. It is more soluble than inulin and easier to measure accurately but not really suitable for routine laboratories. Many researchers developed an accurate measurement of inulin clearance by using a radioactive marker. In 1964 Concannon et al recommended that inulin can be labelled as ^{125}I -allyl-inulin (33). In 1969 Materson et al studied ^{51}Cr -inulin for GFR (34). Results correlate well with inulin clearance but the test has not become popular partly because of expense, limited availability, and instability of label. In 1981 Brauner and Westling showed that it was not necessary to maintain strict bed-rest during determination of ^{51}Cr -EDTA clearance measurement (35). ^{51}Cr -EDTA is readily available and can be stored for a sufficient time to permit departments of medical physics to provide the test on a routine basis in hospitals however this technique is complex. ^{57}Co -Vitamin- B_{12} had a brief vogue before it was discovered that its protein binding was variable. ^{125}I -Hypaque has been used quite widely in the USA but needs to be checked regularly for loss of the iodine label. ^{125}I -sodium-iothalamate has also been used although it has been demonstrated in rats that there can be tubular reabsorption of iothalamate. Although methods have been developed for evaluating GFR with the use of radioisotopes of EDTA, sodium iothalamate and sodium diatrizoate (36-38). The filtration rate measured by these techniques is currently available at only a limited number of centres. In addition, their utility for repeated evaluation of kidney function on a day-to-day basis has not yet been tested.

The reason being that if the constant infusion sufficiently prolonged, the plasma levels of the marker become constant and the infusion rate can be assumed to equal the urinary excretion. No urine samples are required, the infusion rate replacing the quantity $U \times V$ in the $U \times V / P \times$ equation, and the plasma level is

determined in the usual way on two or three occasions only. In 1969 Cote et al proved an accurate and reproducible method with inulin as the marker but the prolonged infusion remains a disadvantage (39).

3.2 Measuring of GFR using endogenous filtration marker

3.2.1 Urea Clearance

Endogenous clearance is the most popular rough test of GFR. Historically, the urea clearance test was developed before the creatinine clearance test, and was the most widely used. In 1921 Austin, Stillman and Van Slyke reported that the main weakness of the urea clearance test is its dependency on the rate of urine flow as well as GFR; it gives reproducible results only during a constant diuresis, which is difficult to obtain in practice (40). For another reason that urea clearance test is a less satisfactory measurement because the plasma concentration of urea is importantly dependent on nitrogen metabolism as well as renal function (6).

Urea is not only filtered by the glomeruli but also partially reabsorbed by the tubules. When fractional tubular reabsorption is constant, the urea clearance is proportionate to GFR, and thus the level of BUN correlates inversely with glomerular function similar to the serum creatinine concentration. The renal mechanisms of urea excretion show interesting similarities to the mechanisms involved in water excretion. Approximately 40 per cent of the filtered urea is reabsorbed in the proximal tubule; this fraction appears to be independent of the presence or absence of antidiuretic hormone and independent of the state of water diuresis. However, in the distal tubule and collecting duct, urea reabsorption roughly parallels water reabsorption (21,22). During antidiuresis (low rate of urine flow) the reabsorption of water from the distal nephron increases the tubular fluid urea concentration, which in turn favors the reabsorption of urea. The movement of urea out of the lumen is further enhanced (as is that for water) in the presence of antidiuretic hormone.

3.2.2 Creatinine clearance

As we know, creatinine released from the muscle is rather constant and only minimally dependent on physical activity, protein intake and protein catabolism. Endogenous creatinine clearance (Ccr) is the most popular rough test of

GFR. In 1976 Cockcroft and Gault studied the relationship between Ccr and age. They found that Ccr in men decreased in young people and in elderly people (41). Rowe et al in 1976 reported a similar result (42). The percentage fall with age is the same in women as in men. The results taken together suggest that there is a progressive reduction in Ccr with age even in the patients without clear-cut evidence of renal disease whose P does not increase with age. The constancy of plasma creatinine in the face of a fall in Ccr is explained by a fall in creatinine production (U_xV) which is proportionally similar to the fall in Ccr. It is generally assumed that there is a relationship between Ccr and body size, but this relationship has not been defined in detail in adults

When the serum creatinine concentration is moderately elevated, the filtration rate can be readily estimated from the serum level, but if the range is between 1.0 and 1.5 mg/dL, interpretation may be difficult. This is because muscular people normally have higher plasma concentration so a value of 1.5 mg/dL may represent normal renal function for an athlete but a definite impairment of function for a smaller sedentary person. It may be possible to clarify the interpretation of borderline values by a measurement of creatinine clearance. Of course, the creatinine clearance also varies with body size, and it is customary to correct the clearance as follows (41):

$$\text{Corrected clearance} = \text{observed clearance} \times \frac{1.73}{\text{Patient's surface (m}^2\text{)}}$$

Where the factor 1.73 represents the surface area appropriate to an observed clearance of 120 ml per minute.

Three arguments form the basis for the widely held view that Ccr is preferable to plasma creatinine as a measure of glomerular function. First, the obvious objection to plasma creatinine is that it is in no sense a measure of glomerular filtration rate whereas Ccr is closely related to GFR and changes in step with and in the same direction as the GFR (43). A much-used argument against plasma creatinine as an assessment of glomerular function has been it is 'insensitive' since it only becomes abnormal when glomerular function is grossly reduced (44).

Another argument is that a wide range of values of Ccr can be found at any particular observed value of plasma creatinine and there will be patients who have a 'low' Ccr but a 'normal' Plasma creatinine (45).

3.2 Prediction of GFR from creatinine clearance

Several authors have suggested that the errors and difficulties in the measurement of CCr can be avoided by predicting it from plasma creatinine (46). The relationship between plasma creatinine and CCr has been made linear either by plotting the logarithms of the two variables or by plotting CCr against the reciprocal of the plasma creatinine (25,42). This approach takes no account of the variation in the urinary excretion of creatinine between people at a given age and weight. Crockroft and Gault have given functions for the prediction of CCr from age and weight. Several formulas have been developed to estimate creatinine clearance from plasma creatinine concentration, age, sex and body size. No formula is more widely used to predict creatinine clearance than that proposed by Crockroft and Gault (41).

$$\begin{aligned} \text{Ccr} &= [(140-\text{age}) \times \text{weight}] / (\text{Pcr} \times 72) \text{ for men} \\ &= [(140-\text{age}) \times \text{weight}] / (\text{Pcr} \times 85) \text{ for women} \end{aligned}$$

This formula is used to detect the onset of renal insufficiency, to adjust the dose of drugs excreted by the kidney, and to evaluate the effectiveness of therapy for progressive kidney disease. More recently it has been used to document eligibility for reimbursement from the Medicare End Stage Renal Disease (ESRD) and for accrual of points for patients on the waiting list for cadaveric renal transplantation. Major clinical decisions in general medicine, geriatrics, and oncology (as well as nephrology) are made by using the Cockcroft-Gault formula and other formulas to predict the level of kidney function. However, the errors of this predictions were 21-27 %, which is not less than the standard deviation of the range of Ccr at a given age and surprisingly, it is not much less for the predictions based on age and weight than for that based on plasma creatinine (46). However, the predictions although inaccurate, are reproducible in the sense that the only source of variation in a series

of measurements is the variation. Brochner-Mortensen and Rodbro discussed this argument in more detail (47).

4. Urinalysis for protein

Urine test strips are an essential part of the nephrologist's practice today (48). They offer diagnostic information within seconds, without having to resort to chemical laboratories. While the patient is still waiting to consult with the doctor, the test strips initiate a truly magnificent array of chemical reactions, and offer up to ten diagnostic parameters of multicoloured results on test zones of only a few square millimetres.

Since Bright's observations the detection of proteinuria has remained a valuable indicator of kidney disease (14). Proteinuria usually refers to "Albustix-positive" proteinuria, corresponding to a protein concentration of at least 300 mg/l. Proteinuria demonstrated by strip testing is usually due to increased filtration through the glomerulus. It is to define the degree of renal dysfunction, the natural history of the disease and especially the response to treatment (49).

Clinical proteinuria refers to an excess of protein excretion sufficient to be detected by reagent strip testing. In an attempt to define some of the problems and improve laboratory performance, surveys of urinary total protein estimation were introduced into the regular activities of the UK External Quality Assessment Scheme for general Clinical Chemistry (UKEQAS) (12). Determination of total protein content of urine has previously been recommended as a useful test in the diagnosis and management of patients with kidney disease. Screening for proteinuria by means of 'dipstick' tests, corresponding to a protein concentration of at least 300 mg/L is widely practised. However, this is a relatively insensitive test, which can usually only identify proteinuria of the order of 300 mg daily. It cannot identify immunoglobulin light chains or many low molecular weight proteins (50).

Urine strip testing is usually highly specific, although it can give false-positive results in some situations such as highly alkaline, concentrated urine, gross hematuria, mucus, semen or leukocytes or contamination with chlorhexidine or benzalkonium. On the other hand, it is not as sensitive as quantitative methods. The

sensitivity of reagent strips is only 32% to 46%, with specificity of 97% to 100% (50).

False negative results can occur if the urine is dilute and protein loss is mild, as the method detects protein concentrations and not absolute amounts. Therefore, strip testing is useful only when urinary protein exceeds 300 to 500 mg/day or albumin excretion more than 20 mg/day (12,49,50).

Laboratory analysis is still necessary for the differentiation of abnormal from normal protein output and the assessment of kidney disease. There is evidence to suggest that a preceding phase of albuminuria, characterized by concentrations of albumin above normal but not detectable by conventional dipstick tests may predict clinical proteinuria.

In 1971, Mogensen found that patients with newly diagnosed IDD had an elevated albumin excretion, which reverted to normal on instituting insulin therapy (51). Later, Parving demonstrated that raised albumin excretion occurred in IDD after short-term cessation of insulin (52). Mogensen's proposal that 'dipstick' testing for urine protein should not be applied to the classification of renal disease. In 1979, Viberti et al. showed that 30-45% of Albustix-negative IDD patients, particularly those with a duration of diabetes between 10 and 20 years, had raised albumin excretion rates, and 3 years later found that microalbuminuria was a powerful predictor of overt nephropathy in IDD (15,53).

5. microalbumin

An early sign of kidney disease is the presence of small amounts of protein in the urine (14). A small increase in proteinuria is called microalbuminuria and occurs in many conditions of non-renal origin as well as those associated with renal disease (14,54). The role of screening for renal disease using sensitive strip testing or by direct sensitive quantitation of albumin is not normally practised (55). Only in diabetes and perhaps in hypertension of non-renal origin, is there an established indication for sensitive quantitation when the urine is negative on the conventional strip test (55,56). Mogensen and Schmitz defined the presence of microalbuminuria as 'the incipient stage of nephropathy' and related it to early glomerular hyperfiltration (57). They concluded that about 80% of microalbuminuric without

intervention develop overt nephropathy. The American Diabetes Association recommends that people with Type 2 diabetes and people with Type 1 diabetes who are past puberty and have had diabetes for more than five years have the amount of protein in their urine measured at least once each year. This test is called urine microalbumin test. There is evidence to suggest that a preceding phase of albuminuria, characterized by concentrations of albumin above normal but not detectable by conventional dipstick test call “microalbuminuria”, may predict clinical proteinuria for early kidney dysfunction (54-57).

When your kidneys first begin to leak, generally only small amounts of protein (albumin) escape. In the early stage of kidney disease, you may lose between 15 to 300 mg of albumin a day (55,59).

5.1 Pathophysiology of microalbuminuria

The glomerular basement membrane (GBM) of the kidney acts as a size- and charge-selective filter for macromolecules in plasma (60). The permeability of GBM is indicated by the glomerular sieving coefficient, GSC. Molecules such as inulin, which pass the GBM freely, have by definition, a GSC of one. Studies using infusions of neutral dextran have shown that fractional clearance of the dextran fall sigmoidally to zero as the size of the molecule, measured by the Stokes radius, increase (60,61). If the molecule has a negative charge, its fractional clearance is smaller than that of a neutral macromolecule of the same size. Rendering the molecule cationic increases the fractional clearance. As an anionic protein, albumin is excreted several fold less than would be predicted for a neutral protein with the same Stokes radius of 3.6 nm. The increased resistance is assumed to arise from electrostatic repulsion by the negatively charge heparan sulphate and sialoproteins which constitute part of the glomerular basement membrane. The amount of protein filtered in a given time depends on the GSC, the glomerular rate and the protein concentration in plasma available for filtration (62). Although the GSC of albumin is very low (63), a considerable quantity of albumin is filtered in consequence of the high albumin concentration in plasma. Some grams of albumin reach the glomerular filtrate every 24 hours, but only a few milligrams are excreted into urine, the rest of filtered albumin being reabsorbed by tubular cells. This tubular reabsorption

mechanism works normally near maximal capacity and therefore a small increase in GBM permeability leads to a clearly increased albumin excretion rate. The pathophysiology underlying microalbuminuria in diabetic nephropathy is still not fully understood, but it is in all likelihood multifactorial intrarenal haemodynamic alteration, accumulation of sorbitol, and glycosylation of glomerular proteins and all thought to be involved in the pathogenesis of diabetic nephropathy.

Microalbuminuria is a major factor of progressive renal disease. There is a close relationship between low level albumin excretion highly sensitive to the presence of any inflammation (64). The kidney is ideally placed to amplify any small change in systemic vascular permeability (65). The glomeruli receive 25% of the cardiac out put. Of the 70 mg of albumin that pass through the kidneys every 24h, less than 0.01% reaches the glomerular ultrafiltrate (i.e. less than 7g/24hrs) and hence enters the renal tubules. Almost all filtered albumin is reabsorbed by the proximal tubule via a high-affinity, low-capacity endocytotic mechanism, with only 10-30 mg/24h appearing in urine. Assuming that 7 g of albumin is filtered every 24 hrs, a 1% increase in systemic vascular permeability in response to an additional 70 mg of albumin passing into the filtrate. Since tubular mechanism for albumin reabsorption are near saturation, urinary albumin excretion would increase from a maximum of 30 to approximately 100 mg/ 24hrs.

It has been proposed that increased intraglomerular pressure causes endothelial damage to the vascular system of the glomerulus. This, in turn, leads to platelet aggregation and attraction of macromolecules into the endothelium and triggers mesangial proliferation (65,66). Mesangial cells produce more matrix, which is a typical structural abnormality in overt nephropathy. Systemic blood pressure plays an important role in the development of diabetic nephropathy. Hypertension predisposes to progression.

5.2 Microalbumin and Diabetes

Diabetes nephropathy is the leading cause of adult end-stage kidney disease in the United States and is responsible for an enormous burden of healthcare costs, including dialysis, transplantation, premature death, and unemployment (64,66-68). Once undergoing dialysis, mortality rates among diabetic exceed those of cancer.

The cost of caring for patients with diabetic end-stage kidney failure in the United States approached \$ 2 billion in 1991. After Mogensen found those patients with newly diagnosed IDD had an elevated albumin excretion, in 1982 Viberti et al. had reported that microalbuminuria predicted clinical nephropathy and death in-patients with type 1 diabetes (15). Mogensen et al. concluded that a significant increase in cardiovascular and total mortality in-patients with type 2 diabetes who had microalbuminuria. In that pioneer study, the authors divided patients with type 2 diabetes into three groups according to urinary albumin concentration (30-140 mg/L), 16-29 mg/L and < 15 mg/L) and also followed a group of normal controls. At 9 years, the highest total mortality rate was in the group with diabetes and the highest rate of albumin excretion (4). In other words, microalbuminuria doubled the risk of having a cardiovascular event. A study of the Heart Outcomes Prevention Evaluation (HOPE) study found that microalbuminuria was a strong predictor of risk for cardiovascular disease even after adjustment for renal function. 30% of the HOPE study patients had diabetes, mostly type 2. In type 1 diabetes, on the other hand, microalbuminuria is a good predictor of kidney disease. In this population, microalbuminuria has also been found to be associated with high blood pressure and poor glycemic control (70).

5.3 The prognostic value of microalbuminuria

Longitudinal studies showed that IDD with microalbuminuria are very much more likely than those without microalbuminuria to develop clinical proteinuria, progress to end-stage kidney disease (67). The prospective studies have shown that microalbuminuria is a powerful 'risk marker' for overt nephropathy in IDD although the cut-off levels of albumin excretion, above which there was an increased risk of developing nephropathy, varied considerably (68). This may be due to differences in the types of sample, the assay method, the population of patients and the duration of follow-up. The selection of any of these discriminatory values for routine clinical use is to some extent arbitrary and the progression from normal to pathological albuminuria is likely to be a continuous process.

5.4 Prevalence and incidence of microalbuminuria

The prevalence figures depend on the method of urine collection, the cut-off level of albumin excretion and the clinical characteristic of the population (71). Most studies have been carried out on insulin-dependent diabetics attending out patient clinics. Those diabetic with microalbuminuria obviously had progression to overt nephropathy manifested as clinically detectable proteinuria, much more frequently than did those who were tested initially without microalbuminuria (71,72).

The incidence of persistent microalbuminuria has not been as well studied as its prevalence. The few studies that are available suggest that the incidence is low (1-4% per year) (71-73). Once microalbuminuria is established the mean annual increase in albumin excretion may vary from 7 to 18.6%. The tendency to progress may be directly dependent on the degree of albuminuria, the glomerular filtration rate and the arterial blood pressure (73).

Considerably more studies have been done on insulin-dependent diabetic subjects, and these have documented the predictive value of microalbuminuria for the development of progressive diabetic nephropathy (74,75). Those diabetics with microalbuminuria obviously had progression to overt nephropathy, manifested as clinically detectable proteinuria, much more frequently than did those who were tested initially without microalbuminuria. The prevalence of microalbuminuria is high in those who develop diabetes and is often associated with hypertension. The prevalence of microalbuminuria is less studied in the general group than in the diabetic group. It showed a prevalence of microalbuminuria in about 22% of the diabetic group and they recommended routine monitoring of urinary excretion of albumin, which is common in health check ups.

In a study of Mexican American, a prevalence of 13% was reported. With increasing age, the prevalence of microalbuminuria rises (76). In 1976 Parving reported that up to 20% of the general population aged 60-74 years have microalbuminuria compared with 2% in younger group (77).

5.5 Urine sampling for microalbumin

There is inconclusive evidence to recommend any particular type of urine specimen as the most suitable for either screening or monitoring. A 24-hr urine

collection is the gold standard for the detection of microalbuminuria. (78). However, obtaining accurate 24-hr urine collections can be quite cumbersome, time consuming, and often unreliable (79).

Screening can be more simply achieved by an early morning specimen to minimise changes in urine volume that occur during the day in a random urine specimen (80,81).

Studied by Olivarius N de F and Mogensen found no correlation between body mass index (BMI) and urine albumin concentration in many obese individuals without diabetes, although slightly increased values have been documented (82). In older literature, massive obesity has been associated with reversible nephrotic syndrome (83,84).

Exercise is known to increase urine albumin concentration in normal individual (85,86). However, the effects of exercise are somewhat unpredictable, and thus exercise should be avoided when one is screened for microalbuminuria. Using an overnight or early morning urine sample easily circumvents this problem. Also it has been reported that menstruation may precipitate a leak of erythrocytes and albumin into the urine (87). This should be considered a confounding factor. Another factors that can transiently increase protein excretion include urinary tract infection, acute febrile illness and congestive heart failure (88). The possibility of a false positive result due to contamination with seminal fluid should be borne in mind. No significant sex difference was observed for albumin concentration, excretion rate or ratio to creatinine (89).

Ulla Derhasching reported that the albumin: creatinine ratio (ACR) did not provide any advantage compared with microalbumin measurement alone (90). Therefore, measurement of microalbuminuria alone in an on the first morning urine test is more convenient in daily clinical practice and should be used as the screening method for patients. Using first morning urine sample has low intra-individual variation. Also in 1999, Ahn CW, et.al reported that the results between urine albumin concentration and urine ACR in first morning urine were not different statistically by McNemar test (91). Spot urine should think about using albumin: creatinine ratio (92).

5.6 Sample stability and storage for microalbumin

There is little evidence that albumin is lost from urine samples through absorption in to the container wall. Glampietro et al. demonstrated that the material of the storage and assay tube such as glass, polystyrene and polypropulene had no effect on urinary albumin measurement by immunoassay (93).

Freezing urine specimens may cause conformational change in urinary proteins, resulting in a partial precipitation. Erman et al. studied the effect of freezing on albumin concentration (94). They found that freezing decreased the result by 20%. Elving et al. measured urinary albumin concentrations with a nephelometer and also noted reductions in albumin concentrations after storing the specimens at -20°C for two or six months (95). Iris Osberg et al. recommended measuring albumin either in fresh urine specimen or in urine stored at 4°C and assay within eight weeks of collection (96). In contrast, Glampietro et al. indicated that if albumin cannot be measured within 1 day of storage at room temperature or within 2-3 days of storage at 4°C , freezing urine samples at -20°C for 4-6months is advisable and should result in no clinically significant albumin loss. For longer storage, they recommended freezing the urine sample at -70°C . Recommendation suggested that the urine sample should keep in aliquot. Centrifugation does not affect the albumin concentration (97). The changes in pH were considered in the formation of precipitate leading to decrease albumin concentration in urine samples (98).

5.7 Measurement of microalbuminuria

A variety of analytical methods is available for measuring urinary albumin, but a highly accurate quantitative immunoassay specific for albumin is idea. The most sensitive techniques, however, are expensive and time consuming, the precise choice of method is often dictated by locally available expertise, equipment, and resources. Immunological methods should be used to measure urinary albumin because dye-binding and protein precipitation methods are insensitive and non-specific. The first trial for measurement of low albumin concentrations in urine was made using radioimmunoassay (RIA) (99). When the importance of microalbuminuria was realised, the reagents for the albumin RIA also became commercially available. Although RIA is still generally considered the reference

method for urinary albumin assays, the ^{125}I as a label have certain disadvantages. It is expensive, it has a relatively short shelf life and its use is possible only in laboratories authorised to handle radioactive substances. Enzyme and fluoroimmunoassay have more stable reagents but these methods were soon replaced by more practical nephelometric and turbidimetric techniques (100-103). Since, However, nephelometer is not a common instrument in clinical laboratories, many immunoturbidimetric (IT) applications have also been developed (104).

The importance of microalbumin in kidney disease is best understood when looking at the various stages of kidney disease.

6. Stage of kidney disease when considering to the order of appearance of biomarkers (1):

6.1 Microalbuminuria occurs when trace amounts of protein called albumin begin to leak through the damaged filtering structure of the kidneys. The presence of micro-albumin in the urine is often an early warning of kidney disease. Normal values on urine testing are less than 15 to 30 mg/l.

6.2 Proteinuria is the spillage of larger quantities of protein. A standard urinalysis will pick up this spillage (normal is less than 100-150 mg/day, depending on the lab). As damage progresses and protein levels reach about 2000-4000 mg/day, proteinuria is followed by:

6.3 A rising blood creatinine. As damaged kidneys have more trouble cleansing the blood creatinine levels rise. After a gradual build-up, toxins in blood reach a critical stage (usually at a creatinine level between 3 and 10). This critical stage requires:

6.4 Dialysis or kidney transplant. These technologies replace the severely damaged kidneys in cleansing the blood. Transplant organs are scanned and the operations are costly. Dialysis is disruptive to one's lifestyle and can cost 1 to 1.8 million baht each year.

In 1994, Walter Hofmann et al. (105) recommended that albumin be a “glomerular” marker. From researcher discovered more than 15 years ago it was found that even slightly elevated level of urine albumin concentration could be a strong predictor of kidney failure. In addition, total protein measured turbidimetrically may serve as a “plausibility control” to detect pre-renal proteinuria by the gap between total protein and albumin.



CHAPTER 3

MATERIALS AND METHODS

1. Materials

1.1 Apparatus

Hitachi 917 Automatic Analyzer Boehringer Mannheim. Roche Diagnostic company, Germany

Centrifuge Kokusan Euroscan H-11n, BIOMED company, Germany

Miditron Junior II Urinalysis Analyzer, Roche Diagnostic company, Germany

Sphygonanometer

Seca Cat No. 0081-42 Supreme company, Thailand

Automatic pipettes, Roche Diagnostic company, Germany

1.2 Reagent

1.2.1 Biosystem microalbumin reagent for detection urine microalbumin, Cat No. COD 31924 1X50mL, Spain.

1.2.1.1 Reagent A : Borate buffer 0.1 mol/L, sodium azide 0.95 g/L, pH10.0

1.2.1.2 Reagent B: Suspension of latex particles coated with antihuman albumin antibodies, sodium azide 0.95 g/L.

1.2.1.3 Albumin standard: Human albumin, concentration value is traceable to the standard reference material BCR 470 (Institute for Reference Materials and Measurement, IRMM)

1.2.2 Biosystem assayed control urine COD 18036, Spain

1.2.3 Biorad Lyphochex control urine microalbumin C-395-10, Roche Diagnostic Company, Germany

1.2.4 Combur¹⁰Test[®]M Cat No. 11379208 Roche Diagnostic Company, Germany. Each tests contains per cm² test patch area the following:

1.2.4.1 Specific gravity: Ethyleneglycol-bis(diaminoethylether) tetraacetic acid 182.8µg: bromthymol blue 3µg

- 1.2.4.2 pH: Bromthymol blue 13.9µg; methyl red 1.2µg;
phenolphthalein 8.6µg
- 1.2.4.3 Leukocytes: Indoxylcarbonic ester 15.5 µg;
metoxymorpholinobenzene diazonium salt 5.5µg
- 1.2.4.4 Nitrite: 3-hydroxy-1,2,3,4-tetrahydro-7,8-benzoquinoline
33.5 µg; sulfanilamide 29.1µg
- 1.2.4.5 Protein: 3',3'',5',5''-tetrachlorophenol-3,4,5,6-
tetrabromosulfophthalein 13.9 µg
- 1.2.4.6 Glucose: 3,3',5,5'-tetramethylbenzidine 103.5µg
GOD6U,POD35U
- 1.2.4.7 Ketone bodies: Sodium nitroprusside 157.2 µg
- 1.2.4.8 Urobilinogen: ethoxybenzene-diazonium-tetrafluoroborate
67.7 µg
- 1.2.4.9 Bilirubin: 2,6-dichlorobenzene-diazonium-tetrafluoroborate
16.7 µg
- 1.2.3.10 Blood: 3,3',5,5'-tetramethylbenzidine 52.8µg; 2,5-
dihydroperoxyhexane 297.2 µg
- 1.2.5 Control-Test M calibration strip, Cat No. 11379194
- 1.2.6 Control level1/2 Lot.A2927f

1.3 Miscellaneous

Paraffin

Labelling stickers

Broken-proof plastic bag

Icebox

2. Methods

2.1 Urine samples collection

The study was performed in the Faculty of Medical Technology, Mahidol University. This study was cross-sectional analysis. Urine samples were collected from 995 people who had come for health check-ups. There were 512 men and 483

women. Age and gender were recorded, and all population was weight without shoes on electronic scale. A morning urine specimen was collected (91). The urine samples were kept in a plastic container (96). Women were not examined during menstruation. Urine test strips were used to measure certain constituents in urine for the semi-quantitative of specific gravity, pH, leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood in urine. Urine with a negative result for the test strip was selected and kept into a glass tube with no additives or preservatives. The tube was covered with paraffin and then kept in a box. To keep the tube at a cool temperature an icebox was placed inside with it. After transferring to the laboratory, it will be kept in the refrigerator at 4°C. The urine microalbumin concentration of each specimen was assayed on the day of collection or within 1 week (96-98). Urine samples were centrifuged at 1500rpm for 5 minutes before analysis.

2.2 Microalbumin testing

The method most commonly used to measure microalbuminuria relies on an early morning urine collection. The microalbumin test is performed by the Hitachi 917 (106).

Albumin in the urine sample causes agglutination of the latex particles coated with anti-human albumin. The agglutination of the particles is proportional to the albumin concentration and can be measured by turbidimetry. Utilizing immunoturbidimetry, it provides the analytical sensitivity and specificity required for detecting and monitoring the low levels of albumin found in microalbuminuria (107). This method is based on the quantitative measurement of agglutination caused by the reaction of goat anti-human serum albumin antibodies to human serum albumin present in the patient's urine sample. The resultant turbidity is measured at 340 nm (primary) and 700 nm (secondary) (101).

The zone effect will cause to obtain falsely low values when albumin is present in the sample at a concentration higher than 1000 mg/L (101).

2.2.1 Reagent preparation

Working reagent: Pour the contents of reagent B vial into reagent A.

Mix thoroughly. Stable for 15 days at 2-8 °C.

Smaller working reagent volumes can be prepared by mixing: 1mL of reagent B and 9 mL of reagent A. Shake the latex vial before pipetting

Albumin Standard (S): Reconstitute with 1 mL of distilled water.

Stable for 1 month at 2-8°C

2.2.2 Metrological Characteristic

Detection limit: 0.9mg/L albumin

Linearity limit: 130mg/L albumin. For higher value dilute sample 1/3 with distilled water and repeat measurement.

Repeatability (within run):

Mean concentration	CV	n
18 mg/L	2,4%	20
57 mg/L	2,2%	20

Reproducibility (run to run):

Mean concentration	CV	n
18 mg/L	5.7%	20
57 mg/L	3.6%	20

2.2.3 Calculation

Calculate the absorbance differences A2-A1 for each albumin calibrator, and plot the values found against the albumin concentration curve. Albumin concentration in the sample is calculated by interpolation its A2-A1 value on the calibration curve.

$$\frac{(A2-A1)S}{(A2-A1)ST} \times \text{Standard Concentration} = \text{mg/L albumin}$$

2.3 Data for Biochemistry test

Biochemistry results were taken from the centre laboratory, the faculty of Medical Technology, Mahidol University. Biochemical parameters monitored including;

Liver function - AST, ALT

- Diabetes - Sugar
- Kidney function - BUN, creatinine , uric acid
- Fat - Cholesterol, HDL
- Total Protein

2.4 Population Selection

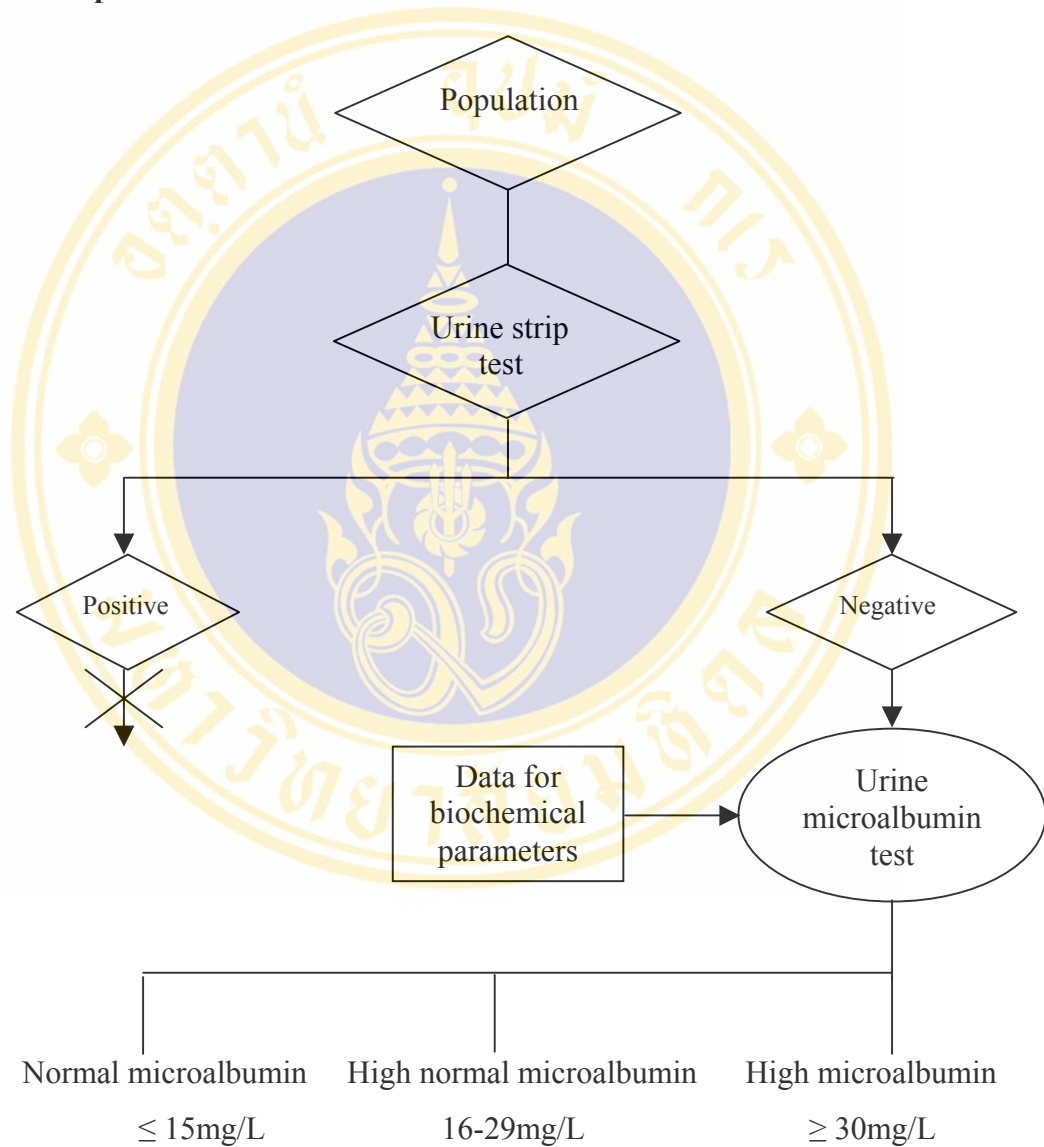


Figure 1. Population selection

Samples were divided into three categories: normal microalbumin ($\leq 15\text{mg/dL}$), high normal microalbumin ($16\text{-}29\text{mg/dL}$) and high microalbumin ($\geq 30\text{mg/dL}$) (108). At each office visit, blood pressure was measured while subjects were seated, with the

use of a mercury sphygomanometer. Subjects were assigned to a category of hypertensive status based on JNC recommendations. Hypertensive was defined by systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. Normal blood pressure was considered to be a systolic reading < 120 mmHg and diastolic reading < 80 mmHg (108). Reading between these levels was classified as pre-hypertensive. BMI was calculated as the ratio between weight (kg) and height squared (m^2). Obesity was defined as a body mass index greater than $30\text{kg}/m^2$.

Creatinine clearance was calculated according to the equation of Crockcraft (41)

$$\begin{aligned} \text{Ccr} &= [(140-\text{age}) \times \text{weight}] / (\text{Pcr} \times 72) \text{ for men} \\ &= [(140-\text{age}) \times \text{weight}] / (\text{Pcr} \times 85) \text{ for women} \end{aligned}$$

2.5 Statistical analysis

Using SPSS software version 11.0 performed all calculations. Continuous data are reported as the mean \pm SD or proportion (%). Skew distributions are reported as the median value with 25th and 75th percentiles. All *P* values are two tailed, and a *P* value less than 0.05 was considered statistically significant. Subjects were divided into three categories, normal microalbumin, high normal microalbumin and high microalbumin on the basis of their urine microalbumin concentration as described above. The significance of between group differences was Mann Whitney U test. The significance of between group differences for microalbumin was Kruskal-wallis H test. Pearson χ^2 and Fisher's Exact Test were used for the examination of the association between variables. Results are expressed as numbers and proportion (%), mean \pm SD or as percentages. The calculated odd ratios and 95% CI are expressed as an approximation of relative risk.

CHAPTER 4

RESULTS

1. General characteristic

Nine hundred and ninety-five samples were included in the study. A total of 342 samples 24.4% had microalbumin in range of 16-29 mg/L in the urine sample and in that group 9.6 % had urine microalbumin concentration higher than 30 mg/L. We categorized microalbumin into three groups; normal albumin, high normal albumin and high microalbumin. Using non-parametric test by Kruskal-Wallis it shows that there are significant differences for age, fasting plasma glucose, cholesterol, triglyceride, systolic and diastolic blood pressure in the high microalbumin group compared to the normal microalbumin group ($P<0.05$). The majority of the study populations were people who came for a health check-up so the majority of these samples were aged over 35 years old. From figure 2 it can be seen that the distribution is not normal but that the peak lies to the left of centre indicating positive skew distribution. Boxplots in figure 3-5 show that there are no significant differences in creatinine, BUN and uric acid test for normal, high normal microalbumin and high microalbumin groups.

Table 1. Clinical data status of samples according to urine microalbumin concentration. (page 1 of 2).

	Normal microalbumin ($\leq 15\text{mg/L}$)	High normal microalbumin (16-29mg/L)	High microalbumin ($\geq 30\text{mg/L}$)
Sex			
M/F	347/309	120/123	45/51
Age (years)	43 \pm 8	44 \pm 9	46 \pm 9 [†]
Urine albumin (mg/L)	6.3(3.7,9.6)	24.5(19.6,26.8)	36.3(32.1,44.6) [†]
Body Mass Index (kg/m ²)	24.3 \pm 5.7	23.6 \pm 3.5	24.9 \pm 3.6
Fasting plasma glucose (mg/dL)	91 \pm 14	91 \pm 17	96 \pm 16 [†]
Total protein (mg/dL)	7.6 \pm 0.7	7.5 \pm 0.9	7.7 \pm 0.6
Liver function (U/L)			
AST	24 \pm 11	25 \pm 15	24 \pm 11
ALT	28 \pm 23	26 \pm 20	28 \pm 22
ALP	70 \pm 22	73 \pm 24	73 \pm 24
Cholesterol (mg/dL)	217 \pm 44	225 \pm 42	229 \pm 36 [†]
Triglyceride (mg/dL)	108 (74,162)	106(75,153)	134 (91,199) [†]
HDL (mg/dL)	53 \pm 12	56 \pm 14	56 \pm 16

Data are mean \pm SD or median (25th and 75th quartile ranges)

[†] $P < 0.05$ compared with normal microalbumin group

Table 1. Clinical data status of samples according to urine microalbumin concentration Continued. (Page 2of 2)

	Normal microalbumin ($\leq 15\text{mg/L}$)	High normal microalbumin (16-29mg/L)	High microalbumin ($\geq 30\text{mg/L}$)
Kidney function			
Creatinine (mg/dL)			
Men	1.0 \pm 0.2	1.0 \pm 0.1	1.1 \pm 0.2
Women	0.8 \pm 0.1	0.8 \pm 0.2	0.8 \pm 0.1
BUN (mg/dL)	13 \pm 3	13 \pm 4	13 \pm 3
Uric acid (mg/dL)	5.5 \pm 1.6	5.4 \pm 1.0	5.7 \pm 1.0
CCr (ml/min ⁻¹ /1.73m ²)	90.7 \pm 23.4	92.1 \pm 21.6	89.6 \pm 21.7
Blood Pressure (mmHg)			
Systolic blood pressure	121 \pm 17	122 \pm 17	135 \pm 20 [†]
Diastolic blood pressure	80 \pm 11	80 \pm 12	88 \pm 11 [†]

Data are mean \pm SD or median (25th and 75th quartile ranges)

[†] $P < 0.05$ compared with normal microalbumin group

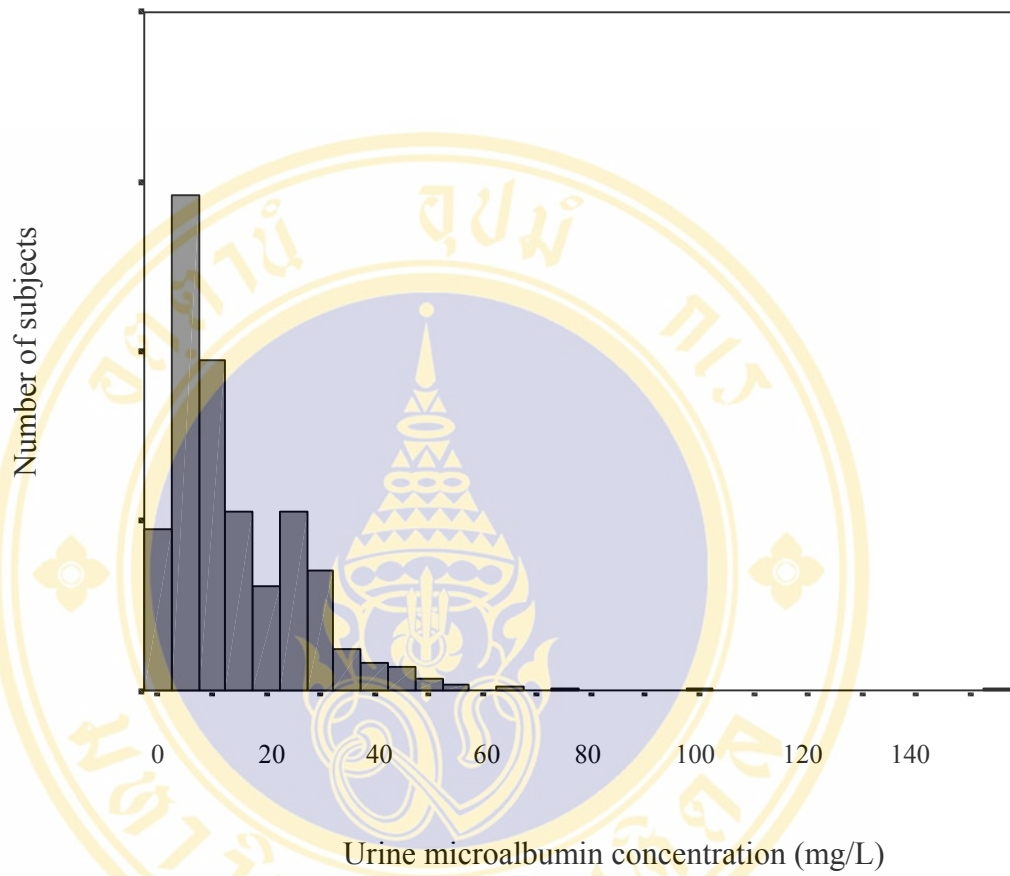


Figure 2. Frequency distribution of urine microalbumin concentration (mg/L).

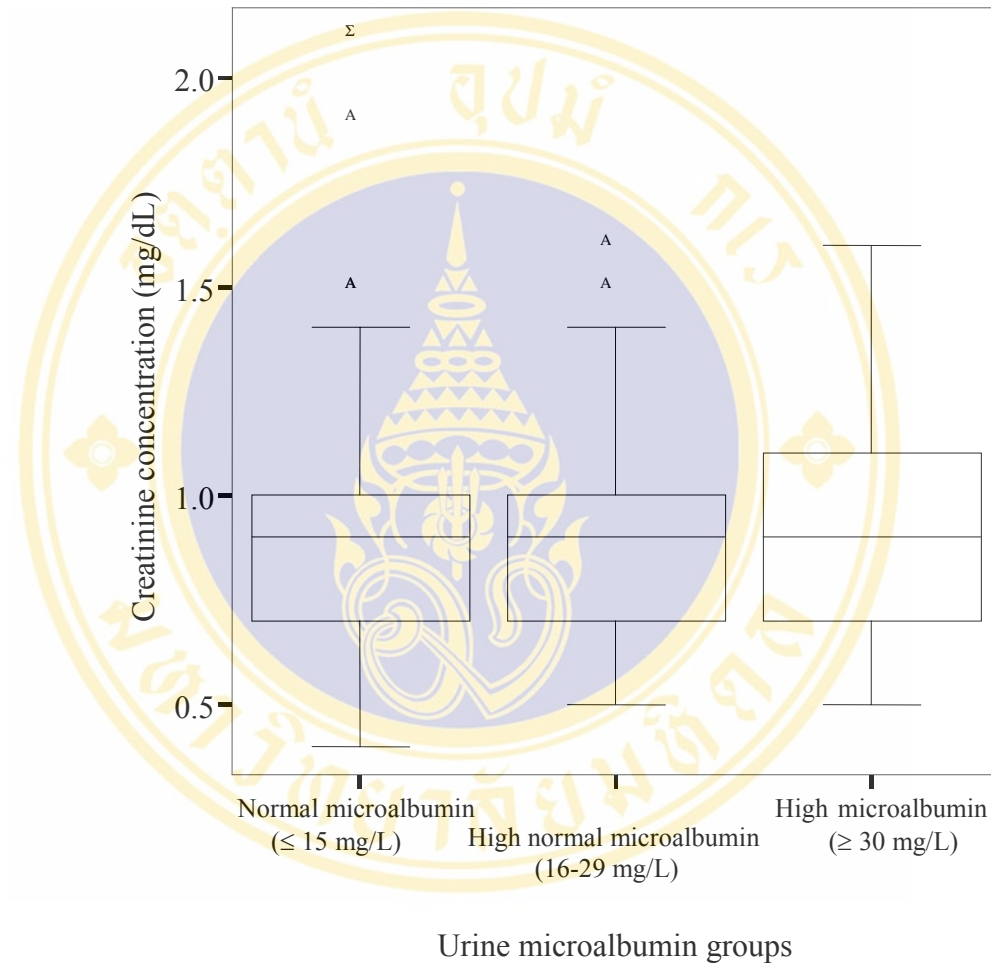


Figure 3. Boxplot showing creatinine concentration (mg/dL) in the microalbumin groups. (The horizontal line inside the box shows median creatinine concentration)

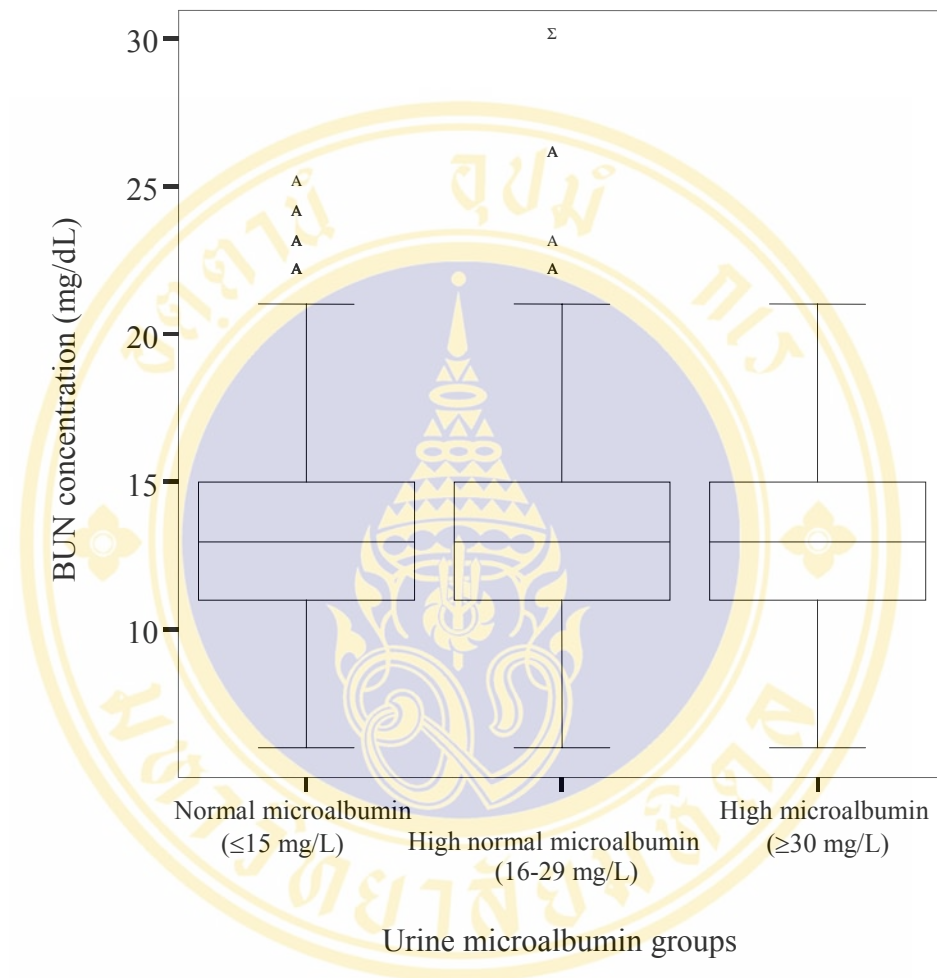


Figure 4. Boxplot showing BUN concentration (mg/dL) in the microalbumin groups.
(The horizontal line inside the box shows median BUN concentration)

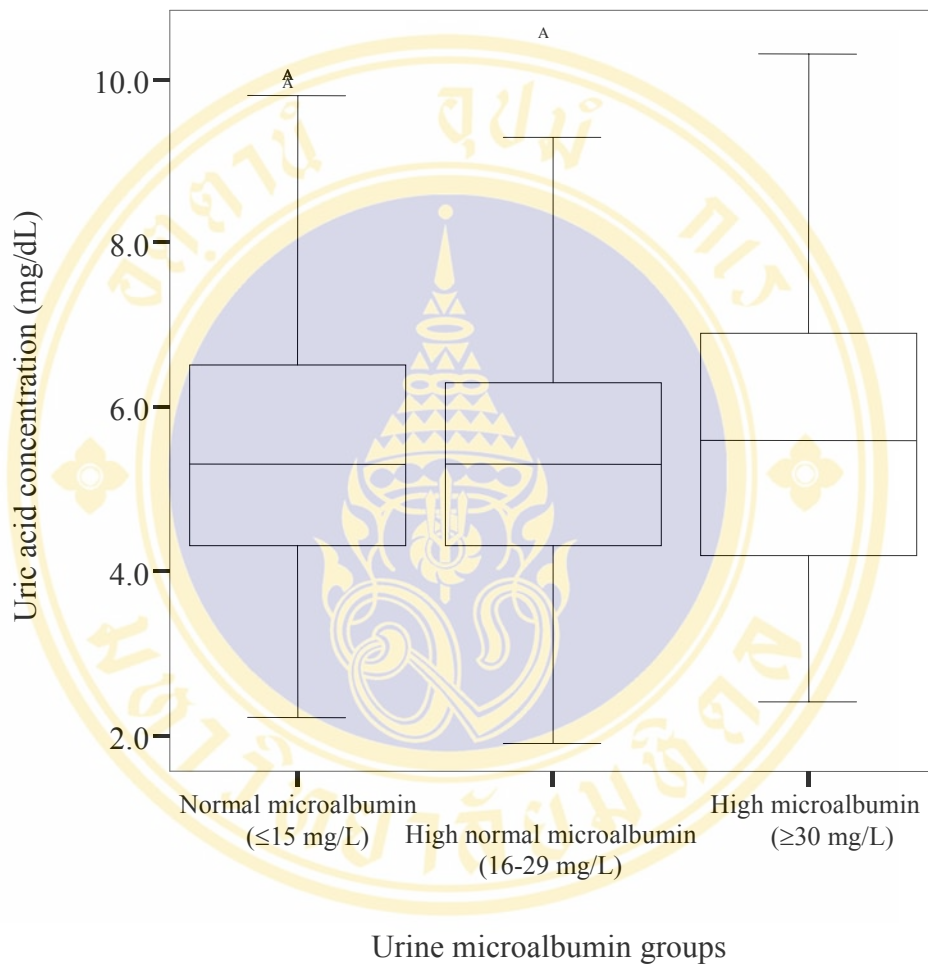


Figure 5. Boxplot showing uric acid concentration (mg/dL) in the microalbumin groups. (The horizontal line inside the box shows median uric concentration)

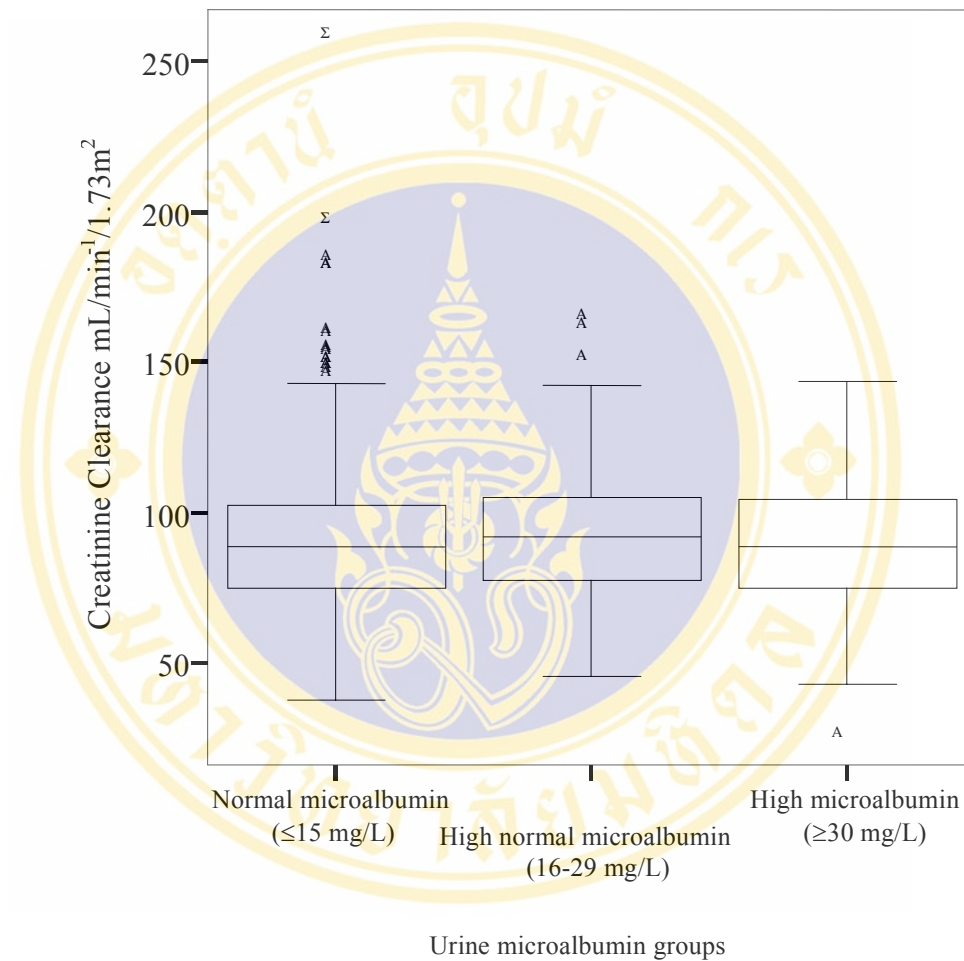


Figure 6. Boxplot showing Creatinine Clearance using Cockcroft-Gault formula (mL/min⁻¹/1.73m²) in the microalbumin groups. (The horizontal line inside the box shows median creatinine clearance)

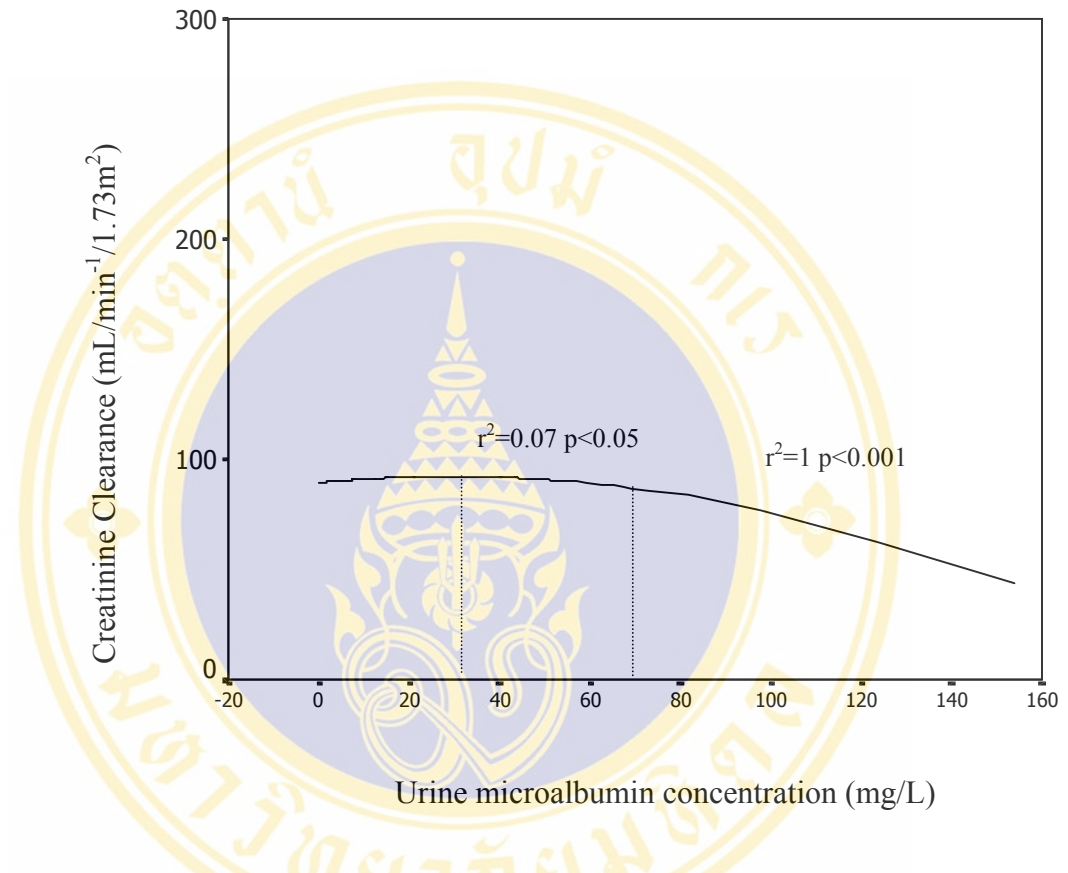


Figure 7. Curve estimate regression between Creatinine clearance using Cockcroft-Gault formula ($\text{mL}/\text{min}^{-1}/1.73\text{m}^2$) and urine microalbumin concentration (mg/L).

Table 2. Prevalence of elevated urine microalbumin levels in age groups

Age (years)	Normal microalbumin ($\leq 15\text{mg/L}$)	High normal microalbumin (16-29mg/L)	High microalbumin ($\geq 30\text{mg/L}$)	Total
25-34	93 (66.0)	38 (27.0)	10 (7.0)	141
35-44	279 (70.5)	88 (22.2)	29 (7.3)	396
45-54	209 (65.1)	72 (22.4)	40 (12.5)	321
55-64	60 (54.5)	35 (31.8)	15 (13.7)	110
More than 65	7 (58.3)	3 (25.0)	2 (16.7)	12
number of subjects (%)				

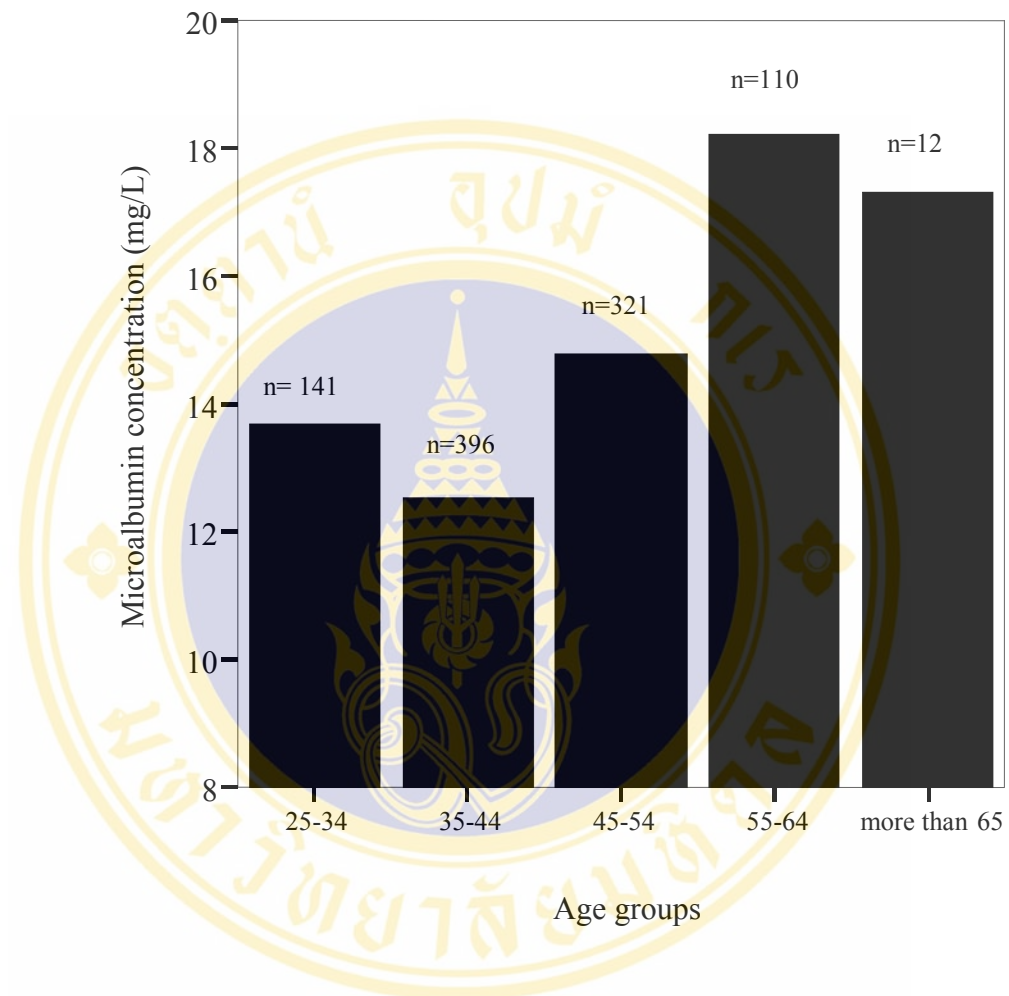


Figure 8. Mean urine microalbumin concentration (mg/L) in age groups

2. Creatinine clearance and urine microalbumin

From Figure 7. curve estimate regression between urine microalbumin concentration and creatinine clearance calculation using Cockcroft-Gault formula. When urine microalbumin was 20-60 mg/mL, creatinine clearance is still in the normal range ($r^2=0.07$ $P<0.05$). After urine microalbumin concentration was higher than 70 mg/L, creatinine clearance was start to decrease ($r^2=1$, $P<0.001$)

3. Urine microalbumin in age groups

From Table 1.it was shown that age in the high microalbumin group was significantly different from normal group ($P<0.05$). Table 2. shows the prevalence of normal microalbumin, high normal microalbumin and high microalbumin groups when categorizing age for 5 groups; 25-34, 35-44, 45-54, 55-64 and more than 65 years old. From 396 people aged between 35-44 years old, 88 people (22.2%) had high normal microalbumin and 29 people (7.3%) had high microalbumin. From 321 people aged between 45-54 years old, 72 people (22.4%) had high normal microalbumin and 40 people (12.5%) had high microalbumin. From 110 people aged between 55-64 years old, 35 people (31.8%) had high normal microalbumin and 15 people (13.7%) had high microalbumin. From 12 people age older than 65 years old, 3 people (25.0%) had high normal microalbumin and 2 people (16.7%) had high microalbumin. Figure 8. shows that there is an obvious increasing trend the higher the age group.

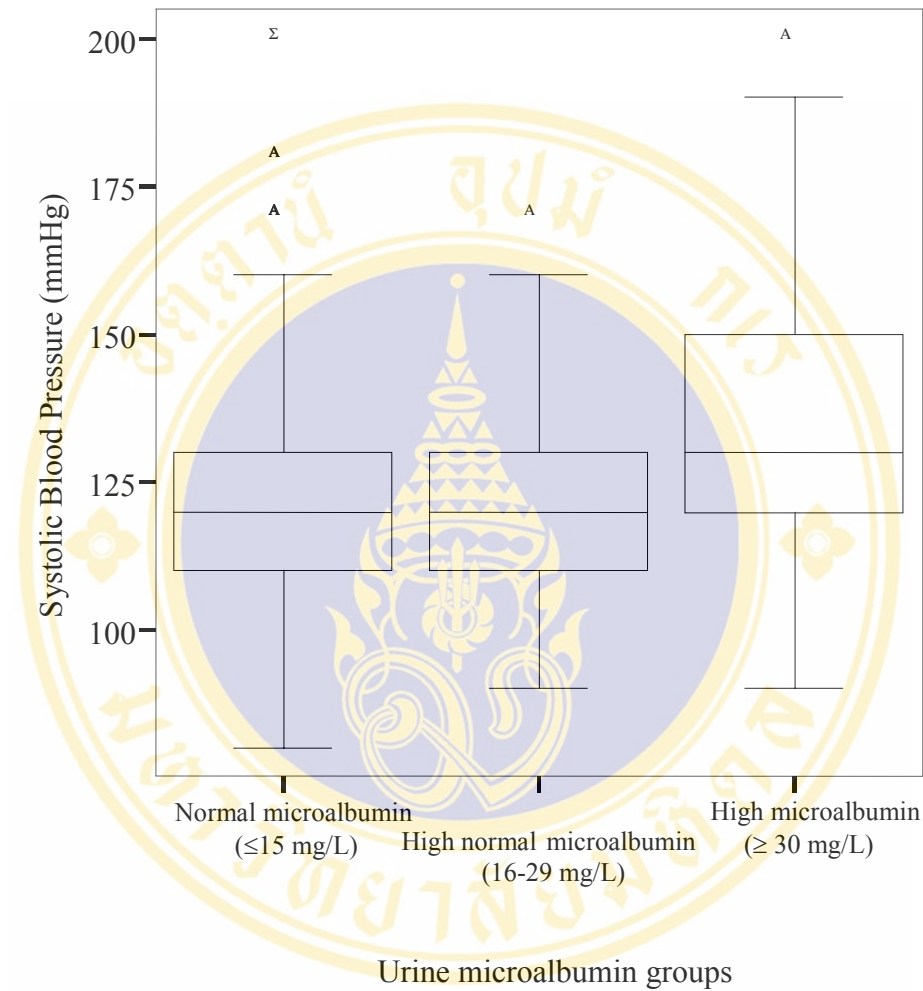


Figure 9. Systolic Blood Pressure (mmHg) in urine microalbumin groups.
 (The horizontal line inside the box shows median systolic blood pressure)

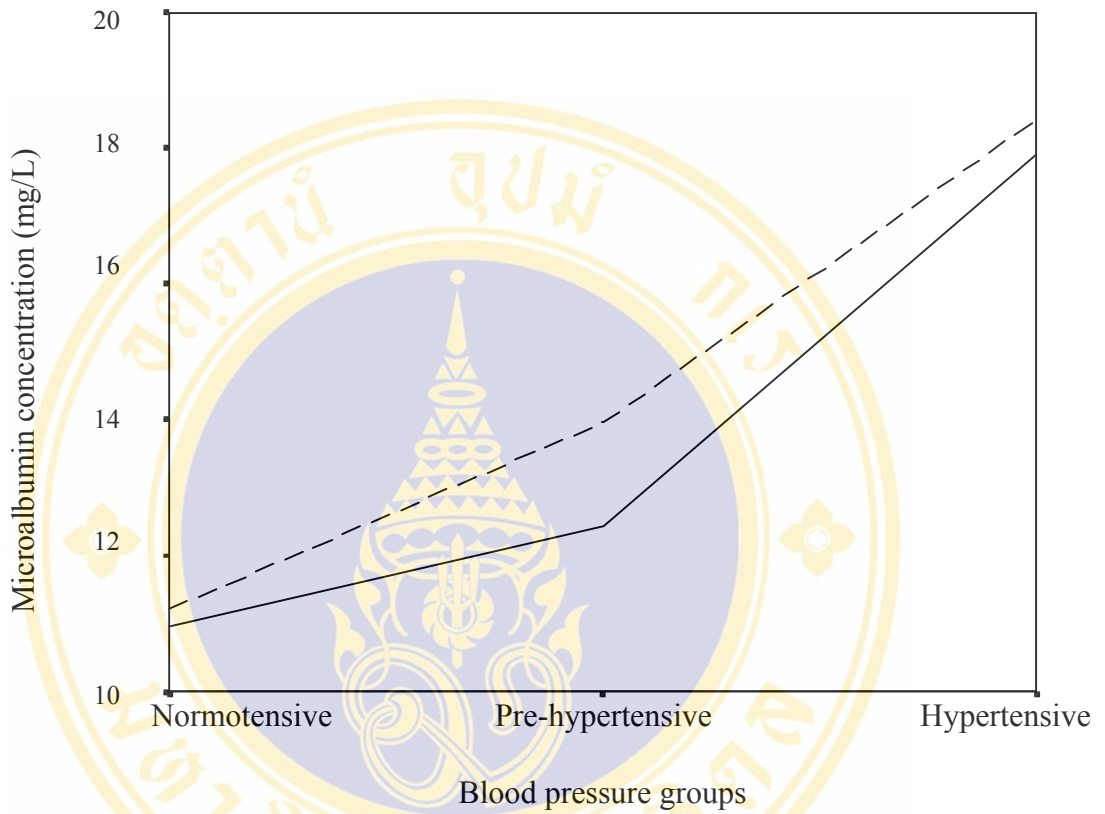


Figure 10. Microalbumin concentration (mg/L) in blood pressure group according to sex
(-----) Female
(___) Male

Table 3. Prevalence of elevated urine microalbumin levels in systolic blood pressure

Systolic BP (mmHg)	Normal microalbumin ($\leq 15\text{mg/L}$)	High normal microalbumin (16-29mg/L)	High microalbumin ($\geq 30\text{mg/L}$)	Total
Women				
Normotensive	183 (73.8)	52 (21.0)	13 (5.2)	248
Pre-hypertensive	31 (66.0)	11 (23.4)	5 (10.6)	47
Hypertensive	41 (60.3)	10 (14.7)	17 (25.0)	68
Men				
Normotensive	171 (76.7)	43 (19.3)	9 (4.0)	223
Pre-hypertensive	70 (73.7)	17 (17.9)	8 (8.4)	95
Hypertensive	69 (63.3)	24 (22.0)	16 (14.7)	109
Both sexes				
Normotensive	354 (75.2)	95 (20.1)	22 (4.7)	471
Pre-hypertensive	101 (71.1)	28 (19.7)	13 (9.2)	142
Hypertensive	110 (62.1)	34 (19.2)	33 (18.7)	177

number of subjects (%)

4. Urine microalbumin and blood pressure

The samples were divided in three groups according to systolic blood pressure, normal blood pressure, pre-hypertensive and hypertensive. In both sex the proportion of high microalbumin in normal blood pressure (4.7%) were lower than pre-hypertensive (9.2%) and hypertensive group (18.7%) see Table 3. From 248 women in normal blood pressure group, 183 people (73.8%) had normal microalbumin, 52 people (21.0%) had high normal microalbumin and 13 people (5.2%) had high microalbumin. 47 women in pre-hypertensive group, 31 (66.0%) had normal microalbumin, 11 people (23.4%) had high normal microalbumin and 5 people (10.6%) had high microalbumin. 68 women in hypertensive group, 41 people (60.3%) had normal microalbumin, 10 people (14.7%) had high normal microalbumin and 17 people (25%) had high microalbumin. From 223 men in normal blood pressure group, 171 people (76.7%) had normal microalbumin, 43 people (19.3%) had high normal microalbumin and 9 people (4.0%) had high microalbumin. 95 men in pre-hypertensive group, 70 people (73.7%) had normal microalbumin, 17 people (17.9%) had high normal microalbumin and 8 people (8.4%) had high microalbumin. 109 men in hypertensive group, 69 people (63.3%) had normal microalbumin, 24 people (22.0%) had high normal microalbumin and 16 people (14.7%) had high microalbumin. Figure 10. shows that there is an obvious increasing trend the higher of microalbumin urine concentration in hypertensive group for both sex.

Table 4. Relative risks for high normal microalbumin and high microalbumin according to systolic blood pressure status and before adjusted for confounders

Variable	Relative Risk for High Normal albuminuria (95%CI)	Relative Risk for Microalbuminuria (95%CI)
Study groups		
Normotensive	1.00 (reference)	1.00 (reference)
Pre-hypertensive	1.06 (0.73-1.53)	2.01 (1.05-3.86) [†]
Hypertensive	1.12 (0.79-1.57)	3.94 (2.38-6.52) [†]

[†] $P < 0.05$

Table 5. Relative risks for high normal microalbumin and high microalbumin according to systolic blood pressure status and after adjusted for confounders

Variable	Relative Risk for High normal microalbumin (95%CI)	Relative Risk for High microalbumin (95%CI)
Study groups		
Normotensive	1.00 (reference)	1.00 (reference)
Pre-hypertensive	1.04 (0.69-1.59)	2.09 (0.97-4.52)
Hypertensive	1.09 (0.73-1.64)	3.68 (1.98-6.85) [†]
Confounders		
BMI	0.57 (0.31-1.05)	0.85 (0.40-1.86)
Glucose level	0.84 (0.50-1.42)	2.09 (1.20-3.60) [†]
Cholesterol level	1.16 (0.91-1.48)	1.21 (0.78-1.86)
TG level	1.01(0.75-1.37)	1.70 (1.10-2.62) [†]

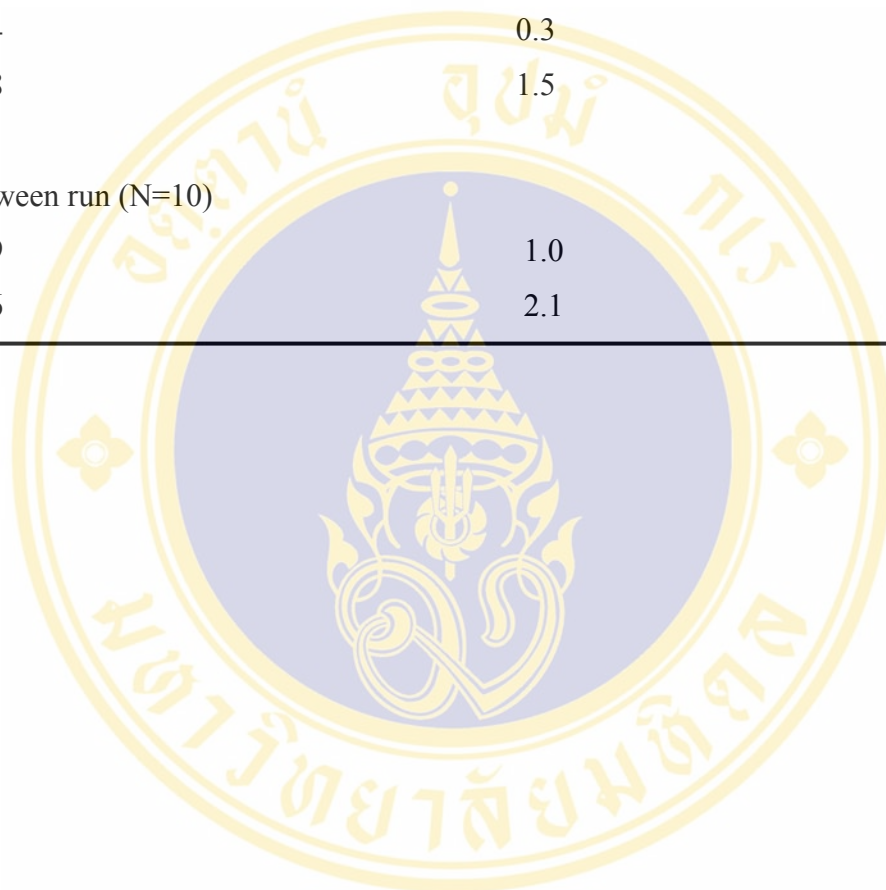
[†] $P < 0.05$

5. Relative risk for high normal microalbumin and high microalbumin

People who have hypertensive had a higher urine microalbumin than normal blood pressure and pre-hypertensive. Before adjust for confounders, people who have pre-hypertensive and hypertensive, respectively showed an independent association with high normal microalbumin (relative risk, 1.06 [95% CI, 0.73 to 1.53] and 1.12 [CI, 0.79 to 1.57]). People who have hypertensive, respectively showed a dependent association with high microalbumin (relative risk, 2.01[95%CI, 1.05 to 3.86] and 3.94 [CI, 2.38 to 6.52]) $P<0.05$. After adjustment for potential confounding factors, such as BMI, glucose, cholesterol and triglyceride level, people who have pre-hypertensive showed an independent with high normal microalbumin (relative risk, 1.04[95%CI, 0.69 to 1.59]) and high microalbumin (relative risk, 2.09[CI, 0.97 to 4.52]). People who have hypertensive showed a dependent association between hypertensive and high microalbumin (relative risk, 3.68[CI, 1.98-6.85]) $P<0.05$ see Table 5.

Table 6. Precision of the urine microalbumin measurement by immunoassay

Mean urine microalbumin (mg/L)	SD	CV%
Within run (N=10)		
14.4	0.3	2.1
42.8	1.5	3.5
Between run (N=10)		
22.9	1.0	4.4
35.6	2.1	5.9



CHAPTER 5

DISCUSSION

In a situation where no single test has clear advantages in terms of sensitivity and specificity, the relative importance of each must be considered. The fact remains that in clinical medicine the utility of a test lies in its ability to tell us whether kidney function is reduced and to what degree, without regard to its dependency on glomerular function. Failure to detect reduced glomerular function at the early stage could result in progression of the disease, further deteriorating kidney function, and irreversible damage. Early detection in the course of kidney disease may be more amenable to therapy; thus it is very important to identify quickly, those is the patients at risk.

The significance of microalbuminuria is the predictor of clinical proteinuria and CKD (49,50). Therefore it is important to assess the validation of microalbumin test as a screening test for kidney function. In this present study, urine albumin concentration was measured in people who come for health check up. They all have negative result for urine dipstick test and normal result for kidney function by creatinine, BUN and uric acid test. Similar to another study found prevalence of people who have high microalbumin from negative result for urine protein dipstick test about 6-40% depend on the cut-off value (71). From 955 people, 243(24.4 %) of them have urine microalbumin in the range 16-29 mg/L and 96 (9.6%) of them have urine microalbumin \geq 30 mg/L

It is known that the GFR test is the gold standard used for the early detection of kidney dysfunction but this test is not suitable for screening in routine laboratory because it is cumbersome and difficult to perform (28,29). The creatinine, BUN and Uric acid test will increase twice fold when GFR was begin decrease and that means 50% of the nephrons would be destroyed (40,43). From this result it means the conventional test for kidney function in Figure 8. When microalbumin was 40-60

mg/L the CCr prediction is still in the normal range, which means the microalbumin test is more sensitive than the conventional test.

It is known that microalbuminuria shows the beginning of kidney dysfunction so it can be said that the microalbumin test has more sensitivity than the conventional screening for kidney function (2,3,80). The high proportion of high microalbumin group in this study indicates the need for think more about screening people for kidney function with urine microalbumin test after the result of urine strip test was negative.

The rates of screening for urine protein were very low, and screening for microalbuminuria was nearly non existent (about 2%). Tests for microalbuminuria are not given to patients instead of urinalysis for proteinuria but should be used in addition to urinalysis. The presence of microalbuminuria is strongly predictive of future development of end stage renal failure. Consequently, the American Diabetes Association (ADA) recommends an annual urinalysis for protein and annual microalbumin measurement if urinalysis is negative for protein (58).

Early detection of microalbuminuria and therapy with angiotensin converting enzyme (ACE) inhibitors have been shown to slow the progression of kidney failure (5,13). The prospective study by Mathisen ER reported that in normal microalbumin group could progression to persistent microalbuminuria especially within 5 years in hypertensive group (71). Study more by Parving has found that a slightly gradual increase in diastolic blood pressure was demonstrated in-patients with microalbuminuria (52). In the present study it was found significant difference for systolic blood pressure in high microalbumin group shown in Figure 9. Trends to have high blood pressure in high microalbumin group. From Parving study that make these result should follow in some of high microalbumin group that have normal blood pressure or pre-hypertensive should be aware of increased blood pressure. Also in the normal microalbumin and high normal microalbumin group but have high blood pressure should be aware for increased of microalbumin.

High blood pressure is a condition that can damage your kidneys. Your kidneys act like a filtering system to get rid of excess water and wastes in the blood. Blood pressure is the force, or pressure, of the blood on the walls of your blood vessels. Overtime, uncontrolled high blood pressure can damage the blood vessels and glomerulus in the kidney. Microalbuminuria appears to be a marker for both the presence and future risk of development of vascular injury. Whatever the reasons responsible for microalbuminuria, once it appears there is a greater likelihood for progressive renal damage and an increased risk of all forms cardiovascular disease. Whereas the progression of nephron loss, shows an increased risk of vascular injury has been attributed to a generalised increase in vascular permeability that results in widespread atherosclerosis. In the present study found the significance difference in cholesterol and triglyceride were found between the normal microalbumin group and high microalbuminuria group. Of interest, the association between blood pressure and microalbumin was found in people who have pre-hypertensive and hypertensive ($P<0.05$). After adjusted for glucose level and triglyceride the relative risk decreased in pre-hypertensive and hypertensive group.

Preliminary data from the Framingham population study indicate that trace proteinuria as determined by standard methods is associated with significant increases in both cardiovascular and overall mortality (1). This information shows a relationship exists when microalbuminuria and hyperfiltration are correlated, with blood pressure. The effects always emerge in the heart and kidney because the most blood volume goes to the heart and secondly to the kidneys. It is of further interest that Shearmann et al (65) have found a positive correlation between GFR and left ventricular mass determined by echocardiography. So blood pressure cannot be considered a confounding factor but rather a part of the natural history of the disease.

In this present study there were significant difference found in mean age for normal urine microalbumin and high microalbumin. The elderly group was found that microalbumin tended to increase and the mean urine microalbumin was significantly different in this group. No relationship between high microalbumin and sex, height, weight and BMI was found.

The usefulness of measuring microalbumin using an overnight or early morning specimen, as an index for estimating quantitative microalbuminuria has been well-established (78,90,91). Urine samples should not be collected after undue exertion nor after an acute fluid load. Reference ranges must specify the type of urine collection (90,91). The possibility of a false positive result due to contamination with menstrual or seminal fluid or due to a urinary tract infection should be borne in mind. In this study we selected the negative result of the urine strip test. Although the stability of microalbumin in urine is good, urine is often stored for long periods at -20°C, particularly in research programs. The study, Erman et al. found a decrease in urine microalbumin concentration after even as little as one week of storage at -20°C (94). It should be said that many researchers have different reports about how to keep urine samples (95-98). The present study was conducted in Thailand which is a tropical country so it is proposed that the best procedure to follow is if it is known that the urine sample cannot be measured within 1 day it should be kept in the refrigerator at 4°C or if the sample is to be collected far away from the laboratory the sample should be transported in a cool box.

The effect of urine pH is also controversial (98). Urine was checked again for pH before and it wasn't different from the beginning urine (pH5-8) analysis and recommended urine pH is not over 9 but in the kit used it can be reported that the buffer had no effect from pH.. It has also been suggested that urinary pH is important in the formation of precipitates leading to a decreased albumin in urine samples, but it was found that there was no relationship between pH and precipitate formation.

Studying in the diabetic group shown that the ability to predict the development of proteinuria was strong (66-70). When the patients with concentrations of less than 15 mg/L were compared to patients with concentrations of 15 to 140 mg/L. In 80% of people with diabetes and microalbuminuria, urinary albumin excretion increases at a rate of 10-20% per year, with the development of clinical proteinuria in 10-15 years. After the development of clinical grade proteinuria, most patients go on to develop decreased glomerular filtration rate and, given enough time,

end-stage renal disease. But still there are no reports for longitudinal study in the healthy group.

Measuring replicates of urine samples with normal and high microalbumin concentrations assessed the precision of immunotubidimetric method. The %CV of within-assay imprecision was 2.1% and 3.5%, respectively and those of between assay imprecision were 4.4% and 5.9% respectively. A detection limit of 0.9 mg/L in this reagent kit suffices for screening kidney function for microalbuminuria. Automatization of the method facilitated analysis of large runs of sample. Smaller runs, however, can also be handled easily and do not make the method more expensive. The kit costs about 20 Baht per sample. Ulla Derhaschnig et al. demonstrated the sensitivity and specificity of the microalbumin concentration in on the urine samples at various cut-off levels. Cut-off levels of more than 30 mg/l will have high specificity but low sensitivity (90).

The impact of test selection screening test has to be evaluated with regard to the prevalence of the disease in the investigated population. The prevalence in the study population was about 30%, which was comparable with other epidemiological studies reporting a prevalence of 6–40%. Additionally urine microalbumin measurement of kidney dysfunction risk screening programmes should be advocated. However, because our study was cross-sectional, it cannot confirm relationship between high microalbumin and hypertension that ultimately results in impaired filtration. The results must be confirmed using a study with a longitudinal design.

CHAPTER 6

CONCLUSION

In order to prevent kidney dysfunction, one must be able to do is early detection through the screening program. The quantitative test of microalbumin has been proposed as a screening test for kidney dysfunction. The potential of microalbumin is presented herein;

1. The microalbumin test is a useful indicator for kidney dysfunction in the screening group rather using the conventional test.

1.1 Conventional screening tests such as the urinalysis, creatinine, BUN and uric acid tests performed in 995 samples result in the normal range. Although a normal result was obtained, however, the urine microalbumin test, a potential marker for early detection of kidney dysfunction, is shown to be high in some of these cases.

1.2 It was also found that abnormal microalbumin was shown before deminishing of creatinine clearance.

1.3 Among the study samples, there were; 24.4% having microalbumin in the range of 16-29mg/L and 9.6% having a higher value than 30 mg/L.

2. Realiability of microalbumin test was studied.

2.1 The %CV of within-assay and between-assay imprecisions, which assessed low and high level of control sample, were 2.1%, 3.5%, 4.4% and 5.9% respectively. The detection limit was 0.9 mg/L.

2.2 Screening for kidney function using the quantitative urine microalbumin test might be an additional test for early detection of kidney dysfunction.

2.3 A urine microalbumin test is a non-invasive technique and easy to perform.

3. It was also found that there is a relationship between microalbumin and other interesting variables such as triglyceride, which requires further study.

4. These studied should be continued and confirmed by a longitudinal design.



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APPENDIX

1. NPar Tests

Kruskal-Wallis Test

Test Statistics^{a,b}

	AGE	HEIGH	WEIGHT	MALB	CREATINI
Chi-Square	13.629	2.206	2.515	693.791	1.853
df	2	2	2	2	2
Asymp. Sig.	.001	.332	.284	.000	.396

a. Kruskal Wallis Test

b. Grouping Variable: ALBNEW

Test Statistics^{a,b}

	BUN	CCR	URIC	PROTEIN	CHOLESTE
Chi-Square	.417	2.000	1.150	4.490	8.246
df	2	2	2	2	2
Asymp. Sig.	.812	.368	.563	.106	.016

a. Kruskal Wallis Test

b. Grouping Variable: ALBNEW

Test Statistics^{a,b}

	TG	HDL	GLUCOSE	AST	ALT
Chi-Square	12.869	8.007	11.093	.798	3.019
df	2	2	2	2	2
Asymp. Sig.	.002	.018	.004	.671	.221

a. Kruskal Wallis Test

b. Grouping Variable: ALBNEW

Test Statistics^{a,b}

	ALP	SYSTOLIC	DIASTOLI	CMMOLI
Chi-Square	3.082	28.866	32.870	1.853
df	2	2	2	2
Asymp. Sig.	.214	.000	.000	.396

a. Kruskal Wallis Test

b. Grouping Variable: ALBNEW

2. Hitachi 917 for microalbumin test

WORKPLACE REAGENTS CALIBRATION Q C

Calculate Test Special Wash To display assay code choices: Touch the Assist Box or press Ctrl+A.

No.	Test	Type	Assay	Calib	Range	Others
45	LDL-C	Ser/PI	assay/Time/Point	Z Point End	4	9 12 0 0
58	CG 2	Ser/PI				
61	HDL-R	Ser/PI	wave(2nd/Primary)	546		
	Urine		S.Vol(Normal)	15.0	14.0	85
	CSF		S.Vol(Decrease)	18.0	14.0	98
63	LDL-R	Ser/PI	S.Vol(Increase)	4.0	0.0	0
	Urine		Diluent	Water	99	Timing
	CSF		Reagent (R1) T1	270	0	00984 1 R1
64	U-ALB	Urine	Reagent (R2) T2	30	0	00984 0 R2
	CSF		Reagent (R3) T3	0	0	00984 0 R3
98	L	Ser/PI	Reagent (R4) T4	0	0	00984 0
	Urine					
	CSF					
	Suprnt		Abs.Limit	32000	Increase	2Tests
01	H	Ser/PI	Prozone Limit	-32000	0 Lower	

Delete Read Cell Detergent Detergent 1

Start Sample Stop Analyzer Stop Sample Track Print Alarm Help

Stand by piroj Mon 07/10/82 16:18

No	Test	Type	Analyze	Calib	Range	Others
38	LDH	Ser/PI				
39	HD	Suprnt				
40	HBA1C	Suprnt				
41	CK-MB	Ser/PI				
45	LDL-C	Ser/PI				
50	CB Z	Ser/PI				
61	HDL-R	Ser/PI				
		Urine				
		CSF				
		Suprnt				
63	LDL-R	Ser/PI				
		Urine				
		CSF				
		Suprnt				
64	U-ALB	Urine				
		CSF				
98	L	Ser/PI				

Application Code: 984 Unit: mg/l

Report Name: URINE-ALB.....

Data Mode: On Board

Control Interval: 1000

Instrument Factor (Y=aX+b): a= 1.0 b= 0.0

Technical Limit: 0.0 | 130.0

Repeat Limit: 0.0 | 130.0

Expected Value: Qualitative

Sex	100	Y	0.00	15.00	Cancel
(Male)	100	Y	0.00	15.00	(1) 0 --
			0.00	15.00	(2) 0 -
(Female)	100	Y	0.00	15.00	(3) 0 +-
	100	Y	0.00	15.00	(4) 0 ++
			0.00	15.00	(5) 0 +++
					(6) 0 ++++

Registration Reagent 99:81:88 piroj Mon 8/7/82 16:16

WORKPLACE REAGENTS CALIBRATION Q C MAINT/UTILITY

Analyzation Calculate Test Special Wash To display calibration method choices: Touch the Assist box or press Ctrl+A.

No.	Test	Type	Analyze	Calib	Range	Others
45	IDL-C	Ser/PI	Calibration Type Linear Point 2 Span Point 2 Weight 0	Auto Calibration Time Out Blank 3 Lot Span 3 Bottle ZPoint 0 Full 0	Change Over 0.1 10 % 50 Abs -9999 9999 -32000 32000	
68	CC 2	Ser/PI				
67	HUL-R	Ser/PI				
		Urine				
		CSF				
		Suprnt				
63	IDL-R	Ser/PI				
		Urine				
		CSF				
		Suprnt				
64	U-ALB	Urine				
		CSF				
90	L	Ser/PI				
		Urine				
		CSF				
		Suprnt				
21	H	Ser/PI				
		SD Limit				
		Duplicate Limit				
		Sensitivity Limit				
		SI Abs Limit				

Delete Read

Start Sample Stop Analyzer Stop Sample Track Print Alarm Help

Stand-by pirej Mon 5/7/10/02 16:22

No.	Test	Type	Analyze	Calib		Range				Others
				(1)	(2)	(3)	(4)	(5)	(6)	
38	LDH	Ser/Pl	<Standard>							
39	HB	Suprnt								
40	HBA1C	Suprnt	Calib.Code	water	366	0	0	0	0	
41	CK-MB	Ser/Pl	Concentration	0.0	48.0					
45	LDL-C	Ser/Pl	Position		18					
50	CO 2	Ser/Pl								
61	HDL-R	Ser/Pl								
		Urine	Sample Volume	14.0	15.0	0.0	0.0	0.0	0.0	
		CSF	Diluted S.Vol	0.0	14.0	0.0	0.0	0.0	0.0	
63	LDL-R	Ser/Pl	Diluent Volume	0	85	0	0	0	0	
		Urine								
		CSF								
		Suprnt								
64	U-ALB	Urine								
		CSF								
90	L	Ser/Pl								

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