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As Senior Paper advisor, I hereby certify that I have read and evaluated this senior paper Prepared under my guidance, by **ALEMAYEHU MICHAEL** entitled: Protein and Albumin to Creatinine ratio and their loss in urine associated with Kidney Diseases.

I recommended that it can be submitted as fulfilling Master's Degree requirements.

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As member of the Board of examiners of senior paper defense examination, we certify that we have read and evaluated the senior paper prepared by **ALEMAYEHU MICHAEL** and examined the candidate. We recommend that the Paper be accepted as full filling the Master's Degree requirement for Medical Biochemistry.

Board of examiners

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Acronym

ACR- Albumin to Creatinine ratio

ADA-American Diabetes Association

AUC-Area under the curve

BMI- Body Mass Index

CKD-Chronic Kidney Diseases

CC- Creatinine Clearance

DBP-diastolic Blood pressure

ERPF-effective renal plasma flow

ESRD- end-stage renal disease

eGFR- estimated glomerular filtration rate

ISSHP-International Society for the study of Hypertension in pregnancy

mg/mmol- milligram per millimole

mg/day- milligram per day

MDRD-Modification of Diet in Renal Disease

NKF-National Kidney Foundation

NKDEP- National Kidney Disease Education Program

NKF-K/DOQI-National Kidney Foundation Kidney Disease Quality Outcomes Initiative

NHANES-National Health and Nutritional Examination Survey

NPV-Negative Predictive Values

NS-not Specified

PPV-Positive Predictive Values

PCR-Protein to Creatinine ratio

ROC- receiver–operator curves

Sn. –Sensitivity

Sp.-Specificity

SBP-systolic Blood pressure

TPCR-Total Protein: Creatinine ratio

TPE -total protein excretion

UAE- urinary albumin excretion

UMACR-Urine Microalbumin: Creatinine ratio

Abstract

Background: Proteinuria is recognized as an independent risk factor for cardiovascular and renal disease and as a predictor of end organ damage. Appearance of albumin in the urine is one of the first sign of deteriorated kidney function. As the kidney functions decreases, the amount of albumin in the urine increases. So, Albumin: Creatinine ratio and/or Protein: Creatinine ratio on random urine sample provides significant results for quantitating proteinuria against conventional 24-hours sample collection.

Objective: - To review and recommend the method(s) for quantitating proteinuria using urinary Albumin: Creatinine ratio and/or Protein: Creatinine ratio on random urine samples vis-a-vis conventional 24-hours urine collection for diagnostic evaluation of Kidney function.

Methods: I performed a systematic review of literatures on measurement of Albumin: Creatinine ratio and/or Protein: Creatinine ratio on a random urine compared with the conventional 24-hours urine collection method.

Results: Data were extracted from 8 studies which investigating proteinuria in several settings. Patient groups in the studied were primarily those with hypertension, diabetic, preeclampsia or renal disease. Urine Microalbumin: Creatinine ratio, Albumin: Creatinine ratio and Protein: Creatinine ratio vs. 24-hours urine specimen has correlation coefficients ($r > 0.84$, $p < 0.001$) except urinary Microalbumin: Creatinine ratio ($r \approx 0.743$) and Sensitivities, specificities for the tests were 83% (ranged 66% to 100%) and 76.5% (ranged 53% to 100%), respectively, whereas positive and negative predictive values were 80.5% (ranged 61% to 100%) and 87.5% (ranged 75% to 100%), respectively.

Conclusion: The use of Albumin: Creatinine ratio and/or Protein: Creatinine on a random urine specimen provides significant correlation with conventional method of a 24-hours urine specimen collection. The analysis of the report reviewed has also revealed that, total Protein: Creatinine ratio has better sensitivity as compared to Albumin: Creatinine ratio in quantitating proteinuria.

Key words: “urinary spot microalbumin: Creatinine ratio (MACR)”, “spot albumin-creatinine ratio (ACR)”, “spot protein-creatinine ratio (PCR)” and/or spot “total Protein: Creatinine ratio (TPCR)” vs. “24-hours urine sample collection”.

1. Introduction

Normally, as blood passes through healthy kidneys, it filters the waste products out and leaves in the things the body needs, like proteins. Protein in the blood is normally unable to pass through the glomerular capsule due to their large size. In normal urine, protein concentration is very low (less than 100mg/day) which cannot be detected by usual tests. These proteins are screened by tubular epithelial cells. However, it may be mentioned that there are a number of conditions such as diabetes mellitus, hypertension, eclampsia, severe febrile illness, immune system disorders, abnormal swelling, malnutrition, or cancer and many other systemic infections which can result in proteinuria [Tietz, 2006 4th ed.].

Disease conditions that affect glomerular basement membrane integrity, which enlarge the filtration spaces and tubular dysfunction, which affect tubular reabsorption are the two major classes of pathological mechanisms which results in proteinuria. The basic pathology of proteinuria is due three major factors. These are: - (a) Glomerular proteinuria, (b) Tubular proteinuria and (c) Overflow proteinuria [Tietz, 2006 4th ed.].

Glomerular proteinuria: the glomerular of kidney are not permeable to substances with molecular weight of more than 69,000 and plasma proteins are absorbed in normal urine. When glomeruli are damaged or diseased, they become more permeable and plasma protein may appear in urine. Tubular proteinuria is seen when tubular reabsorption mechanism is impaired and lower molecular weight protein are excreted. Overflow proteinuria: when small molecular weight proteins are increased in blood and they overflow into glomeruli [Tietz, 2006 4th ed.].

Proteinuria can also be functional proteinuria or organic proteinuria. Functional proteinuria are those conditions that are not related to diseased organ. The amount of protein excreted is usually small, majority of cases show < 0.2% and the condition is usually temporary. Organic proteinuria (pathological proteinuria) may further be due to prerenal, renal and Postrenal. It means that not only due to the primary kidney disease but also variety of other diseases that can be responsible for proteinuria. Some of the diseases are: - diabetes mellitus, hypertension, primary renal disease, or other systemic illnesses [Tietz, 2006 4th ed. and Tietz, 2008 6th ed.].

It may be mentioned that protein appears in the prerenal, renal and postrenal factors such as cardiac diseases, abdominal tumor, cancer, acute glomerulonephritis, chronic glomerulonephritis,

nephrosclerosis, inflammatory, nephrotic syndrome and degenerative or traumatic lesions of the pelvis of the kidney.

Pre-renal proteinuria implies significantly increased concentrations of protein are being presented to the kidney in the plasma that is filtered through a normal glomerulus with normal permeability to macromolecules (i.e., permselectivity) [Tietz, 2006 4th ed. and Katherine M. James 1998]. Renal proteinuria implies the defective renal function and/or inflammation of parenchymal kidney tissue which is the cause of the proteinuria [Tietz, 2006 4th ed. and Adams, LG et al, 1992]. Post-renal proteinuria due to plasma proteins from hemorrhage or inflammation in the urinary tract protein is added to the urine in the urinary tract after formation by the kidney (i.e. in the ureter, bladder or urethra) [Tietz, 2006 4th ed. and Scott A. Brown (2005)].

Generally, different proteins with varying molecular weight can appear in urine. For examples: microalbuminuria is the earliest indicator of cardiovascular diseases and renal disease (nephropathy) attributable to diabetes. “Micro albuminuria” refers to albumin excretion above the normal range but below the level of detection by tests for total protein [Tietz, 2008 and National Institute for Clinical Excellence (2002)].

Albumin is the second and main protein that is most likely to appear in urine, which more likely to escape through the filters of the kidney, called glomeruli. Sometimes the term albuminuria is used when the test detects albumin specifically. Conducting tests for high levels of albumin in the urine of adults helps in identifying diabetes that is vulnerable to kidney disease. [Tietz, 2006 4th ed.] In urine, total protein is which composed predominantly of albumin, but also of physiologic proteins. Proportions of these proteins vary widely in pathologic states. High molecular weight proteinuria correlates more strongly with rate of progression of kidney disease than intermediate- or low-molecular-weight or even total proteinuria. [Paul E. and de Jong, MD, PhD, 2011].

Moreover, when the results are expressed in terms of albumin and/or protein excreted in milligram per gram of creatinine, expression of the results is much more accurate. The ratio of the albumin to creatinine (ACR) and protein: Creatinine ratio (PCR) are measured to check the levels at which the kidneys are functioning. [Tietz, 2008 6th ed. and McIntyre, Natasha J; Taal, Maarten W, 2008]

A test for quantitation of urinary protein excretion in terms of Albumin: Creatinine ratio and/or Protein: Creatinine ratio can be performed to predict accurately the level of proteinuria and becomes increasingly relevant in assessing prognosis and treatments of kidney diseases. Degree of proteinuria reflects the progression of kidney diseases which helps in assessing prognosis of kidney diseases which affecting the normal kidneys functions, and if no treatment is given in time, it could leads to kidney failure. [Caring for Australians with Renal Impairment (CARI) Guidelines, 2004]

Estimation of protein in urine is used for not only monitoring prognosis but also for treatment purposes. Traditionally, the assessment of proteinuria is done by collecting a 24-hours urine sample to measure the amount of protein excreted in mg per 24 hours. This method is fairly accurate but become unreliable due to the problem of 24-hours urinary samples which is never reliable. Also, the collection beyond is too long for the patient of patience. [Amir Said Alizadeh Naderi, MD and Robert F. Reilly, MD, 2008]

For the past many years, the investigators have started collecting short term urinary samples such as 2-hours, 4-hours and 6-hours instead of tedious 24-hours urine collection. They have expressed the results in term of Albumin: Creatinine ratio and/or Protein: Creatinine ratio measurements on a single-voided specimen provide a convenient and reliable alternative method than 24-hours urine measurements. [Caring for Australians with Renal Impairment (CARI) Guidelines, 2004 and Amir Said Alizadeh Naderi, MD and Robert F. Reilly, MD, 2008].

I have conducted a systematic review of the literatures to evaluate the utility of the Albumin: Creatinine ratio and/or Protein: Creatinine ratio in random urinary samples collected over the conventional method of 24-hours urine collections for quantitatively expressing proteinuria to evaluate the kidney function.

2. Literature of Review

2.1 Pathophysiology for Proteinuria

The basic pathology of proteinuria is due three major factors. These are (a) Glomerular proteinuria, (b) Tubular proteinuria and (c) Overflow proteinuria [Tietz, 2006 4th ed.].

2.1.1 Glomerular proteinuria

Normally, the glomerular of kidney are not permeable to substances with molecular weight of more than 69,000 and plasma proteins are absorbed in normal urine. When glomeruli are damaged or diseased, they become more permeable and plasma protein may appear in urine results glomerular proteinuria. That is the smaller molecules of albumin pass through damaged glomeruli than the heavy globulins. So, when proteins appear in urine, albumin predominates. Large quantity of albumin is lost in nephrosis whereas small quantities are seen in acute nephritis, strenuous exercise and pregnancy whereas microalbuminuria is seen in complication of diabetes mellitus and hypertension which is an indicator of future renal failure. [Tietz, 2006 4th ed. and Amir Said Alizadeh Naderi and Robert F. Reilly, 2008].

2.1.2 Tubular Proteinuria

Tubular Proteinuria: is seen when tubular reabsorption mechanism is impaired and lower molecular weight protein are excreted. Tubular proteinuria occurs because small plasma proteins (<15,000 molecular weight) freely traverse the glomerular barrier. There are also small amounts of larger molecular weight proteins (e.g., albumin = 69,000 g/mole) that are filtered through the normal filtration barrier. In a normal kidney, the tubules reabsorb practically all of this filtered protein. In some diseases (e.g., gentamicin nephrotoxicosis) the glomerulus is normal and permits filtering of only small molecular weight proteins and a minor amount of albumin. However, the diseased tubules are unable to metabolize these proteins and tubular proteinuria ensues [Tietz, 2008 6th ed. and Amir Said Alizadeh Naderi and Robert F. Reilly, 2008].

2.1.3 Overflow Proteinuria

Overflow Proteinuria: when small molecular weight proteins are increased in blood and they overflow into glomeruli. For examples, hemoglobin if it exist in free form, appear in urine (hemoglobinuria), myoglobinuria seen muscle crush and injury, Bence-Jones proteinuria. In about 20% cases of multiple myeloma, light chains of immunoglobulins are produced abnormally. Being of smaller molecular weight, they are excreted in urine. These are called

Bence-Jones proteins (monoclonal light chains produced by plasmacytoma). When the urine is heated, at 45⁰C they start precipitating, at 60⁰C there is maximum precipitation, at 80⁰C these proteins start re- dissolving, and will form a clear solution at 100⁰C. [Tietz, 2008 6th ed. and Amir Said Alizadeh Naderi and Robert F. Reilly, 2008]. Proteinuria can also be functional proteinuria or organic proteinuria.

2.1.4 Functional Proteinuria

Functional Proteinuria: - are those conditions that are not related to diseased organ. The amount of protein excreted is usually small, majority of cases show < 0.2% and the condition is usually temporary. Causes of these proteinuria are; Violent exercise and Cold bathing. Alimentary proteinuria (due to after excessive protein ingestion), Pregnancy (here proteinuria may be associated with pressure interfering with the return of blood in renal veins) and Orthostatic or postural proteinuria (mainly occur in children or in adolescents, usually in age between 14 to18 when they are in upright position only) are considered as functional proteinuria. Most adolescents who are diagnosed with proteinuria through screening urinalysis do not have renal disease, and the proteinuria will usually resolve on repeat testing [Tietz, 2006 4th ed.].

2.1.5 Organic Proteinuria

Organic Proteinuria: - is suggestive of kidney disease in patients with diabetes mellitus, hypertension, primary renal disease, or other systemic illnesses. Organic (pathological proteinuria) can be further due to prerenal, renal and postrenal factors. [Tietz, 2006 4th ed.].

Pre-renal proteinuria implies significantly increased concentrations of protein are being presented to the kidney in the plasma that is filtered through a normal glomerulus with normal permeability to macromolecules (i.e., permselectivity). If these proteins are of low molecular weight, they will be filtered only if the concentration in the glomerular filtrate is sufficiently high, tubular reabsorptive processes will be overwhelmed. Examples of proteins that are filtered readily include: - immunoglobulin light chains, hemoglobin, and myoglobin. Some of these low molecular weight proteins are not detected by standard laboratory methods, which generally are more sensitive to albumin. [Tietz, 2008 6th ed. and Amir Said Alizadeh Naderi and Robert F. Reilly, 2008].

Renal proteinuria implies the defective renal function and/or inflammation of parenchymal kidney tissue which is the cause of the proteinuria. Active and acute renal parenchymal

inflammation, is associated with diseases such as pyelonephritis and acute tubular necrosis, may be suspected from the clinical history. Localization of the disease to the kidney may be possible based on physical examination findings (painful swollen kidneys on palpation, fever, renal failure), or resulting from the presence of tubular casts on urine microscopy. So that pathologic renal proteinuria is due to a renal abnormality in protein handling. It may occur from increased leakage of protein across the glomerulus either due to permselectivity defect causing glomerular proteinuria or abnormal tubular handling of filtered protein causing tubular proteinuria, or both [Tietz, 2008 6th ed. , Adams, LG et al, 1992 and Amir Said Alizadeh Naderi, and Robert F. Reilly, 2008].

Post-renal proteinuria is due to plasma proteins from hemorrhage or inflammation in which the urinary tract protein is added to the urine in the urinary tract after formation by the kidney (i.e. in the ureter, bladder or urethra). Inflammation of the urinary tract, resulting most commonly from bacterial infection, should be considered as a possible cause of proteinuria. Other causes include presence of uroliths and tumours, both of which may cause inflammation directly or may be associated with secondary bacterial infection [Tietz, 2008 6th ed. and Amir Said Alizadeh Naderi and Robert F. Reilly, 2008].

2.2 Cause of Proteinuria

Protein in the blood is normally unable to pass through the glomerular capsule due to their large size. In normal urine, protein concentration is very low (less than 100mg/day) which cannot be detected by usual tests. These proteins are screened by tubular epithelial cells. However, proteins can appear due to Prerenal, Renal, and Postrenal factors. It is stated that, there are three main mechanisms that cause proteinuria: disease in glomerulus (glomeruli proteinuria), increased quantity of proteins in serum (overflow proteinuria) and low reabsorption at proximal tubule, fanconi (tubular proteinuria).

Pathology for the proteinuria will not only be due to kidney diseases but also other associated disease conditions such as nephrotic syndromes (i.e. intrinsic renal failure), pre-eclampsia, eclampsia, toxic lesions of kidneys, membranous nephropathy, diabetes mellitus (diabetic nephropathy), Infections (e.g. HIV, syphilis, hepatitis, and poststreptococcal infection), certain biological agents, such as bevacizumab (Avastin) used in cancer treatment .and different type of

chronic kidney diseases. [Tietz, 2006 4th ed. and Tietz, 2008 6th ed.]. Some of the conditions that cause proteinuria are discussed below.

2.2.1 Proteinuria in chronic kidney diseases (CKD)

Accurate identification and quantification of proteinuria are core elements in the diagnosis and management of chronic kidney diseases (CKD). Proteinuria is associated with an increased risk of progressive kidney failure, cardiovascular disease and death; this is used to monitor the prognosis of kidney disease. [Taal MW, Brenner BM, 2007]

Table: 1 Stages of CKD, Irrespective of Cause [source: NKF-K/DOQI]

Stage	Description	GFR (mL/minute/1.73 m ²)
0	Kidney damage with normal or elevated GFR	≥90
1	At increased risk	≤60 (with CKD risk factors)
2	Kidney damage with mild decrease in GFR	60 to 89
3	Moderate decrease in GFR	30 to 59
4	Severe decline in GFR	15 to 29
5	Kidney failure	< 15 or Dialysis.

2.2.2 Proteinuria in Diabetes

People with type 2 diabetes can progress from proteinuria such as microalbuminuria to end-stage renal failure, though this outcome is rare in comparison with cardiovascular mortality and morbidity. Kidney disease in people with diabetes generally progresses from microalbuminuria (loss of small amounts of albumin in the urine) to macroalbuminuria (loss of large amounts of albumin in the urine), and eventually leads to loss of kidney function. However, people with type 2 diabetes do not necessarily follow this progressive and detectable sequence and often present with more advanced kidney disease. [Wisconsin Diabetes Mellitus Essential Care Guidelines, 2011 and National Institute for Clinical Excellence, 2002]

2.2.3 Proteinuria in Hypertension

Detecting an abnormal urinary excretion of protein is an essential part of the screening of hypertensive patients. The presence of increased levels of protein in the urine is associated with a higher rate of cardiovascular events and an increased morbidity and mortality. [Berton G, Cordiano R, Mbaso S, DE-Toni R, Mormino P, Palatini P, 1998]

As stated by Sibai et al., 1983 proteinuria associated with hypertension in pregnancy results in greater adverse maternal and fetal outcome. In pregnant women with mild chronic hypertension but no proteinuria, the outcome is similar to nonhypertensive pregnant women while hypertension together with proteinuria is associated with poor fetal outcome, an increased rate for small gestational age pregnancies, increased perinatal mortality and maternal morbidity.

Measurement of proteinuria in pregnant women with hypertension is important for the diagnosis and criterion for identifying disease severity. Significant proteinuria is defined by the International Society for the Study of Hypertension in Pregnancy as excretion of 300 mg of protein in a 24-hours urine specimen. Thus, the gold standard for diagnosis of significant proteinuria is based on a 24-hours urine collection [Lean~os-Miranda, 2007].

2.2.4 Proteinuria in preeclampsia

The diagnosis of preeclampsia is also determined by the presence of elevated blood pressure and significant proteinuria (≥ 300 mg per 24 hours) after the 20th week of gestation. The gold standard for measuring proteinuria is the 24-hours urine collection. Unfortunately, the 24-hours urine collection takes an entire day to collect and is, therefore, not available to guide clinical decisions upon first evaluation. A rapid screening test to predict 24-hours proteinuria, in combination with other presenting signs and symptoms, can help a clinician to determine the appropriate amount of surveillance and guide care during the initial 24-hours period [Dwyer et al, 2008].

2.3 Types of Protein in Urine

Healthy kidneys take wastes out of the blood but leave protein whereas impaired kidneys may fail to separate a blood protein from the wastes. At first, only small amounts of albumin may leak into the urine, a condition known as microalbuminuria, a sign of deteriorating kidney function. As kidney function worsens, the amount of albumin and other proteins in the urine increases, and the condition is called proteinuria [Paul E. and de Jong, 2011].

In the American Journal of Kidney Diseases, Methven et al shed some light on whether it is urinary albumin or total urinary protein that is more tightly linked to outcomes. “Predictors evaluated in this study indicates, baseline can be checked by measurements of sensitivity and specificity of microalbuminuria, albumin-creatinine ratio (ACR) and total protein-creatinine ratio (PCR) of 1 g/day varied substantially by age, gender and estimated glomerular filtration rate (eGFR) presuming this to be related to muscle mass [Paul E. and de Jong, 2011].

2.3.1 Microalbuminuria

The ADA and NKF define microalbuminuria as an Albumin: Creatinine ratio between 30 to 300 µg/mg in both men and women with 24-hours urine protein excretion or a urinary excretion rate of albumin between 20µg and 200µg/min [Paul E. and de Jong, 2011]. These guidelines do not take into account sex differences in creatinine excretion, and however, currently several researchers have advocated sex-specific cut points of the Albumin: Creatinine ratio to define microalbuminuria [Warram JH, Gearin G, Laffel L, Krolewski AS, 1996 and Connell SJ, Hollis S, Tieszen KL, McMurray JR and Dornan TL, 1994]. That is ACR >2.5mg/mmol (men) or >3.5mg/mmol (women) or albumin concentration >20mg/l.

According to NICE (2008) guideline, “Micro albuminuria” refers to albumin excretion above the normal range but below the level of detection by tests for total protein. When using the urinary Albumin and Creatinine, various factors affecting albumin and creatinine excretion need to be taken into account. Khosla et al. identify factors affecting albumin excretion include blood pressure, time of day, fasting, salt intake and volume status.

Microalbuminuria occurs when the kidney leaks small amounts of albumin into the urine, in other words, when there is an abnormally high permeability for albumin in the renal glomerulus [James GD, Sealey JE, Alderman M, Ljungman S, Mueller FB, Pecker MS, Laragh JH, 1988 and Goldwasser P, Aboul-Magd A, Maru M, 1997]. Microalbuminuria is an independent predictor of cardiovascular disease and CKD where it causes mortality in both diabetic and nondiabetic men and women. [Rossing P, Hougaard P, and Borch-Johnsen K, Parving H, 1996 and Ljungman S, Wikstrand J, Hartford M, Berglund G, 1996].

Table 2 Summary for Microalbuminuria [source: Wikipedia]

Collection Method	Lower Limit	Upper Limit
24-hours urine collection	30	300 mg/24hrs
Short-time urine collection	20	200 µg/min (microgram albumin per minute)
Spot urine sample	30	300 mg/L or µg/g
Spot urine ACR	Women:3.5	25 or 35 mg/mmol
	30	300 µg/mg
	Men: 2.5 or 3.5	25 or 35 mg/mmol

2.3.2 Albuminuria and/or Proteinuria

Albuminuria is having moderate protein in the urine. According to the ADA and NKDEP indicates pathologic albuminuria is equals to Urine (mg/dL) = urinary Albumin: Creatinine ratio in mg/g = (approximate) Albumin excretion in mg/day while inversely with correlated Glomerular filtration rate [Hoy WE, Mathews JD and Pugsley DJ, et al, 1998].

Albuminuria is also regarded a sensitive measure of progression of glomerular disease. When protein seeps into the urine (proteinuria), albumin accounts for about one-third of the total protein [National Institute for Clinical Excellence, 2002]. The presence of a large amount of albumin is associated with kidney disease but also other diseases such as heart failure, diabetes mellitus, high blood pressure (hypertension), lupus (systemic lupus erythematosus), sickle cell disease, infection, pre-eclampsia, HIV, and rheumatoid arthritis [McLaughlin, Kevin, Eds. Frank and C. Brosius, et al., 2001]

The excretion of specific types of protein, such as albumin or low molecular weight globulins, depends on the type of kidney disease that is present. According to NICE (2008) guideline, “proteinuria” defined as increased urinary excretion of albumin, other specific proteins, or total protein where “albuminuria” refers specifically to increased urinary excretion of albumin [National Institute for Clinical Excellence, 2002].

Table: 3 Proteinuria in chronic Kidney Diseases [Source: Susan Simmons Holcomb, 2004]

Collection Method	Normal	Abnormal
24-hour (excretion)	<30 mg/day <300 mg/day	Microalbuminuria = 30 to 300 mg/day Proteinuria ≥300 mg/day
Spot Urine Albumin-Specific Dipstick	<30 mg/dL	>30 mg/dl
Spot urine(ACR)varies by gender	Men≤17mg/g and Women≤25mg/g	Microalbuminuria: Men; 17 to 250 mg/g and Women; 25 to 355 mg/g Albuminuria: Men ≥250 mg/g and Women ≥355 mg/g
Spot urine (PCR)	<200 mg/g	>200 mg/g

2.3.3 Albumin: creatinine ratio (ACR)

According to MeReC Briefing (2004), Albumin: Creatinine ratio >30mg/mmol or albumin concentration >200mg/l. According to NICE, 2008 guidance has recommended that urinary Albumin (mg/dL): Creatinine (g/dL) ratio = urinary ACR in mg/g = (approximate) where Albumin excretion in mg/day. That is in diabetics: ACR >2.5 mg/mmol in men and >3.5 mg/mmol in women is considered clinically significant; in non-diabetics: ACR >30 mg/mmol (approx. equivalent to PCR >50 mg/mmol, 0.5 g/day) is considered clinically significant. Heavy proteinuria: ACR >70 mg/mmol (approx. equivalent to PCR of >100 mg/mmol, 1gm/day). Initial detection of proteinuria can be: if Albumin: Creatinine ratio >30 mg/mmol but <70 mg/mmol this should be confirmed by a subsequent early morning sample. If initial Albumin: Creatinine ratio is >70 mg/mmol (Protein: Creatinine ratio >100 mg/mmol) a repeat sample is not required.

2.3.5 Protein: Creatinine ratio (PCR)

Proteinuria is defined in terms of Protein: Creatinine > 45 mg/mmol (which is equivalent to Albumin: Creatinine > 30 mg/mmol) with very high levels of nephrotic syndrome being for a Protein: Creatinine > 100 mg/mmol [UK Renal Association, 2005]. The UK 2005, chronic kidney disease guidelines states spot Protein: Creatinine ratio measurement is a better test than 24-hours urinary protein measurement. The actual 24-hours protein excretion can be calculated from the urinary Protein: Creatinine ratio at all levels of proteinuria, using a simple formula as urinary PCR g per m² per day) = 0.63 × (urinary PCR) [Abitbol C, Zilleruelo G, Freundlich M, and Strauss J, 1990]

Consequently, the Cockcroft-Gault predicted creatinine excretion could easily have been calculated and the observed Protein: Creatinine ratio adjusted accordingly (i.e. by multiplying by predicted creatinine excretion). Performing such an adjustment should account for difference in muscle mass and remove any need for gender-, age- and weight-specific cut-points. Although adjusting for predicted creatinine excretion removes some of the simplicity of Protein: Creatinine ratio, it would be too high to pay for optimizing assessment of proteinuria. Of note, the first reports of Protein: Creatinine ratio assessment demonstrated correlations with 24-hours proteinuria adjusted for Cockcroft-Gault predicted creatinine excretion or body surface area (as an alternative surrogate for muscle mass) [Ginsberg JM, Chang BS, Matarese RA, et al, 1983 and Hallan SI, Orth SR, 2010].

2.4 Methods for Measurement of Proteinuria

Hillege et al., (2002) and Gerstein et al., (2001), found traditional quantitative 24-hours total urinary protein excretion has been used to quantify proteinuria. New method develop in proteinuria assessment have included the use of urinary albumin measurements. While small amounts of albumin can be detected in the urine of a healthy population, the term microalbuminuria has been used to refer to a range of urinary albumin excretion that is above the reference ranges but below amounts referred to as significant proteinuria [Wilmer WA, Rovin BH, Hebert CJ, Rao SV, Kumor K and Hebert LA, 2003]. There have been several advancements in the detection of proteinuria occurred since 19th century. Some of methods are discussed below.

2.4.1 Twenty-four–hours urine collections

Twenty-four–hours urine collection is the most accurate test for quantification of proteinuria and is also used for qualitative urinalysis. However, the test becomes inaccurate when difficulties with urine collection are encountered. To minimize collection and handling errors, patients should receive detailed instructions about 24-hours urine collection. A timed urine collection is often not collected properly. To determine the completeness of 24-hours urine collection, the total daily urine creatinine is often used as a standard. In patients younger than 50 years, normal daily urinary creatinine range is 15 to 20 mg/kg lean body weight for women and 20 to 25 mg/kg lean body weight for men. In older people, the production of daily creatinine is reduced, primarily because of decreased muscle mass. In the latter case, calculating the ratio of creatinine clearance measured by 24-hours collection to the estimated creatinine clearance from a serum sample can help ensure proper collection. A ratio of 0.9:1.1 suggests a complete 24-hours urine collection because both estimated and measured creatinine clearances are roughly equal. Although in many cases the timed urine collection is incomplete, measurement of urinary Albumin: Creatinine ratio and/or urinary Protein: Creatinine ratio from this urine collection is still helpful and may be more accurate than a spot urine specimen [Wilmer WA, Rovin BH, Hebert CJ, Rao SV, Kumor K and Hebert LA, 2003].

2.4.2 Dipstick Urinalysis (Quantitative)

Traditionally, dipstick protein tests would be quantified by measuring the total quantity of protein in a 24-hours urine collection test, and abnormal globulins by specific requests for protein electrophoresis [Ed. Friedlander, 2007].

Urinary dipstick testing is routinely performed in clinical practice. It is an inexpensive test that is used for screening of different disorders, including urinary, gastrointestinal, metabolic, and hematologic diseases. Besides quantification of proteinuria, urinary dipstick testing provides valuable information about other parameters, including urinary blood, glucose, ketones, pH, specific gravity, leukocyte esterase, and nitrite. The standard dipstick measures albumin concentration in the urine via a colorimetric reaction between albumin and tetrabromophenol blue, causing different color shading depending on the albumin concentration in the sample. If the urine is dilute (low specific gravity), urine albumin concentration is decreased and is occasionally not detected by dipstick testing. The most conservative approach would be to repeat the value and, if positive, to obtain a protein: creatinine ratio and/or albumin: creatinine ratio. Importantly, as dipsticks only measure albuminuria, other proteins like renally excreted immunoglobulin light chains in multiple myeloma are not detected. A positive urinary dipstick is a useful screening test for proteinuria. Its limitations are quantification of proteinuria and its inadequacy to detect immunoglobulin light chains [Amir Said Alizadeh Naderi and Robert F. Reilly, 2008].

Roy et al., (2003) described the first assessment of a Protein: Creatinine ratio dipstick. They tested a midstream urine of 171 hypertensive pregnant women with Protein: Creatinine ratio dipsticks on a validated Urinalyzer (Clinitek 50: Bayer diagnostics) and compared this with the use of visual and automated dipstick analysis using a 24-hours total protein measurement as the gold standard.

2.4.3 Semi-quantitative dipstick urinalysis

To increase the applicability of the use of the Albumin: Creatinine ratio and/or Protein: Creatinine ratio in clinical practice, semi-quantitative Protein: Creatinine ratio dipsticks have been developed.

Halligan et al., (1999) states the most commonly employed screening method for proteinuria antenatally is a semi-quantitative dipstick urinalysis. Studies of Gangaram et al., (2005) show that random semi-quantitative dipstick analysis in the diagnosis of proteinuria in pregnancy is imprecise and its value is questionable. False positive results may be seen in patients and inconvenience of over investigation and unnecessary interventions occur, while false negative results may be jeopardize in the health of the woman and her fetus.

Roy et al., (2003) also found the use of semi-quantitative urinary Albumin: Creatinine ratio dipstick analysis using point of care urinalyzer may offer significant advantages. These include decreasing the need for timed 24-hours urine collections, a reduced need for hospital admission and rapid availability of results with improved accuracy over other forms of dipstick urinalysis for proteinuria [Gangaram et al., 2005].

2.4.4 Random Spot Urine Samples

Price et al's, (2000) systematic literature reviewed that "Protein: Creatinine ratio on a random urine specimen provides significant proteinuria defined by a 24-hours urine specimen measurement." The use of the spot urine protein: Creatinine ratio has supplanted most of the 24-hours urine estimations done in both pediatric and adult nephrologist's clinical practice. The need for a 24-hours collection is due to the variation in protein excretion during the day. Factors that may contribute to this variation include variation in water intake and excretion, rate of diuresis, exercise, recumbency and diet. The major problem with a 24-hours protein collection is that it is often impractical in the outpatient setting with problems of incomplete collection. In order to overcome this, the spot Protein: Creatinine ratio has been proposed [Spencer JS, Silva D, Snelling P and Hoy WE, 1998].

Ginsberg et al., (1983) also found urinary protein and creatinine excretion rates are fairly constant during the day provided the glomerular filtration rate is constant. Thus, a ratio of the concentrations of urinary protein and creatinine in a single voided urine sample would reflect the cumulative excretion during the day since the ratio of two stable rates would cancel out the time factor.

The Protein: Creatinine ratio in random, untimed urine samples correlates with a 24-hours protein excretion in pregnant women with and without hypertension [Lean~os-Miranda, 2007].

Measurements of the Protein: Creatinine ratio in a single urine specimen may be a reliable and quick test to estimate a 24-hours protein excretion in a nonpregnant population because the ratio of 2 stable excretion rates (creatinine and protein) minimizes the time involved, thus providing a faster estimate of a 24-hours protein excretion [Ruggenti P, Gaspari F, Perna A and Remuzzi G 1998].

3. Objectives

3.1 General Objective

To review and recommend separately the usefulness in clinical practice of random spot urine of

1. urinary Microalbumin to Creatinine ratio (MACR) vs. 24-hours urine sample collection
2. urinary Albumin to Creatinine Ratio (ACR) vs. 24-hours urine sample collection
3. urinary Protein to Creatinine Ratio (PCR) vs. 24-hours urine sample collection
4. urinary Albumin: Creatinine ratio and total Protein: Creatinine ratio vs. 24-hours urine sample collection.

3.2 Specific Objectives

To review and recommend the method(s) for quantitating proteinuria using urinary Albumin: Creatinine ratio and/or Protein: Creatinine ratio on random urine samples vis-a-vis conventional 24-hours urine collection for diagnostic evaluation of Kidney function.

4. Materials and Methodology

4.1 Materials

I performed an electronic search of the Medline, using the key terms: - random spot "urinary Microalbumin: Creatinine ratio, Albumin: Creatinine ratio, Protein: Creatinine ratio," vs. "24-hour protein excretion" and "correlation coefficient, sensitivity, specificity, positive and negative predictive values".

4.2 Method

This Systematic review is compare and recommends quantitating proteinuria using urinary Albumin: Creatinine ratio and Protein: Creatinine ratio vis-a-vis proteinuria defined by 24-hours urine sample specimen. For the search, I use the keywords urinary spot “microalbumin (MACR)”, “albumin-creatinine ratio (ACR)”, “protein-creatinine ratio (PCR)” vs. “24-hours Protein excretion”.

Inclusion of papers in the data extraction stage was based on the following criteria: (1) diabetes, (2) hypertension, (3) pregnancy with hypertension, (4) pre-eclampsia (5) chronic kidney diseases. Dataset was limited to “human and women”. Exclusion Criteria: (1) Heart Failure, (2) Children and (3) Lupus Nephritis.

Other sources are the reference lists of Guidelines includes: NKF KDOQI Guidelines (National Kidney Foundation/ Kidney Diseases Outcomes Quality Initiatives, 2000), CARI Guidelines (Caring for Australians with Renal Impairment, 2004) and UK Chronic Kidney Diseases Guidelines for Identification, Management and Referral of Adults, 2005) for pregnancy, Diabetes, Chronic Kidney Diseases and hypertension are also used during the review.

5. Results

The initial electronic search yielded a total of 135 titles. Out of these, Guidelines (n=3), systemic review (n=2), articles with no results (n=39), unrelated (n=28), full electronic copies (n=30), animal model (n=7), genome study (n=4), abstract (n=17) and overlapped titles (n=5). After reviewing these titles for relevance, a total 8 papers were subsequently found to meet the inclusion criteria. The titles of the review were published between 2005 and 2010. A summary of the selection process of papers in the review is illustrated in Fig. 1.

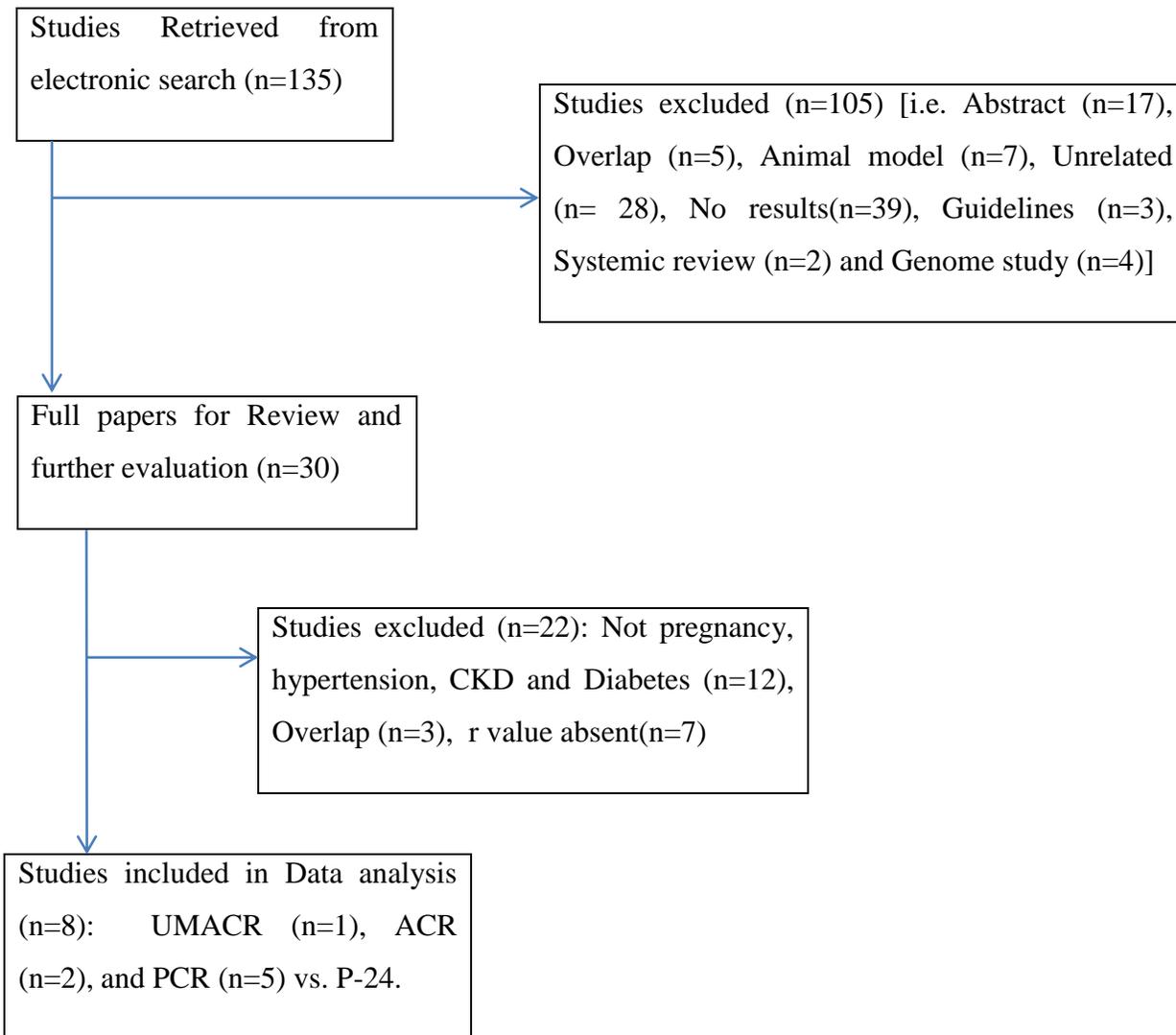


Fig. 1 Details of selection process of papers for Systemic Review from initial electronic search.

UMACR (urinary Microalbumin: Creatinine ratio), ACR (albumin: creatinine ratio), PCR (Protein: Creatinine ratio), P-24 (24-hours proteinuria), and vs. (versus).

Table: 4 Summary statistics of correlation (r) and p values of selected studies given here.

Authors, year (Ref.)	Patient group	No. of patients	Ratio studied	r	P
Horizon Scan Report, 2010 [51]	Diabetic patients	252	ACR vs. P-24-hrs	r = 0.87	P<0.0001
Dwyer et al. 2008 [52]	Pregnant women with hypertension	116	PCR vs. P-24-hrs	r = 0.83	P=0.0001
J.J.S. WAUGH et al. 2005[53]	hypertensive pregnancy	171	ACR vs. P-24-hrs	r = 0.7027	P <0.01
S. Methven et al. 2010[54]	CKD	6842	ACR, TPCR vs. P-24-hrs	r=0.84 for 0.5g/day	p<0.001
				r=0.91 for 1g/day	P=0.004
Rajesh Gangaram, 2008[55]	Normotensive (15)	26	MACR vs. P-24-hrs	r=0.743[0.56-0.927]	P<0.001
	Hypertensive (11)				
Shahbazian and H. Asl 2008[56]	pregnant women with preeclampsia	81	PCR vs. P-24-hrs	r=0.84	P<0.001
BK Yadav et al 2010[57]	Diabetic patient	144	PCR vs. P-24-hrs	r =0.892	P <0.001
Lean~os-Miranda et al.,2007 [58]	Pregnant women with /out hypertension.	927	PCR vs. P-24-hrs	r=0.98	P <0.001

Urinary MACR (Microalbumin: Creatinine ratio), ACR (Albumin: Creatinine ratio), PCR (Protein: Creatinine ratio), TPCR (total Protein: Creatinine ratio) and P-24-hrs (24-hours Protein excretion).

Table: 5 Reported cut-off points and summary measures of diagnostic accuracy are given below.

Authors, year (Ref.)	Reported cut-off point		Sensitivity (%)	Specificity (%)	Predictive Values	
	mg/day	mg/mmol	Sn	Sp	PPV	NPV
Horizon Scan Report, 2010 [51]	30mg/day	3.4mg/mmol(32mg/g)	75%	94%	76%	94%
		20mg/L	92%	100%	93%	100%
Dwyer et al., 2008 [52]	≥300mg/day	≥0.15	96%	53%	66%	94%
		≥0.28	66%	95%	93%	75%
	≥500mg/day	≥2	100%	96%	38%	100%
		≥13.53	67%	100%	100%	99%
J.J.S. WAUGH et al., 2005[53]	≥300mg/day	2.0mg/mmol	94%	94%	92%	95%
S. Methven et al., 2010 [54]	1g/day	TPCR(100 mg/mmol)	93.9	88.5	71.0	98.0
		ACR (70 mg/mmol)	79.0	95.2	83.5	93.8
	0.5g/day	TPCR (50 mg/mmol)	91.3	87.5	82.5	94.0
		ACR (30 mg/mmol)	78.2	94.6	90.0	87.5
Rajesh Gangaram, 2008[55]	30-300 mg/day	< 30mg/g	67%(15)	92%(15)	67%(15)	92%(15)
			100%(11)	83%(11)	83%(11)	100%(11)
Shahbazian& H-A, 2008 [56]	≥300mg/day	0.20	91.2%	87.8%	94.4%	96.8%
BK Yadav et al, 2010 [57]	150 mg/day	0.15mg/mg	95.6	74.5	61.1	97.6
Lean~os-Miranda et al.,2007 [58]	≥300mg/day	≥0.30	98.2%	98.8%	97.2%	99.2%
	≥2g/day	1.45	100%	97%	100%	76.6%

CKD (chronic kidney diseases), Sn. (Sensitivity), Sp. (Specificity), PPV (Positive Predictive Values), NPV (Negative Predictive Values), mg/mmol (milligram per millimole), mg/day (milligram per day),

PCR (Protein: Creatinine ratio), TPCR (total Protein: Creatinine ratio) and ACR (Albumin to Creatinine ratio)

Correlation statistics

From the studies reviewed, calculated correlation coefficients between the spot urinary Microalbumin: Creatinine ratio vs. 24-hours urine specimen collection (n=1) was 0.743[0.56-0.927], Albumin: Creatinine ratio vs. 24-hours urine specimen collection (n=2) was 0.79 (0.87, 0.71), Albumin: Creatinine ratio and total Protein: Creatinine ratio vs. 24-hours urine specimen collection (n=1) was 0.84 for 0.5g/day and $r=0.91$ for 1g/day and protein: Creatinine ratio vs. 24-hours urine specimen collection (n=4) was 0.8855 [0.83, 0.84, 0.892 and 0.98].

Estimates of sensitivity and specificity

The sensitivities and specificities of the 8 studies are given in table 7. Because of dissimilarities in the study patient, ratio used, different cutoff points and unit used, summary estimates of sensitivity, specificity, positive and negative predictive values have been put in reference ranges. Therefore, sensitivities, specificities of 8 studies were 83% (ranged 66% to 100%) and 76.5% (ranged 53% to 100%), respectively, whereas positive and negative predictive values were 80.5% (ranged 61% to 100%) and 87.5% (ranged 75% to 100%), respectively. Positive and Negative predictive values were used to determine the ability of a random urine Albumin: Creatinine ratio and/or Protein: Creatinine ratio to predict significant proteinuria defined by conventional 24-hours urine sample collection.

6. Discussions

An increase in urinary protein and albumin excretion is a widely accepted tool in the detection, diagnosis, and management of people that are considered to be at high risk of developing renal disease and has been advocated as part of a regular check-up.

From the results, the spot urinalysis of hypertensive pregnant women, chronic kidney disease and diabetic patients have different cut-off points, which seemed to be quite different; this is because of the variability in the units used for cut-off points [mg/mmol, mg/ g, mg/mg, and g] and the ratio used for analysis. The ratios of spot Albumin and Protein to Creatinine against 24-hours urine collection used for diagnostic accuracy of kidney diseases were clearly discussed below.

1. Urinary Spot Microalbumin: Creatinine ratio (MACR) vs. 24- hours urine collection

From tables 4 and 5, correlation coefficients between the urinary spot Microalbumin: Creatinine ratio vs. 24-hours urine specimen collection (n=1) was 0.743[0.56, 0.927] and Sensitivity, specificity, positive and negative predictive values of urinary spot Microalbumin: Creatinine ratio vs. 24-hours urine collection in 26 pregnant women [15-normotensive and 11-hypertensive] with cutoff point <30mg/g were 67%, 92%, 67%, and 92% respectively in normotensive pregnant women whereas 100%, 83%, 83%, and 100% in hypertensive pregnant women respectively.

These results is supported by Ulla Derhaschnig, Andreas Bur, Harald Herkner and Michael M. Hirsch (2001), where the diagnostic performance for hypertensive patients, microalbumin measurement alone expressed as area under the curve (AUC) was 0.94 (95% CI 0.90–0.98) and 0.94 (95% CI 0.89–0.97) for Albumin: Creatinine ratio as overall. The PPV and NPV were 44.2 and 97.9% for microalbumin measurement alone and 29.3% and 96.2% for males whereas 42.9 and 98% for females for Albumin: Creatinine ratio when a cut-off value 2.5 mg/mmol and 4.0 mg/mmol was used for males and of for females respectively. That is urinary albumin measurement alone has the recommended threshold of 30 mg/l for microalbumin in the spot urine sample exhibited the highest diagnostic performance, (with highest sensitivity and specificity).

Also J. Incerti et al. (2005) found the first step in the diagnosis of diabetic nephropathy is measuring albumin in a spot urine sample. That is screening for microalbuminuria should be the

measurement of albumin in a urine sample by a reliable method: spot (first-morning or random sample), 24-hours urine collection or timed collection. They found that the correlation coefficient (in 278 urine samples) are 0.76 for urinary Albumin excretion rate vs. urinary Albumin and Creatinine ($P<0.0001$); 0.74 for urinary Albumin excretion rate vs. urinary Albumin: Creatinine ratio ($P<0.0001$); and 0.86 for urinary Albumin: Creatinine ratio vs. urinary Albumin and Creatinine ($P<0.0001$). Sensitivity and specificity values of urinary Albumin and Creatinine and urinary Albumin: Creatinine ratio based on Receiver Operator curve analysis ($n=278$), considering two cutoff points for the diagnosis of microalbuminuria (first point with 100% sensitivity and point of equilibrium between sensitivity and specificity). The specificity of urinary Albumin and Creatinine and urinary Albumin: Creatinine ratio was similar when considering the 100% sensitivity cutoff points.

2. Urinary spot Albumin: Creatinine ratio (ACR) vs. 24-hours urine collection

From tables 4 and 5, correlation coefficients between the urinary spot Albumin: Creatinine ratio ($n=2$) vs. 24-hours urine specimen was $r= 0.79$ (0.87, 0.71) and Sensitivity, specificity, positive and negative predictive values of spot Albumin: Creatinine ratio vs. 24-hours urine collection in 152 diabetic patients with cutoff point 3.4mg/mmol (32mg/L) were 75%, 94%, 76% and 94% respectively whereas with cutoff point 20mg/L 92%, 100%, 93% and 100% respectively.

Sensitivity, specificity, positive and negative predictive values for spot Albumin: Creatinine ratio vs. 24-hours urine collection in 171 hypertensive pregnant women with cutoff point 2mg/mmol were 94%, 94%, 92% and 95% respectively.

These results are supported by T. Pfab, U. Franz; F. Herfeld (2006), with different cut-off points for the randomly chosen samples. For cutoff point >18 mg/l measured by PreventID® Albumin test, a sensitivity of 89.1% and a specificity of 90.9% whereas a positive predictive value of 94.2% and a negative predictive value is not exact but gives an idea of the test performance.

Also Dr. Hiddo J. Lambers Heerspink (2010) found baseline proteinuria measures, a positive continuous relationship with the risk for doubling of serum creatinine (DsCR) or end-stage renal disease (ESRD). The effect size of the first-morning void Albumin: Creatinine ratio was higher than the other proteinuria measures. The first-morning void Albumin: Creatinine ratio

demonstrated the steepest increase in the risk for DsCR or ESRD compared with the other proteinuria measures.

For prediction of DsCR or ESRD, the area under the curve (AUC) of the first-morning void Albumin: Creatinine ratio was significantly higher compared with the three other proteinuria measures. The AUC of the first-morning void Albumin: Creatinine ratio was also largest in age-, gender-, and race specific subgroups, although not statistically significant in all subgroups, which is possibly because of the small number of events in some subgroups. The Area under the curve for urinary albumin concentration in a first-morning void was equal to 24-hours urinary albumin excretion and protein excretion and no difference was observed between 24-hours urinary albumin excretion and protein excretion.

3. Urinary spot Protein: Creatinine ratio (PCR) vs. 24-hours urine collections

From tables 4 and 5, correlation coefficients between the urinary spot protein: Creatinine ratio vs. 24-hours urine specimen are (n=4) was $r=0.8855$ [0.83, 0.84, 0.892 and 0.98] and Sensitivity, specificity, positive and negative predictive values of spot Protein: Creatinine ratio vs. 24-hours urine collection in 144 Diabetic patients with cutoff point 0.15mg/mg were 95.6%, 74.5%, 61.15% and 97.6% respectively.

Sensitivity, specificity, positive and negative predictive values of spot Protein: Creatinine ratio vs. 24-hours urine collection in 1124 pregnant women with/without hypertensive and pregnant women with pre-eclampsia were 83% (ranged 66% to 100%), 76.5% (ranged 53% to 100%), 69% (ranged 38% to 100%) and 87.5% (ranged 75% to 100%) respectively.

These are supported by Dwyer et al. (2008) where spot urinary Protein: Creatinine ratio had better discriminatory power than urinalysis with cutoff ≥ 0.28 had Sensitivity, specificity of 66% and 95%, $P = 0.001$ and with cutoff point (cutoff $\geq 1+$) had 41% and 100% respectively. That is, it had clinically relevant specificity with is more sensitive than urinalysis. They also observed that dipstick urinalysis measures the concentration of protein in the urine and is susceptible to fluctuations in the water content of the urine where dilute urine may underestimate the amount of protein that would be collected in a 24-hours urine collection, whereas concentrated urine may overestimate it.

They also highlight two clinically useful cutoffs, one that maximizes sensitivity and the other that maximizes specificity. For example, cutoff of ≥ 0.15 had a sensitivity of 96% (95% CI 87 to 99%) and a specificity of 53% (95% CI 40 to 66%). A cutoff of ≥ 0.28 had a sensitivity of 66% (95% CI 52 to 78%) and a specificity of 95% (95% CI 86 to 99%) [52]. Therefore, in pregnant women, urinary Protein: Creatinine ratio vs. a 24-hours urine is highly correlated. It compares the spot urine protein excretion to the spot urine creatinine excretion, thereby normalizing protein excretion to the glomerular filtration rate. Thus, urinary Protein: Creatinine ratio is not subjected to variation due to hydration status.

Another study by Shahbazian and Hosseini-Asl (2008) also shows that there was a strong correlation between the spot Protein: Creatinine ratio vs. a 24-hours urine protein excretion ($r = 0.84$; $P < .001$). The optimal spot Protein: Creatinine ratio cutoff point was 0.20 for 300 mg/24-hours of protein excretion (preeclampsia), with a sensitivity, specificity, positive predictive value, and negative predictive value of 91.2%, 87.8%, 94.4%, and 96.8%, respectively.

Other studies by BK Yadav et al. (2010) also found a very good correlation was seen between the 24-hours urine protein and spot Protein: Creatinine ratio, with correlation ($r = 0.892$, $p < 0.001$). Sensitivity and specificity of Protein: Creatinine ratio to detect proteinuria at various cutoffs, for examples, for 0.14mg/mg, sensitivity, specificity, positive predictive value and negative predictive values were 95.6%, 69.1%, 56.4% and 97.4% respectively. For Cutoff 0.22 mg/mg, sensitivity, specificity, positive predictive value and negative predictive values were 82.6%, 76.4%, 59.4% and 91.3% respectively.

4. Urinary spot Albumin: Creatinine ratio (ACR) and Protein: Creatinine ratio (PCR) vs. 24-hours urine collections.

From tables 4 and, correlation coefficients between the urinary spot Albumin: Creatinine ratio and total Protein: Creatinine ratio vs. 24-hours urine specimen collection ($n=1$) was 0.84 for 0.5g/day and 0.91 for 1g/day and Sensitivity, specificity, positive and negative predictive values of spot Albumin: Creatinine ratio and Protein: Creatinine ratio vs. 24-hours urine collections in 6842 patients with Chronic Kidney Diseases had different values. For cutoff point of 1g/day of Protein: Creatinine ratio (100mg/mmol) and Albumin: Creatinine ratio (70mg/mmol) had Sensitivity, specificity, positive and negative predictive values of 86% (93.9% and 79%), 91.85% (88.5% and 95.2%), 77.25% (71% and 83.5%) 96% (98% and 93.8%) respectively. For

cutoff point of 0.5g/day for Protein: Creatinine ratio (50mg/mmol) and Albumin: Creatinine ratio (30mg/mmol) had Sensitivity, specificity, positive and negative predictive values of 85% (91.3% and 78.3%), 91% (87.5% and 94.6%), 86.25% (82.5% and 90%), 90.75% (94% and 87.5%) respectively.

It is also supported by Bhavna Pandya and Katharine Hayden (2006), 100 urinary specimens of nephrology referral of patients for Spot urinary Albumin: Creatinine ratio (ACR) and Protein: Creatinine ratio (PCR) vs. 24-hours urine collections with cutoff point >1 g/day. Sensitivity, specificity, positive predictive and negative predictive value for PCR> 100 were 100%, 81%, 93%, 83% and 88% respectively. Sensitivity, specificity, positive predictive and negative predictive value for Albumin: Creatinine ratio >45mg/mmol were 95%, 100%, 100% and 96% respectively. Sensitivity, Specificity, positive predictive value and negative predictive values for ACR>60mg/mmol were 75%, 100%, 100% and 87% respectively.

Also S. Methven et al. (2010), found that total Protein: Creatinine ratio is highly correlated with 24-hours urine protein ($r = 0.91$), though Albumin: Creatinine ratio also performs well ($r = 0.84$). So for total Protein: Creatinine ratio and Albumin: Creatinine ratio (100 mg/mmol and 70 mg/mmol) to predict 1 g/day of total proteinuria had higher sensitivity (94% and 79%) but lower specificity (88% and 95%) respectively.

7. Conclusions and Recommendations

7.1 Conclusion

On the basis of data analyzed in this systematic review, it can be stated that the method for quantitating proteinuria using spot urine specimen of Microalbumin: Creatinine ratio, Albumin: Creatinine ratio and Protein: Creatinine ratios have correlated significantly with conventional 24-hours urine specimen collection. The analysis has also shows that Protein: Creatinine ratio has better sensitivity compared to Albumin: Creatinine ratio in predicting significant Proteinuria.

It is recommended that the short or spot urine collection method using Microalbumin: Creatinine ratio, Albumin: Creatinine ratio, Protein: Creatinine ratios which can be more sensitive and reliable for the quantitating proteinuria defined by conventional 24-hours urine collection.

7.2 Recommendations

1. It is strongly recommended that using Spot urine sample measurement is more reliable, sensitive and accurate as compared to 24-hours urine collection.
2. It is better to use instruments that have high sensitivity and specificity in detection of low concentrations of urinary albumin (microalbuminuria).
3. Results should be replicated prospectively and examined in other populations and subgroups using other total proteinuria and albuminuria assays as predictors of patient with kidney diseases.
4. Expertise should be involved while collecting urine since patients could have made collection errors during the 24-hours urine collection and patients should oriented before collection.
5. Further research is required to clarify the use of spot urine collection of Albumin: Creatinine ratio and/or Protein: Creatinine ratio which provides enough evidence for Proteinuria as proteinuria defined by a 24-hours urine specimen collection.

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