A STUDY ON ROLE OF SPOT URINE PROTEIN CREATININE RATIO IN QUANTIFICATION OF PROTEINURIA

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BRANCH I

GOVERNMENT MOHAN KUMARAMANGALAM
MEDICAL COLLEGE, SALEM.

APRIL 2012

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LIST OF ABBREVIATIONS

ACE	-	Angiotensin converting enzyme
ANA	-	Anti nuclear antibody
APRI	-	ACE inhibitor in Progressive Renal Insufficiency
CRF	-	Chronic renal failure
ELISA	-	Enzyme linked immunosorbent assay
GBM	-	Glomerular basement membrane
GFR	-	Glomerular Filtration Rate
HIV	-	Human Immuno deficiency virus
HBV	-	Hepatitis B Virus
HCV	-	Hepatitis C virus
HTN	-	Hypertension
IHD	-	Ischemic heart disease
IgA	-	Immunoglobulin A
KDOQI	-	Kidney Disease Outcomes Quality Initiative
MCD	-	Minimal change disease
MDRD	-	Modification of Diet in Renal Disease
MPGN	-	Membranoproliferative glomerulonephritis
NSAIDS	-	Non steroidal anti inflammatory drugs
P/C Ratio	-	Protein-creatnine ratio
RBC	-	Red blood cells
REIN	-	Ramipril Efficacy in Nephropathy
RIA	-	Radio immune assay
SSA	-	Sulpho salycilic acid
SSPS	-	Statistical Package For Social Sciences
UPEP	-	Urine protein electrophoresis
VDRL	-	Venereal disease research laboratory

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ABSTRACT

Back Ground and Objective:

Persistent proteinuria is usually a marker of kidney damage. Quantifying protein in urine is commonly used in the diagnosis of kidney diseases, detection of treatment effects and evaluation of prognosis. Commonly used methods to quantify proteinuria is 24 hours urine collection, which is time consuming cumbersome and often in accurate. The other method of quantifying proteinuria is from Protein-Creatinine ratio. Objective of the study was to compare spot urine protein- creatinine ratio with 24 hours urine protein in the quantitative assessment of proteinuria in patients with varying degrees of renal dysfunction. This study also attempts to assess the best timing for collection of urine for estimation of protein creatinine ratio.

Method:

55 patients with persistent dipstick positive proteinuria with varying degrees of renal dysfunction were included in this study. Two urine samples were collected ,one in the early morning(around 7AM) and other in the evening (7 PM).Both samples were used to estimate protein- creatinine ratio and then a 24 hours urine protein estimation was done and compared.

Results:

There was good positive correlation between spot urine Protein Creatinine ratio of both samples taken at two different times of the day and estimated 24 hours urinary protein though the best correlation was seen in early morning urine sample than evening sample(r=0.931 in 7AM &r=0.872 in 7 PM; $p < 0.01$). The maximum correlation was seen in patients with normal/mild renal dysfunction and non nephrotic range proteinuria. The positive correlation was least in patients with moderate/severe renal dysfunction and nephrotic range proteinuria.

Conclusion :

Urine protein Creatinine ratio is easy to perform, inexpensive and less time consuming method for measuring of proteinuria. It can thus be used in the outpatient setting for screening and quantification of proteinuria.

Key words: Protein - Creatinine ratio, Proteinuria, urine analysis, 24 hours urinary protein.

INTRODUCTION

Proteinuria is a condition in which urine contains an excess amount of proteins. Normal individuals usually excrete very small amounts of protein in the urine (less than 150 mg/day).[1] Evaluation of proteinuria are often triggered by a positive dipstick on routine urine analysis.[2] Proteinuria has fast become a common presentation of renal disease since advent of dipstick testing for protein became widely available, and there is widespread screening of apparently healthy individual. Urine is tested routinely during medical consultations for any complaint, during pregnancy, during insurance examinations, and on entry into many forms of employment such as the armed forces.[3] The prevalence of proteinuria on a routine screening of healthy subjects has been found to be as high as 25%.[4] Considerable evidence accrued over the past decade suggests that the presence of even small quantities of protein or albumin in the urine is an important and early sign of kidney disease and has been shown to be early predictor of increased risk for cardiovascular mortality and morbidity in certain high risk groups.[5] Persistent proteinuria of more than 1.0 gm/day in any adult is not only suggestive of the existence of renal disease but also an increased risk of myocardial infarction and stroke.[6] In particular, an increase in protein excretion is of diagnostic and prognostic significance in the detection and confirmation of renal disease. Quantification of the same may be of considerable value in assessing the effectiveness of therapy and the progression of the disease[7]. So quantification of proteins in urine is very important. It is imperative that all patients with proteinuria be carefully evaluated to identify the etiology of proteinuria.

Current methods for measuring proteinuria vary significantly. Commonly used methods are dipstick urine analysis, 24 hours urine protein estimation and spot urine protein creatinine ratio. The most common method used for the quantification of proteinuria is estimation of 24 hours urinary protein in a urine specimen collected over 24 hours. However, 24 hours urinary protein estimation method has certain pitfalls and thus leads to discarding of nearly 1/3rd of the samples. To obviate these difficulties short timed urine collection have been advocated with the hypothesis that protein excretion is nearly constant throughout the day. Few Indian studies have compared the efficacy of 24 hours urine protein with spot urine protein creatinine ratio , which this study attempts to do.

OBJECTIVES

1. To compare spot urine protein creatinine ratio with 24 hours urine protein in the quantitative assessment of proteinuria in patients with varying degree of renal dysfunction.

2. To assess the ideal time for collection of urine sample for the estimation of spot protein creatinine ratio.

REVIEW OF LITERATURE

Definition:

Proteinuria is a common finding in primary care practice. 2400 years ago Hippocrates noticed the association between "Bubble on the surface of urine" and kidney disease.[8,9] Proteinuria is defined as urinary protein excretion of greater than 150 mg per day.[1] Normally urine contains less than 150 mg protein per day, with only 20% of it being albumin (less than 30 mg/d or 20 μg/min) and 40% being Tamm-Horsfall mucoproteins, which are secreted by the distal nephron.[10] Clinically proteinuria manifest only when the excretion is greater than several grams per 24 hours. Heavy proteinuria causes the urine to be frothy because proteins lower the surface tension of the urine thereby permitting relatively stable foam to form.[3] The appearance of frothing in urine may be a valuable clue in the history in the evaluation of proteinuria.

PHYSIOLOGICAL AND PATHOLOGICAL BASIS OF PROTEINURIA

Glomerular retention and leakage of protein molecules

Normal barriers to protein filtration begin in the glomerulus. The glomerular barrier to filtration consists of three layers: fenestrated endothelial cells, the trilaminar glomerular basement membrane (GBM), and the epithelial cell layer. [11] The glomerular basement membrane has been considered a major barrier to filtration . The epithelium does not constitute a continuous layer; rather, the interdigitating extensions from adjacent epithelial cells or podocytes that are separated by spaces.[12] The glomerular basement membrane traps most large proteins (>100Kda), while the foot process of epithelial cells (podocytes) cover the urinary side of the GBM and produce a series of narrow channels (slit diaphragm) to allow passage of small solutes and water.[2] These slit diaphragm bridges the slits between the foot process of the glomerular basement membrane.[13,14]

Size selectivity

A molecular size greater than 1.5 nm serves as a cut-off of glomerular filtration of proteins with increasing molecular radius. The urinary clearance (sieving co efficient) of albumin is normally less than 0.01 per cent of water, whilst the clearance of proteins such as smaller immunoglobulin light chains approaches that of the glomerular filtration rate (GFR).[3.]

Charge selectivity

Although proteins are handled in a manner similar to that for inert macromolecules, protein clearances tend to be less than those of dextrans of comparable size. Albumin which has an effective molecular radius of 3.6 nm, carries a negative electrostatic charge, and its clearance is only slightly less than that of similarly sized dextran carrying a negative charge. This charge selectivity almost certainly arises from the high density of negative charges present on the structures of the glomerular capillary wall, principally heparan sulphate.[14]

Thus the glomerular wall can be pictured as having two filtration barriers in series; an inner, charge-dependent electrostatic barrier on the surface of endothelial cells and the inner basement membrane; and a more external, mainly size-selective barrier in the outer basement membrane and slit diaphragms of the foot processes.[3]

Tubular Handling of proteins

Renal protein handling also is affected significantly by tubular function. A proximal tubular system has sufficient capacity that, under physiologic conditions, little intact protein from the filtrate is present in the urine.[3] This occurs predominantly , if not exclusively in the proximal tubular cells by endocytosis, with digestion of the absorbed protein within intracellular vesicles. Proteinuria in this disease results from disruption of both receptor-mediated and fluid-phase endocytosis.

PATHOPHYSIOLOGICAL CLASSIFICATION OF PROTEINURIA

A) BENIGN / INTERMITTENT

1. Postural / Orthostatic proteinuria
2. Functional
3. Transient idiopathic

B) PATHOLOGICAL /PERSISTANT

1. Glomerular
2. Tubular
3. Overflow
4. Secretory
5. Post Renal

FIGURE 1 : FUNCTIONAL ANATOMY OF KIDNEY

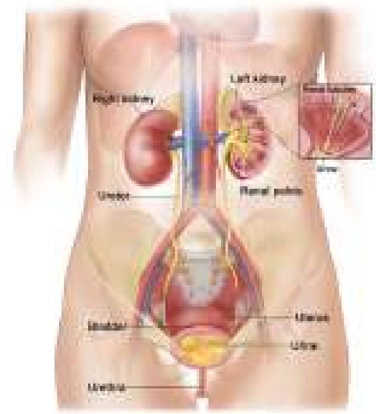


FIG 1-A Anatomical Relations of Kidney

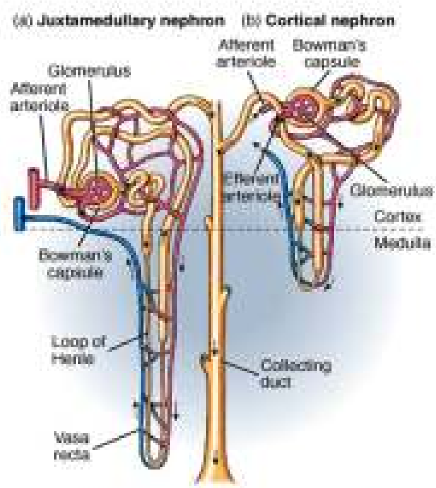


FIG 1 B :Single nephron

FIGURE 2 A: DIAGRAMMATIC REPRESENTATION OF GLOMERULAR BASEMENT MEMBRANE

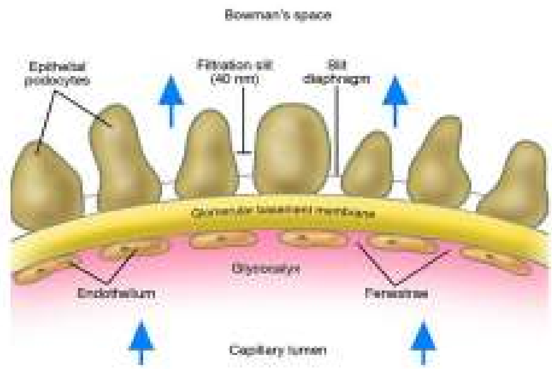


FIGURE 2B: HISTOLOGY OF NORMAL GLOMERULUS

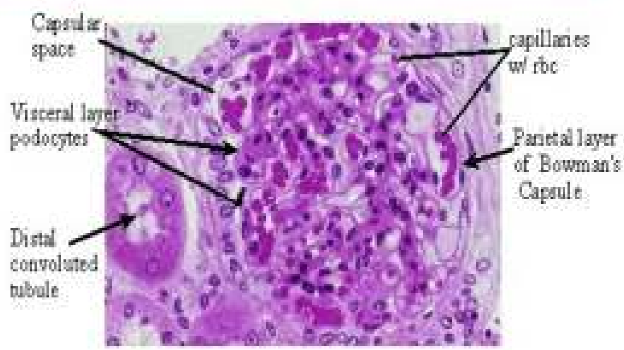
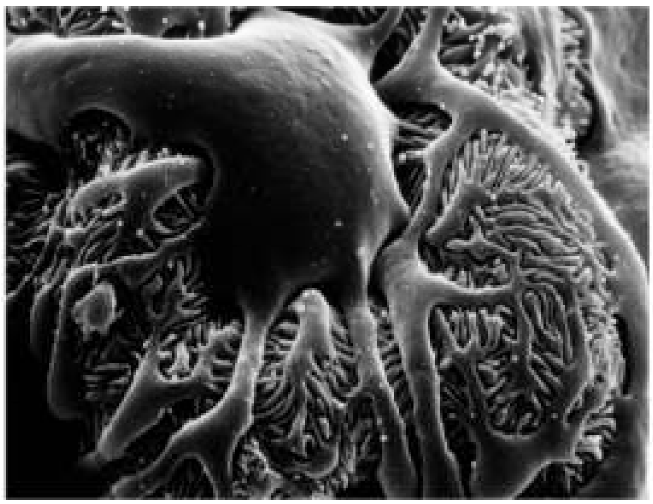


FIGURE 3 : ELECTRON MICROSCOPY OF GLOMERULAR BASEMENT MEMBRANE



A) BENIGN PROTEINURIA

This is a transient proteinuria that occurs despite normal renal function, bland urinary sediment, normal blood pressure and without any significant edema.24 hour urinary albumin is usually less than 1 gm. It may not be indicative of significant renal disease and usually disappears on repeated testing.

1) Orthostatic Proteinuria

Also called POSTURAL PROTEINURIA, it may be seen in 3 to 5% of adolescents[1], especially in young males. Postural or orthostatic proteinuria occurs in individuals only while they are in the upright or lordotic position; the first morning voided specimen is invariably normal in protein content in these individuals.[15] It is diagnosed by split urine protein excretion examination. In orthostatic proteinuria, the urine collected during the day typically has an increased concentration of protein, while nocturnal specimen has a normal concentration . A similar postural variation may be observed in true glomerular disease. However the proteinuria fails to return to normal in the latter. The precise mechanism is unknown, but postural proteinuria is glomerular in origin and may be related to increased renal venous or lymphatic pressures, or both, with the upright position.[16]

Springberg et al [17] concluded that the long term prognosis of orthostatic proteinuria was benign in virtually all cases over years of follow up. Studies have also shown that patients with orthostatic proteinuria retained normal function even at the end of 20-50 years of follow up.[18]

2) Functional Proteinuria

Functional proteinuria is characterized by excess excretion of urinary protein in the absence of renal disease. Functional proteinuria may accompany a febrile illness, strenuous exercise, cold or emotional stress, congestive heart failure, seizures, abdominal operations, and therapy with sympathomimetic drugs. Renal vasoconstriction has been implicated as the primary mechanism of this type of proteinuria.[20] It is usually less than 0.5 gm/day but may be as heavy as 5.0 gm/day (following heavy exercise, marathon running). It disappears with the resolution of causative disorder. [19]

Kallmeyer et al [21] found that recent exercise can induce loss of several gram of protein per liter of urine, sometimes together with accompanying haematuria and casts. Hence the term called jogger's nephritis.[22] **Poortmans et al** [23] found that proteinuria was influenced mostly by the intensity of exercise rather than its duration.

3) Idiopathic Proteinuria

This is seen in young healthy adults. This dipstick positive proteinuria disappears spontaneously by next clinical visit.

B) PATHOLOGICAL PROTEINURIA

Persistent proteinuria that is detected on multiple ambulatory clinical visits is pathological. This is seen in both recumbent and upright position and usually signals a structural renal disease

1) Glomerular Proteinuria

It is the most common cause of proteinuria in clinical practice. The glomerular filter becomes more permeable to proteins of large molecular size in addition to those of low molecular weight. It is characterized by a disproportionate amount of albumin in urine.[24] Due to preservation of selectivity and large concentration of albumin in blood, glomerular proteinuria is 85 to 90 % albumin, accompanied by pre- albumin, orosomucoid, transferrin . They are readily detected by stick or turbidometric methods. Glomerular proteinuria may be only a few hundred mg/24 h. On rare occasions , it may be as much as 100 g/24 h. **McConnell et al** [25] on evaluation of proteinuria found that urinary excretion of more than 2 gm per 24 hours was indicative of glomerular disease. In glomerular proteinuria there is increased glomerular capillary permeability to high molecular weight anionic plasma proteins. The leakage of proteins across the damaged glomerular barrier may be due to[26]

- Increase in glomerular capillary pressure.
- Detachment of epithelial podocytes from basement membrane[27].
- Immune aggregates
- Loss of fixed anionic charge (Congenital nephrotic syndrome, minimal change nephropathy)

The filtered protein, that reach the tubules overwhelm the limited capacity of tubular reabsorption and cause these proteins to appear in urine.

Glomerular proteinuria is of two types:

1. Selective Proteinuria
2. Nonselective Proteinuria

If proteins of moderate molecular mass (40,000-90,000Da) such as albumin are present in the urine, the glomerular defect is termed "selective." However, if moderate and high-molecular-mass proteins are present in the urine, the glomerular defect is termed "non-selective".[3] In selective proteinuria the clearance ratio of immunoglobulin to albumin or transferrin is less than 0.10(<10%).In nonselective proteinuria the clearance ratio of immunoglobulin to albumin or transferrin is more than 0.50(>50%).

Glomerular proteinuria - causes

I. *Hereditary*

- Congenital Nephrotic Syndrome (Podocyte/slit-diaphragm protein mutations)
- Alport Syndrome
- Fabry's disease

II. *Non-Hereditary*

1 Acute Glomerulonephritis(GN)

- Poststreptococcal Glomerulonephritis
- Hemolytic Uremic Syndrome
- Henoch-Schoenlein Purpura

2. Chronic Glomerulonephritis

A) Primary Glomerulonephritis

- Minimal Change Disease
- Focal Segmental Glomerulosclerosis
- Mesangioproliferative GN
- Membranous GN
- Membranoproliferative GN

B) Secondary Glomerulonephritis

- Berger (IgA) Nephropathy
- Goodpastures Nephropathy
- SLE Nephropathy
- Wegeners Nephropathy
- Diabetic Nephropathy
- Renal Vein Thrombosis
- Sickle Cell Disease
- Infections

  HIV,Hepatitis B and C.,Syphilis, Malaria Infective endocarditis
- Drugs and toxins

  NSAIDS, Penicillamine, Lithium, Heroin , Heavy metals

2) Tubular Proteinuria

Tubular protein resorption is an energy-dependent, nonselective, competitive process whereby proteins in the ultrafitrate are reabsorbed and catabolised. If proximal tubular reabsorption is impaired then normally filtered serum proteins appear in the urine in increased amounts. Primary tubular proteinuria is characterized by incomplete reabsorption of low-molecular-mass proteins in the presence of normal glomerular permeability.[28] This usually occurs as part of the Fanconi syndrome of proximal tubular dysfunction. Tubular proteinuria usually does not exceed 2.0 gram per day.[29,30] Beta 2-microglobulin is one of the many micro globulin which make up tubular proteinuria. It can be assessed by Radio Immuno Assay(RIA) or ELISA. The urinary albumin to Beta 2-microglobulin ratio of 10 to 1 suggests the presence of Beta 2-Microglobulin.[28] Evaluation of these proteins can be useful in early detection of renal parenchymal disorders and in monitoring the course of such disorders [28]. Glomerular and tubular proteinuria can be differentiated after electrophoresis of concentrated urine on agarose gel or cellulose acetate

TUBULOINTERSTITIAL CAUSES OF PROTEINURIA

1. Hereditary
   - Cystinosis
   - Galactosemia
   - Lowe Syndrome
   - Medullary Cystic Kidney
   - Proximal Renal Tubular Acidosis(RTA)
   - Wilson Disease
2. Non-Hereditary
   - Acute Tubular Necrosis
   - Drugs
     - Analgesic abuse
     - Antibiotics like penicillin,sulfonamides
     - Penicillamine
   - Cystic diseases
   - Heavy metal poisoning
   - Homograft rejection
   - Hypokalemia
   - Interstitial nephritis
   - Reflux nephropathy

3) Overload / Pre Renal Proteinuria

It is caused by a non renal disorder where excess low molecular mass proteins are filtered by the glomerulus, and exceed the reabsorptive capacity of the tubules.It is characterized by the presence of abnormal spike or peak on urinary electrophoresis.[28] Most common cause being immunoglobulin over production that occurs in multiple myeloma. Bence Jones Protein in multiple myeloma produce a monoclonal spike in the urine electrophresis.[31,32].

Overload Proteinuria Causes

1. Neoplastic
    - Amyloidosis
    - Leukemia (monocytic, monomyelocytic) - lysozymuria
    - Multiple Myeloma
    - Waldenstrom's Macroglobinemia
2. Others
    - Type 1 Diabetes Mellitus (microalbuminuria)
    - Repeated albumin or blood transfusions (FFP)
    - Rhabdomyolysis

4) Secretory Proteinuria

It occurs due to secretion of proteins into the urine after glomerular filtration. About 20 to 30 mg/24 hours of non plasma protein is contributed by renal tubules and lower urinary tract . Mostly these are contributed by Tamm-Horsfall proteins.[32] Some secretory IgA is added by lower urinary tract including the urethral glands together with trace quantity of protein of prostatic or seminal vesicular organ.[33,34] Tamm-Horsfall protein is secreted by the thick ascending limb of loop of Henle and early distal convoluted tubule into the tubular fluid. It is an easily polymerized glycoprotein. They are the predominant constituent of renal tubular casts [35], along with albumin and traces of many plasma proteins, including immunoglobulins. [36]

5) Post Renal Proteinuria

Post-renal proteinuria can be caused by inflammatory or degenerative lesions of the renal pelvis, ureter, bladder, prostate, urethra, or external genitalia. Other causes include genitourinary tract hemorrhage and infections, e.g., cystitis.

Table 1: DEGREE OF PROTEINURIA AND CAUSES [24]

Daily protein excretion	Causes
0.15 to 2.0 g	Mild glomerulopathies Tubular proteinuria Overflow proteinuria
2.0 to 4.0 g	Usually glomerular
>4.0 g	Always glomerular

TABLE -2 : CHARACTERISATION OF PROTEINURIA [37]

	Normal	Primary glomerular disease	Primary tubular disease
Plasma			
Albumin concentration, g/L	40	10-40	40
Low Protein concentration,mg/L	4	1-4	4
Urine			
Total protein,g/24 h	<0.15	>2.5	<2.0
Albumin,mg/24 h	50	>500	<500
β2-Microglobulin, mg/24 h	0.15	0.15	20
Ratio of low Molecular weight/ high Molecular weight protein	0.86	0.17	1.48
Tubular reabsorption of filtered proteins,%	95	3	50

MICROALBUMINURIA

Microalbuminuria (defined as urinary albumin excretion of 30-300 mg/day, or 20-200 µg/min) is an early sign of vascular damage.[38] This level of albumin excretion is above the normal range, yet is undetected by dipstick. Microalbuminuria is caused by glomerular capillary injury and so may serve as a marker for diffuse endothelial dysfunction[39] . Microalbuminuria in patients with renal or cardiac illness signifies worse prognosis. Evidence suggests that microalbuminuria is an independent risk factor for stroke, myocardial infarction and congestive heart failure.[40] The risk for major cardiovascular events increased with increase in level of urinary albumin excretion, including levels within the normal range[40].The appearance of microalbuminuria (incipient nephropathy) in type I diabetes mellitus is an important predictor of progression to overt proteinuria (> 300 mg /dl) or overt nephropathy[41]. The prevalence of microalbuminuria increases to 50-60 % after 20-30 years duration of diabetes[42]. Studies have shown that microalbuminuria in patients with type I diabetes may serve as a useful marker to predict patients at greatest risk for the development of micro vascular and macro vascular disease.[43]

Current recommendations by American Diabetic Association for screening microalbuminuria are [44]

a) Measurement of albumin to creatinine ratio in Random spot collection (preferred method)
b) 24 hour collections with creatinine allowing the simultaneous measurement of creatinine clearance.
c) Timed collection (Eg :- 4 hours or over night)

The analysis of a spot sample for the albumin to creatinine ratio has been strongly recommended by the most authorities. [45]

METHODS OF DETECTING AND MEASURING PROTEINURIA

A) DETECTION OF PROTEINURIA

1 Dipstick analysis

2 Precipitation methods

B) QUANTIFICATION OF PROTEINURIA

1. Biuret method
    - Copper regent
    - Tsuchiya reagent
    - Fowlin-Lowry

2. Turbidimetric method

    - Sulphosalicylic acid
    - Benzethonium chloride
    - Trichloroacetic acid

3. Dye binding technique

    - Pyrogallol red
    - Coomassie brilliant blue
    - Ponceau S

4. Radio immune assay(RIA)

5. Enzyme linked immunosorbent assay (ELISA)

C) CHARACTERIZATION OF PROTEINURIA

1. Immune electrophoresis
2. Column gel chromatography
3. Agarose gel electrophoresis
4. Polyacrylamide gel electrophoresis
5. Isoelectric Focussing

A) DETECTION OF PROTEINURIA

1) Dipstick Method

It is used in most out patient settings to detect proteinuria. It is a semi quantitative method of measuring the urine protein concentration.

Dipsticks for proteins are based on the principle of ‘the protein error of indicators’.[3] Paper strip is impregnated with indicator dye like bromocresol green which changes colour in presence of protein. With increasing concentrations of protein in urine the dye indicators undergo sequential colour changes from pale green to green and blue. In the absence of protein the dipstick panel is yellow. The results are expressed on a scale from 0 to +++ or ++++, each of which correspond to increasing approximate protein concentrations. They preferentially detect negatively charged urinary proteins- Albumin. However albumin levels between 30-300mg/dl are not detected.[2] Light chains and some low molecular weight protein are also not detected by stick tests.

They should be read immediately. Sticks are very sensitive giving a trace or positive reading with many normal urine samples containing only about 100 mg/l of protein.

Ralston et al [46] found that dipstick testing though nearly 100% sensitive, had poor specificity due to high false positive rates. Specificity was 40% with 1 + , 83% with 2 + and 48% with 3 + readings.

Meyer et al [48] in a study found that 66 % of patients with negative or trace protein had significant proteinuria when the sample was compared with 24 hours urine collection.

Davidson et al[47] while evaluating the relation between dipstick positive proteinuria and albumin - Creatinine ratio found that dipstick positive proteinuria of more than or equal to 1 + can substitute for albumin -Creatinine ratio in random urine specimen for proteinuria quantification. .

False Positive Dipstick Proteinuria

- Dipstick immersed too long
- Highly concentrated urine
- Ph > 7
- Gross Haematuria
- Presence of Sulfonamide ,Penicillin or Tolbutamide.
- pus / semen /vaginal secretions

False Negative Dipstick Proteinuria

- Dilute urine (specific gravity > 1.015)
- When the urinary proteins are non albumin or low molecular weight

Table 3 : SEMI QUANTITATIVE ANALYSIS BY DIPSTICK

GRADE	PROTEIN LEVEL
NEGATIVE	<10mg/dl
TRACE	10-20 mg/dl
1+	30 mg/dl
2+	100 mg/dl
3+	300 mg/dl
4+	>2000 mg/dl

2) Precipitation Method

Kjeldahl :

This precipitation method measures protein by measurement of precipitated Nitrogen. Detection limit is 10-20 ng/l.

QUANTIFICATION OF PROTEINURIA

1) Biuret Method:

This method is based on the interaction between copper ions and the carbamide group of proteins .This requires precipitation of protein, by using copper or Tsuchiya reagents. The protein in the urine must be concentrated before the biuret reaction and Tsuchiya reagent (ethanolic hydrochloride phosphotungstic acid) is the best precipitating compound. Its detection limit is 50 mg/l.[49] A modification of the biuret method, which is now the reference method recommended by the American Association for Clinical Chemistry, utilizes gel filtration to exclude small interfering compounds.[18]

2) Turbidimetric method :

The addition of Trichloroacetic acid or Sulphosalicylic acid (SSA) alters colloid properties of protein and produces turbidity to be read in a densitometer. Benzethonium can also be used instead of sulphosalicylic acid. Its detection limit is 50-100 mg/l. The advantage of this easily performed test is its greater sensitivity for Bence Jones proteins. Lack of precision and variance in the reading for albumin and globulin limit the use of this method . Few milliliters of freshly voided, centrifuged urine is added to an equal amount of 3 % SSA. Turbidity will result from protein concentration as low as 4 mg/dl to be read in densitometer. False positive results can occur when a patient is taking sulfonamides or penicillin and within 3 days of administration of radiographic dyes.[51] A false negative result occurs with highly buffered alkaline urine or dilute specimen of urine.

3) Dye binding technique :

This is most widely used technique for quantification of urinary proteins[3]. These are based on the interaction between proteins and a dye, which causes a shift in the absorption maxima (measured photometrically) of the dye. They are reliable, accurate and easy way for assessing proteinuria. Commonly used dyes are pyrogallol red, Coomassie brilliant blue and Ponceau S. Pyrogallol red dye is the most commonly used dye for dye binding technique. Pyrogallol red combines with sodium molybdate to form a red complex. Proteins, in an acidic medium combine with this red complex and form a bluish purple colored complex. The intensity of the color formed is directly proportional to amount of proteins present.

$$\text{Proteins + Pyrogallol red + Molybdate} \xrightarrow{\textit{In acidic medium}} \text{Bluish purple coloured complex}$$

TABLE 4 : DIFFERENT METHODS OF DETECTING AND MEASURING URINE PROTEIN[3]

Method	Description	Detection limit	Comments
Kjeldahl	Remove non-protein nitrogen, digest protein, measure protein nitrogen	10 to 20 mg/l	Reference and research method
Biuret	Copper reagent, measures peptide bonds	50 mg/l	Requires precipitation of proteins, used for 24-h measurement in some laboratories
Turbidimetric	Addition of trichloracetic or sulfosalicylic acids alters colloid properties and produces turbidity to be read in densitometer. Benzethonium also used	50 to100 mg/l	Imprecise, different readings for albumin and globulin
Dye-binding	Indicator changes colour in presence of protein (e.g. Coomassie brilliant blue)	50 to 100 mg/l	Different proteins bind differently; several different dyes in use; used in many laboratories for 24-h excretion
Nephelometric	Specific antialbumin antibody used		Measures albumin excretion not total protein. Does not detect globulins
Stick tests	Impregnated with indicator dye (bromocresol green) which changes colour in the presence of protein	100 mg/l	Reacts poorly with globulins. Usual clinical screening test

COLLECTION OF URINE

There are various methods for collection of urine. Currently acceptable methods are [55]

1) 1-2 hour collection

2) Over night (8-12 hour) collection

3) 24 hour collection

4) Random urine sample for protein Creatinine ratio

The timed specimen (24 hour or over night) is more sensitive but the Protein- Creatinine ratio is more practical and convenient for the patient .[55]

ADEQUACY OF COLLECTION

Creatinine, the metabolic product of skeletal muscle creatine, is produced at constant rate and in quantities directly proportional to skeletal muscle mass. Since concentration of creatinine remains relatively constant on a daily basis it can be used to assess the adequacy of timed urine collections. The urinary creatinine excretion is measured and compared with normal expected ranges of creatinine excreted per day. The normal creatinine excretion per day is as follows.

Males – 16 – 26 mg/kg/day

Female 12-24 mg/kg/day

If expected creatinine is similar to what has been measured in previous timed urine sample the collection is likely to be accurate

TABLE 5 : COMPARISON OF METHODS OF COLLECTION FOR ASSESSMENT OF PROTEINURIA [39]

Random urine for Albumin to Creatinine ratio	First Morning urine for Albumin to Creatinine ratio	Timed overnight urine For albumin excretion	Timed 24-hour albumin excretion
ADVANTAGES:			
A Good estimate of albumin excretion over the whole day	A Good estimate of overnight albumin excretion	Defines albumin excretion overnight	Defines albumin over the entire 24 hours
Easy assays to perform in all laboratories	Easy assays to perform in all laboratories	Easy assays to perform in all laboratories	Easy assays to perform in all laboratories
Directly relates to published results of random A/C	Directly relates to published results of first morning A/C	Directly relates to published results of overnight excretion	Directly relates to published results of 24 hour albumin excretion
Easiest single sample collection	Easier single sample collection	Easier collecion of one or more samples	

DISADVANTAGES:			
Lower creatinine excretion in women:higher values of A/C in women	Lower creatinine excretion in women:higher values of A/C in women	More complex collection of sample(s)	Most complex collection of sample(s)
Lower creatinine excretion with age:higher values of A/C in older people	Lower creatinine excretion with age:higher values of A/C in older people	Frequent incomplete collection of samples.	Frequent incomplete collections of samples

A/C- Albumin/Creatinine ratio

URINE PROTEIN CREATININE RATIO:-

The quantification of proteinuria is commonly used in the diagnosis, assessment and prognosis of glomerular disease.[49] Quantitative estimation of daily urinary protein excretion is usually done by 24 hours urine collections. However such timed collections of urine are inconvenient, cumbersome and at times unreliable because of frequent errors in collection and up to a third of the collected specimens have to be rejected.[51] To overcome these difficulties short timed urine collection have been advocated with the hypothesis that protein excretion is nearly constant throughout the day .Various studies have estimated proteinuria by taking urine samples at 2hrs, 3 hrs and 4 hrs but these studies have not been validated.[52] An alternative to the 24 hours urine estimation is the urine Protein-Creatinine ratio, determined in a random urine specimen while [53,54]the person carries on normal activity .The protein and creatinine concentration in urine are measured by routine biochemical analyzers and the ratio is determined. The ratio is about the same numerical as the number of grams of protein excreted in urine per day. Thus, a ratio of less than 0.2 indicates 0.2gms of protein per day and is considered normal, a ratio of 3.5 is equivalent to 3.5 gms of protein excretion per day and is considered nephrotic range proteinuria.

The randomly obtained urine protein creatinine ratio would be expected to predict 24hour protein for several reasons.[50] First, the concentrations of protein and creatinine in the urine are determined by their excretion rates and by the tubular re absorption of water. Since water reabsorption is the same for both values of the same specimen, the protein – creatinine ratio therefore reflects the excretion rate of protein relative to creatinine. Second, when both urinary protein and urine creatinine values are reported in similar units (mg/dl), the Protein Creatinine ratio can be thought of as the excretion rate of urinary protein in grams relative to the excretion of 1 gm creatinine. Finally, since the average person excretes approximately 1 gm/day creatinine, the ratio there for can be directly used to estimate 24 hours urinary protein in grams/day.

It has been reported that in the presence of stable glomerular filtration rate ,urinary creatinine excretion is fairly constant in a given individual, the fact serving as principle behind the use of Protein Creatinine ratio in quantifying 24 hours proteinuria.

Koopman et al [59] found an excellent correlation between 24 hours protein and spot protein creatinine ratio in random urine sample. Many workers have found out good correlation between 24 hour urinary protein and proteinuria estimated from spot protein creatinine ratio in diverse group of patients such as children, diabetes, nondiabetics, SLE patients, pregnant females, preeclampsia and patients with diverse renal diseases.[50,52,71]

Zelmanovitz et al [56] reported that quantifying proteinuria in a random urine sample using PCR was a reliable and simple method for screening and diagnosis even in case of overt diabetic nephropathy.

Ruggenenti et al [57], on studying about chronic renal disease in non diabetics found excellent correlation between spot urine protein creatinine ratio and 24 hours urinary protein.

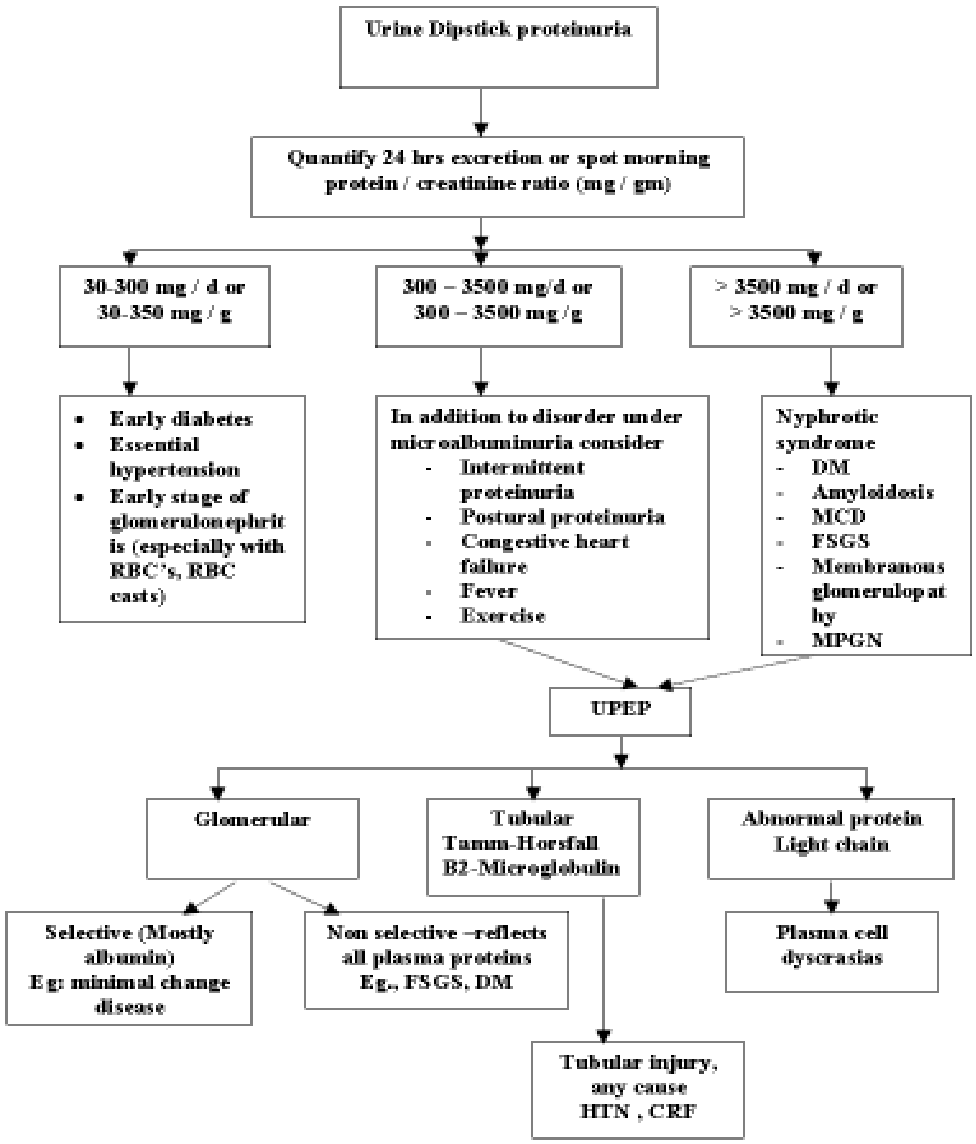
Silink et al [58] on studying relationship between albumin concentration in random urine samples upon 24 hours urinary albumin excretion in patients with type I Diabetes Mellitus showed that the measurement of albumin concentration on the first morning urine sample can be used for a screening test for microalbuminuria in children in type 1 diabetics. Another method for quantification of proteinuria in spot urine sample is by measuring urine Protein – Osmolality ratio.

Wilson et al [61] found that urinary protein osmolality ratio indicated quantitative proteinuria with reasonable prediction and is better than qualitative urine analysis and urinary Protein- Creatinine ratio for detecting or assessing abnormal proteinuria.

Vishwanathan et al [62] in his study showed that estimated proteinuria calculated from urinary Protein- Creatinine ratio in a random urine sample is useful in serial evaluation of kidney function on a follow up basis.

Hence the urine collected from the first morning sample for Protein-Creatinine ratio, is an acceptable alternative to a 24 hours urine collection for proteinuria quantification in clinical follow up and screening[59,60].

FIGURE-4 APPROACH TO A PATIENT WITH PROTEINURIA.[2]



DM- Diabetes Mellitus;MCD-Minimal change disease; UPEP -Urine Protein Electrophoresis; FSGS-Focal Segmental Glomerulo Sclerosis; MPGN-Membranoproliferative Glomerulonephritis; HTN-Hypertension; CRF-chronic renal failure

INVESTIGATION DEPENDING ON TYPE OF PROTEINURIA[31]

Glomerular proteinuria:

- Serum/urine immunoelectrophoresis
- Complements C3, C4
- HIV/HBV/HCV serology
- ANA
- VDRL
- Renal biopsy

Tubular proteinuria:

- β2 Miroglobulin/Albumin excretion ratio
- Urinary electrophoresis
- Heavy metal screening

Over flow Proteinuria: -

- Serum / urine electrophoresis
- Urinary light chains
- Urinary spectrophotometry

URINARY MICROSCOPY

FIGURE 5-A:
Typical morphology of erythrocytes from a urine specimen revealing microscopic hematuria

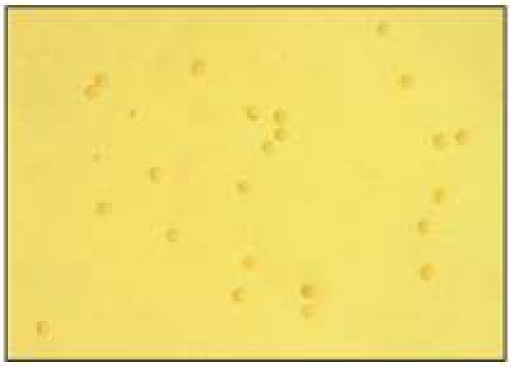


FIGURE 5-B:
Dysmorphic erythrocytes from a urine specimen. These cells suggest a glomerular cause of microscopic hematuria.

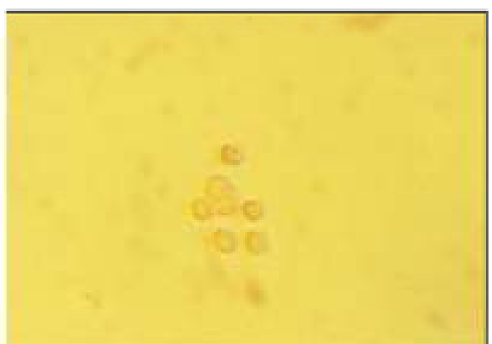
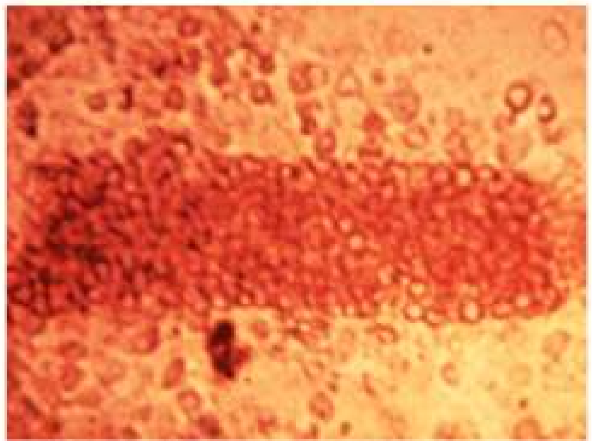


FIGURE -6 :URINE MICROSCOPY

Fatty casts /oval fatty bodies in microscopic study of urine

FIGURE 7 : URINE MICROSCOPY



Urine sediment showing free red cells and a red cell cast that is tightly packed with red cells. Red cell casts are virtually diagnostic of glomerulonephritis or vasculitis.

INFLUENCE OF REDUCING PROTEINURIA ON RENAL OUTCOME

Proteinuria has shown to be an independent risk factor for progression of renal disease. Numerous studies in non diabetic patients have suggested that a reduction in urinary protein excretion may independently predict the extent of preservation of renal function.[63]

The APRI(ACE inhibitor in Progressive Renal Insufficiency) trial, where patients were treated with Benazapril, demonstrated that a greater reduction in risk for the development of a renal end-point was seen in patients who had a larger reduction in the degree of proteinuria.[64]

In the MDRD(Modification of Diet in Renal Disease) study, reduction in blood pressure to approximately 125/75 mm Hg in conjunction with a low-protein diet in patients with greater degrees of proteinuria (>1g/24 hours) was associated with a slow rate of decline of Glomerular Filtration Rate.[65]

The REIN(Ramipril Efficacy in Nephropathy) trial demonstrated that Ramipril reduced the relative risk of developing end stage renal failure 5 fold and decreased the risk of massive proteinuria.[66]

In another study with ACE inhibitors it was found that benefit of therapy with ACE inhibitors was time dependent, with greater benefits being observed in patients treated for longer periods [67] .

Thus the above studies clearly show that drugs and dietary intervention may be needed to reduce the risk of proteinuria.

METHODOLOGY

SOURCE OF DATA:

Total of 55 patients, with persistent dipstick positive proteinuria, admitted from October 2009 to November 2011 in medical and nephrological wards of Government Mohan Kumaramangalam medical college hospital were included in the study.

INCLUSION CRITERIA

1) Patient of either sex

2) Patient above 14 years

3) Patient with persistent dipstick positive proteinuria (on 2 different occasions at least 1 week apart)

EXCLUSION CRITERIA

1) Patients of age less than 14 years

2) Gross Haematuria

3) Patients with febrile illness

4) Dehydration

5) Intense activity

6) Head injury

7) Patients with urinary tract infection.

METHOD OF COLLECTION OF DATA

A study of 55 patients satisfying the inclusion and exclusion criteria managed in medical and nephrological wards of Government Mohan Kumaramangalam Medical College Hospital from October 2009 to November 2011 was done .

A detailed history of the illness was elicited, general physical examination and systemic examination was done. Clinical presentation, past or present medical illness, physical examination findings, baseline laboratory investigations including complete hemogram ,blood urea ,serum creatinine ,urine routine, ultrasound abdomen was done and all these values were recorded in the proforma.

Urine samples were evaluated for albumin, sugar by dipstick method and by microscopy for deposits. Serum biochemical analysis was done using **ROBONIK** auto analyzer and complete blood count by using **SYSMEX KX – 21** cell counter.

For estimation of 24 hours urinary protein, patients were provided with plastic container (5 litre capacity) having 5mL of 10% thymol in isopropanol as a preservative. Time was noted and twenty four hour urine sample was collected by instructing subjects to begin collection immediately after completion of first voiding in morning and to collect all urine into the same container for 24 hours, including final void at completion of 24 hour period. A sample of 2 ml was taken in the morning when the patient first void urine into the container for estimation of protein creatinine ratio. Another urine sample for estimation of urine protein creatinine ratio was obtained around 7 PM in the evening from each patient.

The urine for 24 hours protein and spot urine protein concentration was estimated by using dye binding technique with **Pyrogallol red** in ERBA MANHEIM auto analyzer.

Spot Urine for Creatinine was estimated by using **modified Jaffe's method** in ERBA MANHEIM auto analyzer.

Spot Urine Protein – Creatinine ratio was calculated from the above measured value in the first morning urine sample and in the evening sample by the following formulae.

$$\textbf{Protein-Creatinine ratio} = \frac{\text{urine protein (mg/dl)}}{\text{urine creatinine (mg/dl)}}$$

Expected 24 hour urinary protein was calculated from Protein Creatinine ratio by using the following formula [50]

$$\textbf{Expected 24 hours Urinary Protein(male)} = \frac{[140\text{-Age (yrs)}] \times \text{wt (kg)}}{5000} \times \text{P/C Ratio}$$

$$\textbf{Expected 24 hours Urinary Protein (female)} = \frac{[140\text{-Age (yrs)}] \times \text{wt (kg)}}{5000} \times \text{P/C Ratio} \times 0.85$$

Glomerular filtration rate(GFR) was calculated from age and plasma creatinine by Modified Diet in Renal Disease (**MDRD**) Formula [68]

$$\text{GFR (mL/min/1.73 m}^2) = 186 \times (\text{Pcr})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African - American})$$

The GFR is expressed in mL/min/1.73m^2

All 55 patient were segregated into 3 groups based on protein excretion estimated from 24 hours urinary protein.

TABLE-6 : **Classification of proteinuria**

Group	Degree of proteinuria	Protein excretion/day
1	Minimal	Less than 1 gm
2	Moderate	1 to 3 gm
3	Heavy	More than 3 gm

The results were also analyzed by segregating patients into five groups based on the stages of chronic kidney disease.

TABLE- 7 Kidney Disease Outcomes Quality Initiative (K/DOQI 2002) classification of chronic kidney diseases (CKD)

Stages of renal failure	Glomerular filtration rate
1	more than 90 ml/min
2	b/w 60 to 89 ml/min
3	b/w 30 to 59 ml/min
4	b/w 15 to 29 ml/min
5	less than 15ml/min

Statistical data was analyzed using SPSS computer program version 16. The statistical tests used were

1. Student T test

2. Paired sample correlation test

RESULTS

This study included 55 patients, who had persistent proteinuria with varying degree of renal dysfunction, admitted in nephrology and medical wards of Government Mohan Kumaramangalam Medical College Hospital.

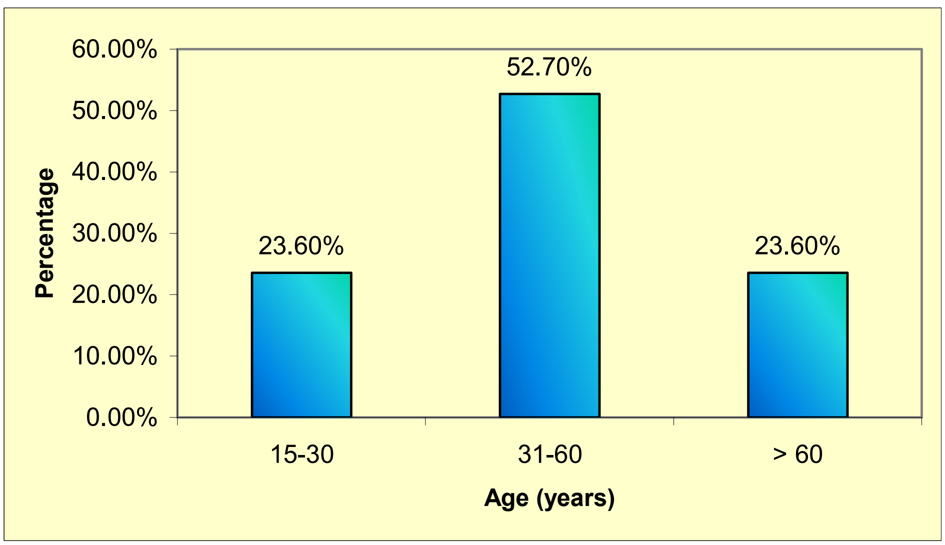
Three kinds of analysis were undertaken based on the results.

1) To assess whether the early morning sample(7AM) or evening sample(7PM) correlates better with 24 hours urine protein.
2) The patients were segregated into 3 groups based on degree of proteinuria and results were analysed.
3) The patients were segregated into 5 different groups based on Kidney Disease Outcomes Quality Initiative (K/DOQI 2002) classification of chronic kidney diseases (CKD) and the results were analysed.

Table 8 : AGE DISTRIBUTION OF PATIENTS WITH PROTEINURIA

Age (Yrs)	No of patients	Percentage
15-30	13	23.6
31-60	29	52.7
> 60	13	23.6

FIGURE 8:AGE DISTRIBUTION OF PATIENTS WITH PROTEINURIA



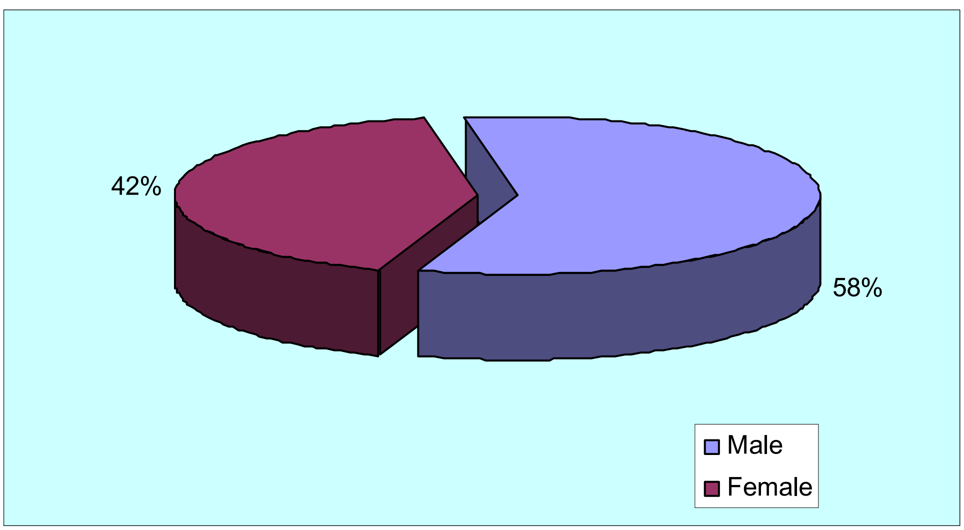
AGE GROUPS

The age of the patients ranges from 15 to 90. The incidence of proteinuria was maximum in the age group of 31-60 years (52.7 %). As the incidence of diabetes and Hypertension increases with age, the microvascular complications of these systemic disorders increase . Hence persistant proteinuria is common as age advances.

TABLE 9 : GENDER DISTRIBUTION IN PATIENTS WITH PROTEINURIA

Gender	No of patients	Percentage
Male	32	58
Female	23	42

FIGURE 9 : GENDER DISTRIBUTION IN PATIENTS WITH PROTEINURIA



Gender Distribution of Cases

In this study, number of males with proteinuria were slightly higher than that of females. The ratio of males to females with persistent proteinuria was 1.38: 1.

TABLE 10: MEDICAL ILLNESS IN PATIENTS WITH PROTEINURIA

Medical illness	No of patients	Percentage
Hypertension(HTN)	31	56%
Diabetes mellitus(DM)	34	61%
Ischemic heart disease(IHD)	12	21%
Cerebrovascular disease	05	09%
DM + HTN	22	40%
DM+HTN+IHD	08	14%
Autoimmune (AI) disease (SLE,RA,Scleroderma)	04	07%
Malignancy(lymphoma,hepatoma)	02	03%

FIGURE 10 : MEDICAL ILLNESS IN PATIENTS WITH PROTEINURIA

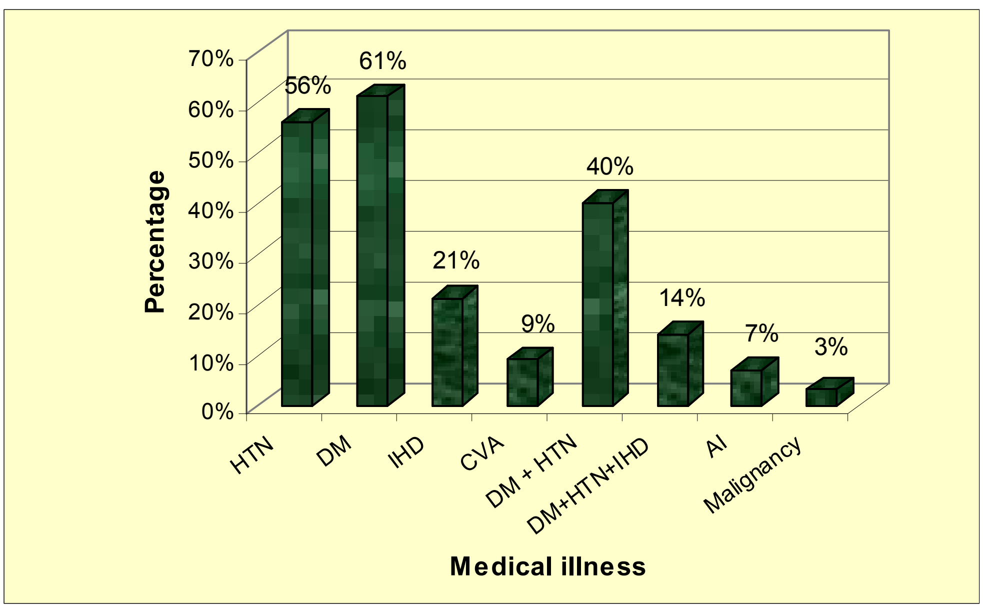
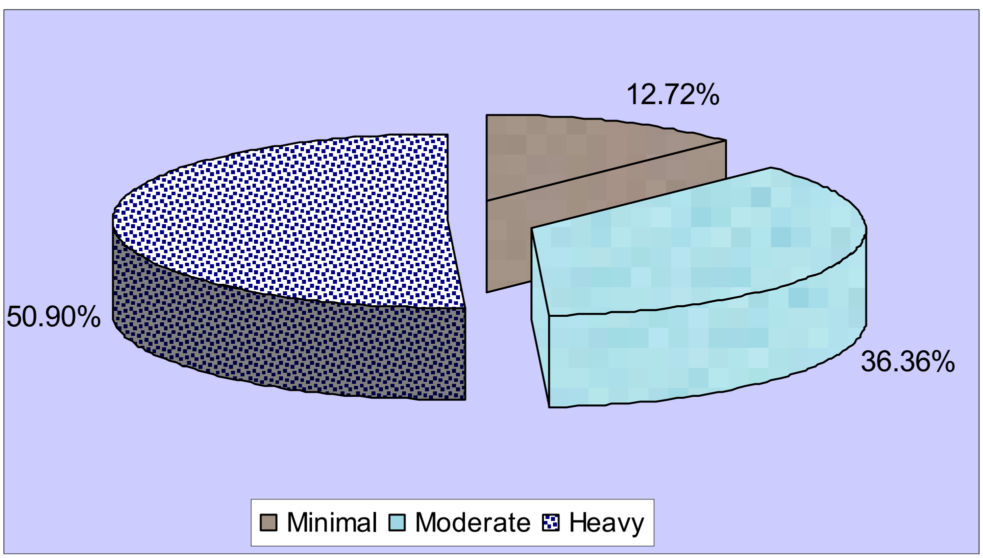


TABLE 11 : DISTRIBUTION OF PATIENTS BASED ON DEGREE OF PROTEINURIA

Degree of proteinuria	Protein excretion/day	No of patients	Percentage
Minimal	Less than 1 gm	7	12.72%
Moderate	1 to 3 gm	20	36.36%
Heavy	More than 3 gm	28	50.90%

FIGURE : 11 DISTRIBUTION OF PATIENTS BASED ON DEGREE OF PROTEINURIA

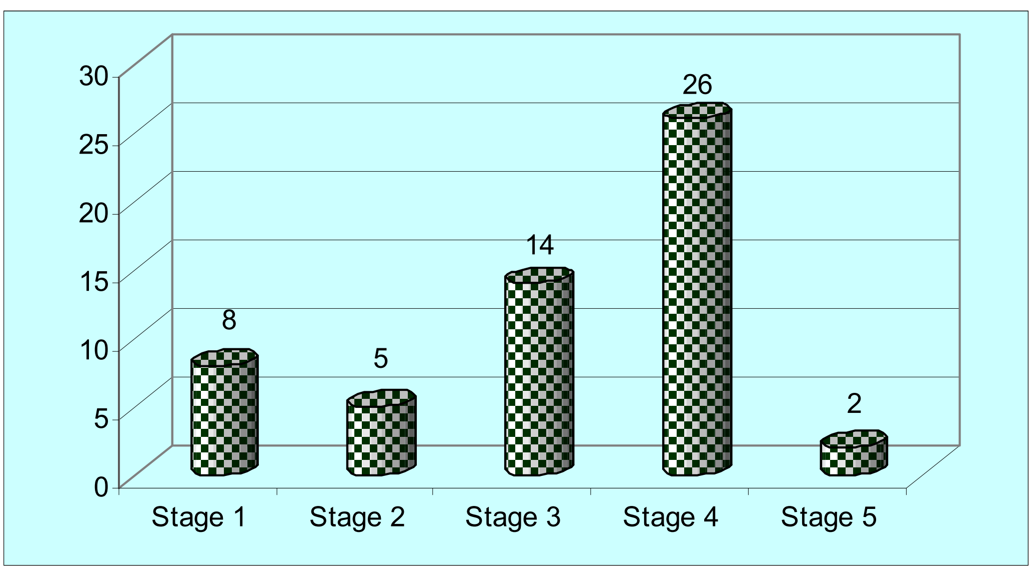


Among 55 patients 7 patients (12.72%) had minimal proteinuria of (<1 g/day), 20 patients(36.36%) had moderate proteinuria (1- 3g/day), 21(50.90%) patients had heavy proteinuria(>3g/day)

Table -12 : DISTRIBUTION OF PATIENTS ACCORDING TO STAGES OF CHRONIC KIDNEY DISEASE (K/DOQI 2002 classification)

Stages	Glomerular filtration rate	No. of patients
1	more than 90 ml/min	8
2	b/w 60 to 89 ml/min	5
3	b/w 30 to 59 ml/min	14
4	b/w 15 to 29 ml/min	26
5	less than 15ml/min	2

FIGURE--12 : DISTRIBUTION OF PATIENTS ACCORDING TO STAGES OF CHRONIC KIDNEY DISEASE



STATISTICAL ANALYSIS

TABLE 13 : OVERALL CORRELATION BETWEEN EXPECTED 24 HOURS URINARY PROTEIN FROM 7AM SAMPLE AND ESTIMATED 24 HOURS URINARY PROTEIN

Pair 1 : *PAIRED SAMPLE STATISTICS*

	Mean	**N**	**Std. deviation**	**Std Error mean**
Expected 24 hours urinary protein(gm) 7AM	3.3635	55	2.45915	0.33159
Estimated 24 hours urinary protein(gm)	3.2545	55	1.96280	0.26466

Pair 1 : *PAIRED SAMPLE CORRELATION*

	N	Correlation	P value
Expected 24 hours urinary protein(gm) - 7 AM & estimated 24 hours urinary protein(gm)	55	0.931	<0.01

TABLE 14 : OVERALL CORRELATION BETWEEN EXPECTED 24 HOURS URINARY PROTEIN FROM 7PM SAMPLE AND ESTIMATED 24 HOURS URINARY PROTEIN

Pair 2 : *PAIRED SAMPLE STATISTICS*

	Mean	**N**	**Std. deviation**	**Std Error mean**
Expected 24 hours urinary protein(gm) 7PM	3.4551	55	2.26523	0.30544
Estimated 24 hours urinary protein(gm)	3.2549	55	1.96300	0.26469

Pair 2 : *PAIRED SAMPLE CORRELATION*

	N	**Correlation**	**P value**
Expected 24 hours urinary protein(gm) - 7 PM & estimated 24 hours urinary protein(gm)	55	0.872	<0.01

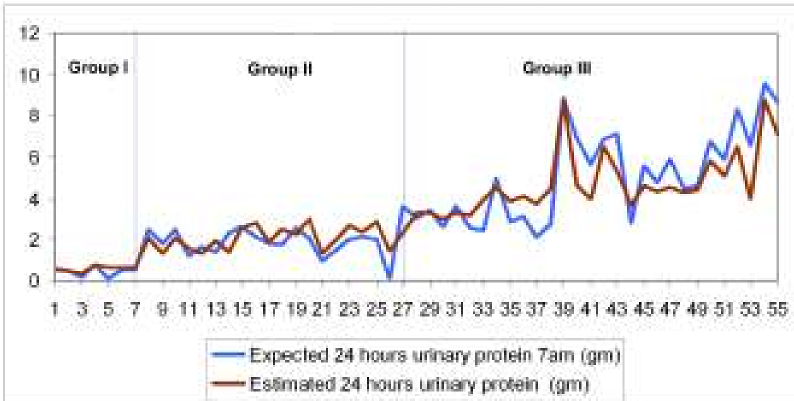
TABLE 15 : correlation between expected 24 hours urinary protein from 7 AM sample and estimated 24 hours urinary protein in patients in various degrees of proteinuria

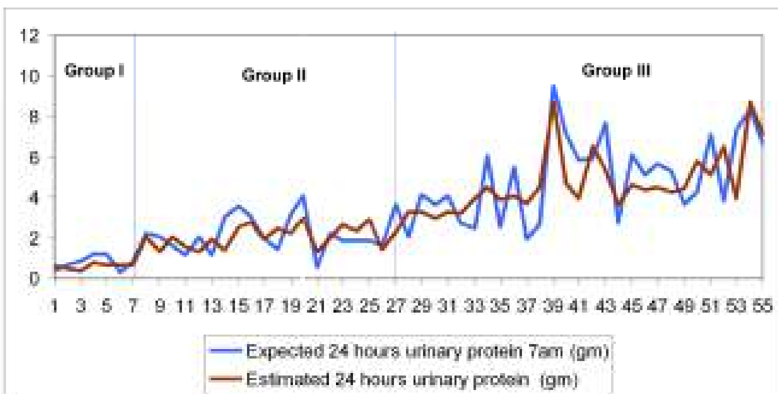
Degree of proteinuria	Protein excretion/day	N	Correlation	P value
Minimal	<1 gm	7	0.881	0.03
Moderate	1-3 gm	20	0.867	<0.01
Heavy	>3 gm	28	0.774	0.05

TABLE 16 : correlation between expected 24 hours urinary protein from 7 PM sample and estimated 24 hours urinary protein in patients in various degrees of proteinuria

Degree of proteinuria	Protein excretion/day	N	Correlation	P value
Minimal	<1 gm	7	0.815	0.01
Moderate	1-3 gm	20	0.863	0.03
Heavy	>3 gm	28	0.567	<0.01

FIGURE-13





Line chart showing comparison between estimated proteinuria using spot PCR in both 7 AM and 7 PM sample and expected 24 hours proteinuria in various degree of proteinuria

TABLE 17 : correlation between expected 24 hours urinary protein from 7 AM sample and estimated 24 hours urinary protein in patients in various stages of renal dysfunction (K/DOQI 2002 classification)

Paired sample correlation of different groups

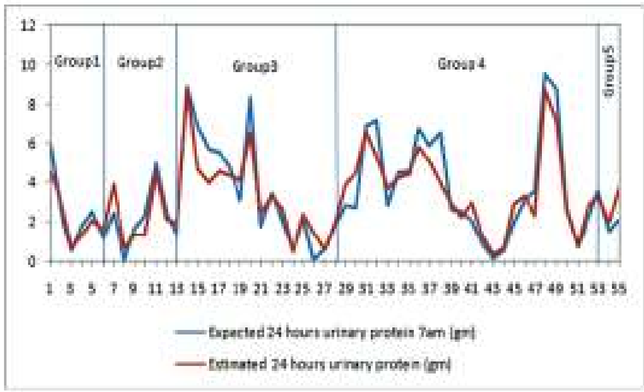
Stages of CKD	N	Correlation	P value
Stage 1	8	0.939	<0.01
Stage 2	5	0.927	0.02
Stage 3	14	0.945	0.06
Stage 4	26	0.837	0.019
Stage 5	2	0.637	<0.01

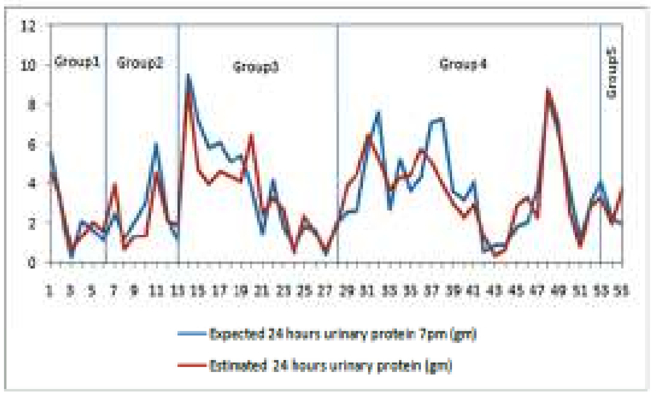
TABLE 18 : correlation between expected 24 hours urinary protein from 7 PM sample and estimated 24 hours urinary protein in patients in various stages of renal dysfunction(K/DOQI 2002 classification)

Paired Sample Correlation of different groups

Stages of CKD	N	Correlation	P value
Stage 1	8	0.941	0.005
Stage 2	5	0.886	0.02
Stage 3	14	0.878	0.01
Stage 4	26	0.820	0.04
Stage 5	2	0.617	0.01

FIGURE-14





Line chart showing comparison between estimated proteinuria using spot PCR in both 7 AM and 7 PM sample and expected 24 hours proteinuria in various stages of chronic kidney disease

DISCUSSION

Measurement of urinary proteins over 24 hours is the definitive method to quantify proteinuria. However, prolonged collections of urine are inconvenient and often inaccurate due to frequent collection errors. [51] In this study, protein creatinine ratio was estimated from two urine samples, an early morning sample and a sample in the evening around 12 hours later. A quantitative estimation of proteinuria was done from both samples using Protein Creatinine Ratio values and analyzed which correlated best with 24 hours urinary protein value . Although both morning and evening sample correlated well with 24 hours urine protein, the degree of correlation was better with early morning urine sample (r=0.931) than with the evening sample (r=0.872).

The result of the study was consistent with **Silink et al** [58] who on studying relationship between albumin concentration in random urine samples upon 24 hours urinary albumin excretion in patients with type I Diabetes Mellitus found that the correlation was best with the early morning first sample.

Ginsberg et al [53] observed that the correlation was most in early morning urine specimen and sample just taken before going to bed. Thus in this study, first morning sample correlated better than the evening sample with 24 hours urine protein.

But **Rodby et al**[50] demonstrated that the timing of collection of urine sample did not have impact on the ability to predict 24 hours urinary protein.

Our study differs from **Koopman**[59] **et al** who observed that the usefulness of the protein:creatinine ratio of a random urine sample for estimation of proteinuria is limited, because of the circadian rhythm of proteinuria. Samples collected at a fixed time of the day are an acceptable alternative for 24-h urine collections in the clinical follow-up of individual patients.

Several authors studied the variation of the relationship of PC ratio during the course of 24 hours and found that the relationship varied by as much as 30% in a day. But samples that were taken during normal daylight activity ,when the patient is ambulatory had minimal variation. The greatest differences were seen during the times when the patients were most likely to be recumbent.

In our study both samples for estimation of protein creatinine ratio were taken when the patients were ambulatory and both correlated well with 24 hours proteinuria.

Analyzing the study by segregating the patients into 3 groups based on degree of proteinuria showed that the best correlation was in patients with minimal to moderate proteinuria in 7 AM sample(r=0.881 in group 1 and r=0.867 in group 2) and 7 PM sample(r=0.815 in group 1 and r=0.863 in group 2). The positive correlation was least in patients with heavy proteinuria in both 7AM sample(r=0.774 in group 3) and 7PM sample(r=0.567 in group 3).

This was in consistent with **Mohan**[70] **et al** who on studying the correlation in type 2 diabetics observed the positive correction was good, but was less with increasing degree of proteinuria. Correlation co efficient(r) values were 0.96, 0.86, 0.74 in groups of patients with proteinuria < 200 mg /day, 201 – 999 mgs/day and more than 1 gm/day respectively.

Leung et al [72] who studied the correlation in lupus nephritis found the correlation was less in higher degrees of proteinuria.

But **Indira Agarwal** [73] et al study found excellent correlation between spot Protein Creatinine ratio and 24 hours proteinuria in all degrees of proteinuria.

Analyzing the study by segregating patients into 5 different groups based on stages of chronic kidney disease shows there was significant positive correlation in patients with normal or milder degrees of renal dysfunction (Pair 1 → *r=0.939* for stage 1 & *r=0.927* for stage 2 ; Pair 2 → *r=0.941* for stage 1 & *r=0.886* for stage 2). However the positive correlation decreased with deterioration of renal function. (Pair 1 r= 0.637 ; Pair 2 r= 0.617 for stage 5)

Siwach et al[74] found that the product of protein creatinine ratio and estimated daily urinary creatinine excretion positively correlated well with the estimated 24 hours urine protein in patient with normal or mild to moderately impaired renal function (r = 0.88 and 0.99), but poorly correlated in patients with advanced renal dysfunction (r= 0.56).

Goldman R[75] found that the possible reason for poor correlation in these patients is that with progression of renal failure the urinary creatinine excretion falls especially after serum creatinine exceeds 6 mg/dl.

Sharma et al[52] observed a good positive correlation even in patients with advanced renal failure. Correlation co efficient (r) values were 0.889, 0.788, 0.375 in patients with serum Creatinine < 1.5 mg/dl, 1.5-4 mg/dl, > 4 mg/dl respectively.

The possible explanation is the erratic and decreased excretion of creatinine in patients with advanced renal failure as observed in numerous studies like **Carrie BJ et al** [77], **Perrone RD et al** [78], **Levey AS et al**[79].

Many studies have compared the 24 hours urinary protein and spot urine protein creatinine ratio in various groups of patients and found good correlation between the two. In our study too, the correlation was significant between 24 hours protein and spot urine protein creatinine of both morning(0.931) and evening samples(0.872).

TABLE-19 : Correlation between spot urine Protein Creatinine ratio versus timed urine protein in adults in various studies

Study	Year	No. of Patients	Time of collection of urine sample for estimation of protein creatinine ratio	Correlation (r)
Schwab et al[54]	1987	101	Midday	0.92
Ginsberg et al[53]	1983	76	Random	0.94
Rodby et al[50]	1995	229	Random	0.81
Zelmanowitz et al[56]	1998	86	Morning	0.83
Steinhauslin and wauters[80]	1995	133	Early morning	0.86

In this study most of the patients who had 24 hour proteinuria > 3.5 gms, had Protein Creatinine ratio of > 3.5 gms in spot urine. Out of the 18 patients in our study with a Protein Creatinine ratio of > 3.5 gms/day, 14 patients (77.7%) had nephrotic range proteinuria by 24 hours estimation. This is similar to 71-94% seen in other studies [53,54,60]

Vijay et al [69] **and Mohan et al**[70] in their study found that prevalence of diabetes related proteinuria was 18.7% and 9.4 % respectively in patients with type 2 diabetes mellitus. In our study 61 % of patients with proteinuria were diabetics.

Thus this study clearly proves that spot urine protein creatinine ratio correlates well with 24 hours urinary protein in patients with varying degrees of renal dysfunction. Urine sample taken in early morning shown to correlate better with 24 hours protein than with evening sample.

SUMMARY

For years twenty-four hours urine collections are often used to quantify proteinuria. However this is cumbersome, subjective to collection errors, required good compliance, and result in a delay of more than 24 hours in diagnosis. This study was undertaken to find if the protein – Creatinine ratio from spot urine sample taken at two different times of a day could reflect the amount of protein excreted in 24 hours in patients with varying degree of renal dysfunction.

55 patients with varying degree of proteinuria and in various stages of renal dysfunction were investigated. An excellent correlation was found between 24 hours urine protein and protein- creatinine ratio taken at two different times of the day although early morning sample(r=0.931) correlated better than evening sample(r=0.872). However best correlation was in patients with non neprotic range proteinuria with normal / mild renal dysfunction. Correlation was least in patients with nephrotic range proteinuria with moderate to severe renal dysfunction. This study supports the use of a Protein-Creatinine ratio from a single voided urine specimen to predict 24 hours urine protein. It avoids collection errors, less time consuming and is suitable for out patient departments.

CONCLUSION

1. Protein Creatinine ratio in the random urine sample is found to be an useful index for quantification of proteinuria in patients with varying degree of proteinuria and renal dysfunction.
2. There was good positive correlation between spot urine Protein Creatinine ratio taken at two different times of the day and 24 hours estimated protein.
3. There was no significant difference between expected and estimated 24 hours urine protein from both samples.
4. The correlation was best when the urine sample for calculating spot protein creatinine ratio was taken in the early morning.
5. The correlation was best in patients with normal or mildly impaired renal dysfunction with non nephrotic proteinuria.
6. The positive correlation was least in patients with moderate to severe renal dysfunction with nephrotic range proteinuria.
7. Urine Protein Creatinine ratio is easy to perform, inexpensive and less time consuming method for measuring of proteinuria. It can thus be used in the outpatient setting for screening and quantification of proteinuria.

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ANNEXURES

PROFORMA

Name	Age	Sex
Address	Hospital No	
Chief Complaints	Duration	
Past history	Duration	

1) DM
2) HTN
3) IHD
4) Renal Disease
5) Others

General examination

Pallor :	Icterus:	Pedal Edema:
Ht :	Wt:	
Temp:	PR:	BP:

Systemic examination:

CVS:

RS:

Abd:

CNS:

Others:

INVESTIGATIONS

Hb:	Tc:	Dc:	Platelet Count:
ESR:	Blood Sugar:	BUN:	

S.Creatinine: S.Na: S.K:

Lipid Profile

CHL: HDL: VLDL:

LDL: TGL:

Liver Function Test

Total Bilirubin:	Sr.Protein :	AST:
Direct Bilirubin:	Sr.Albumin:	ALT:

Alkaline phosphatase:

Urine R/E:

Albumin:	Sugar:	Deposits:

USG Abdomen:

eGFR(ml/min/1.73m^2):

Spot Urine Protein Creatinine Ratio :

Expected 24 Hour Urinary Protein:

Estimated 24 Hour Urinary Protein:

Others:

Diagnosis:

MASTER CHART

Sl No.	Name	I.P. No	Age (years)	Sex	MEDICAL ILLNESS	Blood urea (mg/dl) / Creatinine (mg/dl)	eGFR (ml/min/ 1.73m2)	Urine albumin	7:00 AM Spot Urine Protein/ Creatinine Ratio	7:00 AM Expected 24 hours urinary protein (gm)	7:00 PM Spot Urine Protein/ Creatinine Ratio	7:00 PM Expected 24 hours urinary protein (gm)	Estimated 24 hours urinary protein (gm)
1	Gomathi	38808	18	F	Scleroderma	21/0.6	158	++++	4.01	5.87	3.88	5.64	4.51
2	Chandra	41130	72	F	CVA/SHT	18/1.0	66	++	2.67	2.47	2.68	2.48	3.93
3	Raja	44226	26	M	Post PT sequelae/SHT	32/2.0	49	+++	3.12	4.97	3.78	6.04	4.52
4	Govindaraj	41575	67	M	DM/SHT/CAHD	23/1.6	53	+++	2.08	2.49	1.86	2.23	2.1
5	Salammal	26932	57	F	CAHD/SHT	24/0.6	125	++	3.09	2.58	3.3	2.77	3.21
6	Sakthi	32527	29	M	SHT	8/0.5	125	++	0.5	0.55	0.25	0.28	0.65
7	Siva	54099	30	M	DM	15/0.9	120	++	1.07	1.83	1.22	2.09	1.32
8	shakila	56175	35	F	DM/SHT	18/0.8	99	+++	2	2.52	1.54	1.65	2.06
9	Lavanya	41129	15	F	SLE	26/0.4	252	+++	1.42	1.24	1.56	1.16	1.59
10	Shanthi	41149	42	F	RHD/SHT	18/0.7	111	++	0.108	0.1	1.33	1.2	0.68
11	Paramasivam	31392	44	M	SHT	20/0.9	111	++	1.24	1.66	2.46	2.08	1.32
12	Tirupathi	50769	90	M	DM/SHT/CAHD	20/1.0	85	++	1.781	1.38	1.47	1.15	1.93
13	Krishnaraj	43214	56	M	DM/SHT	16/1.1	84	++	1.69	2.32	2.19	3.02	1.38

14	Thilagam	36808	24	F	SLE	18/3.4	20	++++	2.55	2.86	2.3	2.58	3.88
15	Poovayle	62686	55	F	DM/SHT	26/2.3	27	+++	3.69	3.1	5.27	5.49	4.1
16	Selvipriya	30325	67	F	DM/SHT	20/4.4	12	++	2.76	2.12	2.54	1.96	3.73
17	Selvi	63579	19	F	DM	87/3.4	21	++	2.66	2.73	2.26	2.65	4.5
18	Vadivel	44976	22	M	Seizure Disorder	60/2.8	35	++++	6.96	8.86	7.47	9.53	8.73
19	Aarayee	46632	75	F	DM/SHT/CAHD	22/2.0	29	+++	6.8	6.89	7.02	7.14	4.67
20	Murugan	34890	40	M	CAHD	82/2.8	31	++	4.56	5.65	4.71	5.85	3.95
21	Perumal	45113	45	M	DM/SHT	33/3.5	23	+++	4.77	6.88	4.08	5.89	6.52
22	Anusuya	38699	50	F	DM/SHT	46/2.8	22	+++	6.61	7.13	8.34	7.66	5.33
23	Govindan	45072	52	M	DM/SHT	71/3.2	25	++	2.3	2.83	2.4	2.74	3.67
24	Rangachari	50443	75	M	DM/SHT/CAHD	28/1.9	42	+++	6.1	5.55	6.72	6.12	4.6
25	Venkatesh	52988	40	M	DM	24/3.1	27	+++	2.69	4.75	3.01	5.12	4.36
26	Muniappan	42941	58	M	DM/SHT/CAHD	46/4.1	18	++	3.6	4.48	4.47	5.28	4.26
27	Kandhasamy	50664	56	M	DM/SHT	30/3.8	20	+++	3.6	4.58	2.79	3.65	4.43
28	Rajendiran	51754	40	M	DM/SHT	20/4.1	20	+++	4.5	6.75	2.84	4.26	5.8
29	Gunasekaran	50658	30	M	DM	126/4.2	20	+++	4.3	5.86	5.21	7.12	5.1
30	Padmanaban	31556	22	M	Old PT/DM	38/2.4	41	+++	5.89	8.34	2.72	3.85	6.52
31	Chinnadurai	39467	45	M	DM/SHT	52/3.6	22	+++	4.67	6.56	5.18	7.28	3.96
32	Kandayeeammal	33695	45	F	DM	48/2.8	22	++	2.85	2.66	3.89	3.65	3.01

33	Idhayathullah	41005	74	M	DM/SHT/CAHD	19/1.4	60	+++	2.01	1.8	1.65	1.48	2.48
34	Vengateshwari	42568	35	F	DM	28/3.4	25	++	2.32	2.56	3.53	3.15	2.28
35	Malarkodi	36259	19	F	RHD	65/3.3	22	++	2.6	2.08	5.06	4.06	2.98
36	Sivagami	31823	52	F	DM/CVA	59/2.4	26	++	1.06	0.98	0.74	0.56	1.32
37	Pachaiammal	60292	30	F	DM	103/3.8	17	++	0.14	0.17	0.81	0.88	0.34
38	Duraisamy	63528	55	M	Lymphoma	32/2.4	34	++	2.9	3.45	3.46	4.12	3.31
39	Chinnathayee	26845	75	F	DM/CVA	30/3.6	15	++	1.67	1.51	2.9	2.25	2.01
40	Dhanam	49327	75	F	CAHD/SHT	86/2.8	20	++	0.63	0.53	1.05	0.88	0.69
41	Padmavathy	45589	47	F	Rheumatiod arthritis	32/2.2	29	+++	2.11	2.04	1.93	1.86	2.68
42	Anthonyraj	45786	32	M	SHT	27/1.9	50	++	0.4	0.47	0.55	0.65	0.47
43	Vasudevan	30896	72	M	DM/SHT/CAHD	23/1.4	60	+++	2.1	2.17	2.3	1.88	2.37
44	Pichamuthu	36897	35	M	DM/SHT	14/2.4	38	+++	2.1	2.02	1.92	1.86	2.9
45	Iyyamuthu	40025	79	M	SHT/CVA	11/1.7	47	++	0.13	0.1	2.08	1.65	1.48
46	Chinnapillai	39839	58	F	CVA	55/3.1	19	++	3.5	3.09	2.35	2.08	3.29
47	Arivarasan	41225	22	M	Seizure Disorder	20/2.7	36	++	0.42	0.59	0.27	0.38	0.58
48	Vivekanand	32358	60	M	DM/CAHD	32/2.8	28	++	3.7	3.6	3.75	3.66	2.32
49	Nallammal	36532	65	F	DM	37/4.3	13	++++	10	9.56	8.7	8.32	8.73
50	Shanmugapriyan	42456	47	M	DM/SHT	34/2.6	32	+++	9.35	8.7	7.16	6.66	7.12
51	Danielraj	31228	42	M	Hepatoma	72/2.8	30	++	2.2	2.63	3.03	3.62	2.58

52	Kamar Basha	33125	56	M	DM/SHT	46/3.8	20	++	0.6	0.78	0.95	1.24	0.8
53	Suseela	43626	34	F	RHD	26/3.8	16	++	2.2	2.14	3.15	3.06	2.8
54	Mohanraj	30654	54	M	DM/SHT	52/4.7	16	++	3	3.61	3.31	4.1	3.29
55	Nataraj	50690	75	M	DM/SHT/CAHD	118/1.7	48	+++	1.9	1.83	2.1	2.02	1.92

LEGENDS TO MASTER CHART

eGFR	Estimated Glomerular Filtration Rate
CVA	Cerebrovascular Accident
SHT	Systemic Hypertension
PT	Pulmonary Tuberculosis
DM	Diabetes Mellitus
CAHD	Coronary Artery Heart Disease
SLE	Systemic Lupus Erythematosos
RHD	Rheumatic Heart Disease