Evaluation of Spot Urine Protein Creatinine Ratio as an Index of Quantitative Proteinuria in Varying Renal Disorders

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ABSTRACT

This study is conducted to evaluate the correlation between 24 hrs urine protein and urine Protein Creatinine Ratio (PCR) in spot urine sample and if found comparable the estimation of spot protein creatinine ratio could be adopted as an alternative method for quantification of proteinuria in our clinical lab setting.

Keywords: Protein creatinine Ratio; Proteinuria; Kidney; Chronic kidney disease; Protein

INTRODUCTION

Kidney disease is a worldwide health crisis and it is 12th most common cause of death causing 1.1 million deaths worldwide as per the global burden of disease study done by national kidney foundation in USA [1]. Almost 10% of world population is affected by chronic kidney disease and it is more common in women than in men. 17% of Indians have some form of chronic kidney disease and two lakh patients need dialysis every year in India. Through proper statistics it is assumed as 50% of population having renal disorders see the nephrologists in the last stage of their kidney disease [2]. According to a program conducted for screening of Chronic Kidney Disease (CKD) in Chennai by Kidney Help Trust, the prevalence of CKD was 13.9 percent in a rural population [3].

Proteinuria is an important sign of kidney disease imparting powerful diagnostic and prognostic information. It is a cornerstone of the workup for CKD, AKI, and hematuria and preeclampsia. It is the earliest marker of the glomerular disease occurring before a reduction in GFR [4]. Early screening for kidney diseases by measuring 24 hr urine protein is very useful in prevention of chronic kidney disease. Proteinuria also indicates the progression of kidney disease and also helps to assess the effects of the therapy given to the patient [5]. Routinely proteinuria is estimated along with serum urea and serum creatinine [3]. A normal serum creatinine level does not necessarily mean that all is well with the kidney. It is estimated that a loss of 50% of the functions of nephrons leads to (approximate) doubling of serum creatinine concentration.

Protein estimation in 24 hrs urine is a reference gold standard method for the estimation of proteinuria [6]. However such timed urine collection is a cumbersome task and inconvenient to patients. It has many disadvantages and is prone to errors due to sample collection. Hence an alternate method for estimating proteinuria is essential [6]. Estimating protein creatinine ratio in spot urine sample, will be a convenient method for estimating the proteinuria which is done by estimating spot urine protein, creatinine and calculating protein creatinine ratio from it. This study is conducted to evaluate the correlation between 24 hrs urine protein and urine Protein Creatinine Ratio (PCR) in spot urine sample and if found comparable the estimation of spot protein creatinine ratio could be adopted as an alternative method for quantification of proteinuria in our clinical lab setting.

Aim

To compare the results of spot urine protein creatinine ratio with 24 hrs urine protein

Objective

• To do 24 hrs urine protein
• To do spot urine protein creatinine and calculate the protein creatinine ratio
• To compare both the results

Review of literature

Kidney is regarded as one of the highly differentiated organs in the body. At the end of embryonic development almost 30 different cells will together form a multitude of filtering capillaries and segmented nephrons enveloped by a dynamic equilibrium. This cellular diversity has control over various complex physiologic processes, endocrine functions, intra glomerular hemodynamics, solute and water transport, acid base balance and removal of drugs metabolites and they are all accompanied by intricate mechanism of renal response [7].

Anatomy of kidney

Kidneys are the major excretory organs in our body. They also have Synonyms: Ren: kidney (in Latin); Nephrons: kidney (in Greek). The kidneys are two bean-shaped, reddish-brown organs within the abdomen situated on the posterior abdominal wall [8].

Location

Kidneys lie on the posterior abdominal wall, one on each side of the vertebral column, behind the peritoneum, opposite 12th thoracic and upper three lumbar (T12–L3) vertebrae. They occupy epigastric, hypochondriac, lumbar and umbilical regions

1. The right kidney lies at a slightly lower level than the left one due to the presence of liver on the right side.
2. The left kidney is little nearer to the median plane than the right.
3. Their long axes are slightly oblique (being directed downward and laterally) so that their upper ends or poles are nearer to each other than the lower poles. The upper poles are 2.5 cm away from the midline, the hilum are 5 cm away from the midline, and the lower poles are 7.5 cm away from the midline.
4. Both kidneys move downward in vertical direction for 2.5 cm during respiration.
5. Trans pyloric plane passes through the upper part of the hilum of the right kidney and through the lower part of the hilum of the left kidney [9] (Figure 1).

Figure 1: Location of the kidneys.

A: Surface projection in relation to the anterior abdominal wall. The figure in the inset on the right shows the vertebral levels of the kidneys. The Trans pyloric plane (TPP) passes through the upper part of the hilum of the right kidney and the lower part of the hilum of the left kidney;
B: CT scan in the axial plane showing location of the kidneys in relation to the vertebral column (V) [8].

Shape and measurements

Shape: Bean shaped.
Measurements: Length: 11 cm. (left kidney is slightly longer and narrower).
Width: 6 cm.
Thickness (anteroposterior) 3 cm.
Weight: 150 g in males; 135 g in females (Figure 2).

Figure 2: External Features.

Each kidney presents the following external features:
1. Two poles (superior and inferior).
2. Two surfaces (anterior and posterior).
3. Two borders (medial and lateral).
4. A hilum [10].

Poles

1. The superior (upper) pole than the inferior pole.
2. The inferior (lower) pole is thin and pointed and lies 2.5 cm above the iliac crest.

Surfaces

1. The anterior surface is convex and faces anterolaterally.
2. The posterior surface is flat and faces posteromedially [10].

Borders

1. The medial border of each kidney is convex above and below near the poles and concave in the middle. It slopes downward and laterally, and presents a vertical fissure in its middle part called hilum/hilus which has anterior and posterior lips.
2. The lateral border of each kidney is convex.

Hilum

The medial border (central part) of the kidney presents a deep vertical slit called hilum. It transmits, from before backward, the following structures:
1. Renal vein.
2. Renal artery.
3. Renal pelvis.

Review of literature

Kidney is regarded as one of the highly differentiated organs in the body. At the end of embryonic development almost 30 different cells will together form a multitude of filtering capillaries and segmented nephrons enveloped by a dynamic equilibrium. This cellular diversity has control over various complex physiologic processes, endocrine functions, intra glomerular hemodynamics, solute and water transport, acid base balance and removal of drugs metabolites and they are all accompanied by intricate mechanism of renal response [7].
In addition to the above structures the hilum also transmits lymphatic’s and nerves, the latter being sympathetic and mainly vasomotor in nature [10].

When the kidney is split longitudinally, it presents the kidney proper and the renal sinus [10].

Kidney proper
The naked eye examination of the kidney proper presents an outer cortex and an inner medulla. The cortex is located just below the renal capsule and extends between the renal pyramids (vide infra) as renal columns (columns of Bertini). The cortex appears pale yellow with granular texture. The medulla is composed of 5–11 dark conical masses called renal pyramids (pyramids of Malpighi). The apices of renal pyramids form nipple-like projections—the renal papillae which invaginate the minor calyces. A renal pyramid along with its covering cortical tissue forms a lobe of the kidney.

Macroscopic structure of the kidney as seen in the longitudinal section. Numerous nipple-like elevations (renal papillae) indent the wall of the sinus. The renal pelvis within the sinus is divided into two or three large branches, called major calyces, which further divides to form 5–11 short branches called minor calyces. Each minor calyx expands as it approaches the wall of renal sinus, and its expanded end is indented and molded around the renal papilla (Figure 3).

Figure 3: Macroscopic Structure
The collecting tubules within the renal papilla open into the minor calyx by perforating its wall and capsule lining the Sinus. Thus, the pelvis of ureter (upper funnel-shaped part of the ureter) is connected with the kidney tissue through calyces [8].

Microscopic structure
Histologically, each kidney consists of 1 to 3 million of uriniferous tubules. Each uriniferous tubule consists of two components: nephron and collecting tubule [10].

1. The nephron is the structural and functional unit of kidney. The number of nephrons in each kidney is about 1–3 million. Each nephron consists of a glomerulus and a tubule system. The glomerulus is a tuft of capillaries surrounded by Bowman’s capsule. The tubular system consists of the proximal convoluted tubule, loop of Henle, and distal convoluted tubule.

2. Each collecting tubule begins as a junctional (connecting) tubule from the distal convoluted tubule. Many collecting tubules unite together to form collecting duct (duct of Bellini) which opens on the apex of renal papilla.

The collecting tubules radiate from the renal pyramid into the cortical region to form radial striations called medullary rays. The total capacity of renal pelvis and major and minor calyces is about 8 ml (Figure 4).

Figure 4: Nephron Structure.

Proteinuria
Urine is formed by the ultrafiltration of plasma across glomeruli. Normally glomerular membrane does not allow the filtration of protein into urine because of narrow spaces in the glomerular membrane [3].

Glomerular barrier has three layers
1. Fenestrated endothelium
2. The basement membrane
3. Podocytes
Altogether form a size selective electrostatic filter. Electrostatic barrier consists of negatively charged sialoproteins and proteoglycan [11] (Figure 5).

Figure 5: Glomerular Membranes.
Illustration of glomerular filtration system. Each human kidney contains 1 million glomeruli. An afferent arteriol branches into several capillaries (glomerular tuft), the walls which constitutes filter system. The plasma filtrate is led to the proximal tubule while the unfiltered blood returns to blood circulation.
filtration of the capillary wall contains the inner most fenestrated endothelium, glomerular basement, membrane and the porocytes with their inter digitating foot processes. The slit diaphragm is uniformly wide porous filter between the foot processes. To date the slit diaphragm has been shown to contain distinct components as shown above.

Most proteins such as immunoglobulins both G and M are large to pass through glomerular membrane [11]. Some have charge of confrontation that prevents from traversing through filter. At least one half of the proteins in normal urine are tamm-horsfall proteins, which are localized to the thick ascending limb of loop of henle. The remaining proteins are filtered plasma proteins of different molecular sizes, mostly low molecular weight proteins such as transferrin, macroglobulin and intermediate sized albumin [11].

Most filtered proteins at the glomerulus are reabsorbed in the proximal tubule. Slit diaphragm between podocytes has been recently discovered. These slit diaphragms contribute to the barrier effect. Mutation in slit diaphragm can disrupt normal function and lead to proteinuria. Proteinuria may result from increased glomerular permeability due to damage to the integrity of glomerular filter [12]. Proteinuria can also occur when a reduced number of functioning nephrons leads to increased diffusion of protein across remaining glomeruli.

The normal urinary protein excretion is less than 150 mg/day. Normally only a small amount of protein is excreted (20 mg-150 mg/day) and most of it is albumin. The reminder is almost entirely the Tamm Horsefall Protein Uromucoid a constituent of urinary cast probably secreted by distal tubules [3]. Proteins of molecular weight 15000 Daltons-40000 Daltons filter more easily but in lesser quantities because of their low plasma concentrations. In addition the proportion of individual proteins excreted in urine depend on the extent of their reabsorption by renal tubules, albumin represents approximately 60% of total protein excreted it is not completely removed from the filtrate by tubular cells [3].

Proteinuria is an important sign of kidney disease imparting powerful diagnostic and prognostic information. It is a cornerstone of the work for ckd, aki, and hematuria and pre eclampsia. It is the earliest marker of the glomerular disease occurring before a reduction in GFR. Proteinuria is associated with hypertension obesity and vascular disease it can be used to predict risks of ckd progression, cardiovascular disease. Proteinuria lowering therapies may be Reno protective and monitoring proteinuria is a key aspect of assessing treatment response in a variety of kidney diseases [13].

Changes in glomerular protein filtration and defects in tubular reabsorption cause appearance of proteins in the urine. At values exceeding 300 mg/day, or 200 mg/L, the condition is termed proteinuria. Smaller amounts of protein may appear in the urine in the early stages of progressive disease, such as diabetic nephropathy. Albumin excretion between 30 and 300 mg/day (20-200 mg/L) was previously termed micro albuminuria [7].

Proteinuria is considered severe or in the “nephrotic range” when protein excretion is greater than 3.5 gm/day. When the proteins in the urine have high molecular weight they are considered to have glomerular origin. For proteins with the low molecular weight there is evidence that the defect causing proteinuria is likely related to abnormal proximal tubular reabsorption, often related to toxic damage of tubular cells. Proteinuria associated with progressive kidney disease is predominantly of glomerular origin and mainly composed of plasma albumin [13].

**Causes of proteinuria**

The presence of proteinuria is not always an indicative of renal disease. Proteinuria is broadly classified into

1. **Functional proteinuria**
2. **Organic proteinuria**

**Causes of functional proteinuria**

- Violent exercise
- Cold bathing
- Dehydration
- Emotional stress
- Fever
- Urinary tract infection
- Pregnancy
- Alimentary proteinuria
- Ortho static or postural proteinuria
- Orthostatic proteinuria is a benign condition occurring in young people proteinuria occurs in the upright position due to increased hydrostatic pressure in the renal veins. A diagnosis of ortho static proteinuria is made when an early morning urine sample does not contain protein [13].

**Organic proteinuria**: Proteinuria is seen in different stages of various kidney diseases. Some the kidney diseases in which we can see proteinuria are

- Acute nephritid syndrome
- Nephrotic syndrome

**Asymptomatic hematuria or proteinuria or a combination of these two**

- Diabetic nephropathy
- Chronic kidney disease
- Acute kidney injury [13]

**Mechanism of protein in urine**

The increased proteinuria can result from four mechanisms [14]

- Altered transglomerular passage of proteins
- Decreased tubular reabsorption
- Increased plasma concentration of proteins
- Addition of proteins to the tubular fluid

Therefore important step in the clinical evaluation of proteinuria is the classification of proteinuria.
Types of proteinuria
1. Glomerular proteinuria: Increased filtration of macro molecule across the glomerular filtration barrier due to loss of charge size selectivity. In this type proteinuria will be greater than 1 gm/day.
2. Tubular proteinuria: Tubular damage or dysfunction may inhibit the normal reabsorption capacity of proximal tubule. Lower molecular weight proteins are excreted in urine. Classic causes of tubular proteinuria are fanconis syndrome and dents disease.
3. Over flow proteinuria: Normal or abnormal plasma proteins produced in increased amounts are filtered at the glomerulus and exceeds the resorptive capacity of proximal tubule. Example: myeloma, myoglobin in rhabdomyolysis, hemoglobin in intravascular hemolysis.

Post renal proteinuria
Small amount of IgG or IgA are excreted in UTI or stones.

Proteinuria is the cause and effect of several complications not only at the kidney but also at the systemic level. Proteinuria has been implicated as an effecter of injury process involved in kidney disease progression. Proteinuria is a strong independent determinant of CKD progression, and also an independent risk factor of progression to end stage renal disease (ESKD). Hence proteinuria should be treated accordingly [13].

Detecting and quantifying proteinuria
Screening for proteinuria is done by reagent impregnated dip sticks which detects protein at concentration greater than 200 mg/dl. If proteinuria is detected in this way we have to do a quantitative estimation of protein.

Quantitative estimation of protein can be done by the following methods
1. 24 hours urine protein estimation
2. Random single void urine protein creatinine ratio

24 hours urine protein estimation
Most patients with persistent proteinuria should undergo a quantitative measurement of protein excretion, which can be done with a 24-hour urine specimen. 24 hours urine collection for urine protein estimation is considered to be the Gold standard method for assessment of proteinuria. The patient should be instructed to discard the first morning voidi a specimen of all subsequent voiding should be collected, including the first morning void on the second day. The urinary creatinine concentration should be included in the 24 hour measurement to determine the adequacy of the specimen. Creatinine is excreted in proportion to muscle mass, and its concentration remains relatively constant on a daily basis. Young and middle-aged men excrete 16 to 26 mg per kg per day and women excrete 12 to 24 mg per kg per day. In malnourished and elderly persons, creatinine excretion may be less [15].

However such timed urine collection has many disadvantages and is prone to errors. 24 hours urine protein estimation is a cumbersome task with many errors including incomplete collections, bacterial growth, incorrect timings and incomplete bladder emptying. These errors far exceed those caused by diurnal variation in protein excretion. It also requires hospital admission and cause inconvenience especially for repeated follow up [4].

Random single void urine protein creatinine ratio
Though 24 hours urine protein estimation is the gold standard method it has many limitations. Hence estimation of protein creatinine ratio in random single voided urine sample is used as an alternative method. This approach is based on the fact that in presence of stable glomerular filtration rate urinary creatinine concentration is reported to be fairly constant [16]. As creatinine concentration is fixed its concentration in urine varies with hydration, the random urine protein creatinine ratio nullifies the effect of hydration on Protein estimation [4]. The kidney disease outcomes quality initiative (K/DOQI) of the national kidney foundation practice guide line recommended the use of spot urine/creatinine ratio to detect proteinuria when staging CKD [17]. ADA also recommended a spot urine sample for the quantitative proteinuria in diabetic patients. The concept of UPCR is to use urine creatinine to eliminate the effect of concentration status of urine [17]. To detect and identify proteinuria use acr in reference to PCR because it has greater sensitivity than Protein creatinine Ratio for lower levels of urine protein. For quantification of higher levels of proteinuria, PC RATIO can be used as an alternative [3] UP/UC ratio is a semi quantitative method for estimation of proteinuria with only few studies conducted in children [18].

Moreover random urine collection is a simple procedure can be done at any time of the day [4]. Single void urine protein/ creatinine calculated in mg of protein/mg of creatinine is a convenient method for estimating urine protein excretion without a 24 hrs urine collection [19]. This approach is based on the fact that in the presence of a stable glomerular filtration rate, urinary creatinine excretion has been reported to be fairly constant [16]. As creatinine concentration is fixed its concentration in urine varies with hydration, the random urine protein creatinine ratio nullifies the effect of hydration on Protein estimation [4]. The kidney disease outcomes quality initiative (K/DOQI) of the national kidney foundation practice guide line recommended the use of spot urine/creatinine ratio to detect proteinuria when staging CKD [17]. ADA also recommended a spot urine sample for the quantitative proteinuria in diabetic patients. The concept of UPCR is to use urine creatinine to eliminate the effect of concentration status of urine [17]. For quantification of lower levels of urine protein use albumin creatinine ratio (ACR), as ACR has greater sensitivity than protein creatinine ratio. For quantification of higher levels of proteinuria, Protein Creatinine ratio can be used as an alternative method [3].

Limitations of protein creatinine ratio
1. Variability in the total daily creatinine excretion in and
between individuals
2. Fluctuations in protein excretion that occur throughout the day (exercise posture)

There are many studies which state a relatively high degree of correlation between 24 hr urine protein excretion and protein creatinine ratio in random single voided urine sample in healthy controls and in patients with a variety of kidney disease. But some authors have disapproved it [15].

Hence the study is designed to evaluate the comparison between 24 hours urine protein and protein creatinine ratio and if proved comparable protein creatinine ratio in a random single voided urine specimen could be adopted routinely in our clinical laboratory.

MATERIALS AND METHODS

The present study was conducted in clinical biochemistry laboratory at Saveetha medical college and hospital. The study was carried with the prospective approval of institutional ethical committee (IEC). The study was carried out to determine the correlation between 24 hrs urine protein and spot urine protein creatinine ratio in kidney disease patients. The patients were selected from patients attending outpatient department and in-patient department in Saveetha medical college hospital. Satisfying inclusion and exclusion criteria and informed consent was obtained from all the participants.

Inclusion criteria
Patients having any kind of kidney diseases who are admitted in Saveetha hospital are included in study. Both sexes of the patient are included in study.

Exclusion criteria
Febrile illness, patients having urinary tract infections, excretion of abnormal amount WBC and RBC in urine by microscopic examination are excluded from the study.

Sample size: 50 patients including both sexes

Study design: comparative study

Sampling method: Homogenous sample selection

Specimen collection: After selecting the patient having kidney disease first we must ask for 24 hr urine from the patient. We provided the patient with 5 liters empty can with added preservatives such as thymol (2 gm/can). We must educate the patient about how to collect urine in the containers using plastic mugs to avoid infection.

Armamentarium required:
1. 5 liters urine can
2. Preservatives–thymol crystals in 5 ml of 10% Isopropanol or 10 ml HCl
3. Sterile and non-sterile containers
4. Gloves
5. Test tubes

Specimen used: urine spot and 24 hrs urine

Sample collection
24 hours urine: Instructions to patients for collection of 24 hr urine protein:
1. To collect a 5 litre can with with 2 gms of thymol or 10 ml HCl (urine preservative).
2. To label the collection container (labeling the sample) with name of the patient and date and time of urine collection.
3. To Sample collection: start the collecting the urine sample at 8 am
4. To discard the first urine voided (at 8 am).
5. To record the time and date on the container label (start time)
6. To collect all urine over the next 24 hours (8 am next day).
   To collect all the urine in a separate clean dry container and pour it into 24 hour urine collection container.
7. Even if one sample collection is missed the test must be restarted. To get another sample container from the lab.
8. To obtain two containers from the lab if the first container is filled before 24 hr collection.
9. To keep the container in a cool place (refrigerator)
10. To collect the final urine sample exactly 24 hours after the start of the collection (8 am next day).
11. To record the finish time and date on the container label

Sample transport to lab:
1. To Store the urine container at a cool temperature till it reaches the lab. The container label should have name, start time and date and finish time and date.
2. To transport the 24 hours sample to the lab as early as possible.

Collection of urine for estimation of spot protein and creatinine:
1. To collect Initial morning midstream specimens, particularly for protein analyses. Because they are more concentrated from overnight retention in the bladder.
2. The urine should be freshly collected into a clean, dry container with a tight-fitting cover.
3. It must be analyzed within 1 hour of collection if held at room temperature or else refrigerated at 2°–8°C for not more than 8 hours before analysis.
4. If not assayed within these time limits, several changes will occur. Bacterial multiplication will cause false-positive nitrite tests, and urease-producing organisms will degrade urea to ammonia and alkalinize the pH.

Processing of 24 hours urine protein

Determination of urine protein: Sulphosalicilic Acid Test:
Principle: The proteins in urine will get precipitated and a white precipitate or turbidity is seen when 3% sulphosalicylic acid is added.

Procedure:
Mix well; incubate it for 5 minutes at Room Temperature. Take readings at 670 nm in colorimeter

**Calculation:**

\[
\frac{O.D. \ of \ Test}{O.D. \ of \ Std} \times Concentration \ of \ std
\]

Concentration of standard: 120 mg/dl
Preparation of working standard (conc–120 mg/dl) = mg/dl
Working standard preparation–120 mg/dl (Tables 1 and 2)

**Determination urine protein using semi-automated analyzer:**
1. Take 1 ml of 3% sulphosalicylic acid in a test tube to this add 250 µl of urine mix well. If no precipitate or minimal precipitate aspirate and note the reading.
2. If the precipitate is heavy we have to dilute the urine
3. Dilution of urine
4. 900 µl of distilled water in 100 µl of urine (1 in 10 dilutions)
5. Repeat the test with diluted urine and multiply the value with 10, as we have used 1 in 10 dil urine.

**Table 1: Sulphosalicilic acid test**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
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</thead>
<tbody>
<tr>
<td>3% Sulphosalicylic acid</td>
<td>4 ml</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>Working standard</td>
<td>-</td>
<td>1 ml</td>
<td>-</td>
</tr>
<tr>
<td>Urine</td>
<td>-</td>
<td>1 ml</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Sulphosalicilic acid test**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>4.9 ml or 490 µl</td>
</tr>
<tr>
<td>Protein std (6 gm/dl)</td>
<td>0.1 ml or 10 µl</td>
</tr>
</tbody>
</table>

**Calculation for 24 hrs urine protein:**

Measure the urine volume in the container received from the patient.
24 hours urine protein=Spot urine protein (mg/dl) x 24 hours urine volume (ml)/100
24 hours urine protein=mg/day

**Estimation of urine spot creatinine:**
1. Dilute the urine (1 in 20 dilutions)
2. 1900 µl of distilled water +100 µl of urine
3. Use the diluted urine as sample to estimate urine creatinine by jaffes kinetic method
4. Multiply the obtained value by 20 which is a dilution factor
5. The value obtained after multiplication is the spot urine creatinine in mg/dl.

**Protein creatinine ratio=Spot urine protein/Spot urine creatinine**

Urine protein and urine creatinine are estimated by ERBA CHEM 5 V PLUS.**RESULTS**

In our study 24 hrs urine protein, spot urine protein and spot urine creatinine are estimated for 50 patients having varying kidney diseases like ckd, diabetic nephropathy, nephrotic syndrome etc. Protein creatinine ratio is calculated from spot urine protein and spot urine creatinine 24 hrs urine protein ranged between 0.253 gms/day-3.66 gms/day. Spot urine PCR ranged between 0.2-4.7.

**The observed results are tabulated as follows**

**Descriptive statistics**

There was a good positive correlation between 24 hours urine protein and spot urine protein creatinine ratio with r value-0.7602, showing high significance with p-value 0.001. (r=0.855)- - - - shows a good positive correlation between two method (p<0.001)- - - - shows a high statistical significance (Tables 3 and 4)

**Table 5** the results show a good positive correlation between 24 hrs urine protein and Spot urine creatinine ratio with a (r) value of (0.855) and a high statistical significance with a p value of p<0.001

**Table 3:** Contains the values of 24hrs urine protein <1 gm/day and their corresponding protein creatinine ratio.

<table>
<thead>
<tr>
<th>NO OF PATIENTS</th>
<th>24 hrs URINE PRO</th>
<th>PROTEIN/CREATININE RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIN</td>
<td>MAX</td>
</tr>
<tr>
<td>50</td>
<td>0.253 gms/day</td>
<td>3.66 gms/day</td>
</tr>
</tbody>
</table>

**Table 4:** Correlation between 24 hrs urine protein and spot urine protein creatinine ratio

<table>
<thead>
<tr>
<th>METHOD</th>
<th>r-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24hrs urine protein Vs Spot urine PCR</td>
<td>0.7602</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 5:** Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein <1 gm/day

<table>
<thead>
<tr>
<th>METHODS</th>
<th>SAMPLE SIZE</th>
<th>PEARSON CORRELATION COEFFICIENT(r)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>24hrs urine protein Vs Spot urine PCR</td>
<td>19</td>
<td>0.855</td>
<td>&lt;0.001 Highly Significant</td>
</tr>
</tbody>
</table>
Scatter diagram 1 show there correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein <1 gm/day

Scatter Diagram 1: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein <1 gm/day

(r=0.686)- - -shows a good positive correlation between two method

(p<0.001)- - -shows a high statistical significance

Table 6: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein 1-2 gms/day

Table 6 the results show a good positive correlation between 24 hrs urine protein and Spot urine protein creatinine ratio with a (r) value of (0.686) and a high statistical significance with a p value of p<0.001

Scatter diagram 2 shows that the correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein 1-2 gms/day is good with a highly significant p-value.

Scatter Diagram 2: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein 1-2 gms/day

(r=.311) - - -shows a weak positive correlation between two methods.

(p<0.03) - - -Shows no statistical significance

Table 7 the results show a weak positive correlation between 24 hrs urine protein and Spot urine protein creatinine ratio with a (r) value of (0.311) and no statistical significance with a p value of p<0.03

Table 7: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein >2 gms/day

Scatter diagram 3 shows that there is less correlation found between 24 hours urine protein and spot urine protein creatinine ratio without a significant p-value.

Scatter Diagram 3: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein >2 gms/day

DISCUSSION

In our study 24 hrs urine protein, spot urine protein and spot urine creatinine are estimated for 50 patients having varying degrees of proteinuria due to kidney diseases like CKD, Diabetic nephropathy, Nephrotic Syndrome etc. Protein creatinine ratio is calculated from spot urine protein and spot urine creatinine 24 hrs urine protein ranged between 0.253 gms/day-3.66 gms/day, Protein creatinine ratio ranged between 0.24-7.

We divided proteinuria patients into three groups as follows

Table 5: Correlation between 24 hrs urine protein and spot urine protein creatinine ratio for patients having 24 hrs urine protein <1 gm/day
1. 24 hrs urine protein <1 gm/day (Table 5)
2. 24 hrs urine protein 1-2 gms/day (Table 6)
3. 24 hrs urine protein >2 gms/day (Table 7)

When we analyze the correlation between 24 hours urine protein and protein creatinine ratio Group 1 (patients having less than 1 gms/day) and Group 2 (1-2 gms/day) patients showed a good positive correlation with r-values 0.855 and 0.686, with a high statistical significance with p value less than 0.001.

This is in consistent with the following studies

1. Chu NF et al showed that there was an excellent correlation between 24 hrs urine protein excretion and the single voided urine protein creatinine ratio at various levels of proteinuria. The proteinuria more than 1.0 gm/day group also showed a better relationship than the group with proteinuria of less than 1 gm/day.
2. Karthikeyan et al showed that these 24 hr urine protein values and spot urine protein creatinine ratio values correlated well in patients with 24 hr urine protein in non nephrotic range and in normal mildly impaired renal function.
3. Wai kwan siu et al demonstrated a good positive correlation between 24 hrs urine protein and spot urine protein creatinine ratio over a wide range of proteinuria and most commonly measured range (<3 gms/day).

4. Group 3 patients (24 hr urine protein >2 gms/day) had a weak positive correlation with the r-value of 0.311 which did not have a statistical significance.
5. Naufal Rizwan et al. and karthikeyan et al in their studies found a better positive correlation between 24 hours urine protein and protein creatinine ratio, with minimal to moderate proteinuria (<3 gms/day) than proteinuria of >3 gms/day.
6. But in contrast to our study, Jacob et al has found out that nephrotic range of proteinuria >4 gms/day also exhibited a good positive correlation between both 24 hrs urine protein and protein creatinine ratio.

CONCLUSION

From the results and discussion held so far and by comparison of 24 hrs urine protein and spot protein creatinine ratio for 50 patients having varying degrees of proteinuria due to kidney diseases like CKD, Diabetic nephropathy, Nephrotic Syndrome the following are concluded.

1. 24 hrs urine protein ranged between 0.253 gms/day-3.66 gms/day
2. Protein creatinine ratio ranged between 0.2-4.7.
3. There is a better positive correlation and high statistical significance between 24 hrs urine protein and spot urine protein creatinine ratio with proteinuria of less than 2 gms/day than with proteinuria of greater than 2 gms/day.
4. Hence the urine spot protein creatinine ratio could be used routinely as alternative method to 24 hours protein in our clinical biochemistry lab to quantitate proteinuria.

FUTURE SCOPE OF THE STUDY

1. This study can be extended with larger population.
2. Study can be extended by taking larger sample size with proteinuria >3 gms/day
3. Disease based study can be done i.e., study of different kidney diseases can be done.

REFERENCES