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ORIGINAL ARTICLE

A STUDY ON EGFR, CREATININE CLEARANCE & URINARY PROTEIN TO CREATININE RATIO IN CKD

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ABSTRACT: There was a good correlation between Glomerular Filtration Rate (GFR) values estimated by any of the 3 methods. Crcl correlated better with Cockcroft-Gault (CG eGFR) than Modification of Diet in Renal Disease (MDRD eGFR) and there was also a very good correlation between CG eGFR and MDRD eGFR. Based on this result we feel that in advanced CKD all 3 methods give similar GFR estimates but in earlier stages of Chronic Kidney Disease (CKD) Creatinine clearance (Crcl) gives higher estimate of GFR. Thus use of Crcl may wrongly classify patients into less severe stages of CKD which might lead to delay in initiation of proper treatment. **MATERIALS AND METHODS:** CKD patients were selected on the basis of serum creatinine levels. S.C > 2.5 mg/dl values were considered to be having CKD. 75 Samples were tested. Both males & females patients are taken in this study. Serum Creatinine & Urinary Creatinine by Jaffe's method, Blood Urea by Urease method, Urinary Protein by Pyrogallol red method, estimation of GFR by three methods. Creatinine Clearance &eGFR by using Cockcroft- Gault& MDRD equations. **RESULT:** A total of 75 patients attending the nephrology department (P.E.S Medical College, Kuppam)for treatment and monitoring for chronic kidney disease where included in the present study. Serum creatinine, serum urea and creatinine clearance were measured in all these patients as per methods described under materials and methods. Estimated GFR (eGFR) using CG (Cock croft) and MDRD (Modification of Diet in renal disease) formulae were calculated. Majority of the cases were complications due to long standing diabetes mellitus or hypertension. **CONCLUSION:** Creatinine Clearance over estimates GFR in early stages of CKD. Use of Crcl to assess GFR may not be proper to classify CKD into different stages. Any of the 3 methods (Crcl, CG eGFR and MDRD eGFR) can be used as markers of GFR in advanced stages of CKD. MDRD eGFR is a better indicator of eGFR for staging CKD in early stages of CKD. All patients included in the present study had gross proteinuria even in early stages of CKD. There was no correlation between protein: creatinine ratio and CKD stages. We recommend a larger study reporting eGFR using MDRD equation along with serum creatinine for evaluation of renal function and confirm our findings. Estimated Glomerular Filtration Rate (eGFR), Glomerular Filtration Rate (GFR), Cockcroft-Gault (CG eGFR), Modification of Diet in Renal Disease (MDRD eGFR), Creatinine clearance (Crcl), Chronic Kidney Disease (CKD).

KEYWORDS: GFR- Glomerular Filtration Rate, CKD-Chronic Kidney Disease, Creatinine Clearance.

INTRODUCTION: Chronic renal failure (CRF) is a clinical syndrome resulting in the progressive loss of renal function. The symptoms of chronic renal failure (CRF) result not only from simple excretory failure but also from the onset of regulating failure, the kidneys failure to regulate certain substances, such as sodium and water.[1]

The kidney performs a multitude of essential functions to maintain homeostasis and is intricately involved in the regulation of blood pressure, erythropoiesis and bone mineral metabolism.

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A variety of biological markers are used to assess the functioning status of the kidney. Historically blood urea and serum creatinine levels have been used to assess renal function.[2]

Assessment of renal function represents the commonest core laboratory testing that is performed worldwide. The increasing prevalence of many chronic diseases particularly diabetes mellitus, hypertension, cardiovascular diseases predisposing to renal failure and renal diseases together with the increasing medical care and its impact on improving life expectancy have all centered on the importance of organ functions assessment including most importantly renal function.

Chronic kidney disease (CKD) is also a significant risk factor for vascular disease and early cardiovascular mortality as well as progressive kidney disease.[3]

According to the National Kidney foundation kidney dialysis outcomes quality initiative (NKF-KDOQI) guidelines, chronic kidney diseases are classified into 5 stages.[4]

The NKF – KDOQI guidelines stratify chronic kidney disease from stage 1 at the mild end of the spectrum to stage 5. Stage 1 and 2 CKD are usually not associated with any symptoms arising from the decrement in GFR. If the decline in GFR progresses to stage 3 and 4, clinical and laboratory complications of CKD become more prominent. Virtually all organ systems are affected, but the most evident complications include anemia and associated easy fatigability. If the patient progresses to stage 5 CKD, toxins accumulate such that patients usually experience a marked disturbance in their activities of daily living, well-being, nutritional status, and water and electrolyte homeostasis, culminating in the uremic syndrome.[5]

Quantification of proteinuria is an important parameter of screening and monitoring of kidney diseases. Across every stratum of eGFR higher amounts of proteinuria, signal an increased risk of death, CVS diseases and CKD progression. 24hrs urine collections are generally considered the gold standard for urinary protein quantification, but this procedure has important limitations owing to errors in completeness of collection.[2]

In our clinical practice assessment of renal function is still mainly dependent on serum creatinine levels and estimations of creatinine clearance which are beset with methodological problems. CG and MDRD equations are widely used for calculating eGFR. However these equations for eGFR calculations have not been validated among the Indian population.

The present study aims to evaluate the MDRD and CG creatinine clearance formulae for eGFR calculation among Indian CKD patients and compare these values with creatinine clearance and urinary protein to creatinine ratio from random urine sample to rule in (or) rule out proteinuria in patients with CKD admitted in the medicine department in PESIMSR, Kuppam.

REVIEW OF LITERATURE:

Chronic Kidney Disease: CKD encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function and a progressive decline in GFR.[6]

GFR = Glomerular Filtration Rate: CKD has emerged as a major contributor of human morbidity and mortality globally. Approximately 19 million Americans older than 20 years have chronic kidney disease, and an additional 435, 000 have end-stage renal disease. The incidence of end-stage renal disease, with its annual mortality rate of 24 percent, has doubled every decade since 1980. Chronic kidney disease is 100 times more prevalent than end-stage renal disease, and its incidence is increasing at an even faster rate.[7]

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In the absence of a renal registry the exact disease burden of CKD in our population cannot be assessed. Based on few most representative populations based studies it is estimated that the approximate prevalence of CKD is ~ 800 per million populations (PPM), and the incidence of end stage renal disease is 150-200 PPM in Indian population.[8]

Classification Of Ckd[9]: All individuals with a Glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for 3 months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage.

It is classified into 5 Stages, they are:

Stage 1 CKD: Slightly diminished function; Kidney damage with normal or relatively high GFR (>90 ml/min/1.73 m^2).
Stage 2 CKD: Mild reduction in GFR (60-89 ml/min/1.73 m^2) with kidney damage.
Stage 3 CKD: Moderate reduction in GFR (30-59 ml/min/1.73 m^2).
Stage 4 CKD: Severe reduction in GFR (15-29 ml/min/1.73 m^2).
Stage 5 CKD: Established kidney failure (GFR <15 ml/min/1.73 m^2.

Risk Factors of CKD[4, 10]: It is important to identify factors that increase the risk for CKD, even in individuals with normal GFR. Risk factors include hypertension, diabetes mellitus, autoimmune disease, older age, and African ancestry, a family history of renal disease, a previous episode of acute kidney injury, and the presence of proteinuria, abnormal urinary sediment, or structural abnormalities of the urinary tract.

CRF may be caused by any conditions which destroy the normal structure and function of the kidney. Causes of end stage renal disease, with percentage distribution, are as follows:

1. Diabetes mellitus – 40%.
2. Hypertension – 30%.
3. Glomerulonephritis- 10%.
4. Interstitial nephritis or pyelonephritis – 15%.
5. Polycystic kidney disease – 3%.
6. Secondary glomerulonephritis or vacuities – 2%.
7. Miscellaneous or unknown cause -11%.

Biochemical Assessment of Kidney Function[2]: Historically, urea was the first marker used to formally assess kidney function. Urea is the major form of nitrogenous waste in the body. It is the product of protein and amino acid metabolism and eliminated almost entirely via urinary excretion. Although originally discovered decades earlier, in 1827 Richard Bright was the first to associate an accumulation of urea in the blood with its decrease in the urine among individuals with diseased kidneys.

Serum Creatinine: As a result, an increase in serum creatinine may not be observed until a substantial decrease in GFR has occurred. Additional limitations to the use of serum creatinine to estimate GFR arise from the substantial variability in between-person and within-person creatinine generation. In an attempt to account for this variation, several serum creatinine–based equations

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have been developed to estimate GFR, the most notable being the Cockcroft–Gault, Modification of Diet in Renal Disease (MDRD), and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equations for adults and the Schwartz equation for children. Although these equations generally increase the reliability of estimating the GFR, they all have limitations.

For example, the MDRD equation is known to underestimate the GFR, particularly at lower creatinine concentrations, whereas the Cockcroft–Gault and Schwartz equations have been shown to overestimate the GFR, especially at lower creatinine concentrations. Lastly, the equations do not account for differences that may occur as a result of unusually high or low muscle mass, extreme diets (vegan or excessive meat consumption), or ethnic variation of groups not included in their derivation.

Clearance-Based Markers of Kidney Function[2]: Using the concepts of renal clearance, one may accurately estimate the GFR using endogenous or exogenous substances. The renal clearance of a specific substance is understood to be the volume of plasma that can be completely cleared of that substance in a unit of time.

This is expressed as:

Where C is the clearance of a substance x, U is the urinary concentration of substance x, V is the urine flow rate, and P is the plasma concentration of substance x. Homer Smith is widely credited with introducing renal clearance methodologies and popularizing their utility in the noninvasive measurement of GFR. In his seminal text The Kidney: Structure and Function in Health and Disease**,** Homer Smith described properties of a substance suitable for the clearance-based estimation of GFR, in that it must:

1. Be completely filterable at the glomerulus.
2. Not be synthesized or destroyed by the tubules.
3. Not be reabsorbed or excreted by the tubules.
4. Be physiologically inert, so that its administration does not have any disturbing effect upon the body.

Inulin Clearance[2]: Inulin, a polymer of fructose found in tubers, is an exogenous substance that fulfills the criteria outlined above. The classic method for using inulin clearance to measure GFR described by Homer Smith requires early morning testing in a fasting state, oral fluid loading to promote diuresis, bladder catheterization to ensure complete urine collection, continuous inulin infusion at a constant rate, and multiple urine and blood collections once a steady state has been achieved . Inulin clearance is then calculated from the plasma concentration, urine concentration, and urine flow rate. Inulin clearance is still regarded as the gold standard for the measurement of GFR, although it is rarely used clinically because of the restricted availability of inulin and invasiveness of the procedure. Currently, inulin measurement is not offered in most clinical laboratories.

MATERIALS AND METHODS: The conducted study was a cross section study. Target population was patients admitted in the medicine department of PES HOSPITAL, KUPPAM. 75 Samples were tested.

Among that both Males and Females were included. All patients with CKD attending the nephrology unit of PESIMSR Hospital were included. Patients with Renal Involvement Secondary to

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Trauma & Obstructive diseases were excluded. Blood urea was done manually by DAM METHOD(diacetylmonoxime) reference range 14-40mg/dl,[11]Serum Creatinine reference range 0.6-1.mg/dl, Urinary Creatinine reference range 1-2gm/day was done manually by JAFFE'S METHOD [12] using Colorimeter, and Urinary protein measurement was done using a kit method which is basically colorimetric method. Creatinine Clearance &eGFR were determined by using the formula and calculation.

PROTEIN: CREATININE RATIO: Formula = Volume of urine/ 100 × urinary creatinine.

RESULT: A total of 75 patients attending the nephrology department for treatment and monitoring for chronic kidney disease where included in the present study. Serum creatinine, serum urea and creatinine clearance were measured in all these patients as per methods described under materials and methods. Estimated GFR (eGFR) using CG (Cock croft) and MDRD (Modification of Diet in renal disease) formulae were calculated. Majority of the cases were complications due to long standing diabetes mellitus or hypertension.

Age and Gender distribution of these patients are presented in figure 1 and 2. 80% of the patients were beyond the age of 40 years. A few cases with a lower age with unknown etiology also got included. Among the more than 40 years age group majority of them were in the age range 40 to 80 age range.

73% of the patients were males while 27% were females. There was no significant difference in the age distribution between male and female.

Patients were classified as stage II to V as per the guidelines of national kidney foundation based on Creatinine clearance, CG eGFR and MDRD eGFR. Table 1 show the distribution of cases in different stages using the 3 parameters. Based on creatinine clearance 12% of the cases were in stages II, 20% in stage III, 34% in stage IV and 33% in stage V. as per CG eGFR there were no cases in stage II only 7% were in stage III, 52% of the patients were in stage IV and 40% were in stage V. as per MDRD eGFR 24% of cases were in stage III 33% in stage IV and 43% in stage V there were no cases in stage II.

MDRD eGFR categorised more cases into advanced stages of CKD compared to Crcl or CG eGFR. The number of cases classified as stage V was more or less similar in all the 3 methods used for estimating GFR.

STAGE II			STAGE III			STAGE IV			STAGE V		
CRCL	**CG EGFR**	**MDRD EGFR**	**CRCL**	**CG EGFR**	**MDRD EGFR**	**CRCL**	**CG EGFR**	**MDRD EGFR**	**CRCL**	**CG EGFR**	**MDRD EGFR**
8 [10.6%]	0	0	11 [14.6%]	5 [6.66%]	18 [24%]	16 [21.3%]	28 [37.3%]	16 [21.3%]	20 [26.6%]	21 [28%]	21 [28%]
1 [1.3%]	0	0	4 [5.33%]	0	0	10 [13.3%]	11 [14.6%]	9 [12%]	5 [6.66%]	9 [12%]	11 [14.6%]

TABLE 1: Distribution of cases in stages of CKD AS PER CRCL, CG EGFR & MDRD EGFR

The actual GFR value measured using the 3 methods are presented in table 2 and figure 4.a and 4.b GFR by Creatinine Clearance was 63.85ml/min/1.73m^2 in stage II and there were no patients who showed similar GFR estimate using either CG or MDRD formulae. GFR measurements among stage III patients were slightly higher (41.24 ml/min/1.73m^2) compared to CG eGFR (35.76

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ml/min/1.73m^2) or MDRD eGFR (34.93 ml/min/1.73m^2) In stage IV and stage V patients GFR measurements by all the 3 methods were comparable.

STAGE II			STAGE III			STAGE IV			STAGE V		
CRCL	CG EGFR	MDRD EGFR	CRCL	CG EGFR	MDRD EGFR	CRCL	CG EGFR	MDRD EGFR	CRCL	CG EGFR	MDRD EGFR
63.85	0	0	41.24	35.76	34.93	22.37	20.46	22.88	8.56	9.55	9.86

TABLE 2: GFR by CRCL, CG & MDRD eGFR in stages CKD

CKD STAGES	CRCL	CG EGFR	MDRD EGFR	STATISTICAL SIGNIFICANCE
II	63.85±2.54	29.87 ± 6.827	32.33±5.39	***, +++
III	41.24±7.04	21.63 ± 4.71	30.00±7.68	***, +++
IV	22.37±4.69	17.26±5.43	20.93±7.43	***, +++
V	8.56±3.77	9.23±3.07	9.24±3.72	NS
POOLED	26.52 ±18.72	16.97± 8.200	20.22 ± 10.75	

TABLE 3: Comparison of CG EGFR & MDRD EGFR values with CRCL values in different stages of CKD as per Crcl values

*** Comparison between CRCL & CG EGFR P<0.001

+++ Comparison between CRCL & MDRD EGFR P<0.001

NS – NOT SIGNIFICANT

Table 3 shows the comparison of GFR measured by Crcl with those estimated by CG and MDRD formulae in different stages of CKD as per Crcl values. In stage II to IV the Crcl levels were much higher when compared to GFR estimated by CG or MDRD formulae. In stage V there was no difference in GFR values between creatinine clearance and CG or MDRD eGFR. Even the mean GFR value in all the 75 cases was higher by Creatinine clearance as compared to CG or MDRD eGFR.

CKD STAGES	CRCL	CG EGFR	MDRD EGFR
II	87.11±23.47	0	0
III	78.47±33.35	86.2±9.44	77.55±26.05
IV	84.62±27.08	80.4±30.22	85.4±29.68
V	122.28±62.84	117.77±57.54	115.21±58.37

TABLE 4: Serum urea levels in different stages of CKD as per Crcl, CG & MDRD eGFR

Table .4 shows serum urea levels in different stages of CKD according to Creatinine Clearance, Cg eGFR or MDRD eGFR. Serum urea levels were elevated in all stages of CKD by any of the methods used and there was no significant difference between stages.

Show correlation between the GFR values determined using the 3 methods. The highest correlation of R=0.862 was obtained between CG and MDRD eGFR values. Creatinine clearance values showed a better correlation with CG eGFR (R=0.800) as opposed to MDRD eGFR (R= 0.791).

Protein to creatinine ratio was high in all stages of CKD irrespective of the CKD stages by any of the 3 methods of estimating eGFR and values ranged from 1851 to 2240 mg/gm of creatinine.

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Shows the correlation between PCR and GFR measured using the 3 different methods, there was no significant correlation between PCR and GFR values since PCR was uniformly elevated in all stages of CKD. The lack of correlation between PCR and GFR in the patient studied is not surprising.

DISCUSSION: Assessment of renal function has been based on plasma creatinine levels for a long time. However, serum creatinine has been found to be a poor indicator as levels are influenced by muscle mass, age, gender, race and also methodological variations in techniques of creatinine measurement.

Clearance based indicators using exogenous as well as endogenous markers have been found to be better indicators of renal function. Renal clearance of exogenous substances like inulin, I125 - Iothalamate etc, has been found to closely reflect to exactly GFR. Endogenous substance clearances like creatinine clearance have been found to be more accurate but they are cumbersome, labor intensive and may be associated with disadvantage of the need for 24hrs urine collection with its known drawbacks of wide intra individual variations, inaccuracy and inconvenience and collecting 24hrs urine specimen.

Development of formulae based calculation of eGFR has offered approaches for converting serum creatinine value into GFR result with its advantages in reflecting actual GFR.

Many formulae have been developed for calculating eGFR but among them CG (Cock croft) MDRD (Modification of diet in renal disease) formulae have been used widely both formulae have been validated in western population and MDRD eGFR has been found to be better than CG eGFR. The MDRD eGFR is routinely used in western countries for measurement of eGFR and classification of CKD (Chronic Kidney Disease). These formulae however have not been validated among Indian or other south Asian population and their use is still not widely practiced in these countries.

In the present study we have studied 75 CKD patients and estimated creatinine clearance values using 24hrs urine collection and also calculated eGFR using CG and MDRD equations. We found that using creatinine clearance as a marker of GFR more patients were classified into stage 2 CKD while using CG and MDRD equation none of the patients could be categorized into stage 2.

Creatinine is filtered and also secreted by renal tubules and it is quite possible the amount of creatinine secretion by the tubules increase with onset of CKD. This would result in Crcl values being higher leading to over estimation of GFR. However in patients who were classified as stage 4 and 5 there was no difference in either GFR values or the number of patients who belong to these stages using any of the 3 methods for measurement of GFR.

There was a good correlation between GFR values estimated by any of the 3 methods. Crcl correlated better with CG eGFR than MDRD eGFR and there was also a very good correlation between CG eGFR and MDRD eGFR. Based on this result we feel that in advanced CKD all 3 methods give similar GFR estimates but in earlier stages of CKD Crcl gives higher estimate of GFR. Thus use of Crcl may wrongly classify patients into less severe stages of CKD which might lead to delay in initiation of proper treatment.

Our results suggest that use of MDRD equation for calculating GFR can be adapted among our population and it might provide a better method for staging CKD in our population. In view of the increasing prevalence of diabetes and hypertensive complications among our population it may be necessary to use better methods like MDRD eGFR for assessment of renal functions.

The urea levels were uniformly elevated in all stages of CKD. We are not sure whether stage 4 and 5 CKD patients could have had different urea levels since all these were already on regular dialysis therapy.

Protein to creatinine ratio in our patients was found to be elevated in all patients in all stages of CKD. There was no difference in protein to creatinine ratio values in different stages of CKD. There are reports suggesting that PCR could also correlate with different stages of CKD. However in our patients there was no relationship between PCR and different stages of CKD. [13-18].

The extent of proteinuria depends upon the protein nutritional status. Because of the poor nutritional status of our population proteinuria may not correlate with eGFR particularly in advanced stages of CKD.

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