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Comparative study of glomerular filtration rate estimatation by formulae using two different methods for creatinine estimation in chronic kidney disease

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ABSTRACT

Ckd is rapidly raising epidemic with increasing prevalence in India due to raised diabtes and hypertension cases. This is a Prospective crosssectional study & proven cases of Ckd (n = 100) admitted in the hospital between March 2014- August 2015. at Gandhi Hospital were included in the study. Serum creatinine was measured by Jaffe's and creatininase-creatininase methods and gfr was calculated using conventional formula and Cockcroft-gault formula. 100 patients were selected with male predominance (1.5:1) and average mean age group of 5^{th} to 6^{th} decade are the Ckd cases.This study established that the clearance measured by 24-hour urine collection has inherent errors, and determined that the CGF formula produces better results closest to the clinical symptoms and condition of chronic kidney disease, than the creatinine clearance obtained with conventional method (i.e., 24-hour urine collection) in patients, stating that creatinine clearance obtained by using enzymatic creatinine method with COCKCROFT –GAULT formula correlates better.

1. Introduction

Chronic kidney disease is rapidly assuming epidemic proportions globally.[1–3] The prevalence of kidney failure is estimated to be 8-16% worldwide. In India too, there is significant burden though exact figures vary,[4] 40-60% are attributed to the increasing prevalence of Diabetes mellitus hypertension, ischemic heart disease. In most cases, GFR continues to decline even when the initial insult has been removed.[5,6] Glomerular Filtration Rate (GFR) is the best parameter to assess the overall kidney function.[7] Normal level of GFR varies according to age, sex and body size. Normal GFR in young adults-approximately 120-130mL/min per1.73m^2. It can be measured by calculating plasma clearance of various glomerular filtration markers like creatinine, inulin, etc; in a 24-hr urine sample. These give an accurate measurement but are time consuming and costly.[8,9] Creatine's anhydride is creatinine. Creatine and Creatine phosphate on nonenzymatic spontaneous dehydration gives creatinine. It is excreted into plasma at a constant rate. The amount of creatinine synthesised depends on muscle mass, age, sex, diet and exercise.[10] Plasma creatinine is inversely related to glomerular filtration rate and directly proportional to urinary creatinine. Creatinine clearance is the ratio of the rate of creatinine excretion in urine to its concentration in serum, a value that reflects the body's ability to excrete creatinine in the past, 24 hrs. Urine creatinine clearance has been regarded as a more sensitive tool for the detection of kidney failure than a single plasma creatinine measurement. However, the inconvenience of a timed urine collection, failure to collect the entire specimen, and the wide (11%) intra individual variability, restrict the usefulness of this procedure. While recognising the inadequacies of plasma creatinine and a 24 hr creatinine clearance, the National Kidney Foundation

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Disease Outcomes Quality Initiative (NKF- KDOQI) recommended use of estimates of GFR calculated from prediction equations based on plasma or serum creatinine. Cockcroft and Gault published an equation to predict creatinine clearance based on age, weight, and height and plasma creatinine, together with correction factors. Therefore, the present study was designed to measure creatinine by two different methods i,e jaffe's kinetic method & creatinase-creatininiase method and compare the conventional method i,e 24-hr urinary creatinine clearance method with Cockcroft-Gault formula (CGF) in a patient population with wide range of renal function, to evaluate their clinical utility.

2. Materials and Methods

1. To measure the creatinine by two different methods - jaffe's kinetic method & creatinase-creatininiase method.
2. To compare and determine the accurate method of GFR estimation among the 24-hr urinary creatinine clearance method & Cockcroft-Gault formula in order to measure degree of renal dysfunction and progression of established kidney disease.

The present study was done in March 2014- August 2015 at Gandhi Hospital, Secunderabad, after getting approval from Ethical Committee. It was a Prospective cross sectional study. 100 Patients having chronic kidney disorder receiving dialysis of age group 30-60 years Cases were selected for this study. Jaundice patients, pregnant women, Chronic alcoholics, Patients on drugs like Metamizole, Methyldopa, Ethamsylate were excluded from the study. Blood and 24 hr urine samples were collected for the estimation of creatinine in blood and urine. Blood samples were centrifuged and serum was separated and stored in refrigerator at 2-8 °C and were analyzed in batches. 24-hr urine was collected in a 5 litre container with thymol preservative and analysed as soon as the sample was received by the laboratory. Estimation of creatinine in the samples was done by two methods Jaffe's kinetic method using 750 mmol/L of NaOH which is followed in our laboratory routinely. Enzymatic method using creatinase and creatininase carried out on AGAPPE- MISPA EXCEL semi autoanalyzer which is taken as reference method traceable to IDMS method.

In the present study we have estimated serum creatinine and urinary creatinine in 100 samples by two different methods, out of which enzymatic method was taken as reference method which is traceable to IDMS method. The values obtained are calculated by the conventional creatinine clearance and Cockcroft –Gault formula. The statistical analysis was based on paired t test using the Graphpad prism analysis software and IBM analysis software. The aim was to compute the levels of differences between the methods. The probability of significance (p value) is considered significant less than 0.05 i.e., considering α =5. The values are analyzed in terms of Mean, Standard Deviation, Standard Error of Mean, Paired sample test. A possible relation between the differences and the means was examined by calculating the rank correlation between the absolute differences and the means. The differences between measured Crcl and formula varied in a systematic way over the range of measurements; therefore a logarithmic (log) transformation of the data was used to remove the correlation between the differences and the means. The limits of agreement were considered clinically appropriate if they were between −10% and +10% limits. The Bland and Altman regression approach was used to calculate the limits of agreements (using log transformation of the data did not remove the correlation between the differences and the means, and therefore could not be used).

3. Results

In our study male predominance was observed with 60 males and 40 females with a male to female ratio of 1.5:1. Most of patients of CKD were in the 5^{th} to 6^{th} decade of life.

In females, mean value of serum creatinine by jaffe's method is 4.41±2.56 and mean value by enzymatic is 5.52±2.66 and p value is 0.001, this shows that Enzyamtic is more significant than Jaffe's.In males, mean value of serum creatinine by jaffe's method is 5.54±2.44 and mean value by Enzymatic is 6.61±2.55 and p value is 0.0001, this shows that Enzymatic is more significant than Jaffe's.

In females, mean value of measured creatinine clearance by jaffe's method is 16.11±7.16 and mean value by Enzymatic is 11.40± 4.65 and p value is 0.001, this shows that Enzymatic is more significant than jaffe's. In males, mean value of measured creatinine clearance by jaffe's method is 9.0±3.04 and mean value by Enzymatic is 6.91± 2.21 and p value is 0.0001s, this shows that measured creatinine clearance of Enzymatic is more significant than jaffe's.

In females, mean value of estimated creatinine clearance by jaffe's method is 19.85±7.4 and mean value by Enzymatic is 13.47± 4.2 and p value is 0.0001, this shows that estimated creatinine clearance by Enzymatic is more significant than Jaffe's. In males, mean value of estimated creatinine clearance of CGF by jaffe's method is 19.61±7.2 and mean value by Enzymatic is 14.5± 5.8 and p value is 0.0001, this shows that estimated creatinine clearance of CGF by Enzymatic is more significant than jaffe's (Table 1).

4. Discussion

Chronic kidney disease (CKD) is a major global public health problem. Based on data from the Ausdiab study,[11] it is estimated that one in every 7 adults will have CKD,

Table 1: Comparison of mean values of serum creatinine in males and females by two methods

	Gender	Method	Mean±SD	P value
Creatinine	Females	Jaffe's	4.41±2.56	0.001***
		Enzymatic	5.52±2.66	
	Males	Jaffe's	5.54±2.44	0.0001***
		Enzymatic	6.61±2.55	
Creatinine clearance	Females	Jaffe's	16.11±7.16	0.001***
		Enzymatic	11.40±4.65	
	Males	Jaffe's	9.0±3.04	0.0001***
		Enzymatic	6.91±2.21	
creatinine clearance by CGF	Females	Jaffe's	19.85±7.4	0.001***
		Enzymatic	13.47±4.2	
	Males	Jaffe's	19.61±7.2	0.0001***
		Enzymatic	14.5±5.86	

including one in 10 individuals with at least moderate kidney failure (defined as a glomerular filtration rate ŠGFRĆ $<$ 60 mL/min/1.73m2). Similar findings have also been reported in North America[12] and Europe.[13] Moreover, 1% of adults each year will develop new-onset CKD. Over the last 25 years, while the world's population has grown by approximately 1.5% per annum, the number of individuals being treated with dialysis or kidney transplantation has increased more than 8% per annum.[14]CKD is often not associated with significant symptoms and is unrecognized in 80-90% of cases.[11,15,16] Its presence is a very strong risk factor for cardiovascular disease, such that individuals with CKD have up to a 10- to 20-fold greater risk of cardiac death than age- and sex-matched controls without CKD.[17,18]Furthermore, patients with CKD are at least 20 times more likely to die from cardiovascular disease than survive to the point of needing dialysis or kidney transplantation. Early identification and management of CKD is highly cost-effective and can reduce the risk of kidney failure progression and cardiovascular disease by 20%-50%.[19] Study by NHANES(2006)[20] gives the prevalence of co-morbidites in CKD stage 5 and with age group of 40-59, therefore the present study correlates the same prevalence which is around 24.5%.[20] Since the cases considered in the present study are only 100, the prevalence must be higher, and the actual % should be based on the population study, even though the prevalence is correlating with NHANES study. Ajay K Singh1 et al.[21] (2014)., In India, given its population $>$1 billion, the rising incidence of CKD is likely to pose major problems for both healthcare and the economy in future years. The prevalence of CKD was observed to be 17.2% with ~6% have CKD stage 3 and more. Serum Creatinine, one of the clinically useful analyte has been used by clinicians as a marker of renal function. As creatinine neither secreted nor absorbed by glomerular apparatus, it is considered as best endogenous marker to assess glomerular function. Creatinine clearance varies depending on the method used to measure and the instrument used to estimate creatinine. More the manual equipment and the method used to measure, result in greater error, compared to enzymatic and automated analysers. Most frequently used method for creatinine estimation in various labs is Jaffe's – routine kinetic method, even though it was standardised there is considerable variation from lab to lab. Now- a- days, Enzymatic method isotopically traceable to IDMS was considered as the gold reference standard method for measuring creatinine. Among the many physiological roles of the renal system, GFR is considered the best indicator of overall kidney function and its assessment has become an important clinical tool in the daily care of patients. Estimation of GFR was conventionally done by collecting 24-hr urinary specimen and is calculated by formula

$$GFR = \frac{Us\ Vu}{Ps}$$

U_s – Urine cone of substance 's' P_s– Plasma concentration of substance 's' V_u – Urine volume per minute. estimation of GFR by using creatinine-based equations (ex: CGF and MDRD) is an alternative to the clinician [Martin E Lascano, Emilio D. Poggio][22] to assess the kidney function. In the present study, though MDRD is widely used, it does not consider anthropometric measurement, which is very essential for creatinine estimation. Creatinine values vary in children, males and females and within contingency. It varies depending on muscle mass.

In 1976, Cockcroft DW, GaultMH, formulated Cockcroft gault formula which was the oldest one, but till date it is used in the context of assessing drug dosages for patients with renal impairment. In the present study, serum and urinary creatinine was estimated by 2 different methods in 100 samples. The value of serum and urinary creatinine obtained by enzymatic method (reference method) showed higher creatinine values in all the samples as compared to the routinely used Jaffe's method in our laboratory (i.e. with 90mmol/L NaOH). Measured creatinine clearance ($CrCl_m$) is derived from measurement of creatinine excretion in 24hr urinary output and concomitant SrCr concentration, a procedure that is inconvenient for the patient and inaccurate as a result of potentially unreliable collection

of the 24hr sample. The Cockcroft-Gault(CGF) equation originally published in 1976 was derived in 249 consecutive hospitalised patients (96% male, age range 18–92 years) at the Queen Mary Veterans' Hospital in Canada, based on the means of two 24-hour creatinine clearances. Serum creatinine concentrations were determined by Jaffé reaction using an autoanalyzer (N-11B, Technicon Instruments Corp, NY). The derived formula was then used to predict creatinine clearance in a second validation cohort consisting of 236 patients (206 males, mean creatinine 36.6 mL/min). The Cockcroft-Gault equation has the advantages.clearance 72.7 of being more widely known, easier to remember and more extensively validated than the MDRD formula. Although the equation was developed in hospitalised, white men, many of whom did not have CKD; it has subsequently been extensively validated and found to exhibit satisfactory precision and bias in diverse populations including women and various ethnic groups, and across a broad range of GFRs. The principal disadvantages of the Cockcroft-Gault formula are the requirement to measure weight and height (the latter is required for the purposes of body surface area correction), its estimation of creatinine clearance rather than GFR, and the inability of clinical laboratory creatinine assays to be calibrated to the laboratory that performed the assays on samples used to derive the Cockcroft-Gault equation.

The formula is currently recommended by the American Food and DrugAdministration for pharmacokinetic studies, Plasma creatinine is derived from creatine and phosphocreatine break down in muscle, the reference interval encompasses the range of muscle mass observed in the reference population used. This limitation contributes to the insensitivity of creatinine as a marker of diminshed GFR. In patients with CKD, extra renal clearance of creatinine becomes important when caused by degradation as a result of bacterial overgrowth in the small intestine.[23] According to Michael Peake and Malcolm Whiting,[24] all methods for measuring serum creatinine should have their calibration traceable to an IDMS reference measurement procedure, with low combinations of bias and imprecision, routine methods have the potential to meet the goal of $< 10\%$ total error recommended by NKDEP.

The reference method in the present used is traceable to IDMS, as it is an enzymatic method.

Serum creatinine assay calibration has no influence on the coefficients of the Cockcroft-Gault equation, because the regression did not involve serum creatinine. The study by Ajay K Singh1et al[(21)](2014)., included 5588 subjects. The mean $\pm$ SD age of all participants was 45.22 $\pm$ 15.2 years (range 18–98 years) and 55.1% of them were males and 44.9% were females. The overall prevalence of CKD in the SEEK-India cohort was 17.2% with a mean eGFR of 84.27 $\pm$ 76.46 versus 116.94 $\pm$ 44.65 mL/min/1.73 m2in non-CKD group while 79.5% in the CKD group had proteinuria. Prevalence of CKD stages 1, 2, 3, 4 and 5 was 7%, 4.3%, 4.3%, 0.8% and 0.8%, respectively.

In the present study, mean of estimated GFR by CGF in females is 13.47$\pm$9.2 when compared to measured creatinine clearance 11.40$\pm$10.03 in CKD cases, and estimated creatinine clearance in males is 14.53$\pm$8.8 compared to measured creatinine clearance 6.9$\pm$6.2 in males by enzymatic method, stating prevalence of CKD stages of 3,4 and 5 as 5%, 7% and 88% respectively, the high percentage is due to less number of cases in the study who were admitted in Nephrology department.

In 2012, Vijaya Marakala, et al.,[25] compared analytical performance and practicability of the enzymatic method and kinetic method for serum creatinine for routine use and to compare the effects of some common interfering substances like glucose and bilirubin on the enzymatic method and kinetic Jaffe's method, found that the Mean differences between enzymatic to kinetic Jaffe's methods were -0.042mg/dl, Overall mean difference between the two methods was -0.081 mg/dl. All of the above differences were statistically insignificant ($p>0.05$). The enzymatic creatinine methods appear to be the only assays giving reliable results when specimens take time to reach the laboratory and blood centrifugation is delayed for 24hr or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine by alkaline picrate assays due to the possible interference effect of some metabolites built up in vitro, such as pyruvate or ketones. A minor disadvantage of the enzymatic method is its relatively high cost and estimation of creatinine by enzymatic method showed no statistically significant mean difference (-0.042) with the kinetic Jaffe's method, which is used by several laboratories (including our own centre) in samples without glucose and bilirubin interference. In the presence of glucose interference (glucose $>$ 126 mg/dl), the samples showed no statistical significant mean difference (-0.116) between enzymatic and kinetic Jaffe's methods

5. Conclusion and Summary

A total of 100 samples which are received for serum creatinine and urinary creatinine estimation of chronic kidney disease were selected and measured using the jaffe's fixed kinetic method and Enzymatic method (IDMS traceable). By using this creatinine values, Creatinine clearance was calculated by conventional formula (measured creatinine clearance) and by Cockcroft-Gault formula (estimated creatinine clearance).From this study, it was found that the creatinine estimated by enzymatic method is more significant for calculating creatinine clearance by conventional formula, as well as by Cockcroft Gault formula than with creatinine clearance values obtained by using serum creatinine estimated by jaffe's method. Enzymatic method is best as the sample volume required was lesser, the throughput was higher, the interfering substances

were fewer for this method, the enzymatic method for estimation can be preferred especially in the setting of neonates, patients with diabetes, keto acidosis, jaundice and haemolysis. Whereas jaffe's method was not specific as many compounds produce a Jaffe like chromogen which include bilirubin, haemoglobin, protein, glucose, ascorbic acid, ketone bodies, pyruvate etc. are responsible for false positive results. The estimated creatinine clearance (CGF) is more significant than measured creatinine clearance. This study established that the clearance measured by 24-hour urine collection has inherent errors, and determined that the CGF formula produces better results closest to the clinical symptoms and condition of chronic kidney disease, than the creatinine clearance obtained with conventional method (i.e., 24-hour urine collection) in patients, stating that creatinine clearance obtained by using enzymatic creatinine method with COCKCROFT –GAULT formula correlates better. To harmonise both the methods of creatinine estimation and there by harmonise GFR values more studies are to be performed with greater number of cases. This requires inclusion of non–CKD individuals and individuals with CKD of stage 1 and 2. The limitations of the present study is that only CKD with stage 3,4 and 5 are included. Hence the present study cannot come to a common correction factor for various methods of creatinine estimation (Jaffe's and Enzymatic). Arriving at a correction factor for creatinine methods will help in accurate estimation of GFR and eliminates inter laboratory and instrumental variations of GFR in CKD. Hence, more studies are to be conducted in this direction.

6. Source of Funding

None.

7. Conflict of Interest

None.

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