International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

*Original Research Article*

# Comparison of Various Formulas Used to Calculate eGFR in Type 2 Diabetes Mellitus

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*Received: 05/08/2015* *Revised: 24/08/2015* *Accepted: 28/08/2015*

## ABSTRACT

**Background:** Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both leading to chronic hyperglycemia. Diabetic complications result from the toxic effects of chronic hyperglycemia combined with other metabolic derangements. Diabetic nephropathy eventually leads to loss of kidney function being the most common cause of End stage renal disease (ESRD). Glomerular filtration rate (GFR) is the best overall index of renal function. As Gold standard methods of estimating GFR are impractical for routine use, estimated GFR is calculated using various formulas employing endogenous markers like serum creatinine and serum Cystatin C.
**Aims and Objectives:** To compare the different formulas used for calculating estimated glomerular filtration rate (eGFR) in type 2 diabetes mellitus.
*Methods and Data collection*: The study was carried out in 30 Type-2 Diabetic patients and 30 non-diabetic controls, in the age group of 35 to 75 years. Both the groups were age and gender matched.
**Results:** There was significant difference in the mean eGFR using different equations in cases and controls. The lowest mean eGFR was seen in the cystatin C equation (47.90 ± 15.37 ml/min/1.73m$^2$). Highest prevalence of stage 3 chronic kidney disease (CKD) (< 60 ml/min/1.73m$^2$) was found with the CKD EPI Cystatin C equation (80%) followed by the CKD EPI combined equation (54%) among cases.
**Conclusion:** Inclusion of Cystatin C in equations used to estimate GFR lead to lower estimated GFR, which in diabetic patients may actually reflect a nearer estimate of actual renal function.

***Key words:*** Type 2 Diabetes mellitus, Diabetic nephropathy, eGFR, Cystatin C, Creatinine.

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both and covers a wide range of heterogeneous diseases. [1] Although there is an increase in the prevalence of type 1 diabetes, the major driver of the epidemic is the more common form of diabetes, namely Type 2 diabetes, accounting for more than 90% of all cases. [2]

Diabetic complications result from the toxic effects of chronic hyperglycemia combined with other metabolic derangements. Persons with diabetes are at substantial risk for tissue injury in organs supplied by an endarterial system due to microangiopathy. These microvascular

complications include nephropathy, retinopathy, and neuropathy. [3] Diabetic nephropathy (DN) is the leading cause of chronic renal disease and a major cause of cardiovascular mortality. Diabetic nephropathy has been classically defined as increased protein excretion in urine. [4]

Screening for diabetic nephropathy must be performed when diagnosis of Type 2 DM is made in individuals, since they may have had a silent form of DM for some time already. The first step in screening for DN is to measure albumin in an isolated urine sample. Where quantitative measurements are not available, semi quantitative dipstick measurements of albuminuria can be used, despite being less accurate. [4]

Glomerular Filtration Rate (GFR) is the best parameter of overall kidney function and should be measured or estimated in micro and macroalbuminuric patients. [5] GFR is defined as the clearance of a substance in the plasma which is exclusively metabolized by the kidneys and freely filtered by the glomeruli. [6]

Current Gold standard methods for determining GFR employ the clearance of exogenous radio isotopes like Cr EDTA or non radiolabelled markers like Inulin. They require specialized technical personnel, working over a period of several hours. In addition a number of practical considerations like cumbersome methods of determinations, radio activity and high costs have limited the use of these techniques in routine clinical practice. [7, 8]

The ideal substance to determine GFR must be an endogenous substance having a stable rhythm of production, constant maintenance of circulating levels not affected by other disorders, freely filtered by glomeruli without tubular interference, like secretion or reabsorption. [6]

Serum creatinine is considered relatively specific, but not very sensitive since its levels significantly increase only when more than 50% of the GFR is reduced. Unfortunately, the influence of non renal factors on serum creatinine concentration including age, gender, ethnicity, muscle mass, dietary protein intake and numerous drugs and endogenous substances interfere with its measurement, leading to falsely high or low values, thus limiting its usefulness as an ideal marker of GFR. To overcome the limitations of using creatinine alone, equations to estimate GFR (eGFR) based on serum creatinine have been developed that include variables such as age, sex, race and measurements of body size. [7, 8]

The MDRD (Modification of Diet in Renal Disease) study was based on a multicentre trial to evaluate the effect of dietary protein restriction and blood pressure control on progression of renal disease in 1628 patients with CKD, with the added objective of developing an equation that could improve the prediction of GFR from plasma creatinine. A 6-variable equation was derived, and subsequently a simplified 4-variable version which included age, gender, plasma creatinine value and race differentiation as white or black was published. Results were expressed as per 1.73 $m^2$ of body surface area. To overcome the error from instrument bias, a unified effort to standardize creatinine measurement to the reference isotope dilution mass spectrometry (IDMS) method was encouraged in laboratories around the world. With this, a new factor of '175' (as opposed to '186') was subsequently recommended in the MDRD equation for creatinine assays that are IDMS aligned. [9]

The CKD Epidemiology Collaboration group (CKD-EPI) developed and validated a new equation in 2009 designed to match the accuracy of the MDRD equation at GFR < 60 mL/min/1.73$m^2$ and to offer greater accuracy at higher GFR, minimizing the

over-diagnosis of CKD with the MDRD equation. The new CKD-EPI equation was developed from 8254 data points from six studies and four clinical populations, with original serum creatinine values recalibrated to the Roche enzymatic method. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) is a research group established by the National Institutes of Diabetes, Digestive and Kidney Disease. The CKD-EPI equation was shown to be as accurate as MDRD in the subgroup with eGFR < 60 ml/min/1.73m$^2$ and substantially more accurate in the subgroup with eGFR > 60 ml/min/ 1.73m$^2$. [9, 10]

Cystatin C, a Cysteine protease inhibitor is freely filtered by the renal glomeruli, metabolized by proximal tubule and indentified as a promising marker of renal failure. Cystatin C is produced at a constant rate by nucleated cells and released into the blood stream with a half life of 2 hours. Its concentration is almost totally dependent on GFR, [12] the independence from height, gender, age and muscle mass is advantageous. [11]

Because of the limitations of creatinine, Cystatin C is being considered as a potential replacement for serum creatinine as a filtration marker. GFR-prediction equations based upon cystatin C (eGFR cystatin C) or creatinine (eGFR creatinine) may produce estimated GFR-values, of which 80 – 85% is within ± 30% of GFR measured by invasive gold standard methods. [12, 13]

Hence this study is intended to compare the various formulas using creatinine and cystatin C to calculate eGFR, in diabetic individuals.

## MATERIALS AND METHODS

The study was carried out in 30 Type-2 Diabetic subjects and 30 non-diabetic controls who attended the outpatient and inpatient department of Medicine of our hospital during the year 2012-13. The age of the diabetic subjects ranged from 35 to 70 years and age and gender matched healthy persons were chosen as controls.

Patients with Hypertension, thyroid disorders, Congestive cardiac failure, liver disease, rheumatoid disease, malignancy, fever, dehydration and patients on Glucocorticoids, nephrotoxic drugs, smoking and alcohol users were excluded from the study.

The institutional ethical committee approved the study protocol. History and personal physical data was obtained from both cases and controls.

Informed consent was taken from patient and control subjects. A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age and gender, detailed medical history including conventional risk factors, clinical examinations and relevant investigations were included as part of the methodology.

5 ml of venous blood sample was collected after overnight fasting of 12 hours from both cases and controls and the samples were centrifuged and separated for the estimations. Estimation of serum creatinine (IDMS aligned) was performed using the Jaffe's kinetic method on Roche Hitachi analyser. Estimation of serum Cystatin C was done by Immunoturbidimetric method on Biosystems A25 analyzer. Overnight fasting urine sample was collected in a clean dry container and was tested for albumin immediately using urine albumin dipstick method.

The various eGFRs were calculated using online calculator of National Kidney Foundation.

***Statistical analysis:*** The Statistical software SPSS 17.0 was used for the analysis of the data. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements were presented as

Mean ± SD. P value < 0.05(95% confidence interval) was considered significant. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups. ANOVA was used to study the significance of means between more than two groups. Kappa statistics was used to assess the degree of agreement between different equations.

## RESULTS

The mean serum creatinine was 0.82 ± 0.13 in controls and 0.99 ± 0.26 among cases and was statistically significant. The mean serum Cystatin C was 1.11 ± 0.06 among controls and 1.53 ± 0.34 among cases and was statistically significant. eGFR from CKD-EPI equations using creatinine alone, Cystatin C alone, creatinine and Cystatin C both and MDRD among both controls and cases are statistically significant.( Table 1 )

The diabetic cases were subdivided into three groups based on the duration of the disease and eGFR from CKD-EPI equations using creatinine alone, Cystatin C alone, creatinine and Cystatin C combined and MDRD were compared among the three groups and were all statistically significant. (Table 2)

The diabetic cases were again sub classified based on the urinary albumin excretion assessed by dipstick method into four groups. eGFR from the above equations were compared among these groups and the means were statistically significant. (Table 3)

**Table.1 Comparison of Creatinine, Cystatin C, estimated GFR in diabetics and non diabetics.**

| | Controls | Cases | P Value |
|---|---|---|---|
| Creatinine (mg/dL) | 0.82 ± 0.13 | 0.99 ± 0.26 | 0.003 |
| Cystatin C (mg/dL) | 1.11 ± 0.06 | 1.53 ± 0.34 | < 0.001 |
| CKD-EPI Creatinine | 96.46 ± 12.22 | 80.30 ± 20.42 | 0.002 |
| MDRD | 87.70 ± 13.08 | 73.20 ± 17.95 | 0.002 |
| CKD-EPI Creatinine & Cystatin C | 80.06 ± 8.12 | 60.33 ± 16.70 | < 0.001 |
| CKD-EPI Cystatin C | 68.83 ± 7.83 | 47.90 ± 15.37 | <0.001 |

**Table.2 Distribution of mean estimated GFR by duration of Diabetes**

| Equations | All patients | Duration of diabetes in years | | | |
|---|---|---|---|---|---|
| | | < 1 | 2 - 4 | ≥ 5 | P value |
| CKD-EPI Creatinine | 80.30 ± 20.42 | 106.85 ± 4.94 | 83.84 ± 7.48 | 57.10 ± 9.42 | < 0.001 |
| MDRD | 73.20 ± 17.95 | 96.42 ± 8.14 | 76.15 ± 6.53 | 53.10 ± 7.54 | < 0.001 |
| CKD-EPI Creatinine & Cystatin C | 60.33 ± 16.70 | 83.28 ± 8.78 | 61.23 ± 7.56 | 43.10 ± 5.56 | < 0.001 |
| CKD-EPI CystatinC | 47.90 ± 15.37 | 69.57 ± 11.34 | 47.92 ± 5.90 | 32.70 ± 3.05 | < 0.001 |

**Table.3 Distribution of mean estimated GFR by urinary albumin excretion in diabetes patients**

| Equations | All patients | Urinary Albumin excretion | | | | P Value |
|---|---|---|---|---|---|---|
| | | Nil | + | ++ | +++ | |
| CKD-EPI Creatinine | 80.30 ± 20.42 | 101.63 ± 8.64 | 81.00 ± 3.62 | 63.12 ± 6.59 | 46.00 ± 5.29 | < 0.001 |
| MDRD | 73.20 ± 17.95 | 91.54 ± 9.63 | 74.12 ± 3.68 | 58.00 ± 5.01 | 44.00 ± 4.35 | < 0.001 |
| CKD-EPI Creatinine & Cystatin C | 60.33 ± 16.70 | 78.18 ± 9.92 | 58.57 ± 5.47 | 46.37 ± 3.20 | 36.33 ± 3.20 | < 0.001 |
| CKD-EPI CystatinC | 47.90 ± 15.37 | 63.72 ± 11.95 | 46.37 ± 5.01 | 34.37 ± 3.11 | 30.00 ± 0.00 | < 0.001 |

+ 30mg/dL, ++100mg/dL, +++ 300mg/Dl

**Table.4 Prevalence (%) of CKD stages according to equations used.**

| CKD stages ml/min/1,73 $m^2$ | Controls | | | | Cases | | | |
|---|---|---|---|---|---|---|---|---|
| | CKD-EPI Cr | CKD-EPI CysC | CKD-EPI Cr & Cys C | MDRD | CKD-EPI Cr | CKD-EPI CysC | CKD-EPI Cr & Cys C | MDRD |
| ≥ 90 | 20(67) | 2 | 6(20) | 13(43) | 10(33) | 1(3) | 1(3) | 6(20) |
| 60 - 89 | 10(33) | 28(93) | 24(80) | 17(57) | 14(47) | 5(17) | 13(43) | 16(53) |
| 30 - 59 | 0 | 0 | 0 | 0 | 6(20) | 24(80) | 16(54) | 8(27) |
| 15 - 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| < 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table.5 Kappa Co-efficients between equations among Cases**

| Equations | MDRD | CKD EPI Cr | CKD EPI CysC | CKD EPI Cr & CysC |
|---|---|---|---|---|
| MDRD | ***** | 0.683 | 0.01 | 0.301 |
| CKD EPI Creatinine | | ***** | 0.02 | 0.06 |
| CKD EPI Cystatin C | | | ***** | 0.461 |
| CKD EPI Creatinine & Cystatin C | | | | ***** |

**Table.6 Kappa Co-Efficients between equations among controls**

| Equations | MDRD | CKD EPI Cr | CKD EPI CysC | CKD EPI Cr & CysC |
|---|---|---|---|---|
| MDRD | ***** | 0.553 | 0.171 | 0.493 |
| CKD EPI Creatinine | | ***** | 0.069 | 0.222 |
| CKD EPI Cystatin C | | | ***** | 0.444 |
| CKD EPI Creatinine & Cystatin C | | | | ***** |

There was a downward trend in the eGFR values from different equations as the duration of Diabetes increased and also as the amount of albumin excretion in urine increased. The lowest mean eGFR was seen in the cystatin C equation (47.90 ± 15.37 ml/min/1.73m$^2$) followed by the combined cystatin C – Creatinine equation, MDRD and CKD EPI creatinine equations. Introduction of cystatin C lowered the eGFR values. Highest prevalence of stage 3 CKD (< 60 ml/min/1.73m$^2$) was found with the CKD EPI Cystatin C equation (80%) followed by the CKD EPI combined equation (54%) among cases. (Table 4)

Kappa statistics showed a good concordance between the MDRD and CKD-EPI equations in cases and controls. Moderate agreement was found between MDRD and CKD EPI Creatinine- Cystatin C equation among controls, while it was fair among cases. Moderate agreement was found between CKD EPI Cystatin C and CKD EPI Creatinine – Cystatin C equations among controls and cases. Poor agreement was found between the creatinine equations and the CKD EPI Cystatin C equation among both controls and cases. (Tables 5 and 6)

## DISCUSSION

Diabetes is the most common cause of end stage renal disease (ESRD). Approximately 40% of patients with type 1 and 15% of patients with type 2 diabetes eventually develop ESRD. [14] Indeed type 2 diabetics with ESRD are rapidly increasing because of continuing increase of type 2 diabetes mellitus and the progressively decreasing mortality rate from cardiovascular diseases. [15]

Individuals with mild or moderately decreased renal function are at increased risk for chronic kidney disease and cardiovascular disease. Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment. Although microalbuminuria is the first detectable functional abnormality, glomerular filtration rate (GFR) is the critical renal function. [16] The gold standard for estimation of GFR is clearance of endogenous substances which are incompatible with routine monitoring. Hence estimation of GFR using endogenous substances is much preferred.

In our study we first determined the levels of serum creatinine, Cystatin C and urine albumin among type 2 diabetes cases and matched controls, we then calculated the eGFR among these two groups using various equations. Comparison of these equations was also done in diabetic groups based on duration and urine albumin excretion.

We found a significant increase in the levels of cystatin C among cases which was similar to the results of a study done by Borges et al. [17]

Estimated GFR calculated using the CKD EPI Cystatin C equation was the lowest among the equations followed by the CKD EPI combined equation. In general inclusion of cystatin C resulted in lower eGFR.

About 20-30 % of patients with type 2 DM accompanied by renal insufficiency showed normoalbuminuria. [18] In our study, among diabetic patients with normoalbuminuria the cystatin C equation yielded a lower eGFR followed by the combined equation, while the creatinine equations resulted in higher eGFR. This indicates that equations including cystatin C give a nearer estimate of actual renal function. The highest percentages of estimated GFR-values within ± 30% of measured GFR (gold standard) are obtained using GFR-prediction equations based upon both cystatin C and creatinine (eGFR cystatin C + creatinine ). [13,14]

Highest percentages of stage 3 CKD were found with the equations which included Cystatin C (Cystatin C alone and Cystatin C with creatinine) in our study. Addition of cystatin C as a predictor of GFR may improve the identification of CKD. This would have implications for the identification and treatment of CKD in the individual patient and also for planning and allocation of resources for public health management at the level of public health administration. [19]

Although our study is limited by the population size, it concurs with various other studies found in literature. Hence further studies can be done involving larger Indian population to further assess the accuracy of these equations in comparison to gold standard methods.

## CONCLUSION

Inclusion of Cystatin C in equations used to estimate GFR lead to lower estimated GFR compared to creatinine based equations. Among diabetic patients cystatin C based equations may actually reflect a nearer estimate of actual renal function.

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How to cite this article: Anil Kumar A.S, Ashwin Kumar A.S. Comparison of various formulas used to calculate eGFR in type 2 diabetes mellitus. Int J Health Sci Res. 2015; 5(9):283-289.

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