

## INTRODUCTION

Diabetic nephropathy (DN) is the single most frequent cause of end-stage renal disease in the world and is the most common indication of haemodialysis. However, only about one third patient with diabetes develop nephropathy. It is characterized by microalbuminuria, subsequent macroalbuminuria, and declining glomerular filtration rate (GFR). An increasing number of type 2 diabetic patients live long enough for nephropathy and end-stage renal disease to develop because of the improvement in the treatment of diabetes, hypertension and coronary heart disease. Moreover, presence of stage 3 or higher chronic kidney disease (estimated GFR < 60 mL/1.73m<sup>2</sup>) is associated with high cardiovascular mortality.

Accurate figures regarding prevalence of DN is lacking in India. Screening for DN is traditionally is being done by monitoring patients for the development of microalbuminuria, estimation of e-GFR, determination of serum creatinine (sCr), creatinine clearance (CCr), and nuclear scan. However, there are several limitations to these methods and search for ideal method is constantly being explored.

## PATHOLOGY

It has been known since decades that the GFR is elevated in diabetes mellitus in early stages. Morphologically, the development of diabetic nephropathy is characterized by progressive thickening of the glomerular basement membrane and by expansion of the mesangial matrix which correlates to glomerular filtration function. The hallmark of diabetic glomerulopathy is diffuse mesangial expansion, associated with nodule formation in a minority of patients. Early hemodynamic changes of glomerular hyperperfusion and hyperfiltration are followed by leakage of albumin from the glomerular capillaries and structural changes such as glomerular basement membrane thickening, glomerular hypertrophy, glomerulosclerosis, mesangial cell expansion, and podocyte injury and loss.

## CLINICAL FEATURES

Persisting pedal and periorbital edema, decreasing urine output, increasing blood pressure in a diabetic patient are the features which should prompt physician to look for possible DN. In the initial phase increase in GFR due to glomerular hyperfiltration is common. Persistent albuminuria (>300 mg/24 hr or 200 mg/min) is the hallmark of diabetic nephropathy, which can be diagnosed clinically if the following additional criteria

are fulfilled: presence of diabetic retinopathy and the absence of clinical or laboratory evidence of other kidney or renal tract disease. Systemic hypertension ensues later. This clinical definition of diabetic nephropathy is valid in both type 1 diabetes and type 2 diabetes. Absence of retinopathy almost rules out nephropathy in type 1 DM but in type 2 DM this chronology does not hold good in all cases.

## SCREENING AND DIAGNOSIS

Primary prevention of CKD, early detection of disease and prompt intervention with appropriate, evidence based measures will delay CKD onset and progression, improve kidney and cardiovascular outcomes, and reduce resources utilization. Despite these benefits, CKD is both under-diagnosed and undertreated, and awareness of CKD among both patients and providers is low. CKD is staged based on estimated glomerular filtration rate or eGFR. Detection of CKD in early stage gives treating physician great window of opportunity to preserve and protect kidney from further damage.

Screening of all diabetic patients for detection of nephropathy is mandatory. Several markers and methods are standardised over decades for detection of nephropathy and CKD. Measurement of serum creatinine, blood urea nitrogen, urinary albumin excretion, calculating the estimated GFR using creatinine clearance, inulin clearance and isotopic scans are used for either direct or indirect measure of kidney function. All these methods even though quite reliable, have several limitations. Most markers do not detect early nephropathy. Nuclear scan or Inulin clearance which can accurately detect GFR is either very expensive or cumbersome hence cannot be used in date to day practice. This brings us to the discussion of finding an ideal marker which can detect diabetic nephropathy in early stage.

## MICRO & MACROALBUMINURIA

Albumin excretion up to 30mg/day is considered normal. The earliest clinical evidence of DN is the appearance of low but abnormal levels ( $\geq 30$  mg/day or  $20 \mu\text{g}/\text{min}$ ) of albumin in the urine, referred to as microalbuminuria, and patients with microalbuminuria are referred to as having incipient nephropathy. Microalbuminuria typically occurs after 5 years in type 1 diabetes. Without specific interventions, about 80% of subjects with type 1 diabetes who develop sustained microalbuminuria have their urinary albumin excretion increase at a rate of 10–20% per year to the stage of overt nephropathy or

clinical albuminuria ( $\geq 300$  mg/24 h or  $\geq 200$   $\mu\text{g}/\text{min}$ ) over a period of 10–15 years, with hypertension also developing along the way. ESRD develops in 50% of type 1 diabetic individuals with overt nephropathy within 10 years and in  $>75\%$  by 20 years.

Type 2 diabetes has a more variable course, higher proportion of individuals are found to have microalbuminuria and overt nephropathy at the time of diagnosis of their diabetes but fewer patients with microalbuminuria progress to advanced renal disease. This is because, diabetes is actually present for many years before the diagnosis is made and also because the presence of albuminuria may be less specific for the presence of diabetic nephropathy, as shown by biopsy studies. Without specific interventions, 20–40% of type 2 diabetic patients with microalbuminuria progress to overt nephropathy, but by 20 years after onset of overt nephropathy, only about 20% will have progressed to ESRD.

National kidney Foundation and Kidney Disease Outcomes Quality Initiative (NKF: KDOQI) and professional bodies concerned with management of patient with diabetes recommend that all patients with type 2 Diabetes be screened annually for CKD, starting at diagnosis. Urinary albumin excretion should be evaluated either from 24 hour urine collection or from the albumin to creatinine ratio in a random spot sample. Because of fluctuations in urinary collection excretion, at least 2 of 3 samples collected within a 3 to 6 month time frame should be used to categorize the degree of albuminuria and avoid false positive results.

Measurement of albumin excretion in 24 hour urine and calculation of albumin and creatinine ratio in urine have been used with success in identifying patient who have microalbuminuria.

#### The limitations of estimation of microalbuminuria

1. Evaluation of urinary albumin excretion alone is insufficient to assess the presence of and severity of CKD because some patients with type 2 diabetes can have advanced stage nephropathy in the absence of albuminuria i. e normo albuminuric DN.
2. Few patients may have microalbuminuria but impaired renal function, but not the traditional decline of GFR with the development of proteinuria.
3. Dose not detects DN in early stage (stage of hyperfiltration).
4. Transient proteinuria like orthostatic proteinuria and overflow proteinuria as in case of multiple myeloma may interfere with the measurement of albuminuria.
5. Few conditions other than diabetic nephropathy can cause excretion of albumin in urine in the range of 30–300mg/day. These include fever, high-salt diet, vigorous exercise, marked hyperglycemia,

uncontrolled hypertension, urinary tract infection and dehydration.

### NUCLEAR SCAN

Nuclear scan is considered gold standard in measuring GFR. The radioactive tracers are used for determination of GFR. Glomerular filtration rate and estimated renal perfusion flow may be assessed using dynamic quantitative nuclear imaging techniques. The GFR quantifies the amount of filtrate formed per minute (normal: 125 mL/min in adults).

#### Advantages of the Nuclear scan

- Most accurate method
- Only method in which split GFR for each kidney can be obtained
- Least influence of body weight and age
- Helps in early detection of DN

#### Limitations of Nuclear Scan

1. Complex procedure
2. Needs specially trained individuals in handling and administering the tracers.
3. Time consuming
4. Expensive method, on an average cost of one DTPA scan is Rs. 4000 to 7000, in a resource poor set up, routine use of this method to determine kidney function is not practical.
5. Radiation exposure to patient and individuals who are in close proximity to patient.
6. Needs a dedicated nuclear scan centre with adequate measures to safely store radioactive compounds.

### RENAL BIOPSY

Is one of the invasive means of knowing status of kidney in case traditional means dose not prove conclusive. Renal biopsy is certainly not indicated when a type 1 diabetic patient has retinopathy and when the time course is consistent with DN. Renal biopsy should be considered, however, when proteinuria is present less than 10 years after the onset of type 1 diabetes. In type 2 diabetes, this argument is unreliable because the onset of type 2 diabetes is often not known. The presence of dysmorphic erythrocytes, erythrocyte casts, or cellular casts is not a feature of DN and should prompt investigations to exclude glomerulonephritis or vasculitis, if necessary by renal biopsy. Other indications are rapid deterioration of renal function or elevated serum creatinine without urine abnormalities. Finally, gross proteinuria is not infrequently associated with non-diabetic renal disease, for example, amyloid, focal segmental glomerulosclerosis, etc. Needless to say that prior to renal biopsy renal ultrasonography is indicated which by itself may already yield a diagnosis.

#### Limitations of Renal biopsy

1. Renal biopsy is used as an exception than a rule.

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2. In contemporary practice renal biopsy is obsolete technique to diagnose nephropathy
  3. It is an invasive procedure and hence has all risks involved with invasive procedure.
  4. Not a practical method as a routine use.

## ASSESSMENT OF FUNCTIONAL STATUS OF KIDNEYS USING EGFR

GFR is traditionally measured as the renal clearance of a particular indicator substance, or marker, from plasma. The clearance of an indicator substance is the amount removed from plasma, divided by the average plasma concentration over the time of measurement.

Under the right conditions, measuring the amount of an indicator in both plasma and urine can allow the accurate calculation of GFR. Indeed, if we assume that there is no extrarenal elimination, tubular reabsorption, or tubular secretion of the marker, then GFR can be calculated as follows:

$$\text{Glomerular filtration rate} = (U \cdot V)/(P \cdot T)$$

(U is the urine concentration, V is the urine volume, and P is the average plasma concentration of the marker over the time (T) of the urine collection).

Unfortunately, tubular secretion, tubular reabsorption, or both, of the indicator can cause renal clearance measurements to give estimates of the GFR that are falsely high or falsely low. Under the right conditions, plasma concentrations of an indicator substance can be completely dependent on renal clearance and can accurately reflect GFR. When the amount of an indicator added to the plasma from an exogenous or endogenous source is constant, and when there is no extrarenal elimination, tubular secretion, or tubular reabsorption, then the GFR is equal to the inverse plasma concentration of the indicator multiplied by a constant.

Whether endogenous or exogenous, an ideal indicator would distribute freely and instantaneously throughout the extracellular space. It would not bind to plasma proteins and would be freely filtered at the glomerulus. It would be subject to neither excretion nor reabsorption in the tubules or urinary collecting system. It would be completely resistant to degradation, and its elimination would be entirely dependent on glomerular filtration. It would be easy to measure in plasma and in urine, and nothing would interfere with the assay. Ideally, the inter- and inpatient coefficient of variation would be low.

According to the KDOQI guidelines of the NKF, CKD will be stratified into the following stages based on eGFR:

1. Stage 1: GFR  $\geq$  90 and Albumin excretion rate (AER)  $>$  30mg per 24 hr.
2. Stage 2: GFR 60-89 and AER  $>$ 30mg per 24 hr.
3. Stage 3: GFR 30-59.
4. Stage 4: GFR 15-29.
5. Stage 5: GFR  $<$  15.

(All values of GFR are in ml/min/1.73m<sup>2</sup> BSA)

Hence accurate eGFR measurement gives us stage of CKD. Current markers recognize CKD when at least 50-70% of nephrons in both kidneys are affected. Currently patients are diagnosed in stage 2 or 3 onwards where precious window of opportunity is lost to take necessary steps to either revert or slow the progression of CKD.

### Inulin

Inulin, an inert substance was once considered the gold standard of exogenously administered markers of GFR. It does not bind to plasma proteins. It distributes in extracellular fluid, is freely filtered at the glomerulus, and is neither reabsorbed nor secreted by renal tubules. eGFR is measured by giving a loading dose of Inulin orally and urine samples are collected at regular interval after a steady state is achieved. Currently this method carries only historical importance due to its high cost and complexity of the procedure.

### Urea

Urea was one of the first indicators used to measure eGFR. Unfortunately, it shares few of the attributes of an ideal marker, and plasma urea has been shown to be a poor measure of GFR. Urea production is variable and is largely dependent on protein intake. Although one quarter of the urea produced is metabolized in the intestine, the ammonia produced is reconverted to urea. Thus, most of the urea is ultimately excreted by the kidneys. Because of tubular urea reabsorption, renal urea clearance usually underestimates GFR. Urea clearance can be as little as one half or less of the GFR as measured by other techniques.

### Creatinine

Creatinine is a metabolic product of creatine and phosphocreatine, both of which are found almost exclusively in muscle. Thus, creatinine production is proportional to muscle mass and varies little from day to day. However, production can change over longer periods of time if there is a change in muscle mass. Age- and gender-associated differences in creatinine production are also largely attributable to differences in muscle mass. Hence due to so many confounding factors creatinine levels may not give an accurate functional assessment of kidney function.

## CREATININE CLEARANCE

Measuring creatinine clearance obviates some of the problems of using serum creatinine as a marker of GFR but creates others. Differences in steady-state creatinine production due to differences in muscle mass that affect serum creatinine does not affect creatinine clearance. Extrarenal elimination of creatinine has little influence on the ability of the creatinine clearance to estimate GFR. However, the reliability of creatinine clearance is greatly diminished by variability in tubular secretion of creatinine and by the inability of most patients to accurately collect timed urine samples. The need to collect a urine sample remains a major limitation of the creatinine clearance technique.

## CREATININE BASED EQUATIONS FOR MEASUREMENT OF EGFR

Many creatinine based mathematical equations have been formulated to calculate eGFR based on age weight and gender. Most extensively studied and validated among them are three: the Cockcroft and Gault(CG) equation, Modification of diet in renal disease equation (MDRD) and CKD Epidemiology equation. These have been corner stone of eGFR measurement in several studies and have been successfully replicated across populations.

### Cockcroft-Gault Equation

$$\text{eGFR} = (140 - \text{age in years}) \times \text{Body weight in Kg} / 72 \times \text{serum creatinine (mg/dl)} \times 0.85 \text{ if female.}$$

The Cockcroft-Gault equation is one of the most extensively used equation bedside to calculate eGFR. It estimates creatinine clearance in  $\text{mL} \times \text{min}^{-1}$ , but not GFR, and is not standardized to the body surface area of  $1.73 \text{ m}^2$ . In relation to GFR it systematically overestimates clearance because tubular creatinine secretion is not taken into account. Because this equation includes body weight, it is particularly recommended for the monitoring of renal function during treatment with medications that influence kidney performance.

### Modification of Diet in Renal diseases (MDRD) formula

$$\text{eGFR} = 186 \times (\text{serum creatinine mg/dl})^{-1.154} \times (\text{age in years})^{-0.203}$$

Multiplied by 0.742 if female and multiplied by 1.210 if African American

The GFR has probably never been measured with more accuracy in a large population of patients than it was in the Modification of Diet in Renal Disease (MDRD) Study. A simplified version requiring only serum creatinine value, age, race, and gender was found to similarly correlate with measured GFR. It was standardised mainly in patients with Stage 3 and higher stages and in patients on haemodialysis. This formula over estimates eGFR in early stages hence early CKD is undiagnosed.

### CKD-EPI (creat) Formula

$$\text{GFR} = 141 \times \min(\text{Scr}/\kappa, 1)^\alpha \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 [\text{if female}] \times 1.159 [\text{if black}]$$

$$\kappa = 0.7 \text{ if female } \kappa = 0.9 \text{ if male}$$

$$\alpha = -0.329 \text{ if female}$$

$$\alpha = -0.411 \text{ if male}$$

min = The minimum of  $\text{Scr}/\kappa$  or 1 max = The maximum of  $\text{Scr}/\kappa$  or 1

The CKD-EPI creatinine equation is based on the same four variables as the MDRD Study equation, but uses a 2-slope "spline" to model the relationship between estimated GFR and serum creatinine, and a different relationship for age, sex and race. The equation was reported to perform better and with less bias than the MDRD Study equation, especially in patients with higher GFR. This results in reduced misclassification of CKD.

## Limitations of Creatinine based formulas

1. Serum creatinine formulas to estimate the GFR may not be reliable in certain individuals. Individuals on a vegetarian diet, consuming creatinine supplements, with unusual muscle mass, with unusual weight (morbid obesity, amputation), or pregnant woman were not included in the study populations that were used to generate these formulas.
2. These formulas are not accurate for individuals with normal or near-normal kidney function and ethnic groups.
3. Among healthy individuals such as kidney donors, the MDRD formula underestimated GFR.
4. In kidney transplant recipients, the MDRD provided variable results.
5. For creatinine to raise beyond certain upper limit of normal at least 50-70% of nephrons should be damaged which gives little scope for early intervention and prevention.

## CYSTATIN C

Several low-molecular-weight (LMW) proteins have been evaluated as endogenous markers of GFR, with Cystatin C commanding the most attention. The use of serum Cystatin C as a marker of GFR was first suggested in 1985, when Simonsen and co-workers demonstrated a correlation between reciprocal Cystatin C values and  $^{51}\text{Cr}$ -labeled ethylenediaminetetraacetic acid ( $^{51}\text{Cr}$ -EDTA) clearance. Since then, numerous investigators have shown that cystatin C may be a particularly good marker of GFR. Cystatin C is a 13-kD basic protein of the cystatin superfamily of cysteine proteinase inhibitors. It is synthesized by all nucleated cells at a constant rate, fulfilling an important criterion for any endogenous marker of GFR. In most studies, production of Cystatin C is not altered by inflammatory processes, by muscle mass, or by gender. An increase in levels of Cystatin C after the 5th decade reflects the age-related decline in GFR and contrasts with stable serum creatinine values, presumably due to a decline in muscle mass with age. Because of its low molecular weight and positive charge at physiologic pH, Cystatin C freely passes the glomerular filter. It is not secreted, but proximal tubular cells reabsorb and catabolize the filtered Cystatin C, resulting in very low urinary concentrations. Although calculation of GFR using urinary Cystatin C is not possible, some investigators have speculated that urinary Cystatin C could serve as a marker for renal tubular dysfunction.

Studies in a number of patients have shown that serum Cystatin C may be more sensitive and specific than serum creatinine value for signifying early changes in isotopically determined GFR. ROC analysis of these studies demonstrated superiority of accuracy of Cystatin C over creatinine in patients with reduced GFR. In addition, small reductions in GFR appear to be detected more easily using cystatin C measurement than with creatinine

160 determination. Other studies have indicated that cystatin C determination has a greater ability to detect subclinical kidney dysfunction than using creatinine measurement. Coll and colleagues demonstrated that cystatin C levels rose when GFR fell to 88 mL/min/1.73 m<sup>2</sup> and that creatinine levels did not rise until GFR dropped to 75 mL/min/1.73 m<sup>2</sup>. A meta-analysis incorporating studies published in 46 articles and 8 abstracts and using standard measures of GFR suggested superiority of reciprocal Cystatin C value over reciprocal serum creatinine level as a marker of GFR.

Cystatin C has also been examined in a diverse number of groups. In children, Cystatin C measurement appears to be at least as useful as serum creatinine determination in assessing GFR, although the number of children studied who were younger than 4 years is small. This age subgroup, for which serum creatinine levels have been unreliable, might arguably be most benefited by the measurement of Cystatin C to evaluate GFR. Cystatin C has been favourably evaluated in other similar subgroups, including patients with cirrhosis spinal cord injury, and rheumatoid arthritis, as well as elderly patients.

In kidney transplant recipients, Cystatin C value has been found to be more sensitive than serum creatinine level in detecting decreases in GFR. In one study, levels of Cystatin C were significantly higher in 54 pediatric kidney transplant recipients than in 56 control subjects with similar GFR values.

### **CYSTATIN C BASED FORMULAS:**

Without exception all Cystatin C-based formulae were less biased than the MDRD formula with distinct 95% CIs being observed among these Cystatin C based CKD epidemiology equation is best studied. It includes correction based upon race, which makes it uniquely applicable to most of the races.

#### **CKD-EPI Cys C formula**

$$eGFR = 127.7 \times (\text{Cystatin C in mg/L})^{-1.17} \times (\text{age in years})^{-0.13}$$

multiplied by 0.91 if female.

With regard to accuracy, the proportion of estimated GFR results within 10% of isotopic GFR was greater using Cystatin C-based formulae than the MDRD formula, as evidenced by distinct 95% CIs.

Avinash et al conducted one of the first ever comparative study of Cystatin C based formulas in India in 2010. A total of 172 subjects having diabetes were stratified into different CKD stages based on eGFR calculated using Cystatin C and creatinine based formulas. Both in albuminuric and non-albuminuric subjects, Cystatin C based formulas stratified more subjects in early CKD compared to creatinine based formulas.

Advantages of Cystatin C based eGFR over Creatinine based eGFR in different clinical settings:

1. In children: children have low levels creatinine and determination is unreliable in the lower range of measurement.
2. The Elderly: Owing to physiological reduction

in renal functional and decrease in muscle mass, Cystatin C correlates better than creatinine with inulin clearance.

3. Myasthenic, leg amputees, paraplegics: Because of the lower muscle mass, creatinine synthesis is low and creatinine-based eGFR is late to detect renal failure.
4. Diabetics: Early stages of renal failure are detected more reliably with Cystatin C based than with creatinine based eGFR.
5. Liver cirrhosis: Creatinine methods are slow to detect the decrease in GFR because metabolism in liver is reduced.
6. Cytostatic treatment: The nephrotoxicity of cisplatin is dose dependent and a reduction in GFR is detected earlier by Cystatin C based than by creatinine based eGFR.
7. Contrast induced nephropathy: Cystatin C identified patients in early stages of CIN compared to creatinine. It is especially useful in case of patients undergoing Coronary angiography as these patients are already prone for acute kidney injury.

### **OTHER MARKERS OF KIDNEY INJURY**

Studies have identified a relatively small number of genes that are specifically altered in acute renal tubule injury. The front-runners are genes, such as osteopontin, clusterin, Glutathione S-transferase  $\alpha$ , neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (Kim-1), tissue inhibitor of metalloproteinase-1 (TIMP-1), interleukin 18 (IL-18). It has now become important to validate these as protein markers in urine and to confirm or refute their selectivity and sensitivity for use in preclinical studies and in disease states in humans.

Kim-1 is one of the best-characterized urinary biomarkers to date in both experimental animals and humans with renal disease.

In ischemic injury, Kim-1 expression is most prominent in the S3 segment in the corticomedullary region, which is the part of the nephron most susceptible to ischemic injury. Kim-1 expression is also prominent in the midcortical and superficial tubules in renal disease models, where the primary insult is not directed to the S3 segment. Kim-1 not only functions as a biomarker but also has predictive value for acute renal injury.

#### **Beta 2 microglobulin**

Beta 2 microglobulin is filtered by the glomeruli and reabsorbed by the proximal tubular cells where it is metabolized. Its plasma concentration increases with decreasing renal function. Beta 2 microglobulin was measured in several situations. And few studies have found it as a better marker than other.

Beta 2 microglobulin has been extensively studied in several studies and has been identified as an useful

marker but lacks large studies to use it as a regular marker of kidney disease.

Serum alfa-1 microglobulin, retinol binding protein, atrial natriuretic peptide, serum homocysteine have been tried with variable results.

As of now only serum creatinine and serum Cystatin C have been validated for clinical practice. Search for ideal and affordable marker is still on. Cystatin C being validated for eGFR measurement is a significant step forward. Early detection of kidney injury is possible with Cystatin C with least confounding variables.

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