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**Research Article**

# Assessment of Renal Function of Eritrean Diabetic Patients Using the GFR derived from the Serum levels Creatinine, Cystatin C or Creatinine- Cystatin C

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**Abstract:** Renal insufficiency in one of the most common complication in diabetic patients and in clinical practice renal function status is assessed using serum creatinine or its derivative eGFR. Recently Cystatin C is believed to be more sensitive for assessing renal function. A cross sectional descriptive study on 100 known 51 female ad 49 male diabetic patients whom are on regular follow up at diabetic clinic in Eritrea from Oct 2014 to Jan 2015. The aim of the study was to evaluate the renal function status and glycemic control of diabetic patients based on serum creatinine, cystatin C, and $HbA_1C$, respectively. Demographic data was analyzed using structured questioner and serum creatinine, Cystatin C, $HbA_1C$ and fasting blood glucose was determined. Data was calculated using SPSS version 19. Their age range from 12-89 years with mean (SD) 56(16.3) years and the serum creatinine ranges from 0.5-1.5 mg/dl and only 2 diabetic patients had abnormal serum creatinine, on the other hand serum cystatin C ranges from 0.5 to 2.5mg/dl and about 50 diabetic patients with high level above normal reference value. About 47 cases has poor glycemic control ($HbA_1C$ > 7.6%). Serum creatinine based GFR had shown about 75% of the cases fall on the normal range (>90ml/min/1.73m$^2$) However, based on serum Cystatin C only 22% of the cases fall on normal range and the rest were distributed into minimal, moderate, and high risk group stage. The study concluded that, serum Cystatin C based assessment is more sensitive and accurate to evaluate the kidney function status in diabetic patients and the study recommend that it is high time to introduce it in clinical practice to monitor the diabetic patient for possible risk and early detection of renal complication and further study can be performed in a larger sample size.
**Keywords:** Serum creatinine, Serum Cystatin C, Renal Failure, Eritrean Diabetic Patients.

## INTRODUCTION

Diabetes Mellitus often called 'Diabetics' or same times 'Sugar' is a condition that occur when the body unable to secrete enough insulin or when the body cannot utilize normal amount of insulin properly. Insulin is a hormone that regulates the amount of sugar in the blood. A high blood sugar level can cause problems to many parts of the body. The most common type of diabetes is type I and type II. Type I account 10% of the cases and usually begin in childhood, whereas Type II is the most common type of DM and usually is occurring in individuals of more than 30 years old. Uncontrolled DM can cause damage to many parts of the body, especially Kidney (Diabetic nephropathy, Heart (Atherosclerosis, Eyes (Diabetic retinopathy) and Nerves (Diabetic nephropathy). Uncontrolled DM may damage the renal blood vessels of the kidney. The first sign of renal damage is the presence of albumin in urine [1].

The glomerular filtration rate (GFR) is the most commonly used test of renal function and assessment of kidney disease. The GFR is defined as the rate at which the kidneys filter impurities from the blood stream [2]. It is calculated by measuring the volume of plasma that can be cleared of a particular substance within a unit of time. The gold standard for GFR measurement requires intravenous injections of exogenous radio labeled substances such as Cr- 51-EDTA and Tc-99m-DTPA, or iodinated agents such as iohexol. These procedures are invasive, risky, and time consuming. Alternatively, endogenous substances freely filtered by the glomerulus with minor absorption and secretion by the renal tubules have been used for estimation of GFR. Estimation of glomerular filtration rate (eGFR) is generally performed by the use of serum creatinine. Despite its application, it must be adjusted for factors such as muscle mass, age, gender, diet and race which limit its clinical reliability. Decreased GFR (GFR<60 ml/min per 1.73m$^2$) has found to be associated with increased mortality, cardiovascular

adverse events, hospitalizations, fractures, and unsuccessful aging. International guidelines recommend using the creatinine-based equations to estimate GFR, particularly the modification of diet in renal disease equation [1].

However, all creatinine-based estimating equations have limitations due to non GFR determinants of serum creatinine, largely muscle mass, which cannot be accounted for entirely by age, sex, and race. This is a particular problem was among the elderly, non-white populations, and in the range of mildly reduced GFR, where equations have bias. Therefore, the clinician's reliance on creatinine-based equations for estimating GFR and the risk associated with low GFR could cause misclassification of patients who may be at high risk of CKD and its complications [3].

Cystatin C is independent of these factors and can provide a more precise calculation of GFR in combination with creatinine or alone, without the need of adjustment like serum creatinine. Cystatin C is a low molecular weight (13.3 kDa) protein produced at a constant rate by all human cells containing a cell nucleus. Cystatin C has a stable production rate unaffected by inflammatory processes, gender, age, diet and nutritional status. Cystatin C is freely filtered through the glomerular membrane, and is reabsorbed and almost entirely catabolized in the proximal tubules. Kidney dysfunction will cause a variation in serum Cystatin C and can thus be used to give an immediate and accurate measurement of GFR. The reference interval for Ichroma TM Cystatin C assay is between 0.52 and 1.0 mg/L. In patients with kidney dysfunction the level of Cystatin C may be raised to above 1.1 mg/L. Extensive, well-documented studies comparing creatinine and Cystatin C as markers of GFR have demonstrated Cystatin C is superior to creatinine [4]. In particular, Cystatin C responds more quickly to changes in GFR than creatinine. Normal Cystatin C serum levels are the same for men, women and children, and Cystatin C does not have the "blind-area" from which creatinine suffers. Early signs of kidney disease are general and the use of Cystatin C as a marker is important to confirm the onset of kidney disease. The objective of this study was to introduce Cystatin C as base line for screening of renal function status in clinical practice and ratify diabetic management guidelines in Eritrea.

## MATERIALS AND METHODS

A cross-sectional descriptive study, conducted in Halibet National Referral Hospital and Hazhaz National Referral Hospital which are located in capital city Asmara. The samples were collected in Halibet National Referral and Hazhaz National Referral Hospitals and the sample processed and analyzed in Eritrea and Sudan Wad-medeni renal hospital laboratory from October 1, 2014 to January 10, 2015.

### Study population

Hundred registered diabetes mellitus patients coming from all parts of the Eritrea for routine follow up to two National Diabetic Clinics within national hospitals were included. The patients are males and females with type I and type II diabetes mellitus with different age groups. The duration of diabetes mellitus was from 3 and 32years with no known history of renal problems or dysfunctions.

### Inclusion and Exclusion criteria

Diabetic patients registered in one of the diabetic clinic for follow up with no known previous history of kidney disease or hypofunction and followed up for more than three years. We have excluded the diabetic patients with a previous history of chronic kidney disease. We have also excluded the patients suffering from thyroid disorders or whom under corticosteroid therapy, and the individuals with history of continuing smoking or Hypertension.

### Ethical Consideration

This study has obtained an ethical clearance from the MOH research ethical committee. All volunteers were informed and full explanation was delivered to them verbally on the aim, risks and benefits of the study in simple local language. Then written consent was collected. Anonymity will be maintained by using a form, which bear no name and will be linked with the sample by standard number. The results of the participants will be kept confidential.

### Blood and Data collection

A standardized structured questionnaire was used to collect the demographic data and history of diseases. Blood sample from diabetic patients whom fulfilled inclusion criteria of the study was collected using gold standard BD vacutainer® product. Five milliliter (5ml) venous blood was collected in plain BD vacutainer serum tube container for creatinine, Cystatin C and fasting blood glucose tests. Likewise 4.5ml venous blood collected on the same application in a separate BD test tube container with K3EDTA 3.6mg as anticoagulant for HbA1c test from the same patients. Measurement of serum creatinine, Hemoglobin A1c, glucose was performed using Auto chemical analyzer while Cystatin C measurement was done using Fluorescence immunoassay. Age specific equations [5] were used for the calculation of eGFR where epidemiology collaboration equations with exception to patients with age less than 18 years.

**Table 1: Summary of participant's characteristics parameters according to age categories in diabetic patients**

| Characteristics | | Age category in years | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | <20 | 20-29 | 40-39 | 40-49 | 50-59 | 60-69 | ≥70 | |
| Sex | Female | 0 | 3 | 6 | 7 | 13 | 17 | 5 | 51 |
| | Male | 3 | 1 | 3 | 7 | 9 | 11 | 15 | 49 |
| Total | | 3 | 4 | 9 | 14 | 22 | 28 | 20 | 100 |
| Creatinine level (mg/dl) | 0.00-1.30 | 2 | 4 | 9 | 14 | 22 | 27 | 20 | 98 |
| | >1.31 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| Total | | 3 | 4 | 9 | 14 | 22 | 28 | 20 | 100 |
| Cystatin C level (mg/L) | 0.52-1.10 | 1 | 2 | 8 | 5 | 12 | 15 | 7 | 50 |
| | 1.11-1.70 | 2 | 2 | 1 | 9 | 10 | 11 | 13 | 48 |
| | 1.71-2.50 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| Total | | 3 | 4 | 9 | 14 | 22 | 28 | 20 | 100 |
| HbA1c level (%) | <6.5 | 0 | 0 | 2 | 1 | 2 | 2 | 2 | 9 |
| | 6.5-7.4 | 0 | 0 | 1 | 4 | 5 | 6 | 6 | 22 |
| | 7.5-8.4 | 1 | 1 | 2 | 6 | 4 | 4 | 4 | 22 |
| | 8.5-9.5 | 1 | 2 | 2 | 2 | 8 | 5 | 4 | 24 |
| | >9.5 | 1 | 1 | 2 | 1 | 3 | 11 | 4 | 23 |
| Total | | 3 | 4 | 9 | 14 | 22 | 28 | 20 | 100 |
| Duration of DM (years) | <5 | 2 | 3 | 1 | 5 | 6 | 8 | 2 | 27 |
| | 5-10 | 0 | 1 | 5 | 5 | 9 | 9 | 8 | 37 |
| | 11-15 | 1 | 0 | 2 | 3 | 5 | 4 | 2 | 17 |
| | 16-20 | 0 | 0 | 1 | 1 | 1 | 5 | 5 | 13 |
| | >20 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 6 |
| Total | | 3 | 4 | 9 | 14 | 22 | 28 | 20 | 100 |

**Table 2: Mean values and standard deviation (SD) of eGFR of all participants using the Creatinine Equation (CKD-EPI 2009), Cystatin C Equation (CKD-EPI 2012) and Creatinine–Cystatin C Equation (CKD-EPI mix)**

| Equation used to estimate the GFR (ml/min/1.73m$^2$) | Age categories | | | | | | |
|---|---|---|---|---|---|---|---|
| | <20 | 20-29 | 40-39 | 40-49 | 50-59 | 60-69 | ≥70 |
| Creatinine Eq. | 104 ± 48 | 114± 12.6 | 117 ± 10.2 | 100 ±14.7 | 105± 12.3 | 95± 11 | 79 ±16.4 |
| Cystatin C Eq. | 78 ± 26 | 88± 28.5 | 88± 18.97 | 76 ± 28.7 | 75 ± 21.8 | 64± 21.1 | 64 ± 21.5 |
| Creatinine–Cystatin C Eq. | 93 ± 37 | 98 ± 18.2 | 100± 13.3 | 87± 23.1 | 88 ± 15.6 | 78 ± 17.7 | 72± 19.4 |

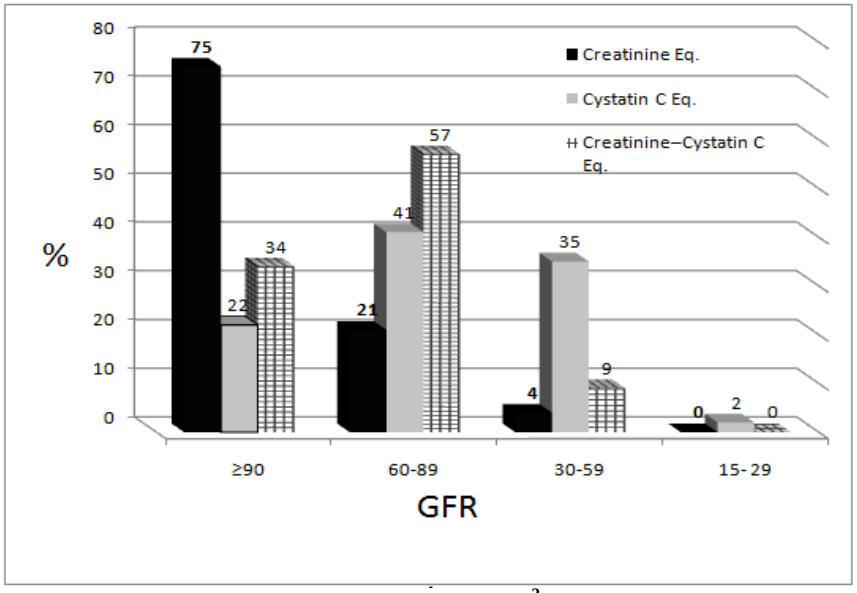


**Fig. 1: The number of participants verses eGFR in ml/min/1.73m$^2$ from 15 to 90 calculated with the 3 equations, the creatinine equation, cystatin c equation and the creatinine–cystatin C equation.**

## RESULTS AND DISCUSSION

The study was conducted in two National Referral Hospitals in Eritrea with the aim to assess the glycemic control (HbA1C level) and renal damage status of known diabetic patients. These patients are on regular follow up in diabetic clinic, and with no previous documented impaired renal function. The renal function was assessed using both serum creatinine and Cystatin C. Based on the serum creatinine and Cystatin C, the eGFR was calculated using epidemiology collaborated equations [5].

The study was done in in both genders. The male to female ratio was almost 1:1 and their ages were ranging from 12 to 89 years old. The study has revealed about 98% of the cases (2 cases had abnormal serum creatinine level) had normal serum creatinine level (Table 1), and 50% had normal serum cystatin C level, whereas the other 50% exhibit higher Cystatin C serum level (Table 1) .This suggests that there is a great variation in detecting the renal disease using the two methods. These results are in line with a recent study [6].

Many previous studies have provided evidences that the serum cystatin C has superiority over serum creatinine as a marker of renal impairment [7-9]. This can be explained by the fact that the serum creatinine as marker of GFR has drawbacks. Firstly, the endogenous production of creatinine is determined by dietary intake and muscle mass. This is probably the reason for different serum creatinine levels observed in various population groups [10, 11]. Secondly, the creatinine is excreted by the proximal tubular cell. This execration has important role in creatinine elimination with reduced GFR. Because of the secretion by proximal tubular cells, serum creatinine concentrations can be kept in the normal range even with a GFR of 60 mls/min/1.73m$^2$. The secretion of creatinine by the proximal tubular cells is regarded as one of the most important limitation of the evaluation of renal status by serum creatinine, and it is called the creatinine blind range [12].

The cystatin C is a small protein with a molecular mass of 13 kDa and a member of the superfamily of cysteine protease inhibitors. Its gene is being expressed in all human nucleated cells. Cystatin has a protective role of connective tissues against intracellular enzyme. It has also antibacterial and antiviral effect [11, 13]. In the normally functioning kidney, the small molecular weight protein, cystatin C is freely filtered in the glomeruli. It is then completely reabsorbed and degraded by proximal tubular cells [11, 14]. In contrast to the serum creatinine, serum cystatin C is not affected by height, gender, age, and muscle mass. Thus cystatin is being produced in all cells at contast rate, and freely filterd by the glomeruli, and its serum level is not influenced by age, gender, or muscle mass. All these factors makes the serum cystatin a better marker for estimation of GFR, and hence for the evaluation of renal function. Special benefit is for the chilren, elderly, and groups with diseases of reduced muscualr mass for example spina bifida, neuromuscular disease, anorexia nervosa, or liver cirrhosis [14]. Therefore, the cystatin C has a constant relationship with GFR; the decline of renal function with aging in diabetic patients in our study is reflected by increasing cystatin C level in elderly DM patients in a sensitive manner. This is very clearly demonstrated in table (Table1). Despite the well-established fact that its superiority over serum creatinine, the cystatin C has not yet introduced into clinical practice and remains still a research tool. The cost of cystatin C might be the only reason that limits its use as a marker of renal function in developing countries such as Eritrea.

Based on the GFR calculated using the serum creatinine, 75% of the patients are in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m$^2$), and 21% of the patient are in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m$^2$. While only 4% of the patients are in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73 m$^2$), and no patients in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m$^2$).

On the other hand according to the eGFR using the serum cystatin C, 22% of the patients are in stage 1 CKD with normal or elevated GFR (≥90 mL/min/1.73 m$^2$), and 41% of the patient are in stage 2 CKD with mild GFR reduction (GFR=60-89 mL/min/1.73 m$^2$. While more that 30% of the patients are in stage 3 with moderate decreased GFR (GFR= 30-59 mL/min/1.73), and 2% patients in stage 4 with severe GFR reduction (GFR= 15-29 mL/min/1.73 m$^2$).

Our result indicates that more DM patients have renal dysfunction when the GFR is calculated by the serum cystatin C than when calculated by serum creatinine (figure 1). This result is consistent with a recent study [15]. Tsai *et al*. [15] have reported that in DM patients, the GFR is lower when calculated by cyctatin C (eGFR cys) than when calculated by creatinine (eGFR cr). Moreover, the GFR calculated with cystatin C is more sensitive in detection of DM complications [15]. Tsai *et al*. [15] have concluded that more studies are needed to address the question of whether eGFR calculated by cystatin C is superior to eGFR calculated by creatinine for evaluation of the renal status of DM patients. Our result is thus provides more evidences prove that the serum cystratin C may be superior to serum creatinine in monitoring the kidney function at least in DM patients. Early detection of renal dysfunction (e.g. by serum cystatin C) will thus enable the intervention to avoid the diabetic nephropathy that would eventually lead to renal failure [16].

Depending on the result and statistical work, our study recommends estimation of using the serum

Cystatin C for all DM patients at least once per year. Because early detection can avoid any possible risk of unnecessary complications, and improve the quality of life of DM patients, reduce mortality due to CKD, and for early therapeutic interventions to slow or prevent the progression toward ESRD. Finally we recommend the introduction of creatinine based eGFR at least and using serum Cystatin C test for better and early detection of impaired renal function or possible CKD and as screening tool in addition to the existing follow up and for effective management, and slowing progression toward ESRD.

## CONCLUSION

As many individuals with DM are at high risk of renal damage, many studies found that, serum creatinine are not sensitive as serum Cystatin C in detection of early renal insufficiency. Similarly, our study revealed that Cystatin C is more sensitive and accurate in assessing the kidney function status in DM.

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