Estimating eGFR using Serum Cystatin C alone and in combination with Serum Creatinine: A Prospective study in individuals with CKD in north Telangana

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ABSTRACT

Introduction

Clinical assessment of kidney function is part of routine medical care for adults. More than 80% of clinical laboratories now report an estimated glomerular filtration rate (GFR) when serum creatinine is measured. Variations in Non-GFR biochemical parameters like creatine can affect the differential diagnosis of renal disorders leading to unnecessary diagnostic interventions. In recent studiesof prognosis, Cystatin C is considered to be a potential alternative marker to serum creatinine for estimating GFR.cystatin C testing has been greatly improved by the release of a certified reference material for calibrating laboratory assays and by the development of new, less expensive methods for automated analyzers.

Aim&Objectives:

To evaluate the role of serum Cystatin-C and serum creatinine for measuring eGFR using CKD-EPI equation in chronic kidney disease patients.

Materials & Methods:

In this study comprising of two comparative groups consisting of 30 study participants each were enrolled in the study. Group-I includes30 normal healthy subjects, Group-II includes 30Chronic kidney disease patients. A random venous blood sample of 5ml was collected from each participant in a plain vaccutainer. Creatinine, Cystatin-C were estimated by using Erba & Accurex kits on XL-640 clinical chemistry analyzer.eGFR was calculated using CKD-EPI equation.

Results: The results of two groups are expressed as Mean±SD. The values of cystatin-C and serum creatinine were significantly increased in Chronic kidney disease (group-II) when compared with group-I (controls).

Statistical Analysis: The data was analyzed by calculating Mean±SD (123.7±36.28),(26.03±12.73), (104.90±29.17),(28.34±22.65) respectively for every parameter and 'p' value (0.0001) was calculated by applying student 't' test using graph pad calculator. Correlation studies were done using Karl Pearson's test. Statistical significance was considered at 'p'-value0.0001 .

Conclusion: The present study suggests that eGFRcalculated using CKD-EPI equation using cystatin-C alone and combination of creatinine and cystatin-c can be used as an indicator for early prognosis of chronic kidney disease .

Keywords: Cystatin-C, Creatinine, Chronic kidney disease,eGFR

INTRODUCTION

Chronic kidney disease (CKD) is a growing public health problem[1]. Chronic kidney disease is defined as when a person suffers from gradual and usually permanent loss of kidney function over time. The kidney function is lost over months or several years. It is best when the disease is diagnosed at an early stage as early intervention may help to slow down the progress of the disease. The stages of CKD (Chronic Kidney Disease) are mainly based on measured or estimated GFR (Glomerular Filtration Rate). There are five stages but kidney function is normal in Stage 1, and minimally reduced in Stage 2. The stages of chronic kidney disease (CKD),are determined by the Glomerular Filtration Rate (GFR). Glomerular filtration rate is a calculation that determines how well the blood is filtered by the kidneys. It is one of the ways to measure kidney function[1].

Glomerular filtration rate (GFR) is calculated using a formula that includes a person's age, gender, race and serum creatinine levels. A GFR under 60 mL/min/1.73 m^2 indicates kidney disease. The lower the GFR number, the worse the kidney function. Chronic kidney disease is defined as either kidney damage or GFR of less than 60 for longer than 3 months. Chronic kidney disease (CKD) typically evolves over many years, with a long latent period when the disease is clinically silent and therefore diagnosis, evaluation and treatment is based

mainly on biomarkers that assess kidney function.The glomerular filtration rate (GFR) is traditionally considered to be the best overall index of the renal function.

The "gold standard" for determining GFR is to measure the clearance of exogenous substances such as inulin. However, the measurement of inulin is time-consuming, labor-intensive, and expensive, which makes it incompatible with routine monitoring.Serum creatinine is a metabolic product of creatine in muscle tissue. As a result, in clinical practice, the measurement of endogenous substances in serum is being done in order to estimate GFR. Serum creatinine has become the most commonly used serum marker of the renal function. Cystatin-c is produced by all nucleated cells with multi polypeptide residues and its catabolic action contributing the renal assessment.

MATERIALS & METHODS

A Prospective study was conducted in the department of Biochemistry, at central laboratory of Prathima Institute of Medical Sciences during the period of June 2016 to July 2017. Two comparative groups consisting of 30 study participants each were enrolled in the study. The study was approved by the ethical committee of the institute. Informed consent was obtained from all the patients participating in the study. We have collected the clinical history and demographic details of the patients by using questionnaire. In each group the subjects were selected by simple random sampling technique.

Inclusion criteria:

- Age above 40 years, and less than 70 years
- Diagnosed cases of CKD

Exclusion criteria:

- Not willing to consent,
- The patients with thyroid disease, or taking the medication due to thyroid disease were excluded. Because thyroid function could affect the levels of cystatin C.[2]

Group-I includes 30 normal healthy subjects (control group), Group-II includes 30 Chronic kidney disease patients. A random venous blood sample was collected from each participant. Under aseptic conditions 5ml of venous blood sample was collected by vein puncture in a plain vaccutainer and Urea, creatinine, cystatin-C were estimated in the serum sample collected from the above subjects on XL640-fully automated analyser. The cystatin-C levels of serum were measured by immuno -turbidimetric method using Accurex kits. Creatinine was estimated by Jaffe's Method, Urea was estimated by Berthelot method .

The eGFRcys level was calculated by the Chronic Kidney Disease Epidemiology (CKD-EPI) equation: eGFR = 127.7 × (cystatin C in mg/L)$^{-1.17}$ × (age in years)$^{-0.13}$ × (0.91 if female) [3].

Healthy controls were enrolled from age and sex matched volunteers from the college and hospital.

RESULTS

The results of two groups are expressed as Mean±SD. Table no:1 shows the comparison of group-I (normal healthy subjects) with group-II (CKD). The values of cystatin-C and serum Creatinine were significantly increased in CKD group-II compared with that of controls group-I. The Urea levels of group-I were (30.69±41.75) as compared to (83.14±41.13) group-II. Creatinine levels were also highly increased in group-II, (5.74±4.25) as compared to group-I. The Mean levels of cystatin-C in group-II were (4.61±3.39) as compared to (0.77±0.45) group-I. The calculated eGFR levels of group –I (controls) by using CKD-EPI equation (cystatin-c alone)were (123.73±36.28) and (104.90±29.17), and the CKD-EPI equation using(cystatin-c &creatinine combination) were significantly decreased in group-II (26.03±12.73) and (28.34±22.65) respectivelyand the 'p' value is 0.0001 which was considered as statistically significant in group-II (CKD). Thus table no:1 reveals that the values of urea, creatinine and cystatin-C were significantly increased in CKD (group-II) compared to group-I (Healthy controls). 'p' value(< 0.0001) for urea, creatinine, cystatin-c which was considered as extremely statistically significant in group-II (CKD).

Table no: 1: Comparison of Control group with CKD

Parameters	Group-I (Control)n=30 Mean± Sd	Group-II (CKD)n=30 Mean± Sd	P- value
Urea(mg/dl)	30.69±41.75	83.14±41.43	<0.0001**
Creatinine(mg/dl)	0.88±0.18	5.74±4.25	<0.0001**
eGFR(alone-cystatin-c) (ml/min/1.732 m2)	123.73±36.28)	26.03±12.73	0.0001*
eGFR (ml/min/1.732 m2) (cystatin-c&creatinine)	104.90±29.17)	28.34±22.65	0.0001*
Cystatin-C (mg/L)	0.77±0.45	4.61±3.39	<0.0001**

DISCUSSION

In this study, The levels of cystatin c were significantly increased in CKD. The increment was due to the tubular phase before glomerular manifestation. This suggests that serum cystatin c levels were related to renal tubular impairment and can be an earlier marker of renal diseases. Thus cystatin C levels would be strictly dependant on renal function. Cystatin C produced by a majority of nuclear cells is a non-glycosylated protein of 120 residue polypeptide chain with a molecular mass of 13 kDa[4]. Because of its small size and basic pH, cystatin C is freely filtered by the glomerulus[5]. After glomerular filtration, it is fully catabolised in the proximal renal tubules and is not

returned into blood circulation, only small amounts of cystatin c are excreted in urine, it indicates that it is produced at a relatively constant rate irrespective of muscle mass. Thus, it was anticipated that cystatin C would provide a better estimate of GFR than estimating equations based on serum creatinine, One of the key criteria that cystatin C needs to meet to be a potential replacement for creatinine is that its production rate should be constant or at least less variable than that of creatinine.[6]

The CKD Epidemiology Collaboration (CKD-EPI) equation was published in 2009 and intended to be more generalizable across various clinical settings than the MDRD equation[7]. Weight, diabetes, and transplant were considered as potential variables, but the final equation uses the same variables as the MDRD equation[8]. The source studies that were used for the CKD-EPI equation can be broken down into two groups: High-risk populations such as patients with clinical CKD, characterized by an average measured GFR (mGFR) <90 ml/ min per 1.73 m2, and low-risk populations such as potential kidney donors, characterized by an average mGFR>90 ml/min per 1.73 m^2. however, this validation did not address the underlying problem with the performance of GFR-estimating equations in different populations. The CKD-EPI equation authors recognized this, stating that "a single equation is unlikely to work equally well in all populations" [7]. For instance, the CKD-EPI equation leads to a lower prevalence of eGFR <60 ml/min per 1.73 m^2 in low-risk white women than the MDRD equation[9], but when demographics in GFR-estimating equations start to model CKD risk, this comes at the cost of less optimally modeling muscle mass [10].

In this issue of CJASN, Michels et al.[11] present a validation analysis of these three equations in a series of clinical patients. Unfortunately, this study adds little insight into equation performance for several reasons: First, the study population was not defined other than as potential kidney donors combined with patients who had amGFR for "clinical reasons," but what are these clinical reasons? What was the breakdown between these two groups? Do equations perform differently in the potential kidney donors than in the other group? Without knowing the reasons for why a person is referred for a direct GFR measurement, it is difficult to interpret the study findings reported.

CONCLUSION

To assess the disease severity after diagnosis of CKD has been made, one can choose the CKD-EPI equation for reducing the risk towards the progression of CKD.

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