Cystatin-C as a Biomarker in Predicting Early Renal Impairment in Normo-Albuminuric Patients with Type 2 Diabetes Mellitus

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Abstract

This study was done to evaluate clinical usefulness of cystatin C levels of serum in predicting renal impairment in normoalbuminuric patients with type 2 diabetes and to evaluate the association between albuminuria and serum cystatin C. Type 2 diabetic patients (n = 239) with Normoalbuminuria (n = 110), Microalbuminuria (n = 81) and Macroalbuminuria (n = 48) were enrolled. Creatinine, urinary albumin levels, serum cystatin C and estimated glomerular filtration rate (eGFR by MDRD [Modification of Diet in Renal Disease] were determined. The cystatin C levels of serum increased with increasing degree of albuminuria, reaching higher levels in macroalbuminuric patients (P < 0.001). The correlation between serum cystatin C and albumin- creatine ratio were analyzed. The serum cystatin levels were directly correlated with albuminuria(r =0.387, p< 0.0001).The serum cystatin C levels were negatively correlate with eGFR (r = - 0.364, p<0.0001). In multiple regression analysis, serum cystatin C was affected by age, sex, albumin-creatinine ratio (ACR) and eGFR. In multivariate logistic analysis, cystatin C levels of serum was identified as independent factors associated with eGFR < 60 mL/min/1.73 m2 estimated by MDRD equation in patients with normoalbuminuria. The cystatin C levels of serum could be useful markers for renal dysfunction in type 2 diabetic patients with normoalbuminuria.

Key Words
Albuminuria, Cystatin C, Diabetic Nephropathies, Estimated Glomerular Filtration   Rate

INTRODUCTION

Diabetic nephropathy is a complication with high morbidity and mortality as well as a major cause of end-stage renal disease. According to the World Health Organization (WHO), the prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. [1] Although glomerular dysfunction is thought to be a major factor for the development and progression of diabetic nephropathy, tubulointerstitial damage may also play an important role in the pathogenesis of diabetic nephropathy (2–4). Several studies have shown that some tubular damage markers have clinical implications as biomarkers for the development and progression of diabetic nephropathy. [5-10]

The sensitivity of serum creatinine in the detection of CKD is poor and it will fail to identify half of the patients with crucial stage 3 CKD (GFR of 30-59 mL/min/1.73m2) [11],[12],[13] as serum creatinine concentration may not change until approximately 50% of the kidney function has been lost. Due to the many problems encountered with measurements of creatinine and its use as a GFR estimate, cystatin C (Cys C) has been proposed as an alternative marker of renal function.

Cystatin C is a 13-kDa, non-glycosylated basic protein belonging to the cystatin super-family of cysteine proteinase inhibitors. Studies have shown that CysC may be more sensitive in identifying mild reductions in kidney function than serum creatinine (S.cr). [14], [15] It is produced at a stable rate, which is unaffected by inflammatory processes, sex, age, diet, and nutritional status. [16]

The study was designed with an aim to evaluate the possibility of serum cystatin C as biomarker of early progression of type 2 diabetic nephropathy and to determine the association between albimunuria and serum cystatin C.

MATERIALS AND METHODS

This was a prospective observational study of patients attending the Department of Endocrinology at Santhi ram General Hospital, Nandyal. The study was conducted with the approval of the Institutional Review Board. A total of 239 Indian type 2 diabetic patients were consecutively enrolled at the outpatient clinics between November 2014 and August 2015. All patients fulfilled the following inclusion criteria: age>18 years and estimated GFR (eGFR) >30 mL/min/1.73 m2. We excluded patients with thyroid disorders or who had been medicated within 6months prior to the study because thyroid function could affect the cystatin C level. [17] Additional exclusion criteria were 1)Active urinary tract infection, 2) Renal disease other than diabetic nephropathy 3) Neoplastic disorders 4) Severe liver dysfunction, 5) Active or chronic infection or inflammatory disorders 6) Pregnancy 7) A recent (within 6 months) history of acute myocardial infarction, stroke, or occlusive peripheral vascular disease.

A random blood sample was obtained from each patient at the clinic visit. Medical histories and anthropometric measurements were also recorded the same day. The eGFR level was calculated using the Modification of Diet in Renal Disease (MDRD) Study formula for the Korean

population: MDRD = 107.904 3 (serum creatinine[mg/dL])21.0093age20.02 (18). A correction factor of 0.667 was used for women. The serum cystatin C levels were measured by the latex agglutination test (Modular P800; Roche Diagnostics, Mannhein, Germany). Finally, a total of 239 patients with type 2 diabetes were enrolled for this study. The two tubular damage markers were measured at intervals of 12 + 1 (mean + SD) months at the outpatient clinic during the follow-up period. Serum creatinine was routinely measured for the estimation of GFR at intervals of 6 +1 (mean +SD) months during the follow-up period using the same methods.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). The data are presented as mean + SD for normally distributed variables and the medians for non normally distributed variables. The distribution of continuous variables was examined for skewness and kurtosis, and logarithm-transformed values of non normally distributed variables were used for analysis. Differences between the groups were analyzed byANOVA, followed by Bonferroni test. Categorical variables are reported as frequencies and proportions. Pearson correlation coefficient was used to test the correlations between individual variables. A P value of, < 0.05 derived from the two-tailed Student t test was considered statistically significant.

RESULTS

A total of 239 patients with type 2 diabetes were participated in the study. The baseline characteristics of the patients are shown in Table 1.

The mean age of the patients was 58.5 (range 45-56 years), and there were 158 males and 81 females. The patients were categorized into three groups according to ACR: those with ACR <30 mg/g creatinine (normoalbuminuria group, n = 110), those with ACR 30–299 mg/g creatinine (microalbuminuria group, n = 81), and those with ACR >300 mg/g creatinine (macroalbuminuria group, n = 48). The correlation between serum cystatin C and albumin creatine ratio were analyzed. The serum cystatin levels were directly correlated with albuminuria (r =0.387, p< 0.0001).The serum cystatin C levels were negatively correlate with eGFR (r = - 0.364, p<0.0001) Table: 2

In pear son's correlation analysis, the serum cystatin C was related to age, duration of diabetes, systolic blood pressure (SBP), HbA1c, ACR, serum creatinine and eGFR. Estimated GFR tend to decrease with increasing degrees of albuminuria (P = < 0.001). The serum cystatin C levels were significantly higher in the macroalbuminuria group than in the normoalbuminuria and microalbuminuria groups (both P=< 0.001), whereas they were not significantly different between the normoalbuminuria and microalbuminuria groups (Table 1).

Table: 1 Characteristics of Laboratory parameters in patients with Type 2 Diabetes mellitus

Parameters	Normoalbuminuria (n=110)	Microalbuminuria (n=81)	Macroalbuminuria (n=48)	P- Value
Age/sex	50.4+3.25	56.4+6.29	59.0+3	0.0001
Duration of DM	5.15+0.46	7.5+0.8	9.03+0.47	0.0001
Systolic BP	111.2+8.2	127.22+6.3	133.9+3.5	0.0001
Diastolic BP	72.45+6.8	78.25+4.8	83.79+4.3	0.0001
Urine albumin	1.08+0.49	5.94+4.4	8.63+0.68	0.0001
Sr.creatinine	0.9+0.19	1.10+0.14	1.37+0.11	0.0001
Sr.cystatin C	0.97+0.23	1.15+0.22	1.61+0.19	0.0001
Albumincreatine ratio(ACR)	16.54+3	71.77+15.09	642.5+144.7	0.0001
HbA1c	7.05+0.28	7.59+0.13	8.11+0.22	0.0001
eGFR	104.6+4.77	90.02+4.47	59.38+5.92	0.0001

Table:2 Correction between Cystatin and Albumin, HbA1C, ACR, eGFR, systolic BP. Pearson correction(r=)

Cystatin c	Albumin	HbA1C	ACR	eGFR	Sys BP	Sr.creatinine
Microalbumin	0.026	0.068	0.16	0.075	0.027	0.246
Microalbumin	0.0321	0.652	0.387	-0.364	0..162	0.2115

DISCUSSION:

In this study, we aimed at evaluating the cystatin C levels of serum in small cohort of patients with type 2 diabetes by categorizing them into 3 groups depending on the different degrees of kidney damage (normoalbuminuria, microalbuminuria and macroalbuminuria). In macroalbuminuric patients the cystatin C levels were significantly increased in patients eGFR< 60 ml/min/1.73 m2 than normoalbuminuric patients. It has been noted that patients with Type 2 Diabetes Mellitus and microangpathy have statistically significant higher levels of cystatin C than healthy individuals. In these patients the cystatin c levels were significantly increased in patients with GFR <60 ml/min/1.73 m2 then those with GFR > 60 ml/min/1.73m2. It was thought that this increament was probably due to the tubular phase before glomerular damage. This suggests that serum cystatin C levels are related to subclinical tubular impairment and can be an earlier marker of renal involment before onset of microalbuminuria.

The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine (19). But, it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR. [20, 21] About 20%-30% of patients with type 2 diabetes, accompanied by renal insufficiency, showed normoalbuminuria.[20-25] To overcome these limitations, many clinicians additionally used creatinine in evaluating such patients. However, serum creatinine also depends on creatinine production, extra renal elimination and tubular handling (26). Moreover, tubular involvement may precede glomerular involvement because several tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and a rise in serum creatinine. [27, 28] Therefore, other biomarkers for estimation of renal function have been searched for and one of them was cystatin C.[29] Our study results confirmed that cystatin C could be one of the additional tubular factors which represent kidney state of diabetic patients. The investigators concluded that serum cystatin C is a useful in early detection of diabetic nephropathy as it reflects reduction in GFR as well as rise in ACR. [30]

CONCLUSION:

The results of this study suggest that cystatin C measurement in serum is useful, non-invasive tool for the evaluation of renal involvement in the course of diabetes, especially in normoalbuminuric patients. It is concluded that cystatin C was best for discriminating between microalbuminuria and normoalbuminuria in those with type-2 diabetes resulting in the conclusion that cystatin C might be a more useful marker than creatinine for detection of early diabetic nephropathy in type 2 patients with diabetes [31]

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