

EARLY PREDICTION OF NEPHROPATHY AND CARDIOVASCULAR DISEASES IN INDIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: It is well established that patients with type 2 diabetes mellitus are at increased risk for chronic kidney and cardiovascular diseases. Diagnosis of diabetic nephropathy and cardiovascular diseases at an early stage in patients with type 2 diabetes mellitus is difficult. Therefore, biomarker for estimation of renal function was chosen.

Aims and Objectives: To evaluate serum cystatin C and its correlation with biochemical, clinical, and anthropometric parameters in patients with type 2 diabetes and healthy controls.

Materials and Methods: The levels of serum cystatin C were evaluated in 100 subjects (50 with type 2 diabetes and 50 controls) of age group 40–70 years and were measured using particle-enhanced immunonephelometry.

Results: The levels of serum cystatin C were found to be significantly increased in patients with diabetes mellitus (0.97 ± 0.19 mg/L; $P < 0.01$) than healthy controls (0.84 ± 0.1 mg/L). In this study, it was found that few diabetes patients with normoalbuminuria with elevated levels of serum cystatin C and cystatin C–based estimated glomerular filtration rate (eGFR). The levels of serum cystatin C correlated positively with eGFR, serum creatinine, and systolic blood pressure and showed negative correlation with estimated creatinine clearance and no association with body mass index, waist-to-hip ratio, and diastolic blood pressure.

Conclusion: Measurement of the levels of serum cystatin C is a useful, practical, noninvasive technique for the evaluation of renal involvement and might be related with a risk for cardiovascular events in patients without nephropathy in the course of diabetes, especially in patients with normoalbuminuria.

Key Words: Serum Cystatin C; Diabetic Nephropathy; Estimated Creatinine Clearance; Estimated Glomerular Filtration Rate

Introduction

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. According to the World Health Organization, the prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and 4.4% in 2030.^[1] It is well established that patients with type 2 diabetes mellitus (T2D) are at increased risk for chronic kidney disease (CKD) and cardiovascular diseases (CVDs). Many studies have reported that diabetic nephropathy develops in 25–35% of patients with T2D. Identifying those at risk is problematic, because even microalbuminuria, often used clinically as an indicator of future renal dysfunction, does not always precede worsening renal function. Previous studies support the concept that patients with renal failure have a high prevalence of CVD, and it has been proposed that atherosclerosis may promote the progression of renal disease. It is commonly accepted that glomerular filtration rate (GFR) assess renal function, the gold standard marker for GFR is to measure the clearance of inulin. But the use of inulin clearance is limited to clinical practice because of the time, labor, and cost involved. Diagnosis of diabetic nephropathy and

CVDs at an early stage in patients with T2D is difficult. Therefore, biomarkers for estimation of renal function have been searched. Recently, few elegant studies showed that biomarker cystatin C has been found to appear in the circulation superior to that of other markers such as albumin and creatinine.^[2–4] In addition, it is important to note that limitation to serum creatinine for GFR measurement is the creatinine blind area. Typically, serum creatinine remains in the normal range until 50% of renal function is lost. Cystatin C is a small protein molecule, a cysteine protease inhibitor freely filtered by the renal glomeruli, produced at a constant rate by nucleated cells, and released into bloodstream. The underlying biological mechanism is the extracellular inhibition of cathepsins. Few recent reviews and studies have implicated that serum cystatin C concentration is totally dependent on GFR, and it is less dependent on age, sex, race, and muscle mass.^[5,6] The medical literature has pointed out that serum cystatin C levels could be useful marker for renal dysfunction in T2D patients with normoalbuminuria and also its role in predicting new onset or deteriorating CVDs.^[7,8] Our aims were to describe the association between serum cystatin C and biochemical, anthropometric parameters in patients with T2D. To the best of our knowledge, there are no reviews

focusing specifically on the association between serum cystatin C and biochemical, clinical, anthropometric parameters so far in Indian population.

Materials and Methods

In this study, 100 subjects (50 patients with T2D and 50 healthy controls) within the age group of 40–70 years from Tagore Medical College and Hospital, Chennai, India, were enrolled as study participants. A detailed questionnaire recorded the medical history of the patients and controls. This study was approved by the ethics committee of the institution. Informed written consent from the patients and healthy controls was obtained before the commencement of the study. The patients with T2D (overt diabetes less than 2 years) were on glucose-lowering drugs. These participants had no sign of renal damage, CVDs, and infectious diseases as observed from their medical history and a physical examination. Patients with uncontrolled hypertension, with thyroid disease, or on the medication due to thyroid disease in 6 months were excluded from the study. Anthropometric measurements such as height, weight, body mass index (BMI), and waist-to-hip ratio (WHR) were recorded. Weight was measured using a beam balance to the nearest 0.1 kg and height to the nearest centimeter, using a tape stuck to the wall. Abdominal girth was measured at the level of umbilicus, with the subject relaxed and in a standing posture. Hip girth was measured at widest point of the hips, at the level of the greater trochanter, with the patient standing with both feet together. WHR was calculated from these measurements. Blood pressure levels were also recorded for all the subjects using mercury sphygmomanometer.

Random blood and urine samples were collected from all the subjects, and serums separated from the blood samples were stored at -20°C till the time of analysis. Cystatin C in human serum was quantitatively determined using particle-enhanced immunonephelometry (Siemens). Cystatin C samples were analyzed in Lister Metropolis Diagnostics Lab (Chennai, India). The serum creatinine levels were analyzed by Jaffe's method using semi-autoanalyzer (ERBA Chem-5 Plus). The urinary albumin levels were measured using urinary strips (SD Bio Standard Diagnostics). Estimated creatinine clearance (eCrCl) rate was calculated by using Cockcroft–Gault formula^[9]:

$$eCrCl = \frac{[(140 - \text{age}) \times \text{weight (kg)} \times \text{constant } t]}{\text{serum creatinine } (\mu\text{mol/L})},$$

where constant t for men and women is 1.23 and 1.04, respectively.

The estimated glomerular filtration rate (eGFR) was calculated using the following formula^[10]:

$$eGFR = 127.7 \times \text{cystatin C}^{-1.17} (\text{mg/L}) \times \text{age}^{-0.13} (\text{years}) \times 0.91 (\text{if female}) \text{ or } 1.06 (\text{if male})$$

Statistical Analysis

Statistical analysis of the data was carried out using SPSS, version 16.0. Results were expressed as mean \pm SD and a value of $P < 0.05$ was considered to be statistically significant. The clinical and biochemical characteristics among the groups were compared statistically using independent t -test. A correlation analysis was done using Pearson's correlation at a 5% level of significance.

Results

The mean levels of the biochemical and anthropometric parameters have been summarized in Table 1. The study was conducted among 100 subjects (50 patients with T2D and 50 healthy controls) in the age group of 40–70 years. The mean serum cystatin C levels were found elevated significantly in patients with T2D (0.97 ± 0.19 mg/L) on comparison with healthy controls (0.84 ± 0.1 mg/L).

On further comparison between men and women, both men and women with T2D showed higher cystatin C levels than their control counterparts. The descriptive statistics are shown in Table 2.

Table 3 shows the relationship between serum cystatin C and biochemical and anthropometric parameters. Notably serum cystatin C levels showed positive correlation with eGFR, serum creatinine, and systolic blood pressure. The negative correlation was apparent between serum cystatin C and eCrCl. However, no association was found with BMI, WHR, and diastolic blood pressure.

Correlation between cystatin C and eGFR is shown in Figure 1. It is important to reiterate that among 50 patients with T2D, 11 had shown trace urinary albumin excretion, 1 had shown 2+ urinary albumin excretion, and 1 has shown 1+ urinary albumin excretion. Of 50 healthy controls, only 15 had shown trace urinary albumin excretion.

Table-1: Comparison of different parameters between type 2 diabetic and control group

Parameters	Diabetic (N=50) (mean ± SD)	Control (N=50) (mean ± SD)
Age	53.3±9.1	48± 8.2
BMI	25.2±3.6 ^{NS}	25.5±3.0
WHR	0.9±0.04 ^{NS}	0.91±0.04
Systolic BP	118.4±11.6 ^{NS}	116.4±10.5
Diastolic BP	76.3±8.9 ^{NS}	75.0±7.4
Creatinine (mg/dL)	1.1±0.2 ^{NS}	1.0±0.3
eCrCl	65.8±20.1 ^{NS}	75.2±27.6
Cystatin C (mg/L)	0.97±0.19 ^{**}	0.84 ±0.1
eGFR	60.96±22.6 [*]	51.7±16.8

BMI, body mass index; WHR, waist-to-hip ratio; eCrCl, estimated creatinine clearance; eGFR, estimated glomerular filtration rate. Results are expressed in mean ± SD. *P < 0.05; **P < 0.01; NS, nonsignificant.

Table-2: Comparison of various parameters between men and women of different groups

Parameters	Diabetic (mean ± SD)		Control (mean ± SD)	
	Men (N=25)	Women (N=25)	Men (N=21)	Women (N=29)
Age	53.72±9.3	52.8±9.18	49.9±8.6	46±7.8
BMI	23.34±3.1 ^{NS}	27.21±3.16 ^{NS}	25.6±1.83	25.45±3.7
WHR	0.94±0.05 ^{NS}	0.9±0.03 ^{NS}	0.91±0.02	0.9±0.04
Systolic BP	117.36±9.2 ^{NS}	119.6±13.7 ^{NS}	118±13.2	115.3±8.23
Diastolic BP	75.84±9.0 ^{NS}	76.88±8.9 ^{NS}	76.4±7.7	74.06±7.3
Creatinine (mg/dL)	1.216±0.23 ^{NS}	1.03±0.29 ^{NS}	1.17±0.36	0.93±0.19
eCrCl	63.65±14.1 ^{NS}	67.97±24.9 ^{NS}	79.7±32.9	72.09±23.2
Cystatin C (mg/L)	0.94 ± 0.18 [*]	0.99±0.20 ^{***}	0.81±0.11	0.86±0.10
eGFR	57.5±23.9 ^{NS}	64.1±21.2 ^{**}	45.3±18.2	56.4±14.3

BMI, body mass index; WHR, waist-to-hip ratio; eCrCl, estimated creatinine clearance; eGFR, estimated glomerular filtration rate. Results are expressed in mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; NS, nonsignificant

Table-3: Pearson's correlation analysis between serum cystatin C and anthropometric and biochemical variables of the study subjects

Parameter	r-Value	P-Value
BMI	-0.38	0.704
WHR	0.022	0.773
Systolic BP	0.354	0.001
Diastolic BP	0.189	0.060
Creatinine (mg/dL)	0.297	0.003
eCrCl	-0.444	0.001
eGFR	0.870	0.001

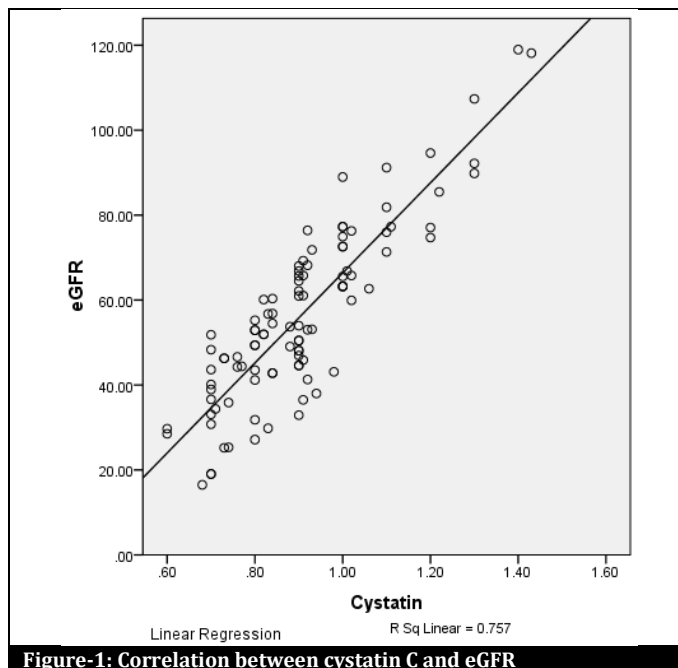
BMI, body mass index; WHR, waist-to-hip ratio; eCrCl, estimated creatinine clearance; eGFR, estimated glomerular filtration rate.

Here, we also noticed that among 50 patients with T2D, 8 patients were found to be hypertensive, 11 regularly smoked and consumed alcohol, and only 11 were regularly involved in physical activity. Similarly we observed that among 50 healthy controls, 3 were found to be hypertensive, 18 had a habit of smoking and tobacco chewing, and 6 consumed alcohol regularly.

Discussion

Reports from different parts of the world suggested that patients with T2D are at increased risk for CVD and CKD. The preceding reports suggested that CKD is significantly associated with CVD. This study was undertaken to find out whether serum cystatin C, eGFR, and eCrCl may have

an impact on the interpretation risk for nephropathy and CVD. Data from the Framingham Study have shown diabetes mellitus to be associated with a two- to fourfold increase in risk for CVD.

**Figure-1: Correlation between cystatin C and eGFR**

Recent researchers revealed that diabetic nephropathy develops in 25–35% of the patients with T2D.^[11] The scientific literature abounds with evidence pointed out the vital role of serum cystatin C in CKD and CVD.^[12,13] However, no evidence is available so far to describe the role of serum cystatin C in patients with diabetes mellitus of the Indian population. We, therefore, aimed to dissect the role of the serum cystatin C levels and their correlation with the anthropometric, clinical and biochemical parameters in Indian patients with diabetes mellitus. We observed for the first time, to the best of our knowledge, an increase in the serum cystatin C levels in Indian patients with T2D. Our results were identical to the reports of earlier studies that were conducted on adults.^[7,14,15] But not all studies have done so.^[16] Our findings reinforced the notion that the serum cystatin C levels are related to subclinical tubular impairment and can be an earlier measurable marker of renal involvement before the onset of albuminuria. Our observations raised the possibility that cystatin C could be an index reflecting renal tubular epithelial cells, and it is novel risk factor for cardiovascular events.^[8,17] The results of our study observed nonsignificant serum creatinine levels between healthy controls and patients with diabetes mellitus. Current data suggest that serum creatinine values are not a direct measure of GFR, which can be reduced as much as 50% while the serum

creatinine value is still within the normal range. These also depend on creatinine production, extrarenal elimination, and tubular handling; our data closely agree with earlier reports.^[10] 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.^[2,18] In this study, the patients with T2D were significantly lower in eCrCl than the healthy controls. Furthermore, our results revealed the inverse association between serum cystatin C and eCrCl. This study confirmed the results of previous studies.^[19] It is generally believed that GFR calculated by Cockcroft–Gault formula correlates well with measured GFR when renal function is within the reference interval, but as renal function declines, it overestimates GFR because creatinine is removed not only by glomerular filtration but also by renal tubular secretion.^[20] This study showed an elevated eGFR in patients with diabetes mellitus, and the positive correlation was also apparent between serum cystatin C and eGFR. The available information suggests that cystatin C–based eGFR were found to be more reliable and sensitive than the earlier markers, particularly in areas where the decrease in GFR is marginal. Hence, it is suggested that cystatin C and cystatin C–based eGFR are better markers for the assessment of renal function, and our data agree with those that have been reported by others.^[21] In this study, nonsignificant BMI, WHR, and systolic and diastolic blood pressures in patients with diabetes mellitus as compared to those in the controls were observed. Thus, it is generally believed that after 1 year of age anthropometric parameters will have minimal effect on the cystatin C levels. Our results were also at par with those of other investigators.^[22] In this study, few diabetes patients with normoalbuminuria with elevated serum cystatin C levels and cystatin C–based eGFR were found. This suggests that the serum cystatin C levels are related to subclinical tubular impairment and can be an earlier measurable marker of renal involvement before the onset of albuminuria. The results of our study also confirm that serum cystatin C could be one of the additional tubular factors that represent kidney state of patients with diabetes mellitus. Our results are in alignment with those of the earlier studies.^[17] Strengths and limitations of our study deserve comments. The potential limitation of our study was lack of detailed measurement of the dietary habits of the patients with T2D and controls and adolescents. Also, the sample size was small to draw valid conclusions. Furthermore, future research should endeavour to build up on the findings of

this study.

Conclusion

From our study, we conclude that cystatin C is an excellent marker of renal function and correlates better to direct measures of GFR more precisely than creatinine, because its serum concentrations are independent of muscle mass and do not seem to be affected by age or sex. The results of this study suggest that cystatin C measurement is a useful, practical, noninvasive tool for the evaluation of renal involvement and might be related with a risk for cardiovascular events in patients without nephropathy in the course of diabetes, especially in patients with normoalbuminuria.

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