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Study on Cystatin C as an Early Biomarker of Nephropathy in Patients with Type-2 Diabetes

Authors
[1]Dr Sanjiv Kumar, [2]Dr Mohd Shafat Imam Siddiqui, [3]Dr Nikhil Sinha
[1]MBBS, MD, Assistant Professor, Department of Medicine
[2]MBBS, MD, Associate Professor, Department of Medicine
[3]MBBS, MD, Assistant Professor, Department of Medicine
[1,2,3]Heritage Institute of Medical Sciences, Varanasi-221311, Uttar Pradesh, India

Abstracts
Diabetes mellitus (DM) is a syndrome associated with chronic hyperglycemia due to relative insulin deficiency, resistance, or both. Our aim to evaluate the clinical usefulness of cystatin C levels in the serum in predicting renal impairment in patients with type 2 diabetes. Thus, we explored the possibility of the cystatin C levels of serum and urine as markers of early renal impairment in normoalbuminuric patients with diabetes. We also evaluated the relationship of albuminuria and serum/urine cystatin C.
Keywords: *Cystatin C, Diabetic Nephropathies and Albuminuria.*

Introduction

In the ancient Sanskrit Literature, diabetes mellitus was described as "honey-urine disease," associated with gross emaciation and wasting. Diabetes is a global endemic with rapidly increasing prevalence in both developing and developed countries.[1] The number of people with diabetes is increasing due to population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity. 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.[2] Estimation of the prevalence of earlier stages of chronic kidney disease (CKD) in the US population and ascertainment of trends over time is central to disease management and prevention planning, particularly given the increased prevalence of obesity and diabetes.[3] To prevent this increase, screening for CKD and early intervention are necessary. In diabetic patients, the early detection of diabetic nephropathy has focused on the measurement of urinary albumin excretion rate. The elevated urinary albumin excretion rate within microalbuminuric level (30-299 mg/24 hr or a spot urine albumin-to-creatinine ratio of 30-299 mg/g) allows the detection of patients with an increased risk for the development of overt diabetic nephropathy with persistent macroalbuminuria. Moreover, impaired renal function may be present even in patients with normal urinary albumin excretion rate.[4] Gold standard procedures for glomerular filtration rate (GFR) measurement, based on the clearance of 51Cr-EDTA or iohexol, are impractical in clinical settings and for larger research studies. Cystatin

C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure.[5] Cystatin C is produced at a constant rate by nucleated cells and released into bloodstream with a half-life of 2 hours. Its concentration is almost totally dependent on GFR. Other studies have demonstrated that serum cystatin C is an early renal marker in diabetic patients,[6-8] but not all studies have done so.[9] Cystatin C is an alternative and more sensitive endogenous marker for the estimation of GFR than serum creatinine.[10]

Thus, we explored the possibility of the cystatin C levels of serum and urine as markers of early renal impairment in normoalbuminuric patients with diabetes. We also evaluated the relationship of albuminuria and serum/urine cystatin C.

Material and Methods

The present study was conducted in the Department of Medicine, Heritage Institute of Medical Sciences, during the period from June 2015 to December 2015. A total of 75 subjects out of which 30 subjects treated as control group and 45 subjects were Type 2 Diabetic nephropathy. The participants were classified into the following groups:

a) Control group: included the 30 healthy individuals. The patients were classified according to the urinary albumin/creatinine ratio (A/C) into the following groups:

b) Normoalbuminuria group:

Included 15 patients with normoalbuminuria: that is, urinary A/C of 30 mg/g or lower.

c) Microalbuminuria group:

Included 15 patients with microalbuminuria: that is, urinary A/C of 30–300 mg/g.

d) Macroalbuminuria group:

Included 15 patients with macroalbuminuria: that is urinary A/C of 300 mg/g or higher.

An overnighy fasting blood sample was collected from both cases and controls and the samples were centrifuged and separated for the estimations. Estimations of fasting blood glucose, blood urea and serum creatine were performed using the serum. Estimation of serum cystatin C was done by immunoturbidimetric method. Data were analyzed by SPSS student t-test and one way ANOVA. A P-value <0.05 was considered statistically significant.

Results and Discussion

Our study enrolled total 75 patients and their distribution of parameters in the case and control groups shown in Table-1&2. Diabetic nephropathy is the leading cause of chronic renal disease in patients starting renal replacement therapy; it is associated with increased cardiovascular mortality.[11]DM has been classically defined by increased protein excretion in urine and decreased GFR thereafter. GFR has been expected to decrease when proteinuria is established.[12] GFR is considered the most accurate measurement of kidney disease and is reduced before the onset of clinical symptoms; it is measured or predicted using different methods.[13] There is no simple and practical way to measure GFR directly; hence, it is estimated. To estimate the GFR, an endogenous substance in the blood that is cleared by the kidney is used; this substance is currently serum creatinine. The Cockcroft–Gault and Modification of Diet in Renal Disease Study equations are serum creatinine based equations that are used to estimate GFR. GFR determinations using creatinine-based equations are not precise; hence, other substances, such as cystatin C, are being explored to estimate GFR.[14]

Table 1. Characteristics of metabolic and laboratory parameters in patients with type 2 diabetes:

Parameters	Normo-albuminuria	Micro-albuminuria	Macro-albuminuria	P-Value
Age (yr)	55.0 ± 12.0	57.2 ± 11.1	66.8 ± 8.8	0.14
ACR (mg/g	11.6 ± 6.8	89.8 ± 64.9	1,403.8 ± 1,001.5	< 0.0001
BMI (kg/m2)	24.5 ± 4.1	24.6 ± 3.1	23.7 ± 3.3	0.381
SBP mmHg	122.9 ± 12.3	125.2 ± 15.6	131.8 ± 22.4	0.007
DPB mmHg	72.1 ± 8.9	72.7 ± 11.4	72.9 ± 12.4	0.836
FBS mg/dl	160.3 ± 57.1	183.0 ± 98.0	192.9 ± 83.1	0.004
HbA1c %	7.1 ± 1.5	7.6 ± 1.9	7.8 ± 1.6	0.014
RAS inhibitors, n (% yes)	5 (33)	8 (53)	7 (47)	< 0.0001
Lipid lowering agent, n (% yes)	8 (53)	7 (47)	4 (27)	0.015
anti-HT Tx, n (% yes)	3 (20)	4 (27)	6 (40)	< 0.0001
eGFR calculated by the MDRD equation (mL/min/1.73 m2)	84.6 ± 23.3	76.0 ± 27.8	44.9 ± 26.6	< 0.0001
eGFR calculated by the CKD-EPI equation (mL/min/1.73 m2)	86.2 ± 22.0	77.2 ± 27.2	43.6 ± 24.4	< 0.0001
BUN (mg/dL)	17.2 ± 13.7	19.2 ± 8.9	34.5 ± 22.5	< 0.0001
Serum Cr (mg/dL)	0.9 ± 0.2	1.1 ± 0.4	2.3 ± 1.9	< 0.0001
Total cholesterol (mg/dL)	172.7 ± 47.4	172.6 ± 43.4	175.2 ± 44.3	0.945
LDL (mg/dL)	95.9 ± 30.5	93.0 ± 31.9	93.1 ± 34.1	0.713
HDL (mg/dL)	46.0 ± 12.6	43.4 ± 11.4	43.1 ± 13.7	0.147
TG (mg/dL)	150.4 ± 88.3	182.9 ± 118.4	187.5 ± 119.2	0.012
CRP (mg/dL)	0.16 ± 0.36	0.19 ± 0.44	0.35 ± 1.22	0.128
Serum cystatin C (mg/L)	0.91 ± 0.26	1.05 ± 0.38	2.04 ± 1.19	< 0.0001
Urine cystatin C (mg/L)	0.06 ± 0.06	0.32 ± 1.14	1.17 ±2.73	< 0.0001
Urine cystatin/Cr × 10-1	1.00 ± 1.49	9.06 ± 40.19	16.84 ± 104.43	< 0.0001
FeCyst (%)	0.09 ± 0.13	1.10 ± 6.27	5.26 ± 12.8	<0.0001

Table 2: Correlation between Cystatin C and different studied parameters:

parameters	Correlation coefficient (r)	
	Cystatin C	serum creatinine
Age	0.075	-0.103
A/C ratio	0.68	0.702
HbA1c	0.54	0.533
FBS	0.48	0.50
2HPP	0.57	0.59
Serum urea	0.17	0.113
Weight	-0.06	0.286
DM duration	0.71	0.584
Serum creatinine	0.74	-
GFR	-0.67	-0.81

The primary limitation of using creatinine level is that the level is determined not only by GFR but also by muscle mass and dietary intake. Lower serum creatinine levels may less reliably detect impaired GFR in patients with certain characteristics like older age, female sex, chronic illness with muscle wasting, amputation, or a vegetarian diet; higher serum creatinine levels are associated with African American race, muscular body habitus, and a high protein diet. Although

estimating equations attempt to adjust for these factors, the result is not precise. Different patients can have the same serum creatinine level with very different GFRs.[15]

Estimation of creatinine clearance requires urine sample collection after a 24-hrs period. A blood sample is drawn during the 24-h period and creatinine clearance can then be calculated. There are several factors that may interfere with the accuracy of the test such as incomplete urine collection, pregnancy, vigorous exercise, and drugs such as cimetidine, trimethoprim, and those that can damage the kidneys; therefore, results may be inaccurate.[16]

Several new biochemical markers have the potential to be markers of chronic kidney disease progression. These new markers might reflect the early diminished GFR compared with traditional markers; these include the following: *N*-acetyl-β-glucosaminidase, β2-microglobulin, α1-microglobulin, retinol-binding protein, human neutrophil gelatinase-associated lipocalin, interleukin-18, clusterin, fatty acid binding protein, and cystatin C.[17]

The aim of this study was to evaluate serum cystatin C as an early marker of renal dysfunction in type 2 DM. Cystatin C is produced at a constant rate by all nucleated cells. Because of its small size, it is freely filtered by the glomerulus and is not secreted but is fully reabsorbed and broken down by the renal tubules. This means that the primary determinate of blood cystatin C levels is the rate at which it is filtered in the glomerulus, making it an excellent GFR marker.[18]

Cystatin C may detect mild-to-moderate decreases in GFR that are not evident with serum creatinine-based measurements. Cystatin C-based estimates of GFR are better than creatinine-based estimates of GFR.[15] Most reports confirm that serum cystatin C concentrations are uninfluenced by age, sex, or muscle mass.[19] However, just recently, a study alleged that serum cystatin C levels appear to be influenced by nonrenal factors such as age, sex, weight, height, current cigarette smoking, and C-reactive protein levels.[20]

In our work, we found that there was a positive correlation between cystatin C level and age, and there was no significant correlation between cystatin C level and sex. A/C is considered to have a useful monitoring role in diabetes with respect to detecting kidney disease progression and evaluating treatment effects.[21]

In this study, we found that there was positive correlation between cystatin C level and age, DM duration, A/C, and levels of HbA1c, FBS, 2HPP, serum urea, and serum creatinine; however, there was a negative correlation between cystatin C level and weight and GFR. In addition, there was positive correlation between serum creatinine level and A/C; levels of serum urea, HbA1c, FBS, and 2HPP; weight; and DM duration; however, there was a negative correlation between serum creatinine level and age and GFR. Jeon *et al.* [22] showed that there was no significant difference in age or in sex between the studied groups, and this was in accordance with our study. In this study, we found that microalbuminuric and macroalbuminuric groups showed higher levels of HbA1c when compared with the normoalbuminuric group. This was supported by the findings of the study by Shehnaz and colleagues, according to which microalbuminuria had a highly significant correlation with duration of diabetes and a high HbA1c level.[20]

In this study, we found a significantly higher concentration of serum creatinine and serum cystatin C in the macroalbuminuric group compared with the normoalbuminuric and microalbuminuric groups, and all groups had higher levels compared with the control group. Herget-Rosenthal *et al.* stated that cystatin C levels can help detect the reduced GFR with higher sensitivity (97 vs. 83%) and higher negative predictive value (96 vs. 87%) compared with creatinine levels; in parallel, sensitivity of cystatin C was significantly higher ($P < 0.05$).[23]

From all of the above, we found that cystatin C is a better indicator of renal functions than creatinine in patients with impaired renal functions; this is because of the unique properties of cystatin C

such as constant production independent of age, sex, and muscle mass, and not being secreted or reabsorbed by the renal tubules. The major advantage of cystatin C over creatinine is its ability to detect a mild reduction in GFR, to which creatinine is insensitive. As there are no specific therapies, early detection of impaired renal functions is crucial to prevent the progression of renal disease and to improve patient outcome; the main disadvantage of cystatin C is the high cost of its immunoassay. Although all the studies reviewed here have demonstrated the distinct advantage of cystatin C over creatinine, it is important to document the advantages of cystatin C to improve patient outcome. Replacement of creatinine, which is the most widely used marker, by a new marker, cystatin C, ultimately depends on the results of patient outcome studies.

Conclusion

The results of this study suggest that cystatin C measurement in urine and serum is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes, especially in normoalbuminuric patients. Further investigations with a larger sample size and a prospective design are required to confirm the potential application of cystatin C as a useful biomarker for the early detection of diabetic nephropathy.

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Corresponding Authors

Dr Sanjiv Kumar, MBBS, MD
Assistant Professor, Department of Medicine Heritage Institute of Medical Sciences, Varanasi-221311, Uttar Pradesh, India
Email: *sanjivsingh315@gmail.com*
Mob No.: +91-9598030100