Assessment of Cystatin C and Microalbumin as Biomarkers for Nephropathy in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

BACKGROUND

Increased levels of urinary biomarkers can be detected in type 2 diabetic patients before the onset of significant albuminuria and may be used as an early marker of renal injury in diabetic nephropathy (DN) which would play a significant role for the effective management and treatment approaches in diabetic care. We wanted to evaluate cystatin C and microalbumin as effective early biomarkers in assessing nephropathy in patients with type 2 diabetes mellitus in this study.

METHODS

A cross-sectional study was conducted among 180 subjects grouped into healthy controls, clinically proven T2DM without nephropathy and type 2 DM with nephropathy comprising 60 participants in each group. Fasting and postprandial blood samples and urine samples were collected and analysed by standard methods. eGFR was calculated using CKD-EPI 2012 equation. IBM - SPSS version 20 was used for statistical analysis.

RESULTS

Diabetic nephropathy patients had significantly elevated serum cystatin C and microalbumin (2.43 ± 0.59, 700.5 ± 591.8 mg / L, respectively), compared to T2DM (0.98 ± 0.26, 63.7 ± 102.9 mg / L, respectively), and the control study subjects (0.81 ± 0.16, 11.15 ± 8.9 mg / L, respectively). Serum cystatin C showed AUC of 0.994 (95 % CI, 0.986 - 1.00) whereas microalbumin showed 0.944 (95 % CI, 0.907 - 0.981). Serum cystatin C showed a sensitivity of 96.7 % and a specificity of 91.7 % at a cut-off point of 1.34 mg / L whereas at a cut-off point of 138.5 mg / L for microalbumin, the sensitivity and specificity were 90 % and 83.3 % respectively.

CONCLUSIONS

Serum cystatin C and microalbumin both could be considered as markers for early detection of nephropathy in T2DM patients. The more prominent rise in serum cystatin C values provide an earlier diagnosis of diabetic nephropathy among T2DM patients.

KEY WORDS

Biomarker, Type 2 Diabetes Mellitus, Cystatin C, Diabetic Nephropathy, Microalbumin

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BACKGROUND

Diabetic nephropathy (DN), a long-term chronic complication of diabetes is a progressive kidney disease caused by glomerular and tubular structural and functional alteration due to disturbance in glucose homeostasis. Incidence of severe macro vascular disease in DN is very high leading to an increased mortality in diabetic patients.[1] In India, DN accounts for about 46 % of chronic renal disease in elderly people and is related to increased cardiovascular mortality and morbidity. A dramatic increase in prevalence of diabetic nephropathy has been seen in Indian diabetic patients, which has grown to be the solitary cause of end-stage kidney disease. DN is the most well-known reason for end stage renal disease in USA, Europe, Japan and other Asian countries, accounting for 25 - 45% of all patients enrolled in End stage renal disease (ESRD) programme.[2] DN is referred to as the chronic kidney disease(CKD) caused by DM, with a persistent eGFR (estimated glomerular filtration rate) of less than 60 ml / minute/ 1.73 m^2 or a urinary albumin / creatinine ratio (ACR) of > 30 mg / gm for over 3 months along with elevated arterial pressure.[3] However these three indicators show no significant variation until patient with DN reaches to stage 3 of CKD and patients remain in stage 1 and stage 2 for five years after diabetes first occurs.[4] Thus studies to discover reliable biomarkers for early diagnosis of this disease aiming to delay its progression and improve the outcome have an important significance.

For kidney function assessment, eGFR and serum creatinine are widely accepted. However, serum creatinine is notable to be influenced by several non-renal factors such as age, weight, nutritional status, race, and gender.[5] It is also known to have low sensitivity to assess mildly reduced renal function i.e., in creatinine blind area (40 - 70 ml / min/ 1.73 m^2).[6] Over the past three decades, microalbuminuria has been assumed to have a central role in diagnosis and management of renal disease among diabetics.[7] Microalbuminuria has been documented to be the earliest clinical evidence of DN in diabetic patients and is characterised by appearance of albumin in urine ≥ 30 mg / day or 20 μg / min.[8] Persistent microalbuminuria turned into a robust risk element for subsequent loss of GFR, which stabilised the importance of sustained increases in urine albumin excretion in the pathogenesis and diagnosis of diabetic kidney disease. However, patients who lost GFR at a high rate did not have overt albuminuria suggesting that it does not always lead to a significant loss of GFR in diabetics and measuring albuminuria alone does not completely seize the scope of early diabetic kidney disease.[9] Cystatin C, a promising marker of renal failure, is a cysteine protease inhibitor. It is a low molecular mass protein (13.4k Da), that is freely filtered at the glomerulus because of its small size and positive charge & then reabsorbed & fully catabolized, but not secreted by proximal renal tubules although they do absorb it.[10] It is constantly produced by all nucleated cells at a stable rate which is unaffected by inflammatory processes, sex, age, diet and nutritional status.[11] Cystatin C is being considered as a competency substitute for serum creatinine as it seems to be less affected by muscle mass and protein intake. These characteristics of cystatin C have made it as an endogenous marker for GFR assessment and have been proposed as a marker of tubular as well as glomerular dysfunction for early DN diagnosis. Previous studies have set up the predominance of serum cystatin C as an early renal marker in patients with diabetes[12,13] but not all studies.[14] Subsequently this study intended to evaluate cystatin C and microalbumin as effective early biomarkers in assessing nephropathy in patients with type 2 diabetes mellitus.

METHODS

This is a hospital based cross-sectional study carried out at R L Jalappa Hospital and Research Centre, Tamaka, Kolar, Karnataka India. Study protocol was approved by the Institutional Ethical Committee. 120 type 2 diabetes mellitus (T2DM) patients diagnosed on the basis of American Diabetes Association (ADA) criteria[15] and 60 age-sex matched apparently healthy controls within the age group of 35 - 70 years were enrolled for the study. It was conducted between July 2019 and March 2020. Study subjects were grouped into: Group I - Healthy Controls; Group II - Clinically proven T2DM without nephropathy and Group III - Type 2 DM with nephropathy. Patients with chronic liver or heart diseases, active urinary tract infections, malignancies, kidney diseases other than DN, acute renal injury along with patient on dialysis were excluded from participation. Informed written consents were obtained from all the study subjects after being informed with the purpose of the study.

Laboratory Evaluation

Clinical details such as anthropometric measurement (Age, gender, height, weight, systolic and diastolic blood pressure, diabetic duration, and pulse rate) of study participants were recorded from face-to-face interviews and the hospital medical records. After fasting for 8 - 10 hours, blood and clean-catch midstream random urine samples were collected from all the study participants. 2 hours' post-prandial blood samples were also collected for measuring post-prandial blood sugar. Routine biochemical parameters including Fasting and post-prandial blood glucose, blood urea, serum creatinine, total protein, uric acid, and serum albumin were analysed using Vitros 5.1 FS dry chemistry auto analyser based on the principle of Reflectance Photometry from Ortho Clinical Diagnosis (OCD), United Kingdom. Glycated haemoglobin (HbA1c) was measured by HPLC (high performance liquid chromatography) method on Bio-Rad D10 automatic glycated haemoglobin analyser. Urinary albumin was measured by quantitative immuno turbidimetric method on 5.1 FS dry chemistry auto analyser. Cystatin C levels were determined using Agappe MISPA i2 based on nephelometry methodology. The eGFR calculation was made using CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration 2012) equation.

Statistical Analysis

IBM-SPSS statistical package 20 program was used for analysis. Data were stated as mean and standard deviation. The one-way analysis of variance (ANOVA) and Pearson's correlation coefficient (R) were used whenever applicable. The specificity and sensitivity of the markers were assessed from receiver operating curve (ROC). The results with $P < 0.05$ were considered statistically significant.

Parameters	Group I (Mean ± SD) (N = 60)	Group II (Mean ± SD) (N = 60)	Group III (Mean ± SD) (N = 60)	F-Value	P - Value
Age(Years)*	46.9 ± 10.01	57.37 ± 8.65	57.67 ± 8.95	26.5	< 0.001[a,c]
BMI (Kg / m²)	24.3 ± 3.87	24.67 ± 4.23	24.56 ± 4.48	0.119	0.888
SBP (mmHg)*	121.1 ± 5.6	125.5 ± 14.84	131.93 ± 19.26	8.58	< 0.001[b,c]
DBP (mmHg)	79 ± 3.4	80.2 ± 8.55	82.17 ± 11.8	2.05	0.132
Diabetic duration (Year)*	-	6.53 ± 5.67	8.98 ± 8.13	-	< 0.005[b]
FBS (mg / dL)*	97.2 ± 7.6	211.78 ± 67.34	155.38 ± 53.05	79.7	< 0.001[a,b,c]
PPBS (mg / dL)*	109.5 ± 19.47	277.9 ± 87.48	249.33 ± 67.25	116.4	< 0.001[a,b,c]
HbA1C (%)*	5.53 ± 0.5	10.88 ± 2.53	8.7 ± 2.33	107.6	< 0.001[a,b,c]
Urea (mg / dL*)	19.78 ± 5.97	25.03 ± 13.5	112.18 ± 42.2	243.3	< 0.001[b,c]
SCr (mg / dL)*	0.72 ± 0.18	0.7 ± 0.31	4.82 ± 2.38	174.5	< 0.001[b,c]
UA (mg / dL)*	4.85 ± 1.37	4.3 ± 1.69	7.77 ± 2.11	68.2	< 0.001[b,c]
Total protein (gm / dL)*	7.22 ± 0.44	6.75 ± 0.7	5.83 ± 0.89	60.9	< 0.001[a,b,c]
Albumin(gm / dL)*	4.09 ± 0.28	3.56 ± 0.51	2.7 ± 0.48	158.7	< 0.001[a,b,c]
Cystatin C (mg / L)*	0.81 ± 0.16	0.98 ± 0.26	2.43 ± 0.59	321.9	< 0.001[a,b,c]
eGFR (mL / min)*	102.6 ± 19.8	82.75 ± 24.4	26.17 ± 9.33	262.4	< 0.001[a,b,c]
µALB (mg /L)*	11.15 ± 8.9	63.7 ± 102.9	700.5 ± 591.8	73.4	< 0.001[b,c]

Table 1. Mean and Standard Deviation of Anthropometric and Biochemical Data in the Study Population

[*P < 0.05 considered as significant; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure, FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1c: Glycated Haemoglobin; SCr: Serum Creatinine; UA: Uric Acid; eGFR: Estimated Glomerular Filtration Rate; µALB: Micro albumin] [a]Group I vs Group II [b]Group II vs Group III [c]Group I vs Group III

RESULTS

A total of 180 participants were included in the study, with 60 healthy controls and 120 T2DM with and without nephropathy. Out of these, 40 % were females and 60 % were males. Significant difference was seen among groups with regard to mean age, Systolic blood pressure (SBP) and diabetic duration among anthropometric measurements. However, Body mass index (BMI) and Diastolic blood pressure (DBP) did not show any significant difference. Study subject's anthropometric and clinical data are shown in Table 1. General diabetic profile and renal profile parameters showed a significant difference in the study groups. Significant high uric acid level was noticed in DN group than in control and T2DM subjects.

Parameters	Cystatin C		Microalbumin		N
	r - Value	P - Value	r - Value	P - Value	
Diabetic Duration	0.189	0.038*	0.184	0.044*	120
Urea	0.808	0.000**	0.501	0.000**	120
SCr	0.865	0.000**	0.612	0.000**	120
Albumin	-0.472	0.000**	-0.363	0.001**	120
eGFR	-0.902	0.000**	-0.568	0.000**	120

Table 2. Correlation of Cystatin C and Microalbumin with Anthropometric and Biochemical Indices in T2DM with and without Nephropathy

*Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed) [BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure, FBS: Fasting Blood Sugar; PPBS: Post Prandial Blood Sugar; HbA1C: Glycated Haemoglobin; SCr: Serum Creatinine; UA: Uric Acid; eGFR: Estimated Glomerular Filtration Rate; µALB: Micro albumin]

Total protein and albumin were lowered in DN group. Diabetic nephropathy patients indicated significantly elevated serum cystatin C and microalbumin (2.43 ± 0.59, 700.5 ± 591.8 mg / L, respectively), compared to T2DM (0.98 ± 0.26, 63.7 ± 102.9 mg / L, respectively), and the control study subjects (0.81 ± 0.16, 11.15 ± 8.9 mg / L, respectively). However, the eGFR was significantly lowered in nephropathy (26.17 ± 9.33 mL / min) group than other groups. Correlation analysis performed with Pearson's correlation coefficient showed a positive significant correlation with urea, creatinine, and duration of diabetes in diabetic patients for both serum cystatin C and microalbumin (Table 2). Also, microalbumin showed a negative correlation with FBS and HbA1C. As expected, a strong inverse association was noted with estimated GFR level and serum cystatin C (R = -0.902, P < 0.001) whereas a negative correlation between eGFR and microalbumin (R = -0.568, P < 0.001) was observed. In all diabetic patients group serum cystatin C had a significant positive correlation with microalbumin (R = 0.64, P < 0.001) (Figure 1).

ROC analysis for evaluation of the diagnostic accuracy of cystatin C and microalbumin in T2DM is shown in Figure 3. Serum cystatin C showed an AUC (area under the curve) of 0.994 (95 % CI, 0.986 - 1.00) whereas the AUC for microalbumin was 0.944 (95 % CI, 0.907 - 0.981). Serum cystatin C showed a sensitivity and specificity of 96.7 % & 91.7 % respectively at a cut-off point of 1.34 mg / L whereas at a cut-off point of 138.5 mg / L for microalbumin, specificity and sensitivity were 90 % and 83.3 % respectively.

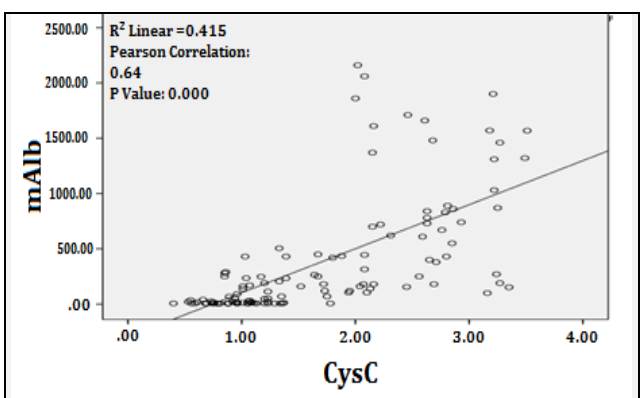

Figure 1. Correlation of Cystatin C with Microalbumin in T2DM Patients with and without Nephropathy

[*P < 0.05 considered as significant; CysC: Cystatin C; µALB: Micro albumin]

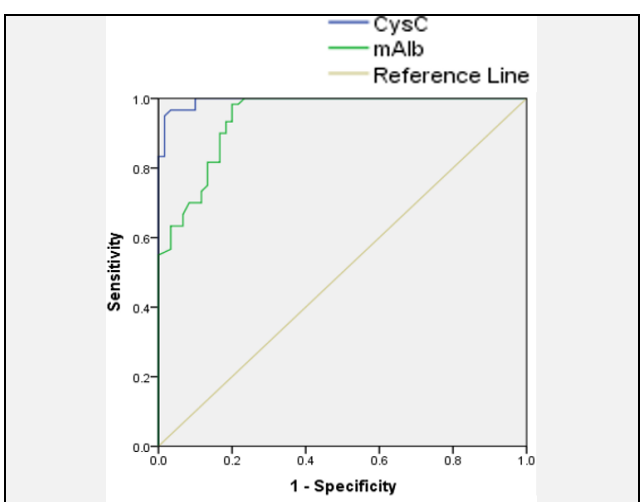

Figure 2. ROC Plots to Assess the Diagnostic Efficiency of Serum Cystatin C and µAlb to Predict Nephropathy in T2DM Patients

DISCUSSION

Assessment of kidney function is important in diabetics as the decline in kidney function results in decline in GFR and a proportionate increase in microalbuminuria in such patients. Serum creatinine is considered specific, but it doesn't increase till the GFR is moderately decreased. This insensitivity for small to moderate decrease in GFR in creatinine blind area (40 - 70 ml / min / 1.73 $m^{2)}$ gives a false sense of security and leads to late detection of kidney damage. In addition, serum creatinine depends on creatinine production, external and tubular handling and also on body mass and protein intake which limits serum creatinine as a reliable marker. GFR which is accepted as the best index reflecting kidney function can be measured directly with the infusion of exogenous markers such as inulin or ^{51}Cr-EDTA. Though these approaches are not practical and cannot be used on a daily basis.[16] Microalbuminuria is used for routine classical evaluation of diabetic nephropathy. Reduced creatinine clearance, appearance of microalbuminuria and elevated serum creatinine helps in the diagnosis of DN with limitations. Cystatin C is being considered as a promising substitution for serum creatinine as it appears to be less influenced by factors known to confound creatinine concentrations such as muscle build and protein intake.

Results revealed that age and SBP were higher in diabetic patients as compared to control subjects. The diabetic duration was almost 1.5 times higher in T2DM with nephropathy than T2DM alone. We observed higher levels of basic diabetic profile and overall renal parameters in T2DM patients than control subjects similar to findings of prior studies conducted by Takir M et al.[16] in 2016 and Patel et al.[17] in 2019. Hyperglycaemia being a crucial factor in the development of diabetic nephropathy induces hemodynamic and metabolic factors which are thought to be the main mediators of this renal injury.

Low level of total protein and albumin in nephropathy patients indicate loss of albumin through urine as a consequence of defective glomerular and tubular functions. We also observed a decline in eGFR in T2DM patients with nephropathy when compared with T2DM and control group. It has been well-known that angiotensin II increases efferent arteriolar pressure and plays a significant role in the autoregulation of renal blood flow and GFR. Prolonged inappropriate increase in angiotensin II leads to decrease in GFR and renal blood flow along with the release of cytokines and growth factors.[18]

Elevated cystatin C was observed in both T2DM and DN groups compared to control subjects and was statistically significant. This alteration in cystatin C levels showed that even with a minor reduction in eGFR, there was an increased cystatin C level. In our study, the variation observed in serum cystatin C levels between diabetics and control subjects suggest that diabetes kidney disease may be identified through serum cystatin C even with normal levels in albuminuria. Thus, nephropathy can be diagnosed in the beginning phase of disease progression.

Result of our study is in accordance with the previous studies.[11,16,19,20,21] However, contrary to our findings few studies showed that cystatin C is not a sensitive marker for diagnosis of early diabetic kidney diseases.[14,22,23] In this study, the microalbumin levels were elevated significantly in T2DM with nephropathy patients than the healthy control and T2DM patients. However, we didn't observe any significant difference among control and T2DM patients with respect to microalbumin level.

In the present study, serum cystatin C as well as microalbumin showed a significant negative correlation with albumin and eGFR level. With eGFR the correlation was stronger for cystatin C (R = -0.902) than the microalbumin (R = -0.568), thus indicating cystatin C as a most promising marker for reduced eGFR than microalbumin. As shown in Figure 1, serum cystatin C is associated with microalbumin reflecting with increase in urinary microalbumin, the serum cystatin C level will be elevated. Positive correlation with serum creatinine and urea was noted for both microalbumin and cystatin C. However, the correlation with respect to cystatin C was stronger than that of microalbumin.

In our study, the optimum cut-off for microalbumin and cystatin C were 138.5 and 1.34 mg / L respectively, as at these points it showed the best diagnostic accuracy. The sensitivity of cystatin C was 96.7 % and specificity was 91.7 %. This is in agreement with several studies with optimum cut-offs ranging between 1.38 to 1.4 mg / L.[19,24] Microalbumin showed a sensitivity of 90% and specificity of 83.3 % at above mentioned cut-off point. Thus both serum cystatin C and microalbumin could be considered for diagnosing nephropathy among T2DM patients. Although the AUC for cystatin C was approximately equivalent to that of microalbumin, our result showed that serum cystatin C had higher sensitivity and specificity meeting the criteria of a screening test for diabetic kidney disease.

CONCLUSIONS

Serum cystatin C and microalbumin both could be considered as markers for early detection of nephropathy in T2DM patients. The more prominent rise in serum cystatin C values provide an earlier diagnosis of diabetic nephropathy among T2DM patients. Serum cystatin C presents with a higher specificity and sensitivity for detecting diabetic nephropathy than microalbumin. Thus, serum cystatin C and microalbumin seems to be alternative markers for diagnosis of diabetic nephropathy in patients with a creatinine blind area.

Limitations

There are some limitations in our study that need to be mentioned. We do not have direct measures of GFR to precisely reflect kidney function. Single centric nature and small sample size of this study are other limitations. Another limitation is that, rather than 24-hour urine sample for microalbumin measure we have used spot urine sample.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
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