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Research Article

Value of Urinary Cystatin C In Early Detection Of Diabeticnephropathy In Type 2 Diabetes Mellitus

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Abstract

This study aimed to assess the value of urinary Cystatin C in early detection of diabetic nephropathy in type 2 DM. 42 T2DM patients were selected &6 normal adults were chosen as a control group. Patientswere divided into 2 groups: group I (20 patients)with microalbuminuria without any other urinary abnormality and with normal serum creatinine; and group II (22 patients) without microalbuminuria or any other urinary abnormality. All patients were subjected to assessment of CBC, serum creatinine, BUN, random blood sugar, HbA1C, CRP, microalbuminuria by ELISA, urinary α-1 microglobulin by ELISA, serum & urinary Cystatin C by ELISA, calculation of the creatinine clearance using Cockcroft-Gault Formula (CGF) & MDRD formula and measurement of glomerular filtration rate. No statistical significant difference was found when comparing serum creatinine, BUN and serum cystatin C between patients with microalbuminuria and those with normoal buminuria. Also, no statistical significant difference was found when comparing urinary α1-microglobulin and urinary cystatin/creatinine ratio between patients with microalbuminuria and those with normoalbuminuria while there was a statistically significant difference found when comparing urinary cystatin C between the three studied groups and when comparing the 2 patients group together. This study demonstrates that urinary cystatin C levels could be a useful marker for detection of microalbuminuria independent on any other tubular markers; in addition, it can be used as a good predictor for the presence of microalbuminuria in early diabetic nephropathy.

Keywords: Cystatin C, Diabetic Nephropathy, Albuminuria

Introduction

Diabetic nephropathy is the single most frequent cause of end-stage renal disease (ESRD in the United States and in Europe [1, 2]and is predominantly due to type2 diabetes mellitus (T2DM). An increasing number of type2 diabetic patients livelong enough for nephropathy and ESRD to develop, since the treatment of diabetes, hypertension and coronary heart disease has improved. Therefore, prevention of diabetic renal disease, or atleast the post ponementors lowing down of the disease process, has emerged as a key issue[3]. Microalbumin uria is the first detectable functional abnormality; aproportion of microalbumin uric patients then progress to overt nephropathy, characterized by the presence of proteinuria [4].

Small amounts of urinary proteins observed at early stages of diabetic nephropathy may result from both glomerular and proximal tubulardys function[5].

Increased urinary excretion of low-molecular-weight proteins (tubularproteins) and enzymes has been shown to indicate proximaltubular injury. Some of the best characterized tubular proteins and enzymes to detect proximal tubular injury areα1- and β2-microglobulin, cystatin C, retinol- binding protein [6, 7], glutathione S- transferase (GST), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and N-acetyl-ß-D- glucosaminidase (NAG)[7-9].

Cystatin C is an onglycosylated basic protease inhibitor that is produced at a constant rate by all nucleated cells[10, 11]. It is freely filtered by the renal glomerulus and primarily catabolized in the renal tubules [11]. Furthermore, levels are reported to be independent of gender, age, and body mass[12-14].
Increased urinary cystatin C concentrations allow the accurate detection of tubular dysfunction among pure and mixed nephropathies. Because of its ability to be processed on automated clinical chemistry analyzers, this assay could easily be used as an adjunct to the standard panel used to screen kidney pathologies, even in emergency situations[15].

In this study we assessed the possible value of urinary Cystatin C in early detection of diabetic nephropathy in type2 diabetes mellitus (T2DM).

Materials and Methods

Forty two patients with T2DM were randomly selected from Diabetes Out-Patient Clinic in Ain Shams University Hospitals (during their regular routine follow up visits), and 6 normal adults were chosen as control group.Patients were divided into 2 groups: group I (20 patients) were patients with detected microalbuminuria without any other urinary abnormality and with normal serum creatinine; and group II (22 patients) were patients without microalbuminuria or any other urinary abnormality.
Microalbuminuria was defined according to the American diabetes association guidelines [16] and the patients were divided into groups according to the result of spot urinary albumin result.

Exclusion criteria included smokers, patients with urinary tract infection, patient with acute inter current infection, patients with history of chronic analgesic abuse, and hypertensive patients.

All patients were subjected to Complete medical history and detailed clinical examination including body mass index(BMI), routine laboratory tests including complete blood count(CBC), serum creatinine, blood urea nitrogen, random blood sugar, glycosylated hemoglobin (HbA1c), C-reactive protein (CRP)using CRP Direct Latex kit from QUIMICA CLINICA APLICADA S.A., measurement of microalbuminuria by ELISA using ORG 5MA Micro-Albumin kit from ORGENTEC Diagnostika GmbH, measurement of urinary α-1microglobulinbyELISA using α1-Microglobulin ELISA Kit from Immundiagnostik AG, measurement of serum& urinary Cystatin C by ELISA using RD191009100 Human Cystatin C ELISAkit from BioVendor - Laboratorní medicína a.s., calculation of the creatinine clearance using Cockcroft-Gault Formula (CGF) & MDRD formula, measurement of glomerular filtration rate using creatinine clearance by the following equation:

$$\frac{U \text{ X } V}{P \text{ X } 1440}$$

U=Urinary Creatinine, V=Urine Volume (mL/24hour), P=Serum Creatinine

Data were analyzed on an IBM personal computer, using Statistical Package for Special Science (SPSS) software computer program version15. Assessment of normality of the studied variables was performed using Kolmogorov-Smirnov test. Data were described as mean±standard deviation(SD) for parametric numerical variables, median & range for non-parametric numerical variables and as frequency & percentage for categorical variables. Independent Student t test was used for comparison of parametric quantitative variables among two independent groups. Mann-Whitney Test was used for comparison of non-parametric variables among two independent groups. Chi-square test (or Fisher'sexact test when appropriate) was used for comparison of distribution of qualitative variables among different group. Kruskal-Wallis Test was used for comparison of non-parametric variables among more than two independent groups. One-way ANOVA test was used for comparison of quantitative variables among more than two independent groups. Least significant difference test (LSD) test was used as post-hoc test. Pearson correlation coefficient was used to for testing association between different parametric variables while Spearman correlation coefficient was used to for testing association between different non-parametric variables. Stepwise linear regression analysis was used as the method for performing multivariate analysis. Significance level(P) value: $P>0.05$is insignificant.(NS); $P\leq0.05$is significant.(S)

Results

There was no statistically significant difference found when comparing age, sex, blood pressure, weight,

height, BMI, between patients with microalbuminuria and those with normoalbuminuria.

There was no statistically significant difference found when comparing diabetes duration, HbA1C, RBS, between patients with microalbuminuria and those with normoalbuminuria.

There was no statistically significant difference found when comparing ALT, AST, Total Proteins, serum albumin Hb, WBCs, platelets, cholesterol, LDL, HDL, CRP between patients with microalbuminuria and those with normoalbuminuria while there was statistically significant difference found when comparing triglycerides between the two groups.

There was no statistically significant difference found when comparing serum creatinine, blood urea nitrogen and serum cystatin C between patients with microalbuminuria and those with normoalbuminuria while there was statistically significant difference found when comparing serum cystatin C between the three studied groups.

There was no statistically significant difference found when comparing urine volume, urinary creatinine, urinary α1-microglobulin and urinary cystatin/creatinine ratio between patients with microalbuminuria and those with normoalbuminuria while there was a statistically significant difference found when comparing urinary cystatin C between the three studied groups and when comparing the 2 patients group together.

There was no statistically significant difference found when comparing measured creatinine clearance, calculated creatinine clearance using CGF and MDRD between the three studied groups.

Plotting of the ROC curve for assessment of use of urinary cystatin C as a predictor for the presence of microalbuminuria resulted in an AUC = 0.701 with p value 0.026

Discussion

Tubulo-interstitial pathology in diabetic nephropathy is thought to be caused by cell injury that is induced by high ambient glucose levels and increased proportions of glycated proteins. Other mechanistic hypotheses engage glomerular ultrafiltration of proteins and bioactive growth factors and their effects on tubular cells. Some scholars promote tubular ischemia due to reduced peritubular blood flow as a response to glomerular injury. All of these mechanisms contribute to renal tubulo-interstitial injury in diabetic nephropathy [17].Cystatin C, a 13-kDa endogenous cysteine proteinase inhibitor, is produced by nucleated cells at a constant rate [18, 19].Cystatin C is excreted by glomerular filtration, and then undergoes essentially complete tubular reabsorption and catabolism (without secretion) [20-22].In normal renal function, tubular reabsorption and catabolism of cystatin C is almost complete and cystatin C is only detected in very small quantities in the urine[23].

In the current study, the urinary cystatin C level was found to be significantly higher in patients with microalbuminuria than patients with normoalbuminuria and than control.This difference in the levels of the urinary cystatin C among the studied groups may be attributed to the difference in the serum cystatin C (as it was found to be higher in the patient groups than the control group) but this postulation was not supported by the finding that there was no correlation between the urinary cystatin c and the serum cystatin c among the studied patients. Early tubular dysfunction among patients with early diabetic nephropathy can be the cause of the increased level of urinary cystatin C in patients with microalbuminuria; but this postulation was faced with the finding that there was no significant correlation urinary cystatin C with the levels of the urinary α1-microglobulin.Also, we found that there is no statistical significant difference between the different studied groups regarding the levels of the urinary α1-microglobulinindicating that there was no detectable tubular dysfunction using urinary α1-microglobulin among the studied groups.

The estimated GFR using MDRD equation and the creatinine clearance using CGF among patient with microalbuminuria and patients with normoalbuminuria in comparison to the control group showed that there was no statistically significant difference suggesting that there is no effect of glomerular hyperfiltration on the urinary levels of the studied variables. So, there is no evidence suggesting that local hyperfiltration in the glomerulus might be the cause of this difference in the level of urinary cystatin C.The catabolism of the cystatin C and its uptake by the tubular cells is altered

by the presence of albuminuria which causes its increase in the urine of patients with albuminuria. This postulation was supported by our finding that showed significant positive correlation between the levels of urinary cystatin C with the level of sport urinary albumin/creatinine ratio, and with 24hr urinary albumin, and withthe timed urinary albumin. This result was confirmed with performing multivariate regression analysis for different variables that might affect the urinary cystatin C level which confirmed that only the urinary spot albumin/creatinine ratio is the variable that has direct effect on the level of urinary cystatin C.

Supporting this hypothesis, Thielemans et al. suggested that there is competition between albumin and low-molecular-weight proteins e.g. cystatin C for renal tubular uptake in experimental nephropathies by the hypothesis that at high filtered loads, albumin decreases the tubular uptake of low-molecular-weight proteins most likely by competition for a common transport mechanism [24]. Also, In 1997 Tian et al. reported that urinary excretion of cystatin C might be increased in adult patients with renal diseases and reduced GFR [25].In 2004, Tkaczyk et al. found that there is increased level of urinary cystatin C in children with active idiopathic nephritic syndrome than normal healthy controls and postulated that proteinuria might have changed tubular cystatin C reabsoption [26].Conti et al. in 2006 observed normal levels of urinary cystatin C in diabetic patients with documented nephropathy by high serum cystatin C, serum creatinine and microalbuminuria [15]. Also, he suggested that for mild to subnormal proteinuria, urinary cystatin C could be a reliable marker of tubular dysfunction as he found that the level of urinary cystatin C is higher in patients with tubular dysfunction than those with glomerular dysfunction and control subjects, while in patients with high proteinuria the clinical value of the urinary cystatin C may worsen. In this context, it will be of great interest to define the proteinuria range in which urinary cystatin C is clinically relevant[15]. Increased urinary cystatin C was noted especially in renal disorders such as the tubular damage associated with Chinese herbs, HIV nephropathy, and acute interstitial nephritis[6, 21, 23, 27, 28].

Assessment of the use of urinary cystatin c to predict the presence of microalbuminuria in early diabetic nephropathy revealed that it has a sensitivity of 90% and specificity of 54.5% when taking the level of 1.25g ng/ml as a cut-off value, above which the patient can be considered to have microalbuminuria. The urinary cystatin C levels might be affected by different variables, but the current study couldn't prove this effect except for the spot urinary albumin/creatinine ratio. However, the small number of cases in the current study may have an implication on the above mentioned findings and postulations, so, studies with larger sample size is needed in order to accurately identify the levels of urinary cystatin C in diabetic patient and the cut-off values for each stage.

Cystatin C has advantages as a urinary marker of tubular injury compared to established markers such as α1-microglobulin, β2-microglobulin and retinol binding protein [22].In contrast to β2-microglobulin, infection, inflammation and malignancy do not increase the urinary concentration of cystatin C as cystatin C is produced and altered at a constant rate [18, 19, 29-32].In addition, cystatin C is more stable in urine than α1-microglobulin, β2-microglobulin or retinol-binding protein,also it may have age and gender-independent reference values [33-35].These advantages may promote the widespread use of urinary cystatin C as a marker of tubular injury in clinical practice[20].Further studies are needed to correlate the levels of the different tubular markers with the degree of tubule-interstitial damage (assessed be renal biopsy) among the patients with different stages of diabetic nephropathy.

The results obtained using urinary cystatin C were not the same when using urinary cystatin C/creatinine ratio.This warrant the use of this ratio and all other tubular markers adjusted to creatinine in the detection of renal pathology.This goes in accordance with Conti et al. 2009 who proposed that adjusting the values of any tubular markers to urinary creatinine, especially in patients with acute or even moderate chronic renal failure, may be inappropriate[36].As in most publications, urine markers of tubular damage are reported following correction for urine creatinine [36].Thus, results are not reported as absolute concentrations. Serum creatinine is filtered through the glomeruli but not catabolized by the renal tubules [22, 37, 38].Thus, urinary creatinine is in large part directly related to the glomerular filtration rate (GFR). However, 10%–40% of urinary creatinine is a consequence of active tubular secretion, with substantial variation among demographic groups

Table 1: Comparison of Demographic data, blood pressure and BMI, diabetes duration, glycemic control, urinary parameters and different laboratory among the studied groups

		Group 1 (n=20)	Group 2 (n=22)	Control Group (n=6)	P value
Age (years)		50.20±7.42	50.18±10.19	32.17±8.84	**0.000***
Sex	**Male**	7 (35%)	12 (54.5%)	2 (33.3)	0.381
	Female	13 (65%)	10 (45.5%	4 (66.7%)	
Weight (Kg)		79.50±9.53	74.78±10.18	70.33±2.25	0.089#
Height (cm)		165.17±10.33	163.03±11.87	167.50±5.01	0.629
BMI		29.21±3.23	28.54±5.92	25.13±1.86	0.176
Systolic BP (mmHg)		121.18±10.54	117.78±12.63	118.33±7.53	0.656
Diastolic BP (mmHg)		78.82±8.57	76.67±9.70	75±5.48	0.605
Mean Arterial BP		92.94±8.73	90.37±10.53	89.44±5.74	0.625
Disease Duration (years)		4 (0.17-15)	4 (0.42-15)	NA	0.704
HbA1C		8.91±1.87	8.91±1.64	6.87±0.79	**0.027****
RBS (mg/dl)		175.90±75.91	181.82±83.17	100.33±23.86	0.067##
ALT		26 (10-43)	18 (10-93)	16.50 (12-21)	0.145
AST		26 (12-72)	25.50 (2-216)	19 (9-24)	0.148
Total Prtn.		7.68±0.66	7.28±0.54	NA	0.111
S.Alb		3.95 (2.60-4.40)	3.95 (2.50-4.30)	NA	0.660
Hb (g/dl)		12.46±1.66	13.09±1.76	13.18±1.16	0.421
WBCs		7.71±2.52	8.30±2.97	7.30±2.12	0.660
Platelets		236.25±97.68	228.70±103.29	233±24.91	0.969
Cholesterol		195.75±57.38	181±60.10	162±23.69	0.402
TG		178.50 (54-233)	103.50 (25-320)	95.50 (34-152)	0.039
LDL		103.11±22.51	122.42±48.79	93.40±30.07	0.302
HDL		42.44±6.69	43.15±11.15	51.80±4.87	0.154
CRP		9.35 (0-180)	9.35 (0-180)	0 (0-0)	0.046
Serum Cr. (mg/dl)		0.80±0.19	0.81±0.18	0.77±0.22	0.907
BUN		14.58±5.41	15.35±7.46	11.67±3.01	0.452
Serum Cystatin (ng/ml)		638 (303-1388)	538 (232-1428)	342.50 (195-539)	**0.025**
Urine Volume		1000 (400-2500)	1000 (700-3000)	NA	0.863
Urinary Cystatin (ng/ml)		1.73 (1.04-8.25)	1.25 (0.92-2.55)	1.25 (1.04-1.85)	**0.041**
Urinary Cr. (mg/dl)		136.90±93.87	130.86±76.74	160.65±72.77	0.745
Urinary albumin (ug/ml)		74.88 (9.25-540)	4.75 (1.20-28)	9.50 (1.50-27)	**0.000**
α1-Microglobulin (mg/l)		5.03±4.18	5.54±3.85	3.63±3.81	0.584
Urinary Cystatin/Cr. ratio (ug/mg)		2.36 (0.61-8.71)	1.15 (0.41-17.12)	0.80 (0.48-3.36)	0.140
Spot Urinary Albumin/Cr. ratio (ug/mg)		56.63 (30.94-290.83)	6.37 (0.84-19.24)	6.65 (0.69-20.87)	**0.000**
24hr Urinary Albumin (mg/24hr)		69.75 (9.25-735)	5.81 (1.20-81)	NA	**0.000**
Timed Urinary Albumin (ug/min)		48.44 (6.42-510.42)	4.04 (0.83-56.25)	NA	**0.000**
Measured Cr. Clearance		131.77 (21.25-300.60)	128.84 (15.42-378.91)	NA	0.694
Calculated Cr. Clearance (CGF)		116.03±27.18	110.30±28.31	133.48±48.64	0.307
Calculated Cr. Clearance (MDRD)		95.39±22.65	99.35±33.76	115.20±53.54	0.432

NA: not available ; * Statistically significant difference was found between control group and both group 1 (p value= 0.000) and group 2 (p value= 0.000) using LSD with no significant difference between group 1 and group 2 ; # Statistically significant difference was found between control group and group 1 (p value= 0.043) using LSD with no significant difference between Control group and group 2 or between group 1 and group 2;** Statistically significant difference was found between control group and both group 1 (p value= 0.012) and group 2 (p value= 0.011) t LSD with no significant difference between group 1 and group 2 ## Statistically significant difference was found between control group and both group 1 (p value= 0.037) and group 2 (p value= 0.024) using LSD with no significant difference between group 1 and group 2

Table 2: Comparison of the TG, CRP, serum cystatin C between the 2 patients groups

	Group 1 (n=20)	Group 2 (n=22)	P value
TG	178.50 (54-233)	103.50 (25-320)	**0.049**
CRP	9.35 (0-180)	9.35 (0-180)	0.886
Serum Cystatin (ng/ml)	638 (303-1388)	538 (232-1428)	0.137
Urinary Cystatin (ng/ml)	1.73 (1.04-8.25)	1.25 (0.92-2.55)	**0.026**

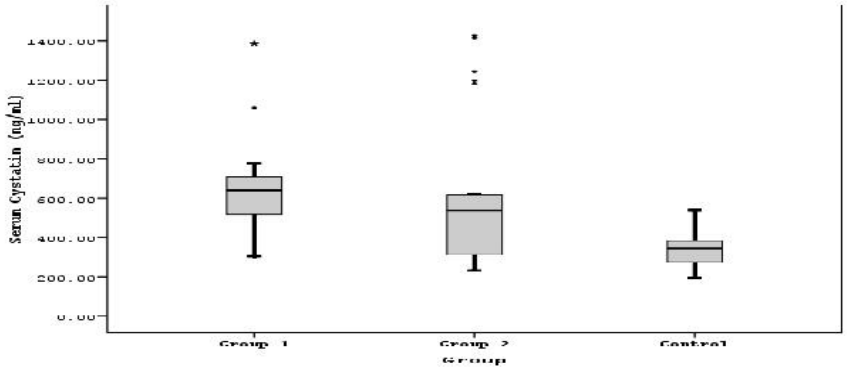

Figure 1: Comparison of serum cystatin C between the studied groups

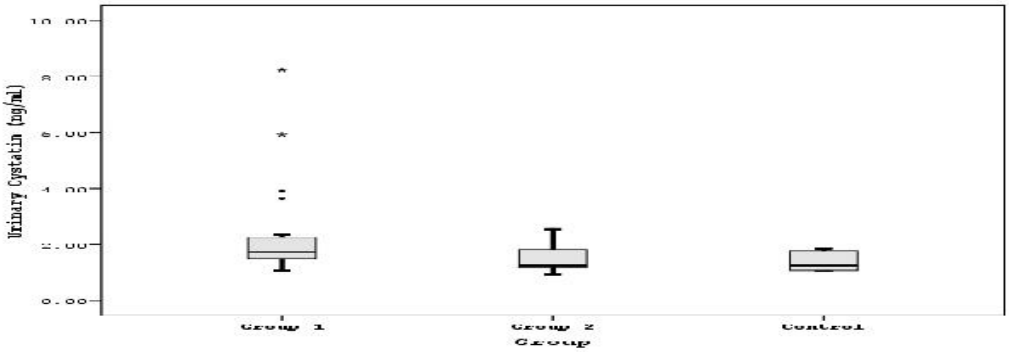

Figure 2: Comparison of urinary cystatin C between the studied groups

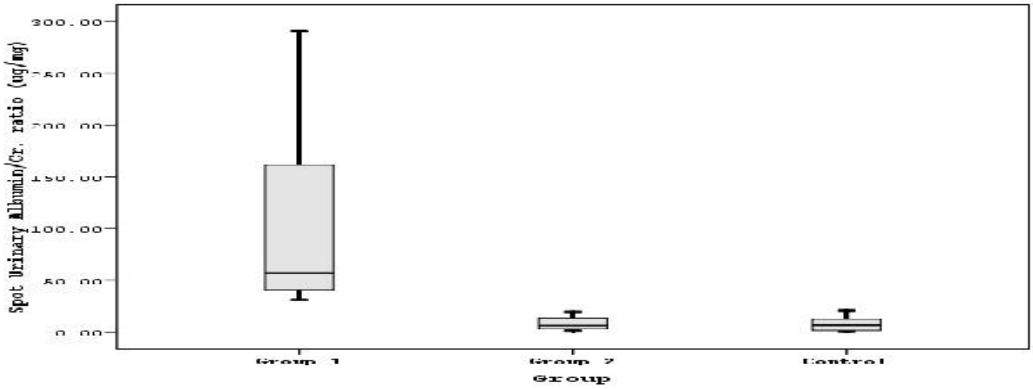

Figure 3: Comparison of Spot urinary albumin/cr. ratio between the studied groups

Table 3: Correlation between Urinary Cystatin C (ng/ml) and different parameters

	Urinary Cystatin C (ng/ml)					
	Group 1 (n=20)		Group 2 (n=22)		Patients (n=43)	
	r	P value	r	P value	r	P value
Age (years)	0.132	0.580	0.064	0.807	0.055	0.747
Disease Duration (years)	-0.057	0.835	0.047	0.861	0.012	0.949
Weight (Kg)	-0.047	0.853	-0.228	0.334	-0.018	0.913
Height (cm)	-0.056	0.826	0.084	0.724	0.085	0.613
BMI	-0.075	0.766	-0.337	0.146	-0.209	0.208
Systolic BP (mmHg)	-0.141	0.590	0.340	0.168	0.186	0.284
Diastolic BP (mmHg)	0.077	0.769	0.434	0.072	0.301	0.079
Mean Arterial BP	-0.006	0.981	0.380	0.120	0.246	0.155
ALT	0.113	0.645	-0.301	0.210	-0.066	0.693
AST	0.235	0.332	-0.243	0.332	-0.044	0.796
Total Prtn.	-0.148	0.647	-0.239	0.432	0.014	0.946
S.Alb	-0.113	0.701	-0.060	0.839	-0.079	0.689
Hb (g/dl)	0.091	0.704	0.044	0.852	0.005	0.975
WBCs	-0.138	0.561	0.250	0.288	0.006	0.972
Platelets	-0.053	0.824	0.071	0.767	0.006	0.969
HbA1C	-0.300	0.199	-0.100	0.658	-0.155	0.328
RBS (mg/dl)	-0.178	0.453	-0.096	0.671	-0.134	0.398
Cholesterol	-0.350	0.130	0.142	0.529	0.061	0.703
TG	0.024	0.918	0.285	0.198	0.350	**0.023**
LDL	-0.500	0.170	-0.390	0.188	-0.436	**0.042**
HDL	-0.183	0.637	-0.201	0.511	-0.232	0.299
CRP	0.373	0.106	-0.015	0.947	0.180	0.255
Serum Cr. (mg/dl)	-0.131	0.582	0.001	0.997	-0.117	0.471
BUN	0.074	0.763	-0.027	0.911	-0.055	0.740
Serum Cystatin (ng/ml)	-0.504	**0.023**	-0.021	0.924	-0.123	0.438
Urine Volume	-0.174	0.476	0.352	0.128	0.111	0.501
Urinary Cr. (mg/dl)	0.365	0.114	-0.108	0.633	0.127	0.421
Urinary albumin (ug/ml)	0.492	**0.028**	0.322	0.143	0.486	**0.001**
α1-Microglobulin (mg/l)	0.111	0.642	0.028	0.902	0.037	0.815
Urinary Cystatin/Cr. ratio (ng/mg)	0.176	0.458	0.479	**0.024**	0.362	**0.018**
Spot Urinary Albumin/Cr. ratio (ug/mg)	0.552	**0.012**	0.420	0.052	0.509	**0.001**
24hr Urinary Albumin (mg/24hr)	0.482	**0.037**	0.357	0.122	0.489	**0.002**
Timed Urinary Albumin (ug/min)	0.482	**0.037**	0.357	0.122	0.489	**0.002**
Measured Cr. Clearance	0.309	0.198	-0.056	0.816	0.121	0.463
Calculated Cr. Clearance (CGF)	-0.113	0.655	-0.162	0.535	0.008	0.965
Calculated Cr. Clearance (MDRD)	0.061	0.797	-0.083	0.750	0.049	0.772

Table 4: Correlation between Urinary Spot Urinary Albumin/Cr. ratio (ug/mg) and different parameters

	Group 1 (n=20)		Group 2 (n=22)		Patients (n=43)	
	r	P value	r	P value	r	P value
Age (years)	0.170	0.474	0.115	0.659	0.053	0.756
Disease Duration (years)	-0.063	0.818	-0.231	0.389	-0.016	0.929
Weight (Kg)	-0.072	0.777	-0.058	0.809	0.163	0.328
Height (cm)	-0.342	0.164	0.387	0.092	0.051	0.761
BMI	0.298	0.229	-0.410	0.073	0.062	0.713
Systolic BP (mmHg)	-0.158	0.544	0.168	0.506	0.123	0.481
Diastolic BP (mmHg)	-0.021	0.937	0.267	0.283	0.155	0.375
Mean Arterial BP	-0.060	0.819	0.197	0.433	0.135	0.441
ALT	0.441	0.059	-0.028	0.909	0.147	0.380
AST	0.380	0.109	0.132	0.601	0.069	0.686
Total Prtn.	-0.074	0.820	-0.185	0.545	0.271	0.190
S.Alb	-0.106	0.718	-0.224	0.442	-0.007	0.972
Hb (g/dl)	0.150	0.528	0.339	0.144	-0.021	0.896
WBCs	-0.005	0.985	0.182	0.442	-0.049	0.764
Platelets	-0.304	0.193	0.096	0.686	-0.037	0.819
HbA1C	-0.537	**0.015**	0.011	0.962	-0.151	0.338
RBS (mg/dl)	-0.216	0.360	0.158	0.484	-0.052	0.744
Cholesterol	-0.573	**0.008**	0.084	0.710	0.069	0.664
TG	-0.141	0.554	0.186	0.407	0.327	**0.035**
LDL	-0.367	0.332	-0.429	0.144	-0.339	0.122
HDL	-0.250	0.516	0.462	0.112	0.070	0.757
CRP	0.140	0.556	0.116	0.607	0.084	0.596
Serum Cr. (mg/dl)	0.189	0.426	0.021	0.929	0.021	0.896
BUN	0.086	0.725	-0.033	0.890	-0.018	0.913
Serum Cystatin (ng/ml)	-0.229	0.331	-0.366	0.093	0.058	0.717
Urine Volume	0.121	0.623	0.603	**0.005**	0.168	0.308
Urinary Cystatin (ng/ml)	0.552	**0.012**	0.420	0.052	0.509	**0.001**
Urinary Cr. (mg/dl)	0.411	0.071	-0.342	0.120	0.031	0.848
Urinary albumin (ug/ml)	0.751	**0.000**	0.584	**0.004**	0.866	**0.000**
α1-Microglobulin (mg/l)	-0.211	0.373	-0.385	0.077	-0.217	0.167
Urinary Cystatin/Cr. ratio (ng/mg)	-0.053	0.826	0.375	0.085	0.266	0.088
24hr Urinary Albumin (mg/24hr)	0.770	**0.000**	0.645	**0.002**	0.849	**0.000**
Timed Urinary Albumin (ug/min)	0.770	**0.000**	0.645	**0.002**	0.849	**0.000**
Measured Cr. Clearance	0.302	0.209	-0.048	0.840	0.014	0.934
Calculated Cr. Clearance (CGF)	-0.344	0.162	-0.032	0.903	0.057	0.746
Calculated Cr. Clearance (MDRD)	-0.328	0.158	0.127	0.626	-0.028	0.871

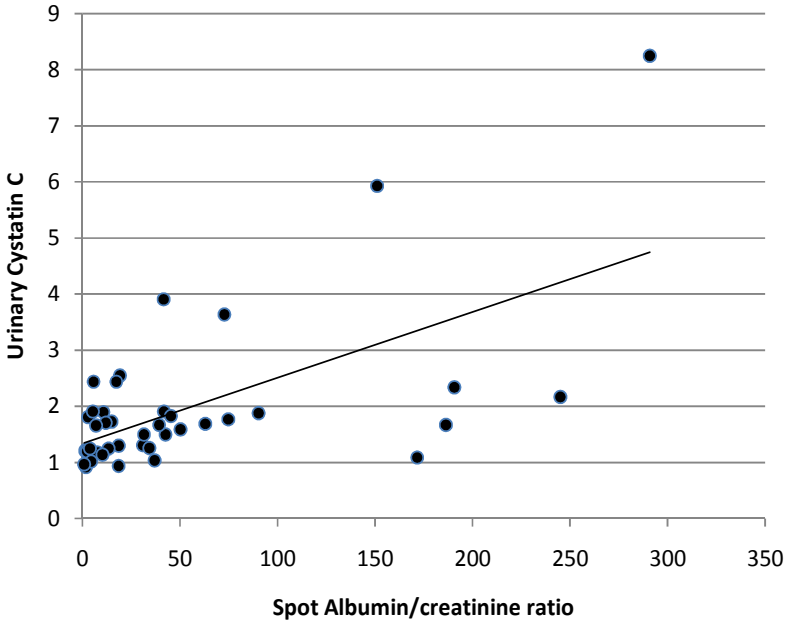

Figure 4: correlation between urinary cystatin C and spot urinary albumin/creatinine ratio

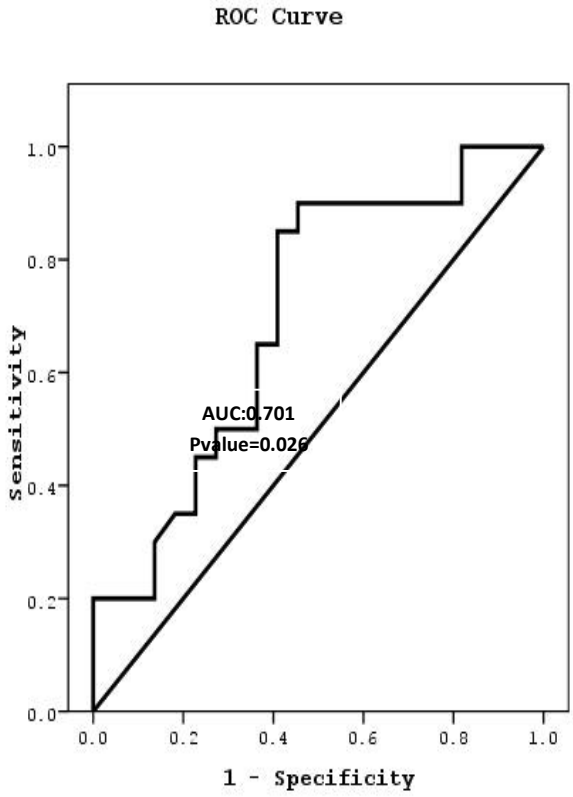

Figure 5: ROC curve for assessment of urinary cystatin C in the assessment of microalbuminuria

Table 5: Estimated different cut-off values for Urinary Cystatin C (ng/ml) in the detection of microalbuminuria

Cut-off value	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
1.2550	0.900	0.545	0.643	0.857	0.714
1.2800	0.850	0.545	0.630	0.800	0.690
1.3050	0.850	0.591	0.654	0.813	0.714

Table 6: Multivariate analysis for the different variables affecting urinary cystatin C

Variable	Beta	P value
Spot Urinary Albumin/Cr. ratio (ug/mg)	0.629	0.001
Age (years)	-0.001	0.995
HbA1C	0.015	0.931
CRP	0.015	0.930
Serum Cystatin (ng/ml)	-0.215	0.202
TG	-0.051	0.776
LDL	-0.146	0.408

[39].Because urinary creatinine varies in parallel with serum creatinine, the latter being highly dependent on age, lean body mass and inflammation status, daily urinary creatinine excretion may vary among individuals (500-2000 mg/day)[40-42].Additionally, creatinine excretion becomes highly variable as renal failure progresses, and this variation influences both timed and spot-urine protein-creatinine ratios [43].

In the present study, we examined urinary α1-microglobulin as a tubular marker between control and patients with normoalbuminuria and patients with microalbuminuria and found that there was no statistically significant difference between the three groups and level of the urinary α1-microglobulin was within normal range among the three groups. This indicates that the degree of tubule-interstitial damage was not high to raise the levels of the urinary α1-microglobulin above normal limits. This is against the postulation of the early tubular dysfunction among patients with early diabetic nephropathy either in the normoalbuminuria phase or in the microalbuminuric phase. On the contrary, some studies performed on Caucasian populations found urinary α1-microglobulin be higher, when compared with normal control subjects, in both type 1 [44] and type 2 diabetic subjects [45] and present even without clinical nephropathy[46, 47].In type 2 diabetic subjects, α1-microglobulin excretion was directly correlated with albuminuria [48] and HbA1c levels and decreased with improved glycemic control[49, 50].Also in 2001, Mahgoub et al. studied the levels of urinary α1-microglobulin and ß2-microglobulin among 83 patients Egyptian patients with type 1 and type 2 diabetes mellitus with different grades of nephropathy and found that α1-microglobulin tend to increase with the progression of the disease[51].Shultz et al found that raised amounts of urine retinol binding protein and N-acetyl-glycosaminidase are related to HbA1c and the duration of diabetes. They occur in the majority of subjects and are not early markers for the risk of microalbuminuria [52].

Traditionally ,the development of renal lesions in patients with type2 diabetes mellitus is attributed to interactions of metabolic and hemodynamic factors. Inflammation is a potential factor in the development and progression of diabetic nephropathy, and recent dataindicate that an inflammatory component contributes to the development of diabetic complications [53-57].The level of CRP was found to be high in both patients group (normoalbuminuria & microalbuminuria) than the control group. This might be due to the inflammatory state associated with the

development of diabetic nephropathy. Also our study showed that the level of CRP was the same among patients with normoalbuminuria in comparison to those with the microalbuminuria.

The present study concluded that urinary cystatin C levels may be valuable for detection of microalbuminuria independent on any other tubular markers and independent of the degree of tubular dysfunction. Urinary cystatin c can be used to predict the presence of microalbuminuria in early diabetic nephropathy with a sensitivity of 90% and specificity of 54.5% at cut-off value of 1.25g ng/ml.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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