Prediction of microalbuminuria by analysing total urine protein-to-creatinine ratio in diabetic nephropathy patients in rural Sri Lanka

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Abstract

Introduction Chronic kidney disease (CKD) is a major complication of diabetes mellitus and it contributes to increased hospital mortality and morbidity. Microalbumin test is used to identify the first sign of deteriorating kidney function but it is an expensive test. Alternatively, measurement of urine total protein-to-creatinine ratio (TPCR) is a simple and inexpensive method.

Objective To find whether the urine TPCR can predict the presence of microalbuminuria in patients with diabetic nephropathy.

Method A cross sectional study was performed on 216 patients with diabetes mellitus at General Hospital, Ampara over a period of 4 weeks. Urine albumin, urine creatinine and urine total protein were analysed on first voided urine samples and urine albumin to creatinine ratio (ACR) and total-protein-to-creatinine ratio were calculated. Regression analysis and Spearman's rank correlation were used to study the linear relationship between two variables.

Results Among 216 patients, 56 (26.1%) were males and 160 (73.9%) were females. The mean urine total-protein-to-creatinine ratio was 89.3 ± 231.6 mg/g and albumin to creatinine ratio was 43.1±76.3 mg/g. Sixty four (29%) patients were newly detected as having microalbuminuria (n=61; 28%,) or macroalbuminuria (n=3; 1%,). There was a significant correlation between urine total-protein-to-creatinine ratio and urine albumin to creatinine ratio (R2 = 0.824, ACR = [TPCR + 18.421]/ 2.5026) in the total sample (p<0.001). The total-protein-to-creatinine ratio showed a significant correlation with urine albumin to creatinine ratio in the range of microalbuminuria (30-300 mg/g creatinine) (R2 = 0.798; p<0.001). The regression equation was ACR = [TPCR - 5.0491]/1.2633.

Conclusion The urine total-protein-to-creatinine ratio showed a positive significant correlation with urine albumin to creatinine ratio, which is clinically important to identify early stage of diabetic nephropathy. This can be used in rural areas as it is inexpensive.

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Introduction

Chronic kidney disease (CKD) is a worldwide public health problem, with adverse outcomes [1, 2]. Several million people worldwide are affected by kidney disease every year. According to a survey conducted by Wijewickrama *et al*., CKD is a main contributor to the increased hospital mortality and morbidity in Sri Lanka [19].

One of the main risk factors for CKD is diabetes mellitus which causes diabetic nephropathy [3-6, 19]. Diabetic nephropathy will develop in about one third of people with Type 1 diabetes and 20 - 40% of people with Type 2 diabetes [7, 6, 23]. The main causes of CKD in Sri Lanka are diabetic nephropathy (88.44%) and hypertension (34.17%) [8,19,22]

Early diagnosis is important to prevent the progression to late stages of CKD. Since those with diabetes have an increased susceptibility to develop diabetic nephropathy, it is important to identify this early [9]. As recommended by NKF KDOQI guidelines, irrespective of the underlying disease, the stage of CKD is based on the combined indices of kidney function which is evaluated by measured or estimated GFR and kidney damage which is evaluated by albuminuria or proteinuria [10]. Although some patients with diabetic nephropathy have no albuminuria there is a high prevalence of microalbuminuria in type 1 (15-40%) and type 2 diabetes (20%) [24, 25]. In patients with diabetes eventual diabetic nephropathy and end-stage renal failure may be predicted by persistent albuminuria in amounts between 30 mg and 300 mg daily [24,25]. Therefore, detection of microalbuminuria is an important screening tool in early diabetes as treatment can be initiated or intensified to slow or stop the progression of kidney disease [11]. Hence micro-

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albuminuria test is a valuable diagnostic tool which is used to identify the first sign of deteriorating kidney function.

In the laboratory microalbuminuria is detected by immuno-turbidimetric methods, radioimmunoassay and high performance liquid chromatography methods (HPLC). All these methods require sophisticated instruments which need special maintenance. Therefore, microalbuminuria is an expensive laboratory test and it is not routinely done in most government hospitals in rural Sri Lanka. As this test is a special laboratory test all samples are analyzed at a particular time of the day or on a particular day of the week. Therefore, the results are delayed. Majority of the at risk population for CKD in Sri Lanka, are farmers in the dry zone. They cannot afford the cost of the test and the government cannot provide funds to screen all at risk persons for microalbuminuria. If total urine protein-to-creatinine ratio (TPCR) can be used rather than the microalbuminuria test to predict microalbuminuria, this can be used as a simple and inexpensive test which can be performed in routine laboratories to detect CKD. The objective of this study was to find whether the total urine protein-to-creatinine ratio can predict the presence of microalbuminuria in patients with diabetic nephropathy.

Methods

A cross sectional study was conducted among patients who were registered in the Diabetic Clinic, District General Hospital, Ampara, Sri Lanka. The hospital caters to low-middle income population in this region and over 70% of the study population are from the farming community.

Diagnosis was confirmed by the consultant at the Diabetic Clinic. Clinical history of the registered patients was evaluated and about 50% of registered patients (n=216) were selected for the study. Inclusion criteria were age 30-65 years, diagnosis of diabetes mellitus and duration of diabetes > 5 years. Patients already diagnosed with CKD, evidence of urinary tract infection, current gestational diabetes, fever or menstruation at the time of sample collection were excluded from the study.

Demographic information, medical history, most recent serum creatinine values (within one month) and other relevant information were collected. A structured instruction sheet was provided to each participant and they were clearly instructed to collect the first voided urine sample into a sterile, wide mouthed bottle on the day of the clinic. Urine 15ml was collected from each patient (during their visit for laboratory investigations) and supernatants were prepared and frozen at -20C°. The urine albumin (immunoturbidimetric method), urine creatinine (Jaffé method) and urine total protein (colorimetric) were analyzed at the laboratory of the Teaching Hospital, Peradeniya within one week by using Konelab prime 30-I biochemistry analyzer. Deposits of all samples were microscopically analyzed on the same day at the laboratory. Patients with evidence of urinary tract infection were excluded from further analysis.

Proteinuria was defined as urinary protein excretion > 50 mg per day [26]. Microalbuminuria was defined as an abnormal increase in albumin excretion rate within the specific range of 30-299 mg of albumin per g of creatinine [27]. Correlation with albumin to creatinine ratio (ACR) range between 30-300 mg/g was carried out. Demographic characteristics and laboratory investigations were summarized and mean, standard deviation, minimum and maximum values. Regression analysis and Spearman's rank correlation were used to study the relationship between microalbuminuria and proteinuria. Regression equations were derived for each relationship to predict the microalbuminuria in study population.

Estimated Glomerular Filtration Rates (eGFR) were calculated the using most recent serum creatinine values of each patient. The MDRD formula was applied and patients were categorized into six stages according to the Kidney Disease Improving Global Outcomes (KDIGO) Guidelines together with microalbuminuria [10].

All statistical tests were carried out using Statistical Package for the Social Sciences (SPSS) version 22. Ethical clearance for the study was obtained from the Ethical Review Committee, Faculty of Medicine, Kotelawala Defence University. Approval was also obtained from the Ministry of Health and the Director, DH Ampara. Written consent was obtained from all patients.

Results

Two hundred and sixteen patients with type 2 diabetes participated in the study. There were 52 (26.1%) males and 147 (73.9%) females. The mean age of the study population was 57.84 ± 10.3 years. The mean age of females was 55.88 ± 9.9 years while the mean age of males was 63.37 ± 9.415 years. Mean duration of diabetes mellitus was 7.6 ± 5.6 years. Description of the study population is shown in Table 1.

Patients were grouped as having normal albuminuria (n=153; 71%), microalbuminuria (n=56; 28%) and macroalbuminuria (n=3;1%) according to diabetic nephropathy diagnostic criteria, (KDIGO, 2016) (Table 2).

There was no significant difference in mean serum creatinine levels of patients or mean duration of diabetes between patients with normal albuminuria and microalbuminuria ($p>0.05$).

Correlation between urine total protein-to-creatinine ratio (TPCR) and urine albumin

There was significant correlation between TPCR and urine albumin ($r= 0.892$; $p<0.001$). The regression equation for the above relationship was, TPCR = 3.1983 Urine albumin -13.976 and R2= 0.7965 ($p<0.001$) (Figure 1).

Correlation between urine total protein to creatinine ratio (TPCR) and urine albumin to creatinine ratio (ACR)

There was significant correlation between urine total protein-to-creatinine ratio (TPCR) and urine albumin to creatinine ratio (ACR) (r= 0.824; p< 0.001). The regression equation of the above relationship was ACR = [TPCR + 18.421]/ 2.5026 and, R2= 0.6794 ($p < 0.001$) (Figure 2).

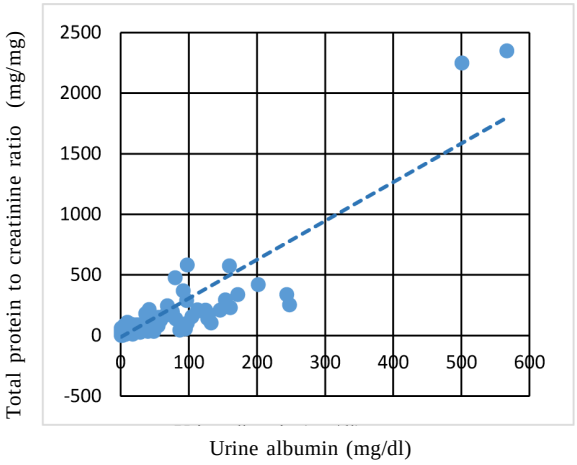

Figure 1. **The correlation between urine total protein to creatinine ratio and urine micro-albumin levels.**

Correlation between urine total protein to creatinine ratio (TPCR) and urine microalbumin to creatinine ratio (ACR) (range of microalbuminuria=30-300 mg/g creatinine)

There was significant correlation between urine total protein-to-creatinine ratio (TPCR) and urine microalbumin to creatinine ratio (ACR) (range of microalbuminuria =30 - 300 mg/g creatinine) (r= 0.798; p<0.001) (n=56). The regression equation of the above relationship was, ACR = [TPCR - 5.0491] / 1.2633and, R2 = 0.6361 ($p < 0.001$) (Figure 3).

Recent serum creatinine values (within one month period) were available only 156 patients and others (n=60) were excluded from the final part of data analysis. eGFR levels were calculated using MDRD formula.

According to the eGFR values and ACR levels, 156 patients were categorized according to the six stage classification criteria and risk of each category was analyzed using a "heat map" [18] (Figure 4).

Table 1. Characteristics of the study populations

	Mean	*SD±*	*Data range*
Age (years)	57.84	10.2	33 - 65
Duration after diagnosis of diabetes (years)	7.6	5.6	5 - 28
Serum creatinine (mg/dl)	0.82	0.25	0.0 - 1.8
Urine creatinine (mg/dl)	84.2	53.0	10.5 - 324.2
Urine total protein (mg/dl)	7.7	22.6	0.012 - 236.9
Urine albumin (mg/l)	32.3	64.6	1.0 - 567.0
Urine total protein-to-creatinine ratio (mg/g)	89.3	231.6	0.7 - 2348.7
Urine albumin to creatinine ratio (mg/g)	43.1	76.3	0.9 - 562.3

Table 2. Mean values of serum creatinine, albumin to creatinine ratio, total protein-to-creatinine ratio and duration of diabetes in the three albumin to creatinine ratio categories

ACR category	*Mean serum creatinine(mg/dl)*	*Mean albumin to creatinine ratio (mg/g)*	*Mean otal protein-to-creatinine ratio (mg/g)*	*Mean Duration of diabetes in years*
Normal (n=153)	0.98 ± 0.23	11.06 ± 7.74	36.08 ± 20.62	7.30 ±5.44
Microalbuminuria (n=56)	0.95 ± 0.38	103.82 ±73.828	136.30 ± 116.86	7.96 ±5.91
Macroalbuminuria (n=3)	1.67 ± 0.57	457.00 ± 145.43	1645.33 ± 1132.44	8.50 ±4.95

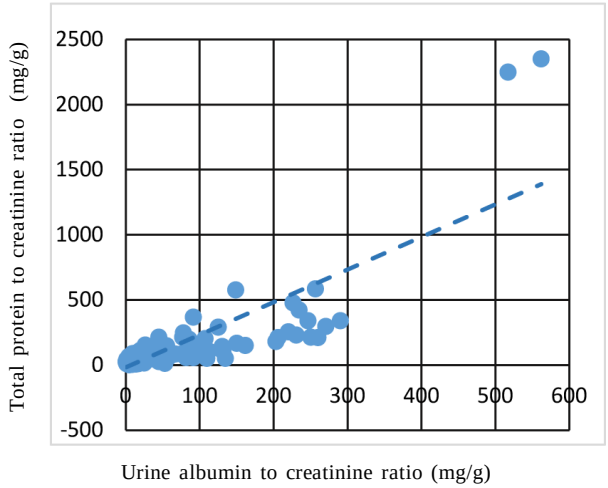

Figure 2. **The correlation between urine total protein to creatinine ratio (TPCR) and urine albumin to creatinine ratio (ACR).**

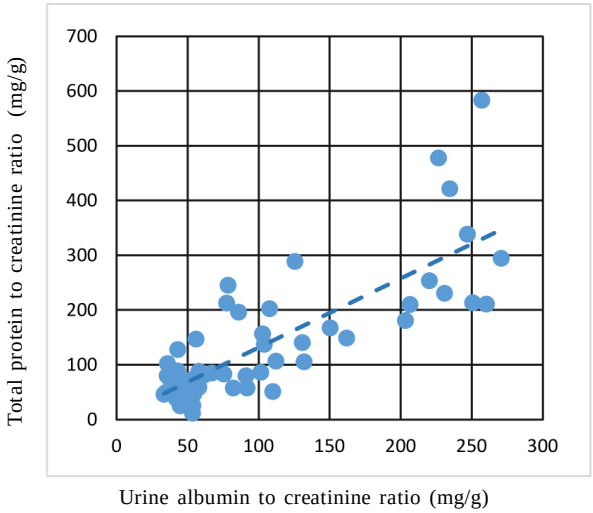

Figure 3. **The correlation between urine total protein to creatinine ratio (TPCR) and urine albumin to creatinine ratio (ACR) in micro-albuminuria range (30-300 mg/g creatinine).**

According to the KDIGO criteria, the green area represented 93 (60%) low risk patients, 44 (30%) had moderately increased risk, 7.5%, had high risk and 2.5 % had very high risk (n=15) for CKD (Figure 4).

The mean duration after diagnosing diabetes was 7.79 years in the low risk, 9.10 years in the moderate risk categories and 9.14 years in high to very high risk populations.

Prognosis of CKD by GFR and albuminuria category				Albuminuria categories Description and Range		
				A1	A2	A3
				Normal albuminuria	Microalbuminuria	Macroalbuminuria
				< 30 mg/g	30-300 mg/g	> 300 mg/g
GFR Categories (ml/min/1.73m^2) Description and Range	G1	Normal or high	90	21% n=33	11% n=17	3% n=5
	G2	Mildly decreased	60-90	39% n=60	13% n=21	0.5% n=1
	G3a	Mildly to moderately decreased	45-59	6% n=9	3% n=4	0.5% n=1
	G3b	Moderately to severely decreased	30-44	1% n=2	2% n=3	- -
	G4	Severely decreased	15-29	-	-	-
	G5	Kidney failure	15	-	-	-

Green areas of the map = low risk, Yellow = moderately increased risk, Orange = high risk, and red = very high risk

Figure 4. **Classification of CKD patients based on eGFR and albuminuria.**

Discussion

The present study included 216 patients with type 2 diabetic mellitus. The mean age of the female patients (55.88 ± 9.85 years) was relatively low compared with males (63.37± 9.41 years). Because we wanted to identify the correlation between ACR and TPCR in microalbuminuria range we included patients with a duration of diabetes > 5 years. Serum creatinine levels are routinely checked in the hospital laboratory and these values were recorded from clinic records. However, 60 (27%) did not have a serum creatinine test done within the month. Therefore, they were excluded from the creatinine based analysis.

We identified albuminuria in 59 (29%) patients who were previously not known to have albuminuria. Of these including 56 (28%) had microalbuminuria and 3 (1%) had macroalbuminuria.

Studies from different countries show wide variation in prevalence of microalbuminuria even within the same community. Three studies published in Singapore demonstrated that the prevalence among patients with diabetes varied from 14%-48% [12-14]. However, majority of these studies used semi-quantitative methods for detection of microabuminuria. In the current study we used immunoturbidimetric method for albumin quantification and patients with other causes such as urinary tract infection were excluded. The mean duration of diabetes increased in the ACR categories from normal albuminuria to microalbuminuria to macroalbuminuria. However, duration after onset of diabetes did not show any significant difference and patients who have had diabetes for 7 years or more should be investigated for TPCR.

A study was conducted by Yamamoto *et al* to predict microalbuminuria by using TPCR by obtaining 1,033 urine samples from patients who visited a cardiovascular clinic. They measured TPCR and ACR from random spot urine samples. The mean age of their study population was 69.61 years. In this study 32.6% of patients had microalbuminuria and 4.1% had macroalbuminuria which was similar to our study. Our sample was relatively younger than theirs.

The main objective of the current study was to identify the feasibility of using TPCR as an alternative method for predicting ACR. Therefore, we considered TPCR versus urine albumin, TPCR versus ACR for total range of albuminuria we detected (1-567 mg/l) as well as for microalbuminuria range (30-300 mg/l) because this range indicated early diabetic nephropathy [15].

The present study showed that there was a strong positive correlation (r=0.892; $p<0.001$) between urine TPCR with urine albumin. TPCR and urine albumin to creatinine ratio (ACR) also showed the significant correlation (r=0.824; $p<0.001$) for the range of 1 to 567 mg/l of albuminuria.

The correlations were significant in the clinically important range of 30-300 mg/l (r=0.798; $p< 0.001$) and regression expression was ACR= (TPCR -5.0491)/ 1.2633 with coefficient of determination $R^2= 0.6361$.

A study by Yamamoto *et al* in 2014 found a strong positive correlation between the ACR and TPCR ($p<0.001$). The regression expression was ACR=1.326ln × TPCR 2.64 [coefficient of determination $R^2= 0.861$]. The optimal cut-off value for the TPCR based on the ROC curve analysis for predicting microalbuminuria was 84 mg/g of creatinine (8.4 mg/mmol). In that study they had calculated the sensitivity (94.4%), specificity (86.1%), and area under the curve (0.903) for this cut-off value [16].

Another study showed a strong positive correlation between ACR and TPCR. In that study they analyzed the correlation coefficient for all cases as well as for a specified range of microalbuminuria (ACR 30- 300 mg/g creatinine) (N= 150). The correlation coefficient for the whole sample was 0.951 and for the specified range of microalbuminuria was 0.911 [17].

Methven *et al*. conducted a large scale observational study to investigate the optimal test to identify and quantify significant proteinuria [28]. They assessed the relationship between TPCR, ACR and 24 hour urine total protein in 6842 patients attending a renal clinic. They found that TPCR is more sensitive than ACR to predict clinically significant proteinuria, at 0.5g/day and 1.0g/day. A cut-off value of 110 mg/g of creatinine had a much lower sensitivity (46.03%) and higher specificity (98.03%). Twenty nine of patients were truly positive based on cut-off value of TPCR and the positive predictive value was 93.54% (29 of true positive cases and 2 of false positive cases).

A similar study found that at a cut-off value of 80 mg/g of creatinine the sensitivity was 95.5% and specificity was 83.7% while at a cut-off level of 84 mg/g creatinine the sensitivity was 94.4% and specificity was 86.1%. At a cut-off value of 90 mg/g creatinine sensitivity was 90.3% and specificity was 88.9%. At a cut-off value of 150 mg/g creatinine, the sensitivity was 58.7% and the specificity was 98.6%. Based on these values 84 mg/g creatinine for TPCR was considered as the optimal cut-off value to predict microalbuminuria [16]. The sensitivity specificity and cut points should be analyzed with reference to the 24hour urine protein/ albumin in future studies.

Conclusions

TPCR was useful in predicting ACR for albuminuria in the range of 1-567 mg/l in this study population. It was also effective in the microalbuminuria range. This is clinically important to identify early stage of diabetic nephropathy. Therefore, evidence from further studies could support the use of TPCR to predict microalbuminuria. This will be a simple, inexpensive measurement with broader applications, leading to earlier intervention and public benefit. Of the patients with duration of diabetes > 5 years we newly diagnosed 30% with microalbuminuria. Thus the determination of either ACR or TPCR is important for study population.

Recommendations

We recommend further studies with larger sample size in diverse populations as this was a single center, hospital-based study. Measures of accuracy as sensitivity, specificity and detection ranges should be calculated by using 24 hour urine sample collection together TPCR and ACR. The patients who are identified as being at high risk for CKD, should have ACR repeated and should be further investigated.

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Conflicts of Interests

Authors declare that there are no conflicts of interest.

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