URINE PROTEIN CREATININE RATIO AS A PREDICTOR OF DIABETIC RETINOPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

BACKGROUND

Evidence of early diabetic nephropathy can be quantified by either urinary Protein Creatinine Ratio (PCR) or 24-hour urinary protein estimation, the latter being the gold standard. Proteinuria is directly linked to diabetic retinopathy and the PCR threshold for onset of diabetic retinopathy needs to be determined.

MATERIALS AND METHODS

Correlation of spot urine protein creatinine ratio (PCR) with 24-hour urine protein estimation in a sample of patients with type 2 diabetes was first assessed. They were grouped by stage of diabetic retinopathy (DR). Receiver Operating Characteristic (ROC) analysis was used to compute the optimum cut-off values of urinary PCR and 24-hour urine protein to predict DR. Clinical and other biochemical parameters were also correlated with proteinuria.

RESULTS

Urinary PCR correlated well with 24-hour urine protein values and were excellent at higher ranges of eGFR. All clinical and biochemical parameters correlated well with urine PCR except for duration of diabetes and HbA1c. The cut off value of 24-hour urine protein and urine PCR for predicting the onset of diabetic retinopathy was 1178 mg/24 hrs and 1.67 respectively. Retinopathy worsens significantly with worsening proteinuria with urine PCR values nearly doubling with each stage of retinopathy.

CONCLUSION

In clinical practice, spot urine PCR needs to be closely monitored from the onset of T2DM for possible early diabetic nephropathy and any value below and near its threshold value necessitates prompt evaluation and intervention for retinopathy. Follow up retinal evaluation is mandated in the face of worsening Urine PCR.

KEYWORDS

Urine Protein Creatinine Ratio, Diabetic Retinopathy.

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BACKGROUND

Diabetic nephropathy accounts for about 20% of cases of chronic renal failure and is the single most common cause of End Stage Renal Disease in many countries.[1] Measurement of protein excretion in a 24-hour urine collection is the gold standard[2] for the quantitative evaluation of proteinuria which is the hallmark of renal damage. However, this method is inconvenient, since it is difficult to collect a complete 24 hr urine sample accurately especially in an outpatient setting. An alternative method for quantitative evaluation of proteinuria is measurement of protein to creatinine ratio in a spot urine sample.[2,3]

Diabetic retinopathy (DR) is a microvascular complication of diabetes, and a leading cause of visual impairment and blindness. DR progresses from mild non-proliferative to moderate and severe non-proliferative DR(NPDR), and to proliferative DR (PDR), which is characterized by increased vascular permeability, retinal nonperfusion, and pathological intraocular proliferation of retinal vessels.[4] Macular oedema, characterized by retinal thickening can develop at any stage of retinopathy[4,5] This complication places a significant burden on society if left untreated; therefore, early detection and identification of the risks for DR are critical.[5] It is well known that glomerular hyperfiltration and proteinuria precedes overt renal damage or early clinically evident diabetic retinopathy.[1] But the exact threshold of proteinuria at which early diabetic retinopathy begins to manifest has not yet been determined. In situations where proper glycaemic control is impossible or renal damage progresses relentlessly despite reasonable glycaemic control and ACE (angiotensin converting enzyme) inhibitor therapy, screening for possible retinopathy is

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critical. This study specifically examines, first the reliability of Urine PCR as a quantitative surrogate to 24-hour urine protein estimation in diabetic nephropathy. Furthermore, we proposed to estimate the threshold values of Urine PCR as well as 24 Hour Urine protein that could predict early DR.

MATERIALS AND METHODS

A random sample of male and female patients with type -2 diabetes mellitus were included in the study. After informed consent, participants were interviewed for the demographic and diabetic history and were requested to provide urine samples for both the tests. Patients with acute febrile illness, Urinary tract infection, glomerulonephritis due to systemic conditions, malignancies, collagen vascular disorders or any other systemic condition causing proteinuria and pregnant women were excluded from the study.

On the test day, in the morning, at the start of the collection period (6.00 a.m.) patients were asked to void urine and discard this sample (as it contains the overnight urine present in the bladder). Subsequently urine was collected for next 24 hours in the bottle provided by the laboratory. The last sample was to be collected on next day at 6.00 a.m. A random sample was also collected on the day of deposition of 24-hour sample (the test day). Urine protein was estimated using Pyrogallol red molybdate method and urine creatinine by modified Jaffe's method. Urine PCR was calculated by dividing the value of spot urine protein (mg/dl) by the spot urine creatinine(mg/dl) and expressed as a ratio. Creatinine clearance was calculated using the MDRD (Modified Diet in Renal Disease) equation: GFR (mL/min/1.73 m^2) = 175 × $(S_{cr})^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American).

Participants underwent ocular examinations, including fundus photographs for reference in doubtful cases. DR was identified by the presence of any characteristic lesion determined by the severity scale: microaneurysm, dot and blot haemorrhages, hard exudates, cotton wool spots, venous beading, intra-retinal microvascular abnormalities, retinal new vessels, vitreous haemorrhage, fibrous proliferation, tractional retinal detachments or previous laser therapy.[4,5] It was classified per the Diabetic Retinopathy Disease Severity Scale into five categories; no DR, mild NPDR, moderate NPDR, severe NPDR, or PDR(4). Data were collected with the aid of a proforma, which included patient history, clinical parameters like duration of diabetes and treatment, prevalence and degree of diabetic retinopathy, biochemical parameters like serum creatinine, eGFR, HbA1c, urine PCR and 24-hour urine protein.

Statistical Analysis

Data were analysed using statistical package-SPSS version 21 for Windows (SPSS Inc, Chicago, USA). Spearman's correlation coefficient was used to find the correlation of numerical variables and Kruskal Wallis test was used to compute the association between retinopathy and urine PCR. ROC curves were used to determine sensitivity, specificity and cut off values of PCR and 24-hour urine protein at different stages of DR.

RESULTS

Gender and Age

There were 49 (67.1%) male and 24 (32.9%) female participants. The mean (SD) age of the patients was 52 ± 10.4 years; range (30-80 years). Mean age of male and female patients were and 52.37 ± 10.5 and 51.2 ± 10.4 respectively.

Clinical and Biochemical Characteristics

Duration of T2 DM ranged from newly detected to 30 years. Median duration was 8 years (IQR 6-12). Majority of the patients, 38 (52.05%) had a duration between 5 and 10 years. 25 (34.25%) patients had nephrotic range proteinuria ≥ 3500 mg/24 hours. 25(34.24%) of the patients had eGFR (calculated by MDRD equation) between 30-60 ml/min/1.73 m^2. 34 (46.6%) was on treatment with OHA's only whereas 39(53.4%) was on treatment with insulin with or without OHA's. Median duration of diabetes was around 6 years and HbA1c was 7.2 reflecting mild uncontrolled DM. Most patients were in diabetic nephropathy given their high S. Creatinine levels and proteinuria. All but 10(13.7%) were in various stages of diabetic retinopathy.

Males Females		n (%) n (%)	49 (67.1%) 24 (32.9%)
Age (Years)		Mean (SD)	52.0(10.4)
Duration (Years) of DM		Median (IQR)	8(6-12)
Duration (Years) Frequency Distribution of Participants	≤ 5	n (%)	14(19.2)
	5-10		38(52.1)
	10-15		13(17.8)
	≥ 15		8(11.0)
Treatment: OHA Only		n (%)	34 (46.6%)
Insulin with/without OHA		n (%)	39(53.4%)
HbA1c (%)		Median (IQR)	7.2(6.8-8.3)
Creatinine (mg/dl)		Median (IQR)	2.4(1.7-3.8)
eGFR (ml/min/1.73 cm^2)		Median (IQR)	27.9(15-42.2)

Frequency within Ranges of eGFR (ml/min/1.73 cm^2)	0-15 15-30 30-60 >60	n (%)	19(26%) 19 (26%) 25 (34.2%) 10(13.7%)
PCR Urine		Median (IQR)	2.9(1.4-6.7)
24-Hour U Protein (mg/24-hrs)		Median (IQR)	2049(1060-4146)
(Any) Retinopathy No Retinopathy Mild NPDR Moderate NPDR Severe NPDR PDR		n (%)	63(86.3%) 10(13.7%) 27(37%) 14(19.2%) 13(17.8%) 9(12.3%)

Table 1

Correlation Between Urine PCR and 24-Hour Urine Protein

Urine PCR correlated well with 24-hour urinary protein excretion (p<0.001: r = 0.870). Correlation was better at higher ranges of eGFR. At eGFR more than 60 ml/min/1.73 m^2 the correlation was excellent (p <0.001; r =0.99). Renal function parameters like S. Creatinine showed a significant positive correlation (p < 0.001; r=0.774) whereas eGFR as expected showed a significant negative correlation (p < 0.001; r= -0.762) with Urine PCR. However, duration of diabetes and glycosylated Hb (HbA1c) showed no significant correlation.

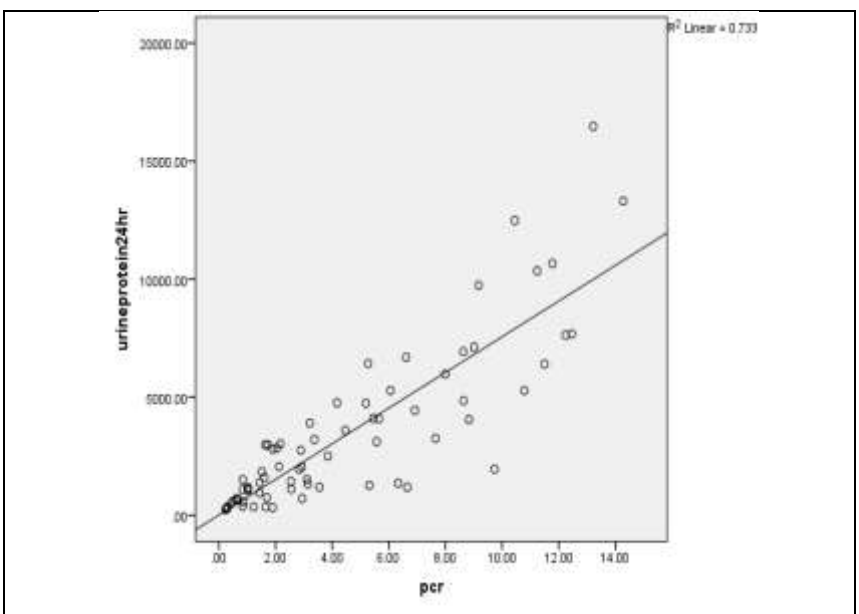

Figure 1. Correlation Between 24-Hour Urine Protein with Urine PCR

PCR & 24-hr urine protein	Spearman rho Corr. Coeff. 0.870	p <0.001

eGFR (ml/min^1/1.73$m^{-2)}$)	Frequency	Corr. Coeff. (Spearman rho)	P value
0 – 15	19	0.800	< 0.001
15 – 30	19	0.817	
30 – 60	25	0.766	
> 60	10	0.999	

Table 2. Correlation Between Urine PCR and 24-Hour Urine Protein at Different Ranges of eGFR

Correlation between Urine PCR and Duration of Diabetes, HbA1c

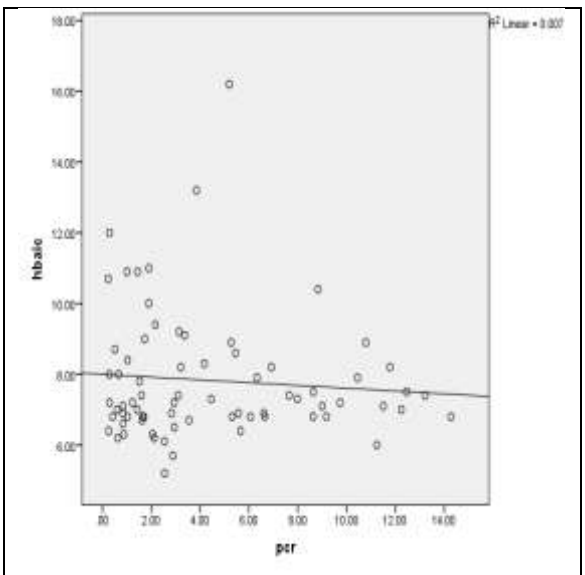

Figure 2 (HbA1c and PCR)

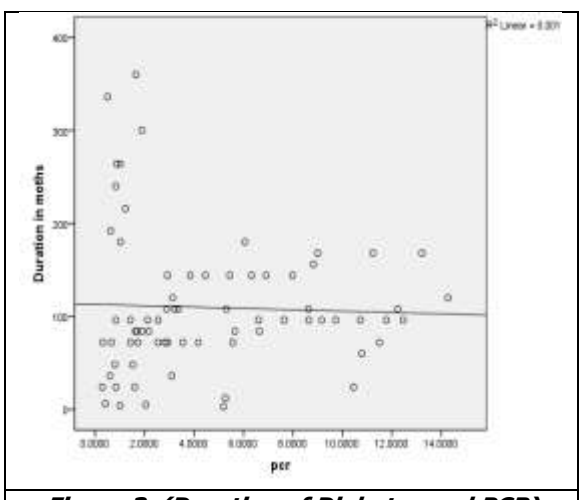

Figure 3. (Duration of Diabetes and PCR)

Urine PCR and HbA1c	(Spearman rho) Corr. Co-eff. 0.007	p=0.956
PCR and Duration of DM	Corr. Co-eff. :0.154	p=0.196

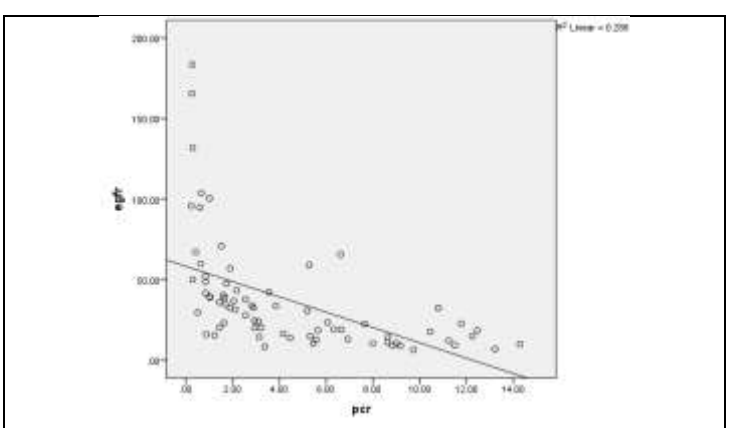

Figure 4. Correlations (eGFR and Urine PCR)

Urine PCR and eGFR	Corr. Coeff. -0.762 (spearman rho)	p <0.001

Association Between Urine PCR, 24-Hour Urinary Protein Excretion and DR

We found that PCR and 24-hour urine estimations significantly correlated with DR and its severity in patients with Type 2 diabetes. PCR levels increased with severity of diabetic retinopathy (DR). ROC curve analysis showed that the optimal PCR cut-of value for predicting the risk of onset DR was 1.67. Since urinary PCR increase was significantly associated with stages of retinopathy, this PCR threshold may predict the risk for no only DR development but also progression. It is well known that DR and Diabetic Kidney Disease are both microvascular complications of diabetes and are characterized by similar pathophysiological mechanisms. The microvascular changes in both the retina and glomerulus are thought to be initiated by chronic hyperglycaemia, followed by the progressive narrowing and eventual occlusion of the vascular lumina.[5] In this study, both PCR levels and 24-hour Urine protein levels were significantly higher at more advanced stages of DR.

	Category	Frequency	Median	Quartiles		p-Value
				1st	3rd	
Retinopathy	No DR	10	0.94	0.47	1.18	<0.001*
	Mild NPDR	27	1.6	0.84	2.11	
	Mod NPDR	14	4.01	3.25	5.28	
	Severe NPDR	13	7.99	6.65	8.83	
	PDR	9	11.78	11.24	12.46	

Table 3. Frequency Distribution of Diabetic Retinopathy

* Kruskal Wallis test.

NO DR	NPDR (p-Values)			PDR (p-Values)
	Mild (p-Value)	Moderate	Severe	
	0.961	0.014	< 0.001	< 0.001
Mild NPDR		0.012	< 0.001	< 0.001
Moderate NPDR			0.368	0.036

Table 4. Urine PCR vs. Stages of Retinopathy

*post hoc Kruskal Wallis test

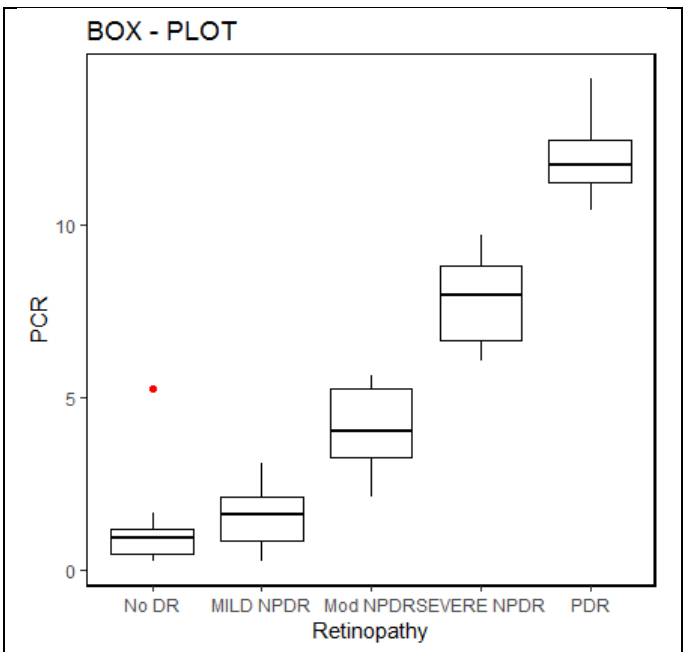

Figure 5. Correlation of Urine PCR with Retinopathy

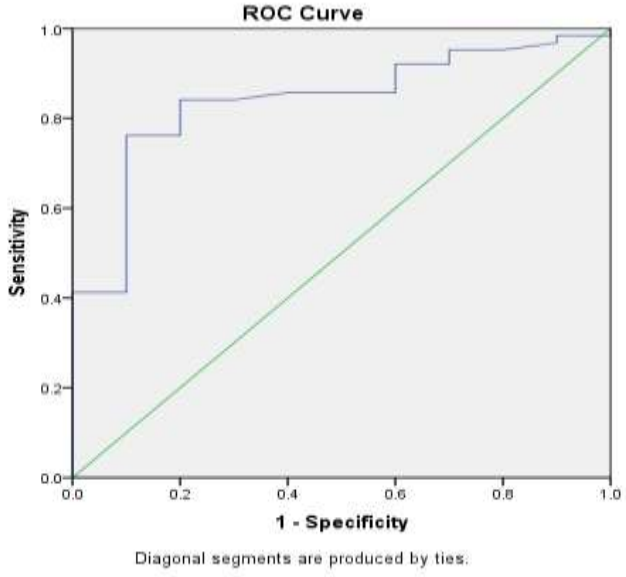

Figure 6. ROC Curve Showing Urine PCR Cut Off for Diabetic Retinopathy

Urine PCR: Area Under Curve			
Area	Std. Error	Asymptotic 95% Confidence Interval	
		Lower Bound	Upper Bound
0.840	0.059	0.724	0.956
Urine PCR Coordinates of the Curve			
Positive if Greater Than or Equal To[a]	Sensitivity	Specificity	
1.670000	0.762	0.900	

Table 5

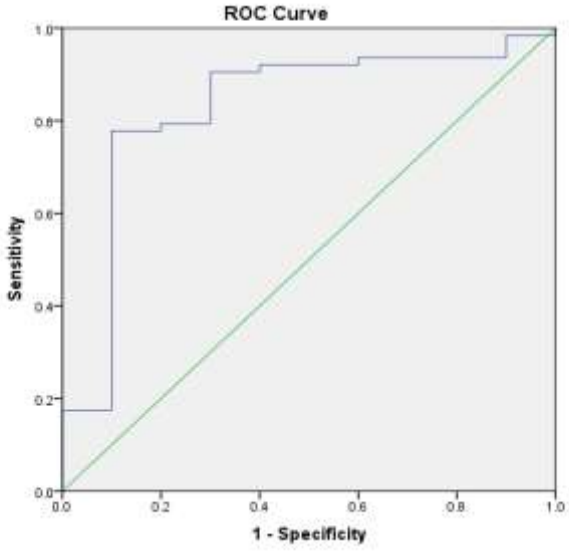

Figure 7. ROC Curve Showing 24-Hour Urine Protein Cut Off for Diabetic Retinopathy

Urine Protein 24 Hour Area Under Curve				
		Asymptotic 95% Confidence Interval		
Area	Std. Error	Lower Bound	Upper Bound	
0829	0.077	0.677	0.980	
Urine Protein in 24 Hour. Coordinates of the Curve				
Positive if Greater Than or Equal To[a]	Sensitivity	Specificity		
1178.6000	0.778	0.900		

Table 6

DISCUSSION

Diabetic nephropathy is associated with structural abnormalities include hypertrophy of the kidney, glomerular basement membrane thickening, nodular and diffuse glomerulosclerosis, tubular atrophy, and interstitial fibrosis. The functional alterations include an early increase in glomerular filtration rate with intraglomerular hypertension, subsequent proteinuria, and eventual loss of renal function.[1,6] The earliest clinical evidence of DN is microalbuminuria defined as urinary albumin excretion of 30-299 mg/24 hours in a 24-hour urinary collection, 20-199ug/min in a timed urine collection, or 30-299 ug/mg creatinine in a spot urine collection on at least two occasions within a three to six-month period.[6] A large number of patients with type-2 diabetes have microalbuminuria and overt nephropathy at or shortly after diagnosis of diabetes. This is because the disease may have been present for several years before the diagnosis is made[1,6] This could be the reason for the absence of association of Urinary 24-hour protein or PCR to the duration of diabetes in our study. We found no association between HbA1c and diabetic nephropathy or retinopathy. Since the average life span of RBC is around 120 days, this value reflects the mean blood glucose concentration over the past three months.[6]

However pathologic mechanisms leading to clinical nephropathy progresses insidiously over a period of 10-20 years after the onset of impaired glucose tolerance and about 5-10 years after onset of microalbuminuria.[1] Hence, in relative terms, HbA1c reflects a relatively short-term diabetic control rather than the years of prolonged hyperglycaemia needed to cause diabetic microvascular disease. In the retina, diabetes induces programmed cell death of Müller and ganglion cells, as well as the loss of endothelial cells in capillaries and the loss of pericytes which leads to progression of DR whereas, in the glomerulus widespread capillary occlusion and podocyte loss triggers urinary protein loss and a decline in renal function.[5] Therefore, early detection of proteinuria and its clinical surrogate, DR is crucial. Improved DR screening rates and appropriate intervention are associated with less frequent visual impairment among patients with diabetes.

CONCLUSION

Urine PCR is simple to perform, and all biochemical and clinical parameters correlate well with spot urine PCR as with 24-hour urine protein estimations. In clinical practice, urine PCR need to be closely monitored from the onset of T2DM for possible early diabetic nephropathy and any value below and near the threshold necessitates prompt evaluation and intervention for retinopathy. Follow up retinal evaluation is mandated in the face of worsening proteinuria.

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