

Comparison of Spot Urine vs 24-Hours Urine in Assessment of Proteinuria in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To assess the correlation between spot urine test (Spot Protein/Creatinine Ratio) and 24-hours urine protein analysis in assessing proteinuria among patients with Type 2 diabetes.

Study Design: Cross-Sectional Study.

Place and Duration of Study: Department of Medicine, Combined Military Hospital, Abbottabad Pakistan, from Oct 2022 to Mar 2023.

Methodology: Eighty patients of either gender with diagnosed Type 2 Diabetes Mellitus presenting to CMH Abbottabad during study period with evidence of proteinuria were included in the study. 24-hours urine sample was obtained for analysis of 24 hours protein excretion. Urine was also tested for spot protein creatinine ratio in all patients. Association of 24-hour urine and spot urine protein levels was observed and represented by ROC curve and Pearson's correlation.

Results: Total 80 diabetic patients with proteinuria analyzed with mean age of 53.51 ± 6.34 years including 45 (56.3%) males and 35 (43.8%) females. The mean duration of diabetes was 7.75 ± 1.95 years with mean fasting sugar 183.88 ± 36.33 mg/dL and HbA1c $9.46 \pm 1.30\%$. The observed mean 24-hour urinary protein was 1194.60 ± 398.39 mg/day and mean spot uPCR was 1005.49 ± 446.84 mg/mmol. An affirmative correlation was noted in 24-hours urine protein and uPCR (r value = 0.604; $p < 0.001$). The ROC curve with respect to age groups showed $p = 0.143$ and $= 0.851$ for 24-hours urine protein and spot uPCR respectively.

Conclusion: 24-hour urinary protein and urine protein/ creatinine ratio (uPCR) were equally reliable for proteinuria detection in patients with Type 2 diabetes mellitus. However, spot uPCR is fast, convenient and easily available substitute for 24-hour urine protein in healthcare facilities.

Keywords: Albuminuria, Diabetes Mellitus, Nephropathy, Proteinuria, Spot uPCR, 24 Hour Urine Protein.

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INTRODUCTION

Diabetes mellitus (DM), silent killer and global health concern, is a chronic metabolic illness affecting rural as well as urban population.¹ As per International Federation of Diabetes (IDF) data, the prevalence of DM is rising in Pakistan with current 28.5% prevalence higher as compared to previous years 11.77% (2016), 16.98% (2018), 17.1% (2019) and 26.7% (2022).² Proteinuria, indicator of diabetic nephropathy, is defined as appearance of excessive amount of protein in urine i.e. 24-hours protein >0.5 g/day, urine protein-creatinine ratio (uPCR) of >50 mg/mmol (>300 mg/mmol Nephrotic range), or urine albumin creatinine ratio (uACR) >30 mg/mmol.³ Nearly half of the protein in urine is Tamm-Horsfall glycoprotein (THG), which is formed in the distal tubule. Other proteins could also be present in urine including: albumin, globulin,

mucoprotein, and Bence-Jones protein.⁴ Consistent proteinuria is an indicator of glomerular damage and kidney injury. Hence, estimation of urinary protein has pivotal role in diagnostic workup for renal disease.⁵ The incidence of diabetic nephropathy is greater in T1DM (15-40%) as compared to T2DM (5-20%) with overall 10-year incidence of 33% in both types.⁶ Proteinuria in the background of type 2 diabetes mellitus (T2DM) is linked with adverse and serious health problems like chronic kidney disease (CKD) leading to cardiovascular, cerebrovascular, hematological and metabolic complications.⁷

In order to minimize the risk of long term complications of proteinuria, early and comprehensive evaluation and treatment plan is necessary for all patients.⁸ Protein electrophoresis and immunoassays can identify the specific protein type responsible for the proteinuria and underlying cause as well as disease.⁹

The conventional and most reliable way to quantify proteinuria is to monitor 24-hours urinary

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protein excretion which is a time-consuming method and inaccurate, wasting about one-third of the urine protein in the sample.¹⁰ Although it is the gold standard method, but it is inconvenient for patients and there are issues of incorrect sample collection and the possibility of infection spreading while determining proteinuria from 24-hour urine samples. Consequently, the protein/creatinine ratio (uPCR) assay is now considered as substitute gold standard for urine testing and PCR of >50mg/mmol is labeled as proteinuria.¹⁰

There are limited studies conducted in local type-2 diabetic population for proteinuria assessment. Hence, the rationale for conducting this study was to assess proteinuria by 24-hours urine protein measurement and spot uPCR method in the background of T2DM in local population.

METHODOLOGY

This was a cross-sectional study, conducted at Combined Military Hospital (CMH) Abbottabad, over a period of 6 months from October 2022 till March 2023 following approval from institutional ethical committee (Ref:CMHAtd-ETH-90-MED-23). Convenience sampling technique was used and sample size of 73 was calculated using WHO online sample size calculator by keeping in view the prevalence of proteinuria in T2DM to be 5%⁶ with a confidence level of 95%, margin of error 5% and 24-hour and spot urine protein agreement proportion of 82.1%.⁶ All T2DM patients presenting to OPD during study periods, were advised urinary test for proteinuria and any patient with evidence of proteinuria (>50mg/mmol) was enrolled in the study after fulfilling inclusion criteria and informed consent was taken from all patients.

Inclusion Criteria: Adults of either gender with age 40-65 years, diagnosed with Type 2 Diabetes Mellitus (minimum 3 years) with evidence of proteinuria visiting the Medical Outpatient department of the hospital were included.

Exclusion Criteria: Patients with UTIs, chronic renal failure, genetic renal disease, Type 1 diabetes mellitus and glomerulonephritis secondary to systemic conditions, hypertension and pregnant women were excluded.

Patients fulfilling inclusion criteria were advised to collect their urine for the following 24 hours in the same container with 5mL of 10% thymol in Iso-propanol as a preservative, starting after their first

bowel movement in the morning. They had their final defecation at the 24-hour mark. Total urine volume of 24-hours was estimated, and 10ml of the mixture were extracted after rigorous mixing and urinary protein concentration was measured. To be classified as substantial proteinuria, the 24-hour urine protein level must be 500mg/day or greater. Another sample of urine, early morning mid-stream urine was collected for estimation of spot urinary PCR in all patients. The value of spot uPCR >50 mg/mmol (>150mg/g) was considered to be significant for proteinuria. The results of both methods of urinary protein assessment were noted and compared for urinary protein quantification. Semiautomatic analyzer (Statfax 3300) and a kit approach was also used to assess glycated hemoglobin (HbA1c) and fasting blood glucose.

The means and standard deviations were computed for variables like age, gender, diabetes duration, HbA1c, 24-hours protein, and spot uPCR. Qualitative factors, such as gender and age groupings, were quantified using percentages and frequencies.

Data were entered and analyzed using Statistical Package for Social Sciences version 25 (SPSS v25). The correlation of the spot urine and 24-hour urine protein was analyzed using Pearson's correlation coefficient and receiver operating characteristic (ROC) curve analysis, with a *p*-value <0.05 being considered statistically significant.

RESULTS

Total 80 diabetic patients with proteinuria were included and analyzed with mean age of 53.51±6.34 years including 45(56.3%) males and 35(43.8%) females. The patients were segregated according to age for analysis purpose in age <55 years (52.5%) and >55 years (47.5%). The mean duration of T2DM in studied patients was noted to be 7.75±1.95 years with mean fasting blood sugar of 183.88±36.33 mg/dL and mean HbA1c of 9.46±1.30%. All patients had urinary protein estimation by 24-hours urinary protein as well as spot urinary PCR. The observed mean 24-hour urinary protein was 1194.60±398.39 mg/day whereas mean spot uPCR was 1005.49±446.84 mg/mmol. (Table-I).

A statistically significant positive correlation of 24-hours urinary protein and spot uPCR was noted with Pearson coefficient of 0.604; *p* <0.001. ROC curve for both methods was also drawn with respect to age groups and area under curve was noted. The area under curve for 24-hours protein which was observed

as 0.405 for age <55 years (Figure-1) and 0.595 for age >55 year ($p=0.143$). (Figure-2).

Table: Baseline Clinical Parameters of Patients. (n=80)

Variables	Results
Age (mean years $\pm$ SD)	53.51 $\pm$ 6.34
Age Groups	≤ 55 Years 42(52.5%)
	>55 Years 38(47.5%)
Gender, n(%)	Male 45(56.3%)
	Female 35(43.8%)
Duration of Diabetes (mean years $\pm$ SD)	7.75 $\pm$ 1.95
Fasting Blood Sugar (mean mg/dl $\pm$ SD)	183.88 $\pm$ 36.33
Glycated Hb (mean% $\pm$ SD)	9.46 $\pm$ 1.30
24-Hour Urinary Protein, (mean mg/day $\pm$ SD)	1194.60 $\pm$ 398.39
Spot Urinary Protein (mean mg/mmol $\pm$ SD)	1005.49 $\pm$ 446.84

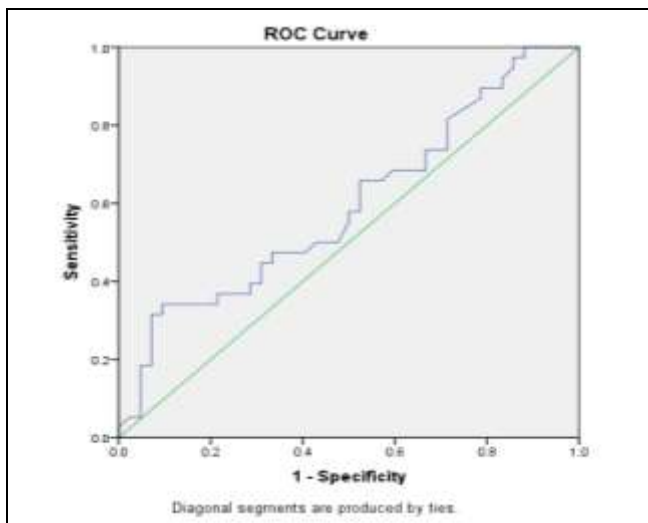
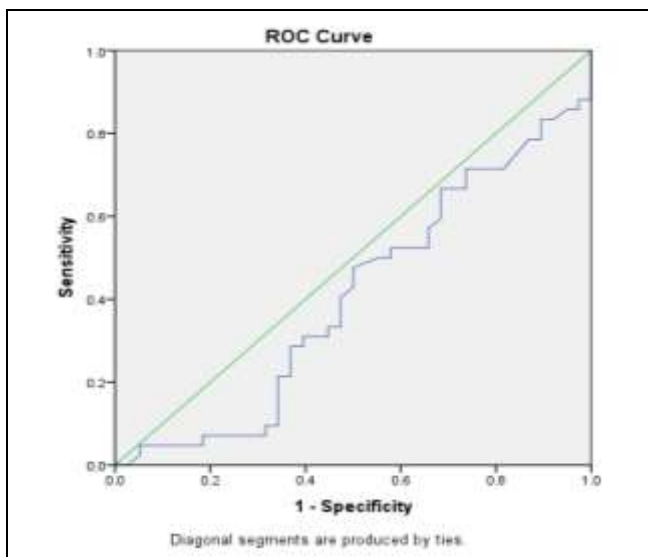


Figure-1 & Figure-2: ROC Curve of 24-hours urinary protein with respect to age groups <55 years and age groups >55 years respectively

Similarly, the area under curve was calculated for spot urinary protein which was 0.488 for age <55 years (Figure-3) and 0.512 for age >55 year ($p=0.851$). (Figure-4).

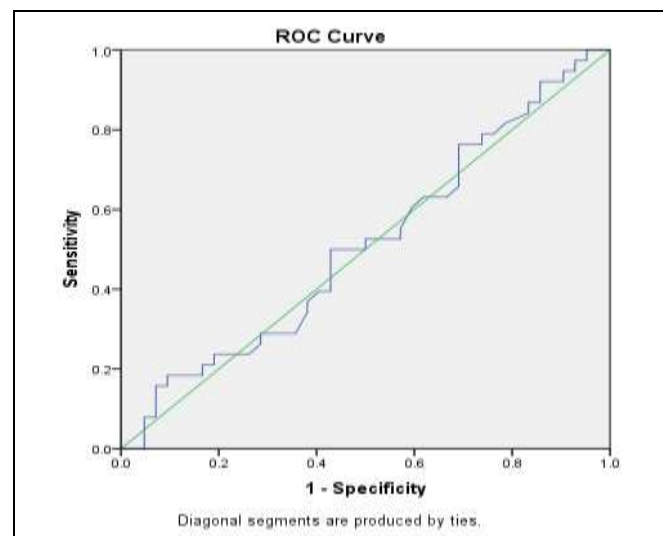
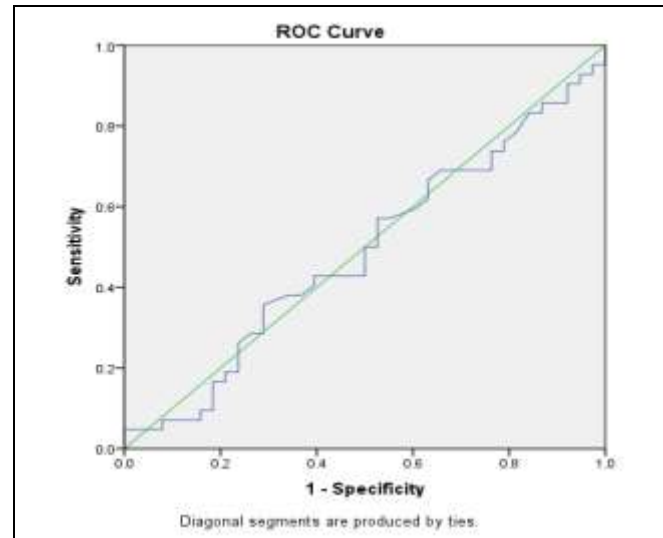


Figure-2 & Figure-4: ROC Curve of spot urinary protein with respect to age groups <55 years and respect to age groups >55 years respectively

DISCUSSION

In our study it was found that 24-hour urinary protein and uPCR were equally reliable for proteinuria detection in patients with Type 2 diabetes mellitus. The current data and past studies confirms that even small amount of proteinuria/albuminuria in the background of diabetes mellitus is concerning and early indicator of kidney disease as explained by Abubacker *et al.*¹¹ It has been documented in literature

that after 5-10 years of T2DM, 18-35% patients have 10-12% loss of renal function and develop micro-albuminuria and proteinuria with prevalence of 15.3 per 1000 person-years.¹² As 24-hours urine protein is "gold standard" for proteinuria detection, it is inconvenient and has sample variability. Therefore, spot uPCR is better alternative option for proteinuria estimation quickly and reliably.¹³ It has been observed in our study that with increasing age there was higher risk of developing proteinuria specifically in the background of underlying diabetes mellitus affecting kidneys as 55% patients with age >55 years developed proteinuria as also explained by Leung *et al.*, that the incidence of proteinuria increases with age.¹⁴

Also it has been observed that, higher levels of HbA1c indicating poorly controlled DM was associated with higher incidence of proteinuria linked to greater risk of chronic kidney disease and renal failure which was in accordance with other studies.¹⁵ This study discovered a favorable correlation of 24-hour urine protein and spot uPCR based on evaluation of proteinuria ($p=0.001$). Yang *et al.*, concluded a strong correlation of uPCR and 24-hours urinary protein with Pearson correlation coefficient =0.85.¹⁶ Ying *et al.*, also demonstrated that 24-hours protein showed strongest associated with >30% reduction in eGFR per log-increment HR=1.54 (1.13-2.09), $p=0.005$.¹⁷

It was observed in this study that observed mean 24-hour urinary protein was 1194.60±398.39 mg/day whereas mean spot uPCR was 1005.49±446.84 mg/mmol in studied diabetic patients. It is noteworthy that, at lower concentrations, the spot uPCR and 24-hour urine protein tend to correspond more closely; however, this relationship begins to decline as the 24-hour urine protein excretion exceeds 2.0 gm. Nazia *et al.*, explained an agreement between spot uPCR and 24-hours urinary protein in 82.1% cases using Kappa statistics as average 24-hours protein in studied patients noted to be 1216.99±949.51 mg/day and spot uPCR was 1919.12±2129.25 mg/mmol.¹⁸

Savith *et al.*, described in a study that there was positive correlation seen between spot uPCR and 24-hours urinary protein ($r=0.805$) with sensitivity and specificity of spot uPCR for detection of proteinuria at cut off value of 0.2, 98.25% and 94.44% respectively and area under curve was 0.996 (95% CI, 0.989-1.0; $p<0.001$).¹⁹ Similarly, study by Vadher *et al.*, showed that the mean 24-hours urinary proteins was 2.972±0.832 g/day with $r=0.851$ and $p=0.03$ and

correlation coefficient decreases with the increase in proteinuria once estimated by spot urinary PCR.²⁰

In an observational study, Raza *et al.*, concluded that a weak but statistically significant correlation was noted between spot uPCR and 24-hours urinary protein ($r=0.342$, $p=0.01$) whereas on age analysis, slight better correlation was noted in age >47 year ($r=0.78$) and in BMI >25 kg/m² ($r=0.45$).²¹

Sahu *et al.*, also explained that spot uPCR is accurate and reliable parameter for proteinuria and to be used as substitute of 24-hours urinary protein estimation but for follow-ups and proteinuria of >0.5 g/day, 24-hours urinary total protein should be considered as accurate measuring tool and gold-standard.²²

LIMITATION OF STUDY

Authors are well aware of limitations of the study most important being the single center study with limited sample size. Only single sample of 24-hours urine and single reading of spot urine PCR was analyzed in this study. The effects of BMI, diet and daily physical routine were not observed which could influence the results. Hence, further multi-center studies are required, preferably with a large sample size before implementing results on general population.

CONCLUSION

This study demonstrated that both, spot uPCR and 24-hour urine protein are reliable methods of detection and estimation of proteinuria in patients with type 2 diabetes mellitus. However, the spot urine analysis is quick and convenient, and therefore, can be used as a substitute to 24-hour urine protein for quantification of proteinuria.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

ZA & RY: Data acquisition, data analysis, critical review, approval of the final version to be published.

ZB & MAK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

NU & MN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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