

SPOT URINE PROTEIN CREATININE RATIO IN COMPARISON TO 24-HOUR URINARY PROTEIN FOR DETECTION OF PROTEINURIA IN TYPE 2 DIABETIC PATIENTS

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Citation:

Paudel GA, Kunwar S, Shakya S, Shakya R, Baral A, Shrestha K. Spot Urine Protein Creatinine Ratio in Comparison to 24-Hour Urinary Protein for Detection of Proteinuria in Type 2 Diabetic Patients. Medphoenix. 2024;9(1):24-28.

DOI: <https://doi.org/10.3126/medphoenix.v9i1.67181>

Conflict of interest: None, **Funding:** None

Publisher: National Medical College Pvt. Ltd. **MedPhoenix - Journal of National Medical College (JNMC); 2024,9(1), available at www.jnmc.com.np**

ISSN:2631-1992 (Online); ISSN:2392-425X (Print)



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**ABSTRACT**

Introduction: Diabetic nephropathy is a disease in which both glucose and protein appears in urine and is characterized by progressive renal damage due to the long-term hyperglycemia affecting the glomerulus and ultimately degrading the filtrating ability of the kidney. Determination of 24-hour urine protein excretion is simple and inoffensive method and is considered to be a gold-standard one. The method is time consuming, tiresome, and imprecise due to collection error. Random urine sample for protein creatinine ratio (PCR) would be more acceptable and less time consuming. Therefore, the present study was carried to compare the efficacy of spot urinary PCR with the 24-hour urinary protein in type 2 diabetic patients.

Materials and Methods: A hospital based cross-sectional study was conducted and total of 66 participants were recruited from department of internal medicine, Star Hospital, Lalitpur, Nepal. Urine samples were analyzed for protein and creatinine by pyrogallol red method and modified Jaffe's reaction. 24-hour urinary protein (24 HUP) was used as a gold-standard method and was compared with spot PCR.

Results: The finding from study depicts strong correlation between spot protein creatinine ratio and 24-hour urinary protein ($r=0.63$, $p=0.001$). A PCR <0.37 ruled out significant proteinuria ($>300\text{mg/day}$) with sensitivity and specificity of 84.4% and 100% respectively. Similarly, PCR value at cut off of 0.9 had sensitivity (86%) and specificity (71%) for detection of severe proteinuria ($>5000\text{mg/day}$).

Conclusion: A strong correlation between spot PCR and 24-hour urine protein was obtained. The present study suggests that random urine PCR can be reasonable alternative to predict the amount of 24-hour urine protein excretion with high accuracy.

Keywords: Diabetic Nephropathy, Proteinuria, Protein Creatinine ratio, Sensitivity, Specificity

INTRODUCTION

Diabetes mellitus (DM) refers to a group of complex metabolic disorders that express the phenotype of hyperglycemia associated with dreadful complications.¹ Diabetic nephropathy is a disease in which both glucose and protein appears in urine and is characterized by progressive renal damage due to the long-term hyperglycemia affecting the glomerulus and ultimately degrading the filtrating ability of the kidney.² Hence, proteinuria is one of the important independent risk factors for the determination of the end stage renal disease.

Normally protein excretion in the urine is critically low, which remains undetectable by the general laboratory technique. Total protein excretion in urine is less than 150 mg/day and the level higher than this is called as proteinuria. Increased urine protein excretion higher than the cut-off value persistently is considered to be a marker for the kidney disease and its progression into

severe form.³ The most widely used screening test is urine dipstick test. Urine dipstick is highly specific method for detecting proteinuria. But, the sensitivity of the test remains questionable compared to the quantitative techniques. Dipstick methods are usually delimited by false positive and false negative results.^{4,5,6}

Determination of 24-hour urine protein excretion is simple and inoffensive method and is considered to be a gold-standard one. Although, no any alternative test has been employed in the place of 24-hour urine protein determination, this method has number of limitations. The method is time consuming, tiresome, and imprecise due to collection error.^{7,8} As a result, clinical decision-making is often delayed. The present scenario demands for a rapid, convenient, and reliable method of proteinuria detection to overcome these limitations. Random urine sample for protein creatinine ratio (PCR) would be more acceptable and less time consuming. The PCR takes

into account the fact that creatinine excretion remains fairly constant in the presence of a stable glomerular filtration rate (GFR).⁹ Recent studies have shown that the correlation between spot urinary protein creatinine ratio and 24-hour urinary protein is statistically significant for all levels of proteinuria. Thus, PCR could be a very useful test as it is quick, cheap and convenient to patients and staff. Therefore, the present study was carried to compare the efficacy of spot urinary PCR with the 24-hour urinary protein in type 2 diabetic patients.

MATERIALS AND METHODS

This hospital based cross-sectional study was conducted by the combined effort of Department of Internal Medicine and Department of Biochemistry at Star Hospital, Lalitpur, Nepal.

Diabetic patients were selected from medicine OPD by convenient sampling technique and the biochemical parameters were assessed in Department of Biochemistry, Star Hospital. The study population includes sixty-six samples in which newly diagnosed cases suspected of having proteinuria and follow-up cases of type-2 diabetes mellitus having proteinuria were covered. However, patients who were excluded from the study were known cases of chronic kidney disease, pregnancy and pre-eclampsia. Data were collected about the age, sex and history.

Spot PCR determination:

We collected 5 mL spot urine sample from each participant irrespective of time during the day. The random urine sample was subjected to spot protein creatinine ratio (PCR) test. Urine protein was measured using a timed endpoint method by reacting with pyrogallol red and molybdate to form a blue color complex (AGAPPE), and urine creatinine was measured by kinetic method by using a modified rate Jaffe reagent (ACCUREX). Urine sample was thoroughly mixed and diluted up to twenty-five times with distill water for creatinine determination. Results for both protein and creatinine were expressed in mg/dL. Spot PCR was calculated by dividing spot urine protein value by spot urine creatinine value.

Calculation:

Urinary protein (mg/dl) = (Absorbance of sample)/(Absorbance of standard) × 100

Urinary creatinine (mg/dl) = (Δ Absorbance of test)/(Δ Absorbance of standard) × 2 × 25

Result is inferred as:

Normal urine protein: < 24mg/dl

Urine creatinine male: 21-26 mg/kg body weight / 24hrs

Urine creatinine female: 16-22 mg/kg body weight / 24hrs

24-hour urinary protein determination:

Patients were asked to collect urine for 24-hour. Collection time starts from 8.00 am on the first day and completed by 8.00 am the next day. 10 ml toluene was used as preservative. 24-hour urinary protein was computed by using following formula.

24-hour urinary Protein (mg/ 24 hour) = (Absorbance of sample × 1000 × total volume of urine in ml)/(Absorbance of standard)

Result is inferred as:

Normal 24-hour urinary protein: <150mg/day

Significant proteinuria: ≥300mg/day

Severe proteinuria: >5000mg/day

Statistical analysis

Firstly, data were managed on an excel spread sheet. Statistical package for social science (SPSS) for window version; 16.0 software was used for statistical analysis. All the results were expressed as Mean ± SD and Median (1st quartile, 3rd Quartile). For variable following non-normal distribution spearman's correlation coefficient was used to calculate the correlation. Sensitivity and specificity of random urine PCR at various cut off values was evaluated using Receiver Operating Characteristic (ROC) curve. P-value <0.05 has been considered statistically significant.

RESULTS

This study included 45 males and 21 females diagnosed with diabetes mellitus suspected of having proteinuria and who already had proteinuria. The mean age of participants was 52.5±13.3 years.

The median PCR was found to be 0.9 (0.44, 1.69). Similarly, the median value of 24-hour urinary protein (mg/day) was 2510.20 (1465.50, 4776.70). Out of total participants, 96% of the patients had significant proteinuria which is greater than equal to 300mg in the 24-hour urine collection, of those 23% had severe proteinuria i.e., more than 5000mg in 24 hours (Table 1).

Table 1: Baseline parameters of the study

Parameters	Mean ± SD	Median (Q1, Q3)
Age (years)	52.58 ± 13.34	51 (44, 60)
Spot Urinary protein (mg/dl)	109 ± 126.24	71.5 (37.5, 127.5)

Urinary Creatinine(mg/dl)	92.77 ± 52	80 (57.5, 121.5)
P:C Ratio	1.31 ± 1.23	0.9 (0.44,1.69)
24-hour Urinary protein (mg/dl)	192.83 ± 270.81	108.5 (66, 171.25)
Volume (ml)	2527.24 ± 818.58	2370 (1937.5, 3025)
24-hour urinary protein (mg/day)	4964 ± 7130	2510.20 (1465.50,4776.70)

Figure 1 demonstrates the relationship between the protein creatinine ratio and the 24-hour urine protein level. There was a strong positive correlation between PCR and 24-hour urinary protein excretion (r=0.633. p=0.001).

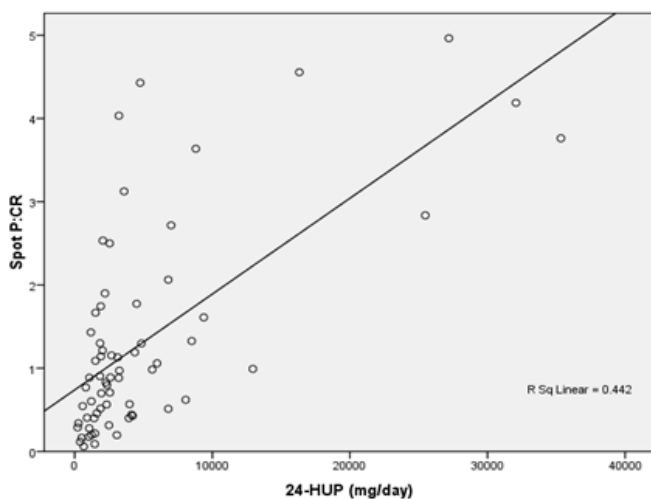


Figure 1: Correlation between 24-hour urinary protein and spot urine P: C ratio in type 2 diabetes mellitus subjects. (r=0.633, P=0.001)

Table 2 demonstrates the sensitivity and specificity of the protein/creatinine ratio at optimal cut-off value. The proteinuria value ≥300 mg protein/day was referred as standard for significant proteinuria.¹⁰

Table 2: Sensitivity and specificity of spot PCR at optimum cutoff value

Cut off Value	Area Under Curve (AUC)	Sensitivity	Specificity
0.37 (Significant proteinuria)	0.852	84.4%	100%
0.90 (Severe proteinuria)	0.833	86%	71%

The area under the curve was 0.852 and PCR with best cut off value of 0.37 had sensitivity and specificity of 84.4%

and 100% respectively. Similarly, the proteinuria ≥ 5000 mg/day was referred as standard for severe proteinuria.¹⁰ The area under the curve was 0.833 and single best value of 0.90 was obtained having sensitivity and specificity of 86% and 71%, respectively.

DISCUSSION

Diabetic nephropathy is the chronic loss of kidney function over a period of time characterized by appearance of protein in urine. Proteinuria is a condition characterized by the presence of excess protein in the urine sample. It is widely used in detection, diagnosis, and management of people having risk of developing renal disease and cardiovascular alteration.¹¹ The detection of proteinuria is known to have both diagnostic and prognostic role in the initial screening and confirmation of renal disease, and the quantification of proteinuria have considerable recognition in assessing the effectiveness of therapy and the betterment of the disease.¹²

The definitive measurement of urinary protein excretion is based on a timed urine collection over a period of 24 hour. It is endorsed that estimation of urinary protein excretion over a 24-hour period is the gold standard method. The use of a 24-hour collection is paramount due to variation of protein excretion throughout the day, which nullifies the use of concentration methods in random urine sample. It is also well known that there are problems associated with the collection of 24-hour urine, with several proclamations identifying poor accomplishment. This further adds the cost to an already expensive procedure.^{13,14,15}

Several authors have reported that protein excretion can vary at a time during the day and found that values can vary from 100% to 500%. This variation is owed to several factors such as hydration of body, diuresis, exercise, lying position, and diet. The variation may be further aggravated by hypertension and renal activity.⁸ An alternative approach that is being practiced is that expressing the protein excretion as a ratio in terms of creatinine. At stable condition of glomerular filtration rate, protein to creatinine ratio in urine at a time can be decisive because excretion of creatinine is fairly constant throughout the day. Newman et al. recently showed that variations in protein excretion in urine samples collected throughout the day are much less when their concentrations are expressed as a ratio to creatinine or specific gravity.^{16,17}

We conducted study on altogether 66 samples including 64 significant proteinuria and 16 severe proteinuria cases. In the present study the median value of 24-hr urinary protein is 108.5mg/day (66, 171.25). Similarly, the median value of protein creatinine ratio is 0.9 (0.44, 1.69). We found significant positive correlation between

spot PCR and 24-hr UP ($r=0.633$, $p=0.0001$). The suitable cut off value of P: C ratio for detection of significant proteinuria was found to be 0.37 with sensitivity and specificity of 84.4% and 100% respectively. Similarly, the appropriate cut off value of 0.90 was found to detect severe proteinuria with sensitivity and specificity of 86% and 71%, respectively.

Several investigators had shown the significant positive relationship between P: C ratio and 24-hr UP. In agreement to our study Durnwald et al and Zhang et al also found positive correlation between the variables ($r=0.64$, $p=0.001$ and $r=0.67$, $p=0.001$ respectively).^{18,19} In most of the cases the correlation coefficient (r) was found to be >0.9 .⁸ Similarly, Robert et al ($r=0.94$), Saudan et al ($r=0.93$), Ramos et al ($r=0.94$) also showed significant positive correlation between P: C ratio and 24-hr urinary protein irrespective of grade of proteinuria.^{20,21,22} However, Birmingham et al found a lower correlation between PCR and 24 HUP ($r=0.5$, $P=0.001$).²³ In our study, the correlation coefficient (r) value is less than 0.9 this may be due to confinement of study to single center with relatively smaller sample size.

Number of studies from pre-eclamptic cases, P: C ratio shows pooled sensitivity and specificity of 90% and 78% respectively. In the lineup with our study Quadri et al, Evans et al shows sensitivity and specificity almost similar to our finding.^{24,25} However, reports from Dyson et al, young et al shows low sensitivities as comparable to our study for detection of severe proteinuria.^{26,27} Since, the sensitivity of the PCR in our study was greater than 80 % in case of both significant and severe proteinuria, it can serve as screening test for detection of proteinuria.

PCR provides evidence to rule out the presence of significant proteinuria, but when the results of PCR are above the cutoff value, a full 24-hour collection is required for accurate quantification. The specific cutoffs of PCR to predict 24HUP may differ for different patient group and laboratory setting. So, it is recommended to determine cutoff value of PCR according to laboratory procedures and setting.

The use of protein: creatinine ratio measurement might be more reliable than the protein concentration measurement when a random urine sample is used. Considering cost effective and laborious issue, spot PCR can be suggested for screening of proteinuria for pursuit of early treatment.

CONCLUSION

PCR correlates well with 24-HUP for different level of proteinuria. The present study depicts that a random urine P: C ratio can predict the amount of 24-hour urine protein excretion value. Therefore, PCR can be used as

an alternative test and all the abnormal results must be followed by the 24-HUP estimation.

LIMITATIONS

This is a cross-sectional study; so, no follow up of patients was done. The study was conducted at single site with minimal sample.

ACKNOWLEDGEMENT

We would like to thank all the people who contributed in some way to the work described in this article. We would like to extend our appreciation to all the study participants, for their participation and cooperation, without whom this study would have been impossible. Finally, we would like to thank Star Hospital for providing us the platform

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