VOLUME-7, ISSUE-3, MARCH-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Biochemistry

ADVANTAGE OF SPOT URINE PROTEIN CREATININE RATIO OVER 24 HOUR URINE PROTEIN FOR PROTEINURIA ASSESSMENT

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ABSTRACT

Background: Proteinuria is a common finding in patients with kidney disease and its presence suggests a poor prognosis. The detection and accurate quantification of protein excretion are key diagnostic and prognostic tools for management of many renal disorders.

Aim and Objective: The main objective of our study was to evaluate the advantage and standardise the method of spot (random) urine protein creatinine ratio (UP/C) over 24 hours urine protein for estimation of proteinuria.

Materials and Methods: The study contained 100 study participants in Government General Hospital ,Kurnool inclusive of 100 cases and 100 normal volunteers. The 24 hour urine protein estimation was done on 24 hour urine samples and UP/C ratio was calculated on random urine samples.

Results: UP/C ratio and 24 hour urine protein estimation had strong correlation with $r = 0.9$ and $p < 0.05$ on Pearson's correlation analysis. Receiver operating characteristic analysis showed random UP/C ratio of 0.1171 reliably predicted 24 hour urine total protein equivalent of >150 mg/24 hrs with sensitivity 100%, specificity 98.1%, positive likelihood ratio 53.5, and negative likelihood ratio 0. UP/C ratio of 3.2 reliably predicted nephrotic range proteinuria at 24 hour urine protein equivalent of >3.5 g/24 hrs with sensitivity 80%, specificity 100%, positive likelihood ratio 154.4, and negative likelihood ratio 0.2.

Conclusion: We conclude that spot/random UP/C ratio is a reliable, simple test to be introduced and adopted in routine practice for monitoring of macro proteinuria.

KEYWORDS : Proteinuria, ROC Curve, Urine Creatinine, Urine Protein.

Introduction

Proteinuria is a common finding in patients with kidney disease and its presence suggests a poor prognosis. The detection and accuarte Quantification of protein excretion are key diagnostic and prognostic tools for management of many renal disorders. Patients with 2 or more positive spot urine tests temporarily spaced by 2 weeks should be diagnosed as having persistent proteinuria and should undergo further evaluation and management for chronic kidney disease.

Protein excretion in urine varies with stress, exercise, hydration status, posture and also diurnally[2]. Hence, the gold standard test is quantitative estimation of protein done on urine collected over 24 hours[2]. Urine protein estimation by 24 hr collection is a cumbersome task with many errors[3] including incomplete collection, bacterial growth, incorrect timings and incomplete bladder emptying. These errors far exceed those caused by diurnal variation in protein excretion. It also requires hospital admission and causes inconvenience, especially for repeated follow up. As creatinine excretion is fixed and its concentration in urine varies with hydration status, the random (spot) urine protein creatinine ratio (UP/C ratio) nullifies the effect of hydration on protein estimation[3].

Random urine sample collection is simple procedure and can be done at any time of the day, though few studies recommend morning samples4. There have been some studies supporting the use of UP/C Ratio[6,7,8]. The cutoff threshold value of UP/C ratio to determine pathologic proteinuria provided by different studies is variable[7,8,11]. This study was done to evaluate and standardize the method of UP/C ratio to determine proteinuria.

Evaluation of proteinuria should be done in renal diseases including Diabetic nephropathy, nephritic syndrome, malignant hypertension, collagen diseases, toxemia of pregnancy, drug nephrotoxicity, SLE, autoimmune diseases, Post transplant rejection.

Subjects and Methods

This was a prospective study done in Government General Hospital ,Kurnool from July 2017 to December 2017. The inpatient and out patients of hospital who were advised 24 hour urine protein estimation within the age group 18-65 years were included in the study. A group of 100 normal volunteers of the same age group with no risk factors for renal impairment on history and examination were taken as controls.

Exclusion Criteria: Inadequate samples were excluded as adjudged by history of incomplete 24 hour collection.[12] Also, patients with urinary tract infection and excretion of abnormal amount of leucocytes in urine- adjudged by presence of > 5 leucocytes/ high power field on urine sediment examination by microscopy13 were not included in the study. Haematuria and excretion of abnormal amounts of RBC's in urine- more than 3 RBC/ high power field on urine sediment examination by microscopy[13] and contaminated samples were excluded.

Sample Collection: The 24 hour urine sample was collected for protein estimation with collection starting from 8 am on first day excluding the first morning urine sample, completing on second day at 8 am including the first morning urine sample. The container was kept in the refrigerator in between the urine collections. Random urine sample was taken either before starting or after completion of the 24 hour collection. Preferably morning sample was collected or sample was taken at any other time of the day. Repeat random urine samples of 100 patients were obtained on the same day or the next day.

Samples were processed as early as possible after collection and were stored in refrigerator in cases of inevitable delays in processing. Urine microscopy was done on random urine samples by sediment preparation. Urine protein analysis was done by Sulphosalicylic acid method and creatinine estimation was done by modified Jaffe's method on a colorimeter provided by ERBACHEM 5Xsemi autoanalyzer. The 24 hr urine samples were evaluated for volume, colour and protein levels. Random urine samples were evaluated for colour, microscopy, protein and creatinine levels. UP/C ratio in random urine samples was calculated by dividing protein in g/L by creatinine in g/L.

Statistical Analysis: The statistical test used for correlation was Pearson's correlation. Chi Square test was used to determine any association of risk factors to proteinuria. Paired t test was used to compare difference between the mean protein excretion of cases and normal volunteers; and to assess repeatability of UP/C ratio. Regression analysis was done to find out the regression formula connecting 24 hr urine protein estimation and UP/C ratio. Data processing was done with statistical software PASW statistics 18 from SPSS for Windows, (Chicago: SPSS Inc). Receiver Operating Characteristic (ROC) analysis done using statistical software Medcalc for Windows, (version 11.4.2.0 MedCalc Software, Mariakerke, Belgium) was used to determine sensitivity, specificity and likelihood ratios of UP/C ratio cutoff values to predict non-nephrotic and nephrotic range proteinuria.

Results

A total of 200 study participants inclusive of 100 cases and 100 normal volunteers were included in the study

The 100 cases when analysed for disease subgroups, 36 subjects had diabetes mellitus (DM), 41 subjects had hypertension (HT), 8 subjects had both diabetes mellitus and hypertension (DMHT), 7 subjects had nephrotic syndrome (NS), and 8 subjects had pregnancy induced hypertension (PIH). Out Of 100 total cases 36(36%) were females and 64(64%) were males. Of 100 normal volunteers 24(24%) were females and 76(76%) were males. In 100 patients, 51(51%) had protein ≤ 0.15 g in 24 hours which was within the normal range and 49(49%) had proteinuria more than 0.15 g (macro) which was in pathologic range. All normal volunteers had urine proteins in the normal range.

No significant correlation was found between gender of the subjects and proteinuria. The p value 0.185 (>0.05) was not significant.

Significant association was found between risk factors (diabetes, hypertension and pregnancy induced hypertension) and abnormal 24 hr protein excretion levels at a $p < 0.05$.

The mean protein excretion in g/24 hr of cases was found to be 0.8207 g (±1.3364) whereas in normal volunteers it was 0.0337 g (±0.0357) as seen in Table 1. There was significant difference in mean 24 hour protein excretion of cases and normal volunteers with a p 0.001 (<0.05).

The mean protein excretion of the 8 cases with two risk factors diabetes and hypertension was more than the mean excretion of those with only one of these risk factors. The mean UP/C ratio of cases was 0.7155 (±1.1151) and of normal volunteers was 0.0269 (±0.0269) as seen in Table 1. The difference between the mean UP/C ratio of cases and normal volunteers was significant with a p 0.001 (<0.05).

The UP/C ratio showed excellent correlation with the 24 hour urine protein values, $p < 0.05$ and correlation coefficient (r) of 0.98. The correlation between 24 hour urine protein and UP/C ratio was significant in all the disease subgroups with p values < 0.05. The value of r was 0.99, 0.96, 0.97, 0.96 and 0.99 in groups DM, DMHT, HT, NS and PIH respectively.

The mean UP/C ratio of first samples was 0.8280 and that of repeat random samples was 0.9078. The difference between means of UP/C ratio of first and repeat samples was not statistically significant with a p value of 0.191 (>0.05). Area under the curve of ROC analysis in Table 2 represents accuracy of the test: a value close to 1 indicates a good test.

The regression coefficient in Figure 1 is 0.94, $p < 0.05$. The formula connecting UP/C ratio (y) to 24 hr urine protein (x) is $y = 1.005x + 0.078$.

Discussion

The National Kidney Foundation (USA),14 the Australasian Society for the Study of Hypertension in Pregnancy and the International Society for the Study of Hypertension in Pregnancy have recommended use of the urinary spot UP/C ratio as an alternative to 24 hour urine collection for urine protein estimation[15,16].

Table 1 : Mean and standard deviation of protein excretion in g/24 hr and in UP/C ratio between cases and normal volunteers

Group	Number	Mean in g/24 hr	Std. Deviation	Mean in UP/C ratio	Standard Deviation
Cases	100	0.8207	1.3364	0.7155	1.1551
Normal	100	0.0337	0.0357	0.0269	0.0269
Paired t Test		*P value 0.001*		*P value 0.001*	

Normal urine albumin excretion is less than 20 mg in 24 hours[17]. Microalbuminuria is defined as albumin excretion more than 30 mg/24hr but below 300 mg/24hr. In diseases like diabetes and hypertension diagnosis of microalbuminuria is important to initiate appropriate treatment. If treatment is not given in time progressive renal failure can develop. Often due to cost constraints patients do not undergo testing for microalbuminuria for diagnosis as well as follow up. Therefore we have taken proteinuria of more than 150 mg/day as abnormal[18] in order to detect >150 mg/24hr of protein which will include microalbuminuria of more than 150 mg/24hr.

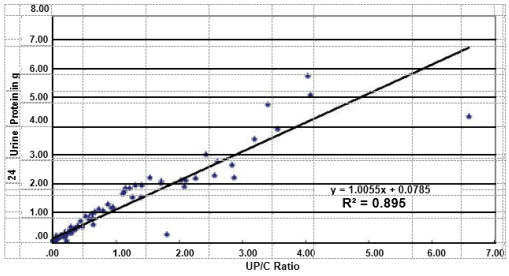

Fig. 1 : Relationship between UP/C Ratio and 24 hr Protein. The regression line and regression equation for UP/C ratio (x) and 24 hr urine protein in g/24 hr (y)

Table 2 : Receiver operating characteristic (ROC) analysis results depicting the sensitivity, specificity and likelihood ratios of various UP/C ratio cutoffs to determine abnormal proteinuria >150 mg/24 hr and nephrotic range proteinuria >3.5 g/24 hr are shown

1 ROC Data for ROC Data for 100 Cases at Proteinuria > 150 mg/24 hr

	Area under the ROC curve 0.9			*p<0.05*		
UP/C Ratio	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>0.1171	100	96.4 - 100.0	98.1	93.4 - 99.7	53.5	0
>0.1481	96	90.2 - 98.9	99.1	94.9 - 99.8	102.76	0.04
>0.1604	95	88.8 - 98.4	100	96.6 - 100	>101.7	0.05

2 ROC Data for 100 Cases at Proteinuria > 3.5 g/24 hr

	Area under the ROC curve 0.9			*p* < 0.05		
UP/C Ratio	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>2.5624	100	78.0 - 100.0	96.4	92.7 - 98.5	27.57	0
>2.867	93.3	68.0 - 98.9	98.4	95.5 - 99.7	60.04	0.07
>3.2318	80	51.9 - 95.4	100	98.1 - 100.0	>154.4	0.2

The excellent correlation between UP/C ratio and 24 hr urinary protein is corroborated by other studies. Chitalia et al7 studying patients with glomerular diseases found correlation between UP/C ratio and 24 hr urine protein was good at $p < 0.05$ and correlation coefficient of 0.97. High correlation coefficients (r=0.91, 0.95 and 0.98) were observed in patients with normal, reduced and severely reduced renal function in a study done by Antunes et al.8

Statistics from Table 2 show UP/C ratio threshold 0.1171 to distinguish normal from abnormal proteinuria is very good for a screening test with sensitivity 100% and 5% false positives. Convincing absence of proteinuria by a good test is important considering the increasing costs involved in treatment of patients with end stage renal diseases, caused by delayed presentation and diagnosis of disease.

UP/C ratio cutoff 0.1604 to distinguish normal from abnormal proteinuria having sensitivity 95% and specificity 100 % (no false positives) can be used when the clinical suspicion of the patient having renal disease is low.

As seen in Table 2 UP/C ratio cutoff 2.5624 is a good criterion to screen for nephrotic proteinuria with sensitivity of 100% and specificity of 96.4%. UP/C ratio 3.2318 when considered compared to other cutoffs has specificity of 100% and sensitivity of 80%. It is recommended as the criterion for determining nephrotic range proteinuria when clinical suspicion is low.

Other studies mention UP/C ratio cutoff values for abnormal proteinuria ranging from 0.2 to 0.3 because the reference threshold of abnormal proteinuria varies ranging from > 0.2 g/24 hr to > 0.3 g/24 hr, in different studies. Authors Ginsberg et al19 and Chitalia et al7 have recommended a cutoff UP/C ratio of 0.2 and 0.26 respectively for abnormal proteinuria; 3.5 and 3.2 respectively for nephrotic proteinuria. Kristal et al11 in their study on 51 patients being followed at the renal and hypertension clinic with stable renal function, have recommended a UP/C ratio threshold of 0.2 and 3.5 for abnormal and nephrotic proteinuria respectively.

The regression formula could be used to predict an approximation of 24 hr protein values from UP/C ratio values. Protein 24 hr g/24 hr = $[1.005 \times (\text{UP/C Ratio g/g})] + 0.078$.

The formula should be used keeping in mind the wide scatter between the values of UP/C ratio and 24 hr urine protein at moderate and high degrees of proteinuria as seen in Figure 1.

Since albumin creatinine ratio is much expensive UP/C ratio can be used as substitute when albumin to creatinine ratio is more than 0.5 as mentioned in guideline five of NKF K/DOQI guidelines[9] Factors which affect creatinine excretion in urine like age, sex, muscle mass also affect the UP/C ratio, should be borne in mind while interpreting the results. The reproducibility of UP/C ratio is important so that it can be used for follow-up.8 Reproducibility is shown in our analysis as there is no significant difference between means of repeat sample testing.

Reduction in UP/C ratio indicates reduction in proteinuria though the absolute value cannot be gauged[9] UP/C ratio is a simple random test, reflects changes in proteinuria over time, also supported by Antunes et al.8 The progressive changes in UP/C ratio can be used to determine therapeutic response and prognosis on follow up. This is supported by the study of authors Ruggenenti et al6 who evaluated glomerular filtration rate in addition to 24 hr urine protein in comparison with UP/C ratio.

Conclusion

UP/C ratio is a simple and convenient test for detecting proteins in urine >150 mg/24 hr and overcomes the pitfalls of 24 hr urine protein estimation. Therefore UP/C ratio estimation should be introduced and adopted in practice in testing for proteinuria.

REFERENCES

1. Bargman JM, Skorecki K. Chapter 274 Chronic Kidney Disease. In, Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J(eds). Harrison's Principles of Internal Medcine, 17th edition. New York, The McGraw-Hill Companies, 2008;1761-71.
2. Rowe DJF, Bagga H, Betts PB. Normal variations in rate of albumin excretion and albumin to creatinine ratios in overnight and daytime urine collections in non-diabetic children. British Medical Journal 1985;291:693-4.
3. Côté AM, Firoz T, Mattman A, Lam EM, von Dadelszen P, Magee LA. The 24-hour urine collection: gold standard or historical practice?. Am J Obstet Gynecol 2008;199:625.e1-6.
4. Newman DJ, Pugia MJ, Lott JA, Wallace JF, Hiar AM. Urinary protein and albumin excretion corrected by creatinine and specific gravity. Clin Chim Acta 2000;294:139-55.
5. Kassirer JP, Harrington JT.Laboratory evaluation of renal function. In, Gottschalk CW, Schrier RW (eds). Diseases of the Kidney, 4th edition. Boston, Little Brown, 1988;393-491.
6. Ruggenenti P, Gaspari F, Perna A, Remuzzi G. Cross-sectional longitudinal study of spot morning urine protein:creatinine ratio, 24-hour urine protein excretion rate, glomerular filtration rate, and end stage renal failure in chronic renal disease in patients without diabetes. BMJ 1998;316:504–9.
7. Chitalia VC, Kothari J, Wells EJ, Livesey JH, Robson RA, Searle M, Lynn KL. Cost-benefit analysis and prediction of 24-hour proteinuria from the spot urine protein-creatinine ratio. Clin Nephrol 2001;55:436-47.
8. Antunes VV, Veronese FJ, Morales JV. Diagnostic accuracy of the protein/creatinine ratio in urine samples to estimate 24-h proteinuria in patients with primary glomerulopathies: a longitudinal study. Nephrol Dial Transplant 2008;23:2242-6.
9. Bolton K, Coresh J, Culleton B, Harvey KS, Ikizler TA, Johnson CA.K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification,The National Kidney Foundation. Am J Kidney Dis 2002;39(2Suppl1):S1-266.
10. Kidney Disease Outcomes Quality Initiative (K/DOQI), Levey AS, Rocco MV, Anderson S, McCullough PA, Andreoli SP. K/DOQI Clinical Practice Guidelines on Hypertension and Antihypertensive Agents in Chronic Kidney Disease. Am J Kidney Dis 2004;43(5 Suppl 1):S1-290.
11. Kristal B, Shasha SM, Labin L, Cohen A.Estimation of Quantitative Proteinuria by Using the Protein-Creatinine Ratio in Random Urine Samples. Am J Nephrol 1988;8:198-203.
12. Edwards OM, Bayliss RIS, Millen S.Urinary creatinine excretion as an index of the completeness of 24-hour urine collections. The Lancet 1969;294:1165-66.
13. McPherson RA, Ben-Ezra J, Zhao S.Basic examination of urine. In, Pincus MR, McPherson RA (eds). Henry's Clinical Diagnosis and Management by Laboratory Methods, 21stedition. Singapore, Saunders, 2007;393-425.
14. Vassalotti JA, Stevens LA, Levey AS. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. Am J Kidney Dis 2007;50:169-80.
15. Brown MA, Hague WM, Higgins J, Lowe S, McCowan L, Oats J, et al. The detection, investigation and management of hypertension in pregnancy: executive summary. Aust N Z J Obstet Gynaecol 2000;40:133-8.
16. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 2001;20:IX-XIV.
17. Gosling P.Chapter 8 Proteinuria. In, Bangert SK, Marshall WJ (eds). Clinical Biochemistry, 1stedition. London, Churchill Livingstone, 1995;143-61.
18. Lewis JB, Neilson EG. Chapter 277 Glomerular Diseases. In, Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J(eds). Harrison's Principles of Internal Medcine, 17th edition. New York, The McGraw-Hill Companies, 2008;1782-96.
19. Ginsberg JM, Chang BS, Matarese RA, Garella S.Use of Single Voided Urine Samples to Estimate Quantitative Proteinuria. N Engl J Med 1983;309:1543–6.