ORIGINAL ARTICLE

Evaluation of Rapid Diagnostic Methods of Urinary Protein Estimation in Patients of Preeclampsia of Advanced Gestational Age

Kumari Archana · Singh Abha · Singh Ritu

Abstract

Background 24-h urine protein is traditionally used as a gold standard method for protein estimation. Because of the operational difficulty, there is the necessity to use rapid, convenient, and reliable method of proteinuria estimation.

Aim We carried out this study to compare the two rapid methods of protein estimation: dipstick method and spot urine protein creatinine ratio (UPCR) with that of 24-h urine protein in patients of preeclampsia with advanced gestational period.

Methodology The values of proteinuria estimated by dipstick method and spot UPCR were compared with that of 24-h urine protein. The strength of correlation was measured by Pearson's correlation coefficient (r). A p value of <0.05 is considered to be statistically significant. The most discriminant spot UPCR value for detecting significant proteinuria ($\geq$300 mg/day) was determined by plotting receiver–operator curve (ROC).

Result The value of spot UPCR strongly correlated with 24-h urine protein ($r = 0.88$ with p value <0.001). The most discriminant spot UPCR value for detecting significant proteinuria ($\geq$300 mg/day) was 0.3. The estimation of proteinuria by dipstick method was poorly correlated with 24-h urine protein with $r = -0.09$.

Conclusion Spot UPCR can be used as a rapid and reliable alternative method in preference to 24-h proteinuria in patients of preeclampsia of advanced gestational age.

Keywords Preeclampsia · Spot UPCR · 24 h urine protein · Dipstick method

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Introduction

Preeclampsia is an important cause of maternal morbidity and mortality as well as a significant contributor to higher incidences of perinatal morbidity and mortality [1, 2]. Proteinuria is a cardinal manifestation of preeclampsia and its quantitation is important not only for making the diagnosis but also in predicting the prognosis of mother and baby [3–5].

Estimation of 24-h urine protein has been considered as the gold standard for quantitation of proteinuria. However, collection of 24-h urine is cumbersome and it is time-consuming. Besides, it may give inaccurate results because of improper collection especially in pregnant ladies with advanced gestational period [6]. There are two rapid methods for quantitative estimation of proteinuria-spot urinary protein:creatinine ratio (UPCR) and dipstick method, which are being used by clinicians of various specialities for the purpose of screening and making diagnosis. In recent years, a number of studies have been performed to compare the gold standard

method of estimation of proteinuria with either of the two rapid diagnostic methods in patients of preeclampsia [7–13], but none has compared the two rapid tests in the same settings in advanced gestational age.

We carried out this study to compare these two rapid methods of proteinuria estimation with 24-h urine protein estimation in patients of preeclampsia with advanced gestational period. We also determined cut-off value of spot UPCR that predicts significant proteinuria on 24-h urine sample.

Materials and Methods

We carried out a prospective, cross-sectional study in the Department of Obstetrics and Gynaecology in collaboration with the Department of Biochemistry in Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi. Four hundred consecutive patients during January 2008–December 2009 with gestational age ≥32 weeks who were admitted in the maternity ward with signs and symptoms suggestive of preeclampsia were enrolled. The study was approved by ethical committee of the Hospital and informed consent was taken before enrolling patients.

In the present study, preeclampsia was diagnosed in patients in whom resting blood pressure was more than or equal to 140/90 mmHg on two occasions at least 4 h apart and significant proteinuria was detected in urine samples (defined as more than or equal to 300 mg/24 h or 30 mg/dl (1+ dipstick) in random urine sample. Women with chronic hypertension, diabetes mellitus, pre-existing renal disease UTI, intrauterine fetal death, multiple gestations, premature rupture of membrane and post term pregnancy were excluded from the study.

At the time of admission, random urine was tested for protein by the semi-quantitative dipstick method using the commercially available dipstick supplied by YD diagnostic India limited. Results were read according to the color scale provided. The first voided morning urine specimen was discarded, and the patients were instructed to rest at least for 1 h in left lateral decubitus to avoid ureteral compression. The 24-h urine collection was done till the first voided urine specimen of the next morning. Urine albumin was measured on six-hourly basis in the maternity ward by dipstick method for monitoring of the patient. 5–10 ml of the voided midstream urine sample was obtained for measurement of protein: creatinine ratio during the 24-h collection. This volume was later adjusted by dilution to make up a total volume of 24-h urine.

Urine protein concentration was estimated on Synchron CX-9 automated analyzer from Beckman, using the kits obtained from Randox. It was based on the principle that the coomassie reagent reacts with protein in an acidic milieu to form a colored complex and that the color intensity is proportional to the protein concentration. Urine creatinine concentration was measured by modified Jaffe's reaction which is based on the principle that creatinine in alkaline solution reacts with picric acid to form a colored complex. The intensity of the color formed is directly proportional to the creatinine concentration. The UPCR was calculated by dividing the quantum of urine protein measured in mg/dl by urine creatinine in mg/dl.

The statistical tests used for analysis were Pearson's correlation coefficient-expressed as "r,", and student Chi square test expressed as χ^2. A p value of <0.05 is considered to be statistically significant.

Results

400 patients of preeclampsia with gestational age ≥32 weeks were included in the study. The general characteristics of patients are mentioned in Table 1. The mean age of the patients was 24.3 years. Majority of the patients were multigravida and had signs and symptoms of impending eclampsia at the time of admission. Almost one third of the patients had severe hypertension on admission. Majority of the patients (74 %) had high Proteinuria (Dipstick 3+ or more).

The quantitative value of proteinuria estimated by spot UPCR and its correlation with 24-h proteinuria is given in Table 2. The maximum number of cases (76 %) had spot UPCR in the range of 0.3–3.0 mg/mg while 14 % had spot UPCR ≥3.0. Only 10 % of the cases had spot UPCR <0.3. The mean spot UPCR was 1.88 ± 0.18. 77.5 % of the patients had significant proteinuria (>300 mg/24 h) while only 22.5 % had non-significant proteinuria (<300 mg/24 h). The mean 24-h urine protein was 1,166 ± 759 mg/24 h.

These values show that there exists a good correlation between 24-h urine protein and spot UPCR <3.0 mg/mg with p value <0.001 whereas for spot UPCR values ≥3.0, there was poor correlation with $r = -0.108$ and $p > 0.05$ which is statistically not significant. However, there was a strong correlation between the two in the range 0.3–3.0 ($r = 0.91$ at p value <0.001 which is highly significant).

The estimation of proteinuria by dipstick method and its correlation with 24-h proteinuria are given in Table 3. The maximum number of cases (76 %) had 3+ proteinuria by dipstick. Overall, the correlation was poor with $r = -0.09$. However, moderate association was seen in patients with 1+ proteinuria.

Determination of Cut-off Value

In the present study, a cut-off value of spot UPCR that predicts significant proteinuria on 24-h urine sample was calculated at different levels of UPCRs (Table 4; Figs. 1, 2).

Table 1 General characteristics of patients

Sr. no.	Parameter	Value
1.	Mean age in years (S.D.)	24.3 (2.6)
2.	Primigravida:multigravida	0.56:1
3.	Mean period of gestation in weeks (S.D.)	36.5 (2.7)
4.	Women signs and symptoms of impending eclampsia (at the time of admission)	54 %
5.	Diastolic blood pressure (at the time of admission)	
	90–99 mm of Hg	38 %
	101–109 mm of Hg	32 %
	DBP ≥110 mmHg	30 %
6.	Proteinuria (at the time of Admission)	
	1+	9 %
	2+	14 %
	3+	42 %
	4+	32 %

On the basis of the ROC, the most discriminant spot UPCR value for detecting significant proteinuria (≥300 mg/day) was 0.3, based on defining the spot UPCR value which gave the best combination of sensitivity, specificity, and positive and negative predictive values. The 0.3 spot UPCR as seen in the table yielded a positive predictive value of 95 % and a negative predictive value of 72 %, while maintaining sensitivity and specificity at 90 and 84 %, respectively.

Discussion

This prospective cross-sectional study was conducted on 400 pregnant women with period of gestation ≥32 weeks who were admitted in maternity ward for the management of preeclampsia. The mean age of the cases was 24.3 ± 2.6 years. The majority of cases (64 %) were multigravida. The mean period of gestation in the study population was 36.5 ± 2.7 weeks. The mean spot UPCR was 1.28 ± 0.18 mg/mg. The spot UPCR correlated very well with 24-h urine protein with overall strength of correlation, $r = 0.88$ with p value 0.001 The strength of correlation was good when spot UPCR was <0.3 ($r = 0.83$ at p value <0.001) and was even better at values of spot UPCR between 0.3 and 3.0 ($r = 0.91$ at p value <0.001). However, at spot UPCR ≥3.0 mg/mg, the degree of correlation was poor ($r = -0.108$ at p value >0.05) which is statistically not significant. Various studies have failed to show consistent results. Some studies have shown a good correlation between the two [7, 8] while others have found it to be of limited use [9–11].

The cut-off value of spot UPCR that predicts significant proteinuria in 24-h urine sample was found to be 0.3. A number of cut-off points of spot urine UPCR that best predicts significant proteinuria have been described in the literatures. The sensitivity, specificity, positive predictive value and negative predictive value of spot UPCR at 0.3 in this study were 90, 84, 95, and 72 % each. A systematic review of 16 studies demonstrated a range of sensitivity and specificity for the test between 69–96 % and 41–97 %, respectively, whereas the positive and negative predictive values ranged between 46 and 95 and 45 and 98 %, respectively [14]. These variations can be due to different settings in terms of ethnicity and gestational ages of the patients of preeclampsia and necessitates the careful interpretation of results in clinical practice.

The dipstick method failed to show significant correlation with the gold standard method in the estimation of proteinuria. Similar results of poor correlations have been seen in the studies involving patients of lupus nephritis and gestational hypertension [12, 13]. There may be many reasons

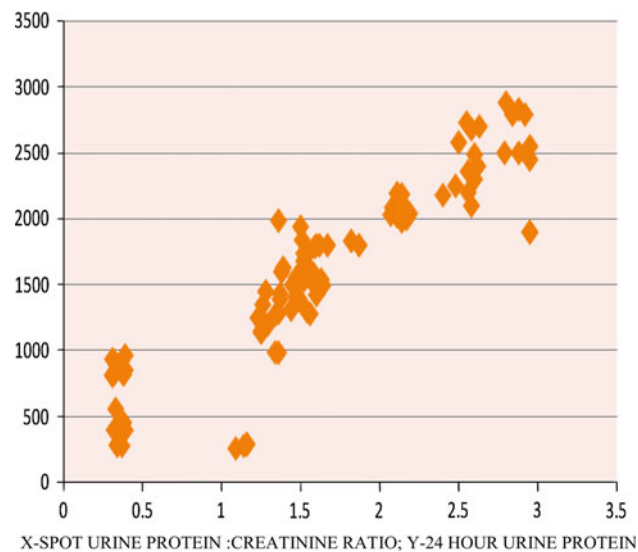**Table 2** Correlation of spot urine protein:creatinine ratio with 24-h proteinuria

Overall correlation coefficient for 400 cases = 0.88 AT p value < 0.001

Urine p:c ratio	No. of cases	24-h proteinuria	range of 24-h urine protein	coefficient of correlation (r)	p value
<0.3	40	124 ± 81	24–315	0.83	$p < 0.001$
0.3–3.0	304	624 ± 281	144–1870	0.91	$p < 0.001$
≥3.0	56	6728 ± 5926	404–10,328	−0.108	$p > 0.05$

Table 3 Correlation of dipstick with 24-h proteinuria

Overall correlation coefficient for 400 cases = −0.09

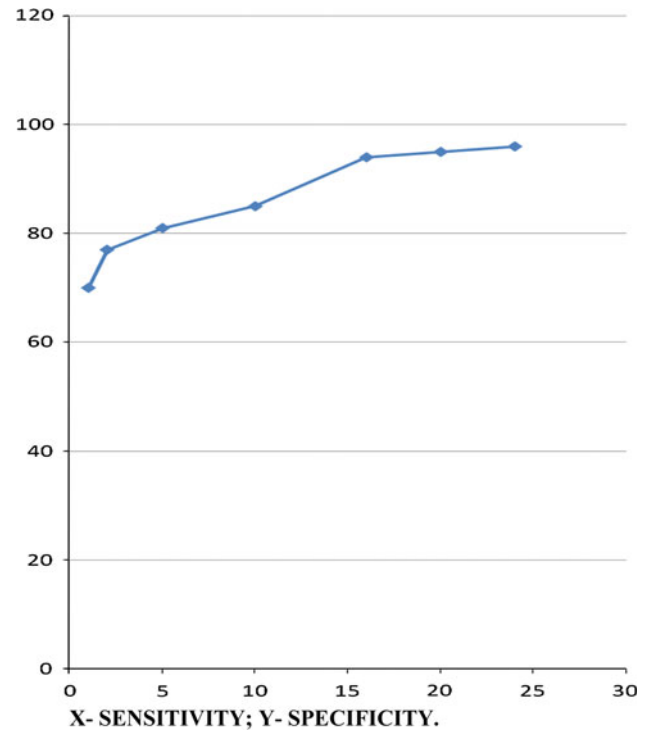| Value of proteinuria dipstick	No. of cases	24-h proteinuria	Range of 24-h urine protein	coefficient of correlation (r)	p value
1+	36	176 ± 75	24–515	0.53	$p < 0.05$
2+	72	924 ± 181	208–1870	0.31	$p > 0.05$
3+	216	3552 ± 1198	876–5743	−0.11	$p > 0.05$
4+	76	5234 ± 2026	698–10,328	−0.108	$p > 0.05$

Table 4 Comparison of different cut-off values of spot urine protein/creatinine (p/c) ratio in prediction of significant proteinuria (≥300 mg/day)

Cut-off value (mg/mg)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0.2	96	76	93	83
0.25	93	80	94	77
0.3	90	84	95	72
0.35	85	88	96	63
0.4	81	92	97	58
0.45	77	96	98	54

X-SPOT URINE PROTEIN :CREATININE RATIO; Y-24 HOUR URINE PROTEIN

Fig. 1 Scatter diagram showing correlation between spot urine protein:creatinine (UPCR) ratio at 0.3–3.0 mg/mg and 24-h proteinuria

X- SENSITIVITY; Y- SPECIFICITY.

Fig. 2 Receiver–operator curve of spot UPCR ratio for 24-h proteinuria

(Gold Standard). Our study has limitation that it compares estimation of proteinuria by different methods in patients of advanced gestational age only. Subsequent multicentric studies involving patient of gestational age ≥20 weeks should be designed to validate its universal use.

why dipstick test of random samples did not correlate completely with 24-h total protein excretion. Observer variation in noting the color of the strip at different points of time could be one of the reasons. At times it is difficult to identify the color accurately, especially when the color is not exactly matching with any one (as 1+, 2+, 3+, or 4+) or having intermediate hue.

The present study demonstrates that the spot UPCR is as good as 24-h urine protein estimation in patients of preeclampsia with advanced gestational age. A poor degree of correlation at severe degrees of proteinuria warrants cautious interpretation of results because a significant fraction of these women will not have severe proteinuria on the 24-h urine collection. A cut-off value of spot UPCR of 0.3 mg/mg showed an excellent predictive value for significant proteinuria in 24-h urine sample (≥300 mg/24 h). The spot UPCR is superior to urinary dipstick tests in screening for significant proteinuria in women with preeclampsia which failed to show correlation with 24-h urine protein estimation

Conclusion

The spot UPCR is reliable, relatively faster, and equally accurate method for detection and quantitation of proteinuria that correlates well with 24-h urine protein estimation can be used as an alternative to 24-h proteinuria in patients of preeclampsia of advanced gestational age. The dipstick method because of its simplicity and cost may be continued to be used as initial screening method though not suitable to be used for quantitation of proteinuria.

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