

The diagnostic accuracy of spot urine protein-creatinine ratio (UPCR) in quantification of proteinuria in women with preeclampsia

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Abstract

Introduction: Estimation of urinary excretion of protein is done by the standard method of collection of urine over 24- hours and the test sample is taken from this collection. Because of its technical difficulty there is the necessity to use quick, convenient and reliable method which is well correlates with 24-h urine protein.

Aim: This study is carried out to compare the dipstick method and spot urine protein creatinine ratio (UPCR) with that of 24-h urine protein (24HUP) and also to determine the role of proteinuria in predicting the pregnancy outcome in preeclamptic patients.

Materials and Methods: We conducted this prospective analytical study in 209 antenatal women in whom 52 were with gestational hypertension and 157 were with preeclampsia. Pearson's correlation coefficient revealed the strength of correlation while the *p* value less than 0.05 was considered to be statistically significant. Receiver-operator curve (ROC) was plotted to find the cut-off value of UPCR and dipstick in detecting significant proteinuria (≥ 300 mg/day).

Results: The value of UPCR strongly correlated with 24-h urine protein ($r=0.941$, $p<0.001$). The cut-off value of 0.25 by UPCR method was considered as significant proteinuria. A strong positive correlation was found between 24HUP test and dipstick test ($r=0.759$, $p<0.001$) results with a cut-off value of 1.5 to detect significant proteinuria.

Conclusions: Spot UPCR can be used as a rapid and reliable alternative method to 24-h urine protein in preeclamptic patients.

Keywords: Preeclampsia, Sspot UPR, 24-h urine protein and dipstick method.

Introduction

Hypertensive disorders constitute most common medical complications of pregnancy and also stand as an important cause of maternal and perinatal morbidity and mortality. In India, they contribute to 12% of maternal deaths. Among this subgroup of disorders, preeclampsia is the most threatening condition occurring in 2 - 10% of all pregnancies world wide.¹ The diagnosis of preeclampsia is considered when the blood pressure is $\geq 140/90$ mm of Hg in 2 sittings in an interval of 6 hours apart after 20th week of pregnancy, with proteinuria ≥ 300 mg in 24- HUP test or $\geq 1+$ reading in dipstick test.² The dipstick analysis done with visual reagent strips is a user-friendly, rapid and portable screening test; but as it has high false positive and false negative rates, the standard 24HUP test is carried out subsequently for confirmation.³ As urine protein concentration varies highly during the course of the day,⁴ the accuracy of random urine protein test (dipstick test) cannot be comparable with that of the 24HUP test.^{5,6} However 24HUP test is a cumbersome, inconvenient, time-consuming process to collect urine and errors may occur due to incomplete collection.⁷ Frequent monitoring of urine protein is required in cases diagnosed as preeclampsia and it is a time taking process causing further delay in the management and sometimes the patients may deliver before completion of urine collection. Therefore, a reliable, quick and easily performable quantitative measurement of urinary protein excretion correlating well with the 24HUP test, is required for frequent monitoring and timely decisions and

during pregnancy in preeclamptic mothers and also for decreasing their admissions.

Urinary creatinine is excreted at a fairly constant rate at stable glomerular filtration rate. Based on this observation in recent years, spot UPCR test has been suggested as a rapid test in quantifying significant proteinuria⁸⁻¹¹. However, the accuracy of the test still remains unclear. Many studies have shown good correlation between UPCR test and 24HUP test^{8,9} while few other studies found no proper correlation.¹⁰⁻¹² In many studies where UPCR was used, the predicting values of test results were not mentioned.⁸⁻¹⁰ Being an exclusive maternal and child health centre, our hospital has a high input of obstetric population and the incidence of hypertensive pregnancies. Our aim was to determine the diagnostic accuracy of UPCR in women with preeclampsia and to predict the outcome of pregnancy in relation to the severity of proteinuria.

Aims and objectives

1. Correlation of proteinuria estimated by 24HUP test with UPCR and to determine its accuracy.
2. Correlation between significant proteinuria by 24 HUP test and random urine protein (dipstick) test for proteinuria.
3. To determine the role of proteinuria as assessed by both the 24HUP (gold standard test) and the UPCR in predicting the outcome of pregnancy in t these participants.

Material and Methods

Our study was a prospective analytical study involving the pregnant women who were diagnosed to have preeclampsia (urine protein with dipstick test 1+ or more) admitted in antenatal ward with the advice of modified bed rest. The exclusion criteria were the subjects with chronic hypertension, intrinsic renal disease, pathological vaginal discharge and urinary tract infection and those planned for termination of pregnancy immediately.

The study was approved by the Scientific and Ethical Committee of the institute. After obtaining informed consent, detailed history including the demographic data, medical, obstetric and menstrual events and thorough clinical examination -both general and obstetric, were performed. Following this all the subjects were evaluated and treated as per the existing protocols of the obstetric unit.

Procedure

All were asked to collect 24-hour urine sample for determination of proteinuria. They were advised to collect urine at 8.00 am of a day till 8.00 am of the next morning. Random sample for UPCR test and dipstick test were collected in the next day morning after the 24-hour urine collection. The adequacy of urine collection was determined by the creatinine excretion. Subjects with inadequate urine sample and those with urinary tract infection were excluded from the final analysis. Only the subjects who had significant proteinuria i.e. ≥ 300 mg in 24 hours urine sample were included in the final analysis.

Methods

Urine dipstick test

The dipstick tests were done using the UROCOLORTM2: SD urinalysis strips. Two random samples of urine collected by clean catch or from a urinary catheter and tested with a reagent strip; if the readings are noted as one plus or more when the urinary specific gravity is < 1030 and pH being less than 8, the condition is considered as significant proteinuria (1+ refers to the urinary albumin level as 0.3g/L and 2+ to 1g/L).

24 HUP

The urine was stirred to ensure homogeneity and a 6-mL aliquot sample was obtained. Analysis for protein was performed using a modified Fujita method (Sigma Diagnostics Microprotein-PR, procedure No. 611). The formula used to determine the amount of urinary protein (U_p) based on the product of concentration of protein in the test sample (T_p) and the total volume of urine (U_v) is expressed as - U_p (mg/day) = U_v (dl) x T_p (mg/dl). Each sample was tested twice to calculate the mean value and each sample was paralleled with a low control and a high control. Comparison of this assay with other similar commercially available reagents shows a correlation coefficient of 0.997 for samples containing 1 mg/dl to 128 mg/dl. For those samples with significant proteinuria that exceed this value, the urine was diluted 1:10 with deionised

water to maintain the sensitivity of the assay. When the 24 hour urine collection has the total protein of 300 mg or greater, the condition is referred as significant proteinuria.

Creatinine

Modified Jaffe Method was used to estimate the urinary creatinine as per the instructions given in the Kit, Quimica Clinical Applicada S.A - Spain 2000. The principle behind this test is that when creatinine reacts with picric acid at alkaline pH values, it forms creatine alkaline picrate which has a distinct colour that can be measured photometrically. The serum creatinine was determined by using the same assay with 300 μ L of serum. Estimation of the creatinine clearance was calculated by using the formula - urine creatinine (mg/dl) x urine flow rate (ml/min) divided by serum creatinine (mg/dl). The tests were performed by a biochemist working in the laboratory of the hospital.

The various factors of outcome measured are:

1. Biochemical parameters: 24hr urine protein, random UPCR, Dipstick urine analysis,
2. Complications of pre-eclampsia:

Maternal: Eclampsia, abruption, HELLP syndrome, ARDS (Adult Respiratory Distress syndrome), Preterm labour, postpartum hemorrhage, other.

Fetal: IUD (Intrauterine Death), still birth, IUGR (Intrauterine Growth Retardation), birth asphyxia, Prematurity, Other

Statistical Analysis

13% Data obtained were entered into MS Excel sheet. The measurements obtained in the study were continuous as well as discrete variables. We used SPSS software version 19.0 for statistical analysis. Categorical variables were analyzed and expressed in percentages and proportions. The association between various categorical variables was found using Chi square test. The continuous variables were analyzed and expressed in descriptive statistics like *mean and SD*. Point estimate of variability was done by *standard error of mean*. The inferential statistics was done and expressed by the application of test of significance to check and estimate if there is any significant difference of mean of various measurements. The test of significance between the dependent variable and other factors as independent variables was done using $P < 0.05$ taken as significant level. Wherever required appropriate modality of graphical presentation was used. Pearson's correlation coefficient and Linear Regression were used to find correlation between the random UPCR and the 24-hour urine protein. Sensitivity and specificity were also calculated. At the end, a receiver operator characteristics (ROC) curve was drawn and area under curve was calculated. Spearman's rank order correlation coefficient and linear regression were used to find correlation between the dipstick test and the 24-hour urine protein; a receiver operator characteristics (ROC) curve was drawn and area under curve was calculated.

Observations and Results

The present prospective study was conducted on 209 pregnant women who were presented with hypertension ($\geq 140/90$ mmHg) and proteinuria (dipstick $\geq 1+$) after 20 weeks of gestation. Among 209 cases 157 were with preeclampsia (24 hr urine protein ≥ 300 mg), 52 cases were with gestational hypertension (24 hr urine protein < 300 mg). Only those 157 patients ($n=157$) who had significant proteinuria (≥ 300 mg in 24 hr urine protein) were included in the final analysis.

The majority of the patients were in the age group of 20 to 30 years (84.1%), more than half of the study group consisted of primigravida and two thirds of them being nulliparous (table 1). The period of gestation was > 37 weeks in half of the study group. The status of preeclampsia was severe in 89 cases (56.7%) and non-severe in 68 cases (43.3%). (Table 2)

Correlation between significant proteinuria by 24HUP test and UPCR (Table 3): The mean and standard deviations for 24HUP test and UPCR are 611.35/0.5900 and 510.462/0.50483 respectively for 209 subjects. There was a near perfect positive linear relationship between 24HUP test and UPCR. The value of $r=0.941$ indicates a good linear relationship. Pearson's correlation test showed a positive correlation between 24HUP test and UPCR ($r = 0.941$) with a $P < 0.001$ which was strongly significant. If 24HUP is an independent variable & the UPCR is a dependent variable, then the equation would be: $PCR = 0.021 + (0.001*24HRUP)$. If UPCR is an independent variable & the 24HUP is a dependent variable, then the equation would be: $24HUP = 50.025 + (951.475*UPCR)$. The ROC curve analysis showed that the optimal cut-off value of spot urine P/C ratio to detect significant proteinuria (>300 mg/day) was ≥ 0.25 , at which, the sensitivity was 100% and specificity was 90.60%. Area under curve was 0.990 (good test).

Correlation between significant proteinuria by 24HUP test and dipstick test (Table 4): A Spearman's rank-order correlation was run to determine the relationship between 24-h urine protein and dipstick test. There was a strong, positive correlation between 24-h urine protein and dipstick test, which was statistically significant ($r_s = 0.759$, $p < 0.001$). If dipstick grade is independent variable & the 24hr urine protein is dependent variable, then the equation would be as follows: $24HUP = -199.840 + (435.832*Dipstick \text{ grade})$. If 24hr urine protein is independent variable & the urine dipstick grade is dependent variable, then the equation would be as follows: $Dipstick \text{ grade} = .972 + (0.001*24HUP)$. The ROC curve analysis showed that the optimal cut-off value of dipstick grade to detect significant proteinuria (>300 mg/day) was >1.5 , at which, the sensitivity was 65.74% and specificity was 79.20%. Area under curve was 0.742.

Pregnancy outcome (maternal and fetal): (Table 5 and 6) Among 157 pre-eclampsia subjects 89 had severe variety 68 had non-severe pre-eclampsia and 68(43%) of all the study group had developed maternal complications, such as

abruptio placenta, antepartum-eclampsia, HELLP syndrome, imminent eclampsia, papilledema, partial HELLP, postpartum-eclampsia, PPH, preterm labour, pulmonary oedema in 11 cases with non-severe pre-eclampsia and 57 cases with severe pre-eclampsia; the most common were preterm labor, PPH and antepartum eclampsia in both the groups. Complications like abruptio placentae, HELLP syndrome, papilledema, postpartum eclampsia and pulmonary oedema were found exclusively in severe preeclampsia. The patients with severe preeclampsia had a greater number of emergency caesarean section (41/89) and preterm vaginal deliveries (35/89) compared to those with non-severe (22/68, 6/68) type with commonest indication being fetal distress and severe IUGR. Hence the number of full-term vaginal deliveries were more in non-severe type (36 vs 5). Among fetal complications, birth asphyxia (BA) and intrauterine growth retardation (IUGR) were the most common (60%) overall and particularly in those with 24HUP > 2 g/day, whereas in cases with 24HUP ≤ 2 g / day, perinatal death (75%)

Discussion

The entity of hypertensive disorders of pregnancy is a major cause of maternal and perinatal mortality worldwide, of which preeclampsia remains the leading cause. Proteinuria with hypertension in pregnancy is associated with greater adverse maternal and fetal outcome. hence detection and frequent monitoring will be helpful in timely decisions. Therefore, reliable quantitative measurement of urinary protein excretion which will be quick and easy to perform and correlate well with the gold standard 24 HUP test is required. Age has an important influence on the incidence of hypertensive disorders of pregnancy with less risk of developing preeclampsia in the age group between 20 to 30 years, whereas the occurrence is more in the teen age and after 30 years of age during pregnancy in general population.¹³ In our study, the mean age of preeclampsia was 25.5 years and the maximum being between 20 to 30 years and hence the confounding effect of age factor has been eliminated in the present study; Majority 87/157 (55.4%) were primigravida and 105/157(66.9%) were nulliparous women similar to the data published by Aggarwal N et al,¹⁴ but in Kumari A et al¹⁵ study majority of the patients were multigravidas. A systematic review of controlled trials by DuckittK et al (2005)¹⁶ observed nulliparity (RR 2.91, 95% CI) as an unadjusted relative risk factor for developing preeclampsia. The mean gestational age was 35 ± 4 weeks similar to previous studies,^{15,17,18} in Aggarwal N et al¹³ study the mean gestational age was 32 weeks which may be due to a greater number of severe cases.

In our study, the UPCR was well correlated with 24HUP with overall strength of correlation $r=0.941$ with p value < 0.001 , similar to previous studies^{15,17,18} and such correlation will reflect the accuracy of UPCR. The optimal cut-off value of UPCR that predicts significant proteinuria in 24-hour urine sample (≥ 300 mg) was found to be ≥ 0.25 as

demonstrated by the area under ROC curve of 0.990, at which the sensitivity was 100% and specificity was 90.6%.

In Aggarwal N et al (2008)¹⁴ study the optimal cut-off value of spot UPCR that predicts significant proteinuria was ≥ 1.14 with 72% sensitivity and 75% specificity which indicates that the UPCR is a poor predictor of 24-hour proteinuria. Few other studies.^{9,15} demonstrated that there was poor correlation between 24-HUP and UPCR at higher degree of proteinuria. These differences could be due to lack of uniformity and variable laboratory methods in estimation of proteinuria and variation in mean 24HUP values which precludes valid comparison among the studies. In our study the strong correlation may be due to majority of cases having proteinuria between 300 to 1000mg/day.

Moreover, the correlation between significant proteinuria by 24HUP & dipstick test was statistically significant ($r_s = 0.759$, $p < 0.001$) in our study whereas with other studies¹⁷ the dipstick method failed to show significant correlation with the 24HUP test in the estimation of proteinuria; similar results of poor correlations have been seen in the studies involving patients of lupus nephritis and gestational hypertension,^{19,20} this may be due to the inter-observer variation in noting the colour of the strip at different points of time could be one of the reasons. At times it is difficult to identify the colour accurately, especially when the colour is not exactly matching with any one (as 1+, 2+, 3+, or 4+) having intermediate hue. In our study 52 subjects out of 209 subjects had false positive

dipstick test, as ours included a greater number of cases (n=209) compared to the other studies and the correlation between dipstick and 24 HUP was high ($r=.759$, $p<0.001$)

Our study shows that the severity of adverse maternal outcome was maximum in subjects who had $>1\text{gm}$ protein in 24-hour urine and preterm delivery and eclampsia were being the most common complications. Among 157 pre-eclampsia subjects 85 patients had adverse foetal outcome and the perinatal mortality was maximum (11 cases out of 15 cases) in subjects who had 24HUP $>1\text{g}$. Waugh et al., (2005a)⁶ predicted adverse foetal outcome in patients with 24HUP $\geq 500\text{mg}$ in their study. Hypertension together with proteinuria is associated with poor foetal outcome, small for gestational age pregnancies, perinatal mortality and maternal morbidity (Chan et al., 2005⁵⁷⁽²⁰⁾; Brown et al., 1996⁵⁸⁽²¹⁾; Ferranzani et al., 1990⁵⁹⁽²²⁾; Chua and Redman, 1992⁶⁰⁽²³⁾; Lin et al., 1982⁶¹⁽²⁴⁾). Newman et al., (2003)^{62 (25)} found in a retrospective review that the magnitude of proteinuria, even when massive ($>10\text{g}/24$ hours), did not correlate with increased maternal or neonatal morbidity in preeclampsia. They concluded that neonatal morbidity appeared to be more a function of prematurity rather than massive proteinuria itself. In our study none of the cases had massive proteinuria. Only 4 cases had proteinuria $>2\text{gm}$. So, we could not conclude the relationship between the proteinuria $>2\text{gm}$ and maternal and foetal outcome.

Table 1:

Demographic character		No. patients (n=157)	%
Age	<20	4	2.5
	20-30	132	84.1
	>30	21	13.4
Gravida	Primi	87	55.4
	Second	42	26.8
	Third	22	14.0
	fourth	6	3.8
Parity	Nulliparous	105	66.9
	P1	40	25.5
	P2	11	7.0
	P3	1	6

Table 2: Distribution of subjects as per gestational age

Period of Gestation (weeks)	Pre-eclampsia					
	Non-severe		Severe		Total	
	N	%	N	%	N	%
<28	1	1.5	9	10.1	10	6.4
28 to 34	2	2.9	28	31.5	30	19.1
34 to 37	10	14.7	28	31.5	38	24.2
>37	55	80.9	24	27	79	50.3
Total	68	100	89	100	157	100
Chi-square=47 df=3 p<0.001 Strongly significant						

Table 3: Correlation of 24HUP with UPCR and regression analysis

		Correlation			24HUP	UPCR		
24HUP test		Pearson Correlation “r”			1	.941**		
		Sig. (2-tailed)				.000		
		N			209	209		
UPCR		Pearson Correlation “r”			.941**	1		
		Sig. (2-tailed)			.000			
		N			209	209		
Coefficients: Dependent Variable - UPCR								
Model		USC		SC	T	Sig.	95.0% CI for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	.021	.019		1.137	.257	-.015	.058
	24HUP	.001	.000	.941	39.997	.000	.001	.001
Coefficients: Dependent Variable – 24HUP								
Model		USC		SC	T	Sig.		
		B	SE	Beta				
1	(Constant)	50.025	18.453		2.711	.007		
	UPCR	951.475	23.789	.941	39.997	.000		

** . Correlation is significant at the 0.01 level (2-tailed), N - Total number of patients, USC – Unstandardized coefficient, SC– Standardized coefficient, CI – confidence interval, SE Standard error

Table 4: Correlation of 24HUP with Dipstick – Spearman’s rho and regression analysis

		Correlation			24HUP	Dipstick
24HUP test		Correlation Coefficient			1.000	.759**
		Sig. (2-tailed)			.	.000
		N			209	209
Dipstick		Correlation Coefficient			.759**	1.000
		Sig. (2-tailed)			.000	.
		N			209	209
Coefficients: Dependent Variable – Dipstick grade						
Model		USC		SC	T	Sig
		B	SE	Beta		
1	(Constant)	.972	.061		15.897	.000
	24HUP	.001	.000	.796	18.947	.000
Coefficients: Dependent Variable – 24HUP						
Model		USC		SC	T	Sig.
		B	SE	Beta		
1	(Constant)	-199.840	47.867		-4.175	.000
	Dipstick	435.832	23.003	.796	18.947	.000

** . Correlation is significant at the 0.01 level (2-tailed), N- Total number of patients, USC– Unstandardized coefficient, SC – Standardized coefficient, CI – confidence interval, SE Standard error

Table 5:

Maternal complications		24 hours urine protein(mg/day)			
		>2000	1000 to 2000	300 to 1000	Total
Abruptio placenta	N (%)	0 (0)	4(66.7%)	2(33.3%)	6(100%)
AP-Eclampsia	N (%)	1(11.1%)	7(77.8%)	1(11.1%)	9(100%)
HELLP syndrome	N (%)	2(66.7%)	1(33.3%)	0(0)	3(100%)
Imminent eclampsia	N (%)	0 (0)	3(42.9%)	4(57.9%)	7(100%)
Papilledema	N (%)	0 (0)	1(100%)	0(0%)	1(100%)
Partial HELLP	N (%)	0 (0)	1(50%)	1(50%)	2(100%)
Postpartum eclampsia	N (%)	0 (0)	1(100%)	0(0)	1(100%)
PPH	N (%)	0 (0)	1(14.3%)	6(85.7%)	7(100%)

Preterm delivery	N (%)	0 (0)	6(19.4%)	25(80.6%)	31(100%)
Pulmonary oedema	N (%)	0 (0)	1(100%)	0(0)	1(100%)
No complications	N (%)	1(1.1%)	7(7.9)	81(91%)	89(100%)
Total	N (%)	4(2.5%)	33(21%)	120(76.4%)	157(100%)
Chi square=106, df=20 p < 0.001 Strongly significant. N- no. of patients					

Table 6: F et al complication relation

Adverse foetal outcome in relation to 24HUP	24 Hour Urine Protein (mg/day)							
	300 to 1000		1000 to 2000		>2000		Total	
	N	%	N	%	N	%	N	%
II trimester abortion	0	0	2	6.5	0	0	2	2.4
BA/IUGR	35	70	15	48.4	1	25	51	60
Perinatal Death	4	8	8	25.8	3	75	15	17.6
Prematurity	11	22	6	19.4	0	0	17	20
Total	50	100	31	100	4	100	85	100
Chi-square=17.9 df=6 p=0.006 Strongly significant								
Birth Weight (Kg)	Non-severe Preeclampsia		Severe Preeclampsia		Total			
	N	%	N	%	N	%	N	%
<1.5 kg	1	1.5	26	29.2	27	17.2		
1.5 to 2.5	14	20.6	46	51.7	60	38.2		
>2.5	53	77.9	17	19.1	70	44.6		
Total	68	100	89	100	157	100		
Chi-square=56 df=2 p < 0.001 strongly significant								
Fetal complications	Non-severe Preeclampsia		Severe Preeclampsia		Total			
	N	%	N	%	N	%	N	%
II trimester abortion	0	0	2	2.8	2	2.4		
BA/IUGR	6	46.2	45	62.5	51	60		
Peri-natal Mortality	0	0	15	20.8	15	17.6		
Prematurity	7	53.8	10	13.9	17	20		
Total	13	100	72	100.0	85	100		
Chi-square=12.3 df=3 p=0.006 strongly significant								

Conclusion

From the results of the present study and review of the earlier studies it can be concluded that, the use of spot UPCR test as alternative test to 24-HUP test can be much more cost effective as shown with many other studies previously. We have also found the 24-hour urine collection to be cumbersome and inconvenient for pregnant women. The dipstick method because of its simplicity and cost may be continued to be used as initial screening method. There was a poor maternal and perinatal outcome in those women with proteinuria >1gm.

Conflict of Interest: None.

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