

Correlation between 24-h urine protein, spot urine protein/creatinine ratio, and serum uric acid and their association with fetomaternal outcomes in preeclamptic women

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Abstract

Background: It is well-known that estimation of 24-h urine protein and spot urine protein/creatinine (P/C) ratio are commonly performed investigations to assess proteinuria in preeclamptic women. Serum uric acid has been shown to correlate well with disease severity in preeclampsia. **Materials and Methods:** A total of 24-h urine protein estimation, spot urine P/C ratio, and serum uric acid measurements were carried out in 75 pregnant preeclamptic women and the correlation between these investigations, as also the association between proteinuria and hyperuricemia with adverse fetomaternal outcomes were studied. **Results:** Pearson's correlation test showed a positive correlation between 24-h urine protein and spot urine P/C ratio. A statistically significant and direct correlation was also found between serum uric acid and spot urine P/C ratio, while there was no statistically significant difference between proteinuria and hyperuricemia with respect to the various fetal and maternal outcome parameters studied. **Conclusion:** In the present study, we found a moderate correlation between 24-h urine protein and spot urine P/C ratio. There was a moderate correlation between spot urine P/C ratio and uric acid, while there was no statistical significance of the association between proteinuria and uric acid with fetomaternal outcomes in preeclampsia.

Key words: Feto-maternal outcomes, hyperuricemia, preeclampsia, 24-h urine protein, serum uric acid, spot urine protein/creatinine ratio

INTRODUCTION

Preeclampsia is a form of hypertension unique to human pregnancy. Though the exact incidence is unknown, it ranges from 3-10% in nulliparous population.^[1] It is a significant public health threat globally contributing greatly to maternal and perinatal mortality and morbidity. It contributes to about 24% of maternal deaths in India.^[2-4] Preeclampsia

is a multisystem disorder characterized by widespread endothelial leak.

Extensive changes occur in the kidneys as part of the end-organ damage in preeclampsia. Proteinuria occurs as a consequence of reduction in the integrity of glomerular barrier or reduced tubular reabsorption. It remains an important objective criterion for diagnosis of preeclampsia and has been used to classify the severity as well as to predict adverse fetomaternal outcomes in preeclampsia.^[1] Among the various methods available to quantify proteinuria, 24-h urinary protein estimation remains the gold standard. Alternative methods like a spot urine sample protein-creatinine (P/C) ratio avoid the influence of variations in urinary solute concentrations and can reduce the delay in diagnosis and management of preeclamptic patients.

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Another laboratory abnormality which arises due to renal affection in preeclampsia is hyperuricemia. Though, serum uric acid level is of limited use in the initial diagnosis of preeclampsia, many investigators have shown that it correlates well with the disease severity.

In this study, we have tried to correlate spot urine P/C ratio with 24-h urine protein estimation and test its diagnostic accuracy for detecting significant proteinuria. We have also correlated spot urine P/C ratio with serum uric acid levels and tried to analyze the association between proteinuria and hyperuricemia with adverse fetomaternal outcomes in preeclamptic women.

MATERIALS AND METHODS

This observational correlation clinical study was conducted after obtaining clearance from the hospital ethics committee. This was a prospective study conducted between November 2010 and May 2012, among pregnant women admitted to the hospital with a suspicion of preeclampsia.

Primigravida with singleton pregnancy, with cephalic presentation, and a diagnosis of preeclampsia were included in the study. Preeclampsia was diagnosed when blood pressure was 140/90 mm Hg or more, on two occasions, at least 4 h apart, or a single diastolic reading of ≥ 110 mm Hg after the 20th week of pregnancy, and the presence of proteinuria of $\geq 1+$ as detected by dipstick urine analysis. Women with previous renal disease, chronic hypertension, urinary tract infection, pathological vaginal discharge, and those who required delivery before the completion of a 24-h urine sample were excluded from the study. A total of 75 pregnant women who satisfied the inclusion and exclusion criteria were recruited after informed written consent for participation.

Participants were asked to collect a random midstream urine sample for estimating the spot urine P/C ratio. They were then instructed to collect the 24-h urine starting from the second urine sample in the morning (i.e., after discarding the first morning specimen) till the first urine sample the next day morning. The urine P/C ratio was obtained by

dividing the urinary protein concentration by the urinary creatinine concentration. Urine protein was measured by the urinary-cerebrospinal fluid protein method, which is an adaptation of the pyrogallol red-molybdate method. Urine creatinine was measured by the CREA method, which is a modification of the Jaffe's reaction. Both the tests were performed by an automated analyzer. Serum uric acid was assessed using automated photospectrometric assay approved by the International Federation of Clinical Chemistry for all the patients. All the patients were followed up till their delivery and the various parameters for maternal and fetal outcomes were noted. Fetal outcomes noted were Apgar scores at 1 and 5 min, neonatal intensive care unit (NICU) admission, birth weight, any other neonatal complications like neonatal sepsis, stillbirth, seizures, and so on. Adverse maternal outcomes were defined as any maternal complications like abruption, eclampsia, postpartum hemorrhage, disseminated intravascular coagulation (DIC), hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, and so on.

Pearson's correlation coefficient expressed as 'r' was used to correlate between spot urine P/C ratio, 24-h urine protein and serum uric acid levels. Chi-square test expressed as 'P' was used to study the levels of spot urine P/C ratio, 24-h urine protein and serum uric acid level in predicting the fetomaternal outcomes. Receiver operating characteristic (ROC) curve analysis was done to evaluate the diagnostic accuracy of spot urine P/C ratio to detect significant proteinuria (>300 mg/day). The statistical software SAS 9.2 was used for the analysis of the data and Microsoft Word and Excel were used to generate graphs, tables, and so on.

RESULTS

A total of 75 pregnant women with preeclampsia were recruited in the study after meeting our criteria. Demographic variables are shown in [Figure 1a-c]. Of these, 73.3% had mild preeclampsia (proteinuria of 1+ using urine dipstick analysis) and 26.7% had severe preeclampsia (proteinuria of $\geq 2+$ with urine dipstick). [Table 1] shows the distribution of the patients according to their 24-h urine protein, spot urine P/C ratio,

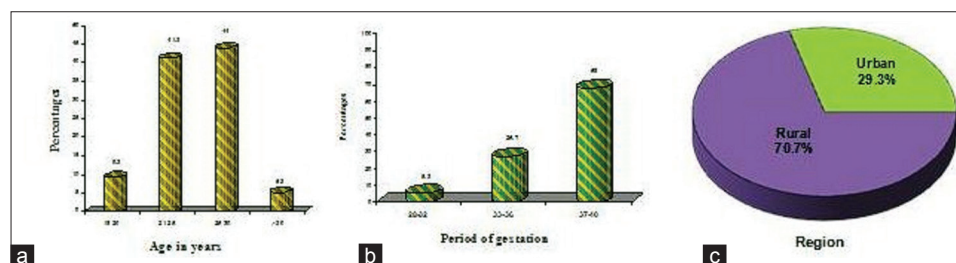


Figure 1: Demographic data: (a) Age in years, (b) Period of gestation and (c) Region-wise distribution

and serum uric acid levels. The Pearson's correlation test showed a positive correlation between 24-h urine protein and spot urine P/C ratio ($r = 0.373$) with a $P = 0.001$ which was strongly significant [Table 2]. Scatter diagram demonstrated a linear correlation between spot urine P/C ratio and 24-h urine protein [Figure 2]. ROC curve analysis showed that the optimal cut-off value of spot urine P/C ratio to detect significant proteinuria (>300 mg/day) was >0.6, at which, the sensitivity was 73.53% and specificity was 65.85%. Area under curve (AUC) was 0.799 (good test) [Figure 3]. Though spot urine P/C ratio had a high sensitivity of 96.9%, but specificity was only 19% at cut-off value of spot urine P/C >0.3 for significant proteinuria. A statistically significant and direct correlation was also found between serum uric acid and spot urine P/C ratio ($r = 0.355$, $P = 0.002$) [Table 2].

Though there was no statistically significant association between proteinuria and hyperuricemia with the various fetal and maternal outcome parameters studied, as shown in Tables 3, 4, and 5, we had three maternal complications, namely, abruptio placentae, intrapartum eclampsia, and HELLP syndrome, which occurred in patients with significant proteinuria and elevated uric acid levels. There were 11 intrauterine growth restrictions (IUGRs) in our study and it was most of the time (89%) associated with significant proteinuria. Only 54.5% of IUGRs was associated with hyperuricemia. A stillbirth occurred in a patient with severe preeclampsia, induced at 30 weeks, who had significant proteinuria (534 g/day), a spot P/C ratio of 0.9 and normal serum uric acid levels.

DISCUSSION

Preeclampsia is a multisystemic disorder with endothelial dysfunction which affects 3-5% of all pregnancies and contributes greatly to fetomaternal morbidity and mortality. Renal dysfunction leading to proteinuria (a diagnostic hallmark of preeclampsia), along with hyperuricemia have been used to predict adverse pregnancy outcomes. 24-h urine protein estimation has been considered the gold standard for testing proteinuria but has the disadvantage of consuming time, delaying diagnosis, and thus delaying initiation of appropriate management. Alternative testing methods like P/C ratio on a single random urine sample

have correlated well with the gold standard. This study was conducted from November 2010-May 2012, to estimate the diagnostic accuracy of spot P/C ratio, to evaluate efficacy of 24-h urinary protein, spot urine P/C ratio and serum uric acid in predicting the outcomes in preeclampsia and to correlate spot urine P/C ratio with serum uric acid levels.

In our study, the mean age was 25.35 years and the mean gestational period was 36.9 weeks among 75 preeclamptic

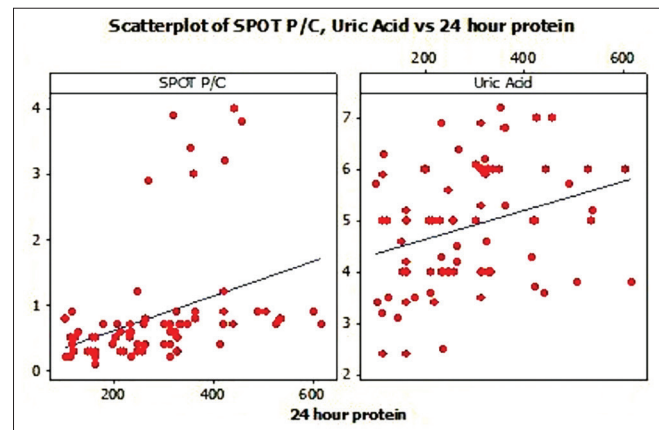


Figure 2: Scatter diagram showing correlation between spot urine protein/creatinine ratio and 24-hour urine protein as well as between serum uric acid and 24-hour urine protein

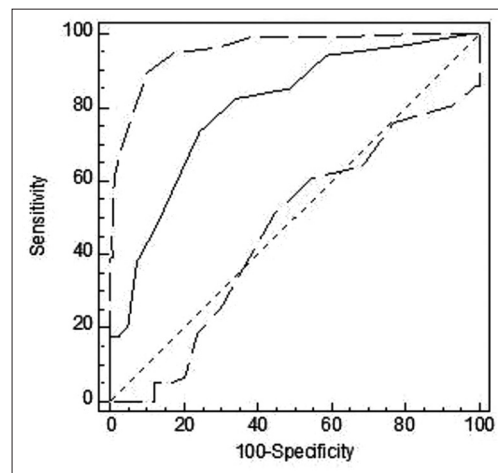


Figure 3: Receiver operating characteristic curves of spot urine protein/creatinine ratio (bold curve) in reference to 24-hour urine protein (upper dashed curve) and serum uric acid (lower dashed curve). The cut-off value of spot P/C ratio is >0.6, for which sensitivity is 73.53% and specificity is 65.85% with ROC Area Under Curve of 0.799 (Good test)

Table 1: Distribution of patients according to 24-h urine protein, spot protein/creatinine ratio, and serum uric acid levels

24-h urine protein (mg/day)			Spot urine P/C ratio		Serum uric acid (mg/dL)		
<300	300-1000	1000	<0.3	>0.3	<3.5	3.5-5.5	>5.5
41 (54.7%)	34 (45.3%)	-	9 (12.3%)	66 (87.7%)	8 (10.6%)	42 (56.2%)	25 (33.2%)
Total=75			Total=75		Total=75		

P/C: Protein/creatinine

Table 2: Pearson's correlation between 24-h urine protein, spot urine protein/creatinine ratio, and serum uric acid

	Pearson's correlation	P value
24-h urine protein vs. serum uric acid	0.118	0.314
24-h protein vs. spot urine P/C ratio	0.373	0.001**
Serum uric acid vs. spot P/C ratio	0.355	0.002**

P/C: Protein/creatinine

Table 3: Fetomaternal outcomes with 24-h urine protein

Fetal outcomes	24-h urine protein (mg/day) (%)		P value
	<300 (n=41)	300-1000 (n=34)	
Birth weight (g)			
<1500	4 (9.8)	6 (17.6)	0.173
1500-2500	9 (22)	12 (35.3)	
2500-3500	27 (65.9)	14 (41.2)	
>3500	1 (2.4)	2 (5.9)	
Apgar score at 1 min			
<7	6 (14.6)	8 (23.5)	0.381
>7	35 (85.4)	26 (76.5)	
5 min			
<7	1 (2.4)	1 (2.9)	1.000
>7	40 (97.6)	33 (97.1)	
NICU admission			
Yes	6 (14.6)	13 (38.2)	0.11
Fetal complications			
Yes	4 (9.8)	9 (26.5)	0.071
Maternal complications			
Yes	0 (0)	3 (8.8)	0.089

NICU: Neonatal intensive care unit

Table 4: Fetomaternal outcomes with spot urine protein/creatinine ratio

Fetal outcome	Spot P/C (%)		P value
	<0.30 (n=9)	>0.30 (n=66)	
Birth weight (g)			
<1500	0 (0)	10 (15.2)	0.645
1500-2500	2 (22.2)	19 (28.8)	
2500-3500	7 (77.8)	34 (51.5)	
>3500	0 (0)	3 (4.5)	
Apgar score at 1 min			
<7	0 (0)	14 (21.2)	0.195
>7	9 (100)	52 (78.8)	
5 min			
<7	0 (0)	2 (3)	1.000
>7	9 (100)	64 (97)	
NICU admission			
Yes	4 (44.4)	25 (37.9)	0.727
Fetal complications			
Yes	0 (0)	13 (19.7)	0.345
Maternal complications			
Yes	0 (0)	3 (4.5)	1.000

NICU: Neonatal intensive care unit, P/C: Protein/creatinine

Table 5: Fetomaternal outcomes with serum uric acid

Fetal outcome	Uric acid (mg/dL) (%)			P value
	<3.5 (n=8)	3.5-5.5 (n=42)	5.5 (n=25)	
Birth weight (g)				
<1500	0 (0)	5 (11.9)	5 (20)	0.670
1500-2500	1 (12.5)	13 (31)	7 (28)	
2500-3500	7 (87.5)	22 (52.4)	12 (48)	
>3500	0 (0)	2 (4.8)	1 (4)	
Apgar score at 1 min				
<7	0 (0)	7 (16.7)	7 (28)	0.232
>7	8 (100)	35 (83.3)	18 (72)	
5 min				
<7	0 (0)	2 (4.8)	0 (0)	0.622
>7	8 (100)	40 (95.2)	25 (100)	
NICU admission				
Yes	3 (37.5)	16 (38.1)	10 (40)	1.000
Fetal complications				
Yes	1 (12.5)	7 (16.7)	5 (20)	0.906
Maternal complications				
Yes	0 (0)	0 (0)	3 (12)	0.063

NICU: Neonatal intensive care unit

women. We found a moderate correlation between 24-h urine protein and spot urine P/C ratio which was statistically significant ($r = 0.373$, $P < 0.001$).

The ROC curve analysis revealed a sensitivity of 73.53% and specificity of 65.85% with AUC 0.799 (good test) for a cut-off value of spot P/C >0.6 to detect significant proteinuria. In a similar study done by Aggarwal *et al.*,^[5] in 120 preeclamptic women, the mean age was 26 years and mean gestational age was 32 weeks. They reported a significant association between the two tests with a correlation coefficient of $r = 0.596$ ($P < 0.01$) and the sensitivity and specificity of spot P/C at a cut-off value >1.14 of 72% and 75%, respectively. But they observed that the values of the spot urine P/C correlated well at higher levels of proteinuria. Thus, they concluded that the test could not rule out mild preeclampsia, and hence should not be used to replace 24-h urine protein estimation.

Wheeler *et al.*,^[6] conducted a study among 126 patients admitted for evaluation of preeclampsia and reported a strong correlation of random spot urine P/C ratio with 24-h urine protein levels (Pearson's $r = 0.88$). The optimal P/C cutoff was 0.21 (300 mg per 24 h) and 3.0 (5000 mg per 24 h). AUC was 0.86 for cut-off values of 0.21 and 1.0 for cut-off values of 3.0. All of them showed excellent accuracy. They concluded that though there is a strong association between the spot urine P/C ratio and 24-h urine protein excretion, the former lacks the ability to measure proteinuria quantitatively.

Other reports have given conflicting results and report that 24-h urine collection should remain the standard for

evaluation of preeclampsia. Durnwald and Mercer^[7] in their comparative study between 24-h urine protein and spot urine P/C ratio among 220 preeclamptic women had a mean age of 26.1 years and gestational age of 36.5 weeks. They reported a poor correlation with coefficient of 0.41 between 24-h urine and spot urine P/C ratio. The ROC analysis revealed no clear shoulder although the AUC was 0.8 with a sensitivity of 55.8% and specificity of 81% at a cut-off value of 0.3 for spot urine P/C ratio. Morris *et al.*,^[8] in their systematic review and meta-analysis concluded that on an average, across all studies the optimal threshold of spot P/C ratio to detect significant proteinuria is between 0.30 and 0.35, relating to sensitivity and specificity values above 75%.

Proteinuria in preeclampsia occurs due to altered glomerular permeability and/or changes in tubular reabsorption of filtered proteins. The severity of proteinuria has been regarded as a predictor for adverse maternal and fetal outcomes. A 24-h urine protein greater than 300 mg has been considered to be significant proteinuria. In our study, incidence of very low birth weight (< 1500 g) increased from 9.8% to 17.6% as the 24-h urine protein exceeded 300 mg.

NICU admission increased from 19.3% to 38.2% and fetal complications like IUGR and neonatal sepsis from 9.8-26.5% at 24-h urine protein values of 300 mg or more. There was one stillborn when the value of 24-h urine was more than 300 mg. There were three maternal complications namely, one abruptio placentae, one intrapartum eclampsia, and one HELLP syndrome, all occurring when 24-h urine protein was >300 mg. But overall, the differences in maternal and fetal outcomes related to 24-h urine protein levels greater than or less than 300 mg did not attain statistical significance. Similar findings were reflected in the prediction of adverse fetomaternal outcomes using spot P/C ratio. There was no NICU admission, very low birth weights, fetal complications in women with spot P/C < 0.3. Whereas 15.2% had very low birth weight, 37.95% had NICU admission and 19.7% had fetal complications with spot urine P/C >0.3. All the three maternal complications were present when spot urine P/C was >0.3. But none of the fetal and maternal parameters had significant association with spot P/C. Literature search revealed only one study which compared spot urine P/C in random urine samples with adverse outcomes in hypertensive pregnant women. This study done by Martins Costa *et al.*,^[9] retrieved the medical charts of 370 hypertensive pregnant women and divided them into three groups based on the spot urine P/C values (group 1: < 0.3, group 2: 0.3-1.99, group 3 \geq 2.0). They compared the groups with composite maternal and perinatal outcomes like, DIC, HELLP, eclampsia, thrombocytopenia, neonatal sepsis, perinatal death, and small for gestational age. They reported that

at random spot urine P/C values >0.3, there was a high probability of having unfavourable maternal and fetal clinical outcomes, but additional increments were not associated with worsening of such outcomes, indicating the usefulness of the test to be diagnostic but not as a monitor of clinical worsening. Newman *et al.*,^[10] concluded in their study that women with preeclampsia and massive proteinuria did not have increased maternal morbidity compared with women with severe or mild proteinuria.

A systematic review of 16 studies for estimation of proteinuria as a predictor of complications of preeclampsia concluded that measure of proteinuria is a poor predictor of either maternal or fetal complications. This study conducted by Thangaratinam *et al.*,^[11] questioned the commonly practised management decisions based on severity of proteinuria in preeclamptic women and highlighted the need for large well-designed prospective studies to address this important issue.

Hyperuricemia is believed to be a contributor to the pathogenesis of preeclampsia. Increase in uric acid levels is a key clinical feature of preeclampsia. Higher levels correlate with significant maternal and fetal morbidity and mortality. The utility of serum uric acid as a marker of severity of preeclampsia has been substantiated by several studies. Yassaee^[12] found a strong correlation between Apgar scores less than 7 and serum uric acid levels \geq 6 mg/dL. In our study there was no statistical significance between Apgar scores and uric acid levels. There were five IUGRs (45%) in women who had uric acid levels more than 5.5 mg/dL and the same number in uric acid levels 3.5-5.5 mg/dL. We observed no differences in the occurrence of fetal complications, NICU admissions between the levels of uric acid 3.5-5.5 and >5.5 mg/dL. Yassaee^[12] reported an (Intra-uterine death) IUD rate of 22% at uric acid levels more than 6 mg/dL. In the present study, no still births were found at levels >5.5mg/dL with only one stillbirth at uric acid level 3.5-5.5 mg/dL (2.3%). Overall, we found no statistical significance between serum uric acid levels and the various parameters of fetal outcome in our study.

The three maternal complications in our study, namely, abruptio placentae, HELLP, and intrapartum eclampsia occurred when the uric acid levels were >5.5 mg/dL, and we thought, this had a suggestive significance ($P < 0.063$). Yaasae^[12] noted that 22% had eclampsia with serum uric acid >6mg/dL in their study. They showed a strong significant ($P < 0.01$) relationship of hyperuricemia with maternal outcomes in their study. A retrospective analysis by Parrish *et al.*,^[13] showed adverse maternal outcomes in 15.3% of 258 persons in their cohort study. In the present study, adverse maternal outcomes occurred in only

4% of the 75 patients studied. In a systematic review of accuracy of serum uric acid in predicting complications of preeclampsia, Thangaratinam *et al.*,^[14] compared 18 studies with a total of 3913 women. He concluded that serum uric acid is a poor predictor of maternal and fetal complications in women with preeclampsia. In this study as well, we could not confirm the association between serum uric acid and fetomaternal outcomes.

The magnitude of proteinuria and hyperuricemia has been correlated with the severity of renal histological finding of glomerular endotheliosis. If so, is there any association between levels of proteinuria and serum uric acid? We tried to correlate proteinuria using spot P/C and 24-h urine protein with serum uric acid levels. Correlation coefficients showed a moderate correlation with urine spot P/C ratio and uric acid ($r = 0.355$, $P < 0.002$) and poor correlation with 24-h urine protein and uric acid level ($r = 0.118$, $P = 314$). However, no studies have been reported till date regarding this and the sample size is very small to generalize the results. Future research can enlighten us in this regard.

There are several limitations in our study. The sample size was small. There was no blinding of the results of spot P/C ratio which might have influenced decisions regarding management of patients to avoid complications. As preeclamptic women on drugs were not excluded, the influence of these drugs on parameters studied might have been obscured. There were no patients with proteinuria more than 1 g, so we could not assess the impact of high levels of proteinuria on pregnancy outcomes.

CONCLUSION

In the present study, we found a moderate correlation between 24-h urine protein and spot urine P/C ratio which was statistically significant ($r = 0.373$, $P < 0.001$). The optimal cut-off value of spot urine P/C ratio for significant proteinuria was >0.6 at which the sensitivity was 73.53% and specificity was 65.85%. The ROC curve analysis for spot urine P/C ratio had an AUC of 0.799 (good test). There was a moderate correlation between spot urine P/C ratio and serum uric acid ($r = 0.355$, $P < 0.002$). There was no statistically significant association between proteinuria and serum uric acid with fetomaternal outcomes in preeclampsia.

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