# Spot urinary protein creatinine ratio with 24 hour urine protein in hypertensive disorders of pregnancy- a comparative study

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**Abstract**
Hypertensive disorders in pregnancy are the leading causes of maternal and fetal morbidity & mortality. Preeclampsia accounts for most of the hypertensive disorders. Quantification of proteinuria is important for making diagnosis and treatment. As the 24 hour urine protein estimation is time consuming and cumbersome, spot urinary protein creatinine ratio had been studied as an alternative method.
**Objectives:** 1. To assess the accuracy of a single spot urinary Protein creatinine ratio (PCR) in proteinuria quantification over 24 hour proteinuria and to assess whether a single spot urinary PCR can be used as a replacement for 24 hour urine protein measurement. 2. To determine the best cut off value of spot PCR for significant proteinuria. 3. To find timing of PCR that correlates well with 24 hour urine protein estimation.
**Methodology:** Prospective study was conducted at the department of obstetrics and gynaecology, Government medical college, Kozhikode, from April 2014 to May 2015. Three hundred antenatal women of >28 weeks gestation who were admitted with
hypertension complicating pregnancy were included in the study. All the women were advised to collect urine for 24 hour period.
Three spot samples of urine were collected following 24 hour collection by noon, night and next day morning. Correlation between PCR and 24 hour urine protein excretion was assessed by Pearson Correlation Coefficient. Receiver Operator Characteristic Curve ROC) analysis was used to find the best cut off value of PCR for predicting significant proteinuria and to know the best time for doing PCR.
**Results:** The PCR value correlated well with 24 hour urine protein estimation, with the correlation coefficient r=0.69(morning PCR),0.76(noon),0.71(night).ROC analysis showed the area under the curve(AUC) for PCR (morning, noon, night) to be 0.79, 0.95, 0.9 respectively with maximum being noon and the optimal cut off for predicting significant proteinuria at morning, noon, and night to be 0.47, 0.40 and 0.45, with sensitivity and specificity values of 89% and 91% with PCR at noon.
**Conclusion:** The present study showed good correlation between PCR and 24 hour urine protein. So PCR can be used as an alternative to 24 hour urine protein. The best cut off value of PCR for

estimating significant proteinuria was 0.4 with sensitivity of 89% and specificity of 91% showing PCR measurement at noon to be taken as best timing of performing PCR.

## Introduction

Hypertensive diseases affect approximately 5-10% of all pregnancies and are the leading causes of maternal and foetal morbidity and mortality.[1]Hypertensive disorders of pregnancy includes Gestational hypertension, preeclampsia and eclampsia, chronic hypertension and preeclampsia superimposed on chronic hypertension. Sixteen percent of maternal deaths in developed countries were reported to be due to hypertensive disorders.[2]

Preeclampsia, which accounts for most hypertensive disorders of pregnancy is defined as gestational hypertension with significant proteinuria (Significant proteinuria is ≥0.3 g /24hours-International society for the study of hypertension in pregnancy).Quantification of proteinuria in pregnancy is important for making diagnosis and also for predicting maternal and fetal outcome. The gold standard for diagnosis of significant proteinuria is 24-hour urine protein, but however, 24-hour urine collection is time consuming, cumbersome and is associated with delayed diagnosis and management. A need therefore exists for a rapid, as well as a valid and accurate test to identify significant proteinuria. Various methods have been used to shorten the time period to diagnose proteinuria in preeclampsia like dipstick method and spot protein creatinine ratio (PCR). International Society for the Study of Hypertension in Pregnancy have proposed use of the urinary spot protein: creatinine ratio (urinary PC ratio) as an alternative to 24hour urine protein. There are various studies on urinary PC ratio compared with 24 hour urine protein but the results are discordant[.3, 4] .Thus, this study was conducted to determine the diagnostic accuracy of urine protein-creatinine ratio in prediction of 24 hour urine protein among women with hypertensive pregnancy disorder.

## Objectives:

1. To assess the accuracy of a single spot urinary protein creatinine ratio (PCR) in proteinuria quantification over 24 hour proteinuria and to assess whether a single spot urinary PCR can be used as a replacement for 24hour urine protein measurement.
2. To determine the best cut-off value of the spot PCR for estimating significant proteinuria.
3. To find out the timing of PCR that correlates well with 24 hour urine total protein estimation.

**Study design:** Prospective study

## Materials and methods

The Prospective study was conducted in patients admitted at Department of Obstetrics and gynaecology, Government medical college, Kozhikode, a tertiary care centre, between April 2014 and May 2015.The study was certified by the Institutional ethics committee and informed consent was obtained from the participants. Three hundred antenatal women of more than 28 weeks gestation who were diagnosed to have hypertension between the age group 18-35 years were selected. Women with chronic hypertension, intrinsic renal disease, urinary tract infection, connective tissue disorders, and diabetes were excluded from the study. After admission, detailed history taken and clinical examination was done. These subjects were investigated and treated according to the existing protocol in the

department. The subjects were advised to collect urine for 24-hour period starting from the second sample after discarding the sample at the time of starting of collection, time is fixed. Three spot samples of urine were collected after the 24 hour collection was over by noon, night and by next day morning (second morning sample).

Urinary protein estimation was done by Biuret method, and urinary creatinine estimation was done by modified Jaffe's method. Urine PCR is obtained by dividing urinary protein concentration by urinary creatinine.

## Statistical Analysis

Correlation between Protein-creatinine ratio and 24-hour urine protein excretion was assessed by Pearson correlation coefficient. The receiver operating characteristic curve (ROC) analysis, with the area under the curve is estimated with a 95% confidence interval (CI).ROC analysis was used to know the best timing of PCR and to find the best cut-off value of PCR. Sensitivity and specificity of the urine protein: creatinine

ratio at best cutoffs for prediction of significant proteinuria was estimated. Diagnostic value of PCR was expressed in terms of specificity and sensitivity. Analysis were performed with the SPSS and MedCalc Software statistics

## Observations and results

Correlation of 24 hour urine protein with spot urinary protein creatinine ratio
In this study, out of 300 patients, 218 (72.6%) patients had significant proteinuria of ≥300mg/24 hour urine. (Table 1)
Using the standard cut-off of 0.3, PCR using morning, noon and night samples showed 240,236 and 255 patients had significant proteinuria respectively.

## Correlation coefficient:

The correlation statistics was done between 24 hour proteinuria and spot protein creatinine ratio (morning, noon and night) showed correlation coefficient (r)values of 0.69,0.76 and 0.71 respectively .This showed a good correlation between the two.

**Table: 1 Significant proteinuria (using 24 hour urine protein)**

| 24 hour Urine Protein | No. of Cases | Percentage |
|---|---|---|
| < 300 mg/day | 82 | 27.3 |
| ≥300 mg/day | 218 | 72.6 |
| Total | 300 | 100 |

Protein creatinine ratio estimated in all these women with morning, noon and night urine sample

**Table: 2 Significant proteinuria (using Protein creatinine ratio at standard cut-off)**

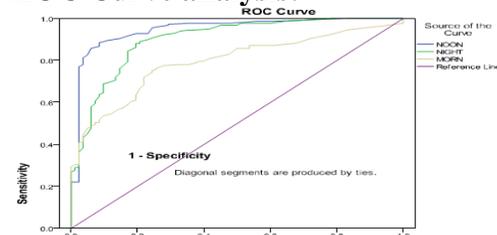| Standard cut off | PCR (morning) $n$ (%) | PCR (noon) $n$ (%) | PCR (night) $n$(%) |
|---|---|---|---|
| <0.3 | 60 (20%) | 64 (21.33%) | 45 (15%) |
| >0.3 | 240 (80%) | 236 (78.66%) | 255 (85%) |
| Total | 300 | 300 | 300 |

## ROC Curve analysis:



**Figure: 1 Best timing of protein creatinine ratio.**

In this study, the area under the curve for protein creatinine ratio (morning, noon and night) are 0.79, 0.95, 0.90 respectively with maximum being with noon. So this study shows that the best timing of performing protein creatinine ratio is at noon. (ii) Cut-off value: Using the ROC analysis, the best cut off value of protein creatinine ratio for significant proteinuria is 0.4(with PCR at noon)

The optimal cut off of PCR (Noon) is 0.40 with sensitivity of 89% and specificity of 91%. Using the ROC analysis, the best cut off value of protein creatinine ratio for significant proteinuria is 0.47(with PCR at morning) with sensitivity of 75% and specificity of 75%.Using the ROC analysis, the best cut off value of protein creatinine ratio for significant proteinuria is 0.45(with PCR at night)with sensitivity of 86% and specificity of 80%. The maximum sensitivity and specificity values were obtained with PCR noon values, compared with the night and morning values.

**Maternal characteristics and proteinuria**

Patients with significant proteinuria of >300mg were having high blood pressure of systolic 154.8± 8.2 mmHg and diastolic of 100.6 ±4.6 mmHg which was statistically significant with p value of <0.001. (Table 4). Out of 300 patients, 252 were having normal body mass index between 19 to 24.9 Kg/m$^2$. Of this 48 (19.04%) had significant proteinuria. Out of 48 overweight patients, 22 (45.83%) had significant proteinuria. (p value 0.32, not statistically significant).

The mean systolic and diastolic blood pressure is high in patients with proteinuria≥300mg, which is statistically significant with p value of <0.001.

**Mode of delivery**

Regarding mode of delivery, there was not much difference in the rate of caesarean section between two groups. Women with significant proteinuria, 60% and among without significant proteinuria, 59% underwent caesarean section.

The maternal complications were more in patients with significant proteinuria. Out of 218 women with significant proteinuria, 20 (9.17%) developed one or other complications shown in table 5 compared to 2 (2.43%) out of 82 women without significant proteinuria (statistically not significant (p value 0.045)

**Table 3: Urine PCR cut off and24 hour urine protein.**

| Urine PCR (noon) | 24 hour urine protein | | |
|---|---|---|---|
| | Significant proteinuria $n$ (%) | No significant proteinuria $n$ (%) | Total |
| ≥0.4 | 195 (89.45%) | 7 (8.54%) | 202 |
| <0.4 | 23 (10.55%) | 75 (91.46%) | 98 |
| TOTAL | 218 | 82 | 300 |

**Table 4: Blood pressure.**

| Blood pressure | 24hr Urine protein | | p value |
|---|---|---|---|
| | <300 | ≥300 | |
| Systolic BP (Mean +/- SD) | 144.8 +/- -8.6 | 154.9 +/- -8.2 | <0.001 |
| Diastolic BP (Mean +/- SD) | 94.8 +/- 6.4 | 100.6 +/- 4.6 | <0.001 |

**Table: 5 Maternal complications**

| Complications | 24hr Urine protein | |
|---|---|---|
| | <300 | ≥300 |
| Eclampsia | 1 | 4 |
| HELLP Syndrome | 1 | 7 |
| Acute Renal Failure | 0 | 2 |
| Abruptio placentae | 0 | 3 |
| PPH | 0 | 3 |
| Pulmonary oedema | 0 | 1 |
| Total | 2 | 20 |

The foetal and the neonatal complications also were more in patients with significant proteinuria.

**Table: 6 Fetal and neonatal complications**

| Complications | 24 hour proteinuria<300mg n (%)    Total- 82 | 24 hour proteinuria>300mg n (%)    Total- 218 | P value |
|---|---|---|---|
| Low birth weight | 20(24.39) | 83(38.07) | <0.026 |
| Preterm delivery | 16(19.51) | 60(27.52) | <0.05 |
| Low APGAR 5 min <7 | 7(8.53) | 31 (14.27) | <0.0016 |
| NICU admission | 15(18.29) | 67 (30.23) | <0.03 |
| Intrauterine death | 0(0.00) | 10 (4.58) | <0.04 |
| Neonatal death | 1(1.21) | 16 (7.33) | <0.04 |

**Discussion**

The 24 hour urine protein measurement is considered as gold standard for proteinuria estimation. Alternative methods of proteinuria estimation are dipstick and urinary PCR.

**Correlation of PCR with 24 hour urine protein:**

The present study showed that the correlation coefficient(r) between protein creatinine ratio (morning, noon and night) and 24 hour proteinuria are 0.69, 0.76 and 0.71 respectively showing good correlation between the two. This is similar to the results obtained from previous studies. Ray Rathindranath et al[5] showed that there was a strong correlation between 24 hour urine protein and PCR (correlation coefficient r=0.9).Shahbazian N et al[7] found that there was a strong correlation between 24 hour urine protein and PCR with a correlation coefficient of 0.84.Similarly study by Bhavana B et al[6]and Choudary J et al[9] found good correlation with correlation coefficient of 0.5 and 0.8 respectively with 24 hour urine protein. But few studies showed results which did not agree with ours. The study by N.Agarwal and colleagues[10] found a poor correlation between the two(r=0.5).Also the study by Durnwald and colleagues[8] found a poor correlation between the two (with r=0.4).

**Timing of protein creatinine ratio:**

With the ROC curve analysis, it was found the AUC (area under the curve) was maximum with the PCR done at noon. (AUC=0.95) The optimal cut off of PCR at morning, noon and at night were 0.47, 0.40 and 0.45 respectively. This showed that for diagnosing clinically relevant proteinuria ,the optimal cut off of PCR with morning sample is 0.47,which is higher than the afternoon and night samples.

**Table7: Comparison with other studies for the validity and the optimal cut-off value of urine PC ratio in predicting proteinuria (using 24 hour urine protein as gold standard).**

|  | Cut off value | Sensitivity | Specificity |
|---|---|---|---|
| Ray Rabindranath | 0.2 | 83.3% | 87.5% |
| Dawnchung | 0.6 | 87% | 100% |
| Oya Demirci[15] | 0.45 | 74.4% | 94.2% |
| Present study | 0.4 | 89% | 91% |

Numerous factors like ambulation, posture, exercise and emotional factors may influence protein excretion, which was in accordance with the high cut-off of morning PCR, which was done following a period of rest. The present study was similar to the result obtained by K.Verdonk et al[11]. Twenty four hour urine collection and simultaneously three single voided urine samples were obtained at 8 am, 12 noon, and 5pm.PCR was measured in each specimen and compared with 24 hour urine protein. The optimal cut off PCR at morning was 0.5(8 am), at 12 noon was 0.4 and at 5pm was 0.3, showing the cut-off to be higher with morning sample to detect significant proteinuria. Gonsales Valerio and colleagues[12] did a cross sectional study comparing 24 hour proteinuria and 4 spot PCR (every 6 hour in 24 hours). This study showed PCR correlated well with 24 hour protein in all 4 spot urine samples of the day, irrespective of sampling time. The study by Annie Lamontagne et al[13] found the validity of PCR test is lower in first morning void sample .The PCR yielded a high positive predictive value and high likelihood ratio in samples other than first void sample. Kooman et al[14] found that the PCR done at fixed time of the day can be an acceptable alternative for 24 hour urine collection rather than doing it randomly.

The present study showed that the optimal cut off for measuring significant proteinuria was 0.4 (PCR at noon) with sensitivity of 89% and specificity of 91%.This was similar to the results obtained from other studies. With the AUC, maximum with PCR at noon (AUC noon -0.95,morning - 0.79,night- 0.90)and with optimal cut off, the sensitivity and specificity of PCR values at noon, morning, night were 89%,91%-noon,75%,75%-morning,86%,80%-at night respectively. This showed that PCR value at noon was the best time for estimating PCR.

**Conclusion**

The present study shows that the correlation coefficient between protein creatinine ratio (morning, noon and night) and 24 hour proteinuria are 0.69, 0.76 and 0.71, implying good correlation between the two. Hence spot urinary protein creatinine ratio can be used as an alternative to the cumbersome and time consuming 24 hour urine protein estimation. The best cut-off value of the protein creatinine ratio is 0.4. The protein creatinine ratio measurement at noon can be taken as the best timing of performing urinary protein creatinine ratio.

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