SIGNIFICANCE OF SPOT URINE PROTEIN CREATININE RATIO VERSUS 24-HOUR URINE PROTEIN ANALYSIS AND THEIR CORRELATION IN QUANTIFICATION OF PROTEINURIA IN HYPERTENSIVE PREGNANT WOMEN- A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

BACKGROUND
We wanted to evaluate the efficacy of protein creatinine ratio in quantification of proteinuria in hypertensive pregnant women between 20 to 40 weeks to study the correlation between protein creatinine ratio of spot urine sample and the protein content of 24 hours urine collection.

MATERIALS AND METHODS
Hypertensive pregnant women between 20 to 40 weeks were included in the study. Protein/creatinine ratio was obtained from urine samples collected soon after 24-hour urine collection. Sensitivity and specificity, positive and negative predictive values of the protein/creatinine ratio for significant proteinuria (≥ 300 mg) were calculated, based on 24-hours urine total protein.

RESULTS
There was a strong correlation between the spot P/C ratio and 24-hour urine protein excretion (r = 0.715; P < .0001). The optimal spot P/C ratio cut-off point was 0.35 for 300 mg/24 h of protein excretion (preeclampsia), with a sensitivity, specificity, positive predictive value, and negative predictive value and accuracy of 91.8%, 93.33%, 98.73%, 66.66% and 92% respectively.

CONCLUSIONS
A significant correlation between the spot urine P/C ratio and 24-hour urine protein excretion in women with preeclampsia was observed during the study. Urine P/C ratio could be used for exclusion of preeclampsia.


BACKGROUND
Hypertensive disorders complicating pregnancy are frequently observed and is a component of the deadly triad along with haemorrhage and infection that result in maternal morbidity and mortality.1

Proteinuria is the surrogate marker essential for the diagnosis of pre-eclampsia. Proteinuria develops late in the course of hypertensive disease and its presence is a sign of worsening disease, specifically pre-eclampsia.2

According to the National Center for Health Statistics in 1998, hypertension with pregnancy is the most common medical risk factor. Pre-eclampsia is a pregnancy specific, hypertensive disorder characterized by hypertension and proteinuria after 20 weeks of gestation and remission of signs after delivery which is unique for human pregnancy. The incidence of pre-eclampsia is 5-10% of all pregnancies. Most cases of pre-eclampsia occur in healthy nulliparous women, the incidence of pre-eclampsia in nulliparous women may be as high as 7.5% worldwide and in India it is around 15%.3 In our institute it is 5-7%.

When proteinuria is overt and persistent, maternal and fetal morbidities are increased. Presence of significant proteinuria predisposes pregnant woman to coagulopathy, liver disease, stroke, serious perinatal morbidity occur in form of preterm delivery and fetal growth restriction.

Complications expand with proteinuria and hence a rapid, accurate detection and quantification of proteinuria are necessary for the management of hypertensive pregnant women.

In pregnancy proteinuria is usually detected and measured either by visual dipstick urine test or by the 24 hrs urinary protein measurement. The visual dipstick urine test serves as a rapid bedside screening test in the initial evaluation of proteinuria.3
The 24 hrs urine collection has been the gold standard in most places for quantifying proteinuria. It requires good patient compliance and there is a delay of 24 hrs from the time of collection until the diagnosis is made.

The protein-creatinine ratio in a single urine specimen has been used for rapid and accurate detection of proteinuria in hypertensive pregnant women. It avoids collection error and gives physiologically more relevant information. In pregnancy, numerous studies have observed a high correlation between the protein-to-creatinine ratio on a spot urine sample and 24-hour urinary protein excretion.

Many national organizations have adopted use of the protein-to-creatinine ratio with a threshold of 30 mg/mmol to diagnose significant proteinuria. However, the use of the protein-to-creatinine ratio and this cut-off are still a matter of debate, in view of the heterogeneity in specificity and sensitivity reported. Hence, there is a need to evaluate these tests which can be used to quantify the proteinuria accurately and rapidly in outpatient settings, overcoming the limitations of routinely performed tests.

National kidney foundation recommendations this test to diagnose proteinuria. International society for study of hypertension in pregnancy has proposed use of urinary spot protein creatinine ratio as an alternative to 24-hour urine collection.

This study was conducted to evaluate the efficacy of protein creatinine ratio in quantification of proteinuria in hypertensive pregnant women from 20 to 40 weeks gestational age and to study the correlation between protein creatinine ratio of spot urine sample and the protein content of 24 hours urine collection.

Pre-eclampsia is prominent from gestational hypertension by the presence of significant proteinuria. An accurate and rapid detection of proteinuria is essential in the management of hypertensive disorders in pregnancy.

The gold standard for the diagnosis of significant proteinuria remains the 24 hours urine protein. The need for a 24-hr collection is because of high degree of variation in the urine protein concentration during the course of the day. However, the method is cumbersome, time consuming and can be inaccurate because of incomplete collection. For these reasons simpler methods which can measure urinary protein in spot samples like urine protein-creatinine ratio are proposed. In our study we correlated urine PCR with the gold standard.

MATERIALS AND METHODS

Study Site
This study was conducted in the Department of OBGY, DDH, Hyderabad with the assistance of laboratory setup of the Department of Biochemistry, DDH, Hyderabad. The study and its conduct were cleared by the ethical committee, DDH, Hyderabad.

Study Population
This was a prospective observational study conducted on Hypertensive pregnant women between 20 to 40 weeks admitted at Durgabai Deshmukh Hospital and Research Centre, during the study period are enrolled for the study.

Period of Study
November 2013 to June 2015.

Study Design
Prospective observational study.

Sample Size
Sample size was calculated on the basis of the primary outcome measure. Using open epi website sample size was calculated using 7% as hypothesized frequency. The Sample size taken as 100 with confidence interval 95%.

Inclusion Criteria
Hypertensive pregnant women between 20 to 40 weeks admitted with a blood pressure of 140/90 mmHg or more on two occasions at least 6 hours apart or a single diastolic reading of ≥110 mmHg and the presence of proteinuria of 1+ or more as detected by a qualitative test done on a random sample of urine.

Exclusion Criteria
Patients with chronic hypertension, diabetes mellitus, or pre-existing renal disease, urinary tract infections, pathological vaginal discharge and who delivered during the day of collection were excluded.

Procedure
1) A detailed history was taken, general physical and systemic examination including the obstetric examination. Per speculum examinations were done to look for any evidence of vaginal infection clinically.
2) Investigations done are: - B. urea, s. creatinine, s. uric acid, urine routine and microscopy, urine dipstick, 24 hrs urine protein, Urine protein/creatinine ratio, Routine Antenatal and PIH investigations.
3) A qualitative test for urinary protein was earned out on a random sample of urine using the dipstick method. Protein by the dipstick method is graded as follows:
   - Traces = <30 mg/dl
   - 1+ = 30 mg/dl
   - 2+ = 100 mg/dl
   - 3+ = 300 mg/dl
   - 4+ = 1000 mg/dl
4) The test was repeated on an additional sample of urine immediately and if the subsequent tests showed 1+ or more, quantitative tests for proteinuria were carried out.

Figure 1. Showing Reagent Urine Strips and Procedure
5) The tests were carried out as follows: The patients were instructed to collect the 24 hours 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. A single voided urine specimen was obtained soon after the 24 hour urine collection, before mid-day.

6) The samples were sent to the Biochemistry laboratory where:
   a. Urine protein was measured by pyrogallol red dye method. The test was performed on semi-automated analyser.
   b. Urine creatinine was measured by Jaffrey’s method. The test was performed on semi-automated analyser.

7) The urine protein and creatinine ratio was obtained by dividing the urine protein concentration (mg/dl) by the urine creatinine (mg/dl).

<table>
<thead>
<tr>
<th>24 hrs Urinary Protein mg/dl</th>
<th>Protein /Creatinine Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>&lt; 300</td>
</tr>
<tr>
<td>Clinically Significant Proteinuria</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Severe Proteinuria</td>
<td>&gt;3000</td>
</tr>
</tbody>
</table>

Table 1. Normal Values of Protein Excretion

Ethics
The study was carried out with Ethics Committee Approval.

Statistical Analysis
Data was analysed using SPSS 17 software. Results were considered significant when p<0.05. Demographic and baseline data were represented as mean± SD and percentage, where appropriate. The correlation between spot urine P/C ratio and 24-hour urine was analysed using Pearson correlation test. Sensitivity, specificity and predictive values of the spot urine P/C ratio were estimated considering 24-hour urine protein as the gold standard. The ROC curve was used and area under curve was calculated. Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS
In the present study of 100 pre eclamptic women, the mean age of the patients was 26.46 years, the mean gestational age was 33.55, 68% constituted primigravidae, while 32% were multigravidae. Except for 29 women, all others were on anti-hypertensives.

The study was limited to hospitalized patients. The ambulatory status of the patients was considered while interpreting the results, as proteinuria is affected by postural change, being higher in the standing than in supine position. Proteinuria more than 300 mg/24 hrs. was taken as clinically significant. Also, the assessment of renal function and other severity predictors of the disease were done.

Urine Dipstick analysis, which is a most commonly used screening tool to detect proteinuria was done. Urine protein-creatinine ratio has been shown as a good and a reliable predictor of proteinuria in a spot sample by various studies. In our study the urine protein-creatinine ratio yielded the sensitivity 91.8%, specificity 93.33%, positive predictive value 98.73%, Negative predictive value 66.66%, and accuracy of 92%.

Observations
Number of Women Studied (n) = 100.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26.46</td>
<td>±3.376</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>POG</td>
<td>33.55</td>
<td>±3.80</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>SBP</td>
<td>157.18</td>
<td>±19.754</td>
<td>130</td>
<td>210</td>
</tr>
<tr>
<td>DBP</td>
<td>101.12</td>
<td>±9.578</td>
<td>80</td>
<td>130</td>
</tr>
</tbody>
</table>

Table 2. Demography of The Subjects

In the present study, it is observed that the mean ±SD of age of the subjects studied was 26.46 ±3.376 yrs., gestational age was 33.55 ± 3.80. Systolic blood pressure was 157.18 ± 19.754 mm of Hg; Diastolic blood pressure was 101.12 ± 9.578 mm of Hg.

<table>
<thead>
<tr>
<th>Age (Yrs.)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>4</td>
</tr>
<tr>
<td>21 -25</td>
<td>35</td>
</tr>
<tr>
<td>26 -30</td>
<td>50</td>
</tr>
<tr>
<td>&gt;30</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3. Age Wise Distribution of Subjects

Among the studied subjects n=100; 4 were below 20 yrs, 35 were of 21-25 yrs. age group, 50 were of 26-30 yrs. age group, 11 were of 30- 35 yrs. age group.
Among the 100 pregnant women included in the study 68% of them were primigravidae and 32% were multigravidae.

Among 100 women, 22 were between 25-28 wks., 15 were 29-32 wks., 48 were 33-36 wks., 15 were 37-40 wks.

Only 22 (22%) subjects among 100 were known to be hypertensive in prior pregnancy and 78 (78%) were not hypertensive.

Out of 100 women studied 71% were on anti-hypertensive drugs and only 29% were not on any anti-hypertensive drugs at the time of collection.

In our study there were 15 patients (15%) gestational hypertensives and 85 patients (85%) were severe preeclamptic.

Different laboratory parameters were assessed to know the severity and complications of the disease.

Mean and standard deviation of 24 hr urine protein and P/C ratio was 228.87 ± 67.55 and 0.21 ± 0.09 in gestational hypertension, 1152.23 ± 657.39 and 3974.47 ± 2362.8 and 5.10± 4.22 in Severe PE.

* Correlation is significant at the 0.05 level (2-tailed) for gestational hypertension.
* Correlation is significant at the 0.01 level (2 tailed) for NSPE.
* Correlation is significant at the 0.01 level (2 tailed) for SPE.
Mean proteinuria was 2283.73±2260.21 when all 100 subjects were considered, and mean P/C ratio was 2.84 ± 3.54.

A fair correlation coefficient of r = 0.715 was observed between the 24 hours urine protein and spot urine protein / creatinine ratio among 100 subjects which was statistically significant at p value <0.001.
The test result variable(s): PCR has at least one tie between the positive actual state group and the negative actual state group.

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Area under curve is 0.969.
Significant at p<0.0001

The cut off value at which both sensitivity and specificity are maximum is 0.35 (sensitivity 91.8% and specificity 93.33%) PPV of 98.73%, NPV of 66.66% and Accuracy is of 92%.

Karl Pearson’s Correlation and Analysis
We found a significant positive correlation in our study, when 24 hours urine protein and spot urine protein-creatinine ratios were correlated with r = 0.715 and p value being highly significant at <0.0001, where all the observations were considered.

All of the previous studies in the table below demonstrate an excellent correlation between the 24 hrs urine protein and the protein-creatinine ratio. The p values are also statistically significant at <0.001.

It was noticed in our study that the correlation coefficient for gestational hypertension is 0.588 and significant at p value 0.05, as compared to NSPE and SPE with correlation coefficient of 0.65 and 0.524 respectively, which are statistically significant at p value 0.01.

We have found the use of this alternative test to 24 hours urine protein to be much more cost effective as shown with many studies previously.

We have also found the 24 hours urine collection to be cumbersome and inconvenient for a pregnant woman. Since the present study included women only with a stable renal function, our study supports the use of the Protein/creatinine ratio in women with normal renal function.

It was noticed in this study that, of 100 subjects studied 15 (15%) were having Gestational HTN and 85 (85%) were having severe pre-eclampsia.

Receiver Operating Characteristic Curves (ROC) Analysis
Urine protein by spot protein-creatinine ratio.

Observation:
The area under the ROC curve – 0.969 (95% CI: 0.939, 0.999) p <0.0001 (highly significant). The optimal cut-off point was 0.35; this cut-off yielded a sensitivity = 91.8% and specificity = 93.33%.

Analysis
With the above results the sensitivity of the spot protein-creatinine ratio remained high at cut-off of 0.35, and also the specificity remained fairly constant. Among 85 of total positive cases, 7 were false negatives and among 15 total negatives 14 were truly negative. Hence by this analysis spot protein-creatinine ratio could be validated both as diagnostic and screening test.

Merits of The Study
- We have found protein to creatinine ratio in single urine sample can be used as an alternative test to 24 hours urine protein and it is much more cost effective as shown with many studies previously.
- The value of protein to creatinine ratio in single urine sample is more accurate since it avoids collection errors.
- This study indicates that, when properly interpreted, the results of measurement of protein and creatinine in single voided urine sample can provide information which for clinical purposes is a satisfactory substitute for 24-hour urine protein in hypertensive pregnant women.

Limitations
- Only 100 cases were available during the study periods that were fulfilling the inclusion criteria.
- The maternal "spot urine" estimate of protein to creatinine ratio shows promising diagnostic value for significant proteinuria in suspected pre-eclampsia. The existing evidence is not, however, sufficient to determine how protein to creatinine ratio should be used in clinical practice, owing to the heterogeneity in test accuracy and prevalence across studies.
- Insufficient evidence is available on the use of protein to creatinine ratio to predict adverse pregnancy outcome.
- We have found the 24 hours urine collection to be cumbersome and inconvenient for pregnant women. Since the present study included women only with a stable renal function, our study supports the use of the Protein/creatinine ratio in women with normal renal function.

DISCUSSION
1. Present study is “a prospective observational study of diagnostic use of spot urine protein creatinine ratio versus 24-hour urine protein analysis and their correlation in quantification of proteinuria in hypertensive pregnant women” carried out at Durgabai Deshmukh Hospital and Research Institute, Hyderabad between November 2013 to June 2015.
2. The objective of the study was to evaluate the efficacy of protein creatinine ratio in quantification of proteinuria in hypertensive pregnant women and to study the correlation between protein creatinine ratio of spot urine sample and the protein content of 24 hours urine collection.
3. Ethical clearance and informed consent were obtained.
4. Study group was selected on the basis of inclusion and exclusion criteria. Urine samples were collected for visual dipstick, 24 hrs urine protein and protein-creatinine ratio estimation, Sample was analysed in Biochemistry Lab, in DDH, Hyderabad.
5. A prospective observational study was conducted on 100 hypertensive pregnant women during the study period.
The data collected were age, parity, POG, SBP, DBP, urine protein by dipstick, urine PCR, 24-hour urine protein and other PIH profile.

7. The mean age of the patients was 26.46 years.

8. 68% of study group were primigravidae and 32% were multigravidae.

9. The mean gestational age was 33.55 weeks.

10. Out of 100 pregnant women, 15 were having gestational hypertension, 40 were having NSPE and 45 were having SPE. 71% were on antihypertensives.

11. The study results were: A fair degree of correlation existed between the two variables (P/C ratio and 24-hour urine protein) with $r = 0.715$ with a highly significant p value = <0.0001 when all the observations were considered.

12. ROC Curve Analysis: Spot P/C ratio - The area under the ROC curve – 0.969 (95% CI: 0.939, 0.999) p < 0.0001 (highly significant) with the optimal cut-off of 0.35 for 300 mg/24 h of protein excretion (preeclampsia), with a sensitivity, specificity, positive predictive value, and negative predictive value and accuracy of 91.8%, 93.33%, 98.73%, 66.66% and 92% respectively.

13. Even though the results were known to clinicians the values were not taken for clinical decision, only by the ratio alone. However, the 24-hour urine protein values were considered for the patient management.

14. For years, 24-hour urine collection has been the gold standard for quantification of proteinuria in the management of women with pre-eclampsia. However, this method necessarily imposes poor patient compliance, cumbersome, a delay of more than 24 hrs on the diagnostic process and sometimes yields inaccurate results because of collection errors.

15. Our contention was that, the value of the protein-creatinine ratio in a single urine sample is potentially more accurate as it avoids collection errors and gives more physiologically relevant information. Also use of the ratio negates the uncertainty associated with the dilute or concentrated urine.

16. Quantification of proteinuria in a random sample is found to be more cost effective and acceptable to the patient than a 24-hour urine collection.

17. The present study indicates that this method for quantification of proteinuria, when properly interpreted, and validated by laboratory can provide valuable information regarding diagnosis and severity of the disease. Hence for the clinical purposes, spot urine protein-creatinine ratio is a satisfactory and reliable substitute for determination of proteinuria than in a 24-hour urine collection. Since pre-eclampsia is a progressive disease, a repeated laboratory examination to quantitate proteinuria is required.

**CONCLUSIONS**

1. The detection of urinary protein excretion has considerable clinical implications in the course of pregnancy and on the perinatal and maternal outcome. Hence, early detection of even minor degrees of proteinuria is important.

2. It is useful in an outpatient setting to predict clinically significant proteinuria and to monitor renal functions especially in women with lesser degrees of proteinuria thus avoiding unnecessary hospital admissions.

3. Based on the results of our study, we conclude that random urine P/C ratios can predict 24-hour urine protein excretion with a high accuracy. This test can also be used as a reasonable alternative to 24-hour urine protein excretion, especially in emergency situations, and, it could also complement the urinary dipstick test in preeclamptic pregnancy.

**REFERENCES**


