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ORIGINAL RESEARCH

Study of Hypertensive Disorders of Pregnancy by Comparision of Spot Urine Protein/Creatinine Ratio and 24hours Urinary Protein toDiagnose Proteinuria

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ABSTRACT

Background:The aim is to compare the spot urine protein/creatinine ratio with 24hour urinary protein in hypertensive disorders of pregnancy.

Materials and Methods: A prospective correlational study was conducted on 100 hypertensive pregnantwomen during the study period. The objective of the study was to know if a spot protein/creatinine ratio would provide an accurate quantification of proteinuria and whether it can replace the use of the 24 hours urine protein in preeclamptic women. Ethical clearance and informed consent were obtained. Urine samples were collected for visual dipstick, spot urine P/C ratio and 24hours urinary protein estimation.

Results: A fair degree of correlation existed between the two variables with r = 0.842with a highly significant p value <0.01 when all the observations were considered. The correlation at lower level of proteinuria was less r = 0.72 compared to higher levels of proteinuria,but is statistically significant. The area under the ROC curve - 0.739 (95% CI: 0.628, 0.849) with p value < 0.01 (significant). The optimal cut off point was 0.5, which yielded a sensitivity of 92% and specificity - 66%. 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. The perinatal outcome in women with higher levels of proteinuria were poor with increased incidences of IUGR, prematurity, low birth weight and the need for NICU care was increased in such babies.

Conclusion: The present study indicates that this method for quantification of proteinuria, when properly interpreted, can provide valuable information, that for clinical purposes is a satisfactory substitute for the determination of protein excretion in a 24 hour collection.

Keywords: IUGR, Perinatal Outcome, NICU, Preeclmpsia, Preganant Woman. Protein/Creatinine ratio.

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INTRODUCTION

Hypertensive disorders complicate 5 to 10 percent of all pregnancies.[1] The preeclampsia syndrome, either alone or superimposed on chronic hypertension, is the major cause of

maternal and perinatal morbidity and mortality, where it forms one of the deadly triad along with hemorrhage and infection.[2,3] Preeclampsia is identified in 3.9 percent of all pregnancies.1

Preeclampsia is a pregnancy specific syndrome. Several studies carried out during recent years have extended the understanding about the pathophysiological basis of disease.[4] However the criteria used to define it are still a matter of controversy. Proteinuria is essential for diagnosis of preeclampsia. Proteinuria develops late in the course of the hypertensive disease and its presence is a sign of worsening hypertensive disease, specifically preeclampsia. As proteinuria increases, the likelihood of complications also increases and hence a rapid and accurate detection and quantification of proteinuria are essential for the management of hypertensive pregnant women.

In pregnancy proteinuria is usually detected and measured either by visual dipstick urine analysis or by the 24hours urinary protein measurement. The visual dip stick urine analysis serves as a rapid bedside screening test in the initial evaluation of proteinuria,[5] but in recent studies it has been found inaccurate, giving a high number of false positives and false negative results.

The 24hours urinary collection has been the gold standard test for quantifying proteinuria. Though reliable indicator, it has the disadvantages of being a cumbersome and time consuming process, for both the patient and laboratory,[6] it is subjected to collection error; requires good patient compliance and there is a delay in the diagnosis and intervention.

Hence, there is a need to evaluate other tests which can be used to quantify the proteinuria accurately and rapidly and at the same time overcome the limitationsof the routinely performed tests.[6] The protein/creatinine ratio in a single urine specimen has been used for the rapid and accurate detection of proteinuria in hypertensive pregnant women,[7] as it avoids collection errors, gives physiologically more relevant information and early intervention can be made.

Aim

To compare the spot urine protein/creatinine ratio with 24hour urinary protein inhypertensivedisorders of pregnancy.

Objectives

- To know if urine protein/creatinine ratio and 24hour urine protein are comparable.
- Reliability of urine protein/creatinine ratio in a random urine sample to confirmpreeclampsia.

MATERIALS & METHODS

Patients and methods:

The present study is a prospective study conducted in the Department of Obstetrics andGynaecology, Dr.Pinnamaneni Siddhartha Institute of Medical sciences and Research foundation, Chinnaoutpalli, from the year 2013 - 2015.

Sample Size: 100 cases

Criteria for selection of patients inclusion criteria:

- Pregnant women with > 20 wks gestation with hypertension.
- Pregnant women with < 20 wks of gestation with hypertension.
- Pregnant women with hypertension and proteinuria.
- Pregnant women with eclampsia.

Exclusion criteria:

- Pre-existing renal disease – Diagnosed prior to pregnancy.
- Normotensive women with proteinuria.

Procedure

All the patients satisfying the above criteria were included in the study. The tests were carried out in hospitalised patients.

A detailed history, general, physical and systemic examination including obstetric examination were done. The following investigations were done.

1. A qualitative test for urinary protein was done on a random sample of urine using sulfosalicyclic method. Protein estimation by the sulfosalicyclic method was graded as follows:

Traces	**Trace perceptible turbidity**	**= ≤ 5mg/dl**
1+	- Distinct turbidity, no discrete granules	= 20mg/dl
2+	- Turbidity with granulations but no flocullations	= 200mg/dl
3+	- Turbidity with granulations with flocculations	= 500mg/dl
4+	- Clumps precipitated protein or solid precipitates	= ≥1gm/dl
2.	24-hour urine protein estimation: The patients	Wereinstructed to collect the 24-hour urine

2. **24-hour urine protein estimation:** The patients Were instructed to collect the 24-hour urine sample starting from the 2nd urine sample in the morning that is after discarding the first morning specimen till the first urine sample the next day morning.

3. **Protein/creatinine ratio:** Random urine for protein/creatinine ratio estimation.
   a) In an emergency at any time of the day.
   b) In an admitted patient, the patient was instructed to void urine at 8AM and 10AM and sample was collected.

The samples were sent to biochemistry laboratory where:

a) Urine protein was measured by pyrogallol red dye method which was performed onan automated analyzer.
b) Urine creatinine was measured by the Jaffe's reaction which was performed on anautomated analyzer.

Proteinuria	**24hour-urinaryprotein (mg/24hr)**	**Spot urine protein /creatinine ratio**
Negative	<300	<0.3
Clinically significantproteinuria	≥300	≥0.3
Severe proteinuria	>3000	>3

The data thus collected were analyzed using appropriate statistical methods.

Statistical tests:

- Mean
- Standard Deviation
- Pearson's correlation coefficient which was expressed as r.
- Student chi square test expressed as p.
- A value of $p < 0.05$ has been considered to be statistically significant.

RESULTS

Table 1:Demography of the subjects studied

Variable	Minimum	Maximum	Mean	SD
Age(years)	18	34	22.17	3.111
Period of gestation	20	40	35.94	3.524
SBP(mm of Hg)	130	180	147.80	9.383
DBP(mm of Hg)	90	110	95.60	7.152
Hb in mg%	8.0	13.7	10.899	1.1243
Platelet count	1.2	3.0	1.983	.4248
Serum urea	10	42	18.36	6.428
Serum uric acid	2.8	6.5	4.249	0.8481
PRIMI			32	
MULTI			68	

In the present study, it was observed that the mean ± SD of age of the subjects studied was 22.17 ± 3.111 (range: 18-34yrs), gestational age was 35.94±3.524 (range: 20- 40weeks), Systolic blood pressure (SBP) was 147.80 ± 9.383 mm of Hg, Diastolic bloodpressure (DBP) was 95.60 ± 7.152 mm ofHg. 32 (32%) of them were primigravidae and 68 (68%) of them were multigravidae.

Table 2: Distribution of subjects according to degree of proteinuria

24 hrs urine protein	Frequency	Percent
<300 mg	36	36.0
300 mg -2000 mg	58	58.0
>2000 mg	6	6.0
Total	100	100.0

In our study, 36 patients (36%) had less than 300mg proteinuria, 58 patients (54%) had 300mg to 2000mg of proteinuria and 6 patients (6%) had more than 2000mg of proteinuria.

Table 3: Distribution of subjects into Gestational HTN, Mild preclampsia and Severe preeclampsia using 24hour protein and Blood pressure recordings

Diagnosis	Frequency	Percent
Gestational hypertension	28	28.0
Mild preeclampsia	48	48.0
Severe preeclampsia	24	24.0
Total	100	100.0

In our study there were 28 patients (28%) with gestational hypertension, 48 patients(48%) were with mild preeclampsia, 24 patients (24%) were with severe preeclampsia.
In our study 61 patients (61%) were primigravidae,38 patients(38%) were multigravidae.

Table 4: Distribution of subjects depending upon the proteinuria

Urine albumin	Frequency	Percent (%)
Nil	36	36
Trace	24	24
1	23	23
2	10	10
3	7	7
Total	100	100

In our study spot urine albumin were nil in 36 patients (36%), traces in 24 patients (24%), 23 patients (23%) with 1+, 10 patients (10%) with 2+, 7 patients (7%) with 3+.

Table 5: Distribution of subjects depending upon number of antihypertensives

Drugs	Frequency	Percent
0	32	32
1	49	49
2	17	17
3	2	2
Total	100	100

Out of 100 women studied, 32 patients (32%) were not on any antihypertensives, 49 patients (49%) were on one antihypertensive drug, 17 patients (17%) were on two antihypertensive drugs, 2 patients (2%) were on three antihypertensive drugs.

Table 6: Mean and SD of 24-hour urinary protein and spot urine Protein/Creatinine ratio in mild PE and severe PE gestation hypertension group

Diagnosis	Variable	Mean	SD
Mild preeclampsia	24 hours urine protein	653.23	588.74
	Protein /creatinine ratio	0.82	0.85
Mild gestational hyzpertension	24 hours urine protein	181.68	62.38
	Protein / Creatinine ratio	0.44	0.64
Severe preeclampsia	24 hrs urine protein	1432.96	929.38
	Protein /creatinine ratio	2.75	2.09

Mean and SD of 24hours urinary protein and urine P/C ratio were 181.68 ± 62.38 and 0.44 ± 0.64 in mild gestational hypertension group.
Mean and SD of 24 hours urine protein and P/C ratio were 653.23± 588.74 and 0.82 ± 0.85 in mild preeclamptic group.
Mean and SD of 24 hours urine protein and P/C ratio were 1432.96 ± 929.38 and 2.75 ± 2.09in severe preeclamptic group.

Table 7: Karl Pearsons correlation coefficient between 24hours urine protein and Protein/Creatinine ratio in mild gestation hypertension group.

Diagnosis	Variable	r-value	P-value	Inference
Mild gestational hypertension	24 hours urine protein	0.402	<0.05	S
	Protein/creatinine ratio			
Mild preeclampsia	24 hours urine protein	0.720	<0.01	HS
	Protein /creatinine ratio			
Severe preeclampsia	24 hours urine protein	0.86	<0.01	HS
	Protein /creatinine ratio			

Pearson's correlation coefficient between 24hour urine protein and P/C ratio in mild gestation hypertension group were r value = 0.402, p value = <0.05 which is significant.
Karl Pearson's correlation coefficient between 24hours urine protein and P/C ratio in mild preeclampsia group were with r value = 0.720, p value = <0.01 which is highly significant.
Karl Pearson's correlation coefficient between 24hours urine protein and P/C ratio in severe preeclampsia group were with r value = 0.86, p value= <0.01 which is highly significant.

Table 8: Mean and SD of 24 hrs urine protein and Protein/Creatinine ratio of all 100subjects

Variable	Mean	SD
24 hours urine protein	707.85	757.654
Protein /creatinine ratio	1.173	1.5122

Mean and SD of 24 hours proteinuria was 707.85 ± 757.654 and P/C ratio was 1.173 ± 1.512 respectively when all 100 subjects were considered.

Table 9: Correlation coefficient between 24 hours urinary protein and spotProtein/Creatinine ratio in all subjects.

Variable	r-value	P-value	Inference
24 hrs urine protein	0.842	<0.01	HS
Protein /creatinine ratio			

Correlation coefficient between 24 hours urinary protein and spot P/C ratio in allsubjects were, with r value 0.842, p value < 0.01 which was highly significant.

Table 10: Sensitivity and Specificity of spot urine P/C ratio

P/C ratio	24hr urine protein		Total
	Negative	Positive	
Negative	20	5	25
	80.0%	20.0%	100.0%
	55.6%	7.8%	25.0%
Positive	16	59	75
	21.3%	78.7%	100.0%
	44.4%	92.2%	75.0%
Total	36	64	100
	36.0%	64.0%	100.0%
	100.0%	100.0%	100.0%

Sensitivity = 92.2%
Specificity = 55.6% Positive
Predictedvalue = 78.7% Negative
Predictedvalue = 80.0%

DISCUSSION

Preeclampsia is a major cause of maternal morbidity and mortality. It accounts for 12.6% of maternal deaths. Since time immortal, urine examination remains one of the important investigations during antenatal checkups. Preeclampsia is distinguished from gestational hypertension by the presence of significant proteinuria. The appearance of proteins in the urine herald's possible onset of hypertensive complication, either proteinuric gestational hypertension or superimposed preeclampsia over preexisting renal disease. An accurate and rapid detection of proteinuria is essential in the diagnosis and management of hypertensive disorders of pregnancy.

Three methods of urine protein estimation have been used amply in the current obstetric practice. The most popular one is urine dipstick analysis which is readily available in most of the hospitals, the second one is so called "gold standard" 24 hours urinary proteins but is limited by its availability and time constraints, and the third one is slowly becoming popular,

that is the estimation of ratio of either protein or albumin to thecreatinine concentration [urinary protein: creatine ratio(UPCR) and urine albumin: creatinine ratio (UACR)]in the random urine sample. This method gives faster and reasonably accurate assessment of significant proteinuria. Of the two, the first oneis preferred as the second ratio is associated with relatively low sensitivity and high false positivity.[8]

The dipstick method is economic and simple to perform.[9] However it is not a recommended test, as studies quote substantial false positive rates, poor sensitivity,and accuracy. Though classically +1 dipstick grade has been considered as a marker ofpathological proteinuria (protein excretion of >300 mg/day) in pregnant women, the grading can vary depending upon maternal hydration status.

The best way to quantify proteinuria is to measure its daily renal excretion. Nonpregnantwomen excrete upto 150 mg per day, whereas in pregnancy the cutoff is 300 mg per day. The gold standard for the diagnosis of significant proteinuria remains the 24hrs urinary protein. The need for 24hrs urinary collection is because of degree of variation inthe urine protein concentration during the course of the day. However, the method is cumbersome, time consuming and can be inaccurate because of incomplete collection, lack of compliance, inconvenience Waiting for the results of protein estimation in a 24-hour urine collection can delay the diagnosis of preeclampsia unnecessarily and potentially put the mother and fetus at risk. As such, the ability to substitute a spot urine PCR for a 24-hour urine collection could have significant clinical implications, including the facilitation of prompt clinical decision making and more expeditious delivery. Such an approach could also impact healthcare costs and improve patient outcome and satisfaction. With easier collection and results available within hours, a spot PCR would be a more efficient test than a 24- hour collection for proteinuria assessment.[10] Moreover, because by definition, the PCR corrects urinary protein concentrations for creatinine, it is independent of the degree of dilution of the urine. Because of the operational difficulty, there is necessity to use rapid, convenient, and reliable method of proteinuria estimation. In our study we correlated the spot urine PCR with the gold standard 24hours urinary protein.

In the present study of 100 women with hypertensive disorders of pregnancy, the mean age of the patients was 22.17 years, The majority of the patients in our study were of young age group of < 30 years. This observation is similar to that of study by Sharma A et al,[11] who observed that 87.92% patients were of age group 21 - 30 years in their study.

The mean gestational age in our study was 35.94 weeks (20-40weeks), in a study by Sharma A et al,[11] it was observed that majority of subjects (42.06%) with preeclampsia belonged to 28 - 32 weeks gestational age.

In our study primigravidae were 62 (62%), while 38 (38%) were multigravidae, Sharma A et al,[11] observed that approximately 46.03% patients in their study were primigravidae.

In our study the mean SBP was 147.80, ranging between 130 - 180mmHg, mean DBP was 95.60, ranging between 90 - 110mmHg.

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

Many studies have evaluated the urine protein/creatinine ratio but, they differ in their recommended cutoffs and differ in their assessment of the test's utility. Different methodologies, such as the spectrum of illness in the population studied, retrospective study design, exclusion of patients with comorbid illness, and non-exclusion of incomplete or infected samples probably account for these discrepancies.[12]

In our study the urine PCR yielded the sensitivity -92.2%, specificity - 55.6%, positive predictive value- 78.7%, negative predictive value - 80.0%.
Urine albumin has sensitivity -75%, specificity - 55.6%, positive predictive value - 75%, negative predictive value - 55.6%.
Previous studies which have been evaluated the role of random protein/creatinine ratio have shown controversial results.
Study by Durnwald and Mercer,[13] in 2003 and Aggarwal N et al,[14] in 2008 showed poor correlation coefficient. Durnwald and colleagues studied the value of urine protein creatinine ratio in prediction of 24hour urine total protein among women with preeclampsia. In this study of 220 women, it was apparent that urine protein creatinine ratio and 24hour urine total protein level showed a poor correlation with negative predictive value of 47.5% and specificity of 55.8%. But it may be noted that it had recruited outpatients while our study had patients being tested after admission. So therewere no dropouts and more accurate sample collection and handling was done.
Kayatas S et al,[15] in 2013, had a prospective study in 200 pregnant women. Theyobserved a significant correlation between 24-hour protein excretion and the urine P/C ratio ($r=0.828$, $p<0.0001$). The cut-off P/C ratio for 300 mg per 24h was 0.28 with sensitivity, specificity, PPV, NPV were 60.4%, 77.9%, 77.5% and 60.9% respectively. The cut-off value of P/C ratio for 2000mg per 24h was 0.77 with sensitivity, specificity, PPV and NPV were 96.8%, 98.6%, 96.8%, 98.6% respectively. They concluded that theSpot P/C ratio is a poor predictor of 24-hour proteinuria but can predict proteinuria >2000 mg better than 300-2000 mg.[15]
Even though only 3 patients in our study had proteinuria ≥ 3000 mg of protein, the urine protein– creatinine ratio had good correlation with 24hr urinary protein. Caution should be used in using spot urine studies rather than 24hour urine collections to make a diagnosis of severe proteinuria because this analysis is limited by the relatively few number of patients with severe proteinuria in this study and, therefore, the inability to accurately describe a range.
In a similar study conducted by Jaschevatzky et al,[16] in 1990, the degree of correlation between the two variables at > 2gms/24 hours proteinuria was lower but still significant at $p<0.05$. This variation in the results at severe degrees of proteinuria, indicates the careful interpretation and validation of the results by the laboratory especially when clinical decisions are based on them.
We found a fair degree of correlation in our study, when the 24 hours urine protein and the random urine protein-creatinine ratios were correlated with $r=0.842$ and the p value being highly significant at<0.01, when all the observations were considered.
Neithardt et al,[17] studied 30 pregnant women, for the prediction of 24 hour protein excretion in pregnancy with a single voided urine protein to creatinine ratio. In their study a significant correlation was found between the 24 hour urine protein and the protein creatinine ratio with a $r = 0.93$ and $p< 0.001$ and the P/C ratio was also helpful in predicting the protein excretion over time.
All of the previous studies demonstrate an excellent correlation between the 24 hrs urine protein and the protein/creatinine ratio. The p values are also statistically significant at <0.01 which is also seen in our study.
It was noticed in our study that the correlation coefficient of mild preeclampsia,at lesservalues of proteinuria was 0.72, which was less compared tosevere preeclampsia, with higher values of proteinuria 0.86, but both are statistically significant with p value <0.01.
This variation in the results at severe degrees of proteinuria, indicates the need for carefulinterpretation of the results especially when clinical decisions are to based on them.

Receiver Operating Characteristic Curves (ROC) analysis: Urine protein by spot protein/creatinine ratio:

Observation:

The area under the ROC curve - 0.739 (95% CI: 0.628, 0.849) with p value <0.01(significant). The optimal cut off point was 0.5, which yielded a sensitivity was 92% and specificity - 66%. Remained high at value 0.3, 0.5, 0.6 cut off points, but the specificity remained fairly constant. Among 75 total positive cases, 16 (21.3%) were false positives and among 25 total negatives, 20 (80%) are true negatives. Hence by this analysis spot urine protein - creatinine ratio could be validated both as diagnostic and screening test.

Karl Pearson's Correlation and Analysis: We found a significant positive correlation in our study, when 24hours urinary protein and spot urine protein/creatinine ratios were correlated with r= 0.842 and p value being highly significant with value< 0.01.

Several studies have established the usefulness of urine protein/creatinine ratio not onlyto predict 300 mg proteinuria, but also to predict the higher range of proteinuria at different cutoffs such as2 grams, 3 grams, and 5 grams there by guiding the physicianto make the diagnosis of severe preeclampsia and thereafter to institute appropriate obstetric management. The cutoff for proteinuria in severe preeclampsia differs from country to country. In China, it is taken as 2.0 g/24 h. in UK it is 3.0 g/24, whereas in the United States, severe preeclampsia by proteinuria is defined as 5.0 g/24 h. A Korean study revealed that the optimal random protein creatinine ratio cutoff points as 0.63 and 4.68 for 300 mg/24 h and 5.0 g/24 h . In our study, the best cut off point for urine protein/creatinine ratio was 0.5 with sensitivity 92% and specificity 66%.

Reported cutoff values for UPCR and diagnostic summary in pregnant women with hypertensive disorders.

However the cutoff values for urine protein: creatinine ratio differs from center to center from 0.18 to1.14. These differences exist because of variation in patient selection, laboratory methods used to estimate urine protein and creatinine levels (various reagents, manual or automated methods), and importantly appropriateness of urine collection. The sensitivity and false positive rates for each type of protein estimation differ significantly. The pyragollal red reaction method used in this study has good range of both sensitivity and specificity and is widely used by laboratories all over the world. Though modified Jaffe's two-point rate method is used to estimate urinary creatinine universally, the exact values depend upon whether manual or automated machines are used for its estimation. Adhering to strict protocols may result in different cutoff values for urine protein/creatinine ratio, which is evident in reviewed studies.

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

LIMITATIONS

- 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.
- The number of patients with severe proteinuriaare less, so we cannot accurately correlate the values.
- Comparison of both couldn't be done in women who needed immediate intervention,only spot urine P/C ratio is done.

CONCLUSION

Since the level of urinary protein excretion has considerable clinical implicationsin the course of pregnancy and on the perinatal and maternal outcome, the early detection of even minor degrees of proteinuria is important. Dipstick analysis and sulfosalicylic acid method as a screening for proteinuria lacks reliability with a high rate of false positives.

For years, 24hour urine collection has been the standard for quantification of proteinuria in the management of women with preeclampsia. However, this method is cumbersome, subjective to collection errors, requires good patient compliance and results in the delay in the diagnosis of > 24 hours from the start of collection. Our contention was, that the value of the protein/creatinine ratio in a single urine sample is potentially more accurate, because it avoids collection errors and may give more physiologically relevant information. Quantification of proteinuria in a random sample was found to be far more cost effective and acceptable to the patient than a 24-hour urine collection.

Since preeclampsia is a progressive disease, repeated laboratory examinations to quantify proteinuria is required. Protein/creatinine ratio has been found to be a superior diagnostic tool compared to the routine urinalysis, which would otherwise be used for daily quantification of proteinuria. It is especially found to be useful in an outpatient setting to predict clinically significant proteinuria and to monitor renal functions in such women with lesser degrees of proteinuria thus avoiding unnecessary hospital admissions.

The present study indicates that this method for quantification of proteinuria, when properly interpreted, can provide valuable information, that for clinical purposes is a satisfactory substitute for the determination of protein excretion in a 24-hour collection.

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