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"A Comparative Study of Urine Dipstick and Urine Protein Creatinine Ratio with 24 Hour Urinary Protein in Estimation of Significant Proteinuria in Pre-Eclampsia"

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I. Introduction

Hypertensive disorders complicate 5-15% of all pregnancies of which pre-eclampsia occurs in 3-10%[1]. Hypertensive disorders complicating pregnancy are common and form one of the deadly triad along with hemorrhage and infection that result in maternal morbidity and mortality [2,3,4].

Pre-eclampsia is a pregnancy specific syndrome that can effect virtually every organ system .

Pre-eclampsia is Gestational Hypertension with proteinuria .

Proteinuria is an important diagnostic criterion.It occurs as consequence of glomerular capillary endotheliosis and reduction in integrity of glomerular barrier or reduced tubular reabsorption.

Proteinuria is essential for diagnosis of Pre-eclampsia[5]. It's presence is a sign of worsening hypertensive disease specifically Pre-eclampsia.

When proteinuria is severe and persistent, maternal and fetal morbidity are increased even more.

As the Proteinuria increases 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.

Among the various methods available to quantify proteinuria, 24 hour urinary protein estimation remains the gold standard. But it is time consuming, cumbersome , inconvenient for the both the patient and laboratory[7] .It is subject to collection errors, requires good patient compliance and there is a delay of 24 hours from the time of collection until the diagnosis is made.

Alternative Random methods like spot urine protein creatinine ratio and urine dipstick can be used.

The visual urine dipstick test serves as a rapid bed side screening test in the initial evaluation of proteinuria[8]. But recent studies have found it to be inaccurate giving high no.of false positive and false negative results, it being influenced by maternal hydration status, diurnal variation, presence of infection ,exercise etc. Hence an alternative method, urine protein creatinine ratio in a single urine specimen has been used for rapid and accurate detection of proteinuria in hypertensive pregnant women[9]. It avoids the influence of variations in urinary solute concentrations, can reduce delay in diagnosis and management of pre-eclampsia patients.

This approach is based on the fact that in the presence of stable GFR, urinary creatinine excretion has been reported to be fairly constant in a given individual.

Pre-eclampsia accounts for 15-20% of maternal mortality and high amount of morbidity. It is a major pregnancy complication causing preterm birth which is iatrogenic IUGR , Abruptio placentae, IUD which contribute significantly to perinatal morbidity and mortality.

Thus early diagnosis of preeclampsia and assessment of severity are important to consider for delivery and to improve maternal and perinatal outcome.

PRE ECLAMPSIA: Minimum Criteria

BP≥140/90 mmHg after 20 weeks of gestation with proteinuria.

Proteinuria :

≥300mg/24 hours

≥1+ dipstick[10](Persistent)

≥ 0.3 (≥30mg/mmol) Urine P/C ratio

URINE PROTEIN-CREATININE RATIO:

Studies have shown that urine protein-creatinine ratio in a random sample has an excellent correlation with the 24hr urinary protein excretion and has been used for rapid detection of proteinuria[6].It is less

cumbersome, less expensive and more convenient alternative to the 24 hr urine protein estimation. It also avoids collection errors. The best correlation has been found in the urine sample collected after the first voided morning specimen & before bedtime[11]. Though there is a marked day to day variation in plasma protein concentrations and protein excretions, certain levels of proteinuria have come to be accepted on the basis of empirical observations as having particularly important clinical implications. In the presence of a stable RFT, a Urine P/C ratio of >3.5 represents a nephrotic range proteinuria & correlates with a 24hr urine protein excretion of >3.5gm and a value <0.2 is said to be within normal limits & correlates with a protein excretion of <0.2 gm/day. Studies have reasoned out that, since urinary creatinine excretion, in the presence of a stable GFR is fairly stable in a given patient, protein excretion rate is also likewise fairly stable during a day. A simple ratio of the concentrations of urinary protein &creatinine in a single voided urine sample, expressed in mg/dl, would reflect the cumulative protein excretion over a day as the ratio of two stable rates would cancel out the time factor[12].In the literature, the spot protein- creatinine ratio performs well at assessing proteinuria in non-pregnant population suffering from systemic lupus erythematosus, glomerular disease, renal transplant[13].Many studies have demonstrated a good correlation between random urine protein creatinine ratio and daily protein excretion in pregnant women with or without preeclampsia

Urinary protein excretion is customarily expressed as the amount of protein excreted per unit time, factored for body surface area. Since creatinine production & excretion are also related to body size, it is possible to expect a good correlation between protein and creatinine ratio & the 24hr protein excretion. It is said to give physiologically more relevant information.

AIMS AND OBJECTIVES

1 .To study the correlationbetween

- visual dipstick urine proteintest,
- The protein content of a 24 hrs urine collection and
- Protein-creatinine ratio of spot urine sample.

2. To Assess the correlation of random methods like urine dipstick and urine spot P/C ratio with that of 24 hr urinary protein (gold standard) and to determine which is more correlating with 24 hr urinary protein.

II. Materials And Methods

SOURCE OF DATA

The study was conducted in 100 antenatal women with pre-eclampsia ,who got admitted in antenatal ward ,Department of Obstetrics and Gynecology ,Gandhi Medical College,Secunderabad.

Study type: observational study

Sample size: 100 cases

Period of study: 18 months (November 2016 - April 2018)

CRITERIA FOR SELECTION OF PATIENTS

Inclusion criteria:

Pregnant woman who were diagnosed as pre-eclampsia are included .

Pre-eclampsia is defined as Blood pressure of ≥ 140mm of Hg systolic or ≥ 90mm of Hg Diastolic recorded on at least two occasions , minimum of 4 -6 hours apart after 20 weeks of gestation along with proteinuria ≥1+ detected by dipstick urine analysis .

Exclusion criteria:

- Chronic Hypertensive pregnant women
- Pre existing renal disease -Stable Renal function ascertained by performing blood urea and serum creatinine.
- Urinary Tract Infection -Urine analysis done for all patients to exclude the presence of microscopic hematuria ,casts and bacteruria.
- Diabetes Mellitus

METHOD OF COLLECTION OF DATA:

All the patients satisfying the inclusion criteria were selected for the study. After getting the informed consent all women were examined. A detailed history was taken, general physical and systemic including obstetric examination was done.

A urinary dipstick was done and women who showed ≥1+ proteinuria along with BP ≥140 and or ≥90 are included .

The following investigations were done:
1) Blood urea and serum creatinine.
2) Urine analysis comprising of microscopy
3) visual urine dipstick test.

Proteinuria was graded as follows

Traces =<30mg/dl

1+ = >30 mg/dl

2+ = 100mg/dl

3+ = 300mg/dl

4+ = ≥1000mg/dl

4) 24hr urine collection for protein

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.

5) Random urine for protein-creatinine ratio estimation

The samples were sent to the biochemistry laboratory where:

Urine protein was measured by pyrogallol red dye method. The test was performed on an automated analyzer-OlympusAU400 Urine creatinine was measured by the Jaffe's reaction[14]. The test was performed on an automated analyzer-Olympus AU400.

The protein concentration in the 24 hrs urine collection sample was measured by the above mentioned method. The urine protein-creatinine ratio in a single voided urine specimen was obtained by dividing the urine protein concentration (mg /dl) by the urine creatinine(mg/dl).

The ISSHP (international society for the study of HTN in pregnancy) defined significant proteinuria as

1. 24 hr urinary protein ≥ 300 mg/day.
2.Random urinary protein/creatinine ≥ 0.3(≥ 30mg/mmol).

The data thus collected were analyzed using appropriate statistical methods

III. Results and Analysis

- NUMBER OF WOMEN STUDIED 100
- n=100

TABLE -1: DEMOGRAPHY OF THE SUBJECTS STUDIED

Variables (n=100)	Range	Mean±SD
Age (yrs)	20-41	28±5.52
POG	21-39	32±4.1
SBP	140-210	154**±15**
DBP	90-120	101**±9**
Primigravida	55	_
Multigravida	45	_

In the present study, it is observed that the mean ±SD of age of the subjects studied was 28±5.52 yrs (range:20-41yrs), gestational age was 32±4.1 , Systolic blood pressure (SBP) was 154**±15** mm of Hg, Diastolic blood pressure (DBP) was 101**±9** mm of Hg. Among them 55 were primigravidae and 45 were multigravidae.

TABLE -2: AGE WISE DISTRIBUTION OF SUBJECTS

Age (yrs)	**Frequency**
20-24	36
25-29	26
30-34	24
35-40	12
40-45	02

Among the studied subjects n=100; 36 were of 20-25yrs age group,26 were of 25- 29yrs age group,24 were of 30-34yrs age group 12 were of 35-40yrs age group and 2 were in 40-45 yrs of age group.

TABLE -3: PARITY WISE DISTRIBUTION OF SUBJECTS

	Frequency	Percentage
Primi	55	55
Multi	45	45
Total	100	100

Among the 100 pregnant women included in the study 55 were primigravidas and 45 were multigravidas

TABLE – 4: NO. OF WOMEN ON ANTI-HYPERTENSIVES

	Frequency	Percentage
Anti-hypertensives	89	89
No Anti-hypertensives	11	11
Total	100	100

Out of 100 women studied 89 were on anti-hypertensive drugs and only 11 were not on any anti hypertensive drugs at the time of collection.

TABLE – 5: NO. OF WOMEN WITH FEATURES OF SEVERE PRE-ECLAMPSIA

Features of severe pre-eclampsia	Frequency	Percentage
Present	41	41
Absent	59	59
Total	100	100

Out of 100 women , 41 patients had features of severe pre-eclampsia and 59 patients had no severe features .

TABLE – 6 : NO. OF WOMEN WITH IUGR

IUGR	Frequency	Percentage
Present	15	15
Absent	85	85
Total	100	100

Out of 100 women , only 15 % patients had associated IUGR.

TABLE -7 : DISTRIBUTION OF SUBJECTS BASED ON DEGREE OF PROTEINURIA (24HRS URINE PROTEIN)

24hr urine protein	Frequency	Percentage
<300mg	17	17
300mg- 2gm	59	59
>2gm (2-5gm)	14	14
>5gm	10	10
Total	100	100

In our study 17 patients had less than 300mg proteinuria , 59 had 300mg to 2gm of proteinuria and 14 patients had 2 -5gm of proteinuria and 10 patients had >5 gm 0f proteinuria

TABLE – 8: MEAN AND SD OF LABORATORY PARAMETERS

Variables (n=54)	Range	Mean±SD
Platelets (lakhs/mm³)	1.2 - 3.5	2.35±**0.56**
Blood urea (mg/dl)	15 - 40	24±**5.3**
Serum creatinine(mg/dl)	0.5-1.2	0.7±**0.13**

Different laboratory parameters were assessed to know the severity and complications of the disease and their mean and SD are as follows:Platelets = 2.35**±0.56,** Blood urea = 24**±5,** Serum creatinine = 0.7**±0.13**

TABLE –9: GHTN, NON SEVERE AND SEVERE PE BASED ON BP RECORDINGS AND PROTEINURIA(24 HR U/P)

Severity of hypertension	Frequency	Percentage
GEST.HTN	**17**	**17**
Non severe pre-eclampsia	42	42

Severe pre-eclampsia	41	41
Total	100	100

Out of 100 women,42 fell in to the category of non severepre-eclampsia and 41 in to severe preeclampsia and 17 in to category of GHTN.

TABLE – 10 : DISTRIBUTION OF SUBJECTS IN TO GEST.HTN , NON SEVERE AND SEVERE PRE-ECLAMPSIA BASED ON PROTEINURIA ONLY

Range of Proteinuria(24 hr U/P)	**Frequency**	**Percentage**	
<300 mg/day	**17**	**17**	**GHTN**
300 mg – 5 gm/day	73	73	NON SEVERE PE
>5gm/day	10	10	SEVERE PE
Total	100	100	

Out of 100 women, only 10 patients had severe proteinuria (>5gm).
73 patients had significant proteinuria i.e.,>= 300mg/ 24 hr but less than 5 gm.
17 patients had no proteinuria in 24 hr U/P but they were ≥ +1 on urine dipstick.

TABLE -11 : SENSITIVITY AND SPECIFICITY OF URINE P/C RATIO

		Urine24hrs (mg)		
		P	**N**	**Total**
Urine P/C ratio	**P**	**76**	**5**	**81**
	N	**4**	**15**	**19**
Total		**80**	**20**	**100**

Sensitivity 95% ,Specificity 75% , Positive Predictive Value = 93%, Negative PredictiveValue = 78%

TABLE -12: MEAN AND SD OF URINE DIPSTICK ,24HR U/P, URINE PCR IN GEST. HTN, NON SEVERE PE, SEVERE PE GROUP.

		N	**Mean**	**Std. Deviation**	**ANOVA F**	**P value**
Urine Alb	Gestational HTN	17	65.882	89.7259		
(dip Stick)	Non Severe PE	42	115.238	162.834		
	SeverePE	41	242.195	276.807	5.795	0.004
	Total	100	158.900	220.050		
Urine P/C Ratio	Gestational HTN	17	0.208	0.090		
	NonSevere	42	1.519	1.259		
	SeverePE	41	2.283	1.732		<0.001
	Total	100	1.609	1.549	13.700	HS
24 hr urine protein	Gestational HTN	17	181.765	46.652		
	Non Severe PE	42	1184.214	1395.438		
	SeverePE	41	1990.976	1751.591	9.862	<0.001
	Total	100	1344.570	1569.2511		HS

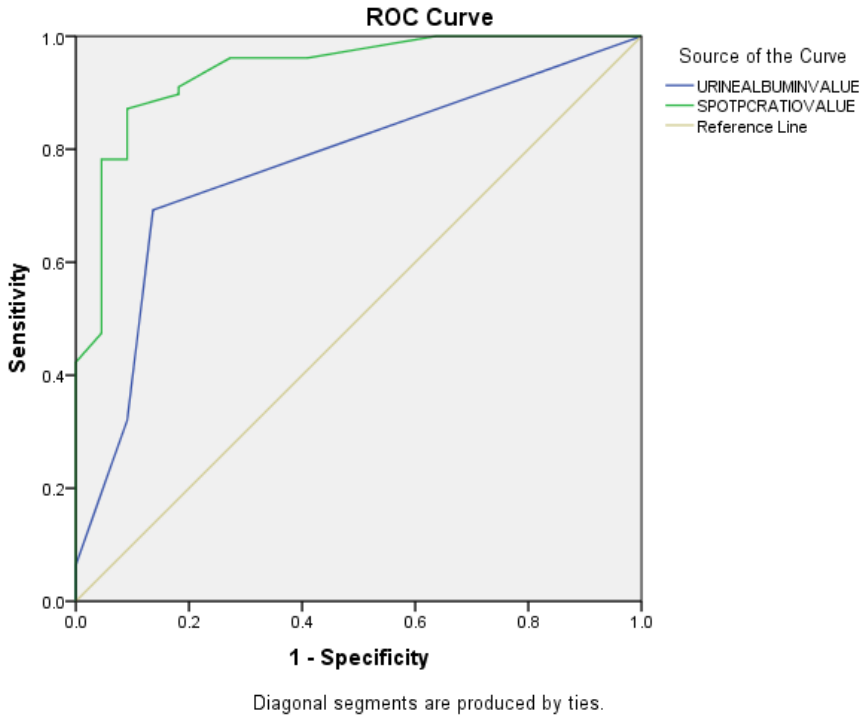
Mean and Std deviation of Urine dipstick, 24 hr urine protein and protein- creatinine ratio in different subgroups and total (n=100) subjects are shown in the table.This table also shows p value of 3 different methods of proteinuria estimation,where it is of statistically high significant ($p < 0.001$) in proteinuria diagnosed by 24hrs

urine protein and spot protein-creatinine ratio.

TABLE -13 : KARL PEARSON CORRELATION OF URINE DIPSTICK AND URINE P/C RATIO WITH 24HRS U/P

Tests		**r value**	**p value**	
Urinealb (dipStick)	**Urine24hrs (mg/24hrs)**	0.49	< 0.004	
UrineP/C ratio	**Urine 24hrs (mg/24hrs)**	0.59	< 0.001	**significant**

We observed a significant positive correlation between 24hrs urine protein and urine P/C ratio (r=0.59, p <0.001) than compared to urine dipstick test r=0.49,p = 0.004



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
URINEALBUMINVALUE	.771	.055	.000	.663	.880
SPOTPCRATIOVALUE	.939	.029	.000	.883	.995

The test result variable(s): URINEALBUMINVALUE, SPOTPCRATIOVALUE has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Urine protein by visual dipstick test:
Observation:
The area under the ROC curve - 0.771(95% CI: 0.663, 0.880) p =0.004 . The optimal cutoff point was 30; this cutoff yielded a sensitivity 90%and 1- Specificity = 70% At the cutoff point of 65, sensitivity dropped to 69% .
Urine protein by spot protein-creatinine ratio: **Observation:**
The area under the ROC curve – 0.939 (95% CI: 0883 ,0.995) p < 0.001 (significant). The optimal cutoff point was 0.315; this cutoff yielded a sensitivity = 84.8% and 1-specificity = 54.8% .But at the cutoff point of 0.2, sensitivity=94%,1-specificity= 62%.

IV. Discussion

Pre-eclampsia is distinguished 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. This can help us to know the severity of condition much earlier which can alter the course of management.

The gold standard for the diagnosis of significant proteinuria remains the 24hours urine protein. The need for a 24hr 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 urinary dipstick and urine protein-creatinine ratio are proposed. In our study we correlated these two methods with the gold standard.

In the present study of 100 women with pre-eclampsia , variables of the study were compared to the other studies. In the present study, women with BP≥140/90 mm of Hg with urine dipstick +1 were included. So, 100 patients had positive urine dipstick. But , when 24 hr urinary protein was done , only 80 patients showed proteinuria. Hence with urine dipstick , false positives were 20. Spot urine P/C ratio is done in all 100 patients, spot P/C ratio is positive that is ≥0.3 in 81 cases. In 5 patients. Spot P/C ratio was positive , but with 24 hr urine protein estimation there was no proteinuria. Hence, with spot P/C , false positives were 5.When both of the random tests are compared, spot P/C ratio has less false positives compared to urine dipstick.

KARL PEARSON CORRELATION AND ANALYSIS:

We found a significant positive correlation in our study, when 24 hr U/P and spot P/C raio were correlated with r = 0.59 and P value being significant at < 0.001, where all the observations were considered. A poor correlation was seen when 24 hr U/P and urine visual dipstick were correlated with r = 0.49 and P value being 0.004.

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 and <0.0001 which is also seen in our study.

Table-14: Comparison of results of previous studies with the present study

Studies	**Correlation Coefficient (r)**	**p-value**
Jaschevatzky et al[15] (1990) n=105	0.94	< 0.001
Coombs et al[16] (1991) n=329	0.98	<0.0001
Quadri et al[17] (1994) n=75	0.92	<0.0001
Steinhauslin et al[13] (1995) n=318	0.93	<0.001
Robert et al[18] (1997)n=71	0.94	< 0.001
Neithardt et al[19] (2000) n=30	0.93	< 0.001
Torng et al[12] (2001) n=289	0.79	<0.0001
Yamasmit et al[20] (2004) n=42	0.95	<0.001
Present study n=100	0.59	< 0.001

Table-15: This table compares the variables of present study, with a study done by Neithardtet al[19].

Variables	**Neithardt et al**	**Present Study**
Mean age	29.4	28
Serum creatinine	0.8	0.7
Mean Period of gestation	29.8	32.9
24hr urine protein	3700	1344
Protein/Creatinine ratio	2.8	1.6
Correlation coefficient 'r'	0.93	0.59

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 studies previously[21].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. But Robert et al in 1997 and Quadri et al in 1994 have proved in their studies that the protein-creatinine ratios are independent of renal function and reliable even in the presence of underlying renal disease and have advocated their use to monitor renal function in pregnancy[22,6]

V. Conclusion

1. The level of urinary protein excretion has considerable clinical implications in the course of pregnancy and on the perinatal and maternal outcome. Hence the early detection of even minor degrees of proteinuria is important.
2. Visual dipstick analysis method used as a screening test for proteinuria lacked reliability with a high rate of falsepositives.
3. 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, a delay of more than 24hrs on the diagnostic process and sometimes yields inaccurate results because of collection errors.
4. Our conclusion 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.
5. Quantification of proteinuria in a random sample is found to be more cost effective and acceptable to the patient than a 24 hour urinecollection.
6. Since pre-eclampsia is a progressive disease, a repeated laboratory examination to quantitate proteinuria is required. Our study demonstrates urine protein-creatinine ratio as a superior diagnostic and screening tool compared to the routine urine dipstick analysis used for daily quantification of proteinuria.
7. 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.

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

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