

To Study the Correlation Between 24 Hour Urinary Protein and Spot Urinary Albumin to Creatinine Ratio and to Determine the Accuracy of Spot Urinary Albumin to Creatinine Ratio as an Indicator to Detect Proteinuria and its Use as a Rapid Alternative Test Over 24 Hour Urinary Protein in Preeclamptic Women

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I. INTRODUCTION

Pregnancy is a unique state of interaction between two dynamic systems, the mother and the baby. Since each baby has a complement of the father's essence, the woman develops a special talent of immunological, physiological and anatomical tolerance to the new arrangement. There are many disease states that are as a result of father's invasion and pregnancy induced hypertension is one of them. It is an autoimmune condition where the mother rejects a foreign body which is the baby.

Preeclampsia is determined by the presence of blood pressure $\geq 140/90$ mm Hg on two occasions at least 4 hours part in a woman with previously normal blood pressure and significant proteinuria defined as the excretion of ≥ 300 mg of protein in a 24 hour urine collection or a timed excretion that is extrapolated to this 24 hour urine value or a protein to creatinine ratio of at least 0.3 (each measured as mg/dL) or a urinary dipstick of 1+ (recommended only if sole test available) after the 20th Week of gestation (ACOG 2013)¹.

Preeclampsia is a significant contributor of maternal and fetal mortality and it affects 2-8% of all pregnancies worldwide. Worldwide about 76,000 pregnant women die each year from preeclampsia and related hypertensive disorders. Hypertensive disorders along with sepsis (10.7%) are the second most leading cause of maternal mortality after hemorrhage (27.1%) (WHO 2014)². Fetal deaths due to maternal hypertensive disorders are thought to be in the order of 500,000 per annum. According to WHO (2007)³, the burden of preeclampsia lies more over developing countries and up to a maximum of 25% results in maternal death.

According to NICE Guidelines (2008)⁴, one of the cornerstones of detection of preeclampsia includes regular measurements of blood pressure and urine analysis for detection and quantification of significant proteinuria which is essential for both diagnosis and assessment of severity of the disease. The presence of significant proteinuria is associated with higher maternal and fetal risks. A need exists for a simple, rapid, convenient and accurate test to identify significant urinary proteinuria. This may lead to timelier decision-making aimed to decrease the patient's anxiety, reduce both fetal and maternal complications, shorten the length of hospital stay with its associated cost savings and detect women with true pathology for treatment.

Sufficient evidence from previous studies show a strong association between random urinary protein to creatinine ratio (PCR) or albumin to creatinine ratio (ACR) and 24 hour urinary protein excretion. However, a consensus has not yet been reached on this ratio's use in detection of fetomaternal outcomes and complications.

II. AIMS AND OBJECTIVES

1. To determine the accuracy of Spot Urinary Albumin to Creatinine Ratio as an indicator to detect proteinuria and its use as a rapid alternative test over 24 Hour Urinary Protein in women with Preeclampsia.
2. To correlate between 24 Hour Urinary Protein and Spot Urinary Albumin to Creatinine Ratio.

III. MATERIALS AND METHODS

Place of Study : Assam Medical College and Hospital, Dibrugarh
Study Design : Prospective Study

Type of Study : Hospital Based Study
Study Period : One Year (June 2016 to June 2017)
Sample Size : 70

Approval was obtained from the Institutional Ethics Committee.

Inclusion Criteria:

1. Primigravida with singleton pregnancy with cephalic presentation with intact membranes.
2. Patients having blood pressure recording of 140/90 mm Hg or more on two occasions 4 hours apart or a single diastolic reading of more than or equal to 110 mm hg after 20th week of pregnancy and the presence of proteinuria $\geq 1+$ as detected by dipstick urine analysis.
3. All booked and unbooked cases willing for a hospital delivery at Assam Medical College and Hospital Dibrugarh.

Exclusion Criteria:

1. Women with Previous Renal Disease, Chronic Hypertension, Urinary Tract Infection, Gestational Diabetes, Connective Tissue Disorders, Epilepsy and Pathological Vaginal Discharge.
2. Women who developed Eclampsia.
3. Women who required delivery before completion of 24 Hour Urine Sample for any reason whatsoever.
4. Cases who would have incomplete 24 Hour Urine Collection and Proteinuria < 300 mg would be excluded.
5. Patients not giving written informed consent.
6. Patients lost to follow up.

IV. METHODOLOGY

A total of 70 consecutive participants depending upon inclusion and exclusion criteria were selected counselled and explained the nature of study. Upon willingness to participate in the study, they were provided with a written and informed consent. A special proforma was made for the study and was filled for every patient who had given full and valid consent to participate in the study. Elaborate History was taken and General Physical and Obstetric Examination were done for every patient in the study. For all cases routine investigations and special investigations like dipstick test for urine albumin in random urine sample, 24 hour urinary protein, spot urinary albumin to creatinine ratio, liver function tests, renal function tests, coagulation profile etc. were done.

V. EVALUATION OF PROTEINURIA

(1) Screening of Proteinuria by Dipstick Method:

For all patients, screening of proteinuria was performed by urinary dipstick method. At the time of admission, random urine samples were tested for protein by a semi-quantitative commercially available dipstick by the name of Siemens Uristiks for Glucose and Protein Urinalysis.

(2) Measurement of 24 Hour Urinary Protein:

Collection and measurement of 24 hour urine collection sample:

After admission, the first voided morning urine specimen was discarded. Instructions were given to patients for proper collection of a 24 hour urine specimen. Patients were asked to rest at least for 1 hour in left lateral decubitus to avoid ureteral compression. Twenty four hour urine collection was started from 8 am in the morning following admission and was taken till next morning 8 am. After the collection was complete, the sample was sent to Department of Pathology at AMCH, Dibrugarh. The sample was measured by decanting in a 1 Litre measuring cylinder and hence, the total volume was noted.

Estimation of 24 hour urinary protein:

Esbach's Albuminometer Method was the method used to estimate 24 hour urinary protein.

This method is based on the principle of cold precipitation by strong acids.

(3) Measurement of Spot Urinary Albumin to Creatinine Ratio (ACR):

Collection of Sample:

5-10 mL mid-stream voided urine sample was collected for calculating spot urinary albumin to creatinine ratio immediately after the 24 hour urine collection. This sample was sent to a NABL laboratory nearby AMCH, Dibrugarh. This was made free of cost to the patient.

(a) Measurement of urinary albumin:

Spot urinary albumin was calculated by MALB Method by an Automated Analyser (Dimension Xpand Plus) by Siemens based on a technique called PETINA (Particle-Enhanced Turbidimetric Inhibition ImmunoAssay) which allows direct quantification of proteins.

(b) Measurement of urinary creatinine:

Spot urinary creatinine was calculated by the CREA Method by an Automated Analyser (Dimension Xpand Plus) by Siemens and was determined by Jaffe’s Reaction. In this method, in the presence of a strong base like sodium hydroxide (NaOH), picrate reacts with creatinine to form a red chromophore.

Calculations:

The spot urinary albumin to creatinine ratio was obtained by dividing the urinary albumin concentration by the urinary creatinine concentration.

$$\text{Urinary Albumin to Creatinine Ratio (mg/g)} = \frac{\text{Concentration of Urinary Albumin (mg/L)}}{\text{Concentration of Urinary Creatinine (mg/dl)}} \times 100$$



SIEMEN’S DIMENSION XPAND PLUS MACHINE FOR CALCULATING URINARY ALBUMIN TO CREATININE RATIO

Labour was recorded in the modified WHO Partograph. Newborns were attended by paediatrician and were examined thoroughly and any complication was duly noted and managed accordingly. Blood pressure record was maintained after delivery till discharge. On discharge, patients were advised to follow up after 6 weeks.

Statistical Methods:

The data collected was analysed by appropriate statistical methods. The mean, standard deviation and range of various parameters were computed. The statistical tests used for analysis were Pearson’s Correlation Co-efficient expressed as ‘r’, Kruskal-Wallis equality-of-population rank test using Dunn’s pairwise comparison expressed as χ^2 and Wilcoxon Rank-Sum (Mann-Whitney) Test. Using analysis of variance, a p value<0.05 was considered to be statistically significant.

VI. RESULTS

All the patients were studied with respect to socio-demographic characteristics and obstetrical parameters and the data has been shown in the following tables. The study aimed at determining the correlation between 24 hour urinary protein and spot urinary albumin to creatinine ratio and their association with fetomaternal outcomes.

Table 1- Socio-demographic, Obstetrical and Laboratory Parameters

Characteristics	Mean ± SD
Mean Age (Years)	23.73 ± 3.65
Mean BMI(kg/m ²)	20.19 ± 1.49
Mean Gestational Age (Weeks)	36.36 ± 1.62
Number of Antenatal Visits	3.46 ± 1.22
Mean Systolic Blood Pressure (mm Hg)	155.26 ±10.08
Mean Systolic Blood Pressure (mm Hg)	102.11±7.04
Mean 24 Hour Urinary Protein (mg/day)	1284.29±844.40
Mean Spot Urinary Albumin to Creatinine Ratio(ACR)(mg/gm)	1812.44±1162.58

Table 2-Tests of Validity of Spot Urinary Albumin to Creatinine Ratio (at 300 mg/g) In Prediction of Significant Proteinuria (=300mg/day)

SPOT URINARY ALBUMIN TO CREATININE RATIO (mg/gm)	24 HOUR URINARY PROTEIN (mg/day)		TOTAL
	≥ 300	< 300	
≥300	57	2	59
<300	3	8	11
TOTAL	60	10	70
Sensitivity	95%		
Specificity	80%		
PPV	96.6%		
NPV	72.7%		

Table 3-Comparison of Urinary Dipstick with 24 Hour Urinary Protein Estimation

URINARY DIPSTICK GRADING	24 HOUR URINARY PROTEIN (mg/day)		TOTAL
	≥300	<300	
=3+ (positive)	51	2	53
=2+ (negative)	9	8	17
TOTAL	60	10	70
Sensitivity	85%		
Specificity	80%		
PPV	96.2%		
NPV	47.0%		

Table 4-Comparison of Tests of Validity between Spot Urinary Albumin to Creatinine Ratio and Urinary Dipstick

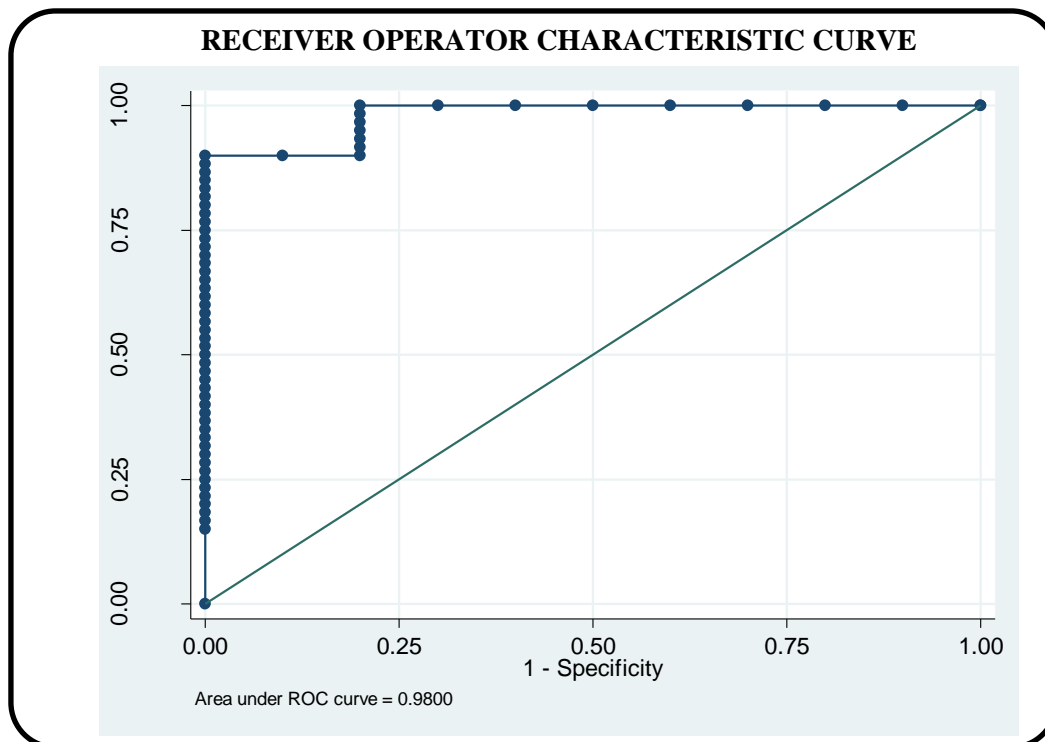
TESTS OF VALIDITY	SPOT URINARY ALBUMIN TO CREATININE RATIO (at 300 mg/g)	URINARY DIPSTICK (at +3 level)
Sensitivity	95%	85%
Specificity	80%	80%
PPV	96.6%	96.2%
NPV	72.7%	47.0%

Tables 2, 3 and 4 compared the two tests-urinary dipstick and spot urinary albumin to creatinine ratio with 24 hour urinary protein. The sensitivity of the two tests showed that spot urinary albumin to creatinine of at 300mg/gm had a better sensitivity of 95% as compared to the urinary dipstick which had a 85% sensitivity. The specificity of both tests was 80%. The positive predictive value of the two tests showed that spot urinary albumin to creatinine at 300mg/gm had a PPV of 96.6% as compared to the urinary dipstick which had a PPV of 96.2%. The negative predictive value of the two tests showed that spot urinary albumin to creatinine at 300mg/gm had a NPV of 72.7% as compared to the urinary dipstick which had a NPV of 47%.

In the present study, a cut-off value of spot urinary albumin to creatinine ratio that predicted significant proteinuria in a 24 hour urine collection was calculated at different levels of spot urinary albumin to creatinine ratio.

Table 5- Determination of Cut-Off Value and Comparison of Different Cut-Off Values of Spot Urinary Albumin to Creatinine Ratio in Prediction of Significant Proteinuria

URINARY ALBUMIN TO CREATININE RATIO CUT-OFF VALUE (mg/gm)	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)
200	98	29	93	67
250	100	60	94	100
300	95	80	97	73
350	95	80	97	73
400	95	80	97	73
450	95	80	97	73



ROC curve gave the most discriminant value for spot urinary albumin to creatinine ratio of 291.9 mg/gm for detecting significant proteinuria (≥ 300 mg/day) which gave the best sensitivity, specificity, positive predictive value and negative predictive values. The spot urinary albumin to creatinine ratio at 291.9 mg/gm yielded a sensitivity of 98.33% and a specificity of 80%. The area under the curve was 0.98 with a standard error of 0.0155 and a confidence interval between 0.94 and 1.00. The discriminant ability of spot urinary albumin to creatinine ratio to differentiate significant proteinuria from insignificant proteinuria in patients with preeclampsia was found to be 98%.

Table 6-Correlation between Spot Urinary Albumin to Creatinine Ratio and 24 Hour Urinary Protein

SPOT URINARY ALBUMIN TO CREATININE RATIO (mg/gm)	TOTAL		24 HOUR URINARY PROTEIN (mg/day)			r	r ²	p value
	n	%	Mean	SD	Range			
<300	11	15.71	381.82	297.72	200.00-1000.00	0.658	0.434	p value =0.027
300-2999	49	70.00	1172.45	420.70	200.00-2250.00	0.906	0.822	p value <0.05
≥ 3000	10	14.29	2825.01	850.2	2250.00-5000.00	0.845	0.715	p value =0.002
All values	70	100.00	1284.29	844.40	200.00- 5000.00	0.922	0.851	p value <0.05

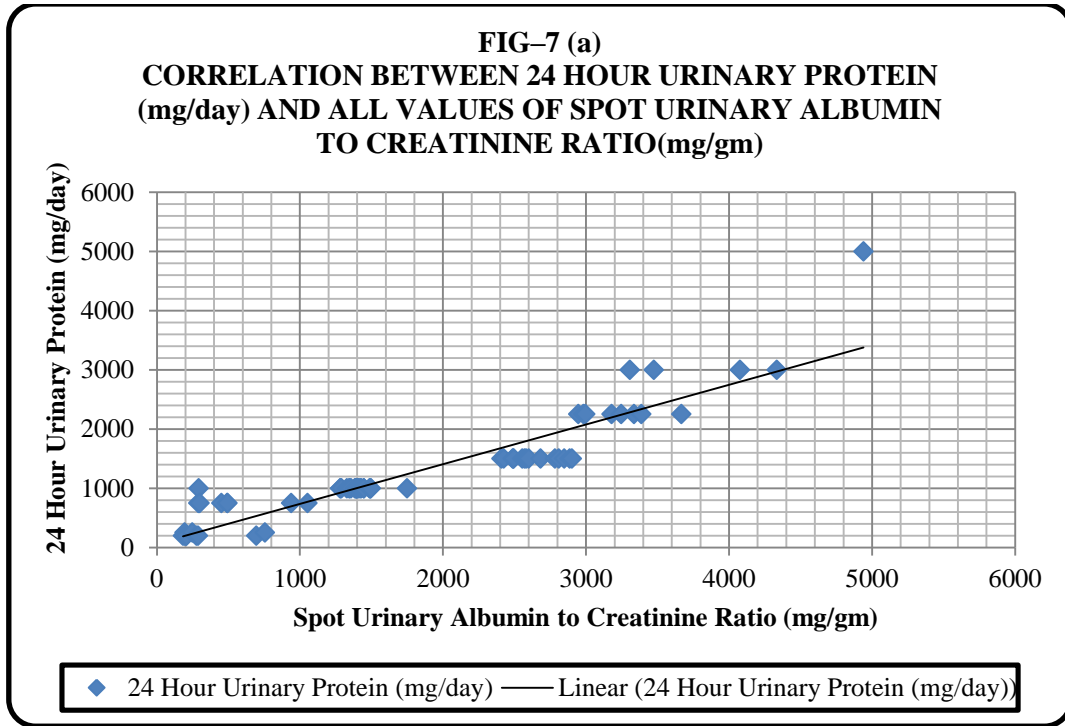
Table 6 shows the correlation between 24 hour urinary protein and different ranges of proteinuria detected by spot urinary albumin to creatinine ratio. Overall a good correlation was achieved between all values of spot urinary albumin to creatinine ratio and 24 hour urinary protein with a correlation coefficient of 0.922 and p value <0.05 which was statistically significant. There was a good correlation between spot urinary albumin to creatinine ratio < 300.0 mg/gm and 24 hour urinary protein with a correlation coefficient of 0.658. The p value was found to be 0.027 which was statistically significant.

Also, there was good correlation between spot urinary albumin to creatinine ratio between 300.0 mg/gm and 2999 mg/gm and 24 hour urinary protein and a correlation coefficient of 0.906 was achieved. The p value was calculated as < 0.05 which was statistically significant.

A good correlation between spot urinary albumin to creatinine ratio ≥ 3000.0 mg/gm and 24 hour urinary protein and was achieved with a correlation coefficient of 0.845. The p value was found to be 0.002 which was statistically significant.

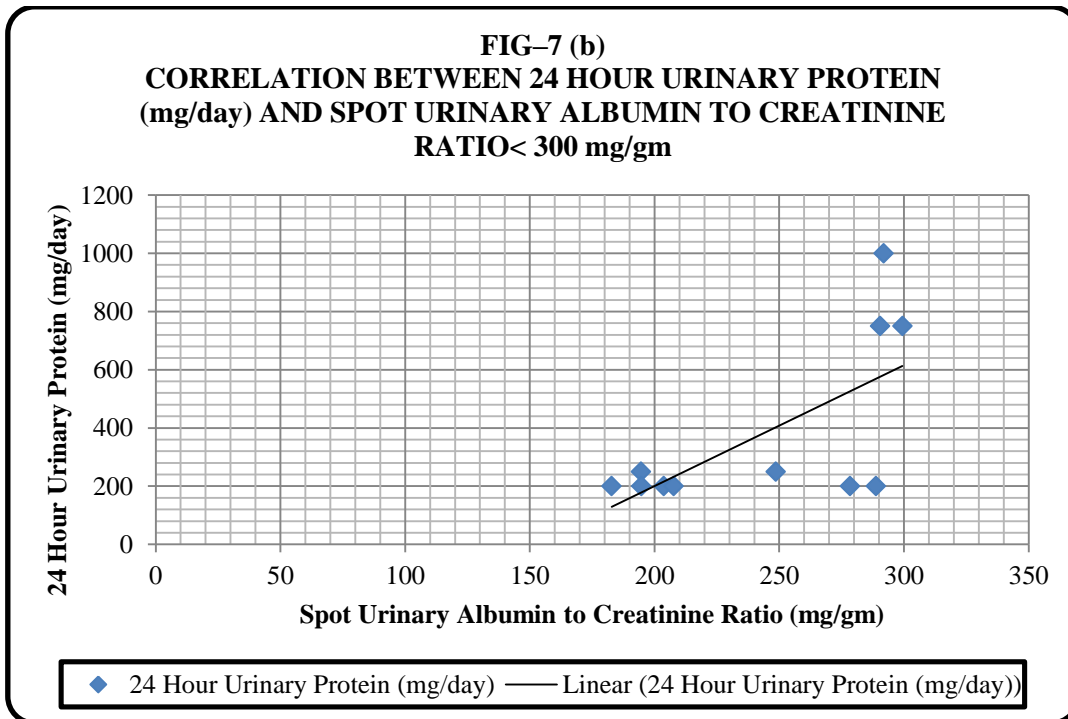
Tables 7- Scatter Diagram Showing Correlation between Spot Urinary Albumin to Creatinine Ratio To 24 Hour Urinary Protein

(a) For all values of spot urinary albumin to creatinine ratio:



The above scatter diagram (Fig-7(a)) shows correlation between all values of spot urinary albumin to creatinine ratio and 24 hour urinary protein. The diagram shows a strong positive correlation which means that high spot urinary albumin to creatinine ratio values (x variable) go with high 24 hour urinary protein values (y variable). The correlation coefficient was 0.922. The p value was < 0.05 which was statistically significant.

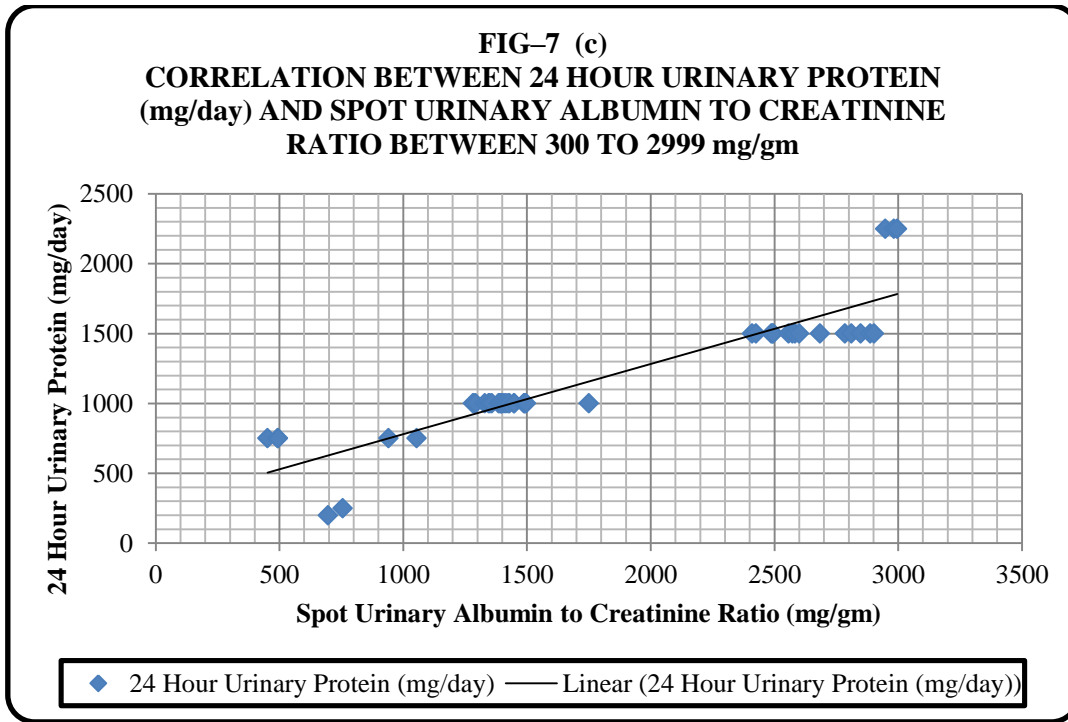
(b) For values of spot urinary albumin to creatinine ratio <300 mg/gm:



The above scatter diagram (Fig-7(b)) shows correlation between all values of spot urinary albumin to creatinine ratio < 300 mg/gm with 24 hour urinary protein. The diagram shows a moderate positive correlation, which means there is a tendency for high spot urinary albumin to creatinine ratio (x variable) values go with high 24 hour urinary protein values (y variable).

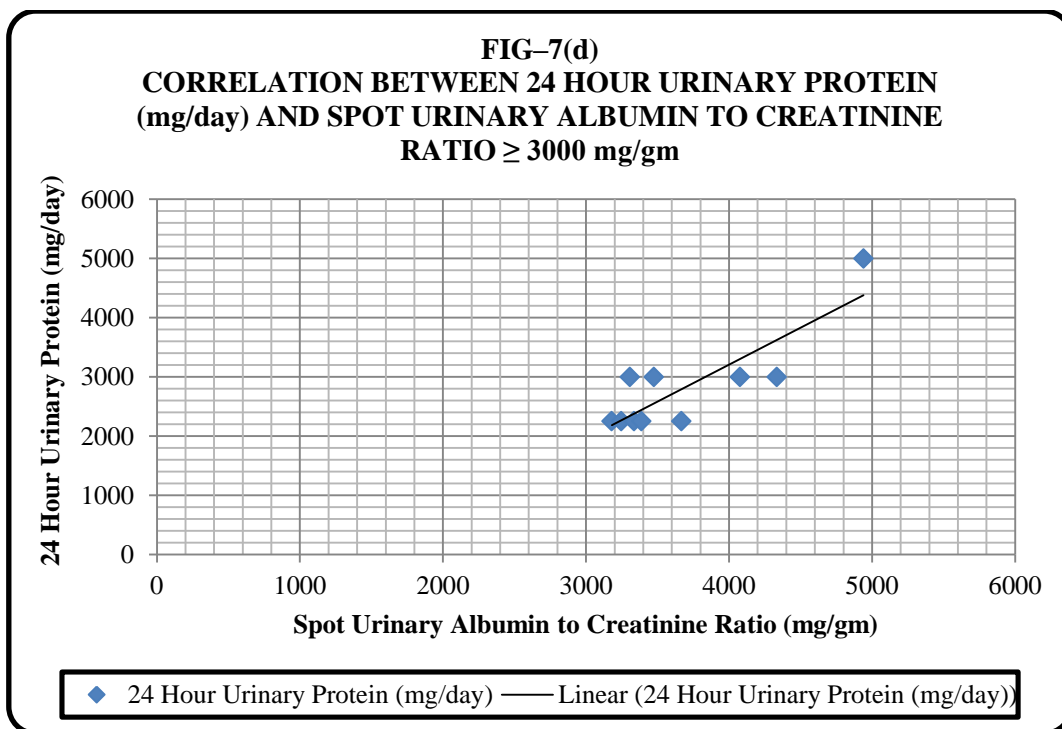
The correlation coefficient was 0.658. The p value was 0.027 which was found to be statistically significant.

(c) For values of spot urinary albumin to creatinine ratio between 300 to 2999 mg/gm:



The above scatter diagram (Fig-7(c)) shows correlation between all values of spot urinary albumin to creatinine ratio 300-2999 mg/mg with 24 hour urinary protein. The diagram shows a strong positive correlation which means that high spot urinary albumin to creatinine ratio values (x variable) go with high 24 hour urinary protein values (y variable). The correlation coefficient was 0.906. The p value was < 0.05 which was statistically significant.

(d) For values of spot urinary albumin to creatinine ratio ≥ 3000 mg/gm:



The above scatter diagram (Fig-7(d)) shows correlation between all values of spot urinary albumin to creatinine ratio ≥ 3000 mg/gm with 24 hour urinary protein. The diagram shows a strong positive correlation which means that high spot urinary albumin to creatinine ratio values (x variable) go with high 24 hour urinary protein values (y variable).

The correlation coefficient was 0.845. The p value was 0.002 which was found to be statistically significant.

VII. DISCUSSION

In the present study, the correlation coefficient between 24 hour urinary protein and spot urinary albumin to creatinine ratio was 0.922 with p value <0.05 which was highly significant. From the ROC curve analysis, the area under the curve was found to be 0.98 with a standard error of 0.0155 with a confidence interval between 0.94 and 1.00 which implied that the discriminant ability of spot urinary albumin to creatinine ratio to differentiate significant proteinuria from insignificant proteinuria in patients with preeclampsia was found to be 98%. Similar studies mentioned in the table below showed a good correlation between 24 hour urinary protein estimation and spot urinary albumin to creatinine ratio. The ROC curve analysis for AUC estimation in the below mentioned studies was also comparable to the present study.

STUDY	R	p value	AUC
Risberg <i>et al</i> (2004) ⁵	0.95	<0.001	0.985
Yamasmith <i>et al</i> (2004) ⁶	0.95	-	0.97
Wheeler <i>et al</i> (2007) ⁷	0.88	-	0.86
Shahbazian <i>et al</i> (2008) ⁸	0.84	<0.001	-
Demirci <i>et al</i> (2015) ⁹	0.758	-	0.89
Shreya <i>et al</i> (2015) ¹⁰	0.84	<0.001	0.92
Present Study (2016-2017)	0.922	<0.05	0.98

Rodriguez *et al* (2001)¹¹ and Huang *et al* (2012)¹² conducted 2 similar studies and found the correlation coefficient between 24 hour urinary protein and spot urinary albumin to creatinine ratio to be 0.80 and 0.938 respectively. The AUC in their studies was 0.91 and 0.918 respectively. Both studies showed a p value of <0.001 . The result of the present study was similar to the results reported by Risberg *et al* (2004)⁵, Yamasmith *et al* (2004)⁶ and Shreya *et al* (2015)¹⁰.

Comparison of Cut-Off Value of Spot Urinary Albumin to Creatinine Ratio in Prediction of Significant Proteinuria

In the present study, the most discriminant value for spot urinary albumin to creatinine ratio for detecting significant proteinuria (≥ 300 mg/day in a 24 hour urine collection) was found to be 291.9 mg/gm which yielded a sensitivity of 98.33% and a specificity of 80%. The table below shows similar studies with the best cut-off values of spot urinary albumin to creatinine ratio for detecting significant proteinuria.

STUDY	24 HOUR URINARY PROTEIN CUT-OFF (mg/day)	SPOT URINARY ALBUMIN TO CREATININE CUT-OFF VALUE (mg/gm)	SENSITIVITY (%)	SPECIFICITY (%)
Risberg <i>et al</i> (2004) ⁵	300	270	95	100
Yamasmith <i>et al</i> (2004) ⁶	300	250	96.6	92.3
Wheeler <i>et al</i> (2007) ⁷	300	210	86.8	77.6
Huang <i>et al</i> (2012) ¹²	300	228	82.4	99.4
Kayatas <i>et al</i> (2013) ¹³	300	280	60.4	77.8
Shreya <i>et al</i> (2015) ¹⁰	300	200	91.2	87.8
Present Study (2016-2017)	300	291.9	98.33	80

Rizk *et al* (2007)¹⁴ from the ROC curve analysis reported the optimal cut-off value for spot urinary protein to creatinine ratio to detect significant proteinuria was >190 mg/gm with a sensitivity of 80.4% and specificity of 68.8%. Demirci *et al* (2015)¹⁵ reported that the optimal cut-off value for spot urinary protein to creatinine ratio to detect significant proteinuria was 450 mg/gm with a sensitivity of 74.4% and specificity of 94.2%. The result of the present study was similar to that found in most of the previous studies in terms of the best cut-off value of spot urinary albumin to creatinine ratio to detect significant proteinuria.

VIII. CONCLUSION

Diagnosing proteinuria and its correlation with fetomaternal outcomes and complications in preeclampsia by 24 hour urinary protein estimation is a frustrating task for the obstetrician due to a long delay for results. Alternative testing methods like spot urinary albumin to creatinine ratios or spot urinary protein to creatinine ratios have correlated well with this gold standard method and the risk of adverse maternal and fetal outcomes was found to increase with higher values of spot urinary albumin to creatinine ratio.

Hence, the spot urinary ACR is a reliable, faster and accurate method for detection of proteinuria in preeclampsia and may allow better management of women with preeclampsia, thereby decreasing maternal as well as perinatal morbidity and mortality. This test could be used as an alternative to 24 hour urinary protein estimation in detection of significant proteinuria and better prediction of maternal and fetal outcomes in women with preeclampsia. However, further studies on a larger population may be required to consolidate the findings of the study.

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