Original Research Article

Protein: Creatinine Ratio is Reliable Indicator in Preeclampsia?

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Abstract

Objectives: This study was undertaken to compare the urine protein to creatinine ratio with 24-hour urine protein estimation in pregnancy complicated by hypertension and to establish the cut-off value of the urine protein to creatinine ratio for predicting significant 24-hour proteinuria. *Design*: This is a comparative study and consists of a single group of 240 subjects. *Setting*: This study was conducted in the Department of Obstetrics and Gynecology in collaboration with the Department of Biochemistry, JIPMER, Pondicherry, India, from February 2011 to January 2013. *Population*: The subjects included 240 pregnant women admitted after 20 weeks of gestation to the JIPMER hospital with hypertension (≥140/90 mmHg). Patients with known cases of renal diseases, diabetes and urinary tract infection were excluded. *Methods*: A first voided morning sample was obtained for urine protein and creatinine estimation and urine culture. Subsequent urine samples were collected for the 24-hour urine protein estimation. *Main outcome measures:* The spot urine protein to creatinine ratio and 24-hour urine protein were significantly correlated (r=0.98; P<0.0001). The cut-off value for the protein to creatinine ratio as an indicator of protein excretion ≥300 mg/day was 0.285. The sensitivity and specificity were 100% and 99.02%, respectively. The positive and negative predictive values were 100% and 99%, respectively. *Results*: The cut-off value for the protein to creatinine ratio as an indicator of protein excretion ≥300 mg/day was 0.285 with significant correlation. *Conclusion*: The spot urine protein to creatinine ratio is valuable for clinical purposes.

Keywords: Protein to creatinine ratio, PIH.

INTRODUCTION

Hypertensive disorders of pregnancy complicate up to 10% of pregnancies and remain a major cause of maternal morbidity and mortality [1]. Antenatal care involves a screening programme, with the measurement of blood pressure and proteinuria performed more frequently towards term, and this information is used to detect hypertensive disorders of pregnancy.

Pre-eclampsia is a multisystem disorder of an unknown aetiology, and it is characterized by the development of hypertension (140/90 mmHg or higher) with proteinuria after 20 weeks of pregnancy in previously normotensive and non-proteinuric patients [1]. Proteinuria is defined as the presence of 300 mg or more of protein in a 24-hour urine specimen [2]. The gold standard 24-hour urine collection method for protein estimation is not without errors, and the most obvious error is variable and incomplete collection. This test is inconvenient and is associated with a delay in laboratory analysis and availability of results. Lack of storage facilities, staff inadequacy and transportation also add to the difficulty. In some cases, delivery may occur before completion of 24-hour urine collection, and the patient often requires hospital admission to complete the test.

Estimation of the urine protein to creatinine ratio has shown promising results and correlates well with the 24-hour protein excretion [3-5]. Therefore, it may be used as an alternative to 24-hour urine protein excretion measurement.

Aim and Objectives
1. To compare the urine protein to creatinine ratio with 24-hour urine protein estimation in pregnancy complicated by hypertension.
2. To establish the cut-off value of the urine protein to creatinine ratio for predicting significant 24-hour proteinuria.

METHODS
This study was conducted in the Department of Obstetrics and Gynecology and in collaboration with

the Department of Biochemistry, JIPMER, Pondicherry, India. This study was approved by the JIPMER Research Committee and Institute Ethics Committee on January 13th, 2011 (IEC No. 2011/1/1 and dated 24/02/2011).

This is a comparative study and consists of a single group of 240 subjects. The sample size was calculated using the standard formula for estimating the sensitivity of a new test. The sample size was estimated by assuming a sensitivity of the spot urine protein to creatinine ratio for true proteinuria of more than or equal to 300 mg/day as 95%, with a 5% of level of significance and 4% desired precision.

Inclusion Criteria
Pregnant women hospitalized after 20 weeks of gestation with hypertension of 140/90 mmHg or higher on two occasions, at least 6 hours apart, were included in the study.

Exclusion Criteria
Patients with known renal disease, diabetes and urinary tract infections were excluded from the study.

We studied the demographic profile, gestational age, blood pressure, urine protein to creatinine ratio and 24-hour urine protein estimation of each patient. The procedure was explained, and consent was obtained from each patient. A first voided morning urine sample was obtained for urine protein and creatinine estimation and urine culture. Subsequent urine samples were collected for 24 hours, including a next day first morning voided sample, which was obtained for the 24-hour urine protein estimation.

Urine protein estimation was performed by the colorimetric method. Urine creatinine estimation was performed by the modified Jaffe's method using a standard autoanalyser. The sensitivity, specificity, and positive predictive and negative predictive values were determined for different protein to creatinine ratios. Receiver operating characteristic (ROC) curves were used for comparisons; values of greater than or equal to 300 mg/day were considered true positive for proteinuria, and values of less than 300 mg/day were considered true negative for proteinuria.

RESULTS
A total of 240 subjects were recruited for the present study. Among them, 27 delivered before collection of the 24-hour urine sample. The 24-hour urine collection was incomplete for 23 subjects. Seven subjects exhibited no continuity between the spot urine collection and 24-hour urine collection, and 7 subjects had a urinary tract infection. Thus, 64 subjects were excluded from the study. Therefore, 176 subjects were studied and followed up to 6 weeks postnatally, and

among them, 7 had chronic hypertension. Ultimately, 169 subjects were included in the study.

The ages of subjects ranged from 18 years to 39 years, with the majority, 131 (87.51%) subjects, in the age group of 21-30 years. The mean age was 25.09 years. Of the 169 subjects, 65 (39%) had gestational hypertension, 100 (59%) had pre-eclampsia and 4 (2%) had eclampsia. The pre-eclampsia group included subjects with mild pre-eclampsia, severe pre-eclampsia and imminent eclampsia.

The subjects included 90 (53.25%) primigravidas and 79 (46.75%) multigravidas. The incidence of hypertensive disorders of pregnancy was similar in the primigravidas and multigravidas. Of the 169 subjects, the majority, 88 (52.07%) subjects, were between 32 and 37 weeks of gestation. The maximum number of subjects in the gestational hypertension (33, 50.77%), pre-eclampsia (53, 31.36%) and eclampsia (2, 50%) groups were also in the gestational age group of 32-37 weeks.

In 61.54% of the gestational hypertension group, 76% of the pre-eclampsia patients and all 4 eclampsia patients, the blood urea levels were >15 mg/dL. In 4.62% of the subjects with gestational hypertension, 16% of those with pre-eclampsia and all patients with eclampsia had serum creatinine levels <0.8 mg/dL. A bilirubin level of 1.2 mg/dL was noted in one case. The incidence of anaemia was 57.98%. Three subjects had an AST level >70, and 6 subjects had an ALT level >70. One subject in the pre-eclampsia group had a platelet level <100,000/mm [3]. Abnormalities were found on examination of the fundus in 13 subjects. Six of these subjects had grade 1 hypertensive retinopathy, and 7 had grade 2 hypertensive retinopathy, while none had papilloedema.

One subject had a BMI of <18, but the majority, 108 (63.91%) subjects, had BMI values between 25 and 29.99. Nine subjects had a BMI>35. Eleven subjects had a history of hypertension during a previous pregnancy. A total of 143 subjects required antihypertensive medications to control their blood pressure. Twenty subjects received antepartum steroids, and 22 subjects received magnesium sulphate (MgSO$_4$).

Labour was induced in 128 subjects by various acceptable methods. Of the 169 subjects, 99 (59%) had term deliveries, and 70 (41%) had preterm deliveries. The subjects included 152 singleton pregnancies, while 15 had twins, and 2 had triplets. One subject had a single foetal demise. Of the 191 babies from the 169 pregnancies, 132 (69.11%) had a low birth weight of <2500 g. Forty-six (25.14%) newborns were transferred to the neonatal intensive care unit. Nine (4.92%) cases of intrauterine foetal death and 9 (4.92%) still births occurred. Furthermore, 9 newborns expired in the neonatal intensive care unit. The incidence of

prematurity was 41%, while the incidence of intrauterine growth retardation was 15.38%.

In our study, 102 (59%) subjects had protein excretion levels of <300 mg/day, while 67 (40%) had significant proteinuria (≥ 300 mg/day). A total of 52.24% of the proteinuria group had preterm deliveries, whereas 46.08% of the non-significant proteinuria group had preterm deliveries. The incidence of low birth weight (<2500 g) was 61.53% in the proteinuria group. Of the 80 babies born to subject with significant proteinuria, 4 (5%) exhibited intrauterine foetal death/still birth, while 20 (25%) babies were transferred to the neonatal intensive care unit. Furthermore, 3 babies died in the neonatal intensive care unit.

During postnatal follow up, 130 (76.92%) subjects had their blood pressure normalized in <48 hours duration, and 37 (21.89%) subjects had normal blood pressure on first follow-up during the 2nd postnatal week. Two subjects had a normal blood pressure on follow-up during the 4th postnatal week. The blood pressure was persistently high beyond the 12 weeks of follow-up for 7 subjects, and they were considered to have chronic hypertension and were excluded from the study.

An excellent correlation exists between the spot urine protein to creatinine ratio (mg/mg) and the 24-hour urine protein (mg/mg) (Figure-1).

The area under the ROC curve is 0.999 (95% confidence interval). The best cut off-value for the protein to creatinine ratio for detecting significant proteinuria according to the ROC curve is 0.285, with 100% sensitivity, 99.02% specificity, 100% positive predictive value and 99% negative predictive value (Figure-2).

DISCUSSION

This study consisting of 169 subjects revealed a P value of <0.0001 (two tailed), which is considered extremely significant, and an excellent correlation coefficient (r=0.9778), with a 95% confidence interval of 0.9700 to 0.9836, for the spot urine protein to creatinine ratio (mg/mg) and 24-hour urine protein (mg/day) calculated by Pearson's method (Table-1).

Table-1: Correlation coefficient between the spot urine protein to creatinine ratio (mg/mg) and the 24-hour urine protein (mg/day) calculated by Pearson's method

Number of subjects	P value (two tailed)	95% confidence interval	Correlation coefficient (r)	Coefficient of determination (r^2)
169	<0.0009	0.9700-0.9836	0.9778	0.9561

The coefficient of determination (r squared) is 0.9561. Table-2 shows the results of similar studies compared to those of the present study in terms of the correlation coefficient for the protein to creatinine ratio with 24-hour protein.

Table-2: Comparision of present study with previous studies

Studies	Correlation coefficient	P-values
Ginsberg *et al.,* [6]	0.93	<0.001
Neithardt *et al.,* [7]	0.93	<0.001
Robert *et al.,* [8]	0.94	<0.001
Boler *et al.,* [9]	0.99	<0.001
Saudan *et al.,* [3]	0.93	<0.001
Young *et al.,* [10]	0.80	<0.001
Jaschevatzky *et al.,* [11]	0.92	<0.001
Present study	0.98	<0.001

ROC curves for the spot urine protein to creatinine ratio show an area under the ROC curve of 0.999 (95% confidence interval) (Figure-2).

The cut-off value of 0.285 results in a sensitivity of 100%, specificity of 99.02%, positive predictive value of 99%, and negative predictive value of 100%, with a 67% likelihood ratio.

In the study by Leanos-Miranda *et al.,* [12] the cut-off value was ≥0.3 with a 98.2% sensitivity, 98.8% specificity, 97.2% positive predictive value and 99.2% negative predictive value. A study by Ramos *et al.,* [13] showed a cut-off value of 0.5 [14] with a sensitivity of 96%, specificity of 96%, positive predictive value of 96% and negative predictive value of 96%, whereas in the study by Rodriguez-Thompson and Lieberman [15] the best cut off value of ≥ 0.19 yielded a sensitivity of 90% and specificity of 70%. In the study by Yamasmit *et al.,* [16] a cut-off value of 0.19 demonstrated a sensitivity of 100% and a specificity of 53.8%.

CONCLUSION

The level of urinary protein excretion has considerable clinical implications for the course of pregnancy and the perinatal and maternal outcomes.

Therefore, early detection of even minor degrees of proteinuria is important.

Dipstick analysis as a screening procedure for proteinuria lacks reliability and has a high rate of false positives. For years, the 24-hour urine collection method has been the gold standard for the quantitation of proteinuria in the management of women with pre-eclampsia. However, this method is cumbersome, is subject to collection errors, requires good patient compliance and results in a delayed diagnosis. The value of the protein to creatinine ratio in a single urine sample is potentially more accurate because it avoids collection errors and may yield more physiologically relevant information.

Quantitating proteinuria in a random sample has been found to be more convenient and acceptable to the patient than a 24-hour urine collection, which often requires hospitalization. Since pre-eclampsia is a progressive disease, repeated laboratory examinations to quantitate proteinuria are required. The repeated collection of 24-hour urine samples is not practical. Therefore, the protein to creatinine ratio is a superior diagnostic tool for predicting significant proteinuria.

The cut-off value for the spot urine protein to creatinine ratio is 0.285 mg protein/mg creatinine. A level below this is not associated with significant proteinuria, and further testing is unnecessary. This method for the quantitation of proteinuria, when properly interpreted, can provide valuable information for clinical purposes and is a satisfactory substitute for 24-hour protein estimation. It is useful in the outpatient setting to predict clinically significant proteinuria without causing an inconvenience to women, and it avoids unnecessary hospitalization. The spot urine protein to creatinine ratio is valuable for clinical purposes and is a satisfactory substitute for 24-hour protein estimation.

Limitations
This study was limited to hospitalized, non-ambulatory patients. Since protein excretion is affected by postural changes, the ambulatory status of the subjects (i.e., patients that are allowed to stand versus those confined to a supine position) may be a confounding factor in the quantitation of proteinuria.

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Author Contributions
1. Dr. Hanumant V. Nipanal – Subject recruitment, data collection, manuscript preperation
2. Dr. Dilip Kumar Maurya : Conceived idea and Protocol preperation
3. Dr. S Susmitha : Manuscript preparation
4. Dr. Ravindra P N: Statistical analysis

Details of Ethics Approval
This study with reference number IEC No. SEC/2011/1/1and dated 24/02/2011, has been approved by Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER). Puducherry, India-605006, research committee and institute ethics sub-committee (human studies) on 13th January 2011. on 24-02-2011.

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