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## OBSTETRICS

# A Correlation between First-void Morning Urinary Protein to Creatinine Ratio (UPCR) and 24 Hours Urinary Protein in Pregnancy with Suspected Preeclampsia

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### ABSTRACT

**Objective:** To evaluate a correlation between first-void morning urinary protein to creatinine ratio (UPCR) and 24-hour urine protein in pregnant women suspected of preeclampsia.

**Materials and Methods:** A total of 40 pregnant women suspected of preeclampsia were enrolled and admitted for 24-hour urine collection. Collected urine was divided into 2 parts, first-void morning urine and the remaining. First-void morning UPCR was determined and 24-hour urine protein was calculated by a combination of protein from both specimens. Significant proteinuria was diagnosed if the total 24-hour urine protein was greater than 300 mg. A correlation between first-void morning UPCR and 24-hour urine protein was estimated using Pearson product-moment correlation coefficient (r).

**Results:** Mean age was 31.0 ± 7.0 years and mean gestational age was 33.2 ± 4.6 weeks. Eight patients (20%) had significant proteinuria and were diagnosed as preeclampsia. A correlation between first-void morning UPCR and 24-hour urine protein showed a significant positive correlation with coefficient (r) of 0.76, $p < 0.001$. At the cut-off value of 0.3, first-void morning UPCR had sensitivity of 87.5% (95% CI 46.7 – 99.3) specificity of 96.9% (95% CI 82.0 – 99.8) for diagnosis of significant proteinuria.

**Conclusions:** First-void morning UPCR significantly correlated with 24-hour urine protein. It should be considered as an alternative method for detecting significant proteinuria in women suspected of preeclampsia.

**Keywords:** preeclampsia, urine protein to creatinine ratio, 24-hour urine protein

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## Introduction

Preeclampsia is a serious complication during pregnancy. It complicates about 2% to 8% of all pregnancies and, together with eclampsia; it causes 10% to 15% of overall maternal death, especially in low resource countries[1-3]. This syndrome diagnosed by a combination of a new onset of hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg) and significant proteinuria (≥ 300 mg in 24 hours) after 20 weeks' gestation in a previous normotensive pregnant woman[2, 4]. Its pathogenesis is related to poor placentation and excessive maternal intravascular systemic inflammatory response. As a result, it finally causes generalized endothelial dysfunction and increased vascular reactivity[3, 4]. In general, the gold standard is based on a collection of total 24 hours urinary protein[4]. However, this collection could be disturbed by many factors, for example, physiological changes of the urinary tract, daily physical activity and an upright or active posture[5-7]. These factors result in an incomplete collection, even a collection from an indwelling Foley's catheter as well as time consuming and less comfortable to patients[8, 9].

Many researchers discovered various alternative methods to substitute a collection of 24 hours urine protein. Urine dipstick ≥ $1^+$ is widely used to diagnose significant proteinuria. Many studies, however, concluded that this method has a poor quality because of its high false positive and false negative result[10-12]. A collection of urine protein to creatinine ratio (UPCR) is another alternative method for prediction of significant proteinuria. Some previous studies concluded that spot or random UPCR is valuable especially in ambulatory patients because it showed high correlation with 24 hours urine protein and can be used as a rule out test for significant proteinuria[13-17]. However, some studies did not support this method because of its poor prediction and poor quality unless in extreme value of proteinuria[18-20]. The data from meta-analysis showed insufficient evidences whether this method should be used in clinical practice[21]. First-void morning UPCR is preferable for detection of significant proteinuria in various areas of medicine, but the data is still lacking in obstetrics[7]. It could be explained that a long period of time overnight and independent from upright posture provided a large amount of protein in the first-void morning urine which could reflect to 24 hours urine protein. The purpose of this study is to demonstrate a correlation between first-void morning UPCR and 24 hours urine protein in pregnancy with suspected preeclampsia.

## Materials and methods

After an approval from the Siriraj Institute Review Board (SIRB), the study was launched between June and December 2013.

Inclusion criteria included inpatient pregnant women who aged over 18 years old and suspected of preeclampsia from a new onset of hypertension after 20 weeks' gestation with or without positive urine dipstick. All patients were admitted for diagnosis of significant proteinuria by a collection of 24 hours urine. Patients with congenital renal anomalies, dependence on drugs that impaired renal function and known medical diseases involving renal function, for example, chronic renal disease or diabetic nephropathy, were excluded. All participants were counseled and written informed consent.

Based on our management protocol, suspected preeclamptic patients were advised to admission for monitoring of blood pressure and collection of 24 hours urine protein. All urine voided within 24 hours were collected and were separate into 2 portions which were the first-void morning urine, or "A" portion and its remaining or "B" portion. After complete 24 hours collection, both specimens (A and B portion) were sent separately and immediately to the standard clinical laboratory center by the ISO 15189. The urine protein was tested by turbidimetric benzethonium chloride method and the urine creatinine was tested by enzymatic method. Both tests were controlled their precision by within-run and between-run coefficient of variation (CV). The cost of both tests is similar and the turn-around time is usually within 1 hour. First-void morning UPCR from "A" portion was reported within 1

decimal place. Twenty-four hours urine protein was calculated by a combination of protein from first-void morning urine and their remaining (Total protein from A and B portion). The significant proteinuria was diagnosed if the total 24 hours urine was more than 300 mg. All data were collected and analyzed by IBM SPSS Statistics Desktop for window version 18.

Characteristics of study population were reported as means, standard deviation, number and percentage. A correlation between first-void morning UPCR and 24 hours urine protein was calculated and reported by Pearson product-moment correlation coefficient (r). An accuracy of first-void morning UPCR using an appropriate cut-off level was reported. P-value of more than 0.05 revealed statistical significant.

The sample size was calculated according to the following formula

$$N = \frac{(z_{\alpha} + z_{\beta})^2}{\frac{1}{4}\left[\log_e\left(\frac{1+\rho}{1-\rho}\right)\right]^2} + 3$$

The data from the pilot study in 10 cases of suspected preeclampsia showed that a correlation coefficient (ρ) between first-void morning UPCR and 24 hours urine protein was 0.48. Type 1 error (α) was 0.05 and type 2 error (β) was 0.1. As a result, a total sample size plus 20 percentage of error was about 40 cases.

**Table 1.** Characteristic of study population (N = 40)

| | Mean (SD) | N (%) |
|---|---|---|
| Age (years) | 31 (7) | |
| BMI (kg/m$^2$) | 27.8 (6.9) | |
| Gestational age (weeks gestation) | 33.2 (4.6) | |
| 24 hour urine protein (mg) | 230.1 (169) | |
| **Blood pressure on diagnosis** | | |
| Systolic blood pressure (mmHg) | 149.1(13.4) | |
| Diastolic blood pressure (mmHg) | 93 (9.9) | |
| **Parity** | | |
| 0 | | 28 (70) |
| ≥1 | | 12 (30) |
| **BMI*** | | |
| Underweight (<18.5 kg/m$^2$) | | 2 (5) |
| Normal (18.5-23 kg/m$^2$) | | 12 (30) |
| Overweight (23–27.5 kg/m$^2$) | | 7 (17.5) |
| Obese (>27.5 kg/m$^2$) | | 19 (47.5) |
| **Smoking** | | |
| Yes | | 38 (95) |
| no | | 2 (5) |

* WHO Appropriate body-mass index for Asian populations[31]

**Table 2.** Characteristic of preeclamptic patients (N = 8)

| Case | Systolic blood pressure (mmHg) | Diastolic blood pressure (mmHg) | Urine dipstick | 24 hours urine protein (mg) | First-void morning UPCR* |
|---|---|---|---|---|---|
| 1 | 155 | 79 | 2+ | 852.40 | 0.3 |
| 2 | 170 | 100 | trace | 741.60 | 0.7 |
| 3 | 150 | 90 | trace | 598.10 | 0.3 |
| 4 | 140 | 90 | negative | 391.20 | 0.3 |
| 5 | 139 | 97 | trace | 377.30 | 0.3 |
| 6 | 160 | 100 | negative | 372.70 | 0.3 |
| 7 | 170 | 110 | negative | 352.20 | 0.5 |
| 8 | 144 | 98 | 1+ | 300.70 | 0.1 |

*each measured as mg/dl

**Table 3.** An accuracy of first-void morning UPCR for diagnosis of preeclampsia (N=40)

| | 24 hours urine protein | |
|---|---|---|
| First-void morning UPCR* | ≥ 300 mg | < 300 mg |
| ≥ 0.3 | 7 | 1 |
| < 0.3 | 1 | 31 |

*each measured as mg/dl

Sensitivity 87.5% (95% CI: 46.7 – 99.3), Specificity 96.9% (95% CI: 82.0 – 99.8), PPV 87.5% (95% CI: 46.7 – 99.3), NPV 96.9% (95% CI: 82.0 – 99.8), LR+ = 28.1, LR- = 0.13

## Results

A total of 40 suspected preeclamptic patients who reached the inclusion criteria were all enrolled. Table 1 revealed baseline characteristic of study population. Majority was obese women (47.5%), nulliparous (70%) and non-smoker (95%). Only 8 patients (20%) of all admission were diagnosed as preeclampsia. The correlation coefficient (r) between first-void morning UPCR and 24 hours urine protein showed a strong correlation ($r = 0.76$, $p < 0.01$). Figure 1 demonstrated the scatter plot between both of them. The table 2 showed characteristic of all preeclamptic patients. Only 2 patients (25%) had positive urine dipstick. Regard to first-void morning UPCR, 7 of 8 patients (87.5%) showed UPCR of 0.3 or more. Only one patient who had 24 hours urine protein overlay on the line (300.7 mg) did not have a high level of first-void morning UPCR.

The table 3 showed an accuracy of first-void morning UPCR when using the cutoff point of 0.3. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 87.5% (95% CI: 0.47 - 0.99), 96.9% (95% CI: 0.82 - 1.0), 87.5% (95% CI: 0.47 - 0.99) and 96.9% (95% CI: 0.82 - 1.0), respectively. Positive and negative likelihood ratios were 28.1 and 0.13, respectively.

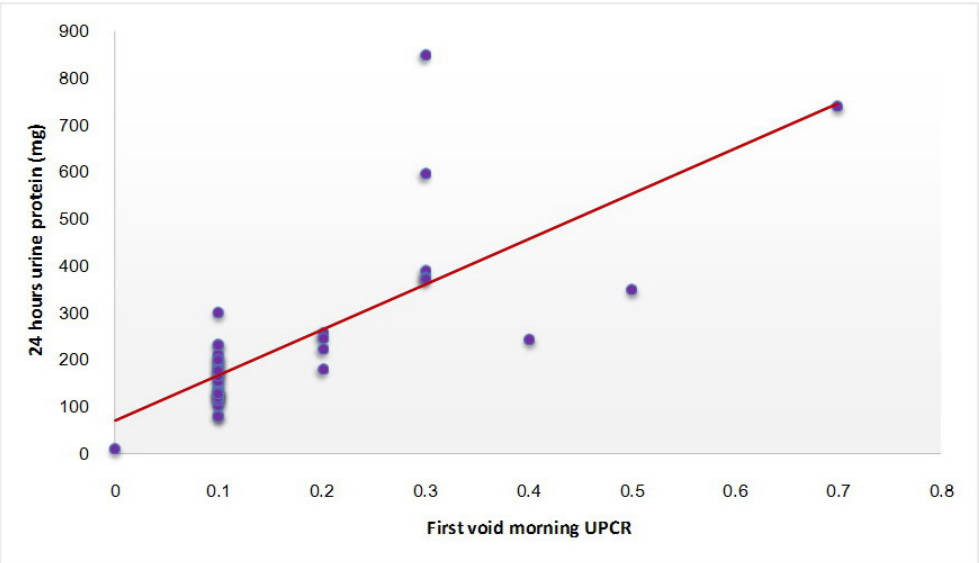


**Fig. 1.** Correlation between the first-void morning UPCR and 24 hours urine protein (N=40), r=0.76

## Discussion

Preeclampsia is the leading cause of maternal mortality and morbidity, especially in developing countries. Significant proteinuria is still a crucial criterion for the diagnosis of preeclampsia[3,22]. The gold standard is a collection of 24 hours urinary protein[4]. However, this method is cumbersome, time consuming and uncomfortable to patients. As a result, many investigators attempted to find out a substitute method to identify significant proteinuria. Urine dipstick was found to be ineffective because of its low accuracy[10-12]. This study confirms that urine dipstick is unreliable to diagnose significant proteinuria because only one-fifth of preeclamptic patients (20%) had a positive result. Random or spot urine protein to creatinine ratio (UPCR) has a significant correlation and was found to be value but was still inconclusive to use in clinical practice[21].

This study focused on the first-void morning UPCR as an alternative method for the diagnosis of significant proteinuria and demonstrated a significant strong correlation between first-void morning UPCR and 24-hour urine protein among women suspected of preeclampsia ($r = 0.76$, $p < 0.001$). The result was similar to other studies. Ruggenenti P, et al concluded that spot morning UPCR is simple, reliable and inexpensive to predict proteinuria in non-diabetic patients with chronic nephropathies[23]. Similar to the study of Morales JV, et al, they found a high significant correlation between morning UPCR and 24 hours urine protein ($r > 0.9$) in all patients that range from normal to serious impaired renal function[24]. Salesi M, et al also studied in patients with lupus nephritis and concluded that morning UPCR is significant correlated with 24 hours urine protein ($r = 0.83$, $p < 0.0001$)[25]. They supported the use of morning UPCR as a valuable predict severity of lupus nephritis. The strength of our study is that it is an initial trial that concentrated the role of using first-void morning UPCR in the prediction of significant proteinuria in obstetric patients. Regard to the result, it could be confirmed that this test is possible to use as an acceptable alternative to diagnose significant proteinuria.

The accuracy of first-void morning UPCR is superior to a spot UPCR and other time. Besides of posture independence, this test is not only timed but also duration related specificity. Depending on glomerular infiltration rate, previous study by Kristal B, et al supported that UPCR is higher in the morning

rather than in the evening and could be used as a screening or a follow-up method of proteinuria[26]. Moreover, a long duration of urine collection (4, 8 or 12-hour urine collection) had a correlation with 24 hours urine protein and, because of its high accuracy; these could be used as a substitute of 24 hours urine protein[27-30]. Our study showed only 20% of suspected patients was diagnosed as preeclampsia while the remaining wasted their time in the hospital for 24 hours urine collection. Although the cost and turn-around time of both tests are similar, those at risk women would need no admission and waste their time in the hospital for urine protein collection, the diagnosis could be made earlier as an outpatient and treatment would not be delayed. In addition, the method could possibly be used for follow-up disease progression as well.

Regard to ACOG 2013, the diagnosis of preeclampsia includes high blood pressure and proteinuria or a new onset of end organ damage (thrombocytopenia, renal insufficiency, liver failure, pulmonary edema, and cerebral or visual symptom) without proteinuria[22]. In addition, protein to creatinine ratio of greater than or equal to 0.3 is still valuable in this recommendation. Similar to this study, we founded a value of first-void morning UPCR of 0.3 in a majority of cases (87.5%). When we used this value as a cut-off point for diagnosis of preeclampsia, it showed a high level of sensitivity, specificity, positive and negative predictive value. Moreover, the results of the study also showed that positive and negative likelihood ratios were 28.1 and 0.13, respectively. This helps confirming that there is a great chance of having significant proteinuria when first-void UPCR is > 0.3 and much less chance when the test result is < 0.3. A low level of first-void morning UPCR was found in the only one patient who exactly had 300.7 mg of 24 hours urine protein. This could be inferred that first-void morning UPCR is not sensitive to detect an early onset of significant proteinuria.

Some limitations of this study are, firstly, the time interval overnight before a collection of first-void morning urine had not been observed and some patients might even urinate during the night. Therefore, this could be a variation among patients. However, this is a similar situation when we use this test as a tool in real clinical practice, especially at an antenatal care clinic. This study showed only the beneficence of the first-void morning UPCR as a predictor of significant proteinuria for the diagnosis of preeclampsia. It did not focus to an efficacy of this test in the diagnosis of the severity of preeclampsia as well as the usefulness of the test to differential diagnosis from other diseases. Further study is needed. Another limitation is the small number of sample size, which could result in less valid estimates, especially diagnostic test performance values. Therefore, it was not possible to present the ROC curve for an appropriate cut-off value in this study. However, the initial sample size calculation was based on the correlation of first-void morning UPCR and 24-hour urine protein, which had 90% power to detect such correlation and its specificity as well as negative predictive value was very high and reliable (96.9% and 95% CI: 0.82- 1.0). Further study with larger samples should be conducted to evaluate the usefulness of first-void morning UPCR in clinical practice.

In conclusion, the first-void morning UPCR significantly correlated with 24-hour urine protein ($r = 0.76$, $p < 0.001$). It should be considered as an alternative method for detecting significant proteinuria in suspected cases of preeclampsia.

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## *ความสัมพันธ์ระหว่างอัตราส่วนโปรตีนต่อครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าและระดับโปรตีนในปัสสาวะ 24 ชั่วโมงในสตรีตั้งครรภ์ที่สงสัยภาวะครรภ์เป็นพิษ*

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***วัตถุประสงค์:*** *เพื่อหาความสัมพันธ์ระหว่างอัตราส่วนโปรตีนต่อครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าและระดับโปรตีนในปัสสาวะ 24 ชั่วโมงในสตรีตั้งครรภ์ที่สงสัยภาวะครรภ์เป็นพิษ*

***เครื่องมือและระเบียบวิธีวิจัย:*** *การศึกษาในสตรีตั้งครรภ์ที่สงสัยภาวะครรภ์เป็นพิษ และนอนโรงพยาบาลเพื่อหาระดับโปรตีนในปัสสาวะ 24 ชั่วโมง จำนวน 40 ราย โดยทำการเก็บปริมาตรปัสสาวะ 24 ชั่วโมง และแบ่งปัสสาวะออกเป็น 2 ส่วน คือ ปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าและปัสสาวะส่วนที่เหลือทั้งหมด จากนั้นนำปัสสาวะทั้งสองส่วนส่งตรวจทางห้องปฏิบัติการเพื่อตรวจหาอัตราส่วนโปรตีนต่อครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าจากปัสสาวะส่วนแรกและระดับโปรตีนในปัสสาวะ 24 ชั่วโมงจากผลรวมของปัสสาวะทั้งสองส่วน การวินิจฉัยภาวะโปรตีนรั่วในปัสสาวะอย่างมีนัยสำคัญใช้เกณฑ์ระดับโปรตีนในปัสสาวะ 24 ชั่วโมงตั้งแต่ 300 มิลลิกรัมขึ้นไป จากนั้นนำค่าที่ตรวจได้ทั้งสองส่วนไปคำนวณหาค่าสัมประสิทธิ์ความสัมพันธ์ (r)*

***ผลการศึกษา:*** *อายุเฉลี่ยของผู้เข้าร่วมวิจัยเท่ากับ 31.0 ± 7.0 ปี และอายุครรภ์เฉลี่ยของผู้เข้าร่วมวิจัยเท่ากับ 33.2 ± 4.6 สัปดาห์ สตรีตั้งครรภ์จำนวน 8 รายหรือคิดเป็นร้อยละ 20 ตรวจพบมีระดับโปรตีนรั่วในปัสสาวะอย่างมีนัยสำคัญและได้รับการวินิจฉัยว่าเป็นครรภ์เป็นพิษ ความสัมพันธ์ระหว่างอัตราส่วนโปรตีนต่อครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าและระดับโปรตีนในปัสสาวะ 24 ชั่วโมง เท่ากับ 0.76 ($p < 0.01$) เมื่อใช้ระดับครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้าเท่ากับ 0.3 เป็นเกณฑ์ในการวินิจฉัยระดับโปรตีนรั่วในปัสสาวะอย่างมีนัยสำคัญพบว่ามีความไวร้อยละ 87.5 (ค่าความเชื่อมั่นร้อยละ 95 เท่ากับ 46.7 – 99.3) และมีความจำเพาะร้อยละ 96.9 (ค่าความเชื่อมั่นร้อยละ 95 เท่ากับ 82 - 99.8)*

***สรุป:*** *อัตราส่วนโปรตีนต่อครีเอทีนีนในปัสสาวะครั้งแรกหลังตื่นนอนตอนเช้ามีความสัมพันธ์กับระดับโปรตีนในปัสสาวะ 24 ชั่วโมงอย่างมีนัยสำคัญทางสถิติ และสามารถพิจารณาใช้เป็นทางเลือกในการตรวจหาระดับโปรตีนรั่วในปัสสาวะอย่างมีนัยสำคัญสำหรับสตรีตั้งครรภ์ที่สงสัยภาวะครรภ์เป็นพิษ*