

# A Comparison of 4- and 24-Hour Urine Samples for the Diagnosis of Proteinuria in Pregnancy

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## Abstract

**Background:** Preeclampsia is a serious complication of pregnancy, and it is vital to diagnosis the condition as early as possible. Proteinuria is an important symptom of preeclampsia, and repeated urine analysis to screen for the condition is part of the standard antenatal care. The purpose of this study was to determine the correlation between 4- and 24-hour urine total protein values to examine whether the 4-hour urine samples could be used for the diagnosis of proteinuria in hypertensive disorders of pregnancy.

**Methods:** A cross-sectional study was performed on 110 pregnant (after gestational week 20 of pregnancy) patients who were hypertensive (blood pressure  $\geq 140/90$  mmHg) and had proteinuria as defined by positive urinary protein of at least 1+ in dipstick. Patients' urine samples were collected over 24 hours; the first 4 hours were collected separately from the next 20-hours. Patients, who did not collect the 24-hour urine, were excluded from the study. One hundred patients met the criteria, and were included in the study. The urine volume, total protein and creatinine levels of 4- and 24-hours samples were measured. The correlation between 4-hour and 24-hour samples was examined using Pearson correlation test.

**Results:** Of the 100 patients, 42 had no proteinuria, 44 had mild proteinuria, and 14 had severe proteinuria. The urine protein values of 4-hour samples correlated with those of the 24-hours samples for patients with mild and severe forms of the disease ( $P < 0.001$ ,  $r = 0.86$ ).

**Conclusion:** This study showed there was a correlation between 4-hour and 24-hour urine proteins. The finding indicates that a random 4-hour sample might be used for the initial assessment of proteinuria.

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**Keywords** • Preeclampsia • proteinuria • hypertension in pregnancy

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## Introduction

Hypertensive disorders complicate 5-10% of all pregnancies, and considering their complications, they are among the major causes of maternal morbidity and mortality.<sup>1,2</sup> The preeclampsia syndrome, either alone or superimposed on the chronic hypertension, is the most serious hypertensive disorder in pregnancy.

Preeclampsia is defined as the development of hypertension

or proteinuria, or both after week 20 in a woman with previously normal blood pressure.<sup>3</sup> Proteinuria is a defining dysfunction of preeclampsia. The degree of proteinuria may fluctuate widely over any 24-hour period due to the circadian variation of urinary albumin excretion.<sup>4,5</sup> It is also influenced by several factors including contamination, urine specific gravity, pH, exercise and posture.<sup>6</sup> However, Douma et al showed that, in comparison with the non-pregnant controls, there was smaller or even absence of circadian variation during pregnancy. Quantification of a timed collection of urine protein has been the gold standard for many decades, and is expressed as the amount of protein excreted in the urine per unit of time. Twenty four-hour specimens have been used on a routine basis.<sup>4,5</sup> The 24-hour period required for the collection of urine may result in a delay in the diagnosis and treatment, or possibly the prolongation of hospital stay. Shortening the period required for the diagnosis of preeclampsia would be valuable for management purposes as well as for decreasing hospital cost and patient inconvenience.<sup>7,8</sup> Several investigators have reported more rapid methods of identifying proteinuria such as the use of protein to creatinine ratios and dipsticks for protein in random urine specimens.<sup>7-9</sup>

The aim of this study was to determine whether 4-hour urine protein values correlate with those of 24-hour values in women with hypertensive disorders in pregnancy.

## Materials and Methods

The study was performed from October 2007 to July 2008 recruiting pregnant women referring to Kosar Obstetrics Hospital, Urmia University of Medical Sciences. All hypertensive patients with a blood pressure (BP) of  $\geq 140/90$  mmHg and a positive proteinuria (at least 1+) who were pregnant for more than 20 weeks and had provided a 24-hour urine samples for urinary protein, as requested by their physicians to rule out preeclampsia, were included in the study. Proteinuria was defined as a 24-hour urinary protein excretion of more than 300 mg, a urine protein to creatinine ratio of  $\geq 0.3$  or persistent proteinuria (30 mg/dl, 1+ dipstick) in random urine samples. Patients with gestational hypertension have only  $<300$  mg, those with mild preeclampsia have 300 mg to 2000 mg, and those with severe preeclampsia have  $>2000$  mg of protein in their 24-hour urine samples.<sup>5</sup> The University Ethics Committee approved that patients' consent were not

required for their participation. Patients were excluded from the study only if they did not complete the 24-hours of collection because of delivery. Urine collection started at 8 am on the first morning after admission to the hospital. Prior to urine collection, all women were carefully instructed regarding the procedure. At 8 am patients were asked to discard the first specimen (start of collection period). In order to increase the accuracy of the test, patients were assisted by a nursing staff for urine collection. The urine samples for each patient were collected in two separate and clearly marked containers. One of the containers was used to collect the first 4-hour urine sample (from 8 am to 12 noon), and the other one was used for the subsequent 20-hour urine sample. The total 24-hour urine volume was calculated by adding up the urine samples in the two containers. The 4-hour urine samples were stirred to ensure homogeneity, and a 6 ml sample was removed from each of them. The remaining 4-hour urine samples were each added to the counterpart 24-hour samples, and stirred for homogeneity. Urine concentrations of creatinine and protein in the two samples were determined using Jaffe,<sup>10</sup> and colorimetric,<sup>11</sup> methods, respectively. The total urinary protein (mg/day) was determined by multiplying the total urine volume (dl) by the concentration of protein in the test sample (mg/dl).

## Statistical Analysis

Based on the concentration of urine protein, the patients were divided into three groups including no proteinuria, mild proteinuria and severe proteinuria. The 24-hour urine protein was used as a gold standard to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 4-hour urine sample. The receiver operating characteristic (ROC) curve was used to determine the cut-off point for predicting mild and severe proteinuria. The data are presented as mean $\pm$ SD. Demographic data were analyzed using Chi-square test or one-way Analysis of Variance. In cases of significant difference with ANOVA, pairwise comparisons were performed using Tukey test. The correlation between the 4-hour and 24-hour urine samples was examined using Pearson correlation test. Data analysis was performed using Statistical Package for Social Sciences (SPSS, version 11), and a P value of  $<0.05$  was considered statistically significant.

## Results

A total of 110 patients participated in the study, and 10 of them were excluded because of

delivery prior to the collection of samples. The remaining 100 patients did finish the study.

The patients were categorized into three groups according to the severity of proteinuria; group I (negative proteinuria) had a 24-hour proteinuria of less than 300 mg, group II (mild proteinuria) had a 24-hour proteinuria of 300-2000 mg, and group III (severe proteinuria) had a 24-hour proteinuria of more than two grams.

Table 1 shows demographic data of patients. There was no statistically significant difference between maternal age, gestational age, mean systolic blood pressure, mean diastolic blood of negative proteinuria, mild proteinuria and severe proteinuria groups. There was a significant correlation between the values of 4-hour urine samples and those of 24-hour urine samples (Pearson Correlation, correlation coefficient (r)=86%, P<0.05).

The area of ROC curve was 0.977 (P<0.001). The ROC curve identified that a value of 62 mg in the 4-hour sample predicted mild proteinuria with a sensitivity of 93.2% , a specificity of 90.2% , a PPV of 93.2%, and a NPV of 90.2% (r=0.9770, P<0.001) (figure 1). All patients with severe proteinuria had a 4-hour protein of 350 mg. Using this value as a cut-off point, severe proteinuria with a sensitivity of 83.3%, a specificity of 92.8%, a PPV of 71.4% and a NPV of 97.5% could be predicted(the area of ROC curve was 0.947, P<0.001) (figure 2).

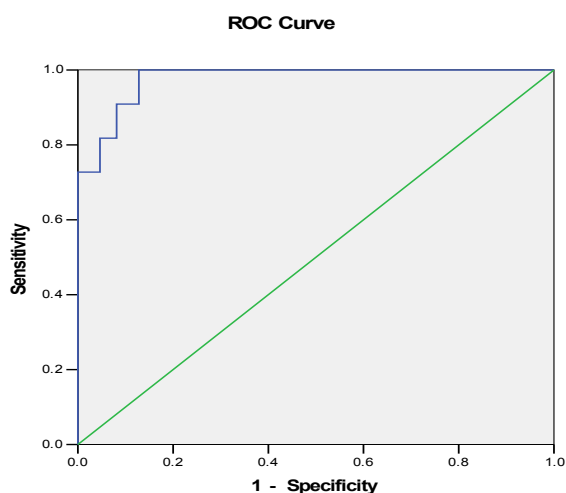


Figure 1: The receiver operating characteristic (ROC) curve of 4-hour urine samples.

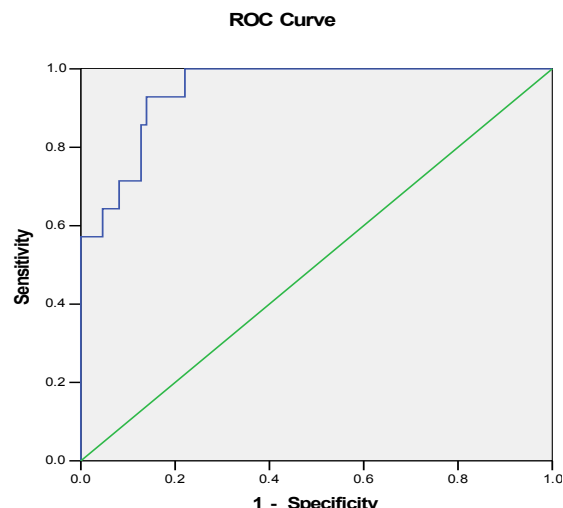


Figure 2: The receiver operating characteristic (ROC) curve of 24-hour urine samples.

### Discussion

At least some degree of proteinuria can establish the diagnosis of preeclampsia, therefore, proteinuria has been proposed to be an indicator of both the severity of disease and the predictor of its outcome.<sup>12</sup> The increased urinary protein excretion in preeclampsia is due to glomerular endotheliosis. However, it might also indicate a generalized increase in capillary permeability in other organ systems of the body.<sup>13</sup>

A major problem in the diagnosis of preeclampsia is that the optimal method of establishing abnormal levels of urine protein is not thoroughly defined.<sup>4</sup> The most common screening method for the detection of proteinuria in preeclampsia is dipstick testing of random urine samples. The dipstick provides a rapid measurement; however, it has been shown to have a low sensitivity and specificity for urinary protein excretion over 24 hours.<sup>13</sup> Thus the assessment may even show a 1+ to 2+ urine protein values for urine specimens from women, who excrete <300 mg/day.<sup>1</sup> Moreover, 24-h urine collection, as a gold standard for titration of proteinuria, is necessary for the confirmation of the results of all dipstick tests and also for the distinction between mild versus severe forms of the disease.<sup>5</sup>

The 24-h urine collection is inconvenient for patients and costly, and may be inaccurate due

Variables	No proteinuria (n=42)	Mild proteinuria (n=44)	Severe proteinuria (n=14)	P value
Age (year)	32.1±1.2	30.4±0.9	29.2±1.8	0.300
Gestational age (week)	32.5±0.7	32.5±0.6	31.5±1.2	0.705
Systolic blood pressure (mmHg)	147.1±1.6	150.5±2	152±2.5	0.782
Diastolic blood pressure (mmHg)	91.7±1.4	95.8±1.6	95±1.5	0.351
4-hour urine protein (mg)	34.8±5.9	194±21.4*	739.6±121.2* <sup>Δ</sup>	<0.001
24-hour urine protein (mg)	131.9±12.4	825±66.8*	3770±300* <sup>Δ</sup>	<0.001

\*Indicate significant difference from group with no proteinuria; <sup>Δ</sup>Indicate significant difference from group with mild proteinuria

to incomplete collection. A shorter period of urine collection to diagnose proteinuria would have clinical benefits such as shortened time of delivery and earlier use of antenatal glucocorticoids. Moreover, a more expedient intervention could decrease prenatal and maternal morbidities. Certainly women without preeclampsia would be discharged earlier if a more rapid and accurate determination of proteinuria is available, therefore, resulting in lower costs of health care. Patient compliance with testing may also improve, if the test for proteinuria can be simplified or shortened.<sup>7</sup>

Several investigators have explored other means of quantifying proteinuria in a shorter period. The protein to creatinine ratio of a single urine sample from pregnant women has been shown to correlate significantly with a 24-hour collection for patients with protein values of less than one gram in 24-hours, but not for those with protein values above one gram in which the variation between the samples was increased.<sup>14,15</sup> Aggarwal et al studied protein to creatinine ratios in pregnant women with preeclampsia, and showed significant correlation ( $r=0.0596$ ,  $P<0.01$ ) between 24-hour protein excretion and the random urine protein-creatinine ratio. With a cut-off protein to creatinine ratio greater than 1.14 as a predictor of significant proteinuria, sensitivity and specificity were 72% and 75%, respectively. The positive predictive value was 94.9% and NPV was 29.9%. They concluded that the random urine protein to creatinine ratio was not a good predictor of significant proteinuria in patients with preeclampsia.<sup>16,17</sup> In addition, others have shown that the results of urine dipstick for protein correlate poorly with 24-hour urine samples for differentiating patients with no disease or severe disease.<sup>7,18</sup>

The results of our study indicate that the protein values for the first 4-hour period do correlate with that of the first 24-hour sample for patients with mild and severe proteinuria. Therefore, it might be taken as evidence to suggest that the 4-hour urine collection might be used to predict or diagnose mild or severe form of the disease. A total urine protein value of more than 62 mg in the 4-hour samples was predictive of mild proteinuria. In this study 4-hour protein values of  $>350$  mg were predictive of severe proteinuria. However, it should be noted that there were only 14 patients in the severe proteinuria group. In this study, we had a small number of patients with severe preeclampsia. Such a small number might be due to the recent improvement of prenatal care. Moreover, because of the need for urgent termination

of pregnancy in severe preeclampsia, there is not significant time for the 24-hour collection.

Several studies have been done for the evaluation of proteinuria in a shorter period (2, 4, 6, 8 and 12 hours), and all of them have revealed that it is possible to determine proteinuria and its severity using a shorter time of urine collections.<sup>7,8,12</sup> However, a number of studies recommend more studies to confirm their own findings, and to generate an exact and reliable cut-off values for predicting mild and specially severe preeclampsia.<sup>13,17-20</sup>

The number of recruited patients (100 patients) in our study was more than those of other studies. They all were inpatients and at bed rest, therefore, there was less or negligible diurnal variation in protein excretion.<sup>6</sup> The sensitivity and cutoff values of mild preeclampsia in the present study were similar to those of Adelberg and colleagues.<sup>7</sup> However, the cut-off values for the diagnosis of severe preeclampsia in this study was significantly different from that the Adelberg et al.<sup>4</sup> This difference could be due to the definition of severe proteinuria. We defined severe proteinuria as a 24-hour urine protein of  $\geq 2$  g protein,<sup>5</sup> whereas Adelberg and colleagues,<sup>4</sup> defined it as a 24-hour urine protein of  $\geq 5$  g.

## Conclusion

The findings of this study indicate that the 4-hour values of urine protein correlated positively with values of 24-hour samples. This might be used as evidence to suggest the values of total urine protein of 4-hour samples might be used for initial assessment of preeclampsia. The use of such samples for the assessment of preeclampsia helps avoid the patients' inconvenience and delay in the treatment of the disease.

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**Conflict of Interest:** None declared

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