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Protein:Creatinine Ratio in Random Urine Samples Is a Reliable Marker of Increased 24-Hour Protein Excretion in Hospitalized Women with Hypertensive Disorders of Pregnancy

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Background: **The protein:creatinine ratio in random, untimed urine samples correlates with 24-h protein excretion in pregnant women with and without hypertension. Nevertheless, whether this ratio is appropriate as a screening test for proteinuria is still unclear, in part because of the paucity of large studies.**

Methods: **We measured protein:creatinine ratios in random urine samples and protein contents of 24-h urine samples in a cross-sectional study of 927 hospitalized pregnant women at ≥20-weeks of gestational age and in a 2nd cohort of 161 pregnant women. In the 2nd group, urine specimens were obtained before and after completion of the 24-h collections, avoiding 1st-morning void specimens.**

Results: **Protein excretion was ≥300 mg/24 h in 282 patients (30.4%). The urine protein:creatinine ratio and the 24-h protein excretion were significantly correlated ($r = 0.98$, $P < 0.001$). The protein:creatinine ratio as an indicator of protein excretion ≥300 mg/24 h was ≥0.3. The sensitivity and specificity were 98.2% and 98.8%, respectively. Positive and negative predictive values were 97.2% and 99.2%, respectively, and positive and negative likelihood ratios were 79.2 and 0.02, respectively. The diagnostic accuracy of the urinary protein:creatinine ratio was corroborated in the 2nd cohort of patients, which also showed no statistically significant difference in protein:creatinine ratio between samples obtained >24 h apart.**

Conclusions: **Random urinary protein:creatinine ratio is a reliable indicator of significant proteinuria (>300 mg/day) in nonambulatory pregnant women, irrespective of sampling time during the daytime. The protein:creatinine ratio may be reasonably used as an alternative to the 24-h urine collection method.**

Hypertension is the most frequent medical complication of pregnancy and is a major cause of perinatal mortality and morbidity *(1, 2)*. Because proteinuria is required for the diagnosis of preeclampsia, and it is also a criterion for identifying disease severity, measurement of proteinuria in pregnant women with hypertension is important. Significant proteinuria is defined by the International Society for the Study of Hypertension in Pregnancy as excretion of ≥300 mg of protein in a 24-h urine specimen *(3)*. Thus, the gold standard for diagnosis of significant proteinuria is based on a 24-h urine collection. The difficulties of 24-h urine collection are well recognized; however, this test is unreliable in up to one-third of cases *(4, 5)*, is time-consuming, and often prolongs the patient's hospital stay. In addition, a rapid test may be needed in clinical practice well before a timed collection of urine is complete. Detection of proteinuria in a single random urine sample may be an alternative to timed urine collections in pregnant women with hypertension.

Measurements of the protein:creatinine ratio in a single urine specimen may be a reliable and quick test to estimate 24-h protein excretion in a nonpregnant population because the ratio of 2 stable excretion rates (creatinine and protein) minimizes the time involved, thus providing a faster estimate of 24-h protein excretion *(6–8)*. The

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clinical utility of the relationship between the gold standard for detecting significant proteinuria and the urine protein:creatinine ratio in hypertensive pregnant women still remains unclear. Although several studies have shown that the urine protein:creatinine ratio is a predictor of significant proteinuria *(9–13)*, others have found a weaker value of this test for this purpose *(14, 15)*. Nevertheless, the appropriateness of the urine protein:creatinine ratio as a screening test for proteinuria is still unclear, in part because of the paucity of large studies on proteinuria in pregnant women with hypertension. We therefore conducted this study in a large number of patients to reassesses whether measurement of urine protein:creatinine ratio in a single urine specimen in clinical practice provides a reliable estimate of significant proteinuria (≥300 mg/24 h) in women with hypertensive disorders of pregnancy.

Patients and Methods

The study protocol was approved by the Human Ethics Committee and Medical Research Council of the Instituto Mexicano del Seguro Social. Written informed consent was obtained from all study participants.

All participating women (1128) were patients admitted to the Hypertensive Diseases of Pregnancy Clinic of our hospital. Study participants were ≥20 weeks gestational age and had new onset of hypertension with or without suspicion of preeclampsia or chronic hypertension (before 20 weeks of gestation) with suspected superimposed preeclampsia. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, measured twice at least 6 h apart. For comparative purposes, 65 hospitalized pregnant women (gestational ages ≥20 weeks) in whom a hypertensive disorder of pregnancy was ruled out were also included in the study. Women in this group were allowed to remain in bed at rest or to ambulate ad libitum. All patients with diagnosed hypertensive disorders of pregnancy were on moderate bed rest and were allowed to intermittently spend a few hours daily outside their rooms.

Random urine samples for determining urinary protein and creatinine excretion were obtained during the daytime before or after the start of the 24-h urine collections. None of the samples were 1st-voided morning urine. Patients with a coexisting urinary tract infection or membrane rupture were not included. Twenty-four hour urine samples were collected at room temperature and without adding preservatives. Protein and creatinine measurements in both (24-h and random) urine specimens were performed immediately after collection.

Urine protein was measured by the Bradford method (Bio-Rad Protein Assay Kit, Bio-Rad Laboratories) using BSA (Bio-Rad) as a calibrator. We performed the assay manually as described by the manufacturer. Briefly, in a 96-well plate the protein calibrator (BSA in 0, 1, 2, 3, and 4 μg/10 μL of isotonic saline solution) or 10 μL of sample (urine sample or a diluted sample as necessary to assess for parallelism with the calibrator curve) were mixed with 200 μL of protein assay solution diluted with 4 volumes of ultrapure water; after 5 min, we measured the absorbance of the assay mixture at 595 nm using an Emax® precision microplate reader (Molecular Devices). The intra- and interassay imprecisions (CVs) were <2.3%. Urine creatinine was measured by the modified kinetic Jaffe reaction in a 96-well plate with a filter at 490 nm. The intra- and interassay CVs were <1.2%. The urine protein:creatinine ratio was obtained by dividing the urinary protein concentration by the urine creatinine concentration, both expressed in mmol/L (mg/dL). We assessed the adequacy of the 24-h urine collection by comparing the total creatinine in the sample to the predicted creatinine estimated by the Cockroft–Gault equation for women *(16, 17)*. Twenty-four-hour urine collections with ≤20% (undercollection) or ≥20% (overcollection) of predicted 24-h urine creatinine excretion were discarded. The attending physicians had no access to the results of the protein:creatinine ratio.

Statistical analyses were performed with the SPSS 11.0 (SPSS Inc.) and MedCal 7.2 (MedCalc Software) statistical packages. Sensitivity, specificity, and predictive values of the random urine protein:creatinine ratio at various cutoffs for prediction of significant proteinuria were estimated using the results from the 24-h urine collection as the gold standard. An ROC curve was constructed, and the area under the curve was calculated. The relationship between the urine protein:creatinine ratio and the 24-h protein excretion was assessed with the Pearson correlation coefficient.

Results

General description of the population studied

A total of 1198 24-h urine specimens were collected from pregnant women. We excluded 271 specimens (22.6%) with inadequate 24-h urine collection; 927 samples from 927 women were included in the final analysis. Among these women, 54 had chronic hypertension (none of the women had preexisting renal disease), 808 had new-onset hypertension (after 20 weeks gestation), and 65 pregnant women at 20 weeks or more of gestation did not meet the criteria for gestational hypertension in further evaluations. The mean (SD) maternal age was 28.6 (6.2) years (range 14–45 years), and the median gestational age was 33 weeks (range 21–40 weeks); 695 women (75%) were nulliparous and 12 had twin pregnancies. Mean (SD) serum creatinine concentration was 61.0 (14.1) mmol/L [0.69 (0.16) mg/dL].

Protein excretion was ≥300 mg/24-h in 282 patients (30.4%); among these, 23 had chronic hypertension with superimposed preeclampsia and 259 had primary preeclampsia. The median protein excretion in a 24-h urine specimen was 985 mg (range 304–15 961 mg). Protein excretion was ≥2 g/24 h in 82 patients (8.8%), and was severe (≥5 g/24 h) in 34 patients (3.7%).

CORRELATION BETWEEN THE URINE PROTEIN:CREATININE RATIO AND 24-H URINE PROTEIN EXCRETION.

We observed a significant positive correlation between the 24-h urine protein excretion and the urine protein:creatinine ratio [$r = 0.98$, $P < 0.001$ ($R^2 = 0.97$)] (Fig. 1). There was no correlation between 24-h urine protein excretion or the urine protein:creatinine ratio and maternal age, gestational age, parity, body mass index, or serum creatinine concentrations ($P \geq 0.53$).

ROC CURVE

The area under the ROC curve was 0.998 (95% CI 0.993–1, $P < 0.001$). The optimal cutoff point was 0.30; this cutoff yielded a sensitivity of 98.2% (95% CI 95.9–99.4), specificity of 98.8% (95% CI 97.6–99.5), and positive and negative predictive values of 97.2% (95% CI 94.6–98.6) and 99.2% (95% CI 98.2–99.7), respectively. At this cutoff, positive and negative likelihood ratios were 79.2 (CI 95% 39.8–157.7) and 0.02 (95% CI 0.008–0.043), respectively. As shown in Fig. 1, there were 5 false-negative samples; however, none had severe proteinuria (304–334 mg/24 h). One of the 8 false-positive samples had a 24-h proteinuria value of 188 mg; the remaining 7 samples were 235–273 mg.

Because the urine protein:creatinine ratio may vary throughout the day, the utility of this measurement as an alternative to 24-h urine protein excretion has been questioned. Therefore we conducted an additional study in a new cohort of 161 patients to evaluate the occurrence of significant variations in the urine protein:creatinine ratio vs the 24-h urine protein excretion. To this end, random urine samples were obtained during the day before the start of 24-h urine collections and after the 24-h urine collections were completed (within 4–6 h). We measured urinary protein and creatinine excretion in each random sample. Again, none of the samples represented 1st-voided morning urine.

Proteinuria was ≥300 mg/24-h in 78 of 161 patients (48.4%). The median protein excretion in 24-h urine specimens was 857 mg (range 331–9 487 mg). There was a significant positive correlation between the 24-h proteinuria values and the urine protein:creatinine ratio from random samples collected either before or after completion of the 24-h urine collection [$r = 0.98$ and $r = 0.97$ before and after completion of the 24-h collection, respectively ($P < 0.001$)]. Similarly, a significant correlation in the urine protein:creatinine ratio between samples obtained before and after completion of the 24-h urine collection was found ($r = 0.99$, $P < 0.001$). As shown in Fig. 2, the urine protein:creatinine ratio before or after completion of the 24-h collection did not vary significantly. The vast majority (82 of 83, 98.8%) of patients with <300 mg protein/24-h exhibited a urine protein:creatinine ratio <0.3 in 2 separate samples, and conversely most (77 of 78, 98.8%) patients with ≥300 mg protein/24-h presented at least 2 value ratios ≥0.3.

According to the International Society for the Study of Hypertension in Pregnancy, a urine protein excretion ≥2 g/24 h is considered a criterion for the diagnosis of severe preeclampsia. Thus, we determined the diagnostic performance of the urine protein:creatinine ratio to estimate a urine protein excretion ≥2 g/day. The area under the ROC curve was 0.998 (95% CI 0.993–1.0, $P < 0.001$). The optimal cutoff point was 1.45, which yielded a sensitivity

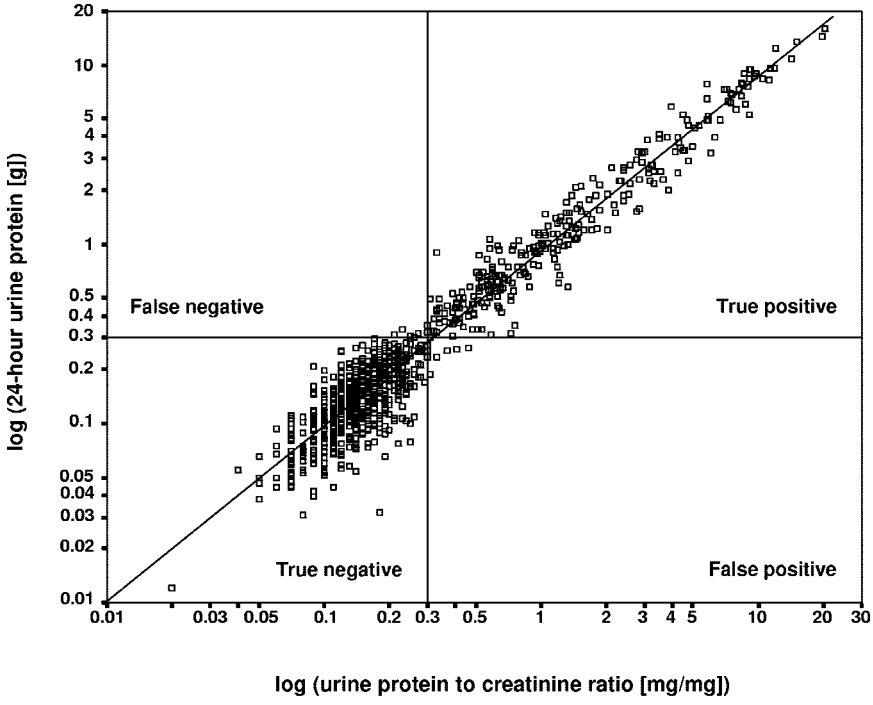

Fig. 1. Relationship between 24-h proteinuria and random urine protein:creatinine ratio in 927 pregnant women.

The *horizontal line* denotes the limit for significant proteinuria, and the *vertical line* is set at 0.3 of the random urine protein:creatinine ratio (see *Results*).

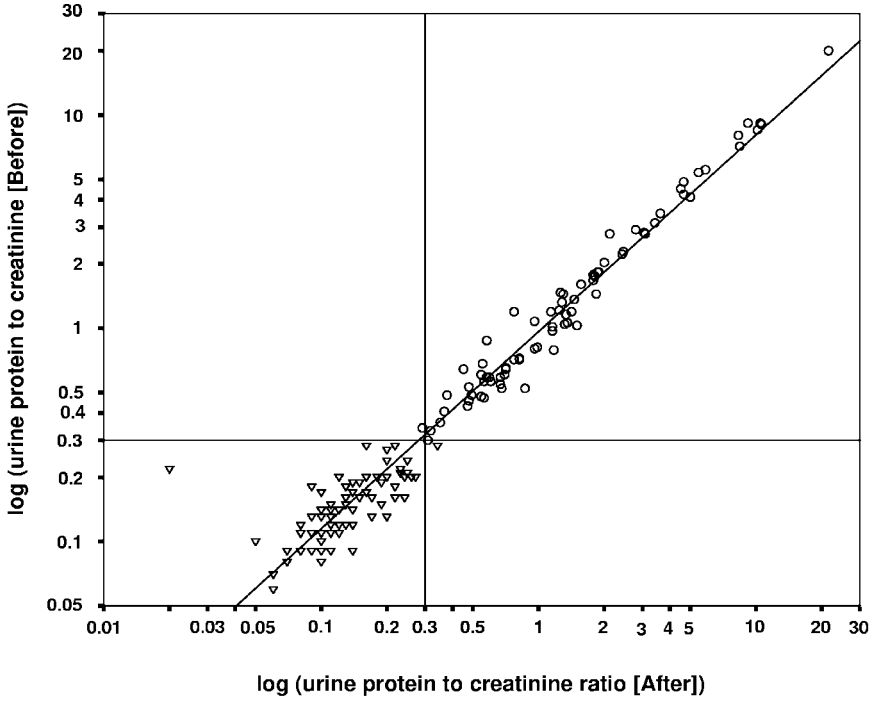

Fig. 2. Relationship between 2 random urine protein:creatinine ratios at different periods (before and after the start the 24-h urine collection).

Data from 83 patients without significant proteinuria (*open triangles*) and 78 patients with significant proteinuria (*open circles*), as determined by 24-h proteinuria are depicted. The *horizontal* and *vertical lines* are set at 0.3 of the random urine protein:creatinine ratio, which is the best cutoff of the test as indicator of significant proteinuria.

of 100% (95% CI 95.6%–100%) and specificity of 97% (95% CI 95.7%–98.1%), with positive and negative predictive values of 100% and 76.6%, respectively. The positive likelihood ratio was 33.8.

Discussion

Measurement of proteinuria is one of the most routinely undertaken laboratory procedures. It is mandatory in evaluating women with hypertensive disorders of pregnancy, and is necessary to establish the diagnosis of preeclampsia, as well as its severity. Urinary protein excretion during a 24-h period, however, is considered to be cumbersome and subject to error due to inadequate collection. In assessing a diagnostic test, the following considerations are important: (*a*) the gold standard should provide the closest approximation to the true status of the disorder under study; (*b*) the clinical spectrum of the disease under study (including individuals with low prior probability to have the disorder); (*c*) the necessity of making blind measurements; and (*d*) the optimal cutoff point taken to lower at maximum both false-positive and false-negative results. All of these are fundamental to determine the diagnostic performance of an assay.

Although several methods may be applied to determine total urinary protein excretion, including the Biuret assay and the sulfosalicylic acid and benzethonium chloride techniques, among others, no existing method can be considered better than the rest. The Coomassie brilliant blue dye-binding technique (Bradford method), when calibrated with albumin standards, is a sensitive and reliable method that offers many advantages as a routine assay for urinary protein *(18–20)*. Moreover, in the absence of a protein calibrator applicable to the wide variety of urinary proteins that are excreted, albumin rather than protein mixtures has been recommended as a calibrator, because albumin is the 1st protein to increase in urine and the main protein excreted in many glomerular disorders, including glomerular endotheliosis. In the present study, we ensured the completeness of 24-h urine collection by excluding those samples in which the predicted and measured creatinine excretion did not agree (i.e., under- or overcollected samples). On the basis of an adequate 24-h urine collection and the Bradford method (as gold standard) to measure protein, we considered that the possibility of misclassification of a urine sample from a pregnant woman as positive or negative for significant proteinuria should be negligible. Consistent with previous studies *(9, 11, 12, 21–23)*, our results demonstrate a strong correlation between 24-h proteinuria and the urine protein:creatinine ratio in pregnant women. The ROC curve analysis showed an area under the curve of 0.99, indicating that the urine protein:creatinine ratio is sufficiently accurate to either detect or rule out significant proteinuria, as confirmed with the optimal cutoff point at $\geq$0.3; this cutoff value yielded a high diagnostic performance with a sensitivity of 98.2% and specificity of 98.8%, as well as high positive and negative predictive values of 97.2% and 99.2%, respectively.

Because of variability in laboratory methods for measuring proteinuria in different reported studies, several cutoff points and different units for the urinary protein: creatinine ratio have been reported, thereby precluding valid comparisons among such studies. Nevertheless, 3 previous studies *(9, 12, 23)* that used optimal cutoff points

similar to the one used by us [≥0.25 to ≥0.30 *(9, 12, 23)*], yielded sensitivities of 83%–96% and specificities of 92%–100%. Despite the smaller sample size used in these studies (42, 100, and 100 participants, respectively) the diagnostic performance of the tests, as indicated by 95% CIs, was similar to that found in the present study. In the present study, we demonstrated a high diagnostic performance of the urine protein:creatinine ratio (at the cutoff point, ≥1.45) as an indicator of proteinuria ≥2 g/24 h.

Because the protein:creatinine ratio may vary throughout the day, its utility as an alternative for measuring 24-h proteinuria has been questioned. For example, Wikström et al. *(24)* assessed the use of the random albumin:creatinine ratio for detecting significant proteinuria in 31 women with preeclampsia and concluded that the random urine albumin:creatinine ratio was not stable during the day and cannot predict 24-h proteinuria accurately; this particular study, however, did not monitor the accuracy of the 24-h urine collections. Nevertheless, as documented in the present study as well as in that previously reported by Valerio et al. *(13)*, the protein:creatinine ratio from single voided urine samples does not actually vary significantly throughout the day and thus may be determined at any time (at least within short periods of time). Similar conclusions were reached in 2 other studies that included small cohorts of patients *(25, 26)*. Furthermore, our study confirms that maternal age, body size, gestational age, and parity are not confounding factors with regard to the urinary protein:creatinine ratio, a finding previously reported by Neithardt et al. *(22)*.

The data obtained in the 2nd cohort of pregnant women studied support the high reliability of using the random urine protein:creatinine ratio to detect or, alternatively, rule out significant proteinuria (even considering that 24-h proteinuria may vary from hour to hour in pregnant women with preeclampsia); 2 values ≥0.3 from 2 samples taken at different time points on the same day are sufficient to detect significant proteinuria, and 2 values ≤0.29 rule out significant proteinuria. The validity of our conclusion is based on the strength of the methods used in the large number of pregnant women studied. The population was clinically appropriate (with a wide spectrum of disease), because it included women in whom the diagnosis of hypertensive disorder of pregnancy was suspected but not confirmed in subsequent evaluations. Moreover, the accuracy of the urine protein:creatinine ratio to detect significant proteinuria was validated by studying obstetric populations with moderate [30.4% (1st cohort)] and high [48.4% (2nd cohort)] prevalence of proteinuria.

In conclusion, a random urine protein:creatinine ratio measurement provides a good estimation of total 24-h proteinuria in hospitalized pregnant women and can replace the time-consuming 24-h urine collection. Nevertheless, the diagnostic performance of the urine protein:creatinine ratio should be validated locally by each laboratory because of the variability in laboratory methods used to measure proteinuria and the criteria to establish cutoff points.

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