Problems in methods for the detection of significant proteinuria in pregnancy

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Abstract

Aim: The aim of this study was to underscore problems associated with the dipstick test and determination of protein concentration alone in spot-urine (P-test) compared with spot-urine protein-to-creatinine ratio (P/Cr test) and to determine whether urine collection for 24-h test was complete.

Material and Methods: Dipstick and P/Cr tests were performed simultaneously in 357 random spot-urine specimens from 145 pregnant women, including 35 with pre-eclampsia. Positive results were defined as ≥1+ on dipstick test, protein concentration ≥30 mg/dL on P-test, and P/Cr ratio ≥ 0.27 (mg/mg) on P/Cr test. Sixty-four 24-h urine tests (quantification of protein in urine collected during 24 h) were performed in 27 of the 145 women. We assumed that P/Cr ratio ≥ 0.27 predicted significant proteinuria (urinary protein ≥ 0.3 g/day). The 24-h urine collection was considered incomplete when urinary creatinine excretion was <11.0 mg/kg/day or >35.0 mg/kg/day.

Results: Forty-four percent (69/156) of specimens with a positive test result on dipstick test contained protein < 30 mg/dL. Dipstick test was positive for 25.7% (69/269) of specimens with protein < 30 mg/dL and for 28.8% (79/274) of specimens with P/Cr ratio < 0.27. P-test results were positive for 7.3% (20/274) and negative for 18.1% (15/83) of specimens with P/Cr ratio < 0.27 and ≥0.27, respectively. Incomplete 24-h urine collection occurred in 15.6% (10/64) of 24-h urine tests. Daily urinary creatinine excretion was 702–1397 mg, while creatinine concentration varied from 16 mg/dL to 475 mg/dL in spot-urine specimens.

Conclusion: Dipstick test and P-test were likely to over- and underestimate risks of significant proteinuria, respectively. The 24-h urine collection was often incomplete.

Key words: creatinine in the urine, protein-to-creatinine ratio, proteinuria.

Introduction

Assessment of proteinuria is an important constituent of antenatal care for pregnant women. The gold standard test for determination of significant proteinuria in pregnancy is currently confirmation of protein ≥ 0.3 g/day in the urine collected for 24 h (24-h urine test). The dipstick test, which can be used for semiquantitative determination of protein concentration in the spot-urine, is used as a screening test for detection of significant proteinuria. However, concerns have been raised regarding the accuracy of dipstick testing.1–7 The amount of proteinuria increases with advancing gestation,8 but we frequently encounter pregnant women who exhibit a negative result after showing a positive result on dipstick testing. Further problems of
24-h urine testing, such as incomplete urine collection and inconvenience for both patients and obstetric service providers, have also been reported.9

The Australian Society for the Study of Hypertension in Pregnancy and the International Society for the Study of Hypertension in Pregnancy have proposed use of the urinary spot protein-to-creatinine ratio (P/Cr test) as an alternative to 24-h urine test.10,11 A systematic review by Cote et al.12 concluded that P/Cr test with a threshold of 0.265 (mg/mg) is a reasonable ‘rule-out’ test for detecting proteinuria ≥0.3 g/day in pregnancy. However, this issue has not been studied extensively among pregnant Japanese women and P/Cr test is not widely used at present in Japan. Accordingly, we conducted this retrospective study to underscore the problems in the dipstick test, determination of protein concentration alone in the spot-urine (P-test), and 24-h urine test.

Methods

This study was conducted after receiving approval from the Institutional Review Board of Hokkaido University Hospital, a tertiary teaching hospital managing mainly high-risk pregnant women. Beginning in 2009, we introduced determination of urinary protein-to-creatinine (P/Cr) ratio (mg/mg) as a routine test for outpatients exhibiting a positive result (≥1+) on dipstick test. Routine laboratory work-up included determination of P/Cr ratio for inpatients with edema and/or hypertension. This method was designated as P/Cr test in this study and a positive test result was defined as a P/Cr ratio ≥ 0.27 (mg/mg).

Of 483 women who gave birth at the Hokkaido University Hospital during the period between January 2011 and July 2012, 145 women underwent both dipstick and P/Cr tests simultaneously in a total of 357 random spot-urine specimens (2.5 ± 1.5 times [range, 1–7]/person) and all of the 145 women were included in this study. A total of 64 × 24-h urine tests were performed in 27 women, two of whom provided aliquots of 5 mL of random spot-urine specimens three times before mixture of their spot-urine with the whole urine for the 24-h urine test. These six spot-urine specimens were used for the P/Cr test.

The dipstick used in the dipstick test was designed to be negative, 1+, 2+, and ≥3+ on visual judgment for protein concentrations in the urine of <30, 30–99, 100–299, and ≥300 mg/dL, respectively, according to the manufacturer’s package insert (Siemens). The protein and creatinine concentrations in the urine were measured using the pyrogallol red method (Wako) and creatinase sarcosine oxidase peroxidase method (Mitsubishi Chemical Medience), respectively, at our institution. Data on age, bodyweight, parity, and clinical outcomes were obtained from the medical records. The term ‘P-test’ was used for the determination of urinary protein concentration alone in spot-urine in this study.

All of the data are presented as the mean or median values. The unpaired t-test, Kruskal–Wallis test, and Mann–Whitney U-test were used to analyze the results. Fisher’s exact test was used for comparison of frequencies. StatView 5.0 for Macintosh (sas) was used for all the statistical analyses, and P < 0.05 was taken to indicate significance.

Results

Accuracy of dipstick test and P-test for prediction of protein concentration ≥ 30 mg/dL and/or P/Cr ratio ≥ 2.7

Of the 357 spot-urine specimens, 201 showed a negative result and the remaining 156 showed a positive result (≥1+) on the dipstick test (Table 1). Dipstick

Table 1 Association between results of dipstick test and concentrations of protein and creatinine in 357 spot urine specimens

<table>
<thead>
<tr>
<th>Dipstick test</th>
<th>No. of tests</th>
<th>[P] (mg/dL)</th>
<th>[Cr] (mg/dL)</th>
<th>P/Cr ≥ 0.27</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>201</td>
<td>3.9 ± 7.1 (0–51)</td>
<td>80.1 ± 46.4 (16–348)</td>
<td>6 (3.0%)</td>
</tr>
<tr>
<td>(+)</td>
<td>88</td>
<td>24.2 ± 14.9 (0–75)</td>
<td>146.6 ± 77.6 (20–440)</td>
<td>22 (25%)</td>
</tr>
<tr>
<td>(2+)</td>
<td>34</td>
<td>91.1 ± 55.0 (0–217)</td>
<td>106.3 ± 87.4 (18–383)</td>
<td>27 (79.4%)</td>
</tr>
<tr>
<td>(≥3+)</td>
<td>34</td>
<td>289.3 ± 243.9 (0–1289)</td>
<td>123.8 ± 102.8 (22–475)</td>
<td>28 (82.4%)</td>
</tr>
</tbody>
</table>

Range is indicated in parentheses. [P], protein concentration in the urine; [Cr], creatinine concentration in the urine. P/Cr, protein-to-creatinine ratio (mg/mg).
test was positive in 87 of the 88 specimens with
[P] ≥ 30 mg/dL, giving a sensitivity of 98.9% for pre-
diction of [P] ≥ 30 mg/dL (Table 2). Sixty-nine (44%) of
the 156 specimens with a dipstick test result ≥ 1+
contained protein < 30 mg/dL. Dipstick test was
falsely positive in 25.7% (69/269) of specimens with
[P] < 30 mg/dL. The mean [P] values were 3.9 ± 7.1,
24.2 ± 14.9 mg/dL, 91.1 ± 55.0 mg/dL and 289.3 ±
243.9 mg/dL for negative, 1+, 2+, and 3+ results on
dipstick test, respectively. Thus, the dipstick test used
in this study was designed to give a positive result in
urine with a far lower [P] than that described on the
package insert.

P/Cr test was positive in 83 specimens from 39
women who developed pre-eclampsia or gestational
proteinuria. The 39 women provided 2.1 ± 1.2 times
(range, 1–5 times) random spot-urine samples with
P/Cr ratio ≥ 0.27. Dipstick test was positive in 77/83
of specimens with P/Cr ratio ≥ 0.27, giving a sensitiv-
ity of 92.8% for prediction of P/Cr ratio ≥ 0.27
(Table 2). Dipstick test was positive in 28.8% (79/274)
of specimens with P/Cr ratio < 0.27. Thus, the dipstick
test overestimated the risk of significant proteinuria in
a significant number of specimens. P-test was positive
in 68/83 of specimens with P/Cr ratio ≥ 0.27, giving a
sensitivity of 81.9% for prediction of P/Cr ratio ≥ 0.27
(Table 2). P-test was positive in 7.3% (20/274) of spec-
imens with P/Cr ratio < 0.27. Thus, P-test underesti-
imated the risk of significant proteinuria in a significant
number of specimens.

### Pitfalls in the dipstick test and its screening
characteristics for detection of P/Cr ratio ≥ 0.27

Although the dipstick test had a high negative predic-
tive value (NPV) of 99.5% for ‘rule out’ of proteinuria ≥ 30 mg/dL, it gave a negative test result in
six (7.2%) of the 83 specimens with a positive result
on the P/Cr test (Table 1). As expected, [Cr] was sig-
nificantly lower in the six specimens with a positive
test result on the P/Cr test than the 79 specimens with
a positive test result on the dipstick test but a negative
result on the P/Cr test in the absence of a difference in
[P] (Table 3). Thus, the dipstick test gave a negative
result in the spot-urine specimens with a P/Cr ≥ 0.27
when [Cr] was low, ranging from 25 to 92 mg/dL.

### Daily urinary creatinine excretion and
completeness of urine collection for 24-h
urine test

A total of 27 women underwent 64 × 24-h urine collec-
tion. Their pre-pregnancy BMI (mean ± SD [range])
and volume of 24-h urine (n = 64) were 23.0 ± 6.0
[16.6–43.3] kg/m$^2$ and 2120 ± 885 [489–5050] mL,
respectively. The distribution of daily urinary creati-
nine excretion corrected by pre-pregnancy bodyweight
is shown in Figure 1. When under- and over-collection
were defined as levels of urinary creatinine (mg/kg/
day) < 11.0 and >25.0, respectively, incomplete urine
collection occurred in 10 (15.6%) of the 64 × 24-h urine
collection. In analysis of 54 complete specimens with

### Table 2 Screening characteristics of dipstick test and P-test for prediction of protein concentration ≥ 30 mg/dL and/or
P/Cr ratio ≥ 2.7

<table>
<thead>
<tr>
<th>Target</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipstick test Protein ≥ 30 mg/dL</td>
<td>99% (87/88)</td>
<td>74% (200/269)</td>
<td>56% (87/156)</td>
<td>99% (200/201)</td>
</tr>
<tr>
<td>Dipstick test P/Cr ratio ≥ 2.7</td>
<td>93% (77/83)</td>
<td>71% (195/274)</td>
<td>49% (77/156)</td>
<td>97% (195/201)</td>
</tr>
<tr>
<td>P-test P/Cr ratio ≥ 2.7</td>
<td>82% (68/83)</td>
<td>93% (254/274)</td>
<td>77% (68/88)</td>
<td>94% (254/269)</td>
</tr>
</tbody>
</table>

95% confidence interval is presented in square brackets. PPV, positive predictive value; NPV, negative predictive value.

### Table 3 Cases with dissociation of results between dipstick and P/Cr tests

<table>
<thead>
<tr>
<th>Test result</th>
<th>Dipstick</th>
<th>P/Cr</th>
<th>No. of tests</th>
<th>[P] (mg/dL)</th>
<th>[Cr] (mg/dL)</th>
<th>P/Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>79</td>
<td>19.1 ± 11.4 (0–42)</td>
<td>172.2 ± 70.4 (20–440)</td>
<td>0.11 ± 0.07 (0–0.25)</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>6</td>
<td>17.2 ± 4.6 (11–25)</td>
<td>46.2 ± 24.5* (25–92)</td>
<td>0.42 ± 0.14 (0.27–0.64)</td>
<td></td>
</tr>
</tbody>
</table>

Range is indicated in parentheses. *P = 0.0002 vs 172.2 ± 70.4. [P], protein concentration in the urine; [Cr], creatinine concentration in the
urine; P/Cr, protein-to-creatinine ratio (mg/mg).
creatinine levels of 11.0–25.0 mg/kg/day, daily creatinine excretion was 965.3 ± 159.6 mg, ranging from 702 to 1397 mg/dL, and that corrected by pre-pregnancy bodyweight was 17.3 ± 2.9 mg/kg, ranging from 11.7 to 24.9 mg/kg. Thus, although daily creatinine excretion in the urine per day was approximately 1000 mg with a relatively narrow range, [Cr] in the random spot-urine specimens varied widely, ranging from 16 mg/dL to 475 mg/dL (Table 1), suggesting limited clinical value of the P-test that determines [P] alone in spot-urine specimens for prediction of significant proteinuria in pregnancy (≥0.3 g/day).

The results of P/Cr test performed within 7 days prior to the 24-h urine tests were available in 39 of the 54 complete 24-h urine tests. One of these 39 tests gave a negative result (<0.3 g/day), while the remaining 38 tests gave a positive result. However, all P/Cr tests exclusively gave a positive result (P/Cr ratio ≥0.27), yielding a sensitivity of 100% (38/38) and positive predictive value (PPV) of 97% (38/39).

Aliquots of 5.0 mL of the spot-urine were obtained from two women three times during the 24-h urine test. These two cases were determined to have significant proteinuria (≥0.3 g/day) with 24-h urine test (Δ, 1.07 g of protein in 2860 mL urine with a P/Cr ratio of 0.97; ○, 0.70 g of protein in 2050 mL urine with a P/Cr ratio of 0.63). Protein concentration increased with increasing creatinine concentration in the spot-urine specimens in both women. Thus, determination of [Cr] in addition to determination of [P] in the spot-urine enhanced the accuracy of detection of significant proteinuria in pregnancy.

Discussion

The dipstick used in this study had a low threshold for showing a ≥1+ result; as many as 69 (44%) of 156 spot-urine samples with a ≥1+ test result on the dipstick contained protein < 30 mg/dL (Table 1). As the dipstick should be associated with a low false negative rate (high sensitivity) to avoid missing significant proteinuria, the screening characteristics of the dipstick test used in this study (high sensitivity of 92.8% and a low PPV of 49.4%) for the detection of P/Cr ≥0.27 may have been reasonable. However, dipsticks employed in other countries seem to have higher thresholds than those used in the present study; in comparison with the results of this study, a lower sensitivity ranging from 51% to 60% and a relatively higher PPV ranging from 64.9% to 96.9% were
reported for the detection of significant proteinuria. An Australian study reported a relatively low PPV of 38–60% and a high NPV of 86–88%, similar to the results of this study. As a screening test with low sensitivity gives a high false negative rate, the diagnosis of pre-eclampsia may be delayed when such a dipstick with a low sensitivity is used. This may explain why proteinuria has been believed to be a late sign in the clinical course of pre-eclampsia in Western countries, whereas we demonstrated previously that significant proteinuria precedes the development of hypertension in approximately 50% of patients with pre-eclampsia.

The dipstick test indeed had a low false negative rate (high sensitivity of 98.9%) for the detection of protein concentration ≥ 30 mg/dL in this study. However, it should be kept in mind that the purpose of the urine test is to detect significant proteinuria ≥ 0.3 g/day. As shown in this study, the dipstick test gave a negative test result in a considerable number of specimens with a positive result on the P/Cr test (7.2% [6/83] of specimens). Therefore, care is required in interpretation of dipstick test results. The dipstick test does not take creatinine concentration into account. Determination of protein concentration alone in the spot-urine appears to be used often for the detection of significant proteinuria as an alternative to the 24-h urine test in Japan. However, as demonstrated in this study (Table 2), the absolute value of protein concentration in the spot-urine specimens would be misleading when creatinine concentration is either too high or too low, leading to over- or underestimation of protein loss per day. Creatinine concentration varied largely from 16 mg/dL to 475 mg/dL in the 357 spot-urine specimens in this study (Table 4), thereby leading to varied P/Cr ratio in the presence of a constant protein concentration as shown in Table 3.

As daily creatinine production is constantly reflecting muscle mass and creatinine is eliminated solely by renal excretion, 24-h urinary creatinine excretion reflects muscle mass, and excretion is relatively constant over time in a given person, ranging from 11.0 mg/kg/day to 25.0 mg/kg/day. However, urinary creatinine excretion was less than 11.0 mg/kg/day or more than 25.0 mg/kg/day in 10 of 64 24-h urine tests, suggesting that under- or over-collection occurred in these 10 cases. Thus, even the 24-h urine test, which is currently considered the gold standard for determination of significant proteinuria, was often inaccurate, as noted by Côté et al. Use of the urinary spot P/Cr ratio is currently recommended in evaluation of protein loss per day outside pregnancy. In addition, the Australian Society for the Study of Hypertension in Pregnancy and the International Society for the Study of Hypertension in Pregnancy have proposed use of the urinary spot P/Cr ratio as an alternative to 24-h urine collection and recommend a threshold of 30 mg/mmol (0.265 mg/mg). Therefore, we used a threshold of 0.27 mg/mg in this study. Although our investigation on the accuracy of P/Cr test for detection of significant proteinuria (≥0.3 g/day) was insufficient because of the limited number of women with borderline proteinuria (5–30 mg/dL) who underwent 24-h urine test, P/Cr test exclusively gave a positive test result in the urine that contained significant levels of protein (≥0.3 g/day). According to a systematic review by Côté et al., the P/Cr test has sensitivity of 83.6% (95% confidence interval 77.5%–89.7%) and specificity of 76.3% (72.6%–80.0%) for the detection of significant proteinuria.

This study has some limitations: this was a retrospective study, multiple data from the same subjects were used as independent data, and the fraction of women with significant proteinuria was larger in the study population than in the general population. These limitations affected our results to some extent regarding screening characteristics of dipstick test and P-test for detecting P/Cr ratio ≥ 0.27, but did not markedly distort our results that dipstick test and P-test were likely to over- and underestimate risks of significant proteinuria, respectively (data not shown).

In conclusion, a high false positive rate (low PPV of 55.8% in this study) on the dipstick test may explain why we often encounter pregnant women with a negative test result after initially showing a positive test result on a previous antenatal visit. Although an even

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**Table 4** Demographic characteristics of 145 study subjects

| Age (years) | 32.1 ± 4.8 |
| Primiparous | 83 (57.2%) |
| Gestational weeks at delivery | 35.3 ± 4.6 |
| <37 | 62 (42.8%) |
| <33 | 32 (22.1%) |
| Definite diagnosis† | |
| Pre-eclampsia‡ | 35 (24.1%) |
| Gestational hypertension | 1 (0.7%) |
| Gestational proteinuria | 4 (2.8%) |

†Diagnosis was made 12 weeks postpartum.‡Among 35 patients with pre-eclampsia, seven showed significant proteinuria defined by a protein-to-creatinine ratio in the spot urine ≥ 0.27 more than 1 week prior to the development of hypertension.
higher false positive rate is expected in the general population of pregnant women, the dipstick test may be appropriate for screening on the basis of both cost and rapidity. However, it must be remembered that a false negative result may occur when creatinine concentration is very low. Generally, detailed investigation should be offered in women with a positive test result on the screening test. The 24-h urine test is currently an option for women with a positive test result on screening. However, as the 24-h urine test is inconvenient for both pregnant women and obstetric service providers, Japanese obstetricians appeared to hesitate in offering the 24-h urine test. The P/Cr test overcomes this disadvantage. As pre-eclampsia is a life-threatening complication and the time interval until delivery after diagnosis of pre-eclampsia is approximately 2 weeks, prompt diagnosis of pre-eclampsia is important. The P/Cr test may be a useful alternative to 24-h urine test in women with a positive test result on the dipstick test.

Disclosure

All authors declare that they have no financial relationship with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript.

References