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A Dipstick Test Combined with Urine Specific Gravity Improved the Accuracy of Proteinuria Determination in Pregnancy Screening

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Proteinuria screening using a semi-quantitative dipstick test of the spot urine in antenatal clinic is known to have high false-positive rates. The aim of this study was to assess availability of a dipstick test combined with the urine specific gravity for the determination of pathological proteinuria.

A dipstick test was performed on 582 urine samples obtained from 283 pregnant women comprising 260 with normal blood pressure and 23 with pregnancy-induced hypertension. The urine protein (P) and creatinine (C) concentrations, specific gravity (SG), P/C ratio were determined, and compared with dipstick test results.

The P concentration increased along the stepwise augmentations in dipstick test result. Frequencies of the urine samples with 0.265 or more P/C ratio were 0.7% with − dipstick test result, 0.7% with the ± result, 3.3% with the 1+ result, and 88.9% with the ≥2+ result. However, if the urine specific gravity was low, frequencies of the high P/C ratio were 5.0% with ± dipstick test result and 9.3% with the 1+ result.

A dipstick test result of ≥2+ seems appropriate for a criterion of positive screening for pathological proteinuria in antenatal care. A dipstick test combined with the urine specific gravity may be useful for outpatient clinic screening.

Assessment of proteinuria during pregnancy is clinically important for the diagnosis and classification of pregnancy-induced hypertension, which consists of gestational hypertension (hypertension only) and preeclampsia (hypertension plus proteinuria) −the latter usually has more severe pregnancy complication. Higby et al., demonstrated that protein levels excreted in the 24 hours urine of normal pregnant women were mean 115 mg/day with a normal upper limit of 260 mg/day 1). On the basis of these results, a number of scientific organizations in the world including the Japan Society of Obstetrics and Gynecology 2) adopt 300 mg/day or more protein excreted in the 24 hours urine as a criterion for pathological proteinuria during pregnancy 3-6).

Measurement of protein concentrations in the 24 hours urine is not a simple or easy procedure at an outpatient clinic, so that proteinuria screening at prenatal visits is normally

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performed using a dipstick test which detects protein levels semi-quantitatively (−, ±, 1+, ≥2+) in spot urine samples. Some studies reported that when compared with results of the protein amount in the 24 hours urine, positive results (≥1+) of a dipstick test in the spot urine presented a high rate of false positive for a criterion of pathological proteinuria (≥300 mg/day) 7, 8). Recent studies noted the usefulness of measuring the protein/creatinine ratio in the spot urine for the diagnosis of pathological proteinuria during pregnancy 9-12). The International Society for the Study of Hypertension in Pregnancy proposed a criterion for pathological proteinuria as 0.265 mg/mg creatinine or more in the spot urine 3). However, measurement of urine creatinine concentrations at every prenatal visits is difficult in light of medical costs.

It is known that urine creatinine concentrations are well correlated with the extent of urine concentrate and the urine specific gravity. The aim of this study was to assess availability of a dipstick test combined with the urine specific gravity for the determination of pathological proteinuria at an outpatient clinic.

MATERIALS AND METHODS

Two hundred eighty-three pregnant women who received prenatal care and gave births at the Kobe University Hospital were enrolled. The 283 subjects consisted of 260 women with normal blood pressure (non PIH group) and 23 with diagnosis of pregnancy-induced hypertension (PIH group). Women with kidney disorders, diabetes mellitus and gestational diabetes were excluded from the enrollment of subjects. Spot urine samples were collected from the 283 women when they were seen for periodic checkups and proteinuria screening of a dipstick test. A total of 582 spot urine samples were used in this study analyses.

Results of a semi-quantitative dipstick test for proteinuria were evaluated by an automated urinometer (Arkray, Kyoto). The urine specific gravity was measured by the pKa change of polyelectrolytes. The protein (P) concentrations in the urine were measured using a pyrogallol red assay; and the creatinine (C) concentrations in the urine were measured using an enzymatic assay. We confirmed that the pH of the urine samples was less than 7.0, and these samples were negative for the white blood cells using the dipstick tests.

Samples were divided into 4 groups according to the dipstick test results as follows; −, ±, 1+, and ≥2+. P and C concentrations, the urine specific gravity, and the P/C ratio were compared among the groups. A correlation and a regression curve between C concentration and urine specific gravity were examined.

In order to clarify an effect of urinary concentration on the dipstick test result, frequencies of 0.265 or more P/C ratio were also compared among the groups, and between in concentrated and diluted urine with the same dipstick result. The criterion for the degree of urinary concentration in each group except that of ≥2 was determined in the following process. 1) The theoretical maximum P concentration in each group was estimated by excluding outliers of P values determined by the Grubbs-Smirnov test for outliers at a level of 1% significance. 2) The C concentration equivalent to yield 0.265 of P/C ratio with each theoretical maximum P was calculated. 3) The urine specific gravity corresponding to each C concentration calculated above was obtained by substituting the C value into the regression curve determined above. 4) Urinary samples with specific gravity higher than the value in each group were regarded as concentrated. The same value of specific gravity determined for the group of 1+ was used for the group of ≥2.

The dipstick test results of −, ±, 1+, ≥2+ were converted to 0, 0.5, 1, and 2, respectively, in statistical analyses. The statistical analyses were performed using the program

Ekuseru-Toukei (Social Survey Research Information Co., Ltd、Tokyo). $P < 0.05$ was considered as statistically significant.

RESULTS

Semi-quantitative determination using a dipstick test

Dipstick test results of a total of 582 spot urine samples were shown in Table I. The frequency of ≥2+ in PIH group was significantly higher than that in non PIH group (x^2 test, $p < 0.0001$).

Association between dipstick test results and the P concentration

The P concentrations (mg/dl, mean ± SD) with dipstick test results of −, ±, 1+, ≥2+ were 2.80 ± 2.39, 7.59 ± 3.65, 14.6 ± 8.80, and 237.4 ± 225.5, respectively.

In terms of the P concentration, there were significant differences among dipstick test results of −, ±, 1+, and ≥2+ ($p<0.0001$); among dipstick test results of ±, 1+, and ≥2+ ($p<0.0001$); and between dipstick test results of 1+ and ≥2 ($p<0.0005$). These significances were determined by multiple comparisons using the Kruskal-Wallis and Steel-Dwass tests.

When outliers in the P concentrations from samples with dipstick test results of −, ± and 1+ were detected at a level of 1% significance, values for 3 samples with − dipstick test result were outliers (12.3, 14.8, and 18.3 mg/dl), values for 2 samples with ± dipstick test result were outliers (20.9 and 23.9 mg/dl) and a value for 1 samples with 1+ dipstick test result was an outlier (57.8 mg/dl). If these outliers were excluded, the theoretical maximum urine P concentrations for −, ± and 1+ samples was 10.6, 19.3, and 35.9 mg/dl, respectively.

Table I. Dipstick test results of a total of 582 spot urine samples

Dipstick test results	Non PIH	PIH	Total
−	278	23	301
±	137	14	151
1+	109	12	121
≥2+	1	8*	9
Total	525	57	582

PIH; pregnancy-induced hypertension
*$p < 0.0001$

Association between dipstick test results and the C concentration

The C concentrations (mg/dl, mean ± SD) with dipstick test results of −, ±, 1+, ≥2+ were 56.8 ± 37.7, 125.8 ± 42.0, 180.3 ± 65.4, and 149.1 ± 85.7, respectively. In terms of the C concentration, there were significant differences among dipstick test results of −, ± and 1+ ($p<0.0001$); between dipstick test results of − and ≥2+ ($p<0.0005$); and between dipstick test results of ± and 1+ ($p<0.0001$, multiple comparisons using the Kruskal-Wallis and Steel-Dwass tests).

Association between dipstick test results and the urine specific gravity

The urine specific gravity (mean ± SD) with dipstick test results of −, ±, 1+, ≥2+ were 1.011 ± 0.007, 1.020 ± 0.006, 1.024 ± 0.007, and 1.020 ± 0.008, respectively. In terms of the

urine specific gravity, there were significant differences among dipstick test results of −, ± and 1+ ($p<0.0001$); between dipstick test results of ± and 1+ ($p<0.005$); and between dipstick test results of − and ≥2+ ($p<0.005$, multiple comparisons using the Kruskal-Wallis and Steel-Dwass tests).

Association between dipstick test results and the P/C ratio

The P/C ratio (mean ± SD) with dipstick test results of −, ±, 1+, ≥2+ were 0.056 ± 0.053, 0.070 ± 0.056, 0.095 ± 0.105, and 1.86 ± 1.41, respectively. In terms of the P/C ratio, there were significant differences between dipstick test results of − and ± ($p<0.01$); between dipstick test results of − and 1+ ($p<0.0001$); between dipstick test results of − and ≥2+ ($p<0.0001$); and between dipstick test results of 1+ and ≥2+ ($p<0.005$, multiple comparisons using the Kruskal-Wallis and Steel-Dwass tests).

Frequencies of the urine samples with 0.265 or more P/C ratio were 2 (0.7%) with − dipstick test result, 1 (0.7%) with ± dipstick test result, 4 (3.3%) with 1+ dipstick test result, and 8 (88.9%) with ≥2+ dipstick test result. A total 15 (2.6%) urine samples exceeded 0.265

Table II. Summary of urine data in 15 women with 0.265 or more P/C ratio

Gestational weeks of urine collection	Hypertension present when collected	Complications during delivery	Final diagnosis	Dipstick test results	P/C ratio	**Specific gravity**	Urine creatinine (mg/dl)	Protein concentration in urine (mg/dl)
28	Y	Y	PIH	−	0.491	**1.01**	30.12	14.8
33	N	N	PIH	−	0.359	**1.005**	28.67	10.3
35	N	N	Non PIH	±	0.513	**1.006**	13.06	6.7
35	Y	Y	PIH	1 +	0.657	**1.01**	51.32	33.7
36	Y	N	PIH	1 +	0.52	**1.01**	61.5	32
37	Y	N	PIH	1 +	0.278	**1.018**	121.19	33.7
33	N	N	Non PIH	1 +	0.763	**1.011**	75.75	57.8
34	Y	Y	PIH	2 +	0.682	**1.016**	114.85	78.3
35	Y	Y	PIH	2 +	0.982	**1.01**	88.47	86.9
36	Y	Y	PIH	2 +	0.619	**1.016**	124.79	77.3
29	Y	Y	PIH	3 +	4.157	**1.021**	45.06	187.3
33	Y	Y	PIH	3 +	3.264	**1.035**	114.56	373.9
37	Y	N	PIH	3 +	1.504	**1.025**	305.9	460
38	Y	N	PIH	3 +	2.805	**1.013**	153.81	431.4
37	Y	N	PIH	4 +	2.663	**1.031**	245.75	654.5

PIH; pregnancy-induced hypertension

P/C ratio. Thirteen of the 15 samples were obtained from PIH women, whereas the other two were from non PIH women (Table II).

Association between the urine specific gravity and the C concentration

There was positive correlation between the urine specific gravity and the C concentration ($r = 0.841$, $p<0.0001$). With the urine specific gravity on the y-axis and urine C concentration on the x-axis, the regression line was y = 1.0061293 + 0.0001199x - 3.9038e-7x^2.

Comparison of frequencies of 0.265 or more P/C ratio based on the urine specific gravity and dipstick test results

As mentioned above, the theoretical maximum urine P concentrations for −, ± and 1+ dipstick test results were 10.6, 19.3, and 35.9 mg/dl, respectively. The corresponding urine C concentrations to result in P/C ratio of 0.265 were 40.0 for − dipstick test result, 72.8 for ±, and 135.5 mg/dl for 1+. When these values were substituted in the regression curve for the urine C concentration and urine specific gravity, the urine specific gravity corresponding to each urine C concentration was 1.0094 for − dipstick test result, 1.0145 for ±, and 1.0219 for 1+.

Frequencies of samples with 0.265 or more P/C ratio were compared according to each group of dipstick test result between low and high urine specific gravity specimens (Table III). There was statistical significance in the frequency between low and high urine specific gravity in the group of 1+ ($p=0.0145$, Fisher's exact test).

DISCUSSION

Brown and Buddle reported that, in pregnant women with hypertension, incidences of false negative (– or ± result on the dipstick test but 300 mg/day or more protein in the 24 hours urine) and false positive (≥1+ result on the dipstick test but less than 300 mg/day in the 24 hours urine) in dipstick test results of the spot urine were 14% and 62%, respectively 7). Therefore, numerous investigators suggested that a collection of 24 hours urine and measurement of the protein concentration are needed for the definite diagnosis of pathological proteinuria in pregnancy.

However, a 24-hour urine collection is not a simple or easy procedure at an outpatient clinic, so a preferable method of assessing whether or not pathological proteinuria is present would be to use spot urine samples. The current study found that when results of screening with a dipstick test were 1+, 96.7% of those came from urine samples with a P/C ratio that did not exceed 0.265, the criterion for diagnosing proteinuria using spot urine samples as proposed by the International Society for the Study of Hypertension in Pregnancy. In contrast, ≥2+ dipstick test results identified 88.9% of samples with a P/C ratio exceeding 0.265. Thus, the ≥2+ dipstick test can be clinically used for the detection of pathological proteinuria in pregnancy screening. With this criterion, however, false negative percentages of −, ±, and 1+ dipstick test results were found to be 0.7, 0.7, and 3.3%, respectively. These frequencies cannot be negligible, especially in the group of 1+.

Endo et al., reported mean urine P concentrations of 2.9 for samples with − dipstick test result, 13.3 for the ± result, 30.4 mg/dl for the 1+result, in non-pregnant individuals 13). In the current study, the mean urine P concentrations with dipstick test results of ± (7.59) and + (14.6 mg/dl) in pregnant women was considerably lower. In pregnant women, color changes on the dipstick may occur at a lower concentration of urine protein than that for non-pregnant individuals. During pregnancy, the partial pressure of carbon dioxide in arterial blood is known to decrease approximately 5-10 mmHg when compared with non pregnant status,

leading to respiratory alkalosis, so that the kidneys compensate by eliminating more bicarbonate 14). Patients with obvious alkaluria were excluded from the current study, but pregnant women may have urine with a higher pH than non-pregnant individuals due to pregnancy-induced changes in the acid-base balance, and this may consequently affect the results of a semi-quantitative determination of urine protein using the dipstick test.

Table III. Comparisons of frequencies of samples with 0.265 or more P/C ratio between low and high urine specific gravity specimens in each dipstick test result

Dipstick(−)			
Specific gravity	1.009 or less	1.010 or more	total
Total sample numbers	142	159	301
High PC ratio sample numbers (%)	1 (0.70%)	1 (0.63%)	2 (0.66%)
Dipstick(±)			
Specific gravity	1.014 or less	1.015 or more	total
Total sample numbers	20	131	151
High PC ratio sample numbers (%)	1 (5%)	0	1 (0.66%)
Dipstick(1+)			
Specific gravity	1.021 or less	1.022 or more	total
Total sample numbers	43	78	121
High PC ratio sample numbers (%)	4 (9.3%)*	0*	4 (3.3%)
Dipstick(2+, more)			
Specific gravity	1.021 or less	1.022 or more	total
Total sample numbers	5	4	9
High PC ratio sample numbers (%)	5 (100%)	3 (75%)	8 (89%)

*: p=0.0145, Fisher's exact test

This study revealed that the extent of urine concentration, i.e. the urine specific gravity and the urine C concentration, were closely related to dipstick test results. The greater the extent to which urine is concentrated, the more likely the dipstick test might be positive. Additionally, the urine specific gravity and the urine C concentration were correlated. Thus, the regression curve for the urine specific gravity and the urine C concentration allowed estimation of the urine C concentration based on urine specific gravity. Urine glucose affects urine specific gravity 15), so women with diabetes mellitus or a pregnancy complicated by

gestational diabetes were excluded from the current study. Detection of statistical outliers in the current study allowed the upper limit for the urine P concentration in samples to be estimated to be 10.6 mg/dl for samples with – dipstick test result, 19.3 mg/dl for the ± result, and 35.9 mg/dl for the 1+ result. On the regression curve, values of urine specific gravity corresponding to the urine C concentrations to result in P/C of 0.265 were calculated to be 1.0094 for samples with – dipstick test result, 1.0145 for the ± test result, and 1.0219 for the 1+ result. According to the comparison of incidence of true proteinuria between samples of high and low specific gravity in each group of the same dipstick test result, urine with higher specific gravity showed significantly higher incidence of negative result for proteinuria in the group of 1+. Therefore, samples with 1+ dipstick test result can be considered to indicate a negative result of screening only if the specific gravity of the sample is 1.022 or more. Conversely, those with 1.021 or less specific gravity should be further assessed for pathological proteinuria. As only one sample with dipstick ± was positive for proteinuria, the comparison between high and low urine specific gravity is not so informative as in those with dipstick 1+. However, it may be reasonable to pay some attention in evaluating the significance of proteinuria screening in cases with dipstick ± and with specific gravity of 1.014 or less. Thus, a dipstick test combined with the urine specific gravity may be useful for outpatient clinic screening.

In this study, 2 samples had a P/C ratio exceeding 0.265 with –dipstick test result. The both samples were obtained from women with final diagnosis of PIH. Therefore, we have to pay attention to possibility of latent proteinuria in women with hypertension and/or other related findings even when a dipstick test result is negative.

In conclusion, first, because women with ≥2+ dipstick test results have a high incidence of 0.265 or more P/C ratio, they should be examined carefully. Second, women with 1+ dipstick test result plus 1.021 or less specific gravity should be further assessed for pathological proteinuria. Third, the potential for 0.265 or more P/C ratio cannot be discounted in women presenting with hypertension at urine sampling even when a dipstick test result is negative.

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