Test Dipstick for Determination of Urinary Protein, Creatinine and Protein/Creatinine Ratio

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A slight increase in urinary albumin level could be an advanced indicator of early stage nephropathy. The aim of this study was to detect the presence of albumin adjusted by creatinine using a dipstick to test urine samples from 301 patients with nephropathy. The dipstick method can also be used to calculate the protein-to-creatinine ratio (P/C ratio) as a valuable index for eliminating false positive or false negative protein reports, which are often caused by abnormal sample concentration variation. The results showed the test data for the dipstick show good agreement with the quantitative measurements of protein, creatinine and P/C ratio. The P/C ratio obtained by the dipstick showed a sensitivity of 98% compared with the protein-only results of 80% in diagnosis of clinical proteinuria/albuminuria. In conclusion, the dipstick is a reliable and practical screening tool for diagnosing the early stage of nephropathy.

Key words: Creatinine, protein/creatinine ratio, Clinitek Atlas PRO 12 Reagent Pak

Introduction

It has recently been shown that even small increases in urinary protein or albumin excretion are early predictors of kidney failure and end-stage renal disease (ESRD) [1,2]. Early intervention has been demonstrated to retard the development or rate of progression of renal disease. Patients with diabetes and/or hypertension are the primary risk groups [3-5]. Proteinuria is a major symptom of renal diseases and the excretive amounts of protein in the urine could be an index for evaluating the pathological stage of renal diseases [6]. Current urinary protein testing is performed by using a urine dipstick in routine urine examinations. The principle behind the reaction is the “protein error of indicators”. Under a fixed pH, indicators will change color when protein exists, and the concentration of urinary protein can be calculated based on the difference of color. However, the test results of urinary protein are often affected by variation in urine samples. One of the main factors in variation lies in the concentrated or diluted samples that lead to urine overestimation or underestimation. In order to solve this problem, 24-hour samples can be used to reduce the variation in the urine concentration [7]. However, inconvenience is encountered and re-testing increases the cost.

Previous studies had revealed urinary creatinine can be used to correct the variation occurring in concentration of random urine samples because the daily excretion of creatinine is consistent [8-11]. The protein-to-creatinine ratio (P/C ratio) can be used to correct the false positive or false negative protein results caused by the variation in urine concentration. Calculation of the P/C ratio may be a more objective method for estimating urinary protein excretion. However, most previous studies used quantitative methods to calculate the P/C ratio. The novel urine dipstick, Clinitek Atlas PRO12 Reagent Pak (PRO12), has the added features of protein-low and creatinine test pads. The protein-low test pad has improved specificity to albumin and the sensitivity has...
been increased from 15mg/dL to 8mg/dL, which also
leads to the display of “trace” results which are replaced
by the indication “15mg/dL”. Creatinine can also be
used to calculate the P/C ratio.

In this study, we examined urine samples from the
outpatient department and evaluated the comparability of
protein, creatinine, and P/C ratio data obtained from the
PRO12 dipstick using quantitative chemical methods.
The results showed that the novel dipstick can eliminate
false negative and false positive reports and provide
more accurate and objective data for clinical physicians
in screening and diagnosis of renal disease.

Materials and Methods

We collected 301 random urine samples from the outpa-
tient department of Taichung Veterans General Hospital,
Taichung, Taiwan. All samples were examined by two
models of automatic urine chemistry analyzers (Roche
Urisys 2400 with the dipsticks, Urisys 2400 cassette and
Siemens/Bayer Clinitek Atlas with the dipsticks, Clinitek
Atlas PRO12 Reagent Pak). The following quantitative
methods were also performed by a Hitachi chemistry
analyzer for each urinary sample: total protein concen-
tration in urine using the total protein dye-binding assay
(pyrogallol red), urinary albumin concentration using the
immuno-turbidimetric method, and urinary creatinine
concentration using Jaffe’s method.

The Clinitek Atlas PRO12 Reagent Pak is enhanced
with a new protein-low pad which uses bis(3',3''-diiodo-
4',4''-dihydroxy-5',5''-dinitrophenyl)-3,4,5,6-tetrabromo-
sulfonephthalein (DIDNTB) dye for the detection of
albumin at > 8 mg/dL. Also present on the dipstick is an
original pad (protein-high pad) that is based on the pro-
tein error of indicator method (tetrabromophenol blue
(TBPB)) for detection of protein at > 30 mg/dL, and a
pad for detection of creatinine that uses the peroxidase
activity of the copper-creatinine complex. The P/C ratio
is automatically calculated by the analyzer based on the
light absorbent readings of the protein and creatinine test
pads.

Results were analyzed by computing the percentage
of same level agreement, ± one-level agreement, sen-
sitivity, specificity and Cohen’s Kappa coefficient.

Results

The correlation of the protein test results between the
dipsticks of the Roche Urisys 2400 and the quantitative
methods was shown in Table 1. The rate of complete
coincidence was 77.7%. Rates of 77.42%, 94.92%, and
87.71% were observed for the agreement rate with the
cutoff point between negative and positive results being
“trace” for the dipsticks and “25 mg/dL” for the quantita-
tive methods.

The correlation of the protein test results between
the PRO12 dipsticks and the quantitative methods was
shown in Table 2. The rate of complete coincidence was
69.44%. Rates of 93.88%, 78.57%, and 86.05% were
observed for the agreement rate with the cutoff point
between negative and positive results being “15 mg/dL”
for the Pro12 test strips and “8 mg/dL” for the quantita-
tive methods. The sensitivity of the PRO12 dipsticks, but
the specificity showed a minor decline.

The correlation of the creatinine test results between
the PRO12 dipsticks and the quantitative methods was
shown in Table 3. The rate of complete coincidence was
52.16%. Agreement was 95.68%, if we extended the
comparison range to ± one level.

Table 4 shows the correlation of the P/C ratio results
between the PRO12 dipsticks and the quantitative
methods. Only 286 samples were compared with the P/C
ratio results of the quantitative methods. (There were 39
over-diluted samples, which were re-collected.) The rate
of complete coincidence was 67.44%. Rates of 98.64%,
76.98%, and 88.11% were observed for the agreement

<table>
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<tr>
<th>Table 1. Correlation between the results of the quantitative method and the protein test portion of the Roche Urisys 2400 with the dipsticks.</th>
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<tbody>
<tr>
<td>Total protein concentration in urine (mg/dL)</td>
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</tr>
<tr>
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<td>25*</td>
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*: Roche Urisys 2400 with the dipsticks (mg/dL); Neg, negative result.
rate with the cutoff point between negative and positive results being “150 mg/gCr” for the Pro12 dipsticks and “80 mg/gCr” for the quantitative methods.

Table 5 shows the correlation between the protein test results obtained with the conventional dipsticks and the P/C ratio results obtained with the quantitative methods. Rates of 70.15% and 93.41% were observed for the agreement rate with the cutoff point between negative and positive results being “<25 mg/dL” for the Roche test strips and “80 mg/gCr” for the quantitative methods.
Discussion

In Taiwan, there are more than 46,000 dialysis patients and the number is increasing. Urine protein examination is the first step in screening for renal diseases. However, the urine dipstick currently used is semi-quantitative. Also, compared with the quantitative method the dipstick has sensitivity limitations (about 15-30 mg/dL). The urinary protein results can also be affected by variations in sample concentrations. Thus, physicians cannot distinguish between pathological proteinuria and false positives caused by concentrated samples when the urinary protein report shows “trace”. Many studies have shown measurement of urinary creatinine could be a good way to correct the variation in sample concentration. The protein-to-creatinine ratio (P/C ratio) can be used to correct the false protein results caused by the physiological differences in urine concentration. Calculating the P/C ratio is a more objective method for assessing urinary protein excretion.

In this study, we evaluated the application of the PRO12 dipstick [12]. The PRO12 is enhanced with a protein-low test pad, which has higher specificity and affinity for detecting lower concentrations of albumin. The protein-low pad has improved specificity to urinary albumin and the sensitivity has been increased to 8~15 mg/dL. The original protein test pad on the PRO12 dipstick is named the “protein-high pad” and it detects the concentration of total urinary protein. The PRO12 test strips have an added feature, a creatinine test, that can detect urinary creatinine and it also acts as a corrective factor for urine concentration variations. We compared both the conventional dipsticks and the PRO12 with the quantitative methods. The sensitivity of the PRO12 for urinary protein tests was 93.88%, but the sensitivity of the conventional dipsticks was only 77.42%. Furthermore, the complete coincidence rate of PRO12 urinary protein tests reached 86.05%.

We also compared urinary creatinine results between the PRO12 dipsticks and the quantitative methods. The complete coincidence rate was 52.16%. By extending the comparison range to one upper or one lower report unit, the overall coincidence rate reached 95.68%. The principle of the creatinine test in the PRO12 dipsticks is based on the peroxidase-like activity of the copper creatinine complex, and vitamin C or blood cells in urine samples can interfere with the reaction. This could be the reason why we did not get a high correlation using the quantitative methods. However, the overall correlation showed that the new creatinine test pad is reliable.

In comparison with the conventional dipsticks, the PRO12 dipsticks can provide both more sensitive protein and P/C ratio results for evaluating clinical proteinuria. Furthermore, the P/C ratio can act as an indicator to detect over-diluted samples which have less than 10 mg/dL of urinary creatinine. When negative protein results are found in over-diluted samples, the samples should be re-collected and re-examined to ensure accurate results. About 10% of routine urine screening samples were diluted samples. That also means without the P/C ratio, the diagnosis of proteinuria will be underestimated because of false negative results. In this study, we ruled out the diluted samples and only compared the P/C ratio data of the other 286 samples between the PRO12 dipsticks and the quantitative methods.

Microalbuminuria has been defined as a urinary albumin-to-creatinine ratio (A/C ratio) in the range of 30~300 mg/gCr. Many studies have revealed that microalbuminuria can be an early warning sign of renal diseases. The high-risk population includes hypertensive and diabetic patients, so we suggest regular monitoring of urinary microalbumin by using the A/C ratio data. In this study, we used 80 mg/gCr as the cutoff point for comparisons with the quantitative results [13]. The results showed that the coincidence rate was 88.11%. The false negative rate was only 1.3%, which indicated the P/C ratio could decrease the chance of false negatives. We also compared the results with those obtained with the Roche Urisys 2400 conventional dipsticks, the original method we used for routine urine tests. The sensitivity was 98.64% and specificity was 76.98%, which means 30% of early nephropathy cases could potentially be misdiagnosed due to negative protein reports. The data shows the P/C ratio of PRO12 could be an effective diagnostic tool for the monitoring of early stage renal diseases, especially for diabetes, hypertension and elderly health screening.

Since conventional dipsticks have sensitivity and specificity limitations, the urinary protein results could be false positive or false negative. If the false results are not detected, the consequences can be serious due to delays in diagnosis and treatment. However, it is costly to retest false negative data. The results of this study show that the new urine dipsticks, which have the added features of the creatinine test and P/C ratio, increase both the sensitivity and specificity of the protein test. These improvements could effectively eliminate most false negative results. We also found that using both the protein and P/C ratio results to evaluate clinical proteinuria...
was better than using the protein-only results. If we use
the routine urine tests to identify patients with potential
early stage renal diseases, treatment could lower the
chances of serious nephropathy. With the current preva-
lence of renal diseases increasing, the PRO12 test strip
could be used as a reliable and effective tool for early
screening.

According to recent data, the population with dia-
betic nephropathy is expanding year by year and is also
the most rapidly growing disease population preceding
end-stage renal disease (ESRD). In Taiwan, the medical
costs for dialysis, which is often required for end-stage
renal disease (ESRD) patients, have reached almost 28
billion New Taiwan Dollars (NT$) per year. The new
dipstick is convenient, rapid, accurate, reliable and eco-
nomical. It can effectively help prevent chronic renal
diseases by early detection and also save medical re-
sources.

In conclusion, we suggest that physicians use the
P/C ratio or A/C ratio to monitor the potential risks of
renal diseases in outpatients, hypertensive, and diabetic
patients. The P/C ratio data, which is easily obtained by
dipsticks, should be included in the routine urine data
and could improve the early diagnosis and monitoring of
renal diseases. Decelerating the prevalence of end-stage
renal disease (ESRD) could effectively relieve the bur-
den on the national health insurance program in Taiwan.

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新式尿液常規試紙檢測尿蛋白、尿肌酸酐及尿蛋白/肌酸酐比值之可用性與臨床檢驗價值研究

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許多文獻顯示，尿液中少量白蛋白的增加可以用來評估早期腎病變及阻止糖尿病或高血壓的高危險族群進展成末期腎病變。然而因目前使用之常規尿液試紙敏感度限制，特別是當尿蛋白濃度介於+/-(trace)的時候，容易因尿液濃度之稀釋或是濃縮的情況而造成錯誤的報告。檢驗室因此需要以其他檢測方式進行確認。本實驗中，我們對門診301位病人之隨機尿液檢體，測試了新的尿液檢驗試紙，新增了白蛋白(albumin)及肌酐酸(creatinine)項目，使原來尿蛋白檢查的檢驗結果加上新的albumin pad得到較精準的protein結果；測試Creatinine的結果則以排除尿液濃度造成的錯誤判斷並提供計算protein/creatinine ratio，藉此可減少尿液檢測偽陽或偽陰性發生，得到較精準之P/C Ratio結果參考。我們比較試紙法所得到的尿蛋白及尿肌酐酸及P/C ratio與定量法之間的相關性，發現兩者間具有很高的相關性，尤其在P/C ratio的項目中，試紙法與定量法比較，具有98%以上的敏感性，相較於傳統僅以尿蛋白結果作判讀的敏感度約80%，有大幅上升。研究顯示，新試紙為更可信的早期診斷初期腎臟病變篩檢疾病之工具。

關鍵詞：肌酸酐、尿蛋白/肌酸酐比值、尿液試紙