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Proteinuria detection in chronic spinal cord
Introduction:

Urinary protein excretion is known to have both diagnostic and prognostic value in detection, confirmation, and the monitoring of progression of renal disease (1-3). Giving the difficulty of 24 hour protein collection, the National Kidney Foundation has recommended the use of simple urine dipstick protein (DSP) measurement for those at lower risk for kidney disease or by determination of the random urine protein:creatinine ratio (UPC) for those at higher risk (4). DSP is highly accurate in predicting clinical proteinuria in the general population (5) and the use of the UPC to quantify proteinuria is considered accurate for normal individuals, pregnant women, patients with diabetes mellitus, renal transplant recipients, and children with nephrotic syndrome (6-9). A recent study even validated its use in patients with glomerulonephritis and impaired renal function (10).

The prevalence of clinical range proteinuria (≥ 0.5 gm/day) in patients with chronic spinal cord injury (SCI) is greater than in the general population (11,12). Proteinuria is recognized as an independent risk factor for cardiovascular disease (13,14), renal disease (15,16), and increased mortality in the general population (13,17). The presence of clinical range proteinuria is associated with increased cardiovascular and non-cardiovascular mortality in the SCI population (12).

24-hour protein excretion is considered the gold standard for quantifying proteinuria in patients with chronic spinal cord injury; however, 24-hour protein measurements have been shown to have significant variability on serial testing in these patients (18). Chronic SCI patients also have clinical characteristics that may decrease the accuracy of DSP and UPC in detecting and quantifying proteinuria. These factors
include dilute urine (5,19) and high rates of urinary tract inflammation, which may lead to increased levels of nonalbuminuric proteins (20-22). As urine dipsticks are more reactive to albumin than nonalbumin proteins (23), dipstick analysis may not be as sensitive in predicting total proteinuria in patients with chronic SCI. Additionally, patients with chronic SCI often have decreased muscle mass with decreased creatinine production and excretion, which may lead to falsely elevated UPC ratios (24). The purpose of this study was to assess the accuracy of DSP and UPC in predicting clinical proteinuria in the SCI population.

Methods

Patient and Urine Sample Selection

Computerized medical records of 219 patients with chronic spinal cord injury were reviewed at the Veterans Administration Medical Center (VAMC) in Memphis, Tennessee. These patients had 24-hour urine studies for both total protein excretion and creatinine clearance measurements as part of routine annual health maintenance evaluations (25). Samples were included if a 24-hour urine collection and random urinalysis were performed within 48 hours of each other. No samples were collected during an active urinary tract infection. To reduce potential collection bias, no more than two samples were collected from an individual patient. This led to the inclusion of 339 samples for study. Data collected from the 24-hour urine included creatinine concentration (mg/dl), protein concentration (mg/dl), and volume. Data collected from the urinalysis included specific gravity, dipstick protein (negative, trace, 1+, 2+, 3+).
A prospective study was also performed in 62 inpatients with chronic SCI to evaluate the accuracy of the UPC in predicting clinical proteinuria. An early morning spot urine sample for UPC and a 24-hour urine collection were performed for each patient under the direct observation of the research team. UPC was calculated as urinary protein (mg/dl) divided by urinary creatinine (mg/dl).

Clinical information obtained at the time of both studies included age, sex, ethnicity, type of injury, duration of injury, and type of bladder management. Type of injury was defined as quadriplegia or paraplegia. Bladder management was one of four types: chronic indwelling Foley catheter, ileal conduit, intermittent catheterization, or spontaneous voiding.

Data and Statistical analysis:

24-hour levels of proteinuria were used as the gold standard for defining the absence or presence of proteinuria. Clinical proteinuria was defined as 0.5 gm/day (12). Sensitivity, specificity, positive predictive value (+PV), negative predictive value (-PV) of DSP and UPC in predicting proteinuria were calculated. Receiver operator characteristic (ROC) curves were created for DSP and UPC as a screening test for clinical proteinuria. Area under the curve (AUC) was calculated and p-values were considered significant if < 0.05.

To assess the effect of urine concentration on the accuracy of DSP, a ROC curve was created using DSP adjusted for specific gravity according to a previously published algorithm (5). Simply, for clinical proteinuria (>0.5 grams/day), all DSP levels were
considered positive except for a) trace proteinuria with a specific gravity >1.015 and b) 1+ proteinuria with a specific gravity >1.025.

Sensitivity, specificity, predictive values and ROC curves of UPC in predicting clinical proteinuria were calculated. The correlation between 24-hour protein and morning spot UPC was analyzed using the concordance correlation coefficient (r) and linear regression. The level of agreement between morning UPC and 24-hour protein was also assessed using the Bland-Altman method (26). Using this method, the mean difference between UPC and measured 24 hour protein directly estimates the global bias. The width of one standard deviation of the mean difference between UPC and 24 hour protein is an estimation of precision, defined as the closeness of agreement between independent test results obtained under stipulated conditions, with a large width indicating low precision.

To determine the potential effect of low creatinine excretion on the level of agreement between morning UPC and 24-hour protein, additional Bland-Altman analysis was performed after categorizing the cohort by daily urine creatinine excretion rate (using 0.6 grams/day as a cutoff point). Statistical analysis was done using Med Calc Version 9.3.0.0 (Mariakerke, Belgium).
Results

Characteristics of the Cohort

Table 1 shows the general characteristics of the retrospective and prospective cohorts of patients that were used to evaluate DPS and UPC as predictors for clinical proteinuria. The majority of patients were male, Caucasian, and paraplegic. Most either used a chronic indwelling Foley catheter or spontaneously voided.

DSP in Predicting Clinical Proteinuria

Table 2 shows the sensitivities, specificities, and predictive values of DSP for 24-hour proteinuria threshold points of 0.5 grams/day. Overall, a positive DSP of any level had a high specificity and positive predictive value for the presence of ≥0.5 grams/day of proteinuria. However, the sensitivity and negative predictive value were low.

The mean and median specific gravity values for DSP measures were 1.011 and 1.010 respectively. The percentage of false negative samples was higher in the dilute urines. For example, in the 90 patients with negative dipsticks and a specific gravity ≤1.005, 24 patients actually had clinical range proteinuria. Negative and trace DSP failed to identify significant numbers of patients with clinical proteinuria.

Figure 1 shows the ROC curve for DSP as a screening test for clinical proteinuria. The area under the curve was 0.749 (0.699 to 0.794, P<0.0001). Adjusting DSP for concentrated urines (i.e. classifying trace protein with a specific gravity of 1.020 or greater and 1+ protein with a specific gravity of 1.030 or greater as negative for clinical proteinuria) slightly improved the specificity of DSP for clinical proteinuria, but also worsened the sensitivity. The AUC decreased to 0.737 (95%CI 0.686 to 0.783, p<0.001) for the corrected dipstick (difference between AUC’s = 0.012, p=0.514). To evaluate the
effect of dilute urine on the accuracy of DSP, a ROC curve was created for 0.5 gram/day proteinuria, excluding urine specimens with a specific gravity of <1.010 (figure not shown). The AUC of the ROC curve showed a mild improvement to 0.784 (95%CI 0.721 to 0.839, p<0.001), secondary to improvement in the sensitivity of the dipstick. When the concentrated and dilute urines were excluded together regardless of their proteinuria predictions, there was a nonsignificant improvement in accuracy (AUC for the receiver-operator curves 0.749 → 0.803, p<0.001) and sensitivity (52.7 % → 65.5%, data not shown).

UPC in Predicting Clinical Proteinuria

Table 4 shows the sensitivity, specificity, and predictive values of UPC in predicting clinical proteinuria. The prevalence of clinical proteinuria in this cohort was 40.4%. A UPC ratio of < 0.3 had a high sensitivity and high negative predictive value for excluding clinical proteinuria. A UPC ratio of > 0.8 had a high specificity and high positive predictive value for detecting clinical proteinuria. A UPC between 0.3 and 0.8 had an intermediate sensitivity and specificity for clinically significant proteinuria. Figure 2 shows the ROC curve of UPC as a screening test for clinical range proteinuria. The AUC was 0.75 (95%CI 0.624 to 0.852, p< 0.0002).

There was a significant correlation between the UPC and the 24-hour urine protein level (n= 62, concordance correlation coefficient (r) = 0.96, 95% CI 0.93 to 0.97, p<0.001). A scatter plot (Figure 3) and regression analysis revealed a strong correlation with few outliers. Excluding patients with 24-hour urine protein > 10g/d (n=60), the concordance correlation coefficient (r) decreased to 0.45, 95%CI 0.227 to 0.635, p<0.0003. The agreement level between morning UPC and 24-hour protein was assessed
using the Bland-Altman method (Figure not shown). The difference between the mean value and its related 95% confidence interval shows that there is an agreement between the UPC and 24 hour protein levels (the bias was 1.19, and the 95% limits of agreements were between (-2.4 -2.3). Bias is the difference between an estimator's expectation and the true value of the parameter being estimated. However, the exclusion of patients with massive proteinuria (≥ 10 grams/day) decreases the bias to to -0.17 (95%CI -1.2 to -0.8) (Figure 4).

To determine the potential effect of low creatinine excretion on the level of agreement between morning UPC and 24-hour protein, Bland-Altman analysis was also performed after excluding samples with ≤0.6 grams/day. Exclusion of these samples did not improve either the global bias or precision (mean – 2* and SD = -2.4; mean + 2* SD = 2.6, Figure not shown), suggesting that the effect of low urine creatinine excretion on UPC was minimal.

Discussion

Accurate determination of urinary protein excretion in patients with chronic SCI is needed as clinical range proteinuria (>500mg/day) is predictive of cardiovascular, noncardiovascular, and all-cause mortality (11,12). Urinary protein excretion’s importance in the chronic SCI population is magnified by its higher prevalence rate compared to the general population (11,12).

The optimal method of measuring proteinuria in chronic SCI patients remains unclear. The measurement of 24-hour urine protein is inconvenient and subject to collection errors. Evidence suggests that approximately 12-15% of 24-hour urine
collections are usually excluded from analysis because of errors during collection (10).

In the general population, measuring the urine creatinine as a function of body mass is used for evaluating the adequacy of 24-hour urine samples. A similar, standardized method is not available for the chronic SCI population, in whom creatinine excretion is highly variable secondary to variable muscle mass (19,27-29). Benefits of 24-hour collections include measurements of sodium and protein intake, and more accurate measurements of creatinine clearance in the chronic SCI population (28).

Given the difficulty and limitations of 24-hour collections, the National Kidney Foundation recommends random urine testing for proteinuria. In the general population, DSP has been shown to be a sensitive but rather nonspecific test for both clinical proteinuria and microalbuminuria (6, 10). In our study, the opposite is true for SCI patients, in whom DSP is very specific but not sensitive for the detection of clinical proteinuria.

There are at least two possible explanations for the lack of sensitivity. The first is urine concentration. In the general population, the exclusion of concentrated urine samples, which may give falsely positive results, improves the specificity of DSP for clinical proteinuria (10). However, the specificity of DSP for clinical proteinuria was quite high in our SCI cohort. Our population overall had many very dilute urines which led to a higher false negative rate of identifying clinical proteinuria. The second possible explanation is the type of protein detected by the dipstick method versus the 24-hour total urine protein. Spinal cord injury patients have a high rate of urinary tract inflammation (29) and chronic pyelonephritis (19), which may lead to increased levels of nonalbuminuric proteins (24). As urine dipsticks are more reactive to albumin than
nonalbumin protein, urine dipsticks may be less sensitive in predicting total proteinuria in patients with spinal cord injury.

A UPC ratio of < 0.3 had a high sensitivity and high negative predictive value for excluding clinical proteinuria and UPC ratio of > 0.8 had a high specificity and high positive predictive value for detecting clinical proteinuria. UPC between 0.3 and 0.8, however, had an intermediate sensitivity and specificity with significant false negative and false positive values. ROC curves showed ~ 25% chance the UPC will lead to misclassification of clinical proteinuria. Bland Altman analysis demonstrated excellent agreement between the UPC and 24-hour proteinuria (low bias and high precision) at levels of proteinuria less than 0.5 g/d; however, global bias increased and precision decreased with progressive increments in proteinuria, thus, limiting the reliability of UPC for detecting clinical proteinuria (6). 24 hour urine collections have also been shown to have greater variability at higher levels of proteinuria (18). Exclusion of patients with reduced muscle mass, defined as urinary creatinine excretion < 600 mg/day per day of creatinine excretion, did not improve the bias or precision of UPC, suggesting that reduced urinary creatinine excretion was not the primary reason for the discrepancies between UPC and 24-hour proteinuria. Given these limitations, UPC should not replace 24 hour protein measurements for detecting clinical significant proteinuria in patients with spinal cord injury.

Limitations of the current study include the inherent limitations of 24 hr urine collections which were used as the “gold standard” for quantifying proteinuria. In the prospective arm of the study, 24 hour urine collections were performed under direct
observation of the research team in an effort to minimize collection errors. The use of an all male population and the single center study design may limit application to the general SCI population.

Conclusion:

24 hr urine collections continue to be the preferred method for quantifying proteinuria in the chronic SCI population. Routine dipstick urinalysis provides clinically relevant information for the SCI patient, but should not be used as the sole method to screen for clinical range proteinuria, even after correction for specific gravity. Spot UPC is of utility for ruling out clinical proteinuria at levels of < 0.3 and in ruling in proteinuria at levels > 0.8; however, intermediate levels (0.3-0.8) require confirmation with 24hr urine collections.

References:


Table 1: Clinical characteristics of the patient population (Retrospective cohort) and (Prospective cohort)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Retrospective study (UDP)</th>
<th>Prospective study (UPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>54 ± 14</td>
<td>59±2</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>153 (70)</td>
<td>34 (55)</td>
</tr>
<tr>
<td>Black</td>
<td>66 (30)</td>
<td>28 (45)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>218 (99.5)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Type of Injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraplegic</td>
<td>127 (58)</td>
<td>41 (66)</td>
</tr>
<tr>
<td>Quadriplegic</td>
<td>92 (42)</td>
<td>21 (34)</td>
</tr>
<tr>
<td>Injury Duration (years)</td>
<td>21 ± 14</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Bladder management</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Indwelling Foley</td>
<td>99 (45)</td>
<td>39 (63)</td>
</tr>
<tr>
<td>Ileal Conduit</td>
<td>7 (3)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Intermittent Catheterization</td>
<td>26 (12)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Spontaneous Voiding</td>
<td>87 (40)</td>
<td>9 (15)</td>
</tr>
</tbody>
</table>

*Mean +/- standard deviation or count with percent in parentheses.*
<table>
<thead>
<tr>
<th>24 Hour Protein</th>
<th>Dipstick</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>^aPV</th>
<th>^b-PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 0.5 gm/day</td>
<td>Trace</td>
<td>52.7 (43.7-61.6)</td>
<td>95.7 (92.0-98.0)</td>
<td>88.3</td>
<td>76.7</td>
</tr>
<tr>
<td>≥ 0.5 gm/day</td>
<td>1+</td>
<td>40.3 (31.8-49.3)</td>
<td>98.6 (95.9-99.7)</td>
<td>94.5</td>
<td>72.9</td>
</tr>
<tr>
<td>≥ 0.5 gm/day</td>
<td>2+</td>
<td>21.7 (14.9-29.8)</td>
<td>100.0 (98.2-100.0)</td>
<td>100.0</td>
<td>67.5</td>
</tr>
<tr>
<td>≥ 0.5 gm/day</td>
<td>3+</td>
<td>0.0 (0.0-2.8)</td>
<td>100.0 (98.2-100.0)</td>
<td>100</td>
<td>61.9</td>
</tr>
</tbody>
</table>

^aPV = Positive Predictive Value  
^b-PV = Negative Predictive Value
### Table 3: Sensitivities, Specificities, and Predictive Values of Random UPC for Clinical Proteinuria

<table>
<thead>
<tr>
<th>UPC ratio</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>(^a+PV)</th>
<th>(^b-PV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.09</td>
<td>100 (83.7-100)</td>
<td>5.13 (1.1-21.1)</td>
<td>44.8</td>
<td>100</td>
</tr>
<tr>
<td>0.1-0.19</td>
<td>100 (83.7-100)</td>
<td>26.92 (11.8-47.7)</td>
<td>51.4</td>
<td>100</td>
</tr>
<tr>
<td>0.2-0.29</td>
<td>93.75 (73.5-99.0)</td>
<td>41.3 (22.7-62.0)</td>
<td>56.2</td>
<td>90.0</td>
</tr>
<tr>
<td>0.3-0.34</td>
<td>90.0 (68.3-98.5)</td>
<td>53.8 (33.4-73.3)</td>
<td>60.1</td>
<td>87.5</td>
</tr>
<tr>
<td>0.35-0.44</td>
<td>86.6 (64.1-97.2)</td>
<td>59.0 (38.1-78.6)</td>
<td>61.3</td>
<td>85.2</td>
</tr>
<tr>
<td>0.45-0.54</td>
<td>80.0 (56.4-94)</td>
<td>66.4 (45.3-83.4)</td>
<td>64.7</td>
<td>81.3</td>
</tr>
<tr>
<td>0.55-0.64</td>
<td>67.5 (43.2-86.2)</td>
<td>76.9 (56.3-91.0)</td>
<td>69.3</td>
<td>75.5</td>
</tr>
<tr>
<td>0.65-0.79</td>
<td>51.6 (28.6-74.2)</td>
<td>85.9 (66.7-96.1)</td>
<td>73.8</td>
<td>69.8</td>
</tr>
<tr>
<td>0.80-0.89</td>
<td>47.5 (25.1-70.6)</td>
<td>92.3 (74.8-98.8)</td>
<td>82.6</td>
<td>69.6</td>
</tr>
<tr>
<td>0.90-0.99</td>
<td>37.5 (17.3-61.55)</td>
<td>92.3 (74-98.8)</td>
<td>78.9</td>
<td>65.7</td>
</tr>
<tr>
<td>1.00-1.49</td>
<td>23.8 (8.2-47.7)</td>
<td>93.3 (76.1-99.0)</td>
<td>73.2</td>
<td>61.3</td>
</tr>
<tr>
<td>1.50-1.99</td>
<td>12.5 (2.5-34.8)</td>
<td>96.2 (80.3-99.4)</td>
<td>70.9</td>
<td>58.8</td>
</tr>
<tr>
<td>&gt;2.00</td>
<td>5.0 (1.7-24.5)</td>
<td>100 (86.7-100)</td>
<td>100</td>
<td>57.8</td>
</tr>
</tbody>
</table>

\(^a+PV\) = Positive Predictive Value  
\(^b-PV\) = Negative Predictive Value
Legend:

**Figure 1.** Receiver operator characteristic curves for clinical proteinuria (0.5 gm/day proteinuria), comparing DSP uncorrected (solid line) and corrected (dotted line) for specific gravity. The curves nearly overlap, with an AUC of 0.749 (0.699 to 0.794, p<0.0001 compared to null hypothesis) for the uncorrected dipstick vs 0.737 (0.686 to 0.783, p<0.001) for the corrected dipstick (difference between AUC’s = 0.012, p=0.514).

**Figure 2.** Receiver operator curves for UPC in detecting clinical proteinuria (0.5 gm/day proteinuria). The best combination of sensitivity and specificity of UPC ratio for diagnosing proteinuria occur at cut-off point of 0.51. AUC= 0.75 (95%CI 0.624 to 0.852, P< 0.0002)

**Figure 3.** Scatter plot and linear regression for UPC versus 24 hour proteinuria.

**Figure 4.** Bland-Altman plot comparing the difference between the 24 hour urine protein (gm/day) and the UPC ratio sample versus the mean of the two methods with massive proteinuria (>10gm/day) excluded. The dashed line represents the mean ± 2 standard deviations (SD). The thick line represents the mean. The agreement between UPC and 24 hour proteinuria is not uniform across the observed range of proteinuria. The agreement between the methods is quite good (low bias and high precision) at levels of proteinuria <0.5 g per day; whereas, the level of agreement progressively decreases with increasing levels of proteinuria (increased bias and decreased precision).