Urine dipsticks must not be used to detect diabetes-induced incipient nephropathy

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Received August 17, 2005
Accepted January 5, 2006

To cite the present paper, use exclusively the following reference:
Free full text in english on www.masson.fr/revues/pm

Summary

Introduction > Microalbuminuria is an early indication of diabetic nephropathy in patients with Type 1 diabetes and a marker of cardiovascular in patients with type 2 diabetes. It must therefore be assessed annually in these patients. We sought to determine whether semiquantitative determination of proteinuria with urinary dipsticks was useful for this purpose.

Method > This analysis of consecutive urinary samples among diabetic patients excluded those with dipstick results positive either for leukocyturia or nitrituria, to avoid false positives due to urinary infection. We assessed the reliability of the dipsticks in comparison with conventional microalbuminuria and proteinuria assays.

Results > The study included 230 patients. Positive dipstick results had good positive (95.7%) and negative (93.9%) predictive values. Low levels of microalbuminuria, however – those that lead to early adjustment of treatment, were much more difficult to identify: the negative predictive value was only 73.7% and proteinuria was no longer correlated with microalbuminuria.

Discussion > Urinary dipsticks cannot replace conventional assays for microalbuminuria or proteinuria.
Practice guidelines stress the need for annual determination of microalbumin levels in the urine of patients with diabetes [1]. Microalbuminuria is a very early indicator of diabetic nephropathy, especially in type 1 diabetes, and it is a marker of cardiovascular risk in type 2 diabetes. Though apparently simple to implement, this recommendation has not followed: practice audits show that this assay is performed in fewer than half of all diabetes patients. Given these circumstances, we wondered whether a semi-quantitative determination of proteinuria with urine dipsticks might be a possible alternative. Such reagent strips have certainly proven highly successful for monitoring diabetes. Although blood tests have now replaced them for measuring glucose and ketone bodies, they are still used to determine proteinuria. This very simple examination, which can be read immediately, may in theory facilitate evaluation of renal and vascular effects, especially for patients with type 2 diabetes, 90% of whom are followed by general practitioners. The aim of this study was to evaluate the reliability of urine dipsticks by comparing their results with those of standard proteinuria and microalbuminuria assays.

Methods

Urine samples of patients consecutively hospitalized for diabetes work-ups were collected from November 2004 to February 2005 in the endocrinology department of Bégin Hospital (Army teaching hospital). For each patient, a urine dipstick (leukocytes, nitrites, proteins) and proteinuria and microalbuminuria assays were performed on the same day. The protocol excluded all patients with a positive dipstick for leukocytes or nitrites because of possible confusion with a urinary tract infection. These dipsticks (Multistix® 10 SG, Bayer), which tested for glucose, bilirubin, ketone bodies, specific gravity, hematuria, pH, proteins, urobilinogen, nitrites, and leukocytes, were read on a Clinitek® 200 Bayer reflectometer, because reflectometry is more accurate than the naked eye. Proteinuria results could either be negative (below the detection limit) or positive (traces, +, ++). The price of each strip was estimated at €0.28 and coded for payment to the hospital at €0.54. The manufacturer set the threshold for a positive reading at 150 mg/L for proteinuria. Leukocyte and nitrite results were checked only to eliminate the risk of false positives. The dipsticks were classified as “positive” or “negative” for proteinuria according to the Clinitek® results. Proteinuria was also determined by a colorimetric reaction with pyrogallol red molybdate on an Olympus AU 600® autoanalyzer with Biogene® reagents (Ref. 840 1501). The detection limit was close to 0.02 g/L and the reaction was linear up to 4 g/L (data from suppliers). The standard values ranged from 0.028 to 0.15 g/L. The cost of each assay was estimated at €0.20 for reagents and coded for payment at €2.16. Proteinuria was considered abnormal at levels > 150 mg/L. All values below that were considered negative and all values above as positive.

Microalbuminuria was assayed by immunoturbidimetric methods by the Olympus AU 600® autoanalyzer with Olympus® reagents (ID: 0.67, ref. : OSR 6167). Albumin forms a complex with a specific antibody and the light transmitted by this antigen-antibody complex was measured at 340 nm. The cost of reagent for each analysis was estimated at €0.40 and coded for payment at €10.80. The threshold for positive microalbuminuria results was set at 20 mg/L (all values below were negative and all higher value positive).

Results

This study included urine samples from 300 patients. Fifty patients had type 1 diabetes and 250 had type 2 diabetes (table I). Because 70 patients had positive dipsticks for nitrites or leukocytes, the analyses only took 230 patients into account: 52 were being treated by angiotensin-converting enzyme (ACE) inhibitors, and 48 by angiotensin receptor II blockers (ARB). The urine dipstick produced positive results (+, ++) for 47 patients, only two of whom had proteinuria < 0.15 g/L; the test thus had a positive predictive value of 95.7% (table II). When the dipstick was negative (164 patients), significant proteinuria was nonetheless observed in 10 patients. The negative predictive value was 93.9%.

The detection limit for proteinuria was near 150 mg/L. The dipsticks performed well in detecting proteinuria > 200 mg/L where sensitivity was 94% and specificity 91%. The false-positive rate was 9%.

Results from comparing the urine dipstick with the microalbuminuria assay appeared less reliable (table III). The dipstick’s negative predictive value was only 73.7%: Accordingly, 43 of 164 patients (26%) whose dipstick was negative did have microalbuminuria exceeding 20 mg/L (up to 88 mg/L).
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On the other hand, in 94% of cases where the dipstick was positive, significant microalbuminuria was observed, and the positive predictive value of the dipstick was thus better, at 95.7%. The dipstick’s detection limit for albumin was around 90 mg/L. As expected, microalbuminuria levels and proteinuria levels were highly correlated for the overall population (r=0.961, p < 0.0001). This correlation was not as clear when considering subjects with proteinuria less than 0.15 g/L. This observation is evidence of the dispersion of microalbuminuria values at low levels of proteinuria (figures 1 and 2).

**Discussion**

Early detection of the renal and cardiovascular complications of diabetes is an important priority because effective early treatment can reduce their consequences [2, 3]. Testing for microalbuminuria is an essential part of this monitoring and should be performed annually in all persons with diabetes.

In type 1 diabetes, microalbuminuria is the first easily discernible marker of the onset of nephropathy and its observation requires initiation of ACE inhibitor treatment even in the absence of hypertension [4]. By helping to prevent progression toward high-flow proteinuria, which contributes to alterations in kidney function, this approach limits the risks of developing end-stage renal failure [5, 6].

In type II diabetes, the observation of microalbuminuria has a more complex meaning. It is not only predictive of diabetic nephropathy but is also a powerful marker of cardiovascular risk and premature mortality [7-9]. Studies show that in this situation, blockage of the renin-angiotensin system, especially with ARBs, can be helpful [10, 11].

Accordingly, patients with diabetes should be tested annually for microalbuminuria [1, 12]. This recommendation is often ignored: only 17.7% of diabetes patients receiving reimbursement from the national health insurance fund in 2003 had a microalbuminuria assay that year [12].

Microalbuminuria is defined as the abnormal presence of low levels of albumin in the urine, ranging from 20 to 200 mg/L or 30 to 300 mg/24 h. False positive results have been reported with urinary infections, fever, physical exercise, and hematuria. Determinations can be made by various techniques, ranging from radioimmunology (reference method) to high performance liquid chromatography. The most common method is liquid phase immunoprecipitation assay [13-15], the technique we used here. Some methods use immunochromatography (Servibio®) that can distinguish samples containing less than 10 mg/L of albumin from those with a greater concentration, which require quantification. These allow rapid bedside evaluation of microalbuminuria [16]. Their unit cost is €3.70.

Evidence of microalbuminuria should be followed up by checking a second sample, because albumin excretion can fluctuate from day to day [17]. The development of urine dipsticks that can identify glycosuria, evidence of urinary infection, hematuria and, naturally, proteinuria should theoretically compensate for the lack of a microalbuminuria assay because of the simplicity and reliability of the method. We correlated the results of the urine dipstick with the weighted proteinuria and microalbuminuria assays. These results showed good positive and negative

<table>
<thead>
<tr>
<th>Table I</th>
<th>Principal clinical and laboratory characteristics of 230 patients</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>Sex ratio (men/women)</td>
<td>154/76</td>
</tr>
<tr>
<td>Type diabetes (n)</td>
<td></td>
</tr>
<tr>
<td>Diabetes type I</td>
<td>38</td>
</tr>
<tr>
<td>Diabetes type II</td>
<td>192</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td></td>
</tr>
<tr>
<td>Diabetes type I</td>
<td>14 ± 12</td>
</tr>
<tr>
<td>Diabetes type II</td>
<td>12 ± 9</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 ± 1.7</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min) (Cockroft’s formula)</td>
<td>80 ± 29</td>
</tr>
<tr>
<td>Creatinine clearance &lt; 60 ml/min (n) (Cockroft’s formula)</td>
<td>56</td>
</tr>
</tbody>
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<tr>
<th>Table II</th>
<th>Comparison of urine dipsticks results and proteinuria</th>
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<tbody>
<tr>
<td>Urine dipsticks (n=230)</td>
<td>Proteinuria &lt; 0.15 g/L</td>
</tr>
<tr>
<td>Negative (n=164)</td>
<td>154</td>
</tr>
<tr>
<td>Traces (n=19)</td>
<td>4</td>
</tr>
<tr>
<td>Positive (+) (n=26)</td>
<td>1</td>
</tr>
<tr>
<td>Positive (++) (n=21)</td>
<td>1</td>
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</table>

The dipstick’s negative predictive value was 93.9% and the positive predictive value of a dipstick positive at + or ++ was 95.7%.

<table>
<thead>
<tr>
<th>Table III</th>
<th>Comparison of urine dipstick results and microalbuminuria</th>
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</thead>
<tbody>
<tr>
<td>Urines dipsticks (n=230)</td>
<td>Microalbuminuria (mg/L)</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>20-200</td>
</tr>
<tr>
<td>Negative (n=164)</td>
<td>121</td>
</tr>
<tr>
<td>Traces (n=19)</td>
<td>2</td>
</tr>
<tr>
<td>Positive (+) (n=26)</td>
<td>1</td>
</tr>
<tr>
<td>Positive (++) (n=21)</td>
<td>1</td>
</tr>
</tbody>
</table>

The dipstick’s negative predictive value was 73.7% and the positive predictive value of a dipstick positive at + or ++ was 93.9%.
predictive values for determination of proteinuria by dipstick. This method works well for proteinuria > 200 mg/L with a sensitivity of 94% and a specificity of 91%. Even though the false-positive rate of 9% appears low, it would lead to overestimating the frequency of renal complications in the overall population of diabetes patients. Determination of proteinuria by laboratory assay thus remains useful.

Dipstick sensitivity and specificity were also insufficient for threshold values of microalbuminuria set at 20 mg/L [18]. Accordingly, given the 26% false negative rate using dipsticks, uncertainty regarding low levels remains too high, and patients with these levels are the ones who need early modification of treatment. The use of more selective dipsticks for albumin might be recommended [19]. In 6% of cases, dipsticks produce false positive readings and lead to unnecessary treatment [5].

Quantitative determination of microalbuminuria levels also make it possible to assess the efficacy of prescribed treatment, by comparing new values to baseline values. Accordingly, urine dipsticks, while highly convenient, cannot substitute for an annual microalbuminuria assay in all diabetes patients. The use of urine dipsticks to determine microalbuminuria also clashes with the technique’s cost and requires dilution of urine in cases of strong flow. Urine dipsticks are therefore useful only in screening for urinary infections. A microalbuminuria assay is essential once traces of proteinuria are detected.

**Conclusion**

Urine dipsticks cannot substitute for microalbuminuria and proteinuria assays. Their systematic use for monitoring diabetes would alert practitioners when it is already too late; they must be replaced by quantitative microalbuminuria assays. Measurement of microalbuminuria allows effective screening for onset of nephropathy by setting a reference for following up on treatment efficacy and in providing information about vascular risk, especially coronary vascular risk.

**References**

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