Brief Report

Rapid microalbuminuria screening in type 2 diabetes mellitus: simplified approach with Micral test strips and specific gravity

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Abstract

Background. Microalbuminuria is known to be a harbinger of serious complications in type 2 diabetes mellitus. Since medical intervention at the onset of microalbuminuria can be critical in reducing these adverse outcomes, it is widely agreed that type 2 diabetic patients should be screened for microalbuminuria. The purpose of the present study is to evaluate Micral test strips in conjunction with a urine specific gravity determination as a rapid and accurate method for detecting microalbuminuria in type 2 diabetic patients.

Methods. In this prospective study, a total of 444 urine samples of type 2 diabetic patients were obtained from the ABCD study cohort for analysis. Urinary albumin concentrations were determined using Micral test strips and compared to results measuring albumin by the immunoturbidimetry method of timed collections. Urine specific gravity was measured by a standard urine dipstick.

Results. The performance characteristics of the Micral test strips for detecting microalbuminuria (30–300 mg albumin/24 h) were adequate but not optimal: sensitivity 88%, specificity 80%, positive predictive value 69%, negative predictive value 92%. A concomitant specific gravity determination was useful in indexing the magnitude of false negative and false positive readings by the Micral test strips.

Conclusions. While the use of Micral test strips provides a rapid approach to detecting microalbuminuria in type 2 diabetic patients, this method has limitations. The simultaneous measurement of specific gravity is helpful in addressing some of the shortcomings of this screening test.

Keywords: micral test; screening test; sensitivity; cost effectiveness; specific gravity

Introduction

The prevalence of type 2 diabetes mellitus has been increasing significantly in all countries during the last century. By 2010, 220 million people in the world are projected to be afflicted by this disease [1]. Microalbuminuria, defined as urinary albumin excretion from 30 to 300 mg per 24 h, is a frequent finding in the diabetic population with an overall prevalence near 30% [2]. Microalbuminuria has been shown to be a harbinger of many of the serious complications in type 2 diabetes mellitus, particularly retinopathy, neuropathy, renal impairment and cardiovascular disease [3,4].

Since medical intervention, including control of blood glucose, cholesterol and hypertension, at the onset of microalbuminuria has been shown to be critical in reducing adverse outcomes, current consensus guidelines recommend that type 2 diabetic patients should be screened annually for microalbuminuria [5]. However, recent studies of primary care physicians in the United States reveal astonishingly low levels of microalbuminuria screening in this patient population [6,7]. Explanations for this poor performance have focused on the lack of immediate results to the physician for medical decision making with both spot urine samples for albumin–creatinine ratios and 24 h urine collections for albumin and creatinine in addition to the associated cost, patient inconvenience, and patient noncompliance with the latter method [6,7].

Consequently, modified urine dipsticks, such as Micral test strips, have been designed to allow a physician to quickly and accurately detect microalbuminuria on a small urine sample from a patient at the time of the visit. Unfortunately, the reported range of sensitivity, specificity and predictive values for this dipstick has raised some concerns of reliability [8,9].
It has been suggested that this suboptimal performance may be due to differences in urine volume that confound dipstick readings [10]. We therefore evaluated whether the performance of the Chemstrip Micral test strip (Micral test strip) to appropriately detect microalbuminuria could be improved by incorporating a urine-specific gravity determination.

Subjects and methods

Patients

In this prospective study, urine samples were collected over a 1-year period from patients with type 2 diabetes mellitus who were participants in the Appropriate Blood Pressure Control in Diabetes (ABCD) study. The complete details of the enrolment criteria, patient follow-up and treatment plan of the ABCD study have been described elsewhere [11].

Sample material and collection

A total of 494 urine samples were collected for albumin analysis, including 277 24-h urine collections, 99 12-h overnight urine collections and 118 mid-stream random spot urine specimens coincident with its timed collection. Importantly, 50 urine samples were excluded from the final analysis for the following reasons: duplicate timed urine collections from the same patients, inadequately collected timed urine samples (urine volume <500 ml, urine creatinine <500 mg/day or >2000 mg/day), and urine samples from patients who were likely to have significant proteinuria due to advanced diabetic nephropathy (serum creatinine >2 mg/dl). Consequently, a total of 444 urine samples from 326 patients were available for final analysis in this study. It is significant to note that subjects in the ABCD study were instructed to avoid strenuous exercise on the day of the urine collection. Moreover, the presence of a urinary tract infection was screened by regular urine dipstick (Multistix 8 SG; Bayer, Elkhart, IN) in all obtained specimens and was not found in any urine samples incorporated into this study.

Sample analysis

The random spot urine samples and aliquots from the 24- and 12-h timed urine collections were evaluated in the following manner: (i) routine urinalysis including specific gravity by regular urine dipstick (Multistix 8 SG); (ii) urinary albumin concentration by Chemstrip Micral test strips (Roche Diagnostics, Indianapolis, IN); (iii) urinary albumin concentration by immunoturbidimetry (Turbidimiter, Dade Behring, Germany); (iv) urinary creatinine concentration by modified Jaffe’s method (colorimetric assay) (Hitachi 911 automatic analyzer, Boehringer Mannheim, Germany). Routine urinalysis was performed using the Multistix 8 SG reagent strip according to the manufacturer’s protocol by a clinically experienced registered nurse. Results were recorded regarding its eight separate reagent areas: glucose, ketones, blood, protein, specific gravity, pH, nitrites and leukocytes. Assessing specific gravity by this instrument has been validated elsewhere [12].

Urinary albumin concentration was performed using Chemstrip Micral test strips (Micral test strips) by a clinically experienced registered nurse. These immunoassay reagents strips function in a similar manner to the previously described Micral-Test II test strips [8]. The distinctive colour scale blocks on the vial label correspond to a range of albumin concentrations as follows: 0, 20, 50 and 100 mg/l. According to the manufacturer’s instructions, albumin concentrations detected ≥20 mg/l are consistent with microalbuminuria (albumin >30 mg/day). It has been shown that the results of the Micral test strips are reproducible between different observers with great accuracy [13].

In order to achieve a comparison analysis, urinary albumin concentrations of all urine specimens were also determined by immunoturbidimetry, a widely regarded quantitative method (gold standard). The interassay coefficient of variation for this assay is 5%. Furthermore, urinary creatinine concentration was measured by the modified Jaffe’s method (colorimetric assay). The interassay coefficient of variation for this assay is 2.3%.

Statistical analysis

Most of the analyses were done using SAS version 8.2 (SAS Institute, Cary, NC). The graphs were prepared using program Prism 3.0 (Graphpad Software, Inc).

Results

The characteristics of the patients in this study included a predominance of men (70%) and elderly subjects (mean age 60) from a variety of ethnic and racial backgrounds (35% non-caucasian). While a significant percentage of study subjects had hypertension (50%), most patients had fairly well preserved renal function (creatinine ≥1.3 mg/dl). As determined by timed urine collections, the majority of subjects had normoalbuminuria (<30 mg/day: 65.6%), while smaller proportions had microalbuminuria (23%) and macroalbuminuria (>300 mg/day: 11.3%). The mean duration of diabetes mellitus in this population was 10 years. Further detailed characteristics of this ABCD population have been described elsewhere [11].

The results of microalbuminuria detection were compared between Micral test strips and timed collections in the following manner. Based on the total amount of urinary albumin found by the timed urine collections using immunoturbidimetry (gold standard), a patient’s albumin excretion was classified with regard to microalbuminuria as follows: positive- meeting/exceeding criteria for microalbuminuria (albumin >30 mg/day); negative- failing to meet criteria for microalbuminuria (albumin <30 mg/day). The results of the amount of urinary albumin of the all the 444 urine specimens (timed collections and random samples) determined by Micral test strips were also classified into the aforementioned categories based
Microalbuminuria screening in type 2 diabetics

Table 1. Performance of Micral test strips by the four different possible concentration readings

<table>
<thead>
<tr>
<th>Micral Test Microalbuminuria</th>
<th>No. of samples</th>
<th>Concordance with immunoturbidimetry on timed collections</th>
<th>Discordance with immunoturbidimetry on timed collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Reading: 0 mg/l 253</td>
<td>True negatives (%) 93</td>
<td>False negatives (%) 7</td>
</tr>
<tr>
<td>Positive</td>
<td>Reading: 20 mg/l 83</td>
<td>True positives (%) 49</td>
<td>False positives (%) 51</td>
</tr>
<tr>
<td></td>
<td>Reading: 50 mg/l 64</td>
<td>True positives (%) 81</td>
<td>False positives (%) 19</td>
</tr>
<tr>
<td></td>
<td>Reading: 100 mg/l 44</td>
<td>True positives (%) 44</td>
<td>False positives (%) 91</td>
</tr>
</tbody>
</table>

on the manufacturer’s instructions, which state that albumin concentrations detected ≥20 mg/l are consistent with microalbuminuria. Assessing the performance of Micral test strips in this manner, we found its test characteristics to be as follows: sensitivity 88%, specificity 80%, positive predictive value 69% and negative predictive value 92%. The test results were unchanged when only the timed urine samples (excluding the spot urine samples) were compared with the gold standard.

A more detailed analysis of the Micral test strips performance in detecting albumin in the urine samples of these study subjects is seen in Table 1, where positive indicates presence of microalbuminuria and negative is absence of microalbuminuria according to Micral test strips. It is apparent from Table 1 that the Micral test strips perform reasonably well at the following concentration readings: 0, 50 and 100 mg/l. This interpretation is evidenced by the relatively high percentage of true negatives and true positives and the low percentage of false negatives and false positive at these levels. Importantly, the Micral test strips do not perform well at a reading of 20 mg/l because of its high false positive rate (51%), whereas for a reading of 50 mg/l the false positives decreased to 19%. For a Micral reading of 100 mg/l the false negative were only 9%. With a reading of 0 mg/l the false positives were only 7%. Thus, microalbuminuria can be excluded with reasonable certainty at a reading of 0 mg/l, and can be reasonably established at a reading of 100 mg/l.

Because the majority (76%) of Micral test strips readings were at 0 mg/l and 20 mg/l, further analysis incorporating specific gravity was performed to evaluate any subsequent improvement in this test at these readings. It appears that the imperfect performance of the Micral test strips at these levels is related, at least in part, to the specific gravity of the urine sample. This relationship between urine-specific gravity and the false negative and false positive rates of this diagnostic test are shown in Table 2. The rate of false negative results for the Micral test strips at a level of 0 mg/l decreases as the specific gravity of the urine sample increases. If the concomitant urine-specific gravity is ≥1.025 at this Micral test strip reading, 98% accuracy for the absence of microalbuminuria exists. At a Micral test strip reading of 20 mg/l, specific gravity is also important. Specifically, the false positive rate increases to 83% when the urine-specific gravity is ≥1.025. However, the false positive rate is reduced to 42% at a Micral test strip reading of 20 mg/l in a dilute urine, a specific gravity ≤1.010. Similarly at a reading of 50 mg/l, the false positives gradually increase as the urine gets concentrated and the false positives readings are 65% at a specific gravity of ≥1.025.

Table 2. Performance of Micral Test at various levels of urine-specific gravity

<table>
<thead>
<tr>
<th>Micral reading of 0 mg/l</th>
<th>True negatives (%)</th>
<th>False negatives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1.010</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>1.015</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>1.020</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>≤1.025</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Micral reading of 20 mg/l</th>
<th>True positives (%)</th>
<th>False positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1.025</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>1.020</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>1.015</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>≤1.010</td>
<td>58</td>
<td>42</td>
</tr>
</tbody>
</table>

At the reading of 0 mg/l (upper panel), as the urine-specific gravity increases (urine becomes more concentrated), the number of false negative results for microalbuminuria decreases. At the reading of 20 mg/l (lower panel), as the urine-specific gravity decreases (urine becomes more dilute), the number of false positive value results for microalbuminuria decreases.

**Discussion**

Microalbuminuria in patients with type 2 diabetes mellitus is a risk factor for cardiovascular complications, progression of renal disease and retinopathy [4]. Macrovascular complications including myocardial infarctions, strokes, and peripheral vascular disease account for roughly 80% of deaths in type 2 diabetes mellitus patients [14]. Moreover, diabetes mellitus, of which 90–95% is type 2, is the most common cause of blindness and end stage renal disease in developed countries. The randomized micro-HOPE trial has recently demonstrated that the angiotensin-converting enzyme (ACE) inhibitor, ramipril, can significantly decrease cardiovascular complications and mortality in type 2 diabetes patients [15]. The randomized hypertensive ABCD study demonstrated a decrease in myocardial infarction with ACE inhibitors and a
decrease in overall mortality with aggressive blood pressure control [16]. Aggressive control of blood pressure (mean 125/75 mmHg) was shown in the normotensive ABCD study to decrease strokes, progression of retinopathy and progression of microalbuminuria to overt albuminuria in diabetic nephropathy [17]. The UKPDS also demonstrated that tight control of blood pressure decreased strokes and diabetes related deaths [18]. The IRMA study recently demonstrated that progression from microalbuminuria to diabetic nephropathy could be decreased with the angiotensin receptor blocker, irbesartan [19]. While intervention with angiotensin receptor blockade in patients with established diabetic nephropathy can slow the progression to end stage renal disease [20], prevention of progressive renal disease and cardiovascular complications no doubt necessitates early detection.

The early detection of microalbuminuria in diabetic patients and non-diabetic hypertensive patients, therefore, has been recommended for allowing for early intervention [4]. Because of the difficulty with accurate timed urine collections, the spot urine albumin to creatinine ratio is now frequently used to detect microalbuminuria (30–300 mg albumin/day). Both approaches, however, fail to provide immediate information at the time of the patient visit and the approximate cost of these tests is US$100.00. Thus, a sensitive dipstick to detect microalbuminuria at the 0 mg/l reading is very attractive. In the present study, a commonly used method known as Micral test strips was analysed. The sensitivity was 88%; specificity 80%, positive predictive value 69% and negative predictive value 92%. At a Micral reading of 0 mg/l, microalbuminuria could be excluded in 93–98% of cases, depending on the urine-specific gravity. The 20 mg/l reading, which is the most likely to detect early microalbuminuria, was also affected by the urine-specific gravity. In fact, the false positives for microalbuminuria increase from 42% in dilute urine to 83% in a concentrated urine. At the Micral reading of 50 mg/l, there were also a considerable percentage (19%) of false positives, which were remedied only partially by specific gravity correction. The 100 mg/l reading had very few false positives thus; the performance of the Micral test strip at this reading is acceptable. Although at 100 mg/l a high level of microalbuminuria is present, the standard urine analysis dipstick would fail to detect this level of albuminuria in a substantial percentage of cases.

In Figure 1 is illustrated a recommended clinical approach for detection of microalbuminuria in the office setting with currently available methods. The Micral readings are significantly affected by urine-specific gravity and are quite sensitive to exclude microalbuminuria at the 0 mg/l reading. The Micral stick also performs adequately at 100 mg/l irrespective of specific gravity correction. Readings of 20 and 50 mg/l need confirmation with a timed urine excretion. More sensitive, rapidly available, cost effective tests, which include correction for specific gravity, are therefore clinically needed to detect microalbuminuria and initiate early prevention strategies.

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Conflict of interest statement. None declared.

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