Does bacteriuria interfere with albuminuria measurements of patients with diabetes?

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Abstract

Background. Urinary albumin is the main parameter employed to diagnose diabetic nephropathy (DN). The exclusion of bacteriuria has been recommended at the time of DN diagnosis. This approach has been debated and information on this suggestion in patients with diabetes is scarce. The present case-control study was conducted to investigate the interference of bacteriuria in the interpretation of urinary albumin measurements in diabetic patients.

Methods. Urinary albumin concentration (UAC) was measured in random urine samples twice in diabetic patients with and without bacteriuria ($\geq 10^5$ colony-forming units/mL). Cases (n = 81) were defined as patients who had baseline UAC measurement in the presence of bacteriuria and had the second UAC measured in a sterile urine sample. Controls (n = 80) had the two UAC measured in sterile urine specimens.

Results. Baseline UAC was not different between case [15.4 (1.5–2148) mg/L; $P = 0.24$], nor was the proportion of patients with normo-, micro- and macroalbuminuria. In cases, UAC measurements in the presence of bacteriuria and in sterile urine specimens were not different [15.4 (1.5–2148) versus 13.7 (1.5–2968) mg/L; $P = 0.14$], nor was the proportion of normo- (51.9% versus 61.5%), micro- (40.7% versus 32.1%) and macroalbuminuria (7.4% versus 6.4%; $P = 0.46$). In the control group, UAC values were also not different in the two urine samples: [14.2 (1.5–1292) versus 9.7 (1.5–1049) mg/L; $P = 0.22$].

Conclusions. The presence of bacteriuria does not interfere significantly with urinary albumin measurements and its exclusion is not necessary to diagnose DN.

Keywords: albuminuria; bacteriuria; diabetic nephropathy

Introduction

Urineary albumin measurement is the main marker employed to diagnose diabetic nephropathy (DN) [1], and increased values are considered a risk factor for DN progression and cardiovascular events [2–4]. The presence of bacteriuria has...
been suggested as a factor that might interfere in urinary albumin measurements [5], and therefore, it has been recommended that urinary albumin should be measured only in sterile urine for the proper diagnosis of DN [6–8]. However, data to support this recommendation, especially in patients with diabetes (DM), are scarce.

The preferred urine sample for DN diagnosis is a random urine sample taken according to the American Diabetes Association [9] and the National Kidney Foundation [10]. The measurement of urinary albumin concentration (UAC) in a random urine specimen seems to be the best choice for the diagnosis of microalbuminuria in diabetic patients, considering its cost and accuracy [11]. In this context, there is no information regarding the role of bacteriuria influence in UAC measurements in patients with diabetes. Therefore, this study was performed to investigate the possible interference of bacteriuria in the interpretation of urinary albumin measurements in random urine samples in diabetic patients.

Subjects and methods

Subjects

Type 2 DM patients [12] who had UAC measurements performed twice in random urine specimens concurrently with urine cultures were identified from the Clinical Pathology Laboratory database (December 2006 to December 2007). From the initial 1814 consecutive recorded samples in the laboratory, 703 had concurrent urine cultures. Of these, only samples from patients who had two UAC measurements, 6 months apart, were included. Exclusion criteria were the presence of persistent bacteriuria or urine samples from patients who had started on ACE inhibitors or new anti-hypertensive medications between the two urine collections. Consecutive diabetic patients who had the first UAC measured in a urine sample with bacteriuria and the second UAC measurement in a sample without bacteriuria were considered cases. The patients whose UAC was measured in the two consecutive urine samples in the absence of bacteriuria were defined as controls. This protocol was approved by Hospital Ethical Committee.

Methods

All urine samples were collected as a midstream specimen in a sterile container and cultured for bacterial growth, quantification and antimicrobial sensitivity. Bacteriuria was defined as pathogen growth of ≥10^5 colony-forming units/mL. Antimicrobial treatment was performed based on antimicrobial sensitivity information. In the cases, eradication of bacteria was documented in the second urine sample concurrently with UAC measurement. The urine specific gravity was measured by a test strip (Combur-Test; Boehringer Mannheim, UK).

Urinary albumin was measured by the immunoturbidimetric method (Microalb; Ames-Bayer, Tarrytown, NY, USA). In our laboratory, using urine samples with the albumin concentration of 30 and 100 mg/L, the intra- and inter-assay CVs were both <6% [13]. Normoalbuminuria was defined as UAC <17 mg/L, microalbuminuria UAC from 17 to 174 mg/L and macroalbuminuria UAC >174 mg/L [14]. Glycated haemoglobin (A1C test) was measured by HPLC (Merck Hitachi 9100, Germany). Fasting plasma glucose was measured by the glucose-peroxidase colorimetric enzymatic method (Biodiagnostica, Germany). Serum creatinine was measured by the Jaffe method, serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boehringer Mannheim, Buenos Aires, Argentina) and HDL cholesterol by the homogeneous direct method (autoanalyzer, ADVIA 1650, Germany). LDL cholesterol was calculated using the Friedewald formula.

Statistical analyses

A sample of 80 patients in each group was estimated to detect a difference of 10 mg/L in urinary albumin between cases and controls, with a power of 80%. Student’s t-test and chi-square tests were used as appropriate. Wilcoxon’s test or Mann–Whitney U tests were used to compare UAC values.

Results

One hundred sixty-one diabetic patients were studied. The case group was formed by 81 patients (18.5% males; aged 58.6 ± 17.1 years) and the control group by 80 patients (31.5% males; aged 56.8 ± 16.1 years).

Table 1 shows baseline clinical and laboratory characteristics of cases and controls. Serum creatinine was the only laboratory data different between cases (83.9 ± 27.4 µmol/L) and controls (76.0 ± 15.9 µmol/L, P = 0.02). The proportion of male sex, age and weight did not differ between patients with and without bacteriuria. Also, blood

<table>
<thead>
<tr>
<th>Bacteriuria</th>
<th>Present</th>
<th>Absent</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>81</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Male subjects, n (%)</td>
<td>15 (18.5)</td>
<td>25 (31.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.6 ± 17.1</td>
<td>56.8 ± 16.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.6 ± 14.8</td>
<td>75.2 ± 13.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129.5 ± 21</td>
<td>129.5 ± 19.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76.4 ± 10.6</td>
<td>76.2 ± 13.2</td>
<td>0.92</td>
</tr>
<tr>
<td>A1C test (%)</td>
<td>8.08 ± 2.25</td>
<td>7.54 ± 1.62</td>
<td>0.08</td>
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<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>7.8 ± 2.7</td>
<td>7.7 ± 3.6</td>
<td>0.91</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8 ± 1.1</td>
<td>4.5 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>High-density cholesterol (mmol/L)</td>
<td>1.45 ± 0.47</td>
<td>1.52 ± 0.45</td>
<td>0.39</td>
</tr>
<tr>
<td>Low-density cholesterol (mmol/L)</td>
<td>2.3 ± 0.42</td>
<td>2.47 ± 1.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.97 (0.9–20)</td>
<td>3.0 (1–38)</td>
<td>0.30</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>83.9 ± 27.4</td>
<td>76.0 ± 15.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Urine specific gravity</td>
<td>1016 ± 6.3</td>
<td>1015 ± 6.5</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, number of subjects with studied characteristic (%) or median (range).
pressure levels, glucose control and lipid profile were not different between the two groups.

UAC [median (range)] was not different between cases [15.4 (1.5–2148) mg/L] and controls [14.2 (1.5–1292) mg/L, \( P = 0.24 \)] as well as the proportion of DN stages (cases: normo- 51.9%, micro- 40.7% and macroalbuminuria 7.4%; controls: normo- 55%, micro- 41.3% and macroalbuminuria 3.8%; \( P = 0.58 \)).

Results from UAC measurements in the first and second urine samples are shown in Figure 1. In cases, baseline UAC values in the presence of bacteriuria [15.4 (1.5–2148) mg/L] were not different from values in sterile urine samples [13.7 (1.5–2968) mg/L, \( P = 0.14 \)], nor was the proportion of normo- (51.9 versus 61.5%), micro- (40.7 versus 32.1%) and macroalbuminuric samples (7.4 versus 6.4%; \( P = 0.46 \)). In the control group, the UAC measurements were also not different between the first and second urine specimens: [14.2 (1.5–1292) mg/L versus 9.7 (1.5–1049) mg/L, \( P = 0.22 \)]. The time elapsed between the two collected urine samples was not different between cases and control groups (data not shown). In addition, no difference was observed between UAC changes from the first to the second measurements both in cases \([-4 (-607–820)] \) and controls \([-1 (-571–223) \text{ mg/L}, P = 0.67]\).

Discussion

This study indicated that the presence of bacteriuria did not significantly interfere with interpretation of UAC measurements in random urine samples in diabetic patients, since UAC values were not modified by the eradication of bacteriuria.

Although evaluation of bacteriuria simultaneously with urinary albumin measurement has been widely used, this practice is still questionable [15]. The absence of interference of bacteriuria in 24-h urinary albumin excretion has already been described in patients with diabetes [16,17]. In a prospective study, Hernandez et al. studied 46 diabetic patients with bacteriuria and found no differences in 24-h urinary albumin excretion from urine samples before and after eradication (11.7 versus 7.1 µg/min, \( P = 0.14 \)) [16]. However, the estimated power to find a difference of 10 µg/min in albuminuria measurements was ~68%. On the other hand, in the present study the power of the observed non-significant difference between UAC measurements in samples with and without bacteriuria was 80%. In a prospective study, 172 type 1 DM patients had 24-h urinary albumin excretion measured six times during 18 months. During the follow-up in the 20 patients who had
bacteriuria or urinary tract infection, the urinary albumin measurements did not change [18]. However, as in the study of Hernandez et al. [16], the small sample of patients with bacteriuria does not allow an accurate conclusion.

As far as we know, the evaluation of bacteriuria interference in albuminuria measurements in spot urine samples has not been studied in diabetic patients. This information is critical for routine practice since the use of spot urine, due to its easy collection, has been recommended as the screening method for the diagnosis of DN [1,7,10]. It was demonstrated that in a random urine spot sample using UAC for DN screening is as accurate as and cheaper than the albumin-to-creatinine ratio [11]. According to the data from the present study, the costs for diagnosis of DN can be further reduced without performing a concomitant urine culture. Other reasons for not routinely performing urine cultures in diabetic patients is that bacteriuria is often found and asymptomatic bacteriuria occurs in up to 17.5% of diabetic women [19]. Furthermore, treatment of patients with asymptomatic bacteriuria is not recommended [20]. Even in patients with increased serum creatinine, the presence of bacteriuria was not associated with the decline of renal function reinforcing the benign role of asymptomatic bacteriuria [21]. Interestingly, in the present study, patients with bacteriuria had higher serum creatinine values than patients without bacteriuria.

A possible limitation of the present study was the measurement of urine albumin concentration instead of the albumin-to-creatinine ratio. Theoretically, isolated albumin concentration measurements could be influenced by urine dilution. Other authors observed that the measurement of the albumin-to-creatinine ratio was more accurate than the albumin concentration for the diagnosis of microalbuminuria when using sex- and age-specific discriminator values [22]. However, there is a strong correlation between the urinary albumin concentration and the urinary albumin-to-creatinine ratio as we [11] and others [22] had already demonstrated, but these data were obtained in the absence of bacteriuria [11]. In the present study, the urine specific gravity was not different between cases and controls (Table 1), and therefore, the absence of creatinine measurements in urine samples probably did not influence our results. Another aspect is the preponderance of the female sex, both in cases and controls. This finding might be explained by increased urine culture requests for women due to a known increased prevalence of urinary infection in diabetic women [18]. However, this feature did not influence the analytical evaluation of urine samples.

In conclusion, the presence of bacteriuria does not interfere significantly in the interpretation of urinary albumin measurements, and a urine culture might not be necessary at the time of DN diagnosis.

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Conflict of interest statement. None declared.

References


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