Comparison between different methods of urine collection for estimation of albumin-creatinine ratio in patients with type-2 diabetes mellitus

Subinay Datta, Mrinal Pal

Department of Biochemistry, Burdwan Medical College, Burdwan, India
Email: *mrinalpal77@rediffmail.com

Received 8 May 2013; revised 7 June 2013; accepted 22 June 2013

Copyright © 2013 Subinay Datta, Mrinal Pal. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT
In patients with diabetes mellitus, urinary albumin-creatinine-ratio (ACR) predicts progressive kidney disease. In order to determine the better urine sample for detecting ACR, we estimated ACR in three modes of urine sample. Two hundred patients of uncontrolled diabetes mellitus with proteinuria irrespective of age and sex were studied for urinary ACR in all the three types of samples over a period of 2 years. The statistical analysis showed that first morning ACR (r = 0.999, p > 0.001) of the subjects was more significantly correlated with their 24 hour urine sample ACR than spot urine (r = 0.995, p < 0.001). We conclude that early morning specimens should be used instead of spot and 24 hour sample.

Keywords: Microalbuminuria; Albumin-Creatinine Ratio; Diabetic Nephropathy

1. INTRODUCTION
Diabetic nephropathy (DN) is the most frequent single cause of end-stage renal disease in many countries [1]. Hyperfiltration and microalbuminuria characterize the clinical stages of DN [2]. Increased levels of albumin in the urine have been clearly established as an important determinant for renal complication of diabetes [3-5]. Screening for increased albumin excretion has therefore been advocated to identify individual at risk for renal disease progression in a timely manner. However, there is still continuing uncertainty as to how urine should be collected and which urinary proteins should be specifically measured for prediction of renal events [6]. So, the present study therefore is to investigate which urine sample such as 24 hour, first morning void or spot urine sample is to detect actual value of microalbuminuria for DN. But measuring only urinary albumin excretion (UAE) is not sufficient as it is influenced by intra-individual variations in urinary volume. That can be overcome by using albumin-creatinine ratio (ACR) that is dividing UAE with urinary creatinine excretion as creatinine excretion in urine has relative constancy over time [7]. So, ACR is used as a parameter to detect albuminuria in the present study.

2. MATERIAL AND METHOD

2.1. Selection of Subjects
The present study was conducted in the department of Biochemistry of Burdwan Medical College, Burdwan, West Bengal. One thousand patients having age > 30 years and suffering from uncontrolled diabetes mellitus (plasma glucose > 110 mg/dl, HbA1C > 8% of total Hb) with proteinuria tested by Dipstick technique were selected. The selected patients were from Burdwan district and adjoining areas. Patients having any concomitant infection and recent history of taking drugs (e.g. NSAIDS, ACE inhibitors) were not included in the study. Then two hundred subjects were selected by a simple random method for this study.

2.2. Collection of Samples
After noting the age and sex, three types of urine samples (24 hour, first morning voided and random) were collected from all subjects. After collection all the tests were done immediately.

2.3. Parameters Assay
Albuminuria was measured by immunoturbidimetric method [8,9] using semiautoanalyser (Chem 5v2 plus). Intra assay CV% was 2.3 and intra assay CV was 3.1 for this method. Urinary creatinine concentration was assayed by
Jaffe’ method [10] using auto analyser (Transasia, XL-600). Intra-assay CV% was 1.8 and inter-assay CV% was 2.9. Then urinary ACR was calculated. Using commercially available Hemoglobin AIC kit supplied by Siemens Company did Hemoglobin AIC test. It implies the principle of turbidimetric inhibition immunoassay (TINIA). This company also supplied total Hb kit for estimation total Hb by alkaline hematin method. Concentration of plasma glucose of the subjects was done by Glucose-Oxidase and Peroxiase method.

2.4. Statistical Analysis

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows.

3. RESULT

The personal profiles and clinical parameters of all the subjects under study are shown in Table 1.

Mean value of 24 hour urine ACR (152.38 μg/mg) are more close to first void urine (151.11 μg/mg) than spot urine sample (154.85 μg/mg). Another observation is that spot urine ACR value has higher mean, SD and has wider confidence interval than other two samples as shown in Table 2.

Table 1. Personal profiles and clinical parameters of the subjects.

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51.54 ± 7.14</td>
</tr>
<tr>
<td>Number of cases</td>
<td>n = 200</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>n = 113</td>
</tr>
<tr>
<td>Female</td>
<td>n = 87</td>
</tr>
<tr>
<td>Demographic data:</td>
<td></td>
</tr>
<tr>
<td>Urban background</td>
<td>n = 129</td>
</tr>
<tr>
<td>Rural background</td>
<td>n = 71</td>
</tr>
<tr>
<td>Fasting plasma glucose level (mg/dl)</td>
<td>168.27 ± 24.40</td>
</tr>
<tr>
<td>HbA1C level (% of total Hb)</td>
<td>9.4965 ± 0.997201</td>
</tr>
</tbody>
</table>

Value are mean ± SD; n = number of cases; Reference range of fasting plasma glucose < 110 mg/dl; HbA1C > 8% of total Hb is considered for revaluation of treatment.

Table 2. Comparison of mean value of three modes of urine samples.

<table>
<thead>
<tr>
<th>Methods of urine collection</th>
<th>Urinary ACR (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours urine sample</td>
<td>152.38 ± 82.26</td>
</tr>
<tr>
<td>First morning voided urine sample</td>
<td>151.11 ± 81.58</td>
</tr>
<tr>
<td>Spot urine sample</td>
<td>154.85 ± 85.75</td>
</tr>
</tbody>
</table>

Regression analysis is performed to evaluate which method of urine collection and which sample shows best correlate with 24 hour sample. Over the whole range of 24 hour sample ACR, the first morning sample shows a higher r value (r = 0.999, p < 0.0001) and spot urine a lower r value (r = 0.995, p < 0.0001) of ACR. That signifies that first morning ACR of the subjects is more significantly correlated with their 24 hour urine sample than spot urine ACR as shown in Table 3 and Figure 1.

The area under the curve (AUC) of ROC curve of between 24 hour and early morning urine sample ACR is more than between 24 hour and spot urine ACR as shown in Table 4 and Figure 2.

Table 3. Pearson’s correlation of 24 hour urinary ACR with early morning and spot urine ACR.

<table>
<thead>
<tr>
<th>Category</th>
<th>r value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour urine vs first morning urine sample</td>
<td>0.999</td>
<td>0.000</td>
</tr>
<tr>
<td>24 hour urine vs spot urine sample</td>
<td>0.995</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are mean ± SD; CI = confidence interval.
4. DISCUSSION

ACR has been proposed as both a screening and diagnostic test for kidney disease [11]. Screening for microalbuminuria is essential as it allows interventions aimed at preventing diabetic nephropathy [12,13] and part of the everyday treatment of diabetic patients [14-16] for detecting kidney disease progression and also evaluation of treatment effect [17] therefore, samples providing immediate and reliable results are highly desirable. With the respect to urine collection procedure 24 hour collection were initially advocated gold standard [18-20] because of circadian rhythm of urinary protein excretion [21] and has found to be the least variable parameter for the measurement of microalbuminuria [22,23]. But 24 hour urine collection is a cumbersome procedure and subject to collection errors [24-27]. More practical and easier alternatives are collection of a first morning void or a spot (random) urine sample [28-32].

Numerous recent studies have been shown that early morning urinary ACR for screening purpose is also a predictor of overt DN and is useful to identify patient at risk [33] as it is less influenced by the factors such as hydration status, physical activity and concentration bias [34,27]. In present study, when cut-off values are used that are advocated in reagent manual, the value of ACR of first void sample are closed to the value 24 hours than spot urine. So, early morning sample can replace the 24 hour sample which is too much lengthy. Various previous studies also give support towards this factor due to easy collection, low cost and high sensitivity [2,35-37].

5. CONCLUSION

The study concluded that early morning sample not the spot urine sample could replace the 24 hour sample for estimation of urinary ACR.

6. ACKNOWLEDGEMENTS

Authors are thankful to Mr. Karthik Roy, Naba Kumar Das and Chandi Charan Das of Burdwan Medical College and Hospital for technical support.

REFERENCES


[31] Witte, E.C., Lambers Heerspink, H.J., de Zeeuw, D., Bak-
First morning voids are more reliable than spot urine samples to assess microalbuminuria. *Journal of the American Society of Nephrology*, 20, 436-443. doi:10.1681/ASN.2008030292


