Establishment of a Method to Detect Microalbuminuria by Measuring the Total Urinary Protein-to-Creatinine Ratio in Diabetic Patients

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Diabetes and chronic kidney disease (CKD) which are risk factors of cardiovascular disease, are increasing global public health problems. Microalbuminuria is an early sign of progressive cardiovascular and renal disease in individuals with or without diabetes. Screening for microalbuminuria and early treatment are recommended for patients with increased cardiovascular and renal risk factors. However, the procedure used to measure urinary albumin is expensive. Alternatively, the measurement of total urinary protein is simple and inexpensive. Thus, we aimed to establish a method that could predict the presence of microalbuminuria by measuring the total protein-to-creatinine ratio. Spot urine samples were obtained from 150 patients with diabetes mellitus, and the total protein-to-creatinine ratio and the albumin-to-creatinine ratio (ACR) were measured. There was a significant positive correlation between the protein-to-creatinine ratio and the ACR ($r = 0.95$). The presence of albuminuria (both micro- and macroalbuminuria) could be predicted from the value of the protein-to-creatinine ratio in more than 90% of patients. A receiver-operating characteristic curve analysis revealed that the protein-to-creatinine ratio had a sensitivity and a specificity of 90.8% and 91.9%, respectively, for the detection of albuminuria and a cutoff value of $0.091 \text{ g/g creatinine}$. These results suggest that screening for microalbuminuria can be replaced by the detection of the protein-to-creatinine ratio, which may be cost-effective for patients with cardiovascular risks as well as for the general population.

Keywords: albumin-to-creatinine ratio; cardiovascular disease; diabetes mellitus; microalbuminuria; protein-to-creatinine ratio


Diabetes and chronic kidney disease (CKD) are increasing global public health problems that affect patients’ physical well-being as well as the national economy (Schoolwerth et al. 2006; Senior 2008; Ohta et al. 2010). The major adverse outcomes for these conditions are an increased risk of cardiovascular disease and end-stage renal disease that requires renal replacement therapy. Microalbuminuria is an early sign of progressive renal and cardiovascular disease in patients with diabetes mellitus or hypertension (Damsgaard et al. 1990; Borch-Johnsen et al. 1999; Weir 2007). Microalbuminuria is also associated with a high incidence of cardiovascular complications in the general population (Romundstad et al. 2003). A community-based cohort study revealed that the risks of mortality, myocardial infarction, and progression to kidney failure increase for patients with a lower estimated glomerular filtration rate (eGFR) and a higher urinary albumin-to-creatinine ratio (ACR) (Hemmelgarn et al. 2010). Therefore, the American Heart Association recommends combined screening for low eGFR and microalbuminuria in patients with risk factors for cardiovascular disease such as diabetes mellitus, hypertension, or cardiovascular disease (Brosius et al. 2006).

The technique used to measure microalbuminuria is still underused due to the lack of awareness of its benefits among physicians as well as its high cost. In Japan, the
measurement of urinary albumin costs approximately 16 times more than the measurement of total urinary protein. Furthermore, the national health insurance reimburses the cost of urinary albumin measurement for patients with diabetes mellitus only. Consequently, individuals without diabetes are rarely screened for microalbuminuria.

Quantitative analysis of total urinary protein is performed routinely, and a level as low as 2 mg/dl of urinary protein can be detected due to recent advances in techniques. Many studies have examined the relationship between proteinuria and albuminuria (Gatling et al. 1988; Sawicki et al. 1989; Marshall 1991; Gazis and Page 1996). However, the relationship between the ACR and the protein-to-creatinine ratio was not sufficiently studied. In this context, Methven and colleagues aimed to identify the optimal test to identify significant proteinuria (Methven et al. 2010), whereas we aimed to test the total urinary protein-to-creatinine ratio to detect microalbuminuria. If the presence of microalbuminuria could be predicted from the value of the total urinary protein-to-creatinine ratio, screening for microalbuminuria could be replaced by this detection method. This simpler and less expensive method to screen for microalbuminuria could enable its widespread use worldwide. In the present study, we established a method to predict the presence of microalbuminuria by measuring the protein-to-creatinine ratio.

**Materials and Methods**

**Subjects**

Urine samples for this investigation were obtained from 228 consecutive adult diabetic patients who were seen at the diabetes clinic of St. Luke's International Hospital in Tokyo from February 8 to April 30 2010. Of these 228 patients, 35 patients with overt proteinuria (a dipstick test result for urine protein of 3+ or a urine albumin concentration of > 600 mg/L) were excluded. In addition, 43 patients with urine albumin levels less than 5 mg/L and/or urine protein levels less than 2 mg/dl were excluded because of the limits of detection. Thus, 150 patients were included in this study (Fig. 1).

**Methods**

Spot urine samples from 150 patients were screened with reagent strips for the presence of protein (Eiken Chemical, Tochigi, Japan). The urine protein content was considered positive when the result of the dipstick test was “1+” to “3+” and was considered negative when it was “–” or “±” (trace). We placed these 150 patients into four groups according to the urine protein results from the dipstick test such that individuals with “–” results were in Group 1 (87 patients), “±” results were in Group 2 (34 patients), “1+” results were in Group 3 (22 patients), and “2+” results were in Group 4 (7 patients). Total protein, albumin, and creatinine levels, as well as serum creatinine and hemoglobin A1c levels, were also measured in each sample. The total protein concentration was quantified using a pyrogallol red molybdate assay (Micro TP-AR; Wako Pure Industrial, Osaka, Japan), and albumin was measured by immunonephelometry (Kanto Chemical, Tokyo, Japan). The inter-assay coefficients of variation (CV) for total protein and albumin were both less than 3% when the concentration of total protein was 2 mg/dl or greater and that of albumin was 5 mg/L or greater, while the CV for total protein was greater than 5% when the concentration of total protein was less than 2 mg/dl. The creatinine level was measured enzymatically (Nittobo Medical, Fukushima, Japan). The protein-to-creatinine ratio is expressed as grams per gram, and the ACR is expressed as milligrams per gram. The urinary albumin-to-total protein ratio is expressed as milligrams per milligram. Additionally, we placed the 150 diabetic patients into four groups according to protein-to-creatinine ratio such that individuals with values < 0.05 g/g creatinine were in Group A (30

![Fig. 1. Inclusion criteria for the study.](image_url)

Of the 228 adult patients from the diabetic clinic at St. Luke's International Hospital, 78 patients who presented with overt proteinuria or minimal levels of albuminuria were excluded from this study.
patients), values of 0.05 to 0.2 g/g creatinine were in Group B (81 patients), values of 0.2 to 0.5 g/g creatinine were in Group C (22 patients), and values ≥ 0.5 g/g creatinine were in Group D (17 patients). The urinary albumin-to-total protein ratio was compared among these groups. Microalbuminuria and macroalbuminuria were defined as ACRs of 30-300 and > 300 mg/g creatinine, respectively. Normoalbuminuria was defined as an ACR less than 30 mg/g creatinine (National Kidney Foundation 2007). The estimated GFR was calculated using the formula shown below, which was been adapted for Japanese individuals and recommended by the Japanese Society of Nephrology (Matsuo et al. 2009).

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eGFR \ (mL/min/1.73 \ m^2) = 194 \times \text{serum creatinine}^{-1.094} \ (mg/dl) \times \text{Age}^{0.20} \ (\text{years}) \times 0.739 \ (\text{if female})
\]

Statistics
The data are expressed as the mean ± the standard deviation (s.d.) for normal distributions and as median values for non-normal distributions. The ACR and protein-to-creatinine ratio data were examined following a log transformation of the values due to the non-normal distribution. The relationship between the protein-to-creatinine ratio and the ACR was examined by regression analysis. The \( \chi^2 \) test was used when appropriate. Receiver-operating characteristic (ROC) curve analysis was used to identify the optimal protein-to-creatinine ratio cutoff value for the detection of albuminuria (both micro- and macroalbuminuria). The Kruskal-Wallis test was used to compare the urinary albumin-to-total protein ratio with the protein-to-creatinine ratio. All of the statistical tests were analyzed using JMP software (JMP version 8, SAS, Cary, USA), and \( P \) values < 0.05 were considered significant.

This study was approved by the Research Ethics Committee of the St. Luke’s International Hospital in Tokyo.

Results
The baseline characteristics and the renal function test results for the 150 patients in the study are listed in Table 1. The urinary protein level was negative (Groups 1 and 2) in 121 patients. Of these, 26 patients (30%) in Group 1 and 18 patients (53%) in Group 2 had microalbuminuria. Furthermore, 4 of the (12%) Group 2 patients were positive for macroalbuminuria. The rate of albuminuria was significantly \( (p < 0.0001) \) higher in Group 2 than in Group 1. Furthermore, 3% of patients with proteinuria (Groups 3 and 4) were negative for albuminuria (Fig. 2).

There was a strong positive correlation between the ACR and the protein-to-creatinine ratio (Fig. 3). The regression expressions were written as follows: (a) \( \log \ ACR = 1.34 \times \log \ [\text{protein-to-creatinine ratio}] + 6.61 \) [correlation coefficient = 0.951 for all cases (\( N = 150 \))] and (b) \( \log \ ACR = 1.05 \times \log \ [\text{protein-to-creatinine ratio}] + 6.30 \) [correlation coefficient = 0.911 for values below the range of microalbuminuria (ACR 30-300 mg/g creatinine) (\( N = 60 \))].

As shown in Figure 4, the optimal cutoff value from the ROC curve analysis for the protein-to-creatinine ratio was 0.091 g/g creatinine. At this cutoff value, the value of the protein-to-creatinine ratio had a sensitivity and a specificity of 90.8% and 91.9%, respectively, for the detection of albuminuria. Additionally, there was a positive predictive value of 92.0%, and a negative predictive value of 90.7%. There were 13 discordant results concerning the ACRs and the protein-to-creatinine ratios (7 false-negative cases and 6 false-positive cases).

As shown in Fig. 5, the urinary albumin-to-total protein ratio increased with increasing protein-to-creatinine ratios, and the regression expression was written as the following: urinary albumin-to-total protein ratio = 0.311 × log [protein-to-creatinine ratio] + 0.703 (correlation coefficient = 0.689).

The median values of the urinary albumin-to-total protein ratios were 0.212 for Group A, 0.352 for Group B, 0.569 for Group C, and 0.708 for Group D. There was a statistically significant difference between the median values of these four groups \( (p < 0.0001) \) (Fig. 6).

| Table 1. Baseline characteristics and renal function test values of the patients. |
|------------------|-------------------------------------------------|---------------------------------|---------------|
| Age (years) | 64.3 ± 12.5 [26-87] |
| Female (%) | 53 |
| Albuminuria (%)* | 51 |
| Serum creatinine (mg/dl) | 0.80 ± 0.35 |
| Estimated glomerular filtration rate (mL/min/1.73 m²) | 71.92 ± 19.80 |
| Hemoglobin A1c (%)** | 8.3 ± 1.3 |
| Hemoglobin A1c (mmol/mol) | 66 ± 14 |
| Urine total protein (mg/dl) | 7.5 [2-83] |
| Urine albumin (mg/L) | 24.35 [5.2-585.4] |
| Total urinary protein-to-creatinine ratio (g/g creatinine) | 0.09 [0.017-2.058] |
| ACR (mg/g creatinine) | 31.55 [2.3-1517.6] |

Note: Data are presented as the mean ± s.d. or as the median [range], as appropriate.

*Albuminuria includes both microalbuminuria and macroalbuminuria.

**The value obtained by the Japan Diabetes Society Standardization Program was transformed into a National Glycohemoglobin Standardization Program value.
Fig. 2. Proportion of micro- and of macroalbuminuria in each group. The proportion of albuminuria (both micro- and macroalbuminuria) significantly increased as the total urinary protein level by dipstick test increased.

Fig. 3. Correlation between the urinary albumin-to-creatinine ratio (ACR) and the total urinary protein-to-creatinine ratio (a) For all cases ($N = 150$), and (b) those below the range of microalbuminuria (ACR 30–300 mg/g creatinine) ($N = 60$). There was a strong positive correlation between the ACR and the protein-to-creatinine ratio. The correlation coefficient was 0.951 for the total cases and was 0.911 for the specified range of microalbuminuria.
Discussion

CKD is growing public health problem worldwide (Schoolwerth et al. 2006). Early detection and treatment of CKD can prevent or delay adverse outcomes such as kidney failure, cardiovascular diseases, or premature death (National Kidney Foundation 2002). Among the various causes of CKD, diabetes is the leading cause and requires renal replacement therapy. Diabetic patients who present with albuminuria, which is defined as an ACR over 30 mg/g creatinine, are diagnosed with diabetic kidney disease (National Kidney Foundation 2007).

Besides serving as a readout for diabetic kidney disease, microalbuminuria is an early marker of cardiovascular disease that can predict myocardial infarction, cerebrovascular disease, and progressive renal failure (Soedamah-Muthu et al. 2008; Yokoyama et al. 2008; Bouchi et al. 2010). A collaborative meta-analysis of the general population that included 1,128,310 participants confirmed that the urine albumin-to-creatinine ratio is associated with the risk for cardiovascular mortality of all causes (Chronic Kidney Disease Prognosis Consortium et al. 2010). The reduction of albuminuria following treatment with either an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker (ARB) is associated with improved renal and cardiovascular outcomes (Mogensen and Cooper 2004; Weir 2009). Because early-stage CKD is unlikely to be symptomatic and because microalbuminuria is not reliably detectable using routine dipstick methods, CKD remains unidentified in many patients. However, the prevalence of microalbuminuria is reported to be high (around 13-14%) in the Japanese general population (Tomura et al. 1999; Konta et al. 2006). The rates of proteinuria according to the results of the urine dipstick test are only one-third as high as those for microalbuminuria (Konta et al. 2006). Therefore, the ACR should be measured for diabetics but also for individuals with hypertension or cardiovascular disease. Repeat screening should be performed if these test results are positive for albumin, and appropriate treatment should be undertaken if this level remains positive. These treatments may offer significant reductions in cardiovascular and/or renal morbidity for many subjects.

Simple and inexpensive method to detect microalbu-
minuria is essential elements in the management of diabetic kidney disease as well as cardiovascular disease. Methven et al. conducted a large scale observational study to investigate the optimal test to identify and quantify significant proteinuria (Methven et al. 2010). They assessed the relationship between total urinary protein-to-creatinine ratio, ACR and 24-hr urine total protein in 6842 patients attending kidney clinic, and found that total urinary protein-to-creatinine ratio is a more sensitive test than ACR to predict clinically significant proteinuria, which they defined 0.5g/day and 1.0g/day proteinuria. While they focused the utility of total urinary protein-to-creatinine ratio in identifying significant proteinuria, our study aims to study the clinical utility of total urinary protein-to-creatinine ratio in detecting microalbuminuria.

As described previously, the main problems associated with using the detection of urinary albumin concentration for the diagnosis of microalbuminuria are its high cost (approximately 14.0 US dollars) and its lack of availability to the general population. A semiquantitative dipstick analysis of urinary protein concentration, which is not expensive (approximately 3.15 US dollars), is routinely used by general practitioners or for screening in a general health checkup system. The clinical significance of trace proteinuria (“±” result of the urine dipstick test) is not well defined, and this result is often ignored by the clinician. In this study, we found that the proportion of individuals with positive albuminuria was significantly greater ($p < 0.0001$) in Group 2 than Group 1. This result indicates that trace proteinuria levels could be a useful indicator of albuminuria in patients with diabetes mellitus, and this has also been previously reported (Sam et al. 2003). However, the sensitivity and the specificity of the urine dipstick test are not always sufficient to detect microalbuminuria. In this case, 30% of the individuals in Group 1 were positive for this condition, and normoalbuminuria was observed in 35% of the patients in Group 2 patients.

The measurement of total urinary protein is simple and inexpensive in Japan (approximately 0.85 US dollars). Therefore, we studied the relationship between the ACR and the protein-to-creatinine ratio and found that there was a strong positive correlation between these ratios. We also found that the presence of albuminuria could be predicted by the protein-to-creatinine ratio in more than 90% of patients. The ROC curve analysis showed that the protein-to-creatinine ratio had a sensitivity and a specificity of 90.8% and 91.9%, respectively, for the detection of albuminuria at a cutoff value of 0.091 g/g creatinine. Periodic screening for the protein-to-creatinine ratio may be more efficient and cost-effective than using the ACR and would likely enable the early identification of progressive renal and cardiovascular disease in many subjects. As a result of this screening, subsequent appropriate treatments may be associated with better outcomes.

The estimation of urinary albumin is straightforward when the albumin percentage of the total urinary protein is constant, as urinary albumin (g/dl) can be estimated as $k$ (constant) $\times$ total urinary protein (g/dl). However, previous studies have shown that the albumin percentage of the total urinary protein varies. For example, albumin constitutes approximately 20% of the total urinary protein in healthy persons, but it can constitute over 60% to 70% of that in patients with extensive proteinuria (Atkins et al. 2003). As shown in Figures 5 and 6, this study found that the percentage of albumin in the total urinary protein increases as the amount of protein increases. The cutoff value of 0.091 g/g creatinine for measuring microalbuminuria and the cutoff values above 30 mg albumin/g creatinine for measuring albuminuria indicate that albumin constitutes approximately 30% or more of the total urinary protein in this population. However, it remains to be determined whether this applies to non-diabetic kidney disease.

For mild proteinuria, proteins such as the Tamm-Horsfall protein lead to tubular proteinuria, whereas albumin protein is the major component of more severe proteinuria. This transition seems to occur at the level of microalbuminuria, which explains the highly variable urinary albumin-to-total protein ratio at the lower range of the total urinary protein-to-creatinine ratio. Furthermore, the measurement of urinary protein is rounded off to the first decimal place, which may increase the variability among the urinary albumin-to-total protein ratios. This variability can lead to false positive results in patients with very low levels of urinary protein or albuminuria when a cutoff value
of 0.091 g/g creatinine is used. Six patients (4%) had false positives for microalbuminuria at the cutoff value of 0.091 g/g creatinine. Each of these 6 cases had urinary albumin-to-total protein ratios of 8.5% to 22.8%, which indicated that their urine had a high level of an unknown non-albumin component. The underlying mechanism responsible for these results in these patients remains to be determined.

This study had a small number of limitations. First, the test that was examined cannot be used in subjects with a total urinary protein level less than 2 mg/dl or a urinary albumin level less than 5 mg/L. Second, the sample size of this study was small, and only patients with diabetes mellitus were tested. Further studies are required to determine whether similar results will be obtained for other patient populations, such as those with hypertension or cardiovascular disease without diabetes, or in the general population.

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**Conflict of Interest**

The authors report no conflict of interest.

**References**


