

Proteinuria is still useful for the screening and diagnosis of overt diabetic nephropathy¹

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OBJECTIVE: To assess the performance of urinary total protein measurements in timed 24-h urine collection and in a diurnal random urine specimen for the screening and diagnosis of overt diabetic nephropathy.

PATIENTS AND METHODS: A total of 167 diabetic patients (20 type 1 and 147 type 2 diabetic patients; 78 women and 89 men), aged 20-84 years, collected 217 timed 24-h urine specimens. Albumin was measured by immunoturbidimetry, total protein by the sulfosalicylic acid technique, and creatinine by Jaffé's method. According to the timed 24-h urinary albumin excretion rate, samples were divided into three groups: normoalbuminuric (urinary albumin excretion rate < 20 $\mu\text{g}/\text{min}$; $n = 84$), microalbuminuric (urinary albumin excretion rate 20-200 $\mu\text{g}/\text{min}$; $n = 78$), and macroalbuminuric (urinary albumin excretion rate > 200 $\mu\text{g}/\text{min}$; $n = 55$). Eight-six patients also collected 105 random urine specimens (normoalbuminuric, $n = 47$; microalbuminuric, $n = 37$; macroalbuminuric, $n = 21$), and urinary protein concentration and urinary protein-to-creatinine ratio were measured. The receiver operating characteristics curve approach was used to analyze the performance of the diagnostic tests.

RESULTS: Spearman's coefficient of correlation of 24-h urinary albumin excretion rate versus 24-h urinary protein was 0.95 ($P < 0.001$), and of 24-h urinary albumin excretion rate versus urinary protein concentration and urinary protein-to-creatinine ratio were 0.77 and 0.72, respectively ($P < 0.001$). The calculated areas (\pm SEM) under the receiver operating characteristics curve for the diagnosis of overt diabetic nephropathy were 0.9987 ± 0.001 for 24-h urinary protein, 0.9926 ± 0.006 for urinary protein concentration, and 0.9751 ± 0.014 for urinary protein-to-creatinine ratio. In the receiver operating characteristics curves, the first points with 100% sensitivity were 541 mg (95.7% specificity) for 24-h urinary protein, 431 mg/l (92.9% specificity) for urinary protein concentration, and 0.2 (76.2% specificity) for urinary protein-to-creatinine ratio.

CONCLUSIONS: Measurements of proteinuria presented almost perfect accuracy for the screening and diagnosis of overt diabetic nephropathy. Protein measurement in spot urine is a reliable and simple method for the screening and diagnosis of overt diabetic nephropathy.

Key-words: Urinary total protein measurements; random urine specimen; overt diabetic nephropathy.

Proteinúria ainda é útil para triagem e diagnóstico de nefropatia diabética sintomática

OBJETIVO: Avaliar a utilização de medições de proteína urinária total em coletas urinárias de 24 horas e em amostras diurnas coletadas aleatoriamente para triagem e diagnóstico de nefropatia diabética sintomática.

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PACIENTES E MÉTODOS: Foram coletadas 217 amostras de urina a cada 24 h de um total de 167 pacientes diabéticos (20 pacientes com diabetes tipo 1 e 147 com diabetes tipo 2; 78 mulheres e 89 homens), com idade entre 20 e 84 anos. A albumina foi medida por imunoturbidimetria, a proteína urinária total foi medida pela técnica do ácido sulfosalicílico e a creatinina, pelo método de Jaffe. As amostras foram divididas em três grupos de acordo com a taxa de 24 h de excreção urinária de albumina: normoalbuminúricos (taxa de excreção urinária de albumina < 20 $\mu\text{g}/\text{min}$; n=84), microalbuminúricos (taxa de excreção urinária de albumina 20-200 $\mu\text{g}/\text{min}$; n=78), e macroalbuminúricos (taxa de excreção urinária de albumina > 200 $\mu\text{g}/\text{min}$; n=55). Foram coletadas ainda 105 amostras aleatórias de urina de 86 pacientes (normoalbuminúricos, n=47; microalbuminúricos, n=37; macroalbuminúricos, n=21), das quais a concentração urinária de proteína e a relação proteína/creatinina urinária foram obtidas. O método da curva de características operacionais do receptor foi utilizado para analisar o desempenho dos testes diagnósticos.

RESULTADOS: O coeficiente de correlação de Spearman para a comparação entre a taxa de 24 h de excreção urinária de albumina e a proteína urinária de 24 h foi 0,95 ($P < 0,001$). O mesmo coeficiente, para a comparação da taxa de 24 h de excreção urinária de albumina com a concentração urinária de proteína, assim como com a relação proteína/creatinina urinária foi 0,77 e 0,72, respectivamente ($P < 0,001$). As áreas calculadas (\pm erro padrão) abaixo da curva de características operacionais do receptor para o diagnóstico de nefropatia diabética sintomática foram: $0,9987 \pm 0,001$ para a proteína urinária de 24 h; $0,9926 \pm 0,006$ para concentração urinária de proteína; e $0,9751 \pm 0,014$ para a relação proteína/creatinina urinária. Nas curvas de características operacionais do receptor os primeiros pontos com 100% de sensibilidade foram: 541mg (95,7% de especificidade) para proteína urinária de 24 h, 431 mg/l (92,9% de especificidade) para concentração urinária, e 0,2 (76,2% de especificidade) para a relação proteína/creatinina urinária.

CONCLUSÕES: As medidas de proteinúria foram extremamente eficazes na triagem e no diagnóstico de nefropatia diabética sintomática. A medição de proteína urinária é um método confiável e simples para a triagem e diagnóstico de nefropatia diabética sintomática.

Unitermos: Medição de proteína urinária total; coleta aleatória de urina ; nefropatia diabética sintomática.

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Introduction

The screening and diagnosis of diabetic nephropathy have been based on the measurement of urinary albumin excretion rate (UAER) in a timed 24-h or overnight urine collection; on albumin-to-creatinine ratio; or on albumin concentration in a random or early-morning urine sample (1-5). The measurement of urinary albumin by accurate methods (immunoturbidimetry or radioimmunoassay) is essential for the diagnosis of the

microalbuminuric phase of diabetic nephropathy, or incipient nephropathy, which can be halted or even reversed if appropriate measures are taken (6). It was demonstrated that screening and intervention for microalbuminuria are worthwhile, can have life-saving effects, and lead to considerable economic savings (7). However, financial constraint in some diabetes clinics has been considered the main reason limiting the use of urinary albumin measurements to screen for diabetic nephropathy (8). Classically, the diagnosis of overt diabetic nephropathy is

established when Albustix results are persistently positive or when total urinary protein (UP) excretion is > 0.5 g/24 h (9). In a recently published position statement, the American Diabetes Association recommended that screening for diabetic nephropathy should start with a routine urinalysis for protein (10). A dipstick test is used by most laboratories and is considered to have high sensitivity. A positive test result ought to be confirmed by quantitative protein measurement. If the test result is negative, measurement of albuminuria is necessary. The measurement of proteinuria by biochemical methods is simple and inexpensive and could increase the accuracy of the dipstick test for the initial screening of diabetic nephropathy. There are few data comparing the dipstick test with measurements of total proteinuria and albuminuria for the diagnosis of overt diabetic nephropathy (11, 12).

The aim of this study was to assess the performance of total UP measurements in timed 24-h urine collection and in a diurnal random urine sample (RUS) for the screening and diagnosis of overt diabetic nephropathy by using the receiver operating characteristics (ROC) curve approach.

Patients and methods

We performed a study of diagnostic tests for overt diabetic nephropathy (macroalbuminuria). The criterion standard was timed 24-h UAER. The study was done at the outpatient diabetes clinic at Hospital de Clínicas de Porto Alegre (HCPA) (a tertiary care center). Informed consent was obtained from each patient, and the protocol was approved by the ethics committee.

Every type 1 and type 2 diabetic patient (defined according to the World Health Organization's criteria) without evidence of cardiac failure or renal tract disease other than nephropathy (urinary tract infection, hematuria, abnormal urinary sediment, plasma creatinine increase without proteinuria) was considered for the study.

The study population consisted of 167 ambulatory diabetic patients (20 type 1 and 147 type 2 diabetic patients; 78 women and 89 men), aged 56.9 ± 12.3 years (range 20-84). Patients were instructed in the collection of timed 24-h

urine for measurement of UAER and total protein and were told to return on the morning after the end of urine collection. No specific recommendation was made about fluid intake, physical exercise, or dietary protein intake. Women were not examined during menstruation. The patients collected 217 timed 24-h urine specimens, all confirmed to be sterile by culture. According to 24-h UAER (criterion standard), samples were divided into three groups: normoalbuminuric (NORMO) (UAER < 20 μ g/min; $n = 84$), microalbuminuric (MICRO) (UAER 20-200 μ g/min; $n = 78$), and macroalbuminuric (MACRO) (UAER ≥ 200 μ g/min; $n = 55$). During the morning visit to the clinic, after completing the timed 24-h urine collection and after the first voided urine sample (not necessarily the second voided urine sample), 86 patients also collected RUSs for measurements of urinary protein concentration (UPC) and urinary protein-to-creatinine ratio (UPCR), as well as for the dipstick test. Fasting was not a criterion for collecting the RUS sample. This procedure was performed twice by 19 patients on different occasions; then 105 urine samples were analyzed. According to the 24-h UAERs, these samples were also divided into NORMO ($n = 47$), MICRO ($n = 37$), and MACRO ($n = 21$) groups.

Urinary albumin was measured by immunoturbidimetry (Sera-Pak, Bayer, Tarrytown NY) in duplicate. The mean intra and interassay coefficients of variation (CVs) are 4.5 and 11.0%, respectively, at our laboratory. Urinary total protein was measured by the quantitative 3% sulfosalicylic acid technique (13). The mean intra-assay CV was 4.8%, and it was calculated from the 24-h samples using three total UPCs: 124, 357, and 893 mg/l. The individual intra-assay CVs of these samples were 1.6, 4.6, and 8.1%, respectively. The semiquantitative dipstick method for proteinuria (Combur M, Boehringer Mannheim, Mannheim Germany) was performed according to the directions of the manufacturer. Urinary creatinine was measured by Jaffe's reaction (CV = 7.2%) (CentrifiChem Auto-Analyser, Hoffman-La Roche, Basel, Switzerland).

The relationship between 24-h UAER and 24-h UP and RUS measurements (UPC and UPCR) was calculated by Spearman's correlation coefficient (r_s). The contribution (%) of urinary albumin to total protein in timed 24-h urine was

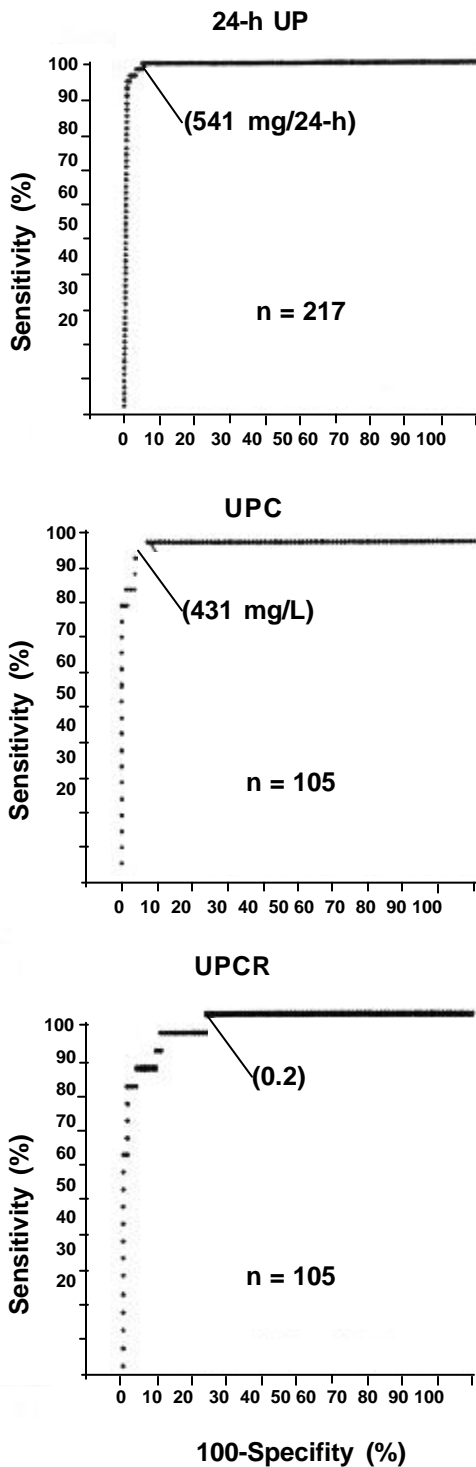


Figure 1. Receiver operating characteristics curves for total urinary protein, urinary protein concentration, and urinary protein-to-creatinine ratio as diagnostic tests for macroalbuminuria. Values in parenthesis correspond to the first point with 100% sensitivity.

determined in NORMO, MICRO and MACRO samples, and the contributions of urinary albumin to total protein were compared using the Kruskal-Wallis analysis of variance (ANOVA) and

nonparametric multiple comparison test. Sensitivities and specificities of total protein measurements in timed 24-h urine and in RUSs (UPCs and UPCR) as a diagnostic test for overt diabetic nephropathy were calculated in each sample. The ROC-curve approach was used to analyze the performance of the screening tests. The true-positive rate (sensitivity) versus the false-positive rate (100 - specificity) was plotted for each measurement. The first point with 100% sensitivity was chosen in each curve, and a second cutoff point was also determined in each curve by the intersection of the curves with the 100%-to-100% diagonal. The latter point represents the best equilibrium between sensitivity and specificity. The statistical analyses of ROC curves were done using a Visicalc program (14) and the ROC Analyser software version 5.0, which calculates the area under the curves (15). In assessing the accuracy of the Combur M test for the diagnosis of overt diabetic nephropathy, the 24-h UAER was considered the criterion standard. The level of significance was set at 0.05.

Results

The correlation of 24-h UAER to 24-h UP was 0.95 (n = 217; $P < 0.001$). When these urine samples were divided into the NORMO, MICRO and MACRO groups, the values for rS were 0.61, 0.80, and 0.95, respectively ($P < 0.001$). The rS values of 24-h UAER versus UPC and UPCR were 0.77 and 0.72, respectively ($P < 0.001$).

The median values and range of 24-h UAER and 24-h UP of 217 samples classified as NORMO, MICRO and MACRO, and the contribution of albumin to total protein in each sample group are shown in Table 1. The mean contribution of urinary albumin to total protein is lower in NORMO samples than in MICRO and MACRO samples. No difference was observed between MICRO and MACRO samples ($P < 0.05$, Kruskal-Wallis ANOVA).

Figure 1 depicts the ROC curves for 24-h UP (n = 217) and UPC and UPCR (n = 105) as diagnostic tests for overt diabetic nephropathy. The calculated area (\pm SEM) under the ROC curves for the 24-h UP was 0.9987 ± 0.001 . The calculated areas under the ROC curves for RUS measurements for UPC (0.9926 ± 0.006) and UPCR (0.9751 ± 0.014) did not differ.

Table 1. Measurements of 24-h urinary albumin excretion rate, 24-h urinary protein, and contribution of albumin to total protein in the 217 urine samples^a

Sample group	n	24-h urinary albumin excretion rate (µg/min)	24-h urinary protein (mg/24-h)	Contribution of albumin to total protein (%)
NORMO	84	4.9 (0.1-19.5)	45.7 (6.5-323.7)	16.8 (0.42-47.5)
MICRO	78	69.6 (20.3-197.8)	230.9 (51.6-625.1)	44.2 (14.3-76.5)
MACRO	55	627.7 (201.2-4,056.8)	2,133.1 (541.2-12,123.5)	47.1 (33.4-79.2)

^aData are expressed as median range, except for contributions of albumin to total protein, which are expressed as mean range.

Table 2 presents the characteristics of the cutoff points for diagnosis of overt diabetic nephropathy according to the first point with a sensitivity of 100% and the nearest point to the intersection of the curves with the 100%-to-100% diagonal. At these two cutoff points, the corresponding values of 24-h UAER were 201.2 and 312.1 µg/min, respectively, for 24-h UP. For the chosen UPC cutoff points, the corresponding 24-h UAER values were 307.1 µg/min (first point with a sensitivity of 100%) and 185.3 µg/min (intersection of 100%-to-100% diagonal). For the UPCR cutoff points these values were 307.1 and 672 µg/min, respectively.

When the performance of 24-h UP as a diagnostic test for microalbuminuria was analyzed, the calculated area under the ROC curve (\pm SEM) was 0.9665 ± 0.0106 . In this curve, the first point with 100% sensitivity for the diagnosis of microalbuminuria was 51.6 mg/24-h (53.6% specificity) and the point determined by the intersection of the curve with the 100%-to-100% diagonal was 134 mg/24-h (90.9% sensitivity; 90.5% specificity).

The Combur M test presented a 100% sensitivity for the diagnosis of overt diabetic nephropathy, according to the 24-h UAER. False-positive results were observed in about 18% of specimens (82.8% specificity). The accuracy of the Combur M test was 86.7%.

Conclusions

The data presented in this study showed that quantitative measurements of total UP in both 24-h and spot urine samples, as analyzed by the area under the ROC curve, are accurate for the screening and diagnosis of overt diabetic nephropathy. The values of the estimated area under the curves were close to 1.0, which represents perfect accuracy. Interestingly, the 100%-sensitivity cutoff value of 541 mg in 24-h urine is very similar to the value of 500 mg/24-h, which was adopted to define overt diabetic nephropathy (9). Few studies have compared the measurements of total proteinuria and albuminuria for the diagnosis of diabetic nephropathy (11, 12). These studies analyzed

Table 2. Characteristics of the cutoff points of 24-hour urinary protein, urinary protein concentration, and urinary protein-to-creatinine ratio as diagnostic tests for overt diabetic nephropathy^a

	24-h urinary protein (mg/24 h)		Urinary protein concentration (mg/l)		Urinary protein-to-creatinine ratio (mg/mg)	
	Cutoff1	Cutoff2	Cutoff1	Cutoff2	Cutoff1	Cutoff2
Cutoff point	541	568	431	490	0.2	0.5
Sensitivity (%)	100	98.2	100	95.2	100	90.5
Specificity (%)	95.7	97.5	92.9	95.2	76.2	90.5
Accuracy (%)	96.8	97.7	94.3	95.2	80.9	90.5

^aCutoff 1 is the first cutoff point with 100% sensitivity; cutoff 2 is the nearest point to the intersection of the curve with the 100%-to-100% diagonal.

only patients with overt diabetic nephropathy (11) or were designed for the diagnosis of microalbuminuria (12) and did not use the ROC-curve approach. The ROC curve has been used to evaluate overall test performance (14, 15) and to compare the discriminating ability of clinical tests (16). As far as we know, no other study used this approach to analyze the performance of proteinuria for the diagnosis of overt diabetic nephropathy. For the screening of a disease, it is desirable to have a test that is highly sensitive and, if possible, highly specific (17). High sensitivity and specificity were found in the present study because the 100%-sensitivity cutoff values of total proteinuria in 24-h and random urine (UPC) were highly specific (95.7 and 92.9%, respectively). This was not observed when proteinuria was corrected for urine creatinine in spot urine (UPCR). The specificity was only 76.2% at the cutoff value with 100% sensitivity. This means that about 25% of patients diagnosed by this test as having overt diabetic nephropathy would go on to be wrongly diagnosed. These errors are probably due to an increase in tubular secretion of creatinine in patients with nephrotic syndrome (18) and the interference of ketone bodies and some drugs in the creatinine assay method (19).

Moreover, the creatinine measurement increases the cost of the screening test. The quantitative measurement of total protein in the same spot sample of urine that is positive on the semiquantitative dipstick test will increase the accuracy of routine urinalysis for the diagnosis of overt diabetic nephropathy. Furthermore, the measurement of total protein in an RUS could be used to follow patients with overt diabetic nephropathy at a lower cost than the measurement of albuminuria. At our institution, the cost per test is as follows (all amounts expressed in U.S. currency): dipstick method, \$0.61; protein measurement by sulfosalicylic method, \$0.71; automated immunoturbidimetry for albumin, \$0.78. These economic data took into account the cost of the materials used (Combur M, \$0.51 per strip; sulfosalicylic method, \$0.001; automated immunoturbidimetry for albumin, \$0.77), the time spent to perform each test (1.00, 1.68, 0.10 min, respectively), and the hourly rate of a technician (\$ 6.00).

This study confirms that 24-h proteinuria is not suitable for the screening of

microalbuminuria because the specificity of the first point with 100% sensitivity on the ROC curve was unacceptably low. Considering that the detection of microalbuminuria is important in forestalling the development of advanced renal disease (5), it is imperative that accurate methods be used to test dipstick negative urine samples for albuminuria.

The use of 24-h UP and UAER produced a positive and significant correlation over a wide range of values. This correlation was stronger in macroalbuminuric (0.95) and microalbuminuric (0.80) samples than in normoalbuminuric samples (0.61). A close correlation between albumin and total protein excretion was also observed by other authors in a small sample ($n = 11$) of type 1 diabetic patients with overt diabetic nephropathy (11). The weaker correlation in normoalbuminuric samples could be explained by a diminutive fraction of albumin in the total protein at those low levels of proteinuria. After the development of diabetic renal disease, there is a progressive increase in urinary albumin and total protein excretion. The mean contribution of albumin to total protein was approximately 45% in both micro- and macroalbuminuric samples, which was significantly different from the proportion found in the normoalbuminuric samples (17%). These results are similar to those previously reported by Viberti et al. (20), who observed that the contribution of albumin to total protein was respectively 7, 15, and 42% in the urine samples of control subjects (UAER range 4.3-26.2 mg/24 h), patients with microproteinuria (UAER <150 mg/24 h; range, 18.7-122.8) and patients with macroproteinuria (UAER > 150 mg/24 h; range 157-6257). The different proportions observed in the microproteinuric group and our microalbuminuric group are probably due to the different criteria used to classify the patients. According to the current classification of stages of diabetic nephropathy (10), patients with UAER levels in the upper range of microalbuminuria were excluded, and normoalbuminuric patients were included, in the group classified as microproteinuric by Viberti et al. (20).

In conclusion, proteinuria demonstrated almost perfect accuracy for the screening and diagnosis of overt diabetic nephropathy. Protein measurement in spot urine is a reliable and simple method for the screening and diagnosis of overt diabetic nephropathy.

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