A New Cutoff for Abnormal Proteinuria in Diabetes Mellitus Patients: Relationship to Albuminuria

Orit Erman MD[1], Arie Erman PhD[2], Alina Vodonos MPH[4], Uzi Gafter MD PhD[2,3] and David J. van Dijk MD[2,3]

Departments of [1]Internal Medicine A and [2]Nephrology and Hypertension, Rabin Medical Center (Beilinson Campus), Petah Tikva, Israel
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[4]Department of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

ABSTRACT: **Background:** Proteinuria and albuminuria are markers of kidney injury and function, serving as a screening test as well as a means of assessing the degree of kidney injury and risk for cardiovascular disease and death in both the diabetic and the non-diabetic general population.

Objectives: To evaluate the association between proteinuria below 300 mg/24 hours and albuminuria, as well as a possible association with kidney function in patients with diabetes mellitus (DM).

Methods: The medical files of patients with type 1 and type 2 DM with proteinuria below 300 mg/24 hours at three different visits to the Diabetic Nephropathy Clinic were screened. This involved 245 patient files and 723 visits. The data collected included demographics; protein, albumin and creatinine levels in urine collections; blood biochemistry; and clinical and treatment data.

Results: The association between proteinuria and albuminuria is non-linear. However, proteinuria in the range of 162–300 mg/24 hours was found to be linearly and significantly correlated to albuminuria ($P < 0.001$, $r = 0.58$). Proteinuria cutoff, based on albuminuria cutoff of 30 mg/24 hours, was found to be 160.5 mg/24 hr. Body mass index (BMI) was the sole independent predictor of proteinuria above 160.5 mg/24 hr. Changes in albuminuria, but not proteinuria, were associated with changes in creatinine clearance.

Conclusions: A new cutoff value of 160.5 mg/hr was set empirically, for the first time, for abnormal proteinuria in diabetic patients. It appears that proteinuria below 300 mg/24 hr is not sufficient as a sole prognostic factor for kidney failure.

IMAJ 2016; 18: 418–421

KEY WORDS: albuminuria, diabetes mellitus (DM), proteinuria, creatinine clearance

Diabetic nephropathy is the leading cause of renal failure and is responsible for morbidity and mortality in diabetes. Proteinuria and albuminuria are markers of kidney injury and function, serving as a screening test as well as a means of assessing the degree of kidney injury and risk for cardiovascular disease and death [1-5] in both the diabetic and the non-diabetic general population.

In the past three decades clinical proteinuria was defined as > 300 mg/day [6]. Values below 300 mg/day were considered by some authors to indicate microproteinuria [7,8] and by others as normal. In recent years sensitive equipment has been developed, enabling detection of urine protein concentration as low as 20 mg/L. Recently, abnormal excretion of protein in the adult was defined as > 150 mg/day [9]. The clinical importance of proteinuria > 150 mg/day, considered to be the upper limit of normal, but below 300 mg/24 hours, defined as borderline proteinuria, has not been established. Yet, early renal disease is reflected by lower levels of proteinuria, particularly increased amounts of albuminuria. Since a universal discriminative value for proteinuria does not exist, Gorriz and Martinez-Castelao [3] suggested that both albuminuria and proteinuria be considered as a continuous variable. However, unlike microalbuminuria which has been shown to be predictive of clinical proteinuria, chronic kidney disease and early mortality [5,10,11], the clinical importance of proteinuria at levels < 300 mg/day in patients with diabetes mellitus is questionable.

Thus, the aim of the present study was to evaluate the association between proteinuria in the range below 300 mg/24 hours and albuminuria, as well as the association with renal function in patients with diabetes mellitus (DM).

MATERIALS AND METHODS

We screened 972 files of patients who visited the diabetic nephropathy clinic in a tertiary hospital during the years 2006–2010. The files selected were of patients with type 1 and 2 DM who presented with proteinuria < 300 mg/day, at three different visits, spaced with a minimal 3 month interval. The following patients were excluded: (i) those with other kidney diseases that may cause overt proteinuria such as lupus, amyloidosis, IgA nephropathy, membranoproliferative glomerulonephritis, etc., (ii) pregnant women, and (iii) those for whom more than one albumin or protein determinations were missing. The study was approved by the Rabin Medical Center Institutional Board.

The study group comprised 245 patients, each having three 24 hr urine samples. At each visit the patient provided 24 hr urine collection for the determination of albumin, protein and creatinine. Blood was withdrawn for determination of creatinine and HbA1c. Creatinine clearance was evaluated using the Cockroft-Gault equation [12]. Blood pressure (BP) was measured, and gender, age and body weight were recorded. Data on hypertension, cardiovascular disease and pharmacological treatment were also documented.

Urine protein and creatinine determinations were performed on an Olympus 2700 analyzer (Olympus Diagnostics, Germany). Albumin was performed on Olympus 2700 and Immulite (DPC) analyzers. Blood creatinine was performed on Roche Integra 400 (Roche Diagnostics Ltd, Switzerland). All assays were performed with specific kits and calibrators supplied by the manufacturers.

Patients with DM were described according to categorized variables presented as percentage and continuous variables presented as mean ± SD, median and range. Normal distribution of the data was examined by the Kolmogorov-Smirnov test. Correlations were assessed using the Spearman test.

Receiver operator characteristic (ROC) curve analysis was used to estimate the discriminative power of 24 hour protein excretion using the 24 hr urine albumin excretion rate of 30 mg/24 hr as the reference method. Changes in protein and albumin excretion and creatinine clearance between the three visits of each patient were analyzed in a model of repeated measurements (generalized estimating equations, GEE), standardizing time with referring to dependable variants using Wald chi-square analysis. Logistic regression analyses were performed to evaluate the influence of independent variables like age, body mass index (BMI), HbA1c, etc., on the prediction of protein excretion. Of 723 urine protein and albumin determinations, 61 protein determinations were reported as 0 (below the level of detection) and therefore were not included in the correlation analyses. In addition, protein values were missing in five visits. Data were analyzed using SPSS 18.

RESULTS

Characteristics of demographic, clinical and biochemical features of 245 diabetic patients are described in Table 1. T2DM patients were significantly older (< 0.001), had greater BMI and higher percentage of hypertension and cardiovascular diseases compared to T1DM patients. Yet, their mean duration of diabetes was 19.2 years, being shorter than that of T1DM patients (27.6 years, $P < 0.001$).

Urine protein excretion was significantly greater in T2DM patients, and creatinine clearance, according to the Cockroft-Gault equation, was significantly lower compared to T1DM patients [Table 1B]. Mean 24 hr urinary albumin excretion in T1DM patients was significantly lower than in T2DM patients (18.0 vs. 45.8 mg/24 hr) and, interestingly, its ratio to the total protein excretion was about half that in T2DM patients (12.5% vs. 23.8%).

Table 1. Demographic and clinical characteristics **[A]** and biochemical features **[B]** of patients with DM

[A]	T1DM patients	T2DM patients	DM patients
No. of patients	141	104	245
Age (years)	40.8 ± 12.1	65.45 ± 13.1*	51.2 ± 17.5
Male, n (%) Female, n (%)	77 (54.6%) 64 (45.4%)	51 (49.0%)* 53 (51.0%)	128 (52.2%) 117 (47.8%)
BMI (kg/m^2)	25.3 ± 4.2	29.6 ± 5.9*	27.2 ± 5.4
SBP (mmHg)	117.6 ± 12.7	125.05 ± 15*	120.7 ± 14.2
DBP (mmHg)	70.1 ± 7.6	70.1 ± 7.4 (NS)	70.1 ± 7.5
Hypertension, n (%)	38 (27.0%)	78 (75.0%)*	116 (47.3%)
Cardiac events, n (%)	9 (6.4%)	57 (54.8%)*	66 (26.9%)
Vascular, n (%)	8 (5.7%)	35 (33.7%)*	43 (17.6%)
Duration diabetes (years)	27.6 ± 11.3	19.2 ± 10.9*	24.0 ± 11.8
[B]	**T1DM patients**	**T2DM patients**	**DM patients**
HbA1C (%)	7.5 ± 1.2 (5.3–12.5)	7.9 ± 1.2* (5.4–12.7)	7.7 ± 1.2 (5.3–12.7)
Creatinine blood (mg/dl)	0.82 ± 0.2 (0.47–1.95)	1.07 ± 0.4* (0.4–3.11)	0.92 ± 0.36 (0.4–3.1)
Protein (mg/24 hr)	141.3 ± 60.6 (3–299)	175.3 ± 65.8* (24–299)	155.5 ± 65 (3–299)
Microalbumin (mg/24 hr)	18.01 ± 26.8 (0.75–263)	45.8 ± 48.3* (1–219)	29.5 ± 39.6 (0.75–263)
Cockroft Gault (ml/min)	124.9 ± 37.3 (51–292)	95.9 ± 56.8* (21.7–338.9)	112.7 ± 8.6 (21.7–338.9)
Albumin/Protein (%)	12.5 ± 13.3 (0.78–91.3)	23.8 ± 19.8* (0.82–101.2)	17.2 ± 17.2 (0.78–101.2)

Data presented are mean ± SD of 245 patients and 723 biochemical determinations. Protein was determined in 718 urine samples. Percentage and range are in parentheses

*P value < 0.001 refers to differences between T1DM and T2DM patients

The relationship between protein excretion, at levels < 300 mg/24 hr, and albumin in the whole diabetic population is shown in Figure 1. The association is non-linear and is presented as a quadratic equation. Yet, two tangential linear plots that overlap the lower and the upper values of proteinuria may be plotted. These plots intersect at the protein value of 162 mg/24 hr. The plots presenting the lower and higher values of proteinuria had two different slopes of 0.140 and 0.530, respectively [Figure 1]. Proteinuria in the range of 162–300 mg/24 hr was linearly correlated with albuminuria ($P < 0.001$, $r = 0.58$). ROC curve analysis was performed in order to predict a cutoff value for protein excretion, relating to albumin excretion of ≥ 30 mg/24 hr [Figure 2]. A protein cutoff point of 160.5 mg/24 hr was obtained, with a sensitivity of 78.3%, specificity 74.3% and area under the curve 0.815.

The contribution of independent variables that may predict increases in protein excretion of ≥ 160.5 mg/day were evalu-

Figure 1. Relationship between albumin and protein in 24 hr urine samples of DM patients. Albumin and protein were determined in 24 hr urine samples (n=657) of DM patients. An increased association at proteinuria levels greater than 162 mg/24 hr (n=268) was observed

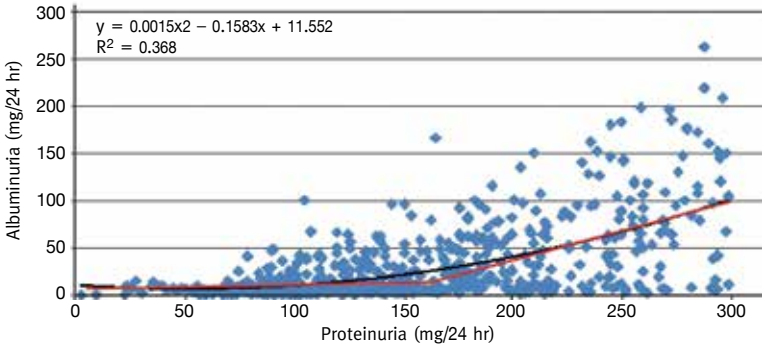

Figure 2. ROC curve for proteinuria predicting albuminuria of 30 mg/24 hr. Data were analyzed using Youden's Index

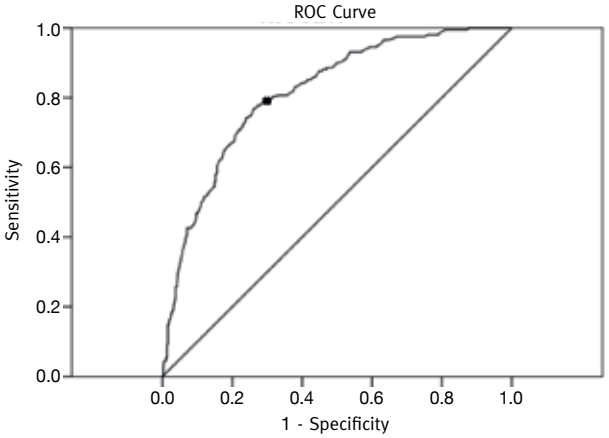

Table 2. Factors associated with protein excretion ≥ 160.5 mg/day

					95%CI for EXP(B)	
	B	SE	Sig	Exp(B)	Lower	Upper
Cockroft-Gault	-0.008	0.006	0.202	0.992	0.980	1.004
Age	-0.025	0.021	0.229	0.975	0.936	1.016
BMI	0.123	0.041	0.003	1.131	1.044	1.225
HTN T2DM	-0.169	0.354	0.632	.844	0.422	1.690
Duration of diabetes	0.019	0.017	0.275	1.019	0.985	1.055
Type of diabetes	0.934	0.604	0.122	2.546	0.780	8.309
ACEI/ARB treatment	0.264	0.230	0.250	1.303	0.831	2.043
HbA1C	-0.188	0.154	0.222	0.829	0.613	1.120
Constant	-2.356	1.631	0.149	0.095		

Contribution of the following independent variables: renal clearance according to Cockfoft-Gault, age, body mass index (BMI), hypertension in T2DM patients (HTN T2DM), duration of diabetes, type of diabetes, angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB), HbA1c to predict protein excretion ≥ 160.5 mg/day was examined using logistic regression analysis.

ated, using logistic regression analysis [Table 2]. BMI was found to be the only significant predictor, so that an increase in one unit of BMI increased the hazard by 13.1% ($P = 0.003$). The other variables examined, such as creatinine clearance, age, hypertension, duration of diabetes, HbA1c, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II blockers, were not predictive.

Creatinine clearance, estimated by the Cockroft-Gault equation, was poorly correlated with proteinuria ($r = -0.164$, $P < 0.001$). A model for prediction of changes in creatinine clearance with time based on repetitive measurements of 24 hr urine protein or albumin revealed that changes in protein excretion had no impact on the differences in creatinine clearance along the years of observation (slope 0.003, $P = 0.775$). In contrast, increases in albuminuria were significantly associated with decreases in creatinine clearances (slope -0.143, $P = 0.014$).

DISCUSSION

The association between protein excretion at levels lower than 300 mg/24 hr and albumin excretion in diabetic patients was non-linear. It was found that urinary albumin excretion of 30 mg/24 hr predicts the excretion of 160.5 mg/24 hr of protein. Thus, a new cutoff point for protein excretion in DM was empirically established.

Many studies have used a 24 hr protein output of greater than 300 mg to indicate overt, clinical or pathological proteinuria [6,7,9,13,14]. In healthy individuals, approximately 40 to 80 mg of protein are excreted in the urine daily [6,7,14]. It was recommended that urinary protein excretion in the normal adult should be less than 150 mg/day [3,14]. This value of 150 mg/day, which is regarded as abnormal proteinuria, is a historical definition [15] and its clinical value is unclear. Thus, the finding of protein excretion of 160.5 mg/day, which corresponds with albuminuria of 30 mg/day, is the first to be experimentally proven.

Albumin in the urine of healthy subjects comprises 7–15% of total protein [13,14]. In the present study, median albumin to protein ratio in the urine of DM patients was 10.1%. These findings are in agreement with findings in patients with various kidney diseases, showing that albumin as a percentage of total protein is highly variable at lower levels of proteinuria. When total protein approaches and exceeds 1 g/24 hr, the relative contribution of albumin increases and the ratio becomes relatively constant [13,16].

Albuminuria and proteinuria at lower values were poorly associated, reflecting the low content of albumin in urine samples (12.7%). At higher values of proteinuria (≥ 162 mg/day), the association was linear, significant and reflects the greater albuminuria content (24.4%) in those urines. These findings are supported by findings demonstrating a convergence toward the line of unity for the association between albuminuria and

proteinuria due to an increased proportion of urine albumin at higher levels of total protein excretion [13,16].

Microalbuminuria is typically defined as urinary albumin excretion ≥ 30 mg/24 hours. The ROC curve analysis shows that using this cutoff for albumin predicts a new cutoff point for protein excretion, that of 160.5 mg/24 hr. This is the first study to define a urinary protein cutoff based on albumin excretion. This, despite the common knowledge that total proteinuria cannot be reliably predicted from albuminuria because of the variable proportion of non-albumin proteins, particularly in the clinically relevant range of 0.3–1 g/day [17]. Many studies refer to protein excretion > 150 mg/24 hr as abnormal [3,9,18].

The performance of different estimating formulas of glomerular filtration rate (GFR) in patients with T2DM both with and without overt nephropathy has been reported [19]. The authors claimed that the GFR and GFR decline cannot be accurately estimated in those patients. Our findings indicate a negative association between proteinuria and creatinine clearance which, although being statistically significant, is very weak. Repetitive measurements of urine protein do not predict changes in creatinine clearance using the Cockroft-Gault equation. Protein is a known predictor of chronic kidney disease and higher levels of proteinuria are associated with increased risk of end-stage renal disease (ESRD), whereas reduction in proteinuria is associated with reduced risk of ESRD development [2,3,7,10,20]. In all these clinical studies, protein excretion was greater than 300 mg/24 hr. Research in African-Americans with hypertensive and renal disease and proteinuria in the range of 25.5–2023 mg/day has shown that change in the level of proteinuria is a predictor of subsequent progression of hypertensive kidney disease at a given GFR. In that study the median proteinuria was 143 mg/day and there was no attempt to distinguish between overt and borderline proteinuria [21].

Increased urinary albumin excretion in the microalbuminuric range predicts the development of cardiovascular and renal disease and the risk of death in both the diabetic and the non-diabetic general population [2,10,11]. Ruggenenti et al. [22] reported that among normoalbuminuric patients with T2DM, any degree of measurable albuminuria bears a significant cardiovascular risk. Our finding that increases in albuminuria, within the normo and microalbuminuric range, was significantly associated with decreases in creatinine clearance over time is consistent with many studies in the diabetic and non-diabetic population and kidney transplant recipients [23]. Nevertheless, changes in proteinuria in the range below 300 mg/day had no impact on the renal risk.

This study provides a new cutoff value of 160.5 mg/day for abnormal proteinuria. Among the variables examined, only BMI predicts this value. Yet, the clinical benefit of this value needs to be further examined. Compared to proteinuria, albuminuria at low levels is a better predictor for kidney disease in the diabetic population.

Correspondence
Dr. O. Erman
Dept. of Internal Medicine A, Rabin Medical Center (Beilinson Campus), Petah Tikva 49100, Israel
Phone: (972-3) 937-7485, **Fax:** (972-3) 937-6481
email: oriterman@gmail.com

References

1. The diabetes pandemic. *Lancet* 2011; 378 (9786): 99.
2. Culleton BF, Larson MG, Parfrey PS, Kannel WB, Levy D. Proteinuria as a risk factor for cardiovascular disease and mortality in older people: a prospective study. *Am J Med* 2000; 109: 1-8.
3. Gorriz JL, Martinez-Castelao A. Proteinuria: detection and role in native renal disease progression. *Transplant Rev* 2012; 26: 3-13.
4. Remuzzi G, Ruggenenti P, Benigni A. Understanding the nature of renal disease progression. *Kidney Int* 1997; 51: 2-15.
5. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984; 310: 356-60.
6. Knoll GA. Proteinuria in kidney transplant recipients: prevalence, prognosis and evidence-based managements. *Am J Kidney Dis* 2009; 54: 1131-44.
7. Shin M, Song SH, Kim JM, et al. Clinical significance of proteinuria at posttransplant year 1 in kidney transplantation. *Transplant Proc* 2012; 44: 610-15.
8. Li J, Liu B, Yan LN, et al. Microproteinuria for detecting calcineurin inhibitor-related nephrotoxicity after liver transplantation. *World J Gastroentrol* 2009; 15: 2913-17.
9. Burton DR, Fletcher SW. Evaluation of isolated proteinuria in adults. UpToDate, Wellesley, MA, 2012.
10. Viberti GC, Hill RD, Jarret RJ, Argropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1982; i: 1430-2.
11. Bigazzi R, Bianchi S, Baldari D, Campese VM. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. *J Hypertens* 1998; 16: 1325-33.
12. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31-41.
13. Guy M, Borzomato JK, Newall RG, Kalra PA, Price CP. Protein and albumin-to-creatinine ratios in random urines accurately predict 24h protein and albumin loss in patients with kidney disease. *Ann Clin Biochem* 2009; 46: 468-76.
14. Rose BD, Post TW. Measurement of urinary protein excretion. UpToDate Wellesley, MA, 2011.
15. Barratt J, Topham P. Urine proteomics: the present and future of measuring urinary protein components in disease. *CMAJ* 2007; 14: 361-8.
16. Atkins RC, Briganti EM, Zimmet PZ, Chadban SJ. Association between albuminuria and proteinuria in the general population: the AusDiab Study. *Nephrol Dial Transplant* 2003; 18: 2170-4.
17. Methven S, MacGregor MS, Traynor JP, O'Reilly DStJ, Deigham CJ. Assessing proteinuria in chronic kidney disease: protein-creatinine ratio versus albumin-creatinine ratio. *Nephrol Dial Transplant* 2010; 25: 2991-6.
18. Miettinen H, Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Proteinuria predicts stroke and other atherosclerotic vascular disease events in non-diabetic and non-insulin-dependent diabetic subjects. *Stroke* 1996; 11: 2033-9.
19. Gaspari F, Ruggenenti P, Porrini N, et al. The GFR and GFR decline cannot be accurately estimated in type 2 diabetics. *Kidney Int* 2013; 84: 164-73.
20. Levey AS, Cattran D, Friedman A, et al. Proteinuria as a surrogate outcome in CKD: report of a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *Am J Kidney Dis* 2009; 54: 205-26.
21. Lea J, Greene T, Hebert L, et al. The relationship between magnitude of proteinuria reduction and the risk of end-stage renal disease: results of the African American Study of kidney disease and hypertension. *Arch Intern Med* 2005; 165: 947-53.
22. Ruggenenti P, Porrini E, Motterlini N, et al. Measureable urinary albumin predicts cardiovascular risk among normoalbuminuric patients with type 2 diabetes. *J Am Soc Nephrol* 2012; 23: 1717-24.
23. Halimi JM, Buchler M, Al Najjar A, et al. Urinary albumin excretion and the risk of graft loss and death in proteinuric renal transplant recipients. *Am J Transplant* 2007; 7: 618-25.