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Urinary κ and λ Immunoglobulin Light Chains in Normoalbuminuric Type 2 Diabetes Mellitus Patients

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Background and Aim: Kidney disease is one of the major chronic microvascular complications of diabetes. Tubular involvement may precede glomerular involvement and the appearance of microalbuminuria. The aim of the study was to evaluate quantitatively immunoglobulin light chains (IgLCs), kappa and lambda excretion in the urine of patients with type 2 diabetes mellitus with normoalbuminuria and with microalbuminuria compared to control group. *Results*: Urinary IgLCs levels of the control group were significantly lower than diabetic patients with normoalbuminuria and diabetic patients with albuminuria. IgLCs were significantly associated with the duration of disease and negatively with estimated glomerular filtration rate. *Conclusion*: Type 2 diabetic patients can have significantly raised concentrations of urinary IgLCs before microalbuminuria or renal disease occurs. Further investigations are recommended to assess LC evaluation in the early management of diabetic renal disease. J. Clin. Lab. Anal. 25:229–232, 2011. © 2011 Wiley-Liss, Inc.

Key words: microalbuminuria; tubules; glomeruli; nephropathy; glomerular filtration rate

BACKGROUND

Kidney disease is one of the major chronic microvascular complications of diabetes. Diabetic nephropathy (DN) has several distinct phases of development and multiple mechanisms contribute to the outcome (1). DN has been classically defined as increased protein excretion in urine. Early stage is characterized by a small increase in urinary albumin excretion (UAE), also called microalbuminuria (2). More advanced disease is defined by the presence of macroalbuminuria or proteinuria.

Proteinuria and decreased gomerular filtration rate (GFR) occur in parallel in most cases. GFR has been expected to decrease when proteinuria is established. However, it is apparent that some patients could have DN without increased UAE (3). About 10% of subjects with type 2 diabetes mellitus will have low GFR without micro- or macroalbuminuria (4). This was also observed among patients with type 1 DM and microalbuminuria (5).

It is well established that the detection of microalbuminuria in a patient with DM indicates the presence of glomerular involvement in early renal damage. However, there is also a tubular component to renal complications of diabetes. Tubular involvement may precede glomerular involvement and the appearance of microalbuminuria (6). Also, some patients with microalbuminuria have advanced renal pathological changes for which therapy is less effective than earlier stages of the disease (7). Thus, novel bimarkers for earlier identification of DN are crucially required.

In the normal kidney, the negatively charged glomerular capillary wall repels negatively charged albumin and prevents its filtration. The slit pores between the podocytes in the glomerular capillary wall are small enough to exclude larger proteins, such as globulins, from the glomerular filtrate. Immunoglobulin light chains (IgLCs) are produced by B cells, slightly in excess of immunoglobulin heavy chains (8), and therefore are present in the serum of healthy adults in free form at low concentrations. Although complete immunoglobulin molecules cannot pass the intact glomerular filtration barrier, both κ- and λ-free IgLCs are filtered through the glomeruli. IgLCs are low-molecular-weight proteins that are nearly completely reabsorbed (95%) and degraded by the proximal tubule (9). Consequently, the urinary excretion of LCs is increased in renal

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diseases characterized by tubular proteinuria and in situations where tubular protein reabsorption is impaired. Increased urinary excretion of IgLCs in a diabetic individual could indicate tubular damage and represent an important stage in the development of DN.

The aim of the study was to: (1) evaluate quantitatively IgLCs, κ, and λ excretion in the urine of patients with type 2 diabetes mellitus; (2) correlate urinary light chain levels and the duration of the disease; and (3) examine the relationship between urinary light chains concentrations and the GFR and UAE in patients with DM.

MATERIALS AND METHODS

Patients and Controls

We studied 35 patients with type 2 diabetes, followed in the Outpatient Clinic of Diabetes, Cairo University, and 20 age and sex-matched healthy volunteers. The 35 diabetes patients were 22 females and 13 males, with mean age of 57.8 years (range: 43–75 years). The period of time elapsed since the diagnosis of diabetes of each patient varied from 2 to 21 years. Informed consent was obtained from both patients and controls.

DM patients had to fulfill diagnostic criteria for diabetes according to the World Health Organization (10). Patients were divided in two groups according to UAE: UAE <30 mg/g (20 patients) and UAE >30 mg/g (15 patients). Patients with causes that may alter UAE were excluded as fever, congestive heart failure, hypertension, coronary artery disease, pregnancy, and smoking. Also, patients with renal disease other than DN were excluded from the study. Healthy volunteers had no previous history of diabetes or kidney disease, and normal urinalysis.

Diabetes patients and controls were subjected to fundus examination, abdominal ultrasonography, and estimation of GFR by using Modification of diet in renal disease formula: $186 \times [\text{plasma creatinine (mg/dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female})]$ (11). Laboratory investigations were performed, including serum creatinine, complete blood picture, urine examination, and 24 hr urinary proteins. Microalbuminuria was estimated by nephlometric method on BN Prospec (Dade Behring, Deerfield, IL).

Quantitative Determination of Light Chains in Urine Samples

Light chains, κ, and λ determination was performed by nephlometric assay on BN Prospec (N Antisera to Human Immunoglobulin/L-chains, Dade Behring, Marburg GmbH). In an immunochemical reaction, the proteins contained in the urine samples form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the protein in the sample. The result was evaluated by comparison with standards of known concentration.

Statistical Analysis

The following statistical tests were applied considering $P<0.05$ as significant. Mann Whitney Wilcoxon U test was used in analysis in non parametric data. The Spearman correlation coefficient was used to rank variables against each other.

RESULTS

Diabetic patients had mean serum creatinine 0.98 ± 0.4 mg/dl, urea 55 ± 42 mg/dl, whereas normal controls showed 0.55 ± 0.33 mg/dl and 29.7 ± 14.9 mg/dl, respectively ($P<0.05$, $P<0.05$). eGFR was 83 ± 39 ml/min in normoalbuminuric diabetic patients and 76 ± 33 ml/min in albuminuric patients.

Urinary κ and λ Light Chains in Diabetic Patients

Urinary LCIg levels of healthy volunteers (median: κ/creatinine ratio (KCR); 13.0, and λ/creatinine ratio (LCR); 3.1), diabetes patients with normoalbuminuria (median: KCR; 16.7, LCR; 7.4), and diabetes patients with microalbuminuria disease (median: KCR: 21.8; LCR: 8.9) were significantly different. The levels of both light chains in controls were lower than those of diabetes patients without albuminuria and with albuminuria ($P<0.01$) (Table 1).

Urinary Light Chains and Duration of Diabetes

There was a significant positive correlation between urinary levels of κ and λ light chains ($r = 0.92$, $P<0.01$). Also, the correlation coefficients between urinary LCIg

TABLE 1. Urinary κ and λ Light Chain Concentrations in Diabetic Patients With and Without Albuminuria Compared With Controls ($P<0.01$)

	Urinary κ/creatinine		Urinary λ/creatinine	
Groups	No	Median	No	Median
Control	20	13.0	20	3.1
Diabetes with normoalbuminuria	20	16.7	20	7.4
Diabetes with microalbuminuria	15	21.8	15	8.9

Controls showed significantly lower urinary levels of κ and λ light chains compared with diabetic patients with normoalbuminuria and with albuminuria ($P<0.01$).

and the duration of diabetes were significant (κ: $r = 0.39$, $P < 0.05$; λ: $r = 0.38$, $P < 0.05$).

Urinary Light Chains and eGFR

Urinary κ and λ LCs had a negative correlation with the estimated glomerular filtration rate (κ: $r = -0.31$, $P < 0.05$; λ: $r = -0.27$, $P < 0.05$, respectively). Urinary κ and λ chain concentrations correlated significantly with urinary albumin (κ: $r = 0.29$, $P < 0.05$; λ: $r = 0.28$, $P < 0.05$, respectively) and serum creatinine (κ: $r = 0.33$, $P < 0.05$; λ: $r = 0.30$, $P < 0.05$, respectively).

DISCUSSION

Protein markers that would augment prediction of DN are highly desirable. During synthesis of Igs by plasma cells, LCs are produced in excess of heavy chains (8). These excess polyclonal LCs are released into the serum, from where they are rapidly removed by the kidneys with a half-life of 2–6 hr (12). Thus, we investigated urinary IgLCs in the progression of type 2 diabetes mellitus.

Both urinary κ and λ light chains were significantly higher in diabetic patients with normoalbuminuria and albuminuria than controls. Furthermore, our study showed a significant inverse correlation between both urinary κ and λ chain levels with eGFR and a significant positive correlation with urinary albumin and serum creatinine. These findings indicate that urinary κ and λ LCs can be observed in DM even when the albumin excretion is normal.

In accordance with our findings, Groop et al. (13) found that diabetic subjects with normal albumin excretion showed increased κ chains compared with controls, indicating that increased urinary excretion of κ chains may be an early sign of DN. Also, despite similar albumin excretion, the excreted κ LCs were significantly higher in diabetic patients than nondiabetic patients associating IgLCs in the differentiation between proteinuria of diabetic and nondiabetic origin (14). Up to 68% of type 2 diabetic patients with normal urinary albumin:creatinine ratios had abnormal urinary LC:creatinine ratios (15). In particular, patients with DN and those with ischemic nephropathy had higher fractional excretions of IgLCs than those with nondiabetic glomerular diseases (16). Interestingly, Type II diabetic patients had significantly raised concentrations of serum polyclonal free LCs before overt renal impairment developed (15).

The origin of urinary κ and λ LCs is likely to result from the combination of raised serum concentrations and altered renal handling of these molecules. Elevated serum IgLCs may represent a marker of inflammation in diabetic patients (16). The presence of raised concentration of LCs in the urine of diabetic patients before albuminuria, as demonstrated in our study, may represent a combination of glomerular and tubular dysfunctions. LCs have lower molecular masses than albumin and will be more sensitive to changes in glomerular permeability. The higher serum concentration observed in diabetics (15) will increase the concentration in the proximal tubules, where albumin is reabsorbed in preference to LCs. As progressive tubular injury occurs, the reabsorption of LCs will become less effective. The proximal tubule is uniquely susceptible to a variety of metabolic and hemodynamic factors associated with diabetes (17). In patients with DN, renal function and prognosis correlate better with structural lesions in the tubules and cortical interstitium than with classical glomerular changes (18). Tubular hypertrophy and reduced organic ion transport are already apparent before the onset of proteinuria in diabetes. Increased tubuloglomerular feedback and defective uptake and lysosomal processing may independently contribute to hyperfiltration and urinary protein loss, respectively (17).

Our study demonstrated a significant positive correlation between LCIgs with the duration of type 2 diabetes. Although Groop et al. (13) found increased urine excretion of κ LCs in insulin-dependent DM of long duration compared with newly diagnosed, there was no increase in LC excretion with longer duration of type 2 DM. Our study has shown that as eGFR decreases, the urinary concentrations of IgLCs increase, and also urinary κ and λ chain concentrations correlated significantly with urinary albumin. Similarly, it has been demonstrated that as renal function decreases, the serum and urinary concentrations of polyclonal free LCs increase up to five-fold in predialysis chronic kidney disease (16) and both urinary κ and λ concentrations correlated with urinary albumin:creatinine ratio in type 2 diabetes (15).

Microalbuminuria is currently the only diagnostic tool available for early diagnosis of DN. The test is based on immunological detection of smaller quantities of albumin in the urinary samples of diabetes patients. However, there are several limitations to the use of microalbuminuria as an index of renal function. The UK Prospective Diabetes Study demonstrated that 51% of patients who progress to chronic renal failure had no preceding albuminuria (19). Also, there may be very significant decreases in GFR even in the microalbuminuric range, and Rachmani et al. (20) showed that in type 2 diabetic patients the renal and cardiovascular risk increased progressively with albumin excretion rates within the normal range. Also, there are a high percentage of microalbuminuric patients who revert to normal urine albumin levels (21). Thus, the interpretation of microalbuminuria has been brought into question.

Our study showed increased urinary κ and λ light chains in diabetic patients with normoalbuminuria compared with controls. These findings provide the basis for future studies to assess the potential of LCs as a useful tool for early diagnosis of diabetic kidney disease.

CONCLUSIONS

Thus, type 2 diabetic patients can have significantly raised concentrations of urinary LCs before microalbuminuria or overt renal disease occurs. Further investigations are recommended to assess LC evaluation in the early management of diabetic renal disease.

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