Glomerular Lesions in Patients with Non-Insulin-Dependent Diabetes Mellitus and Microalbuminuria

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To evaluate the renal structural changes in non-insulin-dependent diabetes mellitus (NIDDM), we studied the renal histological findings and urinary albumin excretion in 75 patients with NIDDM. They were divided into two groups according to excretion of urinary albumin: 40 cases of normoalbuminuria and 35 cases of microalbuminuria. Renal biopsy specimens were evaluated by light microscopy. Diffuse glomerular lesions were graded on a scale of D0 through DIV (Gellman's criteria). The incidence of microalbuminuria was 19.2% in D0, 53.3% in D1, 61.5% in DII and 100% in DIII. Grade IV lesions were not present in either group. Creatinine clearance differed significantly between the groups with and without microalbuminuria. There was no difference between the groups with normoalbuminuria and microalbuminuria in the incidence of retinopathy and hypertension, or in the urinary excretion of β2MG and NAG. We conclude that microalbuminuria in NIDDM indicates the early morphological changes of glomerular lesions.

Key words: diabetic nephropathy, urinary albumin, renal biopsy

Introduction

Microalbuminuria is a good predictor of the development of overt diabetic nephropathy in patients with either insulin-dependent diabetes mellitus (IDDM) (1-4) and non-insulin-dependent diabetes mellitus (NIDDM) (5-7). In Mogensen's classification of diabetic nephropathy, the renal structural lesion at the stage of incipient nephropathy revealed a thickening of the glomerular basement membrane and expansion of the mesangium (8). This classification has been well studied in patients with IDDM, but not in those with NIDDM and microalbuminuria. The morphologic evaluation of diabetic nephropathy in NIDDM with microalbuminuria has not been adequately carried out. Recently, Schmitz and colleagues (9) described the relationship between urinary albumin excretion and glomerular alterations using autopsy materials of NIDDM evaluated by light microscopy. However, there is little information on this relationship using renal biopsy specimens (10). The aim of this study was to clarify the renal structural changes in patients with NIDDM and to investigate the possible relationship to albumin excretion.

Patients and Methods

Patients

Seventy-five patients (37 males and 38 females; age range 33 to 68 years) who were diagnosed with NIDDM at the First Department of Internal Medicine of Nara Medical University were included in this study. They were divided into two groups according to the rate of urinary albumin excretion: 40 cases of normoalbuminuria (urine albumin under 20 μg/min) and 35 cases of microalbuminuria (urine albumin 20-200 μg/min). Renal biopsy was performed in each patient. We explained the purpose, procedures, possible consequences and complications to each patient prior to renal biopsy, and obtained a signed informed consent form.

Laboratory studies

Twenty-four hour urine was collected for the measurement of the excretion rate of albumin, β2-microglobulin (β2MG) and N-acetyl-β-D-glucosaminidase (NAG). Urinary albumin concentration was measured with a solid phase radioimmunoassay as previously described in detail (11). Urinary β2MG was measured using an enzyme immunoassay (12). Urinary NAG was measured by the m-cresol sulphon-phthaleinyl substrate method (13). Serum and urine creatinine were measured by an...
autoanalyzer, and the glomerular filtration rate (GFR) was calculated by creatinine clearance. Hemoglobin A1c (HbA1c) was measured by high performance liquid chromatography.

Classification of glomerular lesions
Tissue specimens obtained by percutaneous renal biopsy were processed for morphometric evaluation by light microscopy. Part of each specimen was fixed and embedded in paraffin by the usual technique. Serial sections 3 μm thick were cut and stained with periodic acid Schiff.

The severity of the glomerular changes was graded on a scale of D0 through DIV according to Gellman's criteria (14) as follows: D0 (all glomeruli appear normal); DI (local lesion present within each glomerulus and focal lesion present within the kidney); DII (mesangial thickening is diffuse within the glomerulus and generalized throughout the kidney); DIII (capillary lumina are narrowed and obliterated only locally); DIV (the lumen is generally narrowed and the entire glomerulus is ischemic and appears to be hyalinized). Glomerular nodular lesions were also evaluated by Gellman's criteria (14).

Classification of tubulo-interstitial lesions
The severity of tubulointerstitial changes was graded on a scale of T0 through TIII as follows: T0 (no morphologic abnormality); TI (focal tubular atrophy and interstitial fibrosis); TII (interstitial fibrosis is moderate with or without infiltration of mononuclear cells); TIII (extensive tubular atrophy and fibrosis).

Classification of diabetic retinopathy
Diabetic retinopathy was evaluated by Scott's classification (15).

Assessment of hypertension
A diagnosis of hypertension was established as a diastolic blood pressure of 95 mmHg or higher.

Statistical analysis
Statistical analysis was performed by Student's t-test and the chi-square method. P-values below 0.05 were considered to be statistically significant.

Results

Clinical features
As shown in Table 1, there were no significant differences between the groups of patients with normoalbuminuria and microalbuminuria with respect to mean age, duration of diabetes, incidence of hypertension and retinopathy, and the urinary excretion of B2MG and NAG. The microalbuminuric had a significantly lower GFR than the normoalbuminuric group.

Relationship between glomerular diffuse lesions and urinary albumin excretion
Figure 1 summarizes the rate of urinary albumin excretion at different stages of glomerular diffuse lesions. In the normoalbuminuric group, the glomerular diffuse lesions were graded as D0 in 21 cases (52.5%), DI in 14 cases (35.0%), and DII in 5 cases (12.5%). In the microalbuminuric group, 5 cases (14.3%) had D0, 16 cases (45.7%) had DI, 8 cases (22.9%) had DII and 6 cases (17.1%) had DIII. The incidence of microalbuminuria was 19.2% in D0, 53.3% in DI, 61.5% in DII and 100% in DIII. Furthermore, we investigated the incidence of urinary albumin excretion above 70 μg/min according to the criteria of Mathiesen et al (4), i.e. the threshold level of microalbuminuria at high risk of developing persistent proteinuria during a 6-year follow-up period. The incidence was 0% in D0, 10.0% in DI, 46.2% in DII and 50.0% in DIII. The incidence of microalbuminuria differed significantly among the four grades (p<0.001). No case of DIV
was observed in either group. Nodular lesions were observed in 1 case (DII) of normoalbuminuria, and in 4 cases (1 case in DII, 3 cases in DIII) with microalbuminuria.

Comparative laboratory findings

In the different stages of glomerular diffuse lesions, the normo- and microalbuminuric groups did not differ as to the GFR and the rate of urinary excretion of $\beta_2$MG. The microalbuminuric group demonstrated a significantly higher rate of urinary excretion of NAG as compared with the normoalbuminuric group of DI class. The microalbuminuric patients exhibited a significantly higher HbA$\text{I}$ level than the normoalbuminuric group of DI class (Fig. 2).

Relationships between tubulo-interstitial lesions and laboratory findings

Table 2 summarizes the GFR and the urinary excretion rate of albumin, $\beta_2$MG and NAG in the different stages of tubulo-interstitial lesions. The normo- and microalbuminuric groups did not differ as to GFR in each stage. However, the microalbuminuric patients in stages TII and TIII exhibited a significantly lower GFR than in T0. Patients in stages TII and TIII also showed a significantly higher level of albuminuria than those in T0. The normo- and microalbuminuric groups did not differ as to urinary $\beta_2$MG excretion in each stage. However, the urinary excretion of $\beta_2$MG was significantly increased in the microalbuminuric patients with TIII as compared with the T0 and T1 stages. The microalbuminuric patients in stage T0 demonstrated a significantly higher rate of urinary NAG excretion as compared with the normoalbuminuric patients in stage T0. In the microalbuminuric group, NAG was significantly lower in TI and TII stages as compared with T0.

Discussion

Albuminuria is an important indicator of glomerular involvement. Urinary albumin levels ranging from 20 to 200 $\mu$g/min indicate the presence of microalbuminuria (7). Mogensen
and colleagues (8) have shown that microalbuminuria commonly occurs in the early stage of diabetic nephropathy in IDDM. Several studies (1–6) recently concluded that the microalbuminuria precedes the development of overt diabetic nephropathy. Mathiesen et al (4) called this phase “incipient” to distinguish it from overt nephropathy. However, little data are available on the incipient phase of renal histopathology.

Osterby and Gundersen (16) reported that in the earliest phase of diabetic nephropathy, i.e. 1 to 6 years after onset of IDDM, glomerular volume is increased with little or no mesangial expansion as compared with normal subjects. Mauer et al (17) examined renal biopsy specimens in 45 patients with IDDM, and demonstrated no relationship between either the thickness of the glomerular basement membrane or mesangial expansion and the duration of diabetes. Chavers et al (18) studied the relation between urinary albumin excretion and renal structure in 48 IDDM patients. They observed a modest structural change between the normo- and microalbuminuric groups in values for mesangial expansion. Nevertheless, the relationship between the glomerular structure and function in patients with NIDDM and microalbuminuria has not been well defined.

Schmitz and colleagues (9) quantitated glomerular structure by light microscopy in 19 subjects with NIDDM who underwent autopsy, and revealed a possible link between morphology and urinary albumin concentration. They found no increase in glomerular volume in NIDDM patients with microalbuminuria or overt proteinuria. Inomata et al (10) examined renal biopsy specimens in 27 patients with NIDDM and determined the relationship between the rate of urinary albumin excretion and the severity of diabetic renal lesions. In that study, the glomerular diffuse lesions graded by Gellman’s classification was noted at DI or DII in the normoalbuminuric patients, whereas it was graded as DII or DIII in the patients with microalbuminuria. The normoalbuminuric and microalbuminuric patients showed similar clinical parameters such as age, duration of diabetes, HbA1 and creatinine clearance except for the frequency of proliferative retinopathy. In the present study, we examined in detail the relationship between the structure and function of the glomerulus in 75 patients with NIDDM, and observed glomerular diffuse lesions in 40 normoalbuminuric patients classified as D0 to DII, and in 35 microalbuminuric patients classified as D0 to DIII. Conversely, positivity for such lesions in those with microalbuminuria was 19.2% in D0, 53.3% in DI, 61.5% in DII, and 100% in DIII. These data demonstrate that overt diabetic nephropathy (as defined by dipstick testing positive for proteinuria) is not manifested until the glomerular lesions are far advanced. Moreover, the present results showed no correlation between urinary albumin excretion and tubulo-interstitial lesions in NIDDM.

We suggest that two mechanisms may be responsible for microalbuminuria in the early stage of diabetic nephropathy. First, the poor control of glycemia may influence the increase in glomerular permeability (19); however, in the present patients hyperglycemia was controlled at the time of renal biopsy. Indeed, a significant difference of HbA1 levels between the normo- and microalbuminuric groups was found only in DI.

Thus, hyperglycemia is not likely to be the main factor that would account for the increased albuminuria in the present cases. Second, the glomerular hyperfiltration may play a role (20). It has been demonstrated that the elevated GFR may be present after a few years of onset in IDDM patients (21), and some studies have shown a close association between the glomerular hyperfiltration and the development of diabetic nephropathy (22). However, some investigators have found an absence of hyperfiltration in NIDDM (23). The present results make it clear that the glomerular filtration rate in both the absence of diabetic nephropathy (D0) and the early stage of diabetic nephropathy (DI) did not differ between the normo- and microalbuminuric groups. Further examinations are needed to elucidate the pathogenesis of the leakage of albumin into the urine in the early stage of diabetic nephropathy. We conclude that an alteration of the glomerular structure is present even at the microalbuminuric phase in patients with NIDDM.

References


