TUBULOINTERSTITIAL LESIONS IN PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS AND DECLINE IN GLOMERULAR FILTRATION RATE

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Abstract: To evaluate the role of tubulointerstitial lesions in diabetic nephropathy, we studied the renal histological findings and various functional parameters in 201 patients with non-insulin-dependent diabetes mellitus (NIDDM). The patients were divided into four groups according to the severity of the tubulointerstitial lesions on a scale of T 0 through TIII. Diffuse glomerular lesions were graded by Gellman's criteria (D 0 through DIV). There was a weak correlation between the severity of glomerular diffuse lesions and that of tubulointerstitial lesions ; discordance between glomerular and tubulointerstitial lesions was presest in 37 patients (18 %). Urinary excretion of β_2 MG and NAG significantly increased with the severity of tubulointerstitial lesions, whereas the creatinine clearance decreased. Multiple regression analysis showed that glomerular and tubulointerstitial lesions independently contribute to the decline of the glomerular filtration rate. These results suggest that tubulointerstitial lesions are involved in the progression of diabetic nephropathy.

Index Terms

diabetic nephropathy, glomerular filtration rate, renal biopsy, tubulointerstitial lesion

INTRODUCTION

Diabetic nephropathy in considered to be mainly a glomerular disease because such patients show a progressive increase in the urinary excretion of albumin followed by a decline in the glomerular filtration rate (GFR). Glomerular diffuse or nodular lesions are typically seen in renal biopsies of patients with advanced diabetic nephropathy[1]. Conversely, some reports suggest a potentially important role for expansion of the interstitium in the development of renal insufficiency in patients with various renal diseases[2,3]. Four studies[4-7] investigated the role of the renal interstitium in insulin-dependent diabetes mellitus (IDDM). However, the relationship of tubulointerstitial damage to the GFR in patients with non-insulin-dependent diabetes mellitus (NIDDM) has not been delineated. The aim of this study was to clarify the tubulointerstitial changes in patients with NIDDM, and to investigate their possible relationship to functional parameters and to the progression of diabetic nephropathy.

PATIENTS AND METHODS

Patients

A total of 201 patients (129 males and 72 femeles) ranging in age from 33 to 78 years and who were diagnosed with NIDDM at First Department of Internal Medicine of Nara Medical

University between 1982 and 1994 were included in this study. Each patients underwent a renal biopsy. We explained the purpose, procedures, possible consequences and complications to each patients before the renal biopsy and obtained from each a signed informed consent form.

Laboratory Studies

Urine was collected over 24 hours from each patients for the measurement of the excretion rate of β_2-microglobulin (β_2 MG) and N-acetyl-β-D-glucosaminidase (NAG). Urinary β_2 MG was measured using an enzyme immunoassay (IMx β_2-Microglobulin Reagent Pack, DAINABOT Co., LTD., Tokyo, Japan). Urinay NAG was measured by the m-cresol sulfonphthaleinyl substrate method[8]. Serum and urine creatinine were measured by an autoanalyzer, and the glomerular filtration rate was calculated by measuring creatinine clearance.

Classification of Glomerular Lesions

The severity of glomerular diffuse lesions was graded on a 5-point scale of D 0 through DIV according to Gellman's criteria[1] as follows : D 0 (all glomeruli appear normal) ; D I (local lesion present within each glomerulus and focal lesion present within the kidney) ; D II (mesangial thickening is diffuse within the glomerulus and generalized throughout the kidney) ; DIII (capillary lumina are narrowed and obliterated only locally) ; and DIV (the lumen is generally narrowed and the entire glomerulus is ischemic and appears to be hyalinized). The severity of the glomerular nodular lesion was graded on a 5-point scale of N 0 through NIV, again using Gellman's criteria[1] as follows : N 0 (no nodule) ; N I (a single nodule in occasional glomeruli) ; N II (many glomeruli contain nodules, but many are spared) ; NIII (almost every glomerulus contains one or more nodules) ; and NIV (almost all glomeruli contain so many nodules as to be rendered almost completely ischemic).

Classification of Tubulointerstitial Lesions

The severity of tubulointerstitial lesions was graded on a 4-point scale of T 0 through TIII according to the previous report[9] as follows : T 0 (no morphologic abnormality) ; T I (focal tubular atrophy and interstitial fibrosis) ; T II (interstitial fibrosis is moderate with or without infiltration of mononuclear cells) ; and TIII (extensive tubular atrophy and fibrosis).

Classification of Vascular Lesions

The severity of vascular lesions was graded on a 4-point scale of V 0 through VIII by Takazakura's criteria[10] as follows : V 0 (normal appearance without PAS-positive deposit) ; V I (a slight PAS-positive thickening in observed but less than half circumference of arteriole) ; V II (most vessel walls are moderately thickened with PAS-positive deposition) ; and V III (a heavy thickening of majority of the vessel walls in seen with luminal narrowing).

Statistical Analysis

Values are represented as mean $\pm$ standard deviation. Statistical analysis was performed by Spearman's correlation coefficient, Kendall's correlation coefficient and Kruskal-Wallis's nonparametric H-test. Differences among the groups were further tested with Scheffe's F-test. Chi square analysis was used to evaluate categorical data. P-values below 0.05 were considered

statistically significant. Multiple regression analysis was applied which the predictor variable was the decline in creatinine clearance. Explanatory variables were selected by integrating the correlations between them by stepwise regression analysis.

RESULTS

Clinical Features

As shown in Table 1, all tubulointerstitial lesion groups except T 0 were similar in age. Patients in groups T II and TIII exhibited a significantly longer duration of diabetes than those in groups T 0 and T I . The prevalence of hypertension increased with the increasing grade of tubulointerstitial lesions ($p<0.01$, chi square analysis).

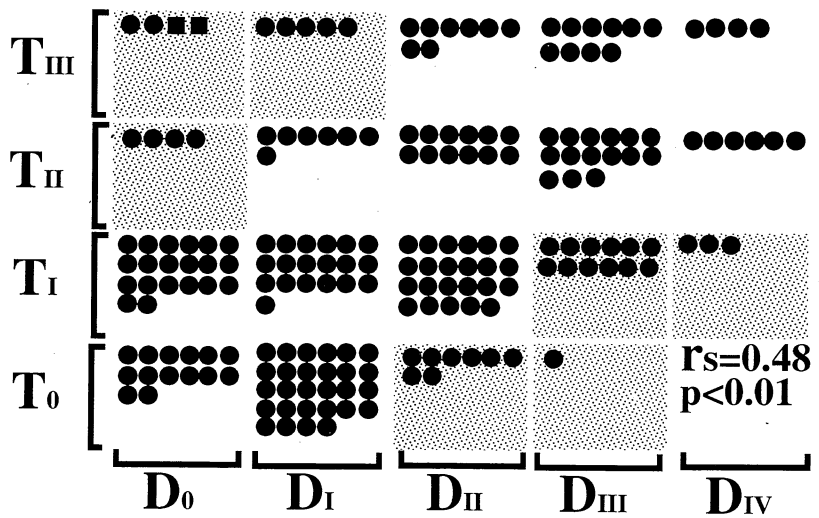

Fig. 1. The relationship between glomerular diffuse lesions (D_0 through D_{IV}) and tubulointerstitial lesions (T_0 through T_{III}). Shaded areas represent the discordance between glomerular and tubulointerstitial lesions. Solid squares indicate cases associated with chronic pyelonephritis.

Table 1. Clinical features

Clinical data	Severity of tubulointerstitial lesion			
	T_0	T_I	T_{II}	T_{III}
Age(years)	52.2±11.1	58.6±7.8	58.9±8.3	59.0±8.1
Gender(male/female)	25/26	56/19	26/18	22/9
Duration(years)	7.0±6.4	7.6±6.1	10.6±6.7[a]	11.0±6.9[a,b]
Treatment(D/A/I)	20/21/10	30/27/18	12/14/18	6/7/18
Hypertension(%)	18	39	43	61

Treatment modalities are : D, diet therapy ; A, anti-hyperglycemic agent ; I, insulin therapy.
a ; $p<0.05$ vs T_0, b ; $p<0.05$ vs T_I

Table 2. Comparison of laboratory data according to tubulointerstitial lesions

Laboratory data	Severity of tubulointerstitial lesions			
	T_0	T_I	T_{II}	T_{III}
β_2MG (μg/day)	98±77	278±768	1,890±4,829	2,967±6,163[a,b]
NAG (U/day)	5.1±2.9	4.6±3.1	6.9±6.5	10.7±9.2[a,b]
Ccr (ml/min)	104±31	87±33[a]	78±32[a]	61±27[a,b]

Abbreviations are : β_2MG, β_2-microglobulin ; NAG, N-acetyl-β-D-glucosaminidase ; Ccr, creatinine clearance
a ; $p<0.05$ vs T_0 b ; $p<0.01$ vs T_I

Table 3. Multiple regression model

Ccr=196.17−1.48(Age)−6.98(D)−1.67(UP)
−4.77(T)−3.75(V)−4.08(MA)−3.09(HT)
R=0.712, $p<0.001$
Variables
Age ; age at renal biopsy (yo)
D ; degree of glomerular diffuse lesions (0, 1, 2, 3 or 4)
UP ; urinary protein (g/day)
T ; degree of tubulointerstitial lesions (0, 1, 2 or 3)
V ; degree of vascular lesions (0, 1, 2 or 3)
MA ; macroangiopathy (yes=1, no=0)
HT ; hypertension (yes=1, no=0)

Interrelationship among Glomerular Diffuse Lesions, Tubulointerstitial Lesions and Vascular Lesions

Figure 1 summarizes the severity of tubulointerstitial lesions at the differing stages of glomerular diffuse lesions. There was a weak correlation between the severity of the glomerular diffuse lesions and the severity of the tubulointerstitial lesions ($r=0.48$, $p<0.01$, Spearman's correlation coefficient). Of the patients with advanced glomerular lesions, 24 did not have marked tubulointerstitial lesions, and 13 patients with moderate to severe tubulointerstitial lesions had no glomerular diffuse lesions. Only 2 of these patients had chronic pyelonephritis. A discordance between the glomerular and tubulointerstitial lesions was present in 37 of the 201 patients (18 %). There was no significant relationship between either the nodular or vascular lesions and the tubulointerstitial lesions ($r=0.35$, $r=0.49$, respectively). Conversely, nodular lesions were significantly correlated with glomerular diffuse lesions ($r=0.72$, $p<0.01$).

Relationships between Tubulointerstitial Lesions and Laboratory Findings

Table 2 summarizes the urinary excretion of β_2 MG and urinary activity of NAG, and creatinine clearance in the different stages of tubulointerstitial lesions. The urinary excretion of β_2 MG was significantly increased in the TIII group as compared with the T 0 and T I groups. Urinary activity of NAG was also significantly increased in the TIII group as compared with the T 0 and T I groups. Creatinine clearance was significantly lower in the T I, TII, and TIII groups than in the T 0 group and was significantly lower in the TIII group than in the T I group.

Multiple Regression Analysis

A multiple regression model using as explanatory variables age, severity of glomerular diffuse lesions, urinary protein excretion, severity of tubulointerstitial lesions, severity of vascular lesions, macroangiopathy, and hypertension, explained the variation in creatinine clearance ($R=0.712$, $p<0.001$)(Table 3). The severity of nodular lesions was removed from the multiple regression model because it was significantly correlated with the glomerular diffuse lesions. The model suggested that the severity of glomerular diffuse lesions and that of

tubulointerstitial lesions contribute independently to the decline in glomerular filtration rate.

DISCUSSION

Although diabetic nephropathy is mainly considered to be a glomerular disease, some observations suggest a potentially important role for tubulointerstitial lesions in the development of renal insufficiency[4-7]. Bader et al.[4] studied 103 biopsies from diabetic patients and found a significant positive correlation between serum creatinine concentration and the severity of interstitial expansion. They hypothesized that when postglomerular vessels are altered by interstitial expansion, the results are an increased resistance to renal cortical blood flow and a subsequent reduction of glomerular perfusion. Thomsen et al.[5] examined autopsy specimens from 34 patients with IDDM and found that the interstitial expansion that accompanied tubular atrophy was correlated with the serum creatinine levels. Mauer et al.[6] evaluated biopsy specimens from 45 IDDM patients and found that both mesangial enlargement and interstitial fibrosis were correlated with a decrease in creatinine clearance, whereas either of those lesions could independently contribute to the deterioration of the glomerular function. Lane et al. [7] also examined the relationship between mesangial and interstitial expansion in 84 patients with IDDM and found a significant correlation between these parameters. They hypothesized that the mesangial and interstitial expansion were probably interrelated mechanisms that led to renal insufficiency in diabetic nephropathy.

While all these studies showed a relationship between interstitial lesions and various measures of renal function in patients with IDDM, none has demonstrated their interaction in NIDDM. In our study, both the urinary excretion of β_2 MG and the urinary activity of NAG were siginificantly increased according to the stage of severity of the tubulointerstitial lesions. Tubular proteinuria in a consequence of the filtered serum protein exceeding the capacity of the renal tubule to reabsorb protein. Advanced diabetic nephropathy is characterized by overt proteinuria associated with increases in the excretion of low molecular weight proteins such as β_2 MG[11] and α_1 -microglobulin[12]. There is also evidence of organic damage to renal tubules because the urinary excretion of NAG in markedly increased in diabetic nephropathy[13].

In our study, a significant negative correlation was found between creatinine clearance and the severity of the tubulointerstitial lesions. Generally, advanced glomerular lesions are accompanied by tubulointerstitial lesions. However, in 18 % of the patients we observed a weak correlation between the severity of glomerular lesions and that of tubulointerstitial lesions. Moreover, 13 patients had a moderate to severe degree of tubulointerstitial lesions without marked glomerular lesions, and only 2 patients had pyelonephritis.

It is not clearly understood how the tubulointerstitial lesions directly contribute to the decline in glomerular filtration rate. Several possible mechanisms could explain renal interstitial lesions in diabetic nephropathy[14]. Extracellular matrix accumulation in the interstitium has been hypothesized as one pathogenic mechanism[15]. Recent evidence suggests that a high concentration of glucose directly promotes interstitial collagen synthesis, such as type I and type III collagens[16]. Such growth factors as insulin-like growth factor-1, transforming growth factor-β, and epidermal growth factor have also been suggested as inducing extracellular matrix synthesis[17]. Another mediators stimulate the glycation of matrix proteins, which alter their structure[18]. Interstitial edema may also contribute to the development diabetic ne-

phropathy. And it is possible that the increased hydrostatic pressure in the interstitium may lead to tubular atrophy[19].

The severity of the arteriolar lesions was directly correlated with the severity of both glomerular sclerosis and tubulointerstitial lesions. Deckert et al. [20], examining renal biopsies of 44 patients with various stages of diabetic nephropathy, found that arteriopathy is an important characteristic in advanced nephropathy. Thus direct arteriolar obliteration could play an important role in the progressive renal failure of diabetic nephropathy through producing chronic tubulointerstitial ischemia. But, in our study, the severity of vascular lesions significantly correlated with that the glomerular diffuse lesions, not with that of tubulointerstitial lesions. Multiple regression analysis also suggested that glomerular diffuse lesions, tubulointerstitial lesions and vascular lesions were independently related to creatinine clearance. It seems likely that glomerular diffuse lesions, tubulointerstitial lesions, and arteriolar hyalinosis represent more than one pathologic mechanism that culminates in renal dysfunction. Study of the structural and biochemical composition of the tubulointerstitium is necessary to further delineate the role of tubulointerstitial lesions in diabetic nephropathy.

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