The Korean Journal of Pathology 2009; 43: 36-42
DOI: 10.4132/KoreanJPathol.2009.43.1.36

The Morphologic Patterns of Diabetic Nephropathy in Koreans

Si-Hyong Jang · Moon Hyang Park

Department of Pathology, College of Medicine, Hanyang University, Seoul, Korea

Received : August 21, 2008
Accepted : October 31, 2008

Corresponding Author
Moon Hyang Park, M.D.
Department of Pathology, College of Medicine, Hanyang University, 17 Haengdang-dong, Sungdong-gu, Seoul 133-791, Korea
Tel: 02-2290-8249
Fax: 02-2296-7502
E-mail: parkmh@hanyang.ac.kr

Background : Diabetic nephropathy is the most common cause of end-stage renal disease and it has various pathologic features. We investigated the clinicopathologic differences between the histologic classes of diabetic nephropathy. **Methods :** A total of 46 patients with diabetic nephropathy were evaluated. Morphologically, the renal lesions were divided into three categories: class 1, diffuse or nodular glomerulosclerosis: class 2, vascular change without evidence of glomerulosclerosis: and class 3, non-diabetic renal disease superimposed on diabetic glomerulosclerosis. We evaluated the laboratory findings and the histologic findings, including mesangial expansion, interstitial fibrosis and inflammation, arteriolar hyalinosis and tubular atrophy. **Results :** The proportion of each class was 32 cases (70%), 4 cases (9%) and 10 cases (21%), respectively. The clinical and laboratory data showed no significant difference among the classes. For the groups of class 1, the group with nodular sclerosis showed a higher serum creatinine level than did the diffuse group (p=0.003). IgA nephropathy was the most common non-diabetic renal disease superimposed on diabetic glomerulosclerosis in our study. **Conclusions :** The patients with nodular glomerulosclerosis presented with a more progressed clinicopathological features than did the patients with class 1 diffuse glomerulosclerosis. We also found 21% of all the patients with diabetic nephropathy had superimposed non-diabetic renal disease in a Korean population.

Key Words : Diabetes mellitus; Diabetic nephropathy; Kidney

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) and it is the largest contributor to the total cost of diabetic care in the world.[1,2] The prevalence of diabetes mellitus (DM), especially type 2 DM, has rapidly increased in the Asian population due to the increasingly westernized diet and lifestyle.[2,3] More than 95% of patients suffering with DM over a 10-year period have renal involvement and this is associated with increased mortality and morbidity. The affected kidney undergoes progressive changes in all renal compartments and it displays a heterogeneous, variable morphology according to the duration of the disease treatment modalities and the disease stage.[1-4] Olson described and classified the typical renal pathologic changes into three classes: 1) glomerulosclerosis (GS), 2) the nonspecific chronic damage that's mostly related to vascular changes and 3) the glomerular diseases superimposed on or even unrelated to diabetic GS.[4] In the aforementioned Olson classification of diabetic nephropathy, it is necessary to diagnose and treat the concurrent glomerular diseases or the unrelated disorders that are superimposed on diabetic nephropathy because of the prognostic and therapeutic importance.[4,5] Although the histologic changes are characteristic, the prevalence of the patterns in these various renal lesions has not yet been established due to the discrepancy among the previously reported articles. This discrepancy is thought to be caused by the small number of cases in the studies, as well as by geographic and/or ethnic factors.[4] This non-diabetic renal disease in DM patients can lead to the earlier, accelerated deterioration of renal function.[5]

Histologically, two typical types of GS can be found in diabetic nephropathy patients: diffuse GS and nodular GS. Diffuse GS is characterized by diffuse mesangial expansion and thickening of the basement membrane in the absence of Kimmelstiel-Wilson (K-W) nodules. On the other hand, nodular GS has acellular PAS positive nodules, the so called K-W nodules. Although this type of nodular lesion has been suggested to be a consequence of the progressive form of diffuse glomerulosclerosis, there is controversy about the pathological relation between the two types of diabetic nephropathy.[1]

We investigated the histologic pattern of diabetic nephropathy, and we described the prevalence of each class and the non-diabetic glomerular diseases that are superimposed on diabetic nephropathy. We also evaluated the relationship between the clinical features and the renal histological findings of the patients with DM.

MATERIALS AND METHODS

Patients and study design

From March 1988 to May 2007, 46 patients with various renal diseases underwent renal biopsy at five general hospitals. All the histologic examination procedures, histologic diagnoses and morphologic assessments of renal damage were done in our pathology department. All the patients had been clinically diagnosed with diabetes mellitus. Two patients had undergone a simple nephrectomy due to acute emphysematous pyelonephritis and the remaining 44 patients had undergone needle biopsy. A renal biopsy was indicated when a renal disease other than diabetic nephropathy was suspected because of the presence of hematuria and/or proteinuria without diabetic retinopathy, rapidly progressive renal failure and renal insufficiency of an unexplained origin.

The clinical information on the patients, including gender, age, the duration of diabetes, the total serum protein, the serum creatinine, the urinary protein excretion, and the systolic and diastolic blood pressures, were obtained by reviewing the clinical records. The male to female composition was 20 to 26 and the age distribution ranged from 23 to 70 years at the time of biopsy. The duration of diabetic illness varied from less than 1 year to 11 years. The serum creatinine levels ranged from 0.5 to 10 mg/dL, and the 24-h urinary protein excretion ranged from 0.8 to 12.7g.

Pathologic studies

The fresh renal tissue was divided into three parts and they were examined by light microscopy, immunofluorescence (IF) and electron microscopy (EM). All 46 cases were examined by light mi-

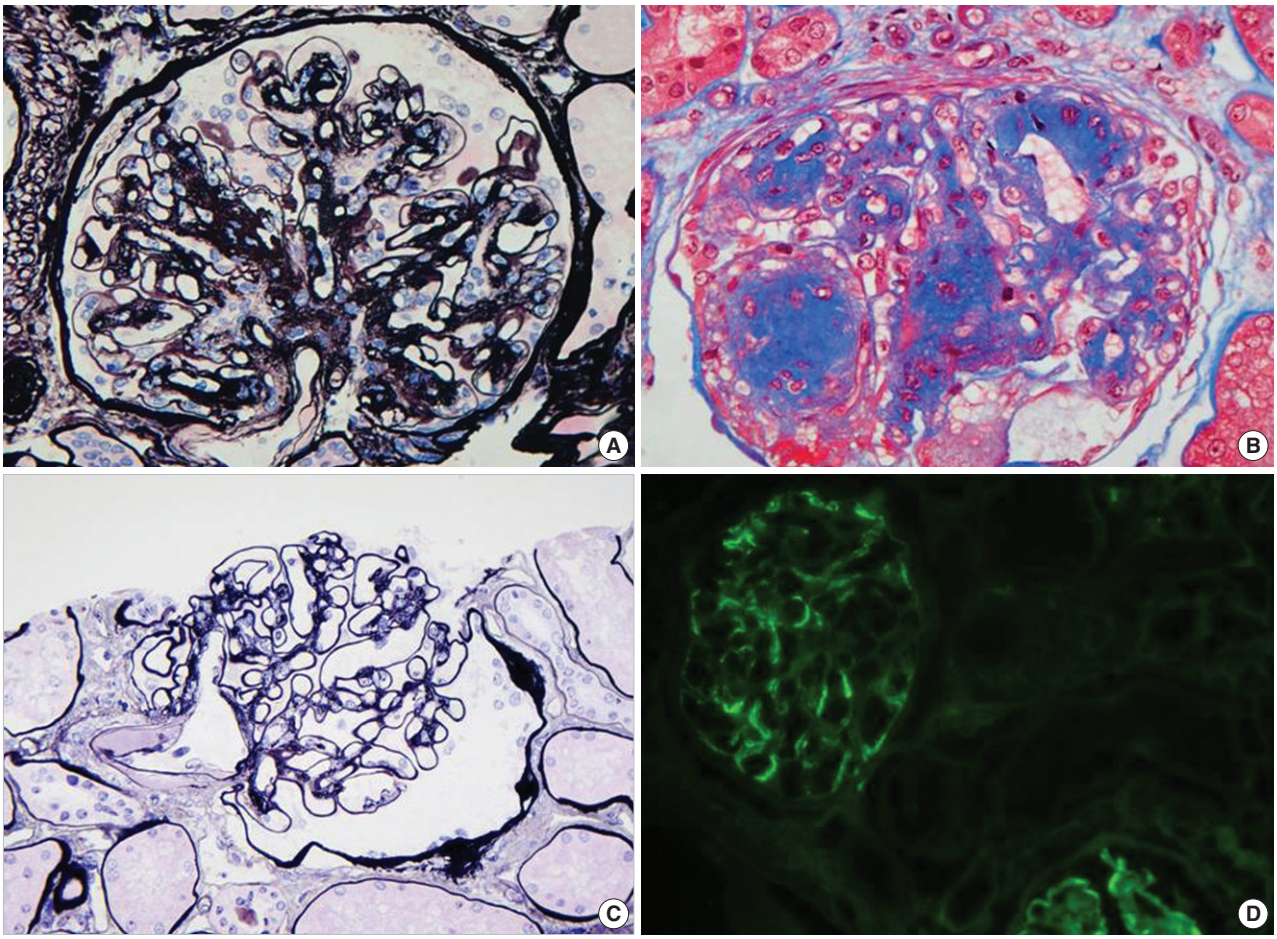

Fig. 1. The histologic pattern of diabetic nephropathy is classified as diffuse glomerulosclerosis (A, methenamine silver) and nodular sclerosis (B, Masson's trichrome). Hyaline arteriosclerosis is visible in preglomerular arterioles in all three photos (A, B, C). The histologic change in class 2 only shows vascular change without evidence of glomerulosclerosis (C, methenamine silver). Immunofluorescence staining demonstrates positive mesangial staining for IgA in the mesangium in the case of IgA nephropathy superimposed on diffuse glomerulosclerosis (D, IgA).

croscopy. Forty-four cases, all except the two nephrectomy specimens, were examined by IF. The glomerular change, as assessed by EM, was studied for the 36 cases that contained glomeruli on the EM.

For light microscopy, the specimens were fixed in Dubosq-Brazil solution, embedded in paraffin, and cut into 2 μm thick sections. The serial sections were stained with hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and Jones' methenamine silver.

An immunofluorescence investigation was performed on the 4-μm thick sections that were obtained from snap-frozen tissues, which were stained with fluoresceinated antiserum that was monospecific for IgG, IgM, IgA, C3, C1q, C4, fibrinogen, albumin and kappa and lambda light chains.

For electron microscopy, the tissue was diced into 1 mm cube fragments, fixed in 2.5% buffered glutaraldehyde, processed by standard techniques and then embedded in Epon. Ultrathin sections were stained with lead citrate and these were examined on a Hitachi H-750 at 75 KV or on a Hitachi H-7600s electron microscope at 80 KV.

Histologic evaluation

All the cases were divided into three classes: diffuse or nodular GS (class 1), vascular change without evidence of GS (class 2) and nondiabetic glomerulopathy superimposed on GS (class 3) (Fig. 1). Class 1 was again subdivided into the diffuse GS and the nodular GS. The total number of glomeruli, the number of glomeruli with global or segmental sclerosis, and the presence of Kimmelstiel-Wilson nodules were evaluated in each biopsy case. The degree of mesangial expansion, the thickness of the glomerular capillary walls, the arterio- and arteriolo-sclerosis, the arteriolar hyalinosis, the tubular atrophy and interstitial inflammation and the fibrosis were graded from zero (absent) to 3+ (maximal expansion).

Statistics

Statistical analysis was carried out using SPSS software (SPSS, version 12.0, Chicago, IL, USA). The clinical and laboratory differences in the classes were examined for statistical significance with using one way ANOVA tests or independent T-tests. The data were expressed as means ± S.E. The differences of the histologic features between the diffuse glomerulosclerosis and the nodular glomerulosclerosis were evaluated with χ^2 tests. A p-value <0.05 denoted a statistically significant difference among the classes.

RESULTS

Clinical pathological correlations

The clinical and functional characteristics of the different histological classes at the time of biopsy are summarized in Table 1. There was no statistical significant difference among classes 1, 2, and 3 for such clinical and laboratory features as gender, age, the duration of disease, serum creatinine, 24-h urinary protein excretion or the blood pressure. Comparison of diffuse GS versus nodular GS (Table 2) showed no significant difference in gender, age, the duration of diabetes, the 24-h urine protein excretion level and the diastolic arterial blood pressure. However, the serum creatinine level and systolic blood pressure were significantly higher in the patients with nodular GS than those in the patients with diffuse GS (p=0.003 and p=0.028, respectively).

Pathology results

The distribution of the histological patterns in the 46 patients was as follows: class 1, 32 cases (70%), class 2, 4 cases (9%) and class 3, 10 cases (21%). Class 1 consisted of 16 cases of diffuse

Table 1. Clinical and laboratory features of each class 1, 2, and 3 in diabetic patients

	Class 1	Class 2	Class 3
No. of patients (%)	32 (70%)	4 (9%)	10 (21%)
Sex ratio (M/F)	0.88	0.33	0.67
Age (year)	50.38±12.21	48.25±17.15	49.30±12.17
Duration of diabetes (month)	142.33±117.69	93.00±87.29	91.00±78.20
Serum creatinine (mg/dL)	2.50±2.48	0.87±0.32	1.74±0.68
24-h urine protein excretion (g)	5.55±3.77	3.37±3.32	3.82±3.35
Systolic arterial blood pressure (mmHg)	149.17±37.04	123.33±11.54	126.00±27.01
Diastolic arterial blood pressure (mmHg)	91.82±13.28	76.67±5.77	78.00±19.24

Table 2. Comparison of clinical and laboratory features of diffuse glomerulosclerosis and nodular glomerulosclerosis (GS)

	Nodular GS	Diffuse GS
No. of patients	16	16
Sex ratio (M/F)	1.00	0.78
Age (year)	52.13±13.35	48.63±11.12
Duration of diabetes (month)	173.33±123.52	116.73±110.26
Serum creatinine (mg/dL)*	3.08±3.12	1.76±1.04
24-h urine protein excretion (g)	7.00±4.11	4.22±2.99
Systolic arterial blood pressure (mmHg)*	155.71±47.91	140.00±12.25
Diastolic arterial blood pressure (mmHg)	98.33±14.72	84.00±5.48

*p-value <0.05 in χ^2 test.

Table 3. Comparison of pathologic changes between diffuse vs nodular glomerulosclerosis in class 1 (n=32)

	Diffuse glomerulosclerosis (n=16)	Nodular glomerulosclerosis (n=16)	p-value
Percentage of global sclerosis	38.03±28.37	23.22±18.29	0.117
Mesangial expansion			**0.041**
0	1	0	
1+	5	0	
2+	5	5	
3+	5	11	
GBM thickness			0.881
1+	4	4	
2+	9	10	
3+	3	2	
Arteriolar hyalinosis			**0.024**
0	2	0	
1+	7	1	
2+	5	11	
3+	2	4	
Arteriosclerosis			0.186
0	1	1	
1+	6	6	
2+	4	9	
3+	3	0	
Arteriolosclerosis			
1+	8	6	
2+	6	8	
3+	2	2	
Tubular atrophy			0.373
1+	3	1	
2+	7	11	
3+	5	4	
Interstitial fibrosis			0.677
0	1	0	
1+	4	5	
2+	5	7	
3+	5	4	
Interstitial inflammation			**0.037**
0	0	1	
1+	13	8	
2+	0	6	
3+	2	1	

Table 4. Non-diabetic renal disease superimposed on diabetic nephropathy in class 3

	Number of patients (n=10)
IgA nephropathy	3
Acute pyelonephritis	2
Membranous glomerulonephropathy	2
Post-streptococcal glomerulonephritis	1
Membranoproliferative glomerulonephritis	1
Batter's syndrome	1

GS and 16 cases of nodular GS (Fig. 1A, B). To assess the difference and severity of the glomerular, tubular, and interstitial change in the patients with solitary diabetic nephropathy, we compared the cases with diffuse GS and the case with nodular GS (Table 3). Mesangial expansion was noted in all 16 nodular GS cases to a moderate to severe degree, and this was more prominent in the nodular GS group than that in the diffuse GS group (p=0.041). Similarly, arteriolar hyalinosis and interstitial inflammation were more severe in the cases with nodular GS than that in the cases with diffuse GS (p=0.024, p=0.037). Class 2 included 4 cases and they showed only arteriolar hyalinosis without evidence of glomerulosclerosis on the light microscopy (Fig. 1C). However, electron microscopy revealed a mild to moderate degree of glomerular basement membrane thickening, ranging from 555 nm to 1,070 nm at the maximum thickness, in all four cases. On reviewing the light microscopic slides of these cases, we could not be certain that there was glomerular capillary wall thickening.

Non-diabetic renal disease

A total of 10 out of 46 cases (21.7%) were classified as class 3, non-diabetic renal disease superimposed on diabetic nephropathy (Table 4). Among these, 7 cases were associated with diffuse GS and the remaining 3 cases were associated with nodular GS as the underlying pathology. IgA nephropathy (3/10 cases, 30%) was the most frequent non-diabetic renal disease (Fig. 1D, 2A,

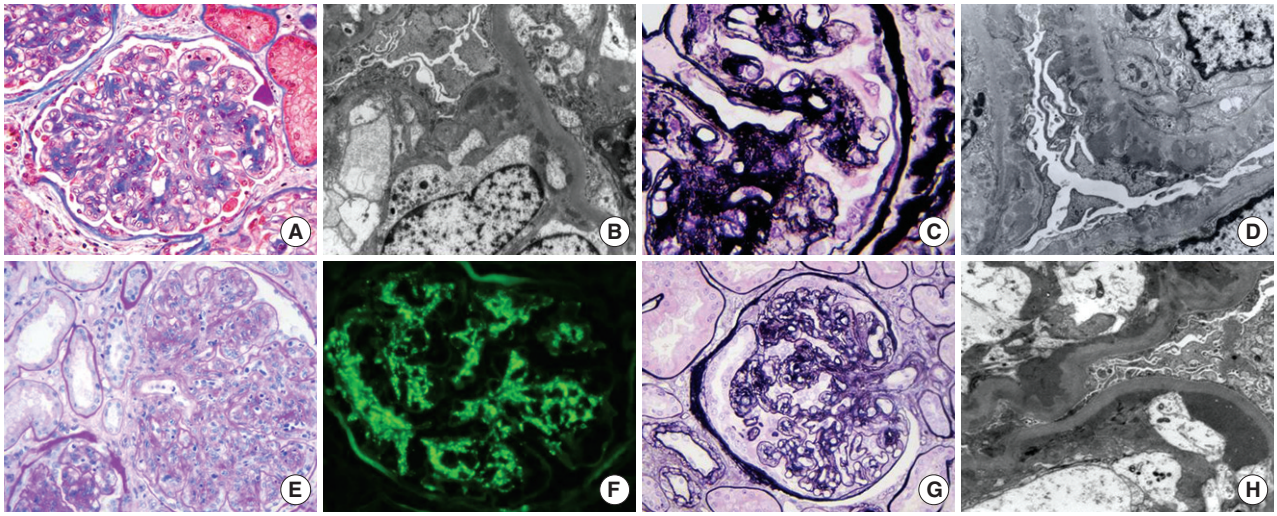

Fig. 2. In class 3, non-diabetic renal diseases superimposed on diabetic nephrosclerosis include IgA nephropathy (A, Masson's trichrome and B, electron micrograph, original magnification ×7,000), membranous glomerulonephropathy (C, methenamine silver ×1,000 and D, electron micrograph, original magnification ×8,000), post-infectious glomerulonephritis (E [PAS] and F [C3]) and membranoproliferative glomerulonephritis (G, methenamine silver). Large subendothelial and mesangial electron dense deposits are noted (H, original magnification ×7,000).

B). Two cases that underwent emergency nephrectomy for acute emphysematous pyelonephritis showed multifocal small or confluent abscesses that were associated with the underlying diffuse GS. The others were two cases of membranous glomerulonephropathy, and one case each of membranoproliferative glomerulonephritis, poststreptococcal glomerulonephritis and Batter syndrome, respectively. As we mentioned above, class 3 showed no difference in the characteristics or laboratory results from the other classes.

DISCUSSION

Diabetic nephropathy exhibits characteristic and unique pathologic changes in all the renal compartments, and this can be summarized as a diffuse form of mesangial sclerosis with an increase in the mesangial matrix, uniform thickening of the glomerular basement membrane, nodular lesion sometimes combined with microaneurysm, insudative or hyalinosis lesion, fibrin caps, capsular drops, and arteriolar hyalinosis. To evaluate the correlation of these histologic findings with their clinical significance, we classified the diabetic nephropathy according to renal histologic patterns, the same as was done in the previous studies[4,6]. class 1 was diabetic GS (32 cases, 69.6%), class 2 showed prevailing vascular change without diffuse or nodular GS on both types of light microscopy (4 cases, 8.7%) and class 3 displayed non-diabetic glomerular disease superimposed on diabetic GS (10 cases, 21.7%). Although a few previous studies have classified the morphologic pattern of diabetic nephropathy and they investigated the frequency of each class, the results were not consistent even within the same ethnic group. Mazzuco *et al*.[4] analyzed the renal pathologic patterns of 393 type 2 Italian DM patients and they reported that the prevalence of class 1, class 2, and class 3 was 39.9%, 15.2%, and 45.03%, respectively. Another study of 52 Italian DM patients demonstrated a relatively high proportion of class 2 (30.8%).[6] As compared with the previous two articles, our study showed features that were similar to the large series of Mazzuco *et al*.[4] The discrepancy in the prevalence rate can be explained by several causes, including the number of cases, the biopsy criteria and geographic and ethnic factors. In particular, the frequency of class 3 in DM patients has been shown to have broad variability, ranging from 12% to 45%.[6-16]

In view of the pathogenesis, a discrepancy exists as to whether the formation of acellular nodules is a distinct developmental pathogenic process or if it is a consequence of disease progression in patients with diffuse GS. Some investigators have reported there is little clinical difference between nodular and diffuse GS and they concluded that these two types of GS were distinctive patterns of GS.[17] On the other hand, others have suggested that nodular GS is a later stage of diabetic nephropathy and it is associated with longer durations of DM, the development of diabetic retinopathy and poor survival rates.[14,18,19] Suzuki *et al*.[12] demonstrated the correlation between the duration of diabetes and the increase of the mesangial matrix, glomerular sclerosis and arte-

riolar hyalinosis in patients with type 2 DM. The previous articles suggest that nodular GS may be the consequence and transformed malignant course of diffuse GS.[4,12] Our previous study[20] showed that in nodular GS, the composition of the early nodules resembled that of diffuse GS. However, the late advanced nodular lesions of the nodular GS revealed decreased reactivity for type IV collagen and fibronectin at the periphery of the nodules, and type VI collagen and interstitial collagen I and III were increased in a laminated pattern in the nodules.[20] In the present study, we subdivided the class 1 into diffuse GS and nodular GS and we compared the clinical and renal pathologic features in both subclasses. The serum creatinine level, which reflects renal function, was significantly higher in the patients with nodular GS than that in the patients with diffuse GS. Although any statistical significance was not evident, the other clinical and laboratory features such as the duration of DM and increased 24-h urine protein excretion tended to be higher in the patients with nodular GS. The histologic findings showed that mesangial expansion and arteriolar hyalinosis were more prominent in the patients with nodular GS, the same as in previous studies. These clinical and pathologic findings suggested that the patients with nodular GS generally present with more progressed clinical and pathological features.

Although the renal function and clinical course of patients with non-diabetic renal disease superimposed on diabetic GS are known to be dependent on the severity and extent of the diabetic GS rather than on the severity and extent of non-diabetic renal disease, recognizing this condition in DM patients is crucial to select the proper treatment modalities.[2,5,21,22] Yet diabetic GS shares similar morphologic features with a wide range of non-diabetic renal disease, including membranoproliferative glomerulonephritis, monoclonal immunoglobulin deposition disease, amyloidosis, membranous glomerulopathy and idiopathic nodular GS. When patients with a short duration of DM abruptly develop hematuria or increased proteinuria, then renal biopsy is essential to determine the possible co-existence of superimposed non-diabetic renal disease. IF, EM and their clinicopathologic correlation can be important diagnostic tools for distinguishing diabetic nephropathy from other clinical conditions.[21,22] Our study revealed that 10 out of 46 patients (21.7%) belonged to class 3 and the most commonly affected renal disease was IgA nephropathy. In Korean and Chinese populations, IgA nephropathy shows a high frequency of coexistence with both diabetic nephropathy and IgA nephritis.[6] Previous studies have evaluated the clinical features and biochemical parameters of the patients with isolated diabetic GS and the patients with non-diabetic renal disease coexisting with diabetes, and they found that there was no statistical difference, except for the level of the blood pressure during follow up period.[3] The other biochemical and clinical features, including serum creatinine, 24-h urine protein excretion and the presence of microscopic hematuria, showed no significant difference.[3,5]

In summary, we presented the prevalence of a histologic pattern in patients with diabetic nephropathy and some evidence that several clinical factors were related to the type of diabetic GS. We found that the nodular GS has more progressed clinical features than that of the diffuse GS. We also found that 21% of all Korean patients with diabetic nephropathy have coexisting non-diabetic renal disease, which is a little lower incidence than was expected when considering that the clinicians performed renal biopsies when other superimposed lesions were clinically suspected. In conclusion, for administering the proper treatment and for exactly evaluating the prognosis of diabetic nephropathy patients, it is necessary to carefully examine the extent and the type of GS, as well as to determine if there are superimposed non-diabetic renal lesions.

REFERENCES

1. Hong D, Zheng T, Jia-qing S, Jian W, Zhi-hong L, Lei-shi L. Nodular glomerular lesion: a later stage of diabetic nephropathy? Diabetes Res Clin Pract 2007; 78: 189-95.
2. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005; 28: 164-76.
3. Wong TY, Choi PC, Szeto CC, *et al.* Renal outcome in type 2 diabetic patients with or without coexisting nondiabetic nephropathies. Diabetes Care 2002; 25: 900-5.
4. Mazzucco G, Bertani T, Fortunato M, *et al.* Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. Am J Kidney Dis 2002; 39: 713-20.
5. Lee EY, Chung CH, Choi SO. Non-diabetic renal disease in patients with non-insulin dependent diabetes mellitus. Yonsei Med J 1999; 40: 321-6.
6. Gambara V, Mecca G, Remuzzi G, Bertani T. Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol 1993; 3: 1458-66.
7. Monga G, Mazzucco G, Barbiano di Belgiojoso G, Confalonieri R, Sacchi G, Bertani T. Pattern of double glomerulopathies. A clinicopathologic study in nine nondiabetic patients. Nephron 1990; 56: 73-80.
8. Amoah E, Glickman JL, Malchoff CD, Sturgill BC, Kaiser DL, Bolton WK. Clinical identification of nondiabetic renal disease in diabetic

patients with type I and type II disease presenting with renal dysfunction. Am J Nephrol 1988; 8: 204-11.
9. Parving HH, Gall MA, Skott P, *et al.* Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. Kidney Int 1992; 41: 758-62.
10. Kleinknecht D, Bennis D, Altman JJ. Increased prevalence of non-diabetic renal pathology in type II diabetes mellitus. Nephrol Dial Transplant 1992; 7: 1258-9.
11. Richards NT, Greaves I, Lee SJ, Howie AJ, Adu D, Michael J. Increased prevalence of renal biopsy findings other than diabetic glomerulopathy in type II diabetes mellitus. Nephrol Dial Transplant 1992; 7: 397-9.
12. Suzuki Y, Ueno M, Hayashi H, *et al.* A light microscopic study of glomerulosclerosis in Japanese patients with noninsulin-dependent diabetes mellitus: the relationship between clinical and histological features. Clin Nephrol 1994; 42: 155-62.
13. John GT, Date A, Korula A, Jeyaseelan L, Shastry JC, Jacob CK. Non-diabetic renal disease in noninsulin-dependent diabetics in a south Indian Hospital. Nephron 1994; 67: 441-3.
14. Olsen S, Mogensen CE. How often is NIDDM complicated with non-diabetic renal disease? An analysis of renal biopsies and the literature. Diabetologia 1996; 39: 1638-45.
15. Mak SK, Gwi E, Chan KW, *et al.* Clinical predictors of non-diabetic renal disease in patients with non-insulin dependent diabetes mellitus. Nephrol Dial Transplant 1997; 12: 2588-91.
16. Wirta O, Helin H, Mustonen J, Kuittinen E, Savela T, Pasternack A. Renal findings and glomerular pathology in diabetic subjects. Nephron 2000; 84: 236-42.
17. Schwartz MM, Lewis EJ, Leonard-Martin T, Lewis JB, Batlle D. Renal pathology patterns in type II diabetes mellitus: relationship with retinopathy. The Collaborative Study Group. Nephrol Dial Transplant 1998; 13: 2547-52.
18. Murussi M, Baglio P, Gross JL, Silveiro SP. Risk factors for microalbuminuria and macroalbuminuria in type 2 diabetic patients: a 9-year follow-up study. Diabetes Care 2002; 25: 1101-3.
19. Gall MA, Hougaard P, Borch-Johnsen K, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. BMJ 1997; 314: 783-8.
20. Paik SS, Park MH. Immunohistochemical localization of extracellular matrix components in diabetic nephropathy. Korean J Pathol 1997; 31: 427-35.
21. Alsaad KO, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: an update. J Clin Pathol 2007; 60: 18-26.
22. Lai FM, Li PK, Pang SW, *et al.* Diabetic patients with IgA nephropathy and diabetic glomerulosclerosis. Mod Pathol 1993; 6: 684-90.