An Evaluation of Renal Biopsy in Type-II Diabetic Patients

Muhammad Arif¹, Muhammad Khubaib Arif² and Muhammad Sohaib Arif³

ABSTRACT
Objective: To determine the renal damage in type-II diabetic patients, who underwent renal biopsy for impaired renal functions and its role in overall patient management.

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: The Kidney Postgraduate Centre, Karachi, Pakistan from January 2000 to May 2005.

Methodology: Histopathological evaluation of 73 patients of type-II Diabetes mellitus were included who underwent renal biopsy. Renal biopsy was performed when a renal disease other than diabetic nephropathy was suspected because of the presence of haematuria, nephrotic syndrome, non-nephrotic proteinuria < 3 gms/day in the absence of retinopathy, rapidly progressive glomerulonephritis and renal insufficiency of unknown origin. On the basis of light microscopy and immunofluorescence, three groups of patients were defined. Group I was characterized by diabetic glomerulosclerosis (DGS) only, group II by the prevalence of vascular changes, while group III had sub-groups IIIa (DGS co-existing with non-diabetic renal diseases) and IIIb (non-diabetic renal diseases without DGS).

Results: Among the 73 patients studied, 20 (27.3%) had diabetic glomerulosclerosis alone (group I), 17 (23.3%) showed occurrence of vascular changes (group II), and 36 (49.3%) had non-diabetic renal diseases (group III). Mean serum creatinine level was significantly greater in group II than in group I and III (p < 0.001). Amount of proteinuria and the presence of haematuria did not show a statistically significant difference in groups I, II and III. The systolic and diastolic blood pressure was higher in groups II as compared to group I and III (p < 0.001). The percentage of sclerotic glomeruli, tubular injury and interstitial inflammation in group II were significantly greater than group I and III (p < 0.001).

Conclusion: Type-II diabetic patients undergoing renal biopsy for impaired renal functions constituted a heterogeneous group of renal damage. This study emphasized the usefulness of renal biopsy for determining the pattern of renal damage that would aid in the overall management of the patients.

Key words: Type-II diabetes. Diabetic nephropathy. Diabetic glomerulosclerosis. Renal biopsy.

INTRODUCTION
Renal involvement is a common complication in type-II Diabetes mellitus and it has emerged as the leading cause of end stage renal disease. The increased incidence of chronic renal failure is related to a rapidly rising prevalence of diabetes worldwide.¹,² Besides diabetic nephropathy, type-II diabetic patients are prone to develop non-diabetic renal disease.³,⁴ Microalbuminuria is the first clinical manifestation of diabetic renal involvement which evolves into asymptomatic overt proteinuria, and later into nephrotic syndrome. Hypertension often accompanies proteinuria that eventually progress within a few years to end stage renal disease in more then 50 percent of the cases.⁵ Renal biopsies from type-II diabetic patients with proteinuria or renal insufficiency showed a heterogeneous pattern of renal disease. It has been estimated that upto one-third of diabetic patients presented with impaired renal functions are suffering from non-diabetic renal diseases, which reported to have better prognosis than diabetic nephropathy.³,⁴ To our knowledge, there is no such study in the local population where histopathological evaluation of renal biopsy has been performed in type-II diabetic patients.

The aim of this study is to determine the outcome of renal damage in type-II diabetic patients who underwent renal biopsy for impaired renal functions and its importance in overall patient management.

METHODOLOGY
All patients of type-II Diabetes mellitus were included who underwent renal biopsy. A total of 73 cases were identified and retrieved from the file of the Histopathology Department of The Kidney Postgraduate Centre, Karachi, during the period of January 2000 to May 2005. Type-II Diabetes mellitus is defined as a “condition in which patients do not depend on insulin for immediate survival and rarely develop ketoacidosis, except under conditions of great physical stress”.⁶

The inclusion criteria of renal biopsies in the present study were a good length tissue core containing 5-10...
glomeruli and also exhibiting obvious morphological changes in the glomeruli, tubulo-interstitial and blood vessels. The biopsies containing less than 4 glomeruli or showing end stage nephrosclerosis were excluded.

Renal biopsies were performed by the clinicians in type-II diabetic patients when a renal disease other than diabetic nephropathy was suspected because of the presence of haematuria, nephrotic syndrome, non-nephrotic proteinuria less than 3 g/d in the absence of retinopathy, rapidly progressive renal failure, and renal insufficiency of unknown origin. However, if a patient had a long history of diabetes with clinical evidence of multi-organ disease, such as retinopathy, the diagnosis of diabetic nephropathy was considered and renal biopsy was not performed.

In most cases, renal tissue was obtained by needle biopsy, and they were separately processes for light microscopy and immunofluorescence microscopy. For light microscope, 2 μm thick serial sections were cut (on 10 glass slides) from paraffin embedded tissue. Sections were stained with hematoxylin and eosin, Masson’s trichome, periodic acid-Schiff, silver methamine, and when required by Congo red stain. The immunofluorescence staining was performed on 4 μm thick cryostat sections obtained from snap-frozen tissue or on paraffin embedded tissue sections. The sections were tested against human immunoglobulin G (IgG), IgA, IgM, C3c, C1q, fibrinogen and kappa (k) and lambda (λ) light chains.

Cases were divided into three groups according to histopathological findings. Group I was characterized by diabetic glomerulosclerosis (DGS) only, group II by the prevalence of vascular changes and group III was subdivided into two sub-groups: IIIa with DGS co-existing with non-diabetic renal disease and IIIb being non-diabetic renal disease without DGS.7

In group I, the Kimmelstiel-Wilson nodule was considered characteristic for DGS. In the absence of the Kimmelstiel-Wilson nodule, DGS was established by a combination of morphological features of diffuse mesangial expansion, basement membrane thickening, insudative glomerular lesions such as fibrin caps and capsular drops, arteriolar hyalinosis and linear IgG positivity along glomerular basement membrane. Immune complex glomerulonephritis was diagnosed separately even if the patient had evidence of diabetic glomerulosclerosis. Group II of this study was characterized by the prevalence of severe ischemic changes affecting glomeruli, associated with marked arteriosclerosis and chronic tubulo-interstitial damage. Typical diabetic changes of diffuse or nodular glomerulosclerosis were not evident. However, the presence of glomerular changes attributable to a mild form of DGS and hyalinosis of arterioles in association with ischemic lesions were included in group II patients. Group III was characterized by the presence of non-diabetic renal disease evident on light microscopy and immunofluorescence microscopy either accompanied with DGS (IIIa) or without features of DGS (IIIb).

Renal tissue damage was assessed by percentage of sclerotic glomeruli, the score of mesangial expansion, tubular injury and infiltrating cells in the interstitium. The percentage of sclerotic glomeruli was calculated by counting the number of sclerotic glomeruli and the total number of glomeruli in the renal biopsy tissues. Tubular injury was also evaluated semi quantitatively using a four grade system as follows. In grade I, only a few injured tubules were noted, grade II represented tissues with damaged tubules limited to less than 10% of the total tubular area, grade III represented those with tubular injury limited to > 10-20% of the total tubular area and grade IV represented severe tubular injury with damaged tubules exceeding 20% of the total tubular area.8 The extent of infiltrating cells in the interstitial area were also evaluated semi quantitatively using a four grade system as follows; grade I represented the presence of only a few infiltrating cells in the interstitial area, grade II represented the presence of infiltrating cells amounting to less than 10% of the total interstitial area, grade III represented the presence of infiltrating cells amounting to > 10-20% of the total interstitial area and grade IV represented the presence of infiltrating cells amounting to > 20% of the total interstitial area. Furthermore, the relationship between clinical parameters and histopathological findings were examined.

Statistical analysis was performed using SPSS version 10.0 for windows. Data are expressed as mean±SD. Frequency and percentage were computed for qualitative and categorical variables (gender and percentage of the patients). For parametric data, Student’s t-test and ANOVA were used to compare two and multiple parameters respectively like age, duration of diabetes, serum creatinine, blood pressure, proteinuria and the percentage of sclerosed glomeruli. For non-parametric data, Mann Whitney and Kruskal Wallis tests were used to compare two or multiple parameters, respectively like degree of tubular injury and degree of cell infiltration. A chi-square test was applied on variables such as gender and haematuria at renal biopsy. P-value < 0.05 was considered as statistically significant.

RESULTS

Table I lists, the clinical and biochemical characteristics which were observed in type-II diabetic patients at the time of renal biopsy. Among the 73 patients studied 20 (27.3%) had diabetic glomerulosclerosis alone (group I), 17 (23.3%) showed prevalence of vascular changes
Renal biopsy in type-II diabetic

glomerulonephritis. MCD: Minimal change disease; TIN: Tubulo-interstitial nephritis; CrGN: Crescentic
renal disease DGS with DGS

Types of non-diabetic Coexisting with Not coexisting Total (%)

** Significant difference between group II and III.
^ Significant difference between group I and III.
* Significant difference between group I and II.
p < 0.05 is considered to be statistically significant.

Significant difference between group I and II.

Significant difference between group I and III.

Significant difference between group II and III.

DISCUSSION

In this study, the pattern of renal damage was observed in patients of type-II diabetes who were biopsied for impaired ren al functions. According to the morphological criteria, the renal biopsies in this series of 73 patients with type-II diabetes permitted the distinction of three groups of renal lesions associated with different prognostic features.

The reported frequency of non-diabetic renal diseases in the literature varies from 9-81%.9 This wide variability is not easy to explain. However, it may relate to selection criteria in different institutions and populations being studied. Some of the earlier studies are not supported by morphological data and, therefore, are not able to clarify this question. When morphological data are available as in two series from Denmark and Finland, the frequency of renal diseases other than diabetic nephropathy alone or superimposed on diabetic glomerulosclerosis (DGS) ranged from 9-18%.3,10 In a study of patients with type-II diabetes in India, the frequency was 81%.9 Similarly, the pattern of distribution of renal damage in type-II diabetes patient is also variable in different studies, and this is also attributed to the design and policy adapted. IgA nephropathy was most frequent (44%) in a study conducted in Japan,8 while non-diabetic glomerulopathy and Tubulo-Interstitial Nephritis (TIN) were almost in equal proportions in a research carried out in India.4 In the present data, Minimal Change Disease (MCD) and/or focal segmental glomerulosclerosis (FSGS) were the most common NDRD. These results are similar to a study conducted in USA, which reported FSGS (21%), the most common lesion in patients with type-II diabetes followed by MCD (15.3%).11 Focal segmental glomerulosclerosis and MCD have also been described as the commonest glomerulopathy in non-diabetic patients who were evaluated for impaired renal functions.12 The adverse renal outcome in type-II diabetic patients in non-diabetic renal diseases depends upon the specific type of renal lesions. Non-diabetic renal diseases with DGS have a significantly worse renal outcome than those without DGS,13,14 Furthermore, it is also known that proliferative glomeru-
lonelphritis, MCD and perhaps membranous glomerulonephritis (MGN), FSGS and some forms of IgA nephropathy might be favourably influence by the therapy if not accompanied with DGS. It is likely that the detection of such histological patterns and the subsequent appropriate therapeutic approach would determine a better outcome in such patients.15

Proteinuria of more than 2 gm/day is known to be associated with disease progression and adverse renal outcome.16 The magnitude of proteinuria probably reflects the severity of renal disease, and proteinuria per se is tubulotoxic. The presence of proteinuria of more than 2 gm/day was noted in all the three groups of type-II diabetic patients in this study, which is in accordance with studies from Italy and China.7-14 They showed that the degree of proteinuria is an independent predictor for adverse renal outcome among type-II diabetic patients, despite potential different patterns of renal disease among different races.

Blood pressure control is of primary importance in the prevention of progression of renal disease in both diabetic and non-diabetic renal disease. In addition, hypertension in the presence of proteinuria can accelerate the decline in the glomerular filtration rate and progress to end stage renal disease.5 The levels of systolic and diastolic blood pressures were high in all three groups of type-II diabetic patients in this study, but they were significantly higher in patients of group I and II. Renal vascular changes also contribute to hypertension in 20% of patients with type-II Diabetes and up to 40% of those with overt nephropathy. High blood pressure accelerates the progressive increase of proteinuria in type-II Diabetes and also increases the loss of renal function in those with overt nephropathy.17

The significantly high systolic and diastolic blood pressures and proteinuria in group II of this type-II diabetic patients may have been related to the presence of vascular changes of Diabetes mellitus. Therefore, emphasis is given on antihypertensive therapy in patients with type-II Diabetes to slow down or prevent the progression of diabetic nephropathy.18,19

The urinary abnormality such as haematuria in the absence of urinary tract infection is one of the indicators of renal damage in diabetic nephropathy as well as in NDRD. However, if haematuria is noted with or without proteinuria in the earlier coarse of type-II Diabetes, it strongly raises the possibility of NDRD.20 This study also showed that the presence of haematuria with short duration of diabetes constitute a sensitive marker of NDRD and is, thus, a strong indicator for renal biopsy.

The pathological hallmarks of diabetic nephropathy are increased thickness of Glomerular Basement Membrane (GBM) and mesangial expansion. The latter is considered more significant than thickening of GBM since expansion has been reported to ultimately lead to renal insufficiency.21 Several studies have demonstrated a close relationship between the extent of glomerular mesangial expansion in Diabetic Nephropathy (DN) and deterioration of serum creatinine level as well as the severity of proteinuria.22,23 This was observed in group II diabetic patients of this study. Furthermore, it has also been shown that tubulo-interstitial lesions such as tubular injury and/or infiltrating cells in the interstitial area play an important role in the progression of DN.24 In this study, the proportion of sclerotic glomeruli, the score of mesangial expansion, tubular injury and infiltrating cells in group II were significantly greater than groups I and III. These morphological changes were correlated with significantly high serum creatinine in group II diabetic patients. In this regard, Bohle et al. indicated that the renal survival rate was highest in type-II diabetic patients with histologically normal renal tubules and interstitial area but it was low in those with interstitial inflammation and fibrosis.25 Taken together, the results of histopathological changes in type-II diabetic patients, the renal biopsy is useful for the detection and prediction of progression of DN.

CONCLUSION

This study has emphasized that among the patients with type-II Diabetes; renal complications may be due to heterogeneous non-diabetic lesions. These non-diabetic lesions may occur alone or may be superimposed on underlined DGS, which are associated with different renal outcome and probably benefited with treatment modalities. Renal biopsies of type-II diabetic patients with DGS and with prevalence of vascular changes are at the highest risk for adverse renal outcome. The results suggest that renal biopsy is useful in the overall management of patients with type-II Diabetes.

REFERENCES

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