Histological Spectrum of Clinical Kidney Disease in Type 2 Diabetes Mellitus Patients with special Reference to nonalbuminuric Diabetic Nephropathy: A Kidney Biopsy-based Study

Jai Prakash1, Prem Shankar Patel2, Mohd Iqbal3, Shiv Shankar Sharma4, Shivendra Singh5, Neeraj K Agrawal6, Usha Singh7

ABSTRACT

Background: Diabetic nephropathy (DN) is an important and catastrophic complication of diabetes mellitus (DM). Kidney disease has heterogeneity in histology in diabetes patients and includes both diabetic kidney disease (DKD) (albuminuric or nonalbuminuric) and nondiabetic kidney disease (NDKD) either in isolation or in coexistence with DN. Diabetic nephropathy is hard to overturn. While NDNKD is treatable and reversible.

Materials and methods: We enrolled a total of 50 type 2 diabetes mellitus (T2DM) patients with clinical kidney disease, of both genders and age >18 years, who underwent kidney biopsy from October 2016 to October 2018. Patients with proteinuria <30 mg per day were excluded from the study. The indications of the renal biopsy were nephrotic syndrome (NS), active urinary sediment, rapid decline in renal function, asymptomatic proteinuria, and hematuria.

Result: A total of 50 (males: 42 and females: eight) patients with T2DM who underwent kidney biopsy were enrolled. The clinical presentation was: NS 26 (52%), chronic kidney disease (CKD) 11 (22%), asymptomatic proteinuria and hematuria six (12%), acute kidney injury (AKI) four (8%), and acute nephritic syndrome (ANS) three (6%). Diabetic retinopathy (DR) was noted in 19 (38%) cases. Kidney biopsy revealed isolated DN, isolated NDNKD, and NDNKD superimposed on DN in 26 (52%), 14 (28%), and 10 (20%) cases, respectively. Idiopathic membranous nephropathy (MN) (4) and amyloidosis (2) were the most common forms of NDNKD, whereas diffuse proliferative glomerulonephritis (DPGN) was the main form of NDNKD superimposed on DN. Diabetic nephropathy was observed in 15 (79%) cases in presence of DR and also in 11 (35.5%) cases even in absence of DR. Of eight patients with microalbuminuria four (50%) cases have biopsy-proven DN.

Conclusion: About 48% of patients had NDNKD either in isolation or in coexistence with DN. Diabetic nephropathy was found in absence of DR and in patients with a low level of proteinuria. The level of proteinuria and presence of DR does not help to distinguish DN vs NDNKD. Hence, renal biopsy may be useful in selected T2DM patients with clinical kidney disease to diagnose NDNKD.

INTRODUCTION

Diabetes mellitus is an important problem globally. The incidence of T2DM is rapidly rising and accounts for 90% of all cases. Type 2 diabetes mellitus leads to damage to multiple organs, out of which DN is a life-threatening complication. Diabetic nephropathy is not the only renal manifestation of DM. The range of kidney diseases in DM includes both DKD (albuminuric and albuminuric) and NDNKD. Recently, a new entity called nondiabetic membranous DKD has been described in diabetes patients and is characterized by nonalbuminuric microalbuminuria with low glomerular filtration rate (GFR). In a patient with long-standing diabetes (both type 1 and type 2) initial GFR loss may occur in the absence of albuminuria and this is more frequent in T2DM. Approximately one-fourth of type 1 diabetes mellitus (T1DM) or T2DM have been found to have nonalbuminuric or microalbuminuric CKD. Nondiabetic kidney disease may occur either in isolation or in coexistence with DN. The prevalence and spectrum of NDNKD in DM are extremely variable. Although the common cause of CKD in diabetes is DKD, the diagnosis of NDNKD is a must because various NDNKD is often plausibly treatable, reversible, and has a good prognosis. Hence, renal biopsy is the gold standard and should be considered in type 2 diabetes patients where the renal manifestation is atypical and with clinical suspicion of NDNKD. At present, data on the prevalence and range of NDNKD in patients with T2DM are limited from our country. With this background knowledge, the present study was conducted to know the histopathological spectrum of kidney disease in T2DM at our center.

This prospective study was carried out from October 2016 to October 2018. The study was approved by the institute’s ethics committee (ECR/526/Inst/UP/2014 Dt. 31.1.14, NO. Dean/2015-16/EC/527 Dated: 19.01.2017). The T2DM patients of both gender (male and female) and age >18 years with clinical kidney disease, who underwent kidney biopsy during the study period were included in the study. Patients aged <18 years, 24-hour urinary protein <30 mg per day, renal dysfunction secondary to extrarenal cause (hypovolemia, ureteric obstruction, or urinary tract infection), having contraindication for kidney biopsy, and lack of informed consent were excluded from the study. Diabetes mellitus was diagnosed as per World Health Organization criteria. The clinical kidney disease was diagnosed by the presence of proteinuria >30 mg per day or acute and chronic renal dysfunction (serum creatinine > 1 mg/dL), estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m², or active urinary sediment. Acute kidney injury, CKD, and NS were defined as per standard guidelines. All patients were subjected to a detailed and meticulous history and physical examination. Optic fundi were examined in all patients for evidence of DR. Urine analysis, 24-hour urinary protein estimation, complete blood count, renal function test, liver function test, lipid profile, immunological profile (RA factor, C3, C4, CRP), and antinuclear antibodies were estimated. The indications of the renal biopsy were nephrotic syndrome (NS), active urinary sediment, acute kidney injury, asymptomatic proteinuria, and hematuria. Acute kidney injury, CKD, and NS were defined as per standard guidelines. All patients were subjected to a detailed and meticulous history and physical examination. Optic fundi were examined in all patients for evidence of DR. Urine analysis, 24-hour urinary protein estimation, complete blood count, renal function test, liver function test, lipid profile, immunological profile (RA factor, C3, C4, 

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ANA, anti-dsDNA antibody, PR3 ANCA, MPO ANCA, and anti-GBM Ab), hepatitis B surface antigen, hepatitis C virus, and HIV were performed in all patients. On basis of 24-hour urinary protein excretion, levels of proteinuria were categorized into microalbuminuria (30–300 mg/day), subnephrotic proteinuria (300–3500 mg/day), and nephrotic range proteinuria (>3500 mg/day). Serum protein electrophoresis was done in the selected case as and when required.

We performed renal biopsy in patients who had been referred to or attended nephrology outpatient department with clinical evidence of kidney disease and met the indications of renal biopsy. The indications of the renal biopsy were: NS, active urinary sediments, rapid decline in renal function, and asymptomatic proteinuria and hematuria. The renal biopsy sample was preserved in a 10% buffered aqueous formaldehyde solution for light microscopy. The sample was studied under light microscopy using hematoxylin and eosin stain, periodic acid-Schiff stain, acid fuchsin orange G, and periodic acid silver methenamine stain. Methyl violet and congo red staining were done on suspicion of amyloidosis on light microscopy (extracellular organized deposits in glomeruli). Electron microscopy and immunofluorescence studies were done. Based on renal histopathology, kidney diseases were categorized into isolated DN, isolated NDKD, and NDKD superimposed on DN (mixed lesion).

**RESULT**

Of 50 patients (males: 42 and females: eight), male to female ratio was 5.2:1. The mean age of the subject was 52 ± 9.8 years with a range of 26–70 years. The average 24-hour urinary protein of 47 patients was 4.37 gm. Three patients had anuria. The clinical presentation in these patients was: NS 26 (52%), CKD 11 (22%), asymptomatic proteinuria and hematuria six (12%), AKI four (8%), and ANS three (6%) (Table 1). Diabetic retinopathy was observed in 19 (38%) cases and 31 (62%) cases had no evidence of DR. The most common indication of kidney biopsy was NS in 26 (52%) patients. Kidney biopsy revealed isolated DN, isolated NDKD, and NDKD superimposed on DN in 26 (52%), 14 (28%), and 10 (20%) cases, respectively. Idiopathic MN (4) and amyloidosis (2) were the most common cause of isolated NDKD (Table 2). Categories of NDKD in the mixed lesion were DPGN (6), followed by thrombotic microangiopathy (TMA) (2), pauci-immune glomerulonephritis (GN), and vasculitis in one case each (Table 2). Of 11 diabetes patients with CKD; six (54.4%) had isolated DN, isolated NDKD in four (36.3%), and one patient had a mixed lesion. Of six diabetic patients with asymptomatic urinary abnormalities, isolated DN was observed in four (66.6%) cases. Isolated NDKD and NDKD superimposed on DN were noted in one patient. Nondiabetic kidney disease superimposed on DN was the most common lesion in patients with AKI in three (75%) and ANS in three (100%) (Table 3, Fig. 1). Of eight cases with microalbuminuria, four (50%) had DN (isolated DN three and mixed lesion one) and remaining four (50%) patients had isolated NDKD. Similarly, in nine cases with subnephrotic proteinuria, isolated DN, isolated NDKD, and NDKD superimposed on DN were observed in four (44.4%), two (22.2%), and three (33.3%) cases, respectively. In 30 cases with nephrotic range proteinuria, 19 (63.3%) patients had isolated DN, eight (26.7%) had NDKD, and the remaining three (10%) cases showed NDKD superimposed on DN (Table 4).

We noted isolated DN in 10 (43.5%) patients with diabetes of <5 years; while in the remaining cases isolated NDKD and NDKD superimposed on DN were observed in nine (39.1%) and four (17.4%) cases, respectively. We observed isolated DN as a predominant lesion in eight (66.7%) patients with diabetes of >10 years. However, we even noted NDKD either alone or superimposed on DN in the remaining four (33.3%) cases with diabetes of >10 years. A majority (79%) of patients with DR had isolated DN. However, NDKD either alone or in mixed form was observed in four (21%) patients in presence of DR. We noted biopsy-proven isolated DN in 11 (35.5%) patients in absence of DR. Twenty patients without DR had isolated NDKD in 12 (38.7%) and NDKD superimposed on DN in eight (25.8%). Thus, DN was the predominant lesion in presence of DR (79%), while a majority (64.5%) of patients had NDKD either alone or in mixed form in the absence of DR (Table 5).

**DISCUSSION**

Although T2DM patients often encounter DKD, they may have other kidney diseases, which are histologically unrelated to diabetes and are called NDKD. Interestingly, the prevalence of NDKD in T2DM patients is widely variable and it depends on demographic features and criteria used for the selection of the population being studied.13−15 The mean age of the patients was 52 ± 9.8 years, which is almost similar to other studies.16−18 Gender distribution was male to female ratio 5.2:1, which is similar to other studies.16 The average 24-hour urinary protein excretions were 4.37 g in our patients. Our observation of 24-hour urine protein excretions was similar to other studies.16,18 In our patients, NS was

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**Table 1:** Renal manifestation of T2DM patients (*n* = 50)

| Sl. no. | Clinical syndrome | *n* (%)
|---------|------------------|-------|
| 1.      | NS               | 26 (52)
| 2.      | CKD              | 11 (22)
| 3.      | Asymptomatic proteinuria and hematuria | 6 (12)
| 4.      | AKI              | 4 (8)
| 5.      | ANS              | 3 (6)

**Table 2:** Spectrum of NDKD in type 2 diabetes patients (*n* = 24)

<table>
<thead>
<tr>
<th>Type of isolated NDKD</th>
<th><em>n</em> (number)</th>
</tr>
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<tbody>
<tr>
<td>MN</td>
<td>4</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>2</td>
</tr>
<tr>
<td>Membranoproliferative GN</td>
<td>1</td>
</tr>
<tr>
<td>Lupus nephritis (LN)</td>
<td>1</td>
</tr>
<tr>
<td>DPGN</td>
<td>1</td>
</tr>
<tr>
<td>Mesangiproliferative GN</td>
<td>1</td>
</tr>
<tr>
<td>Hypertensive nephropathy</td>
<td>1</td>
</tr>
<tr>
<td>Xanthogranulomatous pyelonephritis</td>
<td>1</td>
</tr>
<tr>
<td>Chronic tubulointerstitial nephritis</td>
<td>1</td>
</tr>
<tr>
<td>TMA</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of superimposed NDKD</th>
<th><em>n</em> (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DN + DPGN</td>
<td>6</td>
</tr>
<tr>
<td>DN + TMA</td>
<td>2</td>
</tr>
<tr>
<td>DN + pauci-immune GN</td>
<td>1</td>
</tr>
<tr>
<td>DN + vasculitis</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3:** Type of nephropathy in patients with various renal syndrome (*n* = 50)

<table>
<thead>
<tr>
<th>Clinical renal syndrome</th>
<th>DN <em>n</em> (%)</th>
<th>NDKD <em>n</em> (%)</th>
<th>DN + NDKD <em>n</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (<em>n</em> = 26)</td>
<td>16 (61.5)</td>
<td>8 (30.7)</td>
<td>2 (7.6)</td>
</tr>
<tr>
<td>CKD (<em>n</em> = 11)</td>
<td>6 (54.4)</td>
<td>4 (36.6)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Asymptomatic proteinuria and hematuria (<em>n</em> = 6)</td>
<td>4 (66.6)</td>
<td>1 (16.6)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>AKI (<em>n</em> = 4)</td>
<td>Nil</td>
<td>1 (25)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>ANS (<em>n</em> = 3)</td>
<td>Nil</td>
<td>Nil</td>
<td>3 (100)</td>
</tr>
</tbody>
</table>
the most common presentation noted in 26 (52%) patients. Nephrotic syndrome was present in 60.9% of cases in a previous study from the same center.13 In another study chronic renal failure was the most common clinical presentation (47%) followed by ANS (18.7%) and NS (15.6%) in diabetic patients with NDKD.6 However, in other studies also most frequent clinical presentations of diabetic patients with NDKD were NS, rapidly progressive renal failure, and AKI.20 Our observation with regards to clinical presentation is corresponding with the majority of the published studies.5,9 The decision for renal biopsy in proteinuric T2DM patients has not been defined and a decision is usually individualized.11,22 A systematic analysis of 48 studies, including 4,876 diabetic patients indicates that in diabetes patients with clinical suspicion of DN, the prevalence of NDKD is indeed very high (up to 82.9%) of the overall diagnosis.13 Hence, kidney biopsy should be considered for diagnostic purposes in diabetic patients with atypical clinical presentations and on suspicion of NDKD.

On kidney biopsy, we observed isolated DN in 26 (52%), isolated NDKD in 14 (28%), and NDKD superimposed on DN in 10 (20%) cases. The author had reported that the prevalence of isolated DN ranged from 56.3 to 87.6% in patients with T2DM in his previous study.6,19 In other studies, the reported prevalence of isolated DN in patients with T2DM were ranging from 27.5 to 61.5%.23 In a meta-analysis of 48 studies, it is revealed that the prevalence of DN was extremely variable, ranging from 6.5 to 94%.15 The reported frequency of NDKD either alone or superimposed on DN is widely variable from 13 to 53% of the total kidney biopsies.10,15 The wide variation in the frequency of NDRD in various studies is due to a policy of renal biopsy criteria, and regional and/or racial variations of the different study population.6,11,16,23 In isolated NDKD categories, our observation revealed that idiopathic MN four (28.5%) and amyloidosis two (14.3%) were the most common cause of NDKD. Diﬀuse proliferative glomerulonephritis was the most common [six (60%)] NDKD in mixed lesions. In an earlier study from the same center, the author reported MN as the most common NDKD in 12.9% of proteinuric type 2 diabetic patients.16 The prevalence of MN in patients with T2DM is variable and ranges from 11.9 to 30%.23 However, in other studies IgA nephropathy, focal segmental glomerulosclerosis,18 and minimal change disease were the common NDKD. Hence, variation in prevalence and type of NDKD in diabetes is considerably high. On basis of our observation, it is clear that type 2 diabetes patients presenting with NS or CKD, or asymptomatic proteinuria had isolated DN in approximately two-thirds of cases and isolated NDKD or NDKD superimposed on DN in the remaining one-third cases. We performed kidney biopsy on the patients with T2DM who presented with AKI when renal failure was unexplained and there is clinical suspicion of NDKD characterized by proteinuria >30 mg/day or active urinary sediments. We noted that NDKD either in isolation or in coexistence with DN was the main (up to 100%) cause of rapid deterioration of renal function in type 2 diabetes patients presenting with AKI or ANS. In the four patients with AKI, the presumed diagnosis was NDKD and renal biopsy showed the presence of DPGN in all patients (one isolated NDKD and three superimposed NDKD). Diabetic kidney disease is characterized by slowly progressive renal failure. A rapid decline in renal function over days or weeks is not a feature of DKD and should trigger a search for NDKD.

We observed that isolated DN was more common (66.7%) in patients with diabetes of >10 years than the patients with diabetes of <5 years [10 (43.5%)]. We noted NDKD either alone or superimposed on DN was more common than DN (56.5 vs 43.5%) in patients with diabetes of <5 years. However, the prevalence of NDKD either alone or superimposed on DN was lower (33.3%) in patients with diabetes of >10 years. Similarly in a previous study author had reported that NDKD was more common than DN (12.9 vs 3.2%)
in patients with diabetes of <5 years and DN was more common than NDKD (32.2 vs 6.5%) in patients with diabetes of >10 years.14 Thus, our observation supports the result of other studies, indicating that a longer duration of diabetes is associated with a greater likelihood of DN and a shorter duration of diabetes is associated with a greater likelihood of NDKD.16

It is widely accepted that the first clinical pointer of DN is increased urinary albumin excretion. However, growing evidence has suggested that a significant number of T2DM patients have low GFR without significant albuminuria and are known as nonalbuminuric DKD. Biopsy studies in diabetic patients with normoalbuminuria and low eGFR revealed histological features of advanced diabetic glomerular lesion than in patients with preserved eGFR.27 We observed, that of eight cases with microalbuminuria, four (50%) had DN (isolated three and mixed one) and the other four (50%) had isolated NDKD. Although the prevalence of DKD increases with an increase in the degree of proteinuria, the reverse is not true and biopsy-proven DKD can occur in a patient with normo or microalbuminuria.27 These data do not support the classical model of progression of DKD. Thus, our observation revealed that the level of proteinuria does not help to distinguish between DN and NDKD, and proteinuria is a bad predictor of the type of nephropathy in T2DM patients.

Diabetic retinopathy is found in almost all T1DM with DN, while only 50–60% of cases with T2DM and DN have DR.27 We observed DR was present in 19 (38%) patients; whereas it was absent in 31 (62%) patients with T2DM. Biopsy proved DN was noted in 11 (35.5%) patients in absence of DR. The author observed that four patients (25%) with biopsy-proven DN did not have evidence of DR in the previous study.13 Our observation supports the other published studies.27 Thus, the absence of DR cannot rule out DN because various studies had demonstrated that a high proportion (50–70%) of cases with DN do not have DR.28,29,30 We noted NDKD either in isolation or in coexistence with DN in four (21%) patients in the presence of DR. Other studies also reported that 31% of patients with NDKD had background DR.6 Recently one meta-analysis revealed that the sensitivity and specificity of DR in predicting DN were only 65% (95% CI 0.62–0.68) and 75% (95% CI 0.73–0.78), respectively.30 Thus, DR is a poor/bad predictor of the type of nephropathy in diabetes patients. Hence, the presence of DR may support the diagnosis of DKD but does not exclude NDKD.

**CONCLUSION**

The incidence of NDKD either alone or superimposed on DN was high (48%). Four out of eight (50%) patients with microalbuminuria had biopsy-proven DN. The presence of DR and degree of proteinuria is a poor/bad predictor of DN. Early diagnosis of NDKD in T2DM with help of kidney biopsy is justified; because NDKD is a treatable/curable form of kidney disease with a good prognosis.

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