Histopathologic and clinical features of diabetic nephropathy alone and with concomitant nondiabetic renal diseases

Sk Md Jaynul Islam[1*], Shamoli Yasmin[1], Ishtyiaque Ahmed[1], Wasim Md Mohosinul Haque[2]

[1]*Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka, Bangladesh;* [2]*Department of Nephrology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh*

Abstract

Background and objective: Diabetic nephropathy (DN) is a major complication of diabetes mellitus (DM) and one of the leading causes of end-stage kidney disease. The aim of the present study was to evaluate the histomorphological and clinical profiles of DN and associated non-diabetic renal dieases (NDRD) in diabetic patients.

Materials and methods: The study was carried out at the Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Dhaka, from July 2019 to December 2020. Renal biopsy samples from known diabetic patients were included in the study. The formalin-fixed tissues were stained with haematoxylene & eosin (H&E), Periodic acid Schiff (PAS), Masson Trichrome (MT) and Jones Methanamine Silver (JMS) stains. Tissues were stained for IgG, IgA, IgM, C3, C1q, kappa and lambda for direct immunofluorescence (DIF) study. DN was histologically classified according to Tervaert classification system. Interstitial fibrosis and tubular atrophy (IFTA) as well as arteriolar hyalinization scoring was also done. Clinical information was retrieved from the patient's information sheet.

Results: Total 46 biopsy samples from DN cases were included in the study. The mean age of the cases was 46.76 ± 10.63 years, including 36 males and 10 females. The most common clinical presentation was nephritic range proteinuria (n=32, 69.56%). Among all, 27 (58.69%) patients had haematuria. The mean serum creatinine level was 4.28 ± 2.61 mg/dl, and 80.43% had serum creatinine levels >1.5 mg/dl. Histopathologic examinatiom revealed type III DN in 26 (56.5%) and type IV DN in 11 (23.9%) cases. IFTA score 1 (<25%) was seen in 20 (43.5%), score 2 (25-50%) in 19 (41.3%) and score 3 (>50%) in 7 (15.2%). Vascular hyalinization score-2 in 25 (54.3%), score-1 in 14 (30.4%) and score-0 in 7 (15.2%). DN class II, III and IV were associated with high urinary total protein (UTP) and serum creatinine levels. Among the histologic changes, percentage of glomerular sclerosis, the mean IFTA score and vascular hyalinization score were found to be highest in class IV DN, and all were significantly associated with histologic glomerular DN classes (p= <0.05). Of the total cases, 21 (45.65%) were found with nondiabetic renal diseases (NDRD), the most common feature was focal segmental glomerulosclerosis (FSGS) (26.57%), followed by IgA nephropathy and post-infectious glomerulonephritis (PIGN). Among 46 cases, one post-transplant biopsy was included, which revealed class II DN along with features of calcineurin inhibitor toxicity.

Conclusion: Tervaert's histologic classification of our cases revealed class III DN lesions as the predominant one, and the classes had a significant association with age of the patient, serum creatinine level, mean IFTA, arteriolar hyalinization and NDRD. Among the NDRD, FSGS was the most common pathology.

*Correspondence: *Sk Md Jaynul Islam, Department of Histopathology, Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka, Bangladesh. Email: jaynul.islam@gmail.com*

Introduction

Diabetic nephropathy (DN) is a major complication of diabetes mellitus (DM) and one of the leading causes of end-stage kidney disease [1]. DN develops in 30% of patients with insulin-dependent DM (type-1) and in 40% with non-insulin-dependent type-2 DM [2]. DN is a clinical syndrome characterized by persistent albuminuria and progressive decline in renal function, and the term refers to the presence of a typical pattern of glomerular disease. DN is reported to occur in 20% to 50% of those with diabetes and is the commonest cause of end-stage kidney disease (ESKD) in different populations, accounting for 28% of those commencing renal replacement therapy (RRT) in the United Kingdom, with corresponding figures of 44% in the United States and 38% in Australia [3,4].

Diagnosis of DN is commonly made by clinical findings. Kidney biopsies are performed less frequently in patients with DM than in other patients with proteinuria and are generally carried out in patients with atypical clinical and laboratory features. Indications for kidney biopsy in patients with diabetes are mostly determined by the attending physician and policies of the country or the institution [5]. The natural course of DN has traditionally been initial glomerular hyperperfusion followed by microalbuminuria, overt proteinuria, and eventually progressive renal dysfunction. Isolated proteinuria, which is likely to be caused by DN, is not an indication for renal biopsy, as pathological confirmation of DN rarely provides additional information regarding the management of patients. However, several studies have suggested that non-diabetic renal disease is common in diabetic patients, ranging from 27% to 79% among patients undergoing renal biopsy [6-10].

According to the International Diabetes Federation (IDF) Diabetes Atlas, there are an estimated 8.4 million people with diabetes in Bangladesh. The IDF projected that the number of people with diabetes will increase to 16.8 million by 2030, placing Bangladesh among the top ten countries globally [11]. Several recent studies have reported DN as a major complication of DM, ranging from 6.4% to 8.6% [12,13]. Most of these studies on DN carried out in Bangladesh focused on clinical parameters and the impact of different risk factors [14, 15]. So far, no study has been reported from Bangladesh on renal biopsy findings of DN cases. In the present study, we evaluated the histomorphological and clinical profiles of of DN cases and associated non-diabetic renal diseases.

Materials and methods

This cross-sectional study was carried out at the Department of Histopathology, Armed Forces Institute of Pathology, Dhaka, a referral diagnostic center of Bangladesh, from July 2019 to December 2020. The study was approved by the concerned authority. Renal biopsy samples of known diabetic patients received during the period with a history of proteinuria, haematuria/renal dysfunction were included in the study. For each patient, two samples of the renal core biopsy were received, one in 10% formalin for histopathological examination and another one in cold normal saline/Michel's transport medium for direct immunofluorescence (DIF) study. The formalin-fixed tissues underwent routine tissue processing followed by paraffin block preparation. Tissues were stained with haematoxylene & eosin (H&E), Periodic acid Schiff (PAS), Masson Trichrome (MT) and Jones Methanamine Silver (JMS) stains. In certain suspected cases, Congo red staining was done. For the DIF study, tissue from each sample was stained for IgG, IgA, IgM, C3, C1q, Kappa and Lambda. Clinical presentation and investigation findings were retrieved from the patient's information sheet accompanying the respective sample.

Two competent histopathologists made the final histological diagnosis after meticulous observation of all the stained histopathology slides, DIF study and consideration of clinical presentations and laboratory investigations. The suboptimal number of the glomerulus in the paraffin section and/or no glomerulus in the DIF study were considered as inadequate specimens. DN has been histologically classified into four glomerular classes, class I to IV according to Tervaert classification (Table-1) [16]. Interstitial fibrosis and tubular atrophy (IFTA) scoring was done as, No IFTA= 0, IFTA <25%=1, IFTA 26-50%= 2, IFTA >50%=3. Similarly, vascular hyalinization scoring was done as no hyalinization= 0, single arteriolar involvement=1, more than one arteriolar involvement=2.

Table-1: *Histologic glomerular classes* according to Tervaert classification *[16]*

Class	Description	Inclusion criteria
Class -I	Mild or nonspecific light microscopic (LM) changes and electron microscope (EM) proven GBM thickening	Biopsy does not meet any of the criteria mentioned below for class II, III, or IV GBM 395 nm in female and 430 nm in male individuals
Class- IIa	Mild mesangial expansion	Biopsy does not meet criteria for class III or IV. Mild mesangial expansion in <25% of the observed mesangium
Class- IIb	Severe mesangial expansion	Biopsy does not meet criteria for class III or IV. Severe mesangial expansion in >25% of the observed mesangium
Class- III	Nodular sclerosis (Kimmelstiel–Wilson lesion)	Biopsy does not meet criteria for class IV. At least one convincing Kimmelstiel–Wilson lesion
Class-IV	Advanced diabetic glomerulosclerosis	Global glomerular sclerosis in >50% of glomeruli lesions from classes I through III

Note: GBM: glomerular basement membrane.

Statistical analysis was performed using the statistical package for social studies (SPSS) version 26 (IBM, USA). $p < 0.05$ was considered as significant.

Results

Excluding the inadequate specimens, total DN samples were 46, which was 4.89% of the total renal biopsy samples received at AFIP during the stipulated period. Detail demographic and clinical characteristics of the study patients are shown in Table-2. The mean age of the cases was 46.76 ± 10.63 years, ranging from 23 years to 82 years. Among all, 36 were male, and 10 were female.

Among 46 cases, 32 cases (69.56%) presented with nephrotic range proteinuria, 27 (58.69%) patients had some form of haematuria, which included gross haematuria (>20/HPF) in 13 (28.26%) cases. Mean serum creatinine level was 4.28 ± 2.61 mg/dl, ranging from 0.3 mg/dl to 6.8 mg/dl and 80.43% had serum creatinine level >1.5 mg/dl. Only one (2.2%) patient had retinopathy.

Table-2: *Demographic and clinical characteristics of DN patients (n=46)*

Baseline characteristics	Value
Mean age in years (Range)	46.76 ± 10.63 (23 to 82)
Age groups in years (number, %)	
<30	3 (6.52)
31-40	11 (23.91)
41-50	18 (39.13)
51-60	11 (23.91)
>61	3 (6.52)
Gender (number, %)	
Male	36 (78.26)
Female	10 (21.74)
Male : Female	3.6:1
Haematuria (number, %)	27 (58.69)
Mean urinary total protein (UTP) g/24 hrs	6.44 ± 3.15 (Range- 0.4 to 15.0)
Nephrotic range proteinuria (number, %)	32 (69.56)
Raised serum creatinine level (≥ 1.5 mg/dl; number, %)	37 (80.43)
Mean basal serum creatinine (mg/dl)	4.28 ± 2.61 (Range- 0.3 to 6.8)

Among all the cases, the predominant histologic glomerular class was type III DN in 26 (56.5%) patients, followed by type IV DN in 11 (23.9%) [Table-3]. Only, one class I DN was found in a 82 yrs old patient who presented with nephrotic syndrome with slightly raised serum creatinine level. It was found with acute tubular injury and associated non-diabetic changes. IFTA score 1 (<25%) in 20 (43.5%) cases followed by IFTA score 2 (25-50%) in 19 (41.3%) cases and score 3 (>50%) in 7 (15.2%) cases. Vascular hyalinization score-2 was seen in 25 (54.3%) cases followed by hyalinization score-1 in 14 (30.4%) cases and score-0 in 7 (15.2%) cases.

Table-3: *Histopathologic classes of DN cases (n=46)*

Histologic class of DN	N (%)	95% CI
Type I	1(2.2)	- 2.04, 6.44
Type II	8 (17.4)	6.45, 28.35
Type III	26 (56.5%)	42.18, 70.82
Type IV	11 (23.9%)	11.58, 36.22

Clinico-pathological characteristics of different glomerular classes of DN are shown in Table-4. Different histologic classes of DN were significantly associated with the age of the patient (p=0.041) and with nondiabetic histologic changes (p=0.010). UTP was high in class III DN in comparison to class IV, while serum creatinine level sequentially increased in class II, Class III and highest in class IV. Among the histologic changes percentage of glomerular sclerosis, the mean IFTA score and vascular hyalinization score were found to be highest in class IV DN, and all were significantly associated

with histologic glomerular DN classes (p= <0.05). NDRD was present in 87.5% of DN class II cases.

Among all the cases, 21 (45.65%) biopsy samples had associated nondiabetic renal diseases (NDRD; Table-5). Among the 21 NDRD cases, the most common was focal segmental glomerulosclerosis (FSGS, 28.6%), followed by IgA nephropathy (14.3%) and post-infectious glomerulonephritis (14.3%). Anti-neutrophil cytoplasmic antibody (ANCA) mediated pauci-immune glomerulonephritis, and acute tubular injury were found in 2 (4.3%) cases each.

Table-5: *Pattern of associated non-diabetic renal diseases (NDRD) in study population (n=21)*

Histologic findings	Number	%
FSGS	6	28.6
IgA	3	14.3
PIGN	3	14.3
ANCA mediated nephropathy	2	9.5
ATI	2	9.5
Crystal nephropathy	1	4.8
IC-MPGN	1	4.8
MN	1	4.8
RCN	1	4.8
Transplant CNI	1	4.8
Total	21	100.0

Note: ANCA- Anti neutrophil cytoplasmic antibody, ATI- Acute tubular injury, FSGS- Focal segmental glomerulosclerosis, IC-MPGN- Immune complex mediated membranoproliferative glomerulonephritis, MN- Membranous nephropathy, PIGN- Post infectious glomerulonephritis, RCN- Renal cortical necrosis, CNI- Calcineurin inhibitor

Table- 4: *Clinico-pathological characteristics of cases with different glomerular classes of DN*

Characteristics	Class I (n=1)	Class II (n=8)	Class III (n=26)	Class IV (n=11)	p values
Mean age	82	43.88	45.89	47.73	0.041
Mean UTP, g/24 hrs	5.8	6.52	7.07	5.06	0.274
Mean serum creatinine, mg/dl	1.8	1.59	2.93	6.26	0.098
Sclerosed glomerulus (%)	8.33	30.62	20.94	66.18	0.054
Mean IFTA score	1	1.37	1.5	2.54	0.000
Vascular hyalinization score	0	0.5	1.46	2.0	0.000
Non diabetic renal diseases	1 (100%)	7 (87.5%)	10 (38.46%)	3 (27.27%)	0.010

Note: IFTA: Interstitial fibrosis and tubular atrophy, UTP: urinary total protein.

Crystal nephropathy, immune complex-mediated membranoproliferative glomerulonephritis (IC-MPGN), membranous nephropathy (MN) and renal cortical necrosis (RCN) were other NDRD. Among 46 cases, one post-transplant biopsy was included, revealing class II DN and features of calcineurin inhibitor toxicity.

Discussion

In this study, we investigated 46 cases of diabetic nephropathy diagnosed on renal biopsy during the 1.5 years of study period, accounting for 4.89% of the total 940 renal biopsy samples. The common affected age group was the 4th decade in our study with a mean age of 46.76 $\pm$ 10.63 yrs, and males were predominantly affected. Lee YH et al also reported male aged more than 50 years as the commonly affected group [17]. In contrast, a study from Turkey reported female as the predominantly affected group [18].

In our study, 69.56% cases had nephrotic range proteinuria and only one case had retinopathy. Therefore, it seems that the most pertinent indication for renal biopsy in DN cases is nephritic range proteinuria. Artan et al also reported proteinuria without retinopathy as the commonest indication of renal biopsy in their cohort [3]. In our cohort, 80.43% had raised serum creatinine levels with mean serum creatinine of 4.28$\pm$2.61 mg/dl, which was quite high in comparison to other similar studies [3,17,18]. The mean UTP level was in our cases was 6.44$\pm$3.15 g/24 hrs and 58.69% of cases had some form of haematuria. Sharma et al in their study, reported a median UTP of 5.0 g/24 hrs and haematuria in 27.8% to 33.6% of cases [19].

In our study, we found Class III DN as the most prevalent glomerular histological class comprising 56.52% followed by class-IV, 23.91%. All the class II lesions were of class IIb type, which was 17.39%. Wang J et al also found Class III DN as the most common DN histologic type (45.71%), but their second common histologic classwas class II DN (32.70%)[18] instead of class IV as found in our series. Different histologic classes of DN were significantly associated with the age of the patient (p=0.041) and nondiabetic histologic changes (p=0.010). UTP was high in class III DN in

comparison to class IV, while serum creatinine level sequentially increased in class II, Class III and highest in class IV DN. Among the histologic changes percentage of glomerular sclerosis, the mean IFTA score and vascular hyalinization score were highest in class IV DN, and all were significantly associated with histologic glomerular DN classes (p= <0.05). Afroz et al also reported a significant association with mean age and sequential increasing mean serum creatinine level, mean IFTA score and mean vascular hyalinization score with diabetic glomerular classes [20]. Sahay et al observed higher degree of proteinuria among cases with higher histologic classes [21].

This study found 45.65% cases of NDRD along with DN. In our study, lower glomerular classes had an increasing association with NDRD. The most common NDRD was FSGS (26.57%), followed by IgA nephropathy and PIGN (14.21%). Similar rate of associated NDRD in DN cases have also been reported by others [22,23]. Sanghavi et al [24] and Sharma et al [19] also reported FSGS as the predominant NDRD in their studies. While Lee et al [17] reported MN as the predominant NDRD and several studies in China [22,23,25] observed IgA nephropathy as the most prevalent NDRD. In this study, one post-transplant calcineurin inhibitor toxicity was identified along with DN.

Diabetic nephropathy has become a leading cause of end-stage renal failure, which ultimately needs renal replacement therapy. Renal biopsy is performed only in some selected cases mostly to find out the presence of other underlying causes other than diabetic changes. In our study, as about half of the cases had NDRD along with DN. Tervaert's histologic classification of our cases revealed class III DN lesions as the predominant one and had a significant association with age of the patient, serum creatinine level, IFTA, arteriolar hyalinization and NDRD. However, the study was conducted on limited number of DN cases. A countrywide study with larger number cases would provide more detail information regarding the state of DN, its classes and progression indicators in Bangladeshi diabetic patients.

References

1. Afkarian M, Zelnick LR, Hall YN, Heagerty PJ, Tuttle K, Weiss NS, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA*. 2016; **31**6(6): 602-610.

2. USRDS: United States Renal Data System Annual Data Report. Epidemiology of kidney disease in the United States. National Institute of Diabetes and Digestive and Kidney Diseases; 2015.

3. Byrne C, Caskey F, Castledine C, Davenport A, Dawnay A, Fraser S, et al. UK Renal Registry: 20th annual report of the Renal Association. *Nephron*. 2018; **139** Suppl 1: S24-35.

4. Koye DN, Shaw JE, Reid CM, Atkins RC, Reutens AT, Magliano DJ. Incidence of chronic kidney disease among people with diabetes: a systematic review of observational studies. *Diabet Med*. 2017; **34**(7): 887-901.

5. Artan AS, Gürsu M, Çoban G, Elçioğlu ÖC, Kazancıoğlu R. Renal biopsy in patients with diabetes: indications, results, and clinical predictors of diabetic kidney disease. *Turk J Nephrol*. 2021; **30**(1): 2-8.

6. Byun JM, Lee CH, Lee SR, Moon JY, Lee SH, Lee TW, et al. Renal outcomes and clinical course of nondiabetic renal diseases in patients with type 2 diabetes. *Korean J Intern Med*. 2013; **28**(5): 565–72.

7. Chang TI, Park JT, Kim JK, Kim SJ, Oh HJ, Yoo DE, et al. Renal outcomes in patients with type 2diabetes with or without coexisting nondiabetic renal disease. *Diabetes Res Clin Pract*. 2011; **92**(2): 198–204.

8. Chong YB, Keng TC, Tan LP, Ng KP, Kong WY, Wong CM, et al. Clinical predictors of nondiabetic renal disease and role of renal biopsy in diabetic patients with renal involvement: a single centre review. *Ren Fail*. 2012; **34**(3): 323–328.

9. Lee EY, Chung CH, Choi SO. Nondiabetic renal disease in patients with non-insulin dependent diabetes mellitus. *Yonsei Med J*. 1999; **40**(4): 321–326.

10. Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, et al. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. *Am J Kidney Dis*. 2002; **39**(4): 713–720.

11. International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels, Belgium: International Diabetes Federation; 2013.

12. Kibriya MG, Mahtab H. Micro-vascular complications in type-2 diabetes in Bangladesh: the Diabcare-Asia, Bangladesh project. *Diabetes Res Clin Pract*. 2000; **50**: 135-136.

13. Latif ZA, Jain A, Rahman MM. Evaluation of management, control, complications and psychosocial aspects of diabetics in Bangladesh: DiabCare Bangladesh 2008. *Bangladesh Med Res Counc Bull*. 2011; **37**(1): 11-16.

14. Islam SM, Islam MS, Rawal LB, Mainuddin A, Wahiduzzaman M, Niessen LW. Clinical profile of patients with diabetic nephropathy in a tertiary level hospital in Dhaka, Bangladesh. *Arch Med Health Sci*. 2015; **3**(2): 191-197.

15. Rahim MA, Zaman S, Habib SH, Afsana F, Haque WMMU, Iqbal S. Evaluation of risk factors for diabetic nephropathy among newly diagnosed type 2 diabetic subjects: preliminary report from a tertiary care hospital of Bangladesh. *BIRDEM Med J*. 2020; **10**(2): 88-91.

16. Tervaert TWC, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*. 2010; **21**(4): 556–563.

17. Lee YH, Kim KP, Kim YG, Moon JY, Jung SW, Park E, et al. Clinicopathological features of diabetic and nondiabetic renal diseases in type 2 diabetic patients with nephrotic-range proteinuria. *Medicine*. 2017; **96**(36): e8047.

18. Wang J, Zhao L, Zhang J, Wang Y, Wu Y, Han Q, et al. Clinicopathological features and prognosis of type 2 Diabetes Mellitus and diabetic nephropathy in different age group: more attention to younger patients. *Endocr Prac*. 2020; **26**(1): 51-57.

19. Sharma SG, Bomback AS, Radhakrishnan J, Herlitz LC, Stokes MB, Markowitz GS, et al. The modern spectrum of renal biopsy findings in patients with diabetes. *Clin J Am Soc Nephrol*. 2013; **8**(10): 1718–1724.

20. Afroz T, Sagar R, Reddy S, Gandhe S, Rajaram KG. Clinical and histological correlation of diabetic nephropathy. *Saudi J Kidney Dis Transpl*. 2017; **28**(4): 836-841.

21. Sahay M, Mahankali RK, Ismal K, Vali PS, Sahay RK, Swarnalata G. Renal histology in diabetic nephropathy: a novel perspective. *Indian J Nephrol*. 2014; **24**(4): 226–231.

22. Zhou J, Chen X, Xie Y, Li J, Yamanaka N, Tong X. A differential diagnostic model of diabetic nephropathy and nondiabetic renal diseases. *Nephrol Dial Transplant*. 2008; **23**(6): 1940–1945.

23. Bi H, Chen N, Ling G, Yuan S, Huang G, Liu R. Nondiabetic renal disease in type 2 diabetic patients: a review of our experience in 220 cases. *Ren Fail*. 2011; **33**(1): 26–30.

24. Sanghavi SF, Roark T, Zelnick LR, Nazafian B, Andeen NK, Alpers CE, et al. Histopathologic and clinical features in patients with diabetes and kidney disease. *Kidney 360*. **1**(11): 1217–1225.

25. Mou S, Wang Q, Liu J, Che X, Zhang M, Cao L, et al. Prevalence of nondiabetic renal disease in patients with type 2 diabetes. *Diabetes Res Clin Pract*. 2010; **87**(3): 354–359.