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A clinicopathological study of 36 diabetic patients with proteinuria

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Abstract

Background: About one-fourth to one-third of diabetic patients develop renal manifestations. The commonest renal manifestation of a diabetic patient is proteinuria. Besides, patients may also present with chronic renal failure and hematuria with or without dysmorphic RBC. Renal manifestation in all proteinuric diabetic patients may not be due to diabetic nephropathy but also non-diabetic renal disease (NDRD). For diabetic patients, distinguishing non-diabetic renal disease (NDRD) from diabetic nephropathy (DN) is important in choosing treatment modalities and determining renal prognosis.

Aim of Study: To see the clinic-pathological manifestation of diabetic patients with proteinuria. among diabetic patients. The duration of diabetes in NDRD is significantly shorter than that in DN.

Methodology: A total of 36 Diabetic patients with nephrotic/nonnephritic range proteinuria, with or without dysmorphic RBC were enrolled in this study. Two biopsies from each patient were obtained by percutaneous needle biopsy by experienced nephrologists after explaining the procedure to the patients and obtaining their written consent. The tissue in normal saline was frozen for subsequent cryostat sectioning and stained with fluorescein isothiocyanate (FITC) conjugated antibodies against immunoglobulin (IgG, IgA, IgM), Complement component (C3, C1q), and Fibrinogen.

Result: Most of the patients showed non-diabetic kidney disease (63.0%) vs diabetic nephropathy (37%) on diagnosis. Most of the patients with dysmorphic RBC showed non-diabetic kidney disease in 12 (60.0%) and eight (40.0%) as diabetic nephropathies on final diagnosis. Regarding the prognosis of diabetic nephropathy, about 25% of patients will develop into end-stage renal failure within 6 years and 50 % within 10 years.

Conclusion: To conclude, clinical parameters such as short duration of diabetes, non-nephrotic range proteinuria, hematuria, and acanthocytes or dysmorphic RBCs may be used as a clinical marker for non-diabetic kidney diseases among diabetic patients. Early diagnosis of NDRD poses a favorable renal prognosis because it requires a different approach than DN.

Keywords: Non-diabetic renal disease (NDRD); Diabetic nephropathy (DN); Proteinuria; Dysmorphic RBC

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1. Introduction

Approximately one-fourth to one-third of diabetic patients develop renal manifestations. The commonest renal manifestation with which a diabetic patient comes is proteinuria. Patients also present with chronic renal failure and hematuria with or without dysmorphic RBC. Renal manifestations in all proteinuria diabetic patients may not be due to diabetic nephropathy. In many cases, kidney biopsy from these patients rather reveals different renal pathology other than diabetic nephropathy[1]. Renal diseases that are pathologically unrelated to diabetes are known as non-diabetic renal disease (NDRD). About one-third of the diabetic patients who present with proteinuria are suffering from non-diabetic renal diseases[2].

The renal biopsy may show:

- Nondiabetic renal diseases like Mesangial proliferative glomerulonephritis, IgA nephropathy, Membranous nephropathy, Focal segmental glomerulosclerosis, Mesangiocapillary glomerulonephritis, rapidly progressive glomerulonephritis, Minimal change disease, and lupus nephritis[3].
- Nondiabetic kidney disease superimposed on diabetic nephropathy[1].

Clinical markers for non-diabetic kidney diseases are –

- The short duration of diabetes
- Absence of diabetic neuropathy
- Absence of diabetic retinopathy
- Hematuria
- Acanthocyturia
- Sudden progression of renal failure
- Non-nephrotic proteinuria[1].

The duration of diabetes is usually short in the isolated NDRD. Acanthocytes or dysmorphic RBCs are used as a clinical marker for non-diabetic kidney diseases among patients with diabetes mellitus. These are useful adjuvants in the diagnosis of glomerulonephritis when the percentage exceeds 75% of the urinary RBCs. Renal biopsy should be considered when a diabetic patient with proteinuria shows 5% acanthocyturia at least in one of three urine samples[4]. In full-blown diabetic nephropathy, dysmorphic RBC is an uncommon finding. The prevalence of NDRD among diabetic patients was significantly higher in patients with mild-to-moderate proteinuria than in patients with heavy proteinuria and DN was diagnosed more frequently in patients presenting with heavy proteinuria. The magnitude of diabetes mellitus in Bangladesh is increasing. In Bangladesh, the current prevalence of diabetes (among people 20-79 years of age) is 4.8%. It is supposed to rise to 6.1% in 2025.[5] Early diagnosis of NDRD is crucial as appropriate therapy can prolong survival in this population. The nature of specific therapy will depend on the nature of underlying lesions[6]. On the other hand, diabetic nephropathy has no specific therapy. Good glycemic control, control of hypertension by using ACE inhibitors, and dietary restriction of protein are necessary to slow the progression of kidney damage and to control related complications[7].Regarding the prognosis of diabetic nephropathy, about 25% of patients will develop into end-stage renal failure within 6 years and 50 % within 10 years[8].Therefore, Non-diabetic kidney diseases in diabetic patients should be distinguished from Diabetic nephropathy for treatment and prognostic purposes.

2. Materials and Methods

This is a study conducted in the Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period from July 2012 to September 2014. A total of 36 Diabetic patients with nephrotic/nonnephritic range proteinuria, with or without dysmorphic RBC were enrolled in this study. Diabetic patients with retinopathy and without proteinuria were excluded from the study. These patients were collected from the Nephrology department of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and the Nephrology department of Bangabandhu Sheikh Mujib Medical University (BSMMU). Two biopsies from each patient were obtained by percutaneous needle biopsy by experienced nephrologists after explaining the procedure to the patients and obtaining their written consent. The specimen for light microscopy was preserved in 10% formalin and that for immunofluorescence study in normal saline. After arrival in the pathology department, tissue in 10% neutral buffered formalin was processed routinely and stained with hematoxylin eosin, and periodic acid Schiff. These were examined under a light microscope. The tissue in normal saline was frozen for subsequent cryostat sectioning and stained with fluorescein isothiocyanate (FITC) conjugated antibodies against immunoglobulin (IgG, IgA, IgM), Complement component (C3, C1q), and Fibrinogen. These sections were examined under a fluorescence microscope. A

diagnosis was made from light and fluorescence microscopic findings correlating with the patient's clinical and laboratory data.

3. Results

A total of 36 cases of this study were collected from the Department of Nephrology of BIRDEM and the Nephrology department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The clinical and demographic parameters were recorded for each patient during renal biopsy. These included duration of diabetes, age, sex, and clinical presentation. Laboratory studies included fasting blood sugar, serum creatinine, blood urea, 24-hour urinary protein, and urinary RBCs including dysmorphic RBC. Light and immunofluorescence microscopy were done for the final diagnosis. Findings were correlated with clinical and biochemical data before a definitive diagnosis. The study showed that, besides the duration of diabetes, Nephrotic / Non -nephrotic range of proteinuria, hematuria, and dysmorphic RBC, the other clinical parameters were not significantly different between Non-diabetic kidney diseases and Diabetic nephropathy.

3.1. Sociodemographic profile

Table 1 shows the age of the study patients. Of the total 36 cases, most of the patients (38.9%) belonged to the age group 51-60 years. The mean age was found 48.03±11.1 years with a range from 18 to 72 years. In the study, number of male patients was 17 (47.2%) and female was 19 (52.8%). Male to female ratio was 0.9:1. In the study, most (58.3%) of the patients had more than 5 years of history of DM. Regarding the duration of diabetes, we found 10 days to 10 years duration in non-diabetic kidney disease and 7 years to 24 years in diabetic nephropathy

Table 1 Sociodemographic Characteristics: (n=36)

Age (in years)	**Number of patients (n)**	**Percentage (%)**
≤30	2	5.6
31-40	7	19.4
41-50	10	27.8
51-60	14	38.9
>60	3	8.3
Mean ± SD	48 ±11.3	
Male:Female	09:1	
Duration of diabetes mellitus	Number of patients (n)	Percentage (%)
Up to 5 years	15	41.7
More than 5 years	21	58.3
Diagnostic group	Duration of diabetes mellitus	
Non-diabetic kidney disease	10 days to 10 years	
Diabetic nephropathy	7 years to 24 years	

3.2. Clinical & Biochemical presentation

Nephrotic Syndrome was the major clinical presentation in this study and accounted for 17 (47.2%) of total cases. This was followed, in descending order by, nephritic syndrome (9=25.0), rapidly progressive glomerulonephritis (4=11.1%), hematuria (3=8.3%), and proteinuria (2=5.6%) [Table no 2]. The main biochemical parameters recorded are shown in Table 4. Fasting blood sugar, serum creatinine, and blood urea were estimated in 36 (100.0%), 36(100.0%), and 17(47.2%) cases respectively. Fasting blood sugar was found to rise in all (100.0%) cases. Serum creatinine was done in 36 cases. Out of 36 cases, 05 (13.9%) cases showed normal creatinine levels and 31 (86.1%) cases had raised creatinine levels. Out of 36 cases, blood urea was done in 17 cases. Among these, 1(2.8%) case showed normal urea level and the rest 16(44.4%) cases had raised urea levels. Among 36 patients, 12(33.3%) patients had 2+ urinary albumin followed by 11(30.6%) who had 3+ and 7(19.4%) who had 1+ albumin by heat coagulation test. Trace Albumin was found in 3(8.3%) patients.

Table 2 Clinical & Biochemical presentation of the patients (n=36)

Clinical Presentation	Number of patients (n)	Percentage (%)
Nephrotic Syndrome	17	47.2
Nephritic Syndrome	9	25.0
Rapidly Progressive Glomerulonephritis	4	11.1
Hematuria	3	8.3
Mild Proteinuria	2	5.6
Nephrotic Syndrome with Hypertension	1	2.8
Biochemical Test		
Fasting Blood Sugar (mmol/L)		
Normal	00	00
Raised	36	100.0
S. Creatinine (mg/dL)		
Normal	05	13.9
Raised	31	86.1
B. Urea (mmol/L)		
Normal	01	2.8
Raised	16	44.4
Urinary Albumin		
+	7	19.4
++	12	33.3
+++	11	30.6
++++	3	8.3
Trace	3	8.3

3.3. Degree of proteinuria by 24-hour urinary protein

Among 36 cases UTP was done in 34 cases. Out of 34 cases, nephrotic range proteinuria was found in 15(41.7%) cases, and non-nephrotic range proteinuria was found in 19 (52.8%) cases.

Table 3 Degree of proteinuria by 24hr urinary protein (n=36)

Urinary Total Protein (24gm/24hr)	Number of patients (n)	Percentage (%)
Nephrotic range proteinuria	15	41.7
Non-nephrotic range proteinuria	19	52.8

3.4. Distribution of the diabetic patients according to the nephrotic range of proteinuria

Non- A nephrotic range of proteinuria was found in 12 (63%) cases of Non- Diabetic kidney Disease and 7 (37 %) cases of Diabetic Nephropathy.

Table 4 Distribution of the diabetic patients according to non- nephrotic range of (n=19)

Diagnostic group	Number of patients with non-nephrotic range of proteinuria (n))	Percentage (%)
Non- Diabetic Kidney Disease	12	63.0
Diabetic Nephropathy	07	37.0

3.5. Distribution of the diabetic patients according to a nephrotic range of proteinuria

Nephrotic range of proteinuria was found in 5 (33%) cases of non-diabetic kidney Disease and 10 (67 %) cases of Diabetic Nephropathy.

Table 5 Distribution of the diabetic patients according to nephrotic range of proteinuria (n=15)

Diagnostic group	**Number of patients with nephrotic range of proteinuria (n)**	**Percentage (%)**
Non- Diabetic Kidney Disease	05	33
Diabetic Nephropathy	10	67

3.6. Urinary RBCs

Out of 36 cases, 30 (83.3%) patients showed RBC in their urine.

Table 6 RBC/HPF in urine (n=36)

RBC/HPF	**Number of patients (n)**	**Percentage (%)**
Done	30	83.3
Not available	6	16.7

3.7. Urinary Dysmorphic RBCs

Out of 30 cases, 20 (55.6%) %) patients showed Dysmorphic RBC in their urine.

3.8. Distribution of the diabetic patients according to dysmorphic RBCs in urine

Dysmorphic RBC was found in 12 (60.0%) cases of Non-Diabetic kidney Disease and 8 (40.0%) cases of Diabetic Nephropathy

Table 7 Dysmorphic RBCs in urine (n=30)

Total number of patients (n)	**Patients with Dysmorphic RBCs**	**Percentage (%)**
30	20	55.6

Table 8 Distribution of the diabetic patients according to urinary Dysmorphic RBCs (n=20)

Diagnostic group	**Number of patients with Dysmorphic RBCs (n)**	**Percentage (%)**
Non- Diabetic Kidney Disease	12	60.0
Diabetic Nephropathy	8	40.0

3.9. Histological diagnosis of 36 cases.

Table IX shows the histological diagnosis of the study patients in total number and percentage. Among 36 cases diabetic nephropathy was found in 14(38.9%) cases. Out of 14 cases of diabetic nephropathy diffuse glomerulosclerosis was found in 9 (64.3%) cases and nodular glomerulosclerosis in 5 (35.7%). Among 22 cases of non-diabetic renal disease,

mesangial proliferative glomerulonephritis was found in 13(36.1%) cases, mesangiocapillary glomerulonephritis in 5(13.9%), lupus nephritis RPS/ISN class IV in 2(5.6%), chronic glomerulonephritis in 1(2.8%) and membranous nephropathy in 1(2.8%) cases.

Table 9 Histological diagnosis of the study patients with their relative percentage (n=36)

Histological Diagnosis	Number of patients (n)	Percentage (%)
Diabetic Nephropathy		
Diffuse glomerulosclerosis	9	25.0
Nodular glomerulosclerosis	5	13.9
Non – Diabetic Renal Disease		
Mesangial proliferative glomerulonephritis	13	36.1
Mesangiocapillary glomerulonephritis	5	13.9
Lupus nephritis class IV	2	5.6
Chronic glomerulonephritis	1	2.8
Membranous nephropathy	1	2.8

3.10. Status of Diabetic Nephropathy and Non -Diabetic Kidney Disease

Table 10 shows the total number and percentage of Diabetic Nephropathy and Non-Diabetic Kidney Disease evaluated by histopathology in the study population. Non- Diabetic Kidney Disease was found in 22 (61.1%) cases and Diabetic Nephropathy was found in 14 (38.9%) cases.

Table 10 Status of Diabetic Nephropathy and Non-Diabetic Renal Disease of the study patients (n=36)

Histological Diagnosis	Number of patients (n)	Percentage (%)
Non- Diabetic Kidney Disease	22	61.1
Diabetic Nephropathy	14	38.9

4. Discussion

In the present study, significant differences were observed between Non-Diabetic Renal Disease (NDRD) and Diabetic Nephropathy (DN) in terms of the duration of diabetes, amount of proteinuria, and presence of dysmorphic red blood cells (RBCs). However, other clinical parameters did not show statistically significant differences between the two groups.Similarly, Zhuo et al. (2013) reported that apart from the duration of diabetes, proteinuria, and dysmorphic RBCs, no other clinical parameters differed significantly between NDRD and DN[3]. The majority of patients (38.9%) in this study were in their 6th decade of life, followed by 27.8% in the 5th decade, 19.4% in the 4th decade, 8.3% in the 7th decade, and 5.6% in the 3rd decade. The mean (±SD) age was 48.03 ± 11.1 years (range: 18–72 years). In comparison, Zhou et al. (2008) reported a mean age of 46.3 ± 11.8 years, while Mundi et al. (2014) observed a younger mean age of 36.9 ± 17.27 years (range: 12–79 years), with 38% of cases between 21–40 years—findings broadly comparable to the current study[9-10]. Chi et al. (2011) reported a mean age of 56.31 ± 11.05 years and 53.9 ± 13.2 years in their diabetic cohorts, whereas Haddiya et al. (2009) found a higher mean age of 51.0 ± 21.0 years (range: 33–75 years). The variations may be attributed to geographical, racial, ethnic, genetic, and lifestyle differences among study populations.In the present study, the male-to-female ratio was 0.9:1, indicating a slight female predominance. Chi et al. (2011)similarly found 47% males and 53% females (ratio 1:1.13). Conversely, Mundi et al. (2014), Zhuo et al. (2013), and Zhou et al. (2008) reported a male predominance[3,9,11,12]. The duration of diabetes ranged from 10 days to 10 years among NDRD patients and 7 to 24 years among DN patients. Liang et al. (2013) also found a significantly shorter diabetes duration in NDRD than in DN (WMD: -34.67; 95% CI: -45.23 to -24.11; $p<0.05$). For Asian populations, a similar difference was noted (WMD: -30.37; 95% CI: -42.93 to -17.81; $p<0.05$). The definition of diabetes duration, however, varied among studies—some considered the interval between diabetes onset and biopsy, others from onset to renal disease detection. A shorter duration of diabetes, absence of retinopathy, presence of microscopic hematuria or dysmorphic RBCs and active urinary sediment are recognized markers suggestive of NDRD and serve as strong indications for renal biopsy. As DN usually progresses gradually—from microalbuminuria to macroalbuminuria and

eventually renal failure—patients with a shorter diabetic duration and persistent proteinuria should be evaluated carefully for possible NDRD[13,14,15.].In this study, 17 patients (47.2%) presented with nephrotic syndrome, followed by 9 (25.0%) with nephritic syndrome, 4 (11.1%) with rapidly progressive glomerulonephritis, 3 (8.3%) with isolated hematuria, 2 (5.6%) with mild proteinuria, and 1 (2.8%) with nephrotic syndrome with hypertension. Mundi et al. (2014) reported nephrotic syndrome as the most common presentation (62.0%), followed by sub-nephrotic proteinuria (24.0%) and rapidly progressive renal failure (14.0%)[10]. Microscopic hematuria and hypertension were observed in 16.0% and 46.0% of their patients, respectively. Zhuo et al. (2013) found diabetic retinopathy in 17 cases, hematuria in 78.9%, hypertension in 14 patients, nephrotic syndrome in 4, nephritic syndrome in 6, and renal dysfunction in 9 cases[3]. Zhou et al. (2008)observed nephrotic syndrome in 41.7%, hematuria in 16.7%, and hypertension in 76.7% of cases[9].In the current study, fasting blood sugar (FBS), serum creatinine, and blood urea were assessed as key biochemical parameters. All 36 patients (100%) had elevated FBS levels ranging from 8.0–32.6 mmol/L (mean 8.83 ± 1.44 mmol/L). Raised serum creatinine was found in 31 cases (86.1%), and 16 of 17 cases (44.4%) had elevated blood urea (mean 36.48 ± 24.7 mmol/L). Chi et al. (2011) reported a mean serum creatinine of 3.7 ± 3.2 mg/dL, while Yaqub et al. (2012) observed a higher mean of 4.5 ± 2.56 mg/dL[11,14].Proteinuria was estimated using 24-hour urinary protein measurement, supplemented by the heat coagulation test. Nephrotic-range proteinuria was found in 15 cases (41.7%) and non-nephrotic range in 19 cases (52.8%). Among nephrotic-range proteinuria cases, 5 (33%) were NDRD and 10 (67%) DN. Conversely, among non-nephrotic cases, 12 (63%) were NDRD and 7 (37%) DN. Liu et al. (2016) similarly reported nephrotic-range proteinuria more common in DN and non-nephrotic range in NDRD[20].Microscopic examination revealed RBCs in 30 (83.3%) cases, with dysmorphic RBCs present in 20 (55.6%). Among these, 12 (60%) were NDRD and 8 (40%) DN. Heine et al. (2004) found acanthocyturia (dysmorphic ring-shaped RBCs) significantly more frequent in NDRD than in DN ($p<0.05$)[4]. Hommel et al. (1987) also suggested that hematuria in diabetic patients indicates possible nondiabetic glomerulopathy[20]. The presence of >5% acanthocytes has been shown to be a highly specific (98%) and moderately sensitive (52%) marker of primary glomerulonephritis, distinguishing it from DN.Histopathological diagnoses in the current study included 14 cases (38.9%) of DN and 22 cases (61.1%) of NDRD. Among DN cases, diffuse glomerulosclerosis was found in 9 (25.0%) and nodular glomerulosclerosis in 5 (13.9%). Among NDRD cases, mesangial proliferative glomerulonephritis (MesPGN) was the most common (13 cases; 36.1%), followed by mesangiocapillary GN (5 cases; 13.9%), lupus nephritis class IV (2 cases; 5.6%), chronic GN (1 case; 2.8%), and membranous nephropathy (1 case; 2.8%). Mundi et al. (2014) also found primary glomerular diseases dominant (72.0%), with FSGS (20.0%), membranous GN (18.0%), and minimal change disease (14.0%)most common[14]. Zhou et al. (2008) reported mesangial proliferative GN in 14%, membranous nephropathy in 22%, and lupus nephritis in 16% of cases[9]. Chi et al. (2011) found 24.0% of their cases with coexisting glomerulopathy—mostly FSGS—while Zhuo et al. (2013) reported 84.0% NDRD, 8.2% DN and 7.8% mixed cases[3,11].In Bangladesh, diabetes prevalence among adults (20–79 years) is 4.8%, projected to rise to 6.1% by 2025[5]. Given that 40–60% of end-stage renal disease (ESRD) in diabetic patients is associated with non-diabetic kidney diseases, distinguishing NDRD from DN is essential for accurate treatment and prognosis. In this study, NDRD was found in 61.1% and DN in 38.9% of cases, consistent with Pham et al. (2007) who found 53.2% NDRD and 46.8% DN, and Kveder et al. (2001) who reported 49% and 22%, respectively[18-19].Early and accurate diagnosis of NDRD is crucial, as timely and targeted therapy can significantly improve renal outcomes. Unlike DN, which typically shows progressive and irreversible renal decline, NDRD is often treatable and potentially reversible with appropriate management.

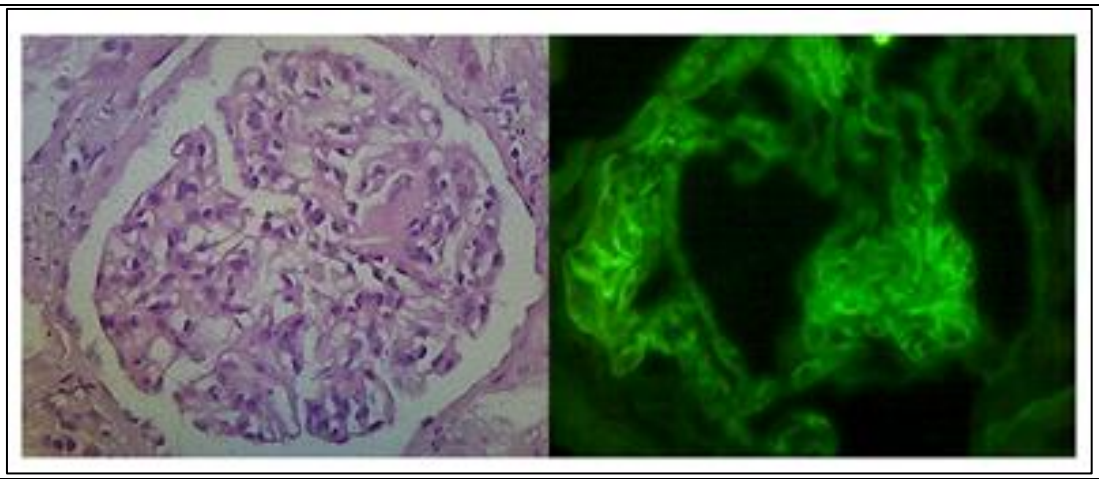

Figure 1 Photomicrograph of MCGN (H&E, x400)

Figure 2 Photomicrograph of MCGN showing deposition of IgG(+) in GBM (DIF,x400)

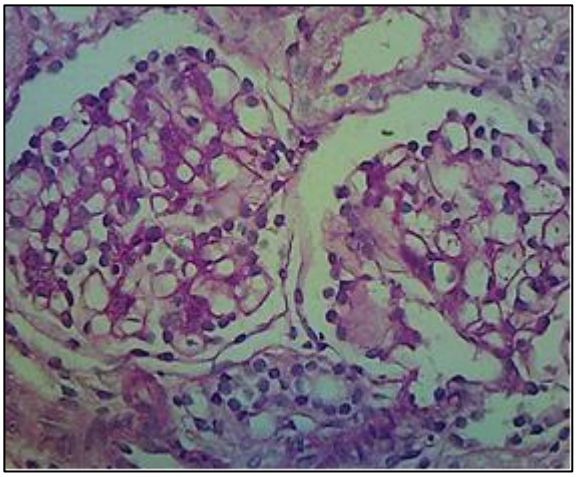

Figure 3 Photomicrograph of MesPGN(PAS, x400)

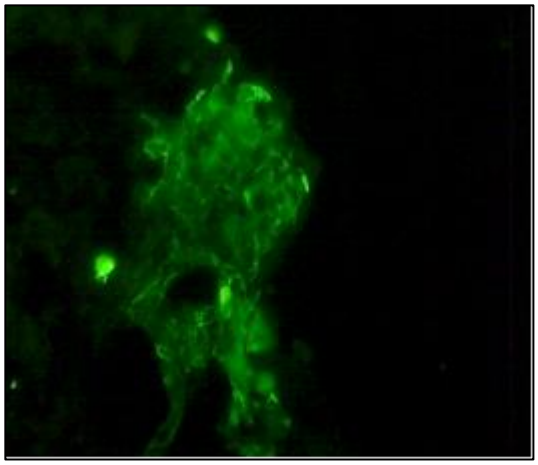

Figure 4 Photomicrograph of l

5. Conclusion

This study was undertaken to evaluate the clinicopathological features of DN and NDRD in diabetic patients with proteinuria. To conclude, clinical parameters such as short duration of diabetes, non-nephrotic range proteinuria, hematuria, and acanthocytes or dysmorphic RBCs may be used as a clinical marker for non-diabetic kidney diseases among diabetic patients. Early diagnosis of NDRD poses a favorable renal prognosis because it requires a different approach than DN.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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