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RENAL BIOPSY PATTERN IN DIABETES MELLITUS PATIENTS AND THEIR CORRELATION WITH CLINICAL PARAMETERS

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РЕФЕРАТ

BACKGROUND. Diabetic nephropathy is a condition marked by persistent proteinuria, hypertension, and a progressive loss of renal function. End-stage kidney disease needing continuous renal replacement treatment is now primarily caused by diabetes. According to Kimmelstiel and Wilson, the hallmark lesion of diabetic nephropathy is nodular glomerulosclerosis. Diabetic nephropathy or Nondiabetic renal disease, or the coexistence of both can be seen in renal histopathology and in differentiating between these diagnostic groups can have an impact on patient care and prognosis. **PATIENTS AND METHODS.** Total of 21 cases of Diabetic nephropathy were included in the study. Clinical details and laboratory parameters like diastolic blood pressure, creatinine level, 24 hrs urinary protein level and HbA1C% were recorded in pretested performa in all cases. The biopsy specimens were stained with hematoxylin & eosin and special stains. **RESULTS.** Among the total DM cases only 21 patients have done renal biopsy, 11 cases (52.3 %) showed KW lesion (Class III) while 06 cases (28.5 %) showed diffuse diabetic glomerulosclerosis (Class IV). The remaining 04 cases (19 %) showed a mild increase in mesangial matrix and slight thickening of glomerular basement membrane (Class II). When compared with clinical parameters, they were more raised in Nodular diabetic glomerulosclerosis type (Class III) lesion as compared to diffuse diabetic glomerulosclerosis. **CONCLUSION.** Nodular diabetic glomerulosclerosis was the most common lesion in renal biopsy of type II diabetes mellitus patients. This KW lesion is responsible for more severe clinical and biochemical renal abnormality in most patients with type II diabetes mellitus.

Keywords: diabetic nephropathy, nodular glomerulosclerosis, diffuse glomerulosclerosis

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BACKGROUND

One in six (17%) people with diabetes worldwide, second only to China, are from India [1]. After China, India is the country with the second-highest prevalence of diabetes, with an estimated 77 million people suffering from the disease. Diabetic nephropathy (DN) is a condition marked by persistent secretion of protein in urine, increase in blood pressure, and a progressive decline in renal function [2]. The diabetic renal disease affects around 20% to 40% of persons with diabetes mellitus [3]. End-stage kidney disease (ESKD) needing continuous renal replacement treatment is now primarily caused by diabetes [4, 5]. According to Kimmelstiel and Wilson, the hallmark lesion of diabetic nephropathy is nodular glomerulosclerosis [6]. Rapid loss of renal function and hematuria (microscopic or

macroscopic) are further clinical predictors of Nondiabetic renal disease (NDRD) in diabetic individuals. In diabetic patients, a kidney biopsy is not usually performed. It is, nevertheless, recommended in patients who are suspected of having NDRD [4].

Patients with diabetes mellitus may manifest with DN or NDRD alone, or the coexistence of both Diabetic nephropathy and non-diabetic renal disease can be seen in renal histopathology findings. Differentiating between these diagnostic groups can have an impact on patient management as well as prognosis, especially the NDRD which leads to specific changes in treatment strategy.

Short disease duration, sudden onset proteinuria, retinopathy, acutely declined kidney function and hematuria have all been demonstrated as predictive

factors for the renal involvement in diabetes mellitus patients by NDRD [7, 8].

In contrast, the longer duration of diabetes (>10 years), retinopathy or neuropathy, are the variables that can predict DN.

It is difficult to distinguish between DN and NDRD in patients with diabetes mellitus without the use of renal biopsies since there is major heterogeneity in clinical details and history [9].

The present study was done to investigate the renal histopathology of diabetes mellitus patients and its correlation with laboratory parameters like diastolic blood pressure, creatinine level, 24 hrs urinary protein level, and HbA1C.

PATIENTS AND METHODS

Patient recruitment and plan of study

This study was approved by the Ethical Committee of Banaras Hindu University (BHU). It was a prospective study. Patients of diabetes mellitus from the Department of Endocrinology and nephrology, SIR SUNDER LAL HOSPITAL, BHU, were consistently contacted for a period of 01 year and 10 months from September 2012 to July 2014. An informed consent was provided during the consultation.

Sample size and study population

Total 21 cases of Diabetic nephropathy were included in this study for the renal biopsy. Clinical details and laboratory parameters like diastolic blood pressure, serum creatinine level, 24 hrs urinary protein level and HbA1C in the patient record were recorded in performa which was pretested in all cases. All diabetes mellitus cases diagnosed by laboratory biochemical test (defined by the WHO criteria) [10] with more than 10 years disease duration, included in this study.

Inclusion criteria

1. Type 2 diabetes mellitus case defined by WHO diagnostic criteria

2. Willing and able to give informed consent

3. Willing and able to follow study rules and regulations.

Exclusion criteria

Cases that were not willing and able to give informed consent.

To investigate the nephropathy status, we have checked diastolic blood pressure, creatinine level, 24 hrs urinary protein level and HbA1C in the patient record.

A competent pathologist examined the renal samples. For light microscopy examination, the renal biopsy specimens were fixed in 10% neutral buffer formalin solution, embedded in paraffin block and section of 2-3 μ m thickness cut by the microtome. Hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and methenamine silver were used to stain the biopsy

samples for light microscopy analysis. When amyloidosis was suspected, Congo red stain was utilized.

Indication of renal biopsy was:

1. Serum creatinine >1.5 mg/dl

2. Proteinuria >0.5 gm/24 hr

3. Diabetes mellitus without retinopathy

Renal Cases of Diabetic nephropathy were classified according to the Renal Pathology Society histologic classification system for diabetic nephropathy proposed in 2010 [Table 4] [11].

Statistical analysis:

Statistical analysis for this study was performed using "SPSS, v.16". The data from renal biopsies was entered into "Microsoft Excelsheet" and personal identifiers were removed to maintain confidentiality of the patients. The measure of central tendency (mean, median and mode), measures of variability (standard deviation and range) were calculated to explain distribution of variables across diabetic renal disease of category II, III and IV. To test the association of clinical parameters and diabetic renal disease the tests of significance were applied. In this particular quantitative or continuous dataset, renal disease was categorised in more than two groups. The parametric tests analysis of variance (ANOVA) was applied to test the significance of difference between the means of clinical parameters. The statistic "Welch" and "Brown- Forsythe" were calculated when the sample size of the three groups of cases were different and assumption of homogeneity of variances was not met. The probability values of less than 0.05 showed significant difference in the present study.

RESULTS

Demographic characteristics: A total of twenty-one renal biopsies from Type II diabetes mellitus (DM) cases are presented in this communication. Among the total patients who came for renal biopsy at the study center; two- third (n=14 or; 66.6 %) were males and one- third (n=7 or; 33.3 %) were females. The mean age was 47.9 ± 10.8 (Mean \pm 2 SD) years for all the participants while the median age was 48 years with the range between 29 and 65 years (Table 1).

Cases having mild changes in glomerular mesangial matrix (Category 2) had mean age of 44.7 ± 10.5 years and median 49.5 years with range between 29 and 51 years. Cases having nodular glomerulosclerosis (Category 3) had mean age of 50.4 ± 10.6 years and median 49 years with range between 35 and 65 years. Cases having diffuse glomerulosclerosis (Category 4) had mean age of 45.6 ± 10.4 years and median 48 years with range between 30 and 61 years. A simple box-plot distribution of age distribution of cases according to the type or renal changes is also presented as Figure 1.



Figure 1. Simple Box-Plot distribution of Age in Type II diabetes cases of category 2,3 and 4. The boxes show the interquartile range with median as straight vertical line and mean as "x" while the whisker shows minimum and maximum values. The black line in the box shows the median value while the black projections show minimum and maximum values.

Clinical Parameters: All cases were evaluated for 24 hours urinary protein, diastolic blood pressure (DBP), serum creatinine and glycosylated hemoglobin (HbA1C). The values of these parameters were noted and average or mean values were calculated for

Table 1
Distribution of cases of renal biopsy according to the age and sex

Age group	No of cases	Male	Female
30–40 years	6	5	1
41–50 years	8	5	3
>50 years	7	4	3
Total	21	14 (66.66 %)	7 (33.33 %)

comparison also shown in table: 3. It was observed that the renal changes of nodular glomerulosclerosis (Category 3) were associated with highest diastolic blood pressure, creatinine, protein and glucose levels in blood. The cases having mild mesangial changes had lowest parameters among the three categories of renal changes in diabetes mellitus cases; though they were higher than the normal ranges.

On comparing the clinical parameter (diastolic blood pressure, creatinine level, 24 hr urinary protein level and HbA1C %) among the three categories of renal changes in diabetes, the difference was found statistically significant between 24 hours protein (Welch F Ratio- 13.6; P Value= 0.001), diastolic blood pressure (Welch F Ratio- 8.7; P Value= 0.008) and Glycosylated hemoglobin (Welch F Ratio- 5.4; P

Table 2
Renal biopsy finding (mean value of clinical parameters) in different stages, Mean \pm 2SD

Parameters	Mild increase in mesangial matrix and GBM thickening N=4	Nodular glomerulosclerosis (KW-lesion) N=11	Diffuse glomerulosclerosis N= 6	P
	Category 2 1	Category 3 2	Category 4 3	
Diastolic blood pressure, mm Hg	87.5 \pm 6.8	96.6 \pm 9.0	90.7 \pm 7.8	1/2=0.091 1/3=0.524 2/3=0.197
Serum Creatinine, mg/dl	1.8 \pm 0.3	2.1 \pm 1.8	1.6 \pm 1.0	1/2 =0.751 1/3=0.712 2/3=0.542
24 hr. urinary protein, gm	0.9 \pm 0.4	2.9 \pm 2.4	2.0 \pm 2.4	1/2 =0.129 1/3=0.399 2/3=0.471
HbA1C, %	8.7 \pm 0.9	10.2 \pm 2.8	8.9 \pm 1.2	1/2 =0.312 1/3=0.784 2/3=0.300

Table 3
Robust tests (ANOVA) of equality of means in renal diabetic cases

Indicators	F- Ratio	Statistic	df1*	df 2 # (within groups)	P
Serum creatinine	Welch	1.0	2	10.7	0,385
	Brown- Forsythe	1.8	2	16.0	0,192
24 hours urinary protein	Welch	13.6	2	9.8	0,001
	Brown-Forsythe	6.7	2	12.4	0,011
Diastolic blood pressure (DBP)	Welch	8.7	2	8.7	0,008
	Brown-Forsythe	9.8	2	14.3	0,002
Glycosylated Hemoglobin (HbA1C).	Welch	5.4	2	10.8	0,024
	Brown-Forsythe	8.2	2	17.0	0,003

df1* Degree of freedom 1 (between groups); df 2 # Degree of freedom 2 (within groups); Post- hoc tests (Games-Howell) were applied to test further significance.

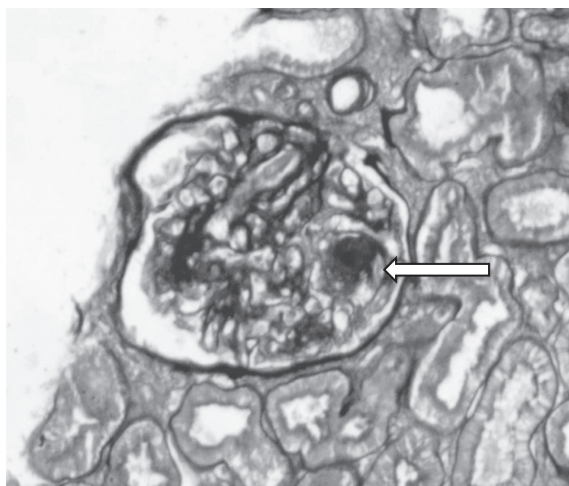


Figure 2. Showing image of glomeruli with Nodular lesion, Class III lesion (Arrow head), PAS stain, 40x.

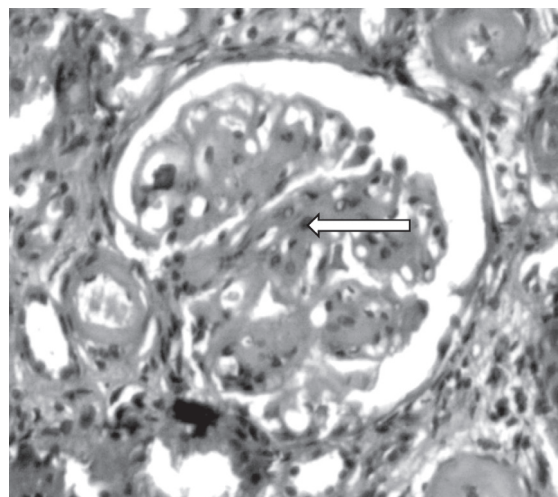


Figure 4. Showing an image of glomeruli with increase in mesangial matrix, Class II lesion (H&E staining, 40x)

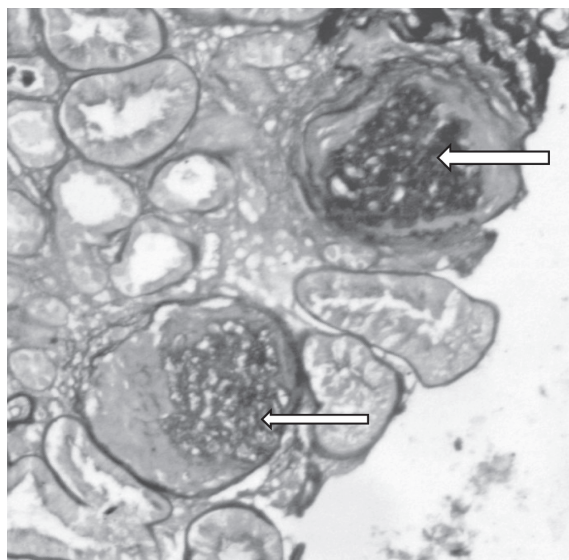


Figure 3. Showing two glomeruli which are completely sclerosed (Diffuse diabetic glomerulosclerosis, Class IV lesion (PAS stain, 100x)

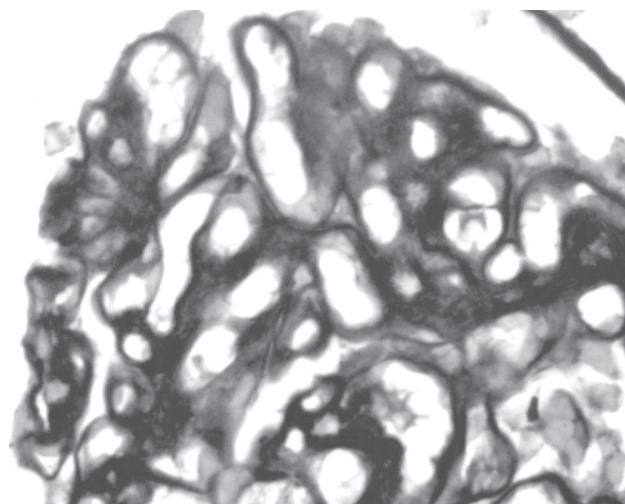


Figure 5. Showing thickening of glomerular basement membrane, Class I lesion (PAS stain, 100x)

Value= 0.02. Both statistic “Welch” and “Brown- For- sythe” values, and degree of freedom are explained in table 3 of robust tests of equality of means.

Post- Hoc tests were applied to verify the difference between the three groups, it was found that 24 hrs urinary protein values were higher in nodular glomerulosclerosis (Cat III) than mesangial changes (Cat II) in renal biopsy cases and this difference was statistically significant (p value=0.019). The diastolic blood pressure (DBP) differed significantly between Cat III and Cat II (p value= 0.004); and Cat III and Cat IV (p value=0.032). The glycosylated hemoglobin was statistically different between Cat III and Cat II and Cat III and Cat IV (p value= 0.013 and 0.039 respectively. (Table 3).

Renal biopsy findings

On microscopic examination in renal biopsy findings, 11 cases (52.3 %) showed KW lesion (Class III) in their renal biopsy, which is characterized by central nodular

area of matrix accumulation and surrounding capillary restriction (Figure 2) while 06 cases (28.5 %) showed diffuse diabetic glomerulosclerosis (Class IV) (Figure 3). Remaining 04 cases (19%) showed mild increase in mesangial matrix and slight thickening of glomerular basement membrane (Class II) (Figure 4 and 4 respectively). 03 cases (14.28%) show associated interstitial nephritis along with Diabetic nephropathy. Among which two were associated with Diffuse diabetic glomerulosclerosis and one with Nodular diabetic glomerulosclerosis. Almost all the patients show changes in blood vessels ranging from intimal thickening to hyaline arteriosclerosis of small to medium sized blood vessels.

DISCUSSION

Diabetic nephropathy is considered one of the most common and clinically significant consequences of diabetes. It affects roughly 40% of individuals

Table 4

Renal Pathology Society histologic classification system for diabetic nephropathy [11]

1	Class: I	Mild or nonspecific changes on light microscopy and confirmed GBM thickening proven by electron microscopy > 395 nm in women and > 430 nm in men
2	Class: II	Diffuse mesangial expansion IIa: Mild mesangial expansion in > 25 % of the observed mesangium Area of mesangial expansion < area of the capillary cavity IIb: Severe mesangial expansion in > 25 % of the observed mesangium Area of mesangial expansion > area of the capillary cavity
3	Class: III	Nodular sclerosis (Kimmelstiel-Wilson lesions) At least 1 Kimmelstiel-Wilson lesion and none of the changes described in class IV, without > 50 % globally sclerosed glomeruli on biopsy
4	Class: IV	Advanced diabetic glomerulosclerosis > 50 % globally sclerosed glomeruli on biopsy with clinical or pathologic evidence indicating that the sclerosis stems from diabetic nephropathy

with diabetes mellitus which have more than 20 years of disease duration and leading to a significant proportion of patients requiring ESRD treatment [12].

Tervaert TW et al. divided diabetic nephropathy (DN) into four stages based on basal membrane involvement, mesangial proliferation, nodular sclerosis, or advanced glomerulosclerosis [11].

The prevalence of diabetic glomerulosclerosis and other form of glomerular pathology include diffuse mesangial sclerosis and other clinical correlate to type II diabetes are less well known.

Schwartz M et al, showed that diabetic glomerulosclerosis is responsible for the clinical renal abnormality in most of the patient in type II DM [16].

Pham T et al, found that in kidney biopsy of Type II Diabetic patients 53.2% cases showed NDRD, pure diabetic glomerulosclerosis (DGS) was seen in 27.5% of cases and concurrent NDRD and DGS in 19.3% cases [7].

Some studies have found no statistically significant clinicopathological correlation between clinical and biochemical parameters in patients with the two most common histological variant of diabetic nephropathies, mainly diffuse and nodular glomerulosclerosis, in terms of age, sex, diabetes duration, systolic blood pressure, HbA1c, 24-hour urinary protein, and serum creatinine [13].

While other have suggested that nodular diabetic glomerulosclerosis is associated with poorer clinical course and longer duration of diabetes mellitus [14, 15].

Suzuki Y et al. studied the relationships between renal lesions, clinical features, and renal prognosis in 128 Japanese type 2 diabetes patients. Diabetes-related glomerulosclerosis (DMGS) was found in 108

cases (84.4%), while DM-associated glomerulonephritis (GN), IgA nephropathy, and membranous nephropathy were found in 20 cases (15.6%). In terms of histological markers, the increase in mesangial matrix was more closely associated with DM duration, GFR, and urine protein than with the degree of glomerular sclerosis, but the prognosis of renal function after renal biopsy in patients with a blood creatinine level of less than 1.2 mg/dl was worse in nodular lesions than in diffuse lesions. The tubulo-interstitial lesion grade was also higher in these patients with nodular lesions [17].

Jang SH et al. investigated diabetic nephropathy patterns in Koreans and clinicopathologic differences between diabetic nephropathy histologic subtypes. The renal lesions of 46 diabetic nephropathy patients were divided into three categories: class one, diffuse or nodular glomerulosclerosis, class two, vascular change without evidence of glomerulosclerosis, and class three, non-diabetic renal disease superimposed on diabetic glomerulosclerosis. In each class, 32 instances (70%), 4 cases (9%), and 10 cases (21%), respectively, were discovered. The clinical and laboratory data did not show a significant difference between the classes, but the nodular sclerosis group had a higher blood creatinine level than the diffuse group ($p=0.003$) in class 1 lesions. The most frequent nondiabetic renal condition superimposed on diabetic glomerulosclerosis was IgA nephropathy. They came to the conclusion that patients with nodular glomerulosclerosis had more advanced clinicopathological characteristics than patients with class 1 diffuse glomerulosclerosis [18].

Hong D et al, find that, in contrast to patients with diffuse glomerulosclerosis, those with nodular glomerular lesions exhibit higher levels of proteinuria, a longer disease duration, and lower BMI, creatinine clearance, and plasma albumin levels ($p 0.01$). There were 90% of instances with nodular glomerular lesions that also had diabetic retinopathy, compared to 14% of those with diffuse glomerulosclerosis. Patients with nodular glomerular lesions had worse renal prognoses, more severe renal damage, and longer periods of diabetes [19].

In their study, Ghani AA et al, divided the participants into two groups: one with standalone DGS and the other with Non-diabetic retinopathy superimposed on DGS. Proteinuria was considerably greater in group I with isolated DGS, 4.97 (2.08) gm/24 hrs urine, than in group II, 2.72 (1.09) gm/24 hrs urine ($P=.003$). Age, diabetes duration, gender, hypertension, GFR, hematuria and level of serum creatinine did not show significant difference between the two groups [20].

In present study majority of the cases (52.3%) showed nodular form of diabetic glomerulosclerosis while only 28.5% cases showed diffuse mesangial sclerosis and remaining 19% cases showed mild in-

crease in mesangial matrix with slight thickening of glomerular basement membrane. When compared with clinical parameters they were more raised in nodular type of diabetic glomerulosclerosis (KW-lesion) as compared to another lesion. This suggest that diabetic glomerulosclerosis is responsible for most of the clinical abnormality in type II DM.

CONCLUSION

Nodular form of diabetic glomerulosclerosis (KW lesion) was most common lesion in renal biopsy of type II diabetes mellitus patients. These KW lesion is responsible for more sever clinical and biochemical renal abnormality in most of the patient with type II diabetes mellitus. Vascular changes ranging from intimal thickening to hyaline arteriosclerosis of small to medium sized blood vessels was seen in almost all patients of diabetic nephropathy included in the study.

Limitation of study

The main shortcoming of the study was the small cohort size. However, larger cohort studies are needed to corroborate the findings.

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