Research Article

Correlation of Demographic and Biochemical Features with Histological Classification of Diabetic Nephropathy

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Abstract:

Background: Diabetic Nephropathy (DN) is a renal disease that leads to progressive renal failure across the world and it is among the most challenging of all renal diseases. The categorization of DN is centered on the lesions of glomeruli and exhibits varied morphological features. These patients outcome is also affected by evaluating the lesions of tubules, interstitium and renal vessels.

Objective: The objective of this study was to apply the current pathological categorization of DN by Tervaert et al to evaluate the lesions of kidney in DN and to correlate clinical and histopathological features.

Methods: This was a retrospective study of 10 years' duration from 2010-2019 at the Histopathology Department, Chughtai Institute of Pathology, Lahore. Fifty two cases of DN were included in the study. Parameters which were assessed included age, gender, duration of diabetes, 24 hours' urinary proteinuria, serum creatinine, histologic classes of diabetic nephropathy, Interstitial inflammation Score, interstitial fibrosis & tubular Atrophy (IFTA) score, Arteriolar Hyalinosis (AH) score and large vessel arteriolosclerosis.

Results: Out of 52 cases, 28 were males and 24 were females. Mean age of patients was 51.40 years. Mean duration of diabetes was 11.54 years. Mean serum creatinine level was 5.42 mg/dl. Mean proteinuria was 4.72 g/24 hours. The most frequent glomerular lesions were in Class III comprising 48% of all classes.

Conclusion: There was a significant correlation of glomerular classes with serum creatinine levels and also with proteinuria. There was also correlation of IFTA with rising serum levels of creatinine and proteinuria.

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Key words: Diabetic Nephropathy (DN), Interstitial Fibrosis & Tubular Atrophy (IFTA), Arteriolar Hyalinosis (AH), Light Microscopy (LM), Immunofluorescence (IF).

Introduction:

Diabetic Nephropathy (DN) is a renal disease that leads to progressive renal failure across the world and it is among the most challenging of all renal diseases[1]. Type 2 diabetes mellitus (DM) is becoming more common and rapidly evolving in the Asian population. It is estimated that proteinuria develops within 20 years of onset of diabetes in approximately 50% of the diabetic patients. Asian population is affected by DM in a younger age as compared to the Western population[2].

DN is the most common complication in patients with DM of more than 5 years duration and presents with proteinuria with or without hematuria. In our routine clinical practice, renal biopsy can sometimes be helpful in patients with short duration of diabetes with no diabetic retinopathy or those which present with massive hematuria, since it is known that proper early treatment can prevent progression to DN[3-5].

Several studies have described different pathological changes of DN mostly in type 2 diabetes mellitus patients[6-8], but the current pathological categorization accepted by the Renal Pathology Society represents more

comprehensive perspective towards histopathological features of DN[9]. The glomerular lesions are classified as I to IV with class I showing glomerular capillary wall thickening. There is mesangial cellularity and matrix expansion in class IIa and IIb based on severity. Nodular lesions are imparted in class III and globally sclerosed glomeruli in class IV. Similarly, tubulointerstitial and vascular compartment lesions are also scored. These lesions include Interstitial Fibrosis & Tubular Atrophy (IFTA), inflammation, Arteriolar Hyalinosis (AH) and Arteriosclerosis.

Until now, Tervaert et al classification has been used only in few studies of DN. This classification includes morphologic differences in all four compartments of the kidney biopsy.

Objective:

The main objective and goal of the current study was to categorize the lesions of kidney in keeping with Tervaert et al classification of DN and to correlate the demographic and biochemical findings with histopathological features.

Methods:

After approval from the Institutional Review Board, a retrospective study was conducted at Histopathology Department, Chughtai Institute of Pathology, Lahore, Pakistan. The data for this study was taken retrospectively from archives of Chughtai Institute of Pathology over a period of 10 years (2010 to 2019). Out of total 65 cases of diabetic nephropathy, 52 cases (n=52) were included in the study. Other cases (n=13) were excluded from the study due to inadequate biopsies and incomplete clinical details. According to the recorded clinical details, the reasons for the kidney biopsy included proteinuria, rapid decline in kidney functions, and active urine sediments. Biopsy was done to rule in/out DN.

Two biopsies were taken from each patient, one for light microscopy (LM) and second biopsy for purpose of immunofluorescence (IF). Electron microscopy (EM) was not available at our center; hence it was not done. Fixation solution was 10% buffered formalin in all the kidney biopsies for LM evaluation and in normal saline for IF studies. Hematoxylin and eosin and special stains, Periodic Acid-Schiff (PAS), Masson's Trichrome, and Jones Methenamine Silver (JMS) were applied in all cases.

Frozen tissue was used for IF study with IgG, IgM, IgA, and complements C1q and C3 in all cases. Tervaert et al classification of diabetic nephropathy was applied to classify glomerular lesions, similarly tubulointerstitial and vascular lesions were also graded according to this classification. All these cases were independently assessed by two consultant pathologists with complete agreement in all cases.

SPSS version 26 was used to analyze all data. Means and standard deviation were calculated for all variables including age, duration of diabetes, proteinuria level, serum creatinine, interstitial inflammation, IFTA score, AH score, large vessel arteriosclerosis score according to histological classes. Number and percentages were used for other variables including classes and gender. One way Anova and Tukey's Test were used for mean comparison. Correlation between different parameters was found by Pearson correlation coefficient and a p-value of <0.05 was considered to be statistically significant.

Results:

Out of 52 patients, type 1 diabetes patients were 2 and 50 of type 2 diabetes. Out of 52 subjects selected for study, 54% were males (n=28) and 46% were females (n=24). According to the clinical history, 34 patients had associated hypertension while 18 had no history of hypertension. Patient's age ranged from 16 to 75 years. The mean age in this study was 51.40±12.04 years. Diabetes duration ranged from 4 to 25 years. Mean duration of the diabetes mellitus in this study was 11.54± 5.16 years.

Tervaert et al classification of DN was used to categorize lesions of glomeruli (Table 1). Our study revealed class III, the most common class accounting for 48.1% (n=25) of all classes. No case of class I was seen in our study. 5.8% (n=3) cases fell into class IIA, 15.4% (n=8) came in class IIB while class IV comprised of 30.7% (n=16) cases (Table 1).

Overall, there was significant difference between mean serum creatinine (p value 0.005) and 24 hour proteinuria (p value 0.001) in all histological classes. Moreover, among all classes significant difference was observed between mean serum creatinine (p value 0.016) and

Table1: *Demographic, Biochemical and pathological features in different classes of diabetic nephropathy (n=52)*

	Class IIA	Class IIB	Class III	Class IV
Number of cases (with percentages)	3(5.8%)	8(15.4%)	25(48.1%)	16(30.7%)
Mean age of patients (Years)	50.66±30.61	55.37±4.71	48.24±12.45	54.50±8.59
Mean duration of diabetes (Years)	9.00±6.24	9.96±5.64	11.20±5.43	13.34±4.12
Mean proteinuria level (g/day)	3.26±1.00	3.62±0.80	4.72±1.03	5.54±1.40
Mean serum creatinine level (mg/dl)	4.10±3.50	4.01±2.75	4.79±2.23	7.35±2.54
Mean interstitial inflammation score	0.67	1.13	1.60	1.87
Mean IFTA score	0.67	1.63	2.12	2.81
Mean arteriolar hyalinosis score	0.33	1.25	1.20	1.87
Mean large vessels arteriosclerosis score	0.33	0.38	0.88	1.31

Table 2: *Correlation of demographic and biochemical features with different classes of diabetes mellitus*

	Pearson correlation coefficient	*p-value*
Correlation of age with classes of diabetic nephropathy	0.043	0.76
Correlation of duration of diabetes with classes of diabetic nephropathy	0.256	0.06
Correlation of proteinuria with classes of diabetic nephropathy	0.543	0.000
Correlation of creatinine with classes of diabetic nephropathy	0.426	0.002
Correlation of IFTA with 24 hour Proteinuria	0.386	0.005
Correlation of IFTA with creatinine	0.372	0.007
Correlation of arteriolar hyalinosis with 24 hour proteinuria	0.279	0.045
Correlation of arteriolar hyalinosis with creatinine	0.372	0.007

Correlation is significant at 0.05 level.

24-hour proteinuria (p value 0.002) of class IIB & class IV. The duration and age means did not show any significant difference (p value of 0.324 and 0.306 respectively).

24-hour urinary protein (in g/day) levels steadily raised from class IIA to Class IV with mean value of 4.72 g/day. The creatinine levels also showed increasing trend with successive histological classes of DN with a mean creatinine value of 5.42mg/dl. Inflammation, IFTA and vascular scores also showed increasing trend with increasing classes of diabetic nephropathy (Table 1).

A positive correlation was found between classes of DN with serum creatinine levels (Pearson coefficient 0.426 and p value of 0.002). Classes of DN also showed a significant positive correlation with 24-hour urinary proteinuria (Pearson coefficient of 0.543 and p-value of 0.000). However, age & duration of diabetes did not show any significant correlation with histological classes of DN (Table 2).

IFTA positively correlated with proteinuria (p-value 0.005) and creatinine (p-value 0.007). IFTA did not reveal any significant correlation with age and duration of diabetes (p-value 0.614 and 0.899 respectively). AH also showed marked positive correlation with proteinuria (p-value 0.045) and creatinine (p-value 0.007) (Table 2).

Figure 1 shows the morphology of different glomerular classes, interstitial and vascular lesions. Class IIA glomeruli showed mildly increased mesangial cells proliferation while class IIB glomeruli revealed markedly increased mesangial cells proliferation, glomerulus in class III showing Kimmelstiel Wilson Lesion, and glomeruli in class IV show advanced diabetic glomerulosclerosis, Figure (E) shows interstitial fibrosis and tubular atrophy (Score 2), and figure (F) shows arteriolar hyalinosis (Score 1).

In both males and females, Class III lesions were the most common (46.4% and 50% respectively) followed by class IV and II. Among males 3 cases (10.7%) were reported as class IIA while no case of this class was seen in females. The means of serum creatinine levels, duration of diabetes, inflammation, AH and arteriosclerosis

in both males and females did not reveal much difference. Slight difference was seen in means of age, 24-hour proteinuria and IFTA score. Mean age in males and females was 52.57±12.85 and 50.04±11.13 years respectively. Mean 24-hour proteinuria was 4.47±1.16 and 5.01±1.44 in males and females respectively. Mean IFTA score in males was 1.96 while in females it was 2.42.

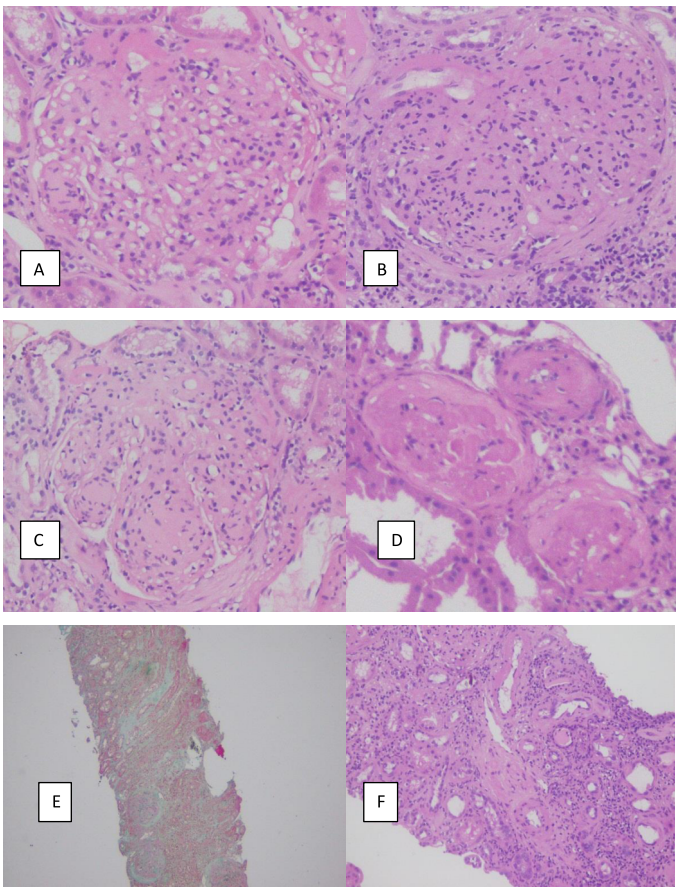

Figure 1: *(A) Glomerulus in Class IIA showing mild mesangial cellularity (H and E, x200). (B) Glomerulus in Class IIB showing marked mesangial cellularity(H and E, x200). (C) Glomerulus showing Kimmelstiel Wilson Lesion (H and E, x200). (D) Advanced diabetic glomerulosclerosis (H and E, x100). (E) Interstitial fibrosis and tubular atrophy (Score 2) (Masson's Trichrome x200). (F) Arteriolar Hyalinosis (Score 1) (H and E, x200).*

Discussion:

DN shows spectrum of characteristic morphological features that can be interpreted on LM and EM. Besides glomerular morphology, changes in tubulointerstitial and vascular compartments are equally important. Pathological classification is available for different diseases of kidney, however a reliable classification for DN was missing, and so Research Committee of Renal Pathology Society inculcated a novel classification of DN[9]. In this study, we have applied this current classification presented by Tervaert et al to categorize pathologic injuries in kidney and evaluated the correlation between different clinical and pathological features.

Using Tervaert classification to the present study, there was a significantly positive correlation between increasing classes of DN with serum creatinine and 24-hour urinary proteinuria. The age and duration of diabetes did not reveal remarkable correlation with different classes of DN. IFTA positively correlated with proteinuria and creatinine. IFTA did not reveal any significant correlation with age and duration of diabetes. AH also showed marked positive correlation with proteinuria and creatinine.

In this study, class III was the top most accounting for 48.1% (n=25) of all classes. According to a study by Afroz et al[1] in diabetic patients, glomerular lesions of class III were frequently seen with 50 percent of total renal lesions. In another study by Klessens et al[10], Class III was also the most common class of DN comprising 42.45% of all cases.

Regarding gender, in both males and females, Class III was the most common (46.4% and 50% respectively). There was no notable difference between means of serum creatinine levels, duration of diabetes, inflammation, AH and arteriosclerosis in males and females. Mean age in males and females was 52.57±12.85 and 50.04±11.13 years respectively. Mean 24-hour proteinuria was 4.47±1.16 and 5.01±1.44 in males and females respectively. Mean IFTA score in males was 1.96 while in females it was 2.42.

In Classes IIA, IIB, III and IV, the mean duration of DM was 9.00 ± 6.24, 9.96 ± 5.64, 11.20 ± 5.43 and 13.34 ± 4.12 years respectively. Classes of DN and the duration of diabetes did not show reliable correlation with each other (P = 0.06). In our study, mean duration of DM was less in class II as compared to class IV and it progressively increased from class II to IV. Similar findings were reported by Afroze T et al[1] that duration of DM was markedly less in class II than in class IV lesions.

In a study by Stefan G et al[11] 69 patients of type II DM were included and they found that apart from glomerular lesions, IFTA was also an important factor in end stage renal disease. Our study also showed similar findings. In contrast, Mottl et al[12] found no association between histological changes of DN and end stage renal disease.

In the study of Mise et al[2] with sample size of 205 DN patients, they found that as the glomerular class increased, tubulointerstitial and vascular lesions were associated with progressive renal failure. In this context, histological classification of DN imparts paramount importance in evaluation of renal prognosis. Similarly, in our study as glomerular histological classes increased, it correlated with more score of interstitial inflammation, IFTA and arteriosclerosis. Therefore, our study is comparable with the research study of Mise et al[2].

The present study had a few limitations. First, sample size was small, second, this was a retrospective study and all clinical and biochemical parameters were not available. Finally, electron microscopy could not be done due to unavailability at the center. Therefore, more cross sectional studies can be helpful for determining the prognosis of DN.

Conclusion:

DN ultimately leads to end stage renal disease requiring renal replacement therapy in most cases. Similar to other diseases, histological changes in different compartments of renal biopsy have utmost significance for prognosis and treatment of DN.

In this study, class III constituted the top most class. There was a significantly positive correlation between increasing classes of DN with serum creatinine and 24-hour urinary proteinuria. Duration of diabetes and age did not show any notable correlation with different classes of DN. IFTA and AH positively correlated with proteinuria and creatinine.

Ethical Approval: Given
Conflict of Interest: The authors declare no conflict of interest.
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