

Prevalence of non-diabetic kidney disease and inability of clinical predictors to differentiate it from diabetic kidney disease: results from a prospectively performed renal biopsy study

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ABSTRACT

Introduction Renal involvement in type 2 diabetes mellitus (T2DM) may be due to diabetes (diabetic kidney disease (DKD)), causes other than diabetes (non-diabetic kidney disease (NDKD)) or overlap of DKD and NDKD (mixed kidney disease group). Prevalence of NDKD and predictive value of clinical or biochemical indicators have been explored in retrospective cohorts with preselection biases warranting the need for prospectively conducted unbiased renal biopsy study.

Research design and methods Consecutive subjects aged >18 years with T2DM and renal involvement with estimated glomerular filtration rate of 30–60 mL/min/m² and/or albumin:creatinine ratio of >300 mg/g were offered renal biopsy. Prevalence of DKD, NDKD and mixed kidney disease was documented. Clinical/laboratory parameters of subjects were recorded and compared between groups and were tested for ability to predict histopathological diagnosis.

Results We screened 6247 subjects with T2DM of which 869 fulfilled inclusion criteria for biopsy. Of the 869 subjects, biopsy was feasible in 818 subjects. Out of 818, we recruited first 110 subjects who agreed to undergo renal biopsy. Among those 110 subjects, 73 (66.4%) had DKD; 20 (18.2%) had NDKD; and 17 (15.4%) had mixed kidney disease. Subjects with NDKD as compared with DKD had shorter duration of diabetes ($p<0.001$), absence of retinopathy ($p<0.001$) and absence of neuropathy ($p<0.001$). Logistic regression revealed that only presence of retinopathy and duration of diabetes were statistically significant to predict histopathological diagnosis of DKD. 30% of DKD did not have retinopathy, thereby limiting the utility of the same as a discriminator. Use of traditional indicators of biopsy would have indicated a need for renal biopsy in 87.2% of subjects, though 64.5% of the subjects had DKD, who would not have benefitted from biopsy.

Conclusion NDKD and mixed kidney disease in T2DM with renal involvement are very common and traditionally used parameters to select biopsies are of limited value in clinical decision making.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ True prevalence of diabetic kidney disease and non-diabetic kidney disease (NDKD) in adults with type 2 diabetes remains largely unknown. Published medical literature is predominantly derived from retrospective analysis of biopsy studies. In these studies, biopsies have been performed in a targeted manner with its own inherent biases.
- ⇒ Various predictors have been suggested to predict the likelihood of NDKD, but these have not been tested in prospectively conducted biopsy studies.

WHAT THIS STUDY ADDS

- ⇒ In this prospectively conducted unselected kidney biopsy study, we have highlighted the high prevalence of kidney disease in subjects with type 2 diabetes mellitus (T2DM) from causes other than diabetes (NDKD in 18.2% and mixed kidney disease in 15.4%).
- ⇒ The commonly used clinical/biochemical markers used to predict NDKD were suboptimal at an individual patient level to help clinical decision making (either to preselect or exclude the need for renal biopsy).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ High prevalence of NDKD (either pure NDKD or mixed kidney disease) in subjects with T2DM reveals the possible potential for treatment of NDKD with resultant improvement in clinical outcomes. The study highlights the limitations of currently used predictors of NDKD and opens up the scope for research to identify better markers to help preselect subjects for biopsy.

Worldwide 537 million adults are estimated to be living with diabetes.¹ Of subjects with diabetes, 20%–40% may have some form of renal involvement,³ highlighting

a huge global burden of kidney disease in subjects with diabetes. Renal involvement in individuals with type 2 diabetes mellitus (T2DM) includes causes due to diabetes per se, that is, diabetic kidney disease (DKD), or may be due to causes other than diabetes commonly referred to as non-diabetic kidney disease (NDKD). In addition, some patients may have renal involvement due to diabetes, as well as causes other than diabetes (mixed kidney disease group, having features of both DKD and NDKD).

The treatment of DKD entails tight blood pressure and glycemic control, often with preference of certain class of drugs over others. However, in a significant proportion of NDKD, there are certain specific treatment options (including steroids and other immunosuppressant) which could significantly improve patient outcomes. In addition, appropriate diagnosis could help in prognostication.

Histopathology is currently accepted as gold standard to differentiate DKD from NDKD. Published literature suggest that subjects with diabetes have varying proportion of DKD and NDKD.³ It may be noted that this difference in prevalence could be because of differences in clinical practice, namely, differences in criteria followed by clinicians to subject a patient to renal biopsy. Most of the published studies are retrospective in nature, with preselection biases of targeted biopsies performed in those with a high index of suspicion of NDKD.³⁻⁵

Considering the invasiveness, limited availability of expertise, infrastructure and cost of the procedure, widespread use of renal biopsy is not practical to perform in all subjects with T2DM with renal involvement.⁶⁻⁸

Traditionally, several clinical parameters have been used to help identify individuals at greater risk of NDKD. These parameters have often been used by clinicians to preselect subjects for renal biopsy. Unfortunately, data are sparse to support the use of the same. Results from meta-analysis of several studies (retrospective analysis of targeted biopsies) have suggested roles of certain clinical pointers to help identify subjects with higher possibility of NDKD.³ Yet there is very little consensus even among nephrologists on indications of renal biopsy.^{8,9} The study reported that decision to perform kidney biopsy is usually based on physicians' personal opinion and protocol/policy of the institute.⁹

Hence, we embarked on to undertake a study of patients with type 2 diabetes with renal involvement and offered them renal biopsies in an unselected manner prospectively to determine what proportion of subjects had DKD, NDKD and mixed kidney disease (having features of both DKD and NDKD).

In addition, we wanted to test how well the currently used clinical and laboratory indicators were in helping differentiating DKD from NDKD and hence their usefulness in helping preselecting subjects for renal biopsy.

MATERIALS AND METHODS

This was a single-centre, cross-sectional study with subjects recruited from the outpatient clinic of the department of endocrinology of the institute.

Consecutive subjects aged more than 18 years with type 2 diabetes with estimated glomerular filtration rate (eGFR) between 30 and 60 mL/min/1.73 m² by Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation and/or morning-spot urine albumin:creatinine ratio (ACR) more than 300 mg/gm (on two occasions, 3 months apart after adequate glycemic control and blood pressure control) were included. We excluded subjects with eGFR of <30 mL/min/m² as they are likely to have advanced disease and histopathology was likely to be of burnt-out disease and also excluded subjects with eGFR of >60 mL/min/m² as it was ethically difficult to justify performing renal biopsies in them.

Subjects were excluded from the study if they had recent or recurrent urinary tract infection, calculus renal disease, obstructive uropathy, renal tumors, bleeding diathesis, bilateral contracted kidneys or single kidney, hydronephrosis, on antiplatelet therapy, unexplained coagulopathy or those who had stages of renal disease, other than those specified in the inclusion criteria. Additionally, those who qualified for the inclusion and exclusion criteria but declined to give consent for the biopsy were also excluded. The protocol for inclusion of the study subjects is described in [figure 1](#).

Clinical/demographic and biochemical parameters from all subjects were documented. This included age, gender and duration of diabetes, family history of diabetes and/or renal involvement, medication history, and anthropometric parameters including height and weight. Body mass index was calculated from them. Hypertension was defined as systolic blood pressure of >140 mm Hg and diastolic pressure of >90 mm Hg or the use of antihypertensive medication. Blood pressure was recorded in right arm supine position with appropriate precautions.

Morning urine samples and fasting blood samples were collected from subjects at baseline. Serum creatinine (by Jaffe's method traced to IDMS (isotope dilution mass spectrometry)), urea, lipid profile, liver enzymes, serum albumin, uric acid and 24-hour urinary albumin were measured by an autoanalyzer (Cobas, Integra 400 Plus; Roche). Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography method (Bio-Rad D10-HbA1c Analyzer). Complete blood count was measured by an automated hematology analyzer. Presence of markers of possible collagen vascular/connective tissue disorders and autoimmune disorders, that is, antineutrophil cytoplasmic antibody (ANCA) (1:10 dilution) and antinuclear antibody (ANA) (1:160 dilutions), was measured by indirect immune fluorescence method, and PR3, myeloperoxidase (MPO) and anti-glomerular basement membrane (GBM), double-stranded DNA (dsDNA) was measured by ELISA (using

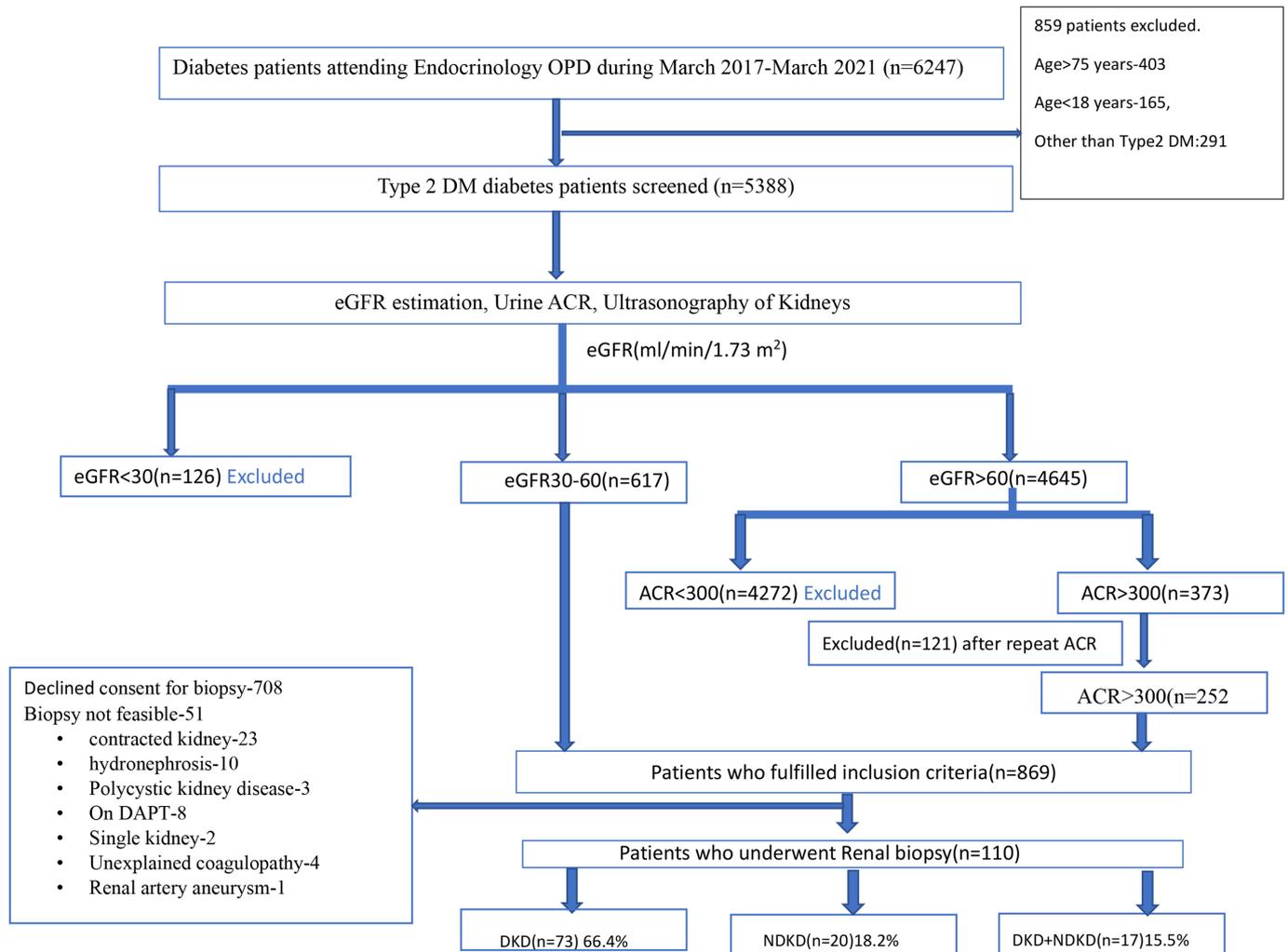


Figure 1 Protocol for inclusion of the study subjects (figure 1). ACR, albumin:creatinine ratio; DKD, diabetic kidney disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; NDKD, non-diabetic kidney disease.

kits Euroimmun US). Complement components (C3 and C4) were measured by nephelometry. The reference range for the above parameters was as follows: C3 (90–180 mg/dL), C4 (10–40 mg/dL), MPO (<20 RU/mL), PR3 (<20 RU/mL), dsDNA (<20 RU/mL) and anti-GBM (<20 RU/mL). eGFR was calculated by the CKD-EPI equation using QxMD calculator.

Urine for routine and microscopic examination test was also performed in all. Presence of more than three red blood cells (RBCs)/high-power field was defined as microscopic hematuria.¹⁰

Urine for ACR was done by an autoanalyzer (Cobas, Integra 400 plus; Roche). ACR value of less than 30 µg/mg of creatinine was taken as normoalbuminuric. Microalbuminuria was defined as an albumin excretion of 30–300 µg/mg of creatinine on more than one occasion. Proteinuria was defined as an albumin excretion more than 300 µg/mg of creatinine on more than one occasion.¹¹

All subjects were also evaluated for 24-hour urinary protein estimation (by calorimetric using pyrogallol red method).

Retinal screening was done by a trained ophthalmologist, with digital fundus photography (Topcon non-mydiatic retinal camera) with pupils adequately dilated. Gradation of retinopathy was done as follows, based on Early Treatment Diabetic Retinopathy Study classification: no retinopathy, early retinopathy: when there was non-proliferative background retinopathy, moderate: preproliferative retinopathy with/without maculopathy without center involvement, severe retinopathy: there is proliferative retinopathy with/without maculopathy with involvement of the area in and around the fovea.¹²

Vibration perception threshold (VPT) for detection of neuropathy was measured by a sensitometer. An average of three measurements was taken. The VPT was measured at six positions and the worst score was taken.⁷ The VPT score for defining neuropathy was graded as normal (<15 V), mild (15–20 V), moderate (20–25 V) and severe (>25 V).¹³

Kidney size was measured by ultrasonography. Unequal kidney size was defined if the difference between two kidneys is more than 1.5 cm; the length of the kidney in the longer axis and size less than 9 cm were considered as

small kidney.^{14 15} Kidney biopsy, however, was avoided if kidney size in the long axis was less than 7 cm.

Subjects who had given informed consent and had no contradictions were subjected to ultrasonography-guided renal biopsy using an automated biopsy gun by two trained nephrologists of the institute. Biopsy specimens were sent for light microscopy, immunofluorescence microscopy and electron microscopy as per standard protocol. Histopathological classification was done by two independent expert renal pathologists following the guideline of the International Society of Nephrology (ISN) and the Renal Pathology Society (ISN/RPS classification). DKD was diagnosed by the presence of mesangial expansion with or without the nodular Kimmelsteil-Wilson formation, basement membrane thickening, fibrin caps or capsular drops. Glomerular lesions were classified according to RPS classification. Scoring system of interstitial lesions interstitial fibrosis and tubular atrophy (IFTA), interstitial inflammation, vascular lesion and arteriosclerosis followed.¹⁶ Depending on pathological findings, the subjects were divided into three groups: diabetes kidney disease (isolated DKD), non-kidney disease (isolated NDKD) and mixed kidney disease group (having features of both DKD and NDKD).

Sample size and statistical analysis

Different studies have reported different prevalence of DKD and NDKD in subjects with T2DM and renal involvement.³ To get the most conservative/largest sample size, we assumed the prevalence of DKD to be 50% in type 2 diabetes. With a 95% confidence level and margin of error of 10%, we calculated that at least 96 subjects would be required to fulfill the first objective for this study. However, assuming a possibility of dropout rate of 15%, we calculated the sample size to be 110.

For our second objective, that is, to establish the ability of various clinical and laboratory parameters to differentiate DKD from NDKD, we considered evaluating traditionally used discriminators including duration of T2DM, absence of retinopathy, presence of hematuria, RBC cast, presence of markers of possible collagen vascular/connective tissue disorders and autoimmune disorders (c3, c4, ANA, ANCA, PR3 and dsDNA). We hoped to identify additional parameters from our study, which may help discriminate DKD from NDKD (anticipating additional three or four parameters). Hence, we assume that the total number of covariates to be tested for validity of prediction is likely to be no more than nine, and hence the minimum number of subjects needed would be no more than 90 (ie, at least 10 times the number of covariates).¹⁷

The data were tested for normality using the Kolmogorov-Smirnov test. Categorical variables are expressed as frequencies and percentages. In the case of a normal distribution, continuous data were presented as mean±SD. Otherwise, median and IQRs were reported. Mann-Whitney U test and Kruskal-Wallis tests were performed for comparing continuous data that did not

conform to normal distribution. Comparison of categorical variables between groups was done by χ^2 test.

To test the validity of clinical and laboratory discriminators to differentiate DKD from NDKD, we excluded the mixed kidney disease group from our analysis and included only subjects with DKD and NDKD to keep the analysis clean from interference of mixed disease. We ran a binary logistic regression to find predictors of DKD. We tested the model for prediction for DKD and not NDKD as NDKD is a group of heterogeneous diseases.

We tested the models for covariates which are traditionally used (duration of diabetes since diagnosis, presence/absence of retinopathy, presence/absence of hematuria, presence/absence of markers of possible collagen vascular/connective tissue disorders and autoimmune disorders, 24-hour urinary protein excretion and kidney size) and also included parameters which were significantly different between DKD and NDKD (with a liberal p value of <0.1)¹⁸ and which were likely to have a biological plausibility of being different between the groups. All statistical analyses were performed using SPSS software V.21.0.

RESULTS

We screened 6247 subjects with T2DM, and of these, 869 fulfilled the inclusion criteria for biopsy. Of the 869 subjects, biopsy was feasible in 818 subjects. Out of 818, we recruited first 110 subjects who agreed to undergo renal biopsy (figure 1). The clinical and biochemical parameters of subjects who declined biopsy and those who underwent biopsy were compared (online supplemental table 1), which indicates that the subjects who underwent biopsy were similar/representative of the total eligible population.

Among the 110 subjects who underwent biopsy, 75 (68.2%) were male and 35 (31.8%) were female. The mean (±SD) age of the subjects was 50.17±8.58 years. The age of subjects ranged from 24 years to 74 years. The mean duration of known diabetes was 103.3±74.1 months, and the mean eGFR was 57.1±21.9 mL/min/1.73 m².

Histopathological diagnosis of DKD was made in 73 (66.4%) subjects, and 20 subjects (18.2%) had NDKD, that is, kidney disease not related to diabetes. Seventeen subjects (15.4%) had features of both DKD and NDKD (mixed kidney disease).

The most common diagnosis in the NDKD was IgA nephropathy (n=5). The other subtypes in the pure NDKD group were as follows: glomerular disease, for example, focal segmental glomerulosclerosis (n=4), minimal change disease (n=3) amyloidosis (n=1), membranous nephropathy (n=4), membranous-proliferative glomerulonephritis (n=1), tubule-interstitial disease, for example, acute tubular necrosis (n=1) and vascular disease, and hypertensive arteriosclerosis (n=1). In the mixed group, the most common subtype of NDKD is focal segmental glomerulosclerosis (n=5).

Fundus examination revealed no retinopathy in 50 (45.5%) subjects, early retinopathy in 31 (28.1%), moderate in 22 (20.1%) and severe in 7 (6.3%) subjects. Evaluation of patients for neuropathy revealed that 22 (20%) had no neuropathy; mild neuropathy was found in 7 (6.36%); moderate neuropathy was found in 23 (20.9%); and 58 (52.7%) had severe neuropathy.

Analysis revealed that 12.5% of subjects with DKD were non-albuminuric DKD and those without albuminuria; 69.2% had true DKD; 15.3% had true NDKD; and 15.3% had mixed kidney disease.

The clinical characteristic and biochemical parameters of DKD, NDKD and mixed kidney disease groups are shown in [table 1](#). A statistically significant difference was found in the duration of T2DM diabetes ($p<0.001$), absence of retinopathy ($p<0.001$), HbA1c ($p=0.003$), level of hemoglobin (%) ($p=0.01$) and absence of neuropathy ($p<0.001$) between the DKD and NDKD groups ([table 1](#)).

Hence, when we choose parameters for testing in a logistic regression model, we additionally chose presence of neuropathy (in addition to the conventionally used clinical/laboratory discriminators) but did not include hemoglobin levels and HbA1c in the model as we felt that decline in renal function itself may be associated with alteration in hemoglobin levels, and the degree of glycemic control might be highly variable across the study population and might not necessarily reflect the etiology of renal dysfunction.

To determine predictors for histopathological diagnosis of DKD, we ran a logistic regression model. The covariates used in this model were the duration of diabetes, presence of retinopathy, presence of neuropathy, absence of hematuria, absence of markers of possible collagen vascular/connective tissue disorders and autoimmune disorders, 24-hour urinary protein excretion and absence of unequal kidneys. Among them, duration of diabetes (in months) (OR 1.02, 95% CI 1.0 to 1.05; $p=0.03$) and presence of retinopathy (OR 18.85, 95% CI 1.2–2.77; $p=0.03$) were found to be statistically significant. It may also be noted that 30% of subjects with DKD had no retinopathy

([table 2](#)).

Our data revealed the presence of at least one traditional clinical/laboratory parameters (indicating possibility of NDKD or mixed kidney disease) and warranting biopsy in 96 (87.2%) out of 110 patients. It was found that 62 (64.5%) of those 96 subjects in whom biopsy would have been indicated had a histological diagnosis of DKD and would not ultimately benefit from biopsy.

The results indicate that the clinical/laboratory parameters are suboptimal in helping us preselect individuals who would benefit from renal biopsy.

DISCUSSION

Previously published studies on renal biopsies in subjects with type 2 diabetes are mostly retrospective in

nature and include data of biopsies which have been performed selectively, in a targeted manner in those in whom the clinician felt that there was greater likelihood of diagnosis of NDKD. Data from prospectively performed renal biopsies in an untargeted (without preselection bias) manner have not been offered to consecutive patients attending a diabetes clinic in any previous study (to the best of our knowledge). In day-to-day clinical practice, it is often difficult to perform renal biopsies. This could be because of several reasons as biopsies are fraught with possible complications, and logistically, it is not possible to perform biopsies in all clinical set-ups. It is true that biopsy is truly needed in subjects with NDKD as it may alter patient outcomes. Previously published studies based on retrospective data of renal biopsies have great differences in prevalence of DKD, NDKD and mixed kidney disease.³ In our study, we found that the prevalence of DKD was 66.4%, and that of NDKD was 18.2%. The prevalence of mixed kidney disease was 15.4%. This highlights the huge burden of NDKD either in isolation or in combination with DKD (ie, mixed kidney disease group) among subjects with T2DM and kidney involvement who might benefit from renal biopsy.

Binary logistic regression analysis of our data suggests that duration of disease and presence of retinopathy were statistically significant in predicting DKD. However, duration of T2DM is often unreliable for subjects with T2DM, as the disease may have been pre-existing long before the diagnosis of the disease. Diabetic retinopathy and DKD are generally believed to develop together as common microvascular complication of diabetes. Previous studies showed different results regarding the renoretinal association. In our study, absence of retinopathy was found in 95% of subjects with NDKD, which suggests that absence of DR could be a possible predictor for NDKD; however, it may be noted that 30% of subjects with DKD had no retinopathy. Interestingly, the ORs of these parameters were either not strong or had very wide CI. Thus, both statistically significant parameters have limited value in making clinical decisions at individual patient level.

In most of the previous studies, the investigators have found difference in certain parameters between DKD and NDKD, but they have mostly not tested the same in the logistic regression model.^{19–21}

In few studies (retrospective studies of targeted renal biopsies), regression analysis was performed,^{22–23} and some even came up with equations^{24–25} to predict probability of either DKD or NDKD. However, it is important to appreciate that these equations were of little use for decision making at individual patient level in day-to-day clinical practice.

Of patients with type 2 diabetes and renal involvement, 87.2% had presence of at least one traditional indicator for biopsy, even though ultimately 64.5% of them had a histopathological diagnosis of DKD. This highlights the limitations and impracticalities of the currently used parameters.

Table 1 Baseline clinical and biochemical parameters according to histopathological classification (N=110)

	DKD (n=73)	NDKD (n=20)	Mixed kidney disease (n=17)	P value (calculated between DKD and NDKD groups)
Age (year)	51 (45.5–56.5)	52.5 (45.25–58.75)	47 (40.0–54.5)	0.607
Age of onset (year)	40 (33.5–46.5)	50.12 (41.62–53.37)	38 (34–50)	0.001
Known duration of disease (months)	120 (60–180)	36 (6–57)	78 (48–120)	<0.001
BMI (kg/m ²)	24.87 (22.9–27.09)	23.24 (21.77–25.35)	22.76 (20.83–24.8)	0.118
Cholesterol (mg/dL)	179 (140.0–221.75)	194 (163–337)	166 (135.7–223.0)	0.06
Triglyceride (mg/dL)	176 (117.2–243.5)	158 (133–280)	160 (96–70)	0.93
HDL (mg/dL)	49 (42.25–58.0)	55 (45–81)	46.5 (32.0–57.75)	0.1
LDL (mg/dL)	88 (59–120)	110 (74–223)	78 (58.0–120.1)	0.07
Albumin (g/dL)	3.6 (2.9–4)	3.7 (2.45–4.12)	3.9 (3.5–4.1)	0.992
Uric acid (mg/dL)	6.7 (5.1–8.6)	6.6 (5.2–7.8)	8 (7.4–9.2)	0.784
HbA1c (mmol/mol)	7.7 (6.7–9.7)	6.4 (5.7–6.8)	9 (6.9–10.8)	0.003
Urea (mg/dL)	38(27–56)	31(23–52)	39(25–56)	0.226
Creatinine (mg/dL)	1.45 (1.2–1.7)	1.1 (0.8–1.6)	1.48 (1.08–1.76)	0.02
eGFR (CKD-EPI) (mL/min/1.73 m ²)	50 (39.3–65.9)	60 (46.8–91.5)	42.6 (52.9–79)	0.05
Hb (%)	10.8 (9.8–12.45)	12.14 (11.2–13.45)	12.95 (10.42–12.95)	0.01
24-hour protein (mg)	2544.5 (714.2–5832.7)	2215 (942.6–5034.8)	2573.3 (1104–9428.8)	0.932
RBC (/HPF)				0.07
Family history of diabetes (%)	57.5	40.0	12.3	0.15
Family history of kidney disease (%)	13.6	5.0	23.5	0.22
Unequal kidney (%)	6.8	10	0	0.64
Small kidney (%)	13.69	15.0	17.6	0.56
High C3 level (%)	0	4	0	0.215
High C4 level (%)	17.8	30.0	11.76	0.186
ANA positive (%)	8.2	4.0	5.8	0.531
ANCA positive	1.36	4.0	0	0.386
PR3/MPO/anti-GBM positive	0	0	0	
High-level dsDNA (%)	2.7	0	0	
Presence of nephrotic range proteinuria (%)	42.4	53.8	47.05	0.61
Presence of hematuria (%)	16.43	35.0	29.4	0.113
Urinary cast (%)	8.2	10.0	29.4	0.68
Presence of hypertension (%)	76.7	55.0	76.4	0.08
Absence of retinopathy (%)	30	95	30.3	<0.001
Absence of neuropathy (%)	13.4	66.0	18.18	<0.001

Continuous data presented as median (IQR), and compared by Mann–Whitney U test and categorical data presented as percentage (%) and compared by Chi-Square Test. As approximately 30 variables were tested, the p value for significance was taken as <0.001 using Bonferroni's correction.

ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DKD, diabetic kidney disease; dsDNA, double-stranded DNA; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HPF, high-power field; LDL, low-density lipoprotein; NDKD, non-diabetic kidney disease; RBC, red blood cell.

As clinicians, we need better clinical/laboratory markers, including newer biomarkers which could help discriminate DKD from NDKD and help exclude

the need of biopsy in those with pure DKD and better help preselect individuals for biopsy with greater likelihood of NDKD who could benefit from other forms

Table 2 Binary logistic regression analysis of factors related to diagnosis of DKD

Variables	B	SE	Sig	OR (95% CI)
Duration of diabetes (months)	0.029	0.014	0.03	1.02 (1.0 to 1.05)
Presence of retinopathy	2.937	1.371	0.03	18.85 (1.2 to 277.009)
Presence of neuropathy	1.952	1.099	0.07	7.04 (0.818 to 60.68)
24-hour urinary protein	0.00	0.00	0.451	1 (1 to 1)
Unequal kidney size	1.835	2.562	0.474	6.26 (0.041 to 949.58)
Hematuria	2.165	1.412	0.125	8.713 (0.547 to 138.66)
Presence of autoantibody makers	1.46	1.336	0.91	1.1 (0.08 to 15.88)

DKD, diabetic kidney disease.

of therapy which could possibly change their clinical outcomes.

Limitations

The study results may have been different had we conducted the study at community level and included all subjects of type 2 diabetes with renal involvement irrespective of stage of kidney disease. Additionally, we could conduct biopsies only in those who gave consent, which meant many eligible subjects did not undergo biopsy. However, this was not possible because of ethical concerns and difficulty in interpretation of histopathology at the extremes of stages of renal involvement. We compared baseline data of those who consented to undergo biopsy and those who declined to undergo biopsy (in spite of fulfilling study criteria) and reassuringly found that there was no differences between the groups. The results of the study may additionally be cross-validated in a future study in a multicenter multinational and multiethnic population.

CONCLUSION

NDKD is very common among subjects with T2DM and renal involvement. Currently used predictors to preselect biopsy for detecting NDKD have limited value at making clinical decisions at individual patient level.

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Supplementary table:1. Comparison of clinical and biochemical parameters of subjects who underwent biopsy versus those who declined to undergo biopsy

	Biopsy subjects' group (n=110)	Decline Biopsy group (n=708)	P value
Age(year)	50(45-56.25)	52.5(43-61.5)	NS
Age of onset(year)	41(35-50.5)	42(34-54)	NS
Known duration of disease (Months)	96(48-150)	92(44-155)	NS
BMI(kg/m ²)	24.3(22.3-26.83)	23.5(22-26)	NS
Cholesterol(mg/dl)	181(143-230)	190(140-250)	NS
Triglyceride(mg/dl)	170(117-244)	208(98-306)	NS
HDL(mg/dl)	49(42-59)	60(44-70)	0.04
LDL(mg/dl)	94(61-125)	109(58-159.5)	NS
Albumin(gm/dl)	3.6(2.9-4)	4.4(2.1-5.1)	NS
Uric Acid(mg/dl)	6.7(5.2-8.6)	8.1(4-9)	0.04
HbA1C(mmol/mol)	7.4(6.7-9.7)	7.5(6-10.2)	NS
Urea(mg/dl)	37(25.5-55.5)	40(25.5-52.5)	NS
Creatinine(mg/Dl)	1.43(1.1-1.7)	1.5(1-1.9)	NS
eGFR (CKD-EPI) (mL/min/1.73m ²)	53.15(41-68.77)	51.2(39-64.8)	NS
Hb	11.5(10-12.85)	10(9-12.2)	NS
24 hr protein(mg)	2544.5(761-5382.7)	3012.5(700-5423.4)	NS
RBC(/HPF)	1(0-2)	1(0-2)	NS
Family history of diabetes (%)	36%	40.8%	NS
Family history of kidney disease (%)	14.03%	15.25%	NS
Unequal kidney (%)	5.6%	7%	NS
Small kidney(%)	15.43%	16.21%	NS
High C3 level(%)	0.09%	0%	NS
High C4 level(%)	19.85%	20.6%	NS
ANA positive(%)	6%	5.6%	NS
ANCA positive	1.5%	2.6%	NS
PR3/MPO/Anti GBM positive	0	0	NS
High level dsDNA%	0.9%	0.1%	NS
Presence of nephrotic range proteinuria%	47.75%	45%	NS
Presence of Haematuria (%)	26.94%	28.9%	NS
Urinary Cast (%)	15.8%	12%	NS
Presence of Hypertension (%)	69.3%	72%	NS
Absence of retinopathy (%)	53.4%	60%	NS
Absence of neuropathy (%)	32.5%	30.6%	NS

Continuous data represented as median (IQR), P value was calculated by Mann–Whitney U test and categorical data represented in %, P value was calculate by Chi square Test

