The effect of non-albumin proteinuria on renal outcomes in patients with biopsy proven diabetic nephropathy

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Abstract. – OBJECTIVE: We aimed at investigating the effect of non-albumin proteinuria on renal outcomes in patients with biopsy-proven diabetic nephropathy.

PATIENTS AND METHODS: The files of all patients who underwent kidney biopsy between January 2010 and January 2020 were reviewed retrospectively. Non-albumin proteinuria was calculated by subtracting albumin from total protein in 24-hour urine samples. The patients were divided into 2 groups, according to the presence of composite kidney outcomes.

RESULTS: The study included 23 patients with diabetic nephropathy. The kidney endpoint was achieved in 34.8% (n=8) of the patients. Hypertension, duration of diabetes mellitus, creatinine level at the date of biopsy, microalbuminuria and non-albumin proteinuria were found to be independent predictors for composite kidney outcome (p=0.002, p=0.007, p=0.004, p=0.006, and p=0.001, respectively).

CONCLUSIONS: NAP was found to be an independent risk factor for doubling the serum creatinine level from the date of biopsy, for starting hemodialysis or peritoneal dialysis, for kidney transplantation, and kidney-related death.

Key Words:
　Diabetes mellitus, Microalbuminuria, Proteinuria.

Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) all around the world[1,2]. According to the Turkish Registry Report, diabetes mellitus (DM) is the most common cause of ESRD with a rate of 36.8%[3]. The American Diabetes Association (ADA) recommends starting DN screening at the time of diagnosis in patients diagnosed with type 2 DM, and 5 years after diagnosis in patients diagnosed with type 1 DM[4]. DN diagnosis is made clinically but, in some circumstances, to exclude primary glomerular diseases a kidney biopsy may be performed. There are 3 methods for screening microalbuminuria; albumin to creatinine ratio in spot urine, microalbuminuria in 24-h urine, and microalbuminuria in timed (4-h or overnight) collected urine. The presence and severity of microalbuminuria is an independent risk factor for the progression of DN, as well as an independent risk factor for cardiovascular diseases and mortality[5-7]. Even high albuminuria levels within normal limits are a cardiovascular risk factor[8].

Non-albumin proteinuria (NAP), together with microalbuminuria, is a frequently requested but often overlooked laboratory test in clinical practice. It can be easily calculated as the NAP-creatinine ratio in spot urine, or it can be found by subtracting albuminuria from the total proteinuria in 24-hour urine samples. NAP is a simple and practical predictor for DN[9]. In studies[10-12] investigating the NAP-DN relationship in the literature, the diagnosis of DN was made clinically. In our literature review, we could not find any study investigating the relationship between NAP and kidney outcomes in patients with biopsy-proven DN. In this study, we aimed at investigating the effect of NAP on kidney outcomes in patients with biopsy-proven DN.

Patients and Methods

Patients

The files of all patients (n=244) who underwent kidney biopsy in our clinic between January 2010 and January 2020 were reviewed retrospectively. Patients whose biopsy results were reported as isolated DN (n=23) were included in the study. Patients who were diagnosed with the non-diabetic glomerular disease did not have proteinuria data before the biopsy date, had a history of kid-

ney transplantation before the biopsy, and did not have sufficient data for kidney outcomes were excluded from the study. Patients who died except for kidney complications were also excluded. Figure 1 shows the inclusion and exclusion criteria of patients and the study design. Demographic and clinical characteristics of the patients, indications for biopsy, duration of DM before biopsy, and presence of diabetic retinopathy before biopsy date were recorded. Body mass index (BMI) was calculated by dividing the patient's weight (kg) by the square of the height in meters. The duration of DM was determined with the information obtained from the patient and/or by backward scanning of the recorded diagnostic ICD codes for the patients. The most recent laboratory parameters before the biopsy date were also recorded.

Kidney Pathology and Outcomes

All kidney biopsy specimens were evaluated together by a nephrologist and a single experienced nephropathologist. Kidney biopsies with thickening of the glomerular basement membrane, hyaline exudative lesions, Kimmelstiel-Wilson nodules, and mesangial enlargement were reported as DN. The primary endpoints were determined as follows: a doubling of the serum creatinine level after the biopsy date, initiation of hemodialysis or peritoneal dialysis as kidney replacement therapy, a kidney transplant due to ESRD, and death due to kidney complications.

Assessment of NAP and Groups

Since all patients in this study had 24-hour urine samples before biopsy, NAP values were calculated by subtracting albuminuria from total proteinuria in 24-hour urine samples. Written information about 24-hour urine collection was given to all patients. Patients who achieved any kidney endpoint were included in the composite kidney outcome group, while patients who did not develop any kidney endpoint were included in the control group.

Statistical Analysis

Categorical variables were presented as frequency and percentages. The Chi-square test was used to compare categorical variables between groups. Numerical variables were checked for normal distribution with the Shapiro-Wilk test. Normally distributed numerical variables were presented as mean±standard deviation (SD). Non-normally distributed numerical variables were presented as median and interquartile range-1 and interquartile range-3 (Q1-Q3). Independent samples *t*-test was used to compare normally distributed numerical variables between groups. Mann-Whitney U test was used to compare non-normally distributed numerical variables between groups. Logistic regression analysis was used to determine the risk factors for kidney composite outcomes. Variables statistically different between groups were included in the logistic re-
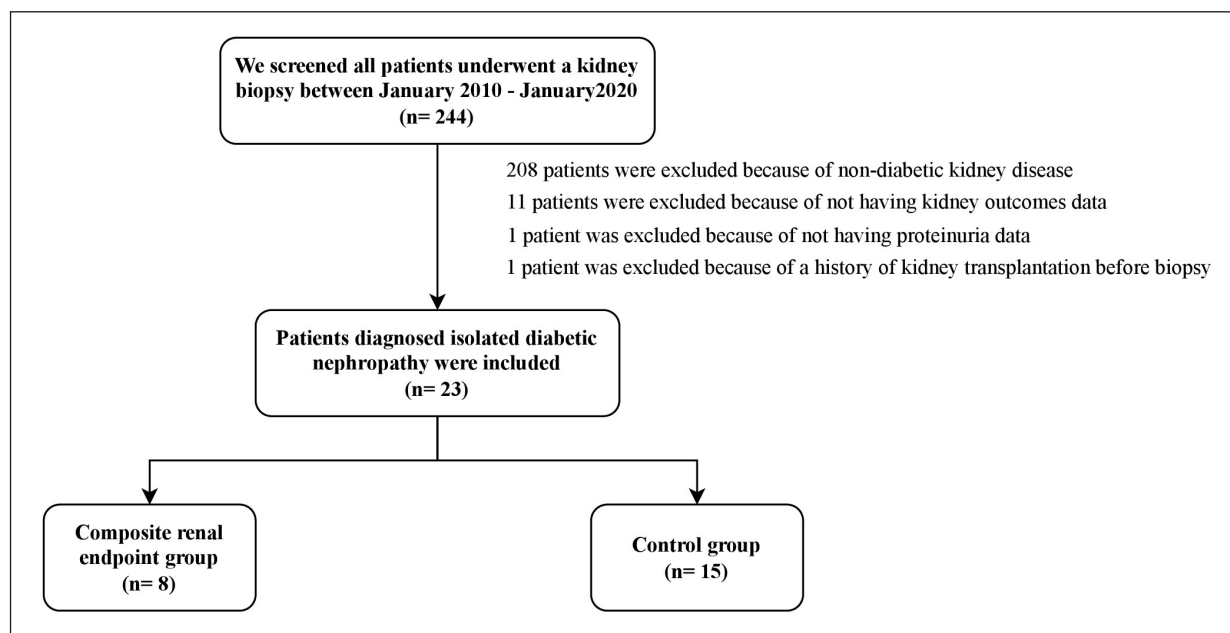

Figure 1. Study design.

gression model. Statistical analyzes were done with SPSS 26.0 (IBM Corp., Armonk, NY, USA) package program. All *p*-values presented were bi-directional and the values lower than 0.05 were expressed as statistically significant.

Results

The study included 23 patients with diabetic nephropathy. The median age of the patients was 45 years (IQR25-75 = 42-50 years). Of the patients, 65.2% were female (n=15). The median follow-up was 86 months (IQR25-75 = 69-128). Of the patients, 52.2% (n=12) was hypertensive. Biopsy was performed in 73.9% (n=17) of the patients because of the nephrotic syndrome clinic and in 26.1% (n=6) because of findings supporting non-DN kidney disease. The kidney endpoint was achieved in 34.8% (n=8) of the patients during follow-up; serum creatinine level had doubled in 3 patients, hemodialysis was started in 2 patients and peritoneal dialysis was started in 1 patient, 1 patient had been transplanted due to ESRD and 1 patient died as a result of kidney complications. Age, BMI, duration of DM, and prevalence of hypertension were higher in patients in the composite kidney outcome group ($p<0.001$, $p=0.027$, $p=0.028$ and $p=0.001$, respectively). Table I shows the demographical and clinical features of the groups. Fasting plasma glucose (FPG), creatinine, HbA1c, proteinuria, microalbuminuria, and NAPs of the patients in the composite kidney outcome group were found to be significantly higher, and eGFR levels were significantly lower than the control group ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.015$ and $p<0.001$, respectively). Table II shows a comparison of groups in terms of laboratory parameters.

In multivariate logistic regression analysis, hypertension, duration of DM, creatinine level at the date of biopsy, microalbuminuria and NAP were found to be independent predictors for composite kidney outcome ($p=0.002$, $p=0.007$, $p=0.004$, $p=0.006$ and $p=0.001$, respectively). Table III shows univariate and multivariate logistic regression analysis for composite kidney outcomes.

Discussion

NAP is an important urinalysis finding. In a comparative study, Kim et al[9] found that NAP could better predict annual eGFR decline when compared to 6 urinary biomarkers, including Kidney Injury Molecule-1 (KIM-1), Interleukin-18 and Neutrophil Gelatinase-Associated Lipocalin (NGAL), which are not widely used in clinical practice.

The present study is the first study investigating the relationship between NAP and kidney outcomes in biopsy-proven DN. In this study, we showed that the presence of hypertension, longer duration of DM, higher serum creatinine level at the time of biopsy, higher microalbuminuria, and higher NAP are independent predictors for worse kidney outcomes. Hypertension in type 2 diabetic patients often already exists before DN develops. The frequency of hypertension varies between 40-60% in newly diagnosed type 2 DM patients. In a study[13] from China, it has been shown that hypertension prevalence was as high as 40% in newly diagnosed type 2 DM. Muddu et al[14] has showed that hypertension is present in over 60% of newly diagnosed type 2 DM patients. While hypertension can be a risk factor for DN itself, it is also a risk factor for DN progression[15,16]. In a prospective observational study, Rossing et

Table I. Comparison of the groups in terms of demographic and clinical features.

Features	Composite Renal Outcome (n=8)	Control (n=15)	*P*
Age (years)	52 (49.25-53)	43 (40-45)	<0.001
Female gender, %-n	62.5-5	66.7-10	0.842
Hypertension, %-n	87.5-7	33.3-5	0.027
BMI (kg/m^2)	26.5±1.1	24.7±2.2	0.028
Duration of DM (years)	10 (9.25-11)	8 (7-9)	0.001
Biopsy indication, %-n			
Nephrotic syndrome	87.5-7	66.7-10	0.369
Features other than DN	12.5-1	33.3-5	
Diabetic retinopathy, %-n	62.5-5	33.3-5	0.221

BMI=Body Mass Index, DM=Diabetes Mellitus, DN=Diabetic Nephropathy.

Table II. Comparison of the groups in terms of laboratory parameters.

Features	Composite Renal Outcome (n=8)	Control (n=15)	p
FPG (mg/dl)	165 (156.75-174.25)	122 (94-134)	**<0.001**
Urea (mg/dl)	53.5±7.3	53.6±9.6	0.993
Creatinine (mg/dl)	1.51±0.04	1.22±0.04	**<0.001**
eGFR (ml/min/1.73m^2)	62.4±5.3	74.2±3.9	**<0.001**
HbA1c (%)	8.88 (8.73-8.9)	7.4 (7.2-8)	**<0.001**
Albumin (g/dL)	3.57 (3.3-3.8)	3.78 (3.23-4.2)	0.087
Proteinuria (mg/24h)	5,476.1±2,019.4	2,686.3±1,074.8	**<0.001**
Microalbuminuria (mg/24h)	3,669.8±1,399.5	2,334.8±1,009.3	**0.015**
NAP (mg/24h)	1,931.3±1,016.7	351.5±95.7	**<0.001**

FPG=Fasting Plasma Glucose, eGFR=estimated-Glomerular Filtration Rate, NAP=Non-Albumin Proteinuria.

al[17] showed that for every 10 mmHg increase in systolic blood pressure, the risk of reaching the kidney endpoints increases 1.23-fold. In terms of hypertension, our findings were similar to the literature data. In our study, it was determined that hypertension is an independent risk factor for kidney outcomes in patients with biopsy-proven DN.

Duration of DM is a well-known risk factor for DN. Because there is a prediabetic process in type 2 DM, it is recommended that screening for DN should be done at the time of diagnosis in patients with type2 DM. In a study by Zoungas et al[18], they found that the duration of DM is a risk factor for microvascular and macrovascular complications as well as a risk factor for mortality. In a large study involving 54,670 patients with type 2 DM, the duration of DM was found to be the strongest risk factor contributing to the development of DN[19]. Our results are similar to the studies in the literature for the duration of DM.

An increase in creatinine is a risk factor for the progression of chronic kidney disease[20].

During the course of DN, elevated creatinine is not an expected finding in the microalbuminuria stage. However, when overt proteinuria occurs in the patient, the serum creatinine level starts to increase and a progressive decrease in eGFR is experienced[21]. The high serum creatinine level in the group that reached the kidney endpoint in our study may be explained by the presence of more severe DN in this group with already increased creatinine. Our study showed that increased serum creatinine level at the time of biopsy is an independent risk factor for worse kidney outcomes.

There are several reviews and meta-analyses[22-24] on microalbuminuria and adverse kidney outcomes. Since microalbuminuria is accepted as a kidney marker of systemic endothelial damage, an annual follow-up of microalbuminuria is recommended in DN screening[25,26]. Patients with early-stage chronic kidney disease with albuminuria have been shown to have a higher risk of kidney failure than those without albuminuria[27]. In this study patients, in the composite kidney outcome, the group had higher al-

Table III. Logistic regression analysis for determining risk factors of composite renal outcome.

Parameters	Univariate OR (95% CI)	p	Multivariate OR (95% CI)	p
Age	4.797 (0.015-9.896)	0.992		
Hypertension	2.872 (1.329-3.612)	**0.001**	2.764 (1.308-3.548)	**0.002**
BMI	1.752 (0.988-3.109)	0.055		
Duration of DM	8.993 (1.261-64.148)	**0.028**	6.789- (1.467-40.561)	**0.007**
FPG	1.214 (0.964-1.528)	0.099		
Creatinine	2.126 (1.652-3.174)	**0.004**	1.785 (1.239-2.167)	**0.004**
Microalbuminuria	1.568 (1.296-2.151)	**0.009**	1.457 (1.325-1.976)	**0.006**
NAP	3.471 (2.875-4.986)	**0.001**	2.891 (2.113-4.342)	**0.001**

BMI=Body Mass Index, DM=Diabetes Mellitus, FPG=Fasting Plasma Glucose, NAP=Non-Albumin Proteinuria.

buminuria and lower eGFR than the control group. This finding is similar to the studies in the literature investigating the relationship between microalbuminuria and kidney outcome.

Two mechanisms prevent proteinuria in normal glomerular hemodynamics. In the early stages of proteinuria, albuminuria occurs due to the deterioration of charge selectivity, while in cases of increased glomerular damage, size selectivity is also impaired, and non-albumin proteins begin to be excreted in the urine. NAP excretion is also observed in patients with severe DN as a result of the occurrence of proteinuria mostly due to increased glomerular permeability in DN[28]. NAP is accepted as an indicator of tubular dysfunction that can be seen in the course of DM as well as glomerular damage[29]. In a recent review[30], NAP is shown as a complementary marker for DN. The most important result of our study is that the patients in the composite kidney outcome group had higher NAP than the control group. There are many publications[31-33] in the literature investigating the effects of NAP on kidney outcomes. Considering the distribution of studies in the literature, we think that NAP is overshadowed by microalbuminuria. Moreover, we believe that NAP is not evaluated in clinical practice, even though it is requested as a test. There are also markers that can predict DN progression but are not commonly used in clinical practice[34]. In this study, we showed that NAP is an independent risk factor for adverse kidney outcomes in biopsy-proven DN.

Limitations

The biggest limitation of our study is that it did not include the drug information of the patients. However, since all patients have a definite diagnosis of DN, we think that most of our patients have been using one of the renin-angiotensin-aldosterone system inhibitors unless no other contraindication has developed. The small number of patients is another limitation of our study. However, our study is the first in the literature to investigate the effect of NAP on kidney outcomes in patients with biopsy-proven DN.

Conclusions

In our study, NAP was found to be an independent risk factor for doubling the serum creatinine level from the date of biopsy, for starting hemodialysis or peritoneal dialysis, for kidney transplantation, and for kidney-related death.

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