Дослідницька стаття
Research Article

НИРКИ KIDNEYS

DOI: 10.65327/kidneys.v15i3.677

Dr. Yagnik Prafulchandra Tank[1], Dr. Bansari Yagnik Tank[2], Dr. Avaniben Sharadbhai Nimavat[3], Dr. Nisha jayantilal parmar[4], Shivam Agarwal[5]

[1]*Associate Professor, Department of Pathology, Specialization in MD Pathology, Dr. N. D. Desai Faculty of Medical Science and Research, Dharmsinh Desai University, Nadiad, Gujarat, India*
Email ID: yagniktank.medical@ddu.ac.in, ORCID ID: 0000-0003-0516-6459
[2]*Associate Professor, Department of Microbiology, Specialization: MD Microbiology, Dr. N. D. Desai Faculty of Medical Science and Research, Dharmsinh Desai University, Nadiad, Gujarat, India*
ORCID ID: 0000-0002-8026-2697
[3]*Assistant professor, Department of Pathology, Specialization in MD Pathology, Dr. N D Desai medical science and research, Dharmsinh Desai University, Nadiad, Gujarat, India, Email ID: avani.nimavat99@gmail.com*
[4]*Assistant professor, Department of Pathology, Specialization in MD Pathology Dr. N D Desai medical science and research, Dharmsinh desai University, Nadiad, Gujarat, India, Email ID:* nisha.parmar1188@gmail.com, *Orcid id: 0009-0000-2686-4016*
[5]*Assistant Professor,College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India.Email ID: shivamagarwal50283@gmail.com, ORCID ID: 0000-0002-7891-9389*

Renal Function and Proteinuria as Indicators of Adverse Outcomes in Biopsy-Confirmed Diabetic Kidney Disease

For citation: Kidneys. 2026;15(3): 50-57. Acceptance- 03/09/2026
Received- 28/07/2026 Doi: 10.65327/kidneys.v15i3.677

ABSTRACT
Diabetic kidney disease (DKD) is a major cause of chronic kidney disease and adverse renal outcomes. Renal function and proteinuria are routinely assessed markers, but their prognostic importance in biopsy-confirmed DKD requires further evaluation. To assess renal function and proteinuria, compare these parameters between patients with and without adverse outcomes and determined their association with adverse clinical outcomes in biopsy-confirmed DKD. A retrospective secondary-data-based observational study was conducted using a publicly available dataset of 168 patients with biopsy-confirmed DKD. Renal function markers included estimated glomerular filtration rate (eGFR), serum creatinine, and blood urea nitrogen, while proteinuria was assessed using 24-hour urinary protein and urinary microalbumin. Adverse outcome was defined as a composite of all-cause mortality, initiation of regular dialysis, or renal transplantation. Descriptive statistics, independent-samples t-test, chi-square test, correlation analysis, and logistic regression were applied. Patients with adverse outcomes showed significantly lower eGFR and higher serum creatinine, blood urea nitrogen, 24-hour urinary protein, and urinary microalbumin. eGFR was negatively correlated with proteinuria, while serum creatinine showed positive correlations with urinary protein measures. Univariate regression identified several renal markers and serum albumin as significant factors. In multivariable analysis, eGFR remained independently associated with adverse outcome, while 24-hour urinary protein lost statistical significance after adjustment. Serum albumin also remained independently protective. Reduced renal function and increased proteinuria were associated with adverse outcomes in biopsy-confirmed DKD, with eGFR emerging as the strongest independent renal indicator.

Keywords: Diabetic kidney disease; eGFR; Proteinuria; Renal function; Adverse outcomes

1. Introduction

Diabetic kidney disease (DKD) is a leading cause of chronic kidney disease (CKD), end-stage kidney disease, and is one of the most significant microvascular complications of diabetes mellitus (DM). As DKD progresses to renal impairment, this can cause significant morbidity, mortality and requirement for renal replacement therapy. Several clinical, biochemical and pathological findings impact the prognosis in patients with DKD; the combination of these parameters may help to assess renal survival in biopsy-proven disease [1]. Histological changes seen on kidney biopsy can also be important to predict renal progression, and may give prognostic details beyond routine laboratory results [2].

Renal biopsy is especially important in the diabetic patient, in whom renal dysfunction could be due to diabetic nephropathy, non-in diabetic renal disease, or both. Histopathological confirmation increases

Full list of authors information is available at the end of the article.

diagnostic diagnosis and aids in the recognition of structural lesions linked with prognosis. However, in patients with type 2 diabetes and biopsy-proven chronic kidney disease, the outcomes of patients with diabetic nephropathy have varied widely, suggesting the need for reliable markers of disease progression [3]. Different prediction methods have thus been created to predict the probability of progression to end-stage renal disease (ESRD) in patients with diabetic kidney disease [4].

Estimated glomerular filtration rate (eGFR), serum creatinine and blood urea nitrogen are widely used tools for the assessment of renal function. Of these, a change in serum creatinine and blood urea nitrogen (BUN) is a good general indicator of decreased renal function, while eGFR is a good indicator of overall filtration capacity. There is variability in the rate of kidney function loss among individuals with type 2 diabetes, and it is important to recognize those who are at higher risk for more rapid kidney disease progression [5]. Other biochemical parameters could also be of importance in terms of renal prognosis. For instance, reduced bile acid content is observed independently as a risk factor for the development of adverse renal outcomes in patients with biopsy-proven DKD [6]; serum alkaline phosphatase is also reported to be associated with adverse renal outcomes in people with type 2 diabetes [7]. Another important feature of DKD is proteinuria, which is also a key indicator of kidney injury. A rise in urinary protein loss is indicative of glomerular damage and is generally associated with progressive structural and functional damage. 24-hour urinary protein, urinary albumin, etc., can therefore give important clues as to the severity of the disease. Early recognition of high risk patients is clinically important as progressive DKD eventually may progress to dialysis, kidney transplantation, cardiovascular complications or death. Nutritional and clinical scoring systems have also proven to be prognostic for poor outcomes in biopsy-proven DKD [8].

Although there has been a growing interest in prognostic biomarkers, many studies have been devoted to special inflammatory or molecular markers, rather than commonly available renal markers. Although plasma inflammatory biomarkers have been linked to histopathological lesions, progression of kidney disease, and mortality, it may not always be practical to utilize these markers in routine care [9]. Likewise, plasma markers in biopsy-proven kidney disease have yielded valuable insights into the biology but need to be evaluated in a more specialized manner [10]. Other studies have shown nutritional status to be associated with renal progression in biopsy-proven DKD, as well as cardiovascular events and mortality, suggesting that prognosis is dependent on several factors [11]. Kidney biopsy studies are also showing the relationship between clinical, laboratory and histopathological findings in diabetic patients with nephrotic-range proteinuria [12]. While new tubular markers are promising, they are not as well accepted or used clinically as standard markers like eGFR and proteinuria [13]. Thus, the role of proteinuria and renal function in terms of prognosis in DKD with biopsy confirmation needs further investigation.

Early risk stratification is clinically important as DKD progression is mediated by complex metabolic, inflammatory, vascular and microbiome pathways [14]. There are several novel tubular injury and inflammation biomarkers that are being investigated, but many of these require special laboratory testing [15]. By comparison, eGFR and proteinuria are cheap, routinely ordered and readily available. Kidney outcomes are also primary outcome measures in key chronic kidney disease (CKD) trials, underscoring the clinical need for reliable predictors of disease progression [16]. Moreover, kidney biopsy findings vary broadly, suggesting that when using clinical markers to diagnose kidney disease, it's important to have pathological confirmation [17]. Combining the assessment of renal function with that of proteinuria may thus offer a convenient way to identify biopsy-proven DKD patients at higher risk for poor outcomes.

Research Objectives

1. To assess renal function and proteinuria levels among patients with biopsy-confirmed diabetic kidney disease.
2. To compare renal function and proteinuria parameters between patients with and without adverse clinical outcomes.
3. To determine the association of renal function and proteinuria with adverse outcomes in biopsy-confirmed diabetic kidney disease.

2. Methodology

2.1 Study Design

The study design was an observational study using a secondary data source with a retrospective approach, and aimed to assess the association of proteinuria and renal function with poor clinical outcomes in DKD patients with biopsy confirmation.

2.2 Data Source

The secondary data were collected from the publicly available dataset [18], which was deposited on Mendeley Data. The data comprises demographic, clinical, biochemical, urinary, pathological and follow-up data of the patients who were diagnosed with diabetic kidney disease and were the only source of data for the current analysis.

2.3 Study Population and Sample Size

The study population comprised patients with diabetic kidney disease (DKD) with renal biopsy-proven diagnosis from the original data set who had complete clinical, biochemical, urinary, pathological, and follow-up data. One hundred and sixty-eight patients were available in the dataset, met the inclusion criteria, and were analyzed.

2.4 Inclusion and Exclusion Criteria

Patients were selected when they had biopsy documented diabetic kidney disease with available renal function measurements, proteinuria related measurements, and outcome status. Records were excluded when a renal biopsy was not obtained, or if there were missing or incomplete data regarding adverse clinical outcomes or renal function or proteinuria.

2.5 Study Variables

The primary predictor variables were renal function markers, including estimated glomerular filtration rate, serum creatinine, and blood urea nitrogen, together with proteinuria-related variables including 24-hour urinary protein and 24-hour urinary microalbumin. The primary outcome was adverse clinical outcome status, defined as a composite endpoint comprising all-cause mortality, initiation of regular dialysis, or renal transplantation. Additional covariates included age, sex, body mass index, diabetes duration, hypertension, HbA1c, serum albumin, hemoglobin, uric acid, lipid parameters, and other clinically relevant variables available in the dataset.

2.6 Data Management

The data set was preanalyzed to evaluate completeness, data duplication, coding consistency, and implausible data values. Based on the measurement type, variables were categorized as continuous or categorical and the outcome status was categorized as adverse outcome or no adverse outcome for comparative and regression analysis.

2.7 Statistical Analysis

Categorical variables were expressed as counts and percentages and continuous variables were expressed as mean ± standard deviation or median and interquartile range depending on distribution. Independent-samples t-tests were used to compare continuous variables between the adverse outcome and non-adverse outcome groups and chi-square tests were used to investigate associations between categorical variables and adverse outcomes. Correlation analysis was done to evaluate the relationship between renal function and proteinuria parameters. The associations between renal function markers and proteinuria with adverse clinical outcomes was evaluated using regression analysis. A p-value of < 0.05 was considered statistically significant.

2.8 Ethical Considerations

The study was conducted with publicly available, de-identified secondary data only, with no direct recruitment of patients, intervention or contact. The source of the original data was properly acknowledged and cited; and the analysis was performed with previously collected, anonymised data.

3. Results

3.1 Demographic and Clinical Profile of the Study Population

There were 168 patients with biopsy-proven diabetic kidney disease. Most were male and the rest were female. The majority of the cases were in the intermediate pathologically involved group, whereas small proportion of cases were in the lower pathological involved group and even smaller proportion in the higher pathological involved group. The single largest group of patients was the highest classification group of hypertension. Almost half of the cohort in the study had an unfavourable clinical outcome at follow-up. Frequency and percentage distribution of the demographic and clinical characteristics are shown in Table 1.

Table 1. Demographic and clinical profile of patients with biopsy-confirmed diabetic kidney disease (N = 168)

Characteristic	Classification	Frequency (n)	Percentage (%)
Sex	Female	72	42.9
	Male	96	57.1
Pathological involvement	Lower-grade	73	43.5
	Intermediate-grade	85	50.6
	Higher-grade	10	6.0
Hypertension classification	Lowest group	22	13.1
	Second group	3	1.8
	Third group	35	20.8
	Highest group	108	64.3
Clinical outcome	No adverse outcome	89	53.0
	Adverse outcome	79	47.0

Figure 1 shows the demographic and clinical characteristics of the study population. The distribution indicates an excess of males compared to females, an excess of the intermediate pathological group compared to the other pathological groups, and an excess of the highest classification of hypertension compared to the lower classifications.

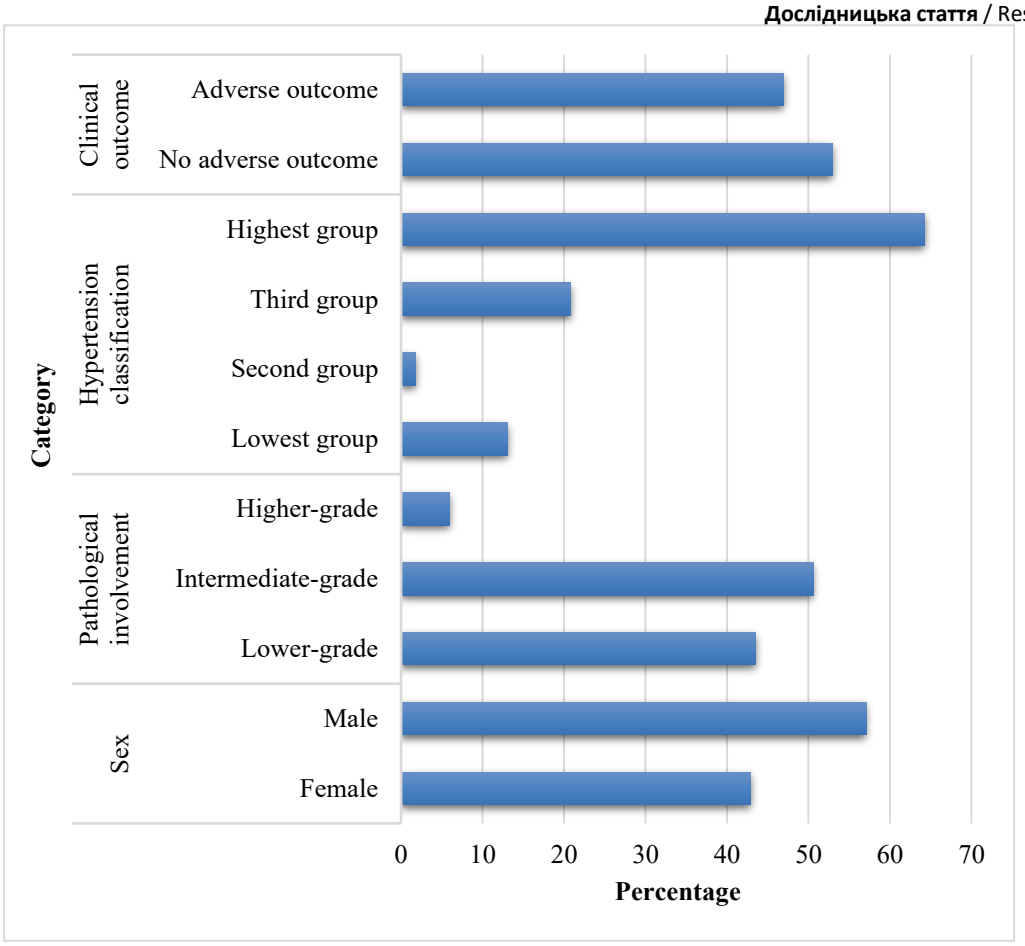

Figure 1. Demographic and clinical distribution of the study population

Inspection of the figure further illustrates that the adverse and non-adverse outcome groups were fairly well balanced, providing a good foundation for further comparative and association analysis.

3.2 Renal Function and Proteinuria According to Adverse Outcome Status

The patient group with adverse outcomes had significantly lower renal function than the group without adverse outcomes. The adverse-outcome group had significantly lower eGFR, and higher serum creatinine and blood urea nitrogen. Proteinuria was also more significant in patients who had adverse outcomes, and both 24-hour urinary protein and urinary microalbumin were significantly different between the groups. Table 2 presents all 5 renal and proteinuria parameters, which were significant different between the two outcome groups.

Table 2. Comparison of renal function and proteinuria between patients with and without adverse outcomes

Parameter	**No adverse outcome, Mean ± SD**	**Adverse outcome, Mean ± SD**	**t-value**	**p-value**
eGFR	66.23 ± 26.67	40.26 ± 24.17	6.620	<0.001
Serum creatinine	116.03 ± 60.87	218.30 ± 174.28	-4.955	<0.001
Blood urea nitrogen	8.11 ± 3.29	10.77 ± 4.17	-4.554	<0.001
24-hour urinary protein	4.91 ± 4.34	8.68 ± 5.37	-4.958	<0.001
24-hour urinary microalbumin	3426.29 ± 2758.52	6036.15 ± 3870.11	-4.976	<0.001

The overall pattern of impaired renal function was seen in the adverse-outcome group as illustrated in Figure 2. This was most apparent for serum creatinine and eGFR was significantly lowered in patients with adverse outcomes.

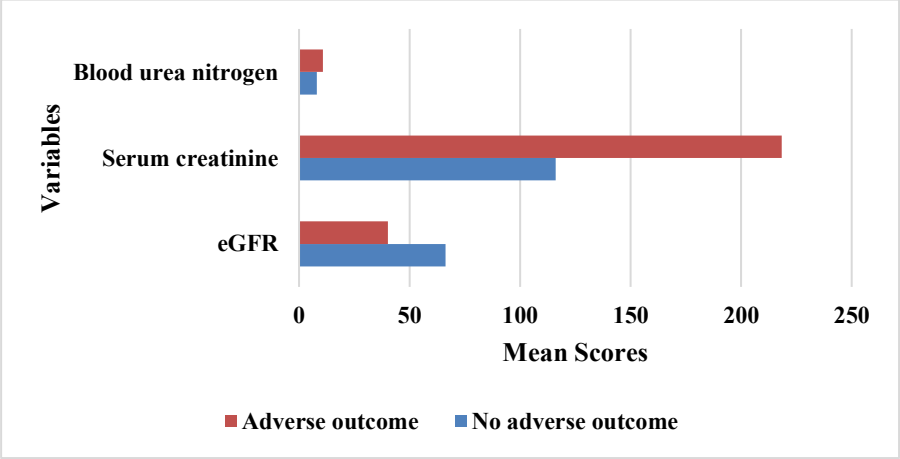

Figure 2. Mean renal function parameters according to adverse outcome status

The visual comparison in Figure 2 indicates a uniform decline in renal functional status with a poor outcome. These results underscore the need for routine renal function indices for stratification of patients with a less favorable clinical course.

3.3 Association of Clinical Characteristics with Adverse Outcomes

There was no significant difference in adverse outcome status between sex (chi-square analysis). By contrast pathological involvement showed a significant association with adverse outcomes with a higher proportion of adverse outcomes in the higher pathological groups. The adverse outcome status was also significantly associated with the hypertension classification. The results of the categorical association analyses are summarized in Table 3.

Table 3. Association of categorical clinical characteristics with adverse outcomes

Characteristic	χ^2	df	p-value	Interpretation
Sex	0.263	1	0.608	Not significant
Pathological involvement	26.074	2	<0.001	Significant
Hypertension classification	7.848	3	0.049	Significant

3.4 Correlation Between Renal Function and Proteinuria

The correlation analysis revealed clearly different interrelationships between the renal function parameters and proteinuria. eGFR was negatively associated with serum creatinine, BUN, 24-hour urinary protein and urinary microalbumin, while serum creatinine had positive associations with the other measures of renal impairment and proteinuria. The highest positive correlation was seen between the two proteinuria markers as shown in Figure 3.

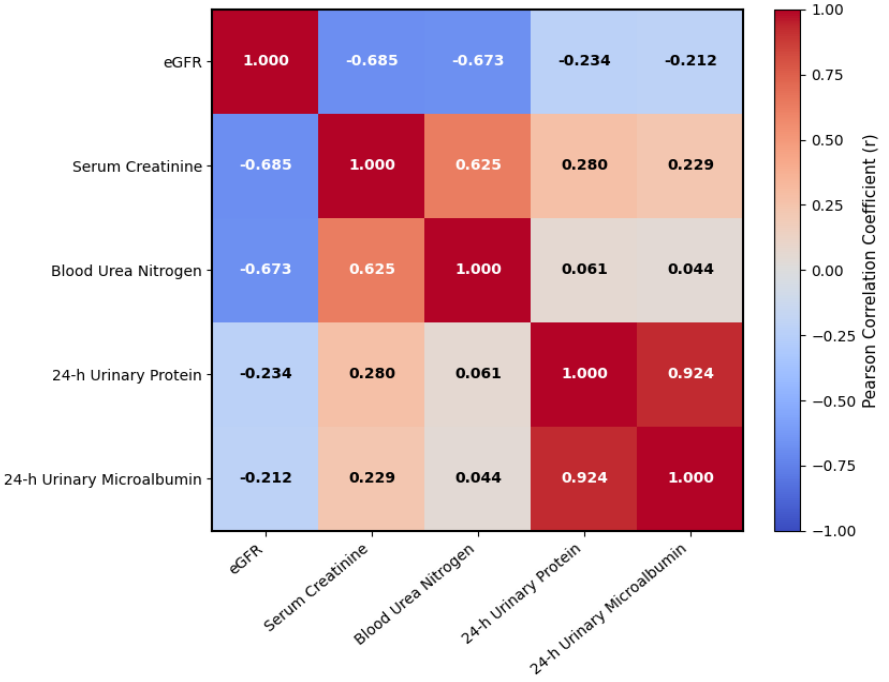

Figure 3. Correlation heat map of renal function and proteinuria parameters

The heat map indicates strong negative correlation between eGFR and serum creatinine, blood urea nitrogen and close correlation between 24-hour urinary protein and urinary microalbumin. Correlations with renal function markers were weaker, indicating that these parameters represent related but partly distinct aspects of the severity of the disease in biopsy confirmed diabetic kidney disease.

3.5 Univariate Factors Associated with Adverse Outcomes

Univariate logistic regression analysis revealed that there were some renal and biochemical variables that were significantly associated with adverse outcomes. A higher eGFR and serum albumin level were associated with lower odds of an adverse outcome, while a higher serum creatinine level, blood urea nitrogen, 24-hour urinary protein, and urinary microalbumin were associated with higher odds of an adverse outcome. Neither age, nor sex, nor diabetes duration, nor HbA1c was statistically significant predictors. The results of the univariate regressions are summarized in Table 5.

Table 5. Univariate logistic regression analysis of factors associated with adverse outcomes

Predictor	Odds ratio	95% CI	p-value
eGFR	0.959	0.944–0.974	<0.001
Serum creatinine	1.013	1.007–1.019	<0.001
Blood urea nitrogen	1.215	1.107–1.334	<0.001
24-hour urinary protein	1.191	1.101–1.288	<0.001
24-hour urinary microalbumin	1.0003	1.0002–1.0004	<0.001
Age	0.990	0.962–1.018	0.462
Sex	0.811	0.440–1.497	0.503
Diabetes duration	1.008	0.971–1.046	0.670
HbA1c	0.933	0.787–1.105	0.423
Serum albumin	0.892	0.845–0.941	<0.001

3.6 Independent Indicators of Adverse Outcomes

In the multivariable logistic regression model, eGFR was a significant independent predictor of adverse outcome, after controlling for proteinuria and pertinent clinical covariates. In addition, serum albumin was independently associated with decreased risk for adverse outcome. In contrast, 24-hour urinary protein lost statistical significance after adjustment indicating that its association with outcome was partially contributed by other clinical factors. No association with adverse outcome status was found with age, sex, diabetes duration, or HbA1c alone. These regression results have been adjusted and are presented in Table 6.

Table 6. Multivariable logistic regression analysis of factors associated with adverse outcomes

Predictor	Adjusted OR	95% CI	p-value
eGFR	0.957	0.941–0.974	<0.001
24-hour urinary protein	1.089	0.975–1.215	0.131
Age	0.989	0.953–1.027	0.578
Sex	0.822	0.371–1.818	0.628
Diabetes duration	0.993	0.944–1.044	0.776
HbA1c	1.077	0.874–1.327	0.486
Serum albumin	0.921	0.851–0.996	0.041

4. Discussion

This study assessed the association of renal function and proteinuria to adverse outcomes in patients with biopsy proven diabetic renal disease. All of these findings were consistently such that patients who had adverse outcomes had worse renal function and higher urinary protein loss than the patients without adverse outcomes. In initial analysis, lower eGFR, higher serum creatinine, higher BUN, higher 24-hour urinary protein and higher urinary microalbumin were all associated with adverse outcome status. These results suggest that the loss of filtration function and the protein loss are associated with a more adverse clinical outcome in DKD. The strong associations found for pathological involvement and hypertension classification also indicate that pathological outcome is not only dependent on renal filtration markers, but also on the extent of renal and vascular involvement.

The correlation results aid in the understanding of the relationship between renal function and proteinuria. Both urinary protein and urinary microalbumin were weakly negatively correlated with eGFR, and weakly positively correlated with serum creatinine. This makes sense on a clinical basis because as the glomerular filtration barrier function deteriorates, there would be an increase in the amount of urinary protein lost and progressive structural damage to the kidneys. The magnitude of these correlations, however, is relatively weak, suggesting that the two factors, renal function and proteinuria are related but not mutually interchangeable. They seem to reflect various aspects of disease severity. There was no significant association between BUN and proteinuria, possibly because it is more influenced by non-renal factors than are eGFR and serum creatinine. These results are given an important interpretation by the regression analyses. All parameters of the univariate analysis were significant: eGFR, serum creatinine,

blood urea nitrogen, 24-hour urinary protein, urinary microalbumin and serum albumin. Once adjusted for, however, eGFR was still independently associated with adverse outcome, and 24-hour urinary protein was no longer significant. This implies that the more a filtration capacity is reduced, the more independent prognostic information is provided than proteinuria, taking into account other clinical characteristics. The serum albumin was also independently protective, suggesting that nutritional status, systemic sickness and protein loss may be playing a role in the overall risk profile.

The present results corroborate previous data that diabetic and non-diabetic kidney disease, confirmed by biopsy, are important sources of difference in renal prognosis and long-term outcomes, highlighting the benefits of biopsy-based risk assessment [19].Preservation of kidney function was also shown to be tightly associated with improvements in major kidney and cardiovascular outcomes in the DAPA-CKD trial across chronic kidney disease populations [20], further supporting the importance of kidney function decline as an adverse prognosis marker. The importance of renal biopsy to help guide on the etiology and severity of renal injury has also been highlighted in clinical contexts where a clinical assessment alone may be inadequate to make a correct etiological diagnosis [21]. Moreover, vascular and renal biomarkers have shown important associations between systemic vascular abnormalities and impaired kidney function, thus further strengthening the multi-dimensional nature of chronic kidney disease progression [22]. The current results also resonate with the longitudinal data that further metabolic factors may play a role in the progression to end-stage kidney disease among patients with diabetic nephropathy, underscoring the interplay among renal, metabolic, and systemic factors that influence the outcomes [23].

The results have clinical relevance as eGFR, serum creatinine and proteinuria are simple, readily accessible and cheap measures. Their collective evaluation can assist in the identification of those patients who need increased nephrology follow-up, more intense blood pressure control, better glycemic control and early renal replacement therapy planning if needed. The independent association of eGFR with adverse outcomes indicates that special consideration should be given to a decreasing filtration function, while proteinuria continues to be a marker of kidney damage and disease burden.

There are a number of limitations to this study. It relied on secondary retrospective data which does not allow control over the original data collecting process and does not allow for causal explanations. The sample size was moderate and data were from a single clinical source to limit generalizability. Multivariable adjustment may also fail to capture all the confounding. Furthermore, the analysis largely relied on routine renal and clinical characteristics and did not use comprehensive molecular, therapeutic or longitudinal trajectory information. These findings need to be validated in larger, multicenter cohorts and extended follow-up. Multiple eGFR and proteinuria measurements might be useful to assess changes over time and trajectories of progression. It would also be worthwhile to investigate the potential for combining renal function, proteinuria, pathological and metabolic measures for better risk stratification and earlier intervention in biopsy proven diabetic kidney disease.

5. Conclusion

This study shows that renal function and proteinuria have a significant impact as predictors of poor outcome in patients with biopsy proven diabetic kidney disease. Patients with adverse outcomes had a definite trend towards lower estimated glomerular filtration rate, and higher serum creatinine and blood urea nitrogen, as well as higher 24-hour urinary protein and urinary microalbumin excretion. These results suggest that progressive decline in filtration function and more proteinuria is tightly linked to an adverse course of diabetic kidney disease. Correlation also revealed that there was a positive correlation between decreasing eGFR with increasing proteinuria and serum creatinine and urinary protein excretion showed a positive correlation. While these relations were limited in size, they do indicate a clinical importance of an integrated evaluation of protein loss and renal function. Univariate regression analysis showed that eGFR, serum creatinine, blood urea nitrogen, proteinuria, urinary microalbumin and serum albumin were significant factors associated to adverse outcomes. Importantly, after adjusting for clinical covariates, eGFR was also independently associated with adverse outcome and there was a protective association between serum albumin and adverse outcome. However, 24-h urinary protein was not an independent predictor in the adjusted model. The results overall emphasize the importance of eGFR as a specific marker of prognosis in biopsy-proven diabetic kidney disease, and proteinuria as a marker of renal damage and disease burden. The periodic evaluation of these parameters can help to risk-stratify patients earlier, monitor patients more closely, and manage patients with a high risk of progression more promptly.

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