Role of Kidney Biopsies for Biomarker Discovery in Diabetic Kidney Disease

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Although estimated glomerular filtration rate and albuminuria are well-established biomarkers of diabetic kidney disease (DKD), additional biomarkers are needed, especially for the early stages of the disease when both albuminuria and estimated glomerular filtration rate may still be in the normal range and are less helpful for identifying those at risk of progression. Traditional biomarker studies for early DKD are challenging because of a lack of good early clinical end points, and most rely on changes in existing imprecise biomarkers to assess the value of new biomarkers. There are well-characterized changes in kidney structure, however, that are highly correlated with kidney function, always precede the clinical findings of DKD and, at preclinical stages, predict DKD progression. These structural parameters may thus serve as clinically useful end points for identifying new biomarkers of early DKD. In addition, investigators are analyzing tissue transcriptomic data to identify pathways involved in early DKD which may have associated candidate biomarkers measurable in blood or urine, and differentially expressed microRNAs and epigenetic modifications in kidney tissue are beginning to yield important observations which may be useful in identifying new clinically useful biomarkers. This review examines the emerging literature on the use of kidney tissue in biomarker discovery in DKD.

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INTRODUCTION

Diabetic kidney disease (DKD) has largely been a clinical diagnosis based on the presence of proteinuria and impaired kidney function in the setting of diabetes.[1] As such, kidney biopsies are not a routine part of management for DKD. However, kidney tissue has been invaluable for determining the structural changes underlying DKD and showing how these structural changes relate to clinical findings. Glomerular basement membrane (GBM) width in normoalbuminuric people with type 1 diabetes, for example, predicts development of microalbuminuria, proteinuria, ESRD, and cardiovascular death.[2-4] Moreover, in multivariate piece-wise regression models, glomerular structural parameters classically associated with DKD, including increased GBM width, increased mesangial fractional volume, and reduced glomerular filtration surface correlate strongly with albuminuria and with kidney functional changes throughout much of the clinical natural history of DKD, and kidney interstitial changes are responsible for loss of kidney function in the later stages of the disease.[5-7]

The principal biomarkers presently used to predict DKD progression are albuminuria and estimated glomerular filtration rate (eGFR). However, not all cases of classical DKD are accompanied by increases in albuminuria,[8-11] which reduces the value of this biomarker, particularly in early DKD. Moreover, so-called "persistent microalbuminuria" defined as 2 of 3 consecutive urine samples in the microalbuminuria range, often spontaneously normalizes or stabilizes, limiting the value of this nonetheless useful biomarker.[2,12-14] Thus, it is important to develop additional biomarkers to supplement albuminuria.[14] The search for new biomarkers of DKD has centered primarily on identifying analytes in urine and blood that improve prediction of later established end points, including ESRD, a GFR loss of >40%, or death. There is also an urgent need to identify biomarkers of earlier stages of DKD when advances in treatment may have the greatest chance of attenuating disease progression, yet this is a time when eGFR is often still in the normal range and before the onset of strongly predictive levels of albuminuria.

Much of what is written here is predicated on the simple but very important notion that the earlier preclinical lesions of diabetic nephropathy are the necessary precursors of the later more severe lesions that are the underpinning of the loss of GFR leading to ESRD. There are several ways in which kidney biopsy tissue may advance DKD biomarker discovery. The structural lesions associated with DKD can be reproducibly quantified and always precede changes in kidney function. As such, they may be used as end points in biomarker studies. Searches are presently underway for proteins, peptides, or metabolites in the blood or urine that are reliably associated with earlier structural damage or predict changes in kidney structure and would therefore be useful biomarkers of early tissue injury and subsequent clinical progression of DKD. Such markers are likely interconnected in large networks which may be perturbed in the presence of disease, leading to changes in their concentrations in biological specimens such as urine or blood. Gene expression profiles derived from diseased kidney tissue may reflect these dynamic molecular perturbations underlying diabetic kidney structural injury. Their differential expression relative to healthy

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tissues or to tissues from people with diabetes who have very slow or no development of these lesions will likely lead to the identification of candidate biomarkers for progression and/or protection from DKD as well as to new treatment targets.[15,16]

Changes in expression patterns of microRNAs (miRNAs) in kidney tissue may provide another means to identify key pathogenetic or protective processes underlying DKD risk and could be used to monitor development and progression of DKD.[17,18] In addition to being detectable in tissue, miRNAs can also be detected in blood and urine, raising the prospect that miRNA concentrations in these easily accessible biospecimens might reflect the underlying tissue changes and therefore be clinically useful biomarkers for DKD.[19,20]

Epigenetic factors may also impact gene expression,[21,22] and differences in gene methylation and histone modification in kidney tissue from affected and unaffected individuals may provide insight into genes and pathway modifications involved in DKD which may identify new biomarkers. As with miRNAs, differential gene methylation and histone modifications may themselves be useful biomarkers, as gene methylation and histone modification patterns can be identified in other, more accessible cells, including peripheral blood cells, which may reflect changes in the underlying kidney tissue.[23]

This review examines the emerging role of kidney biopsies in biomarker research and considers potential future developments. Research kidney biopsies are the most suitable source of tissue for these types of studies, as clinically indicated kidney biopsies are typically performed to identify non-DKD in the setting of diabetes,[24] so they are not as useful when attempting to predict progression of DKD.

CLINICAL SUMMARY

- Changes in kidney structure precede the clinical findings of diabetic kidney disease and provide useful end points for biomarker discovery in early diabetic kidney disease.
- Examination of differentially expressed genes, microRNAs, and epigenetic modifications in kidney tissue may also yield clinically useful biomarkers.
- Advances in imaging may replace the need for kidney tissue in the future, but for the time being access to kidney tissue remains a powerful but underutilized tool for biomarker discovery.

WHAT IS DKD?

The Kidney Disease Improving Global Outcomes group defines CKD as "abnormalities of kidney structure or function, present for greater than 3 months with implications for health."[25] They further subdivide CKD based on underlying cause, range of GFR, and degree of albuminuria.[26] Figure 1 illustrates the current stages of CKD along with their relationship to the risk of CKD progression to kidney failure, cardiovascular morbidity, and death. The Kidney Disease Improving Global Outcomes proposes that the terms "microalbuminuria" and "macroalbuminuria" be replaced with "moderately increased" and "severely increased" albuminuria to better fit the distinction in degree of albumin loss.

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative defines DKD as the presence of elevated urine albumin excretion associated with progressive decline in GFR, increased systolic blood pressure, and high risk of kidney failure in people with diabetes.[27] Kidney biopsy is not routinely used to confirm the diagnosis of DKD, unless there is reason to believe that another kidney disease is involved, so it is not part of routine clinical management in most diabetic patients. Ascertaining DKD based solely on clinical assessment, however, inhibits biomarker discovery and the pathogenetic insights available from kidney tissue. This issue is particularly relevant in type 2 diabetes, where CKD is more often due to nondiabetic causes than in type 1 diabetes.[28] In type 1 diabetes, biopsy studies have confirmed that DKD without albuminuria is associated with the traditional structural changes,[29] whereas this is less well established in type 2 diabetes.[30,31]

In the early stages of DKD, GFR may be normal or even higher than normal (hyperfiltration). Hyperfiltration is seen in both type 1 and type 2 diabetes, though estimates of prevalence based on measured GFR vary markedly from as low as 6% to as high as 73%.[32] Hyperfiltration is associated with an increased risk of moderately elevated albuminuria,[33,34] but at present, there are limited data examining its effect on later stages of DKD.[32] Nevertheless, because hyperfiltration is a common feature of early DKD, a person with a GFR above 90 mL/min may already have experienced a substantive decline in GFR. In nonlinear (piecewise) analyses of relationships between measured GFR and diabetic glomerulopathy parameters in people with type 1 diabetes, the point at which further reductions in GFR become strongly associated with these parameters is at 99 mL/min/1.73 m^2.[35] In such settings, where the CKD level would at most be Stage 1 or 2, it is necessary to consider rate of change in GFR, albuminuria, and other evidence of kidney damage for diagnosis of DKD. As treatment may modify both GFR and albuminuria, previous degrees of albuminuria should be considered. Moreover, the diagnosis of DKD might be doubtful, despite the combination of albuminuria and reduced GFR, in the absence of diabetic retinopathy, when there are signs of other systemic diseases, or when heavy proteinuria, rapidly falling GFR, or refractory hypertension are present.[1]

While eGFR and albuminuria remain useful biomarkers of DKD, there are times when they are not sufficient. For instance, DKD may occur in the absence of sustained albuminuria.[8-11] Moreover, these markers are clearly not sufficient when trying to assess early DKD, when eGFR is still in the normal range and before the onset of elevated albuminuria.