Diagnostic Value of Sensitive Biomarkers for Early Kidney Damage in Diabetic Patients with Normoalbuminuria

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To the Editor: Diabetic kidney disease (DKD) is the most common cause of end-stage renal disease (ESRD); however, the onset of DKD is difficult to detect.[1] When persistent microalbuminuria becomes detectable, DKD has already progressed to the third disease stage, and finding biomarkers that are more sensitive than microalbuminuria is therefore necessary to indicate kidney damage at an earlier stage of DKD.[2] Both glomerular and tubulointerstitial damages have been repeatedly demonstrated to be important factors in the pathophysiology of DKD.[3] Therefore, we investigated the expression levels of six markers closely related to the glomerulus and renal tubule.

A total of 51 type 2 diabetic patients (diabetes duration >10 years) with normoalbuminuria and 27 healthy participants were enrolled in a cross-sectional study. The urine levels of the glomerular and tubular injury biomarkers such as podocalyxin, nephrin, podocin, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and tissue inhibitor of metalloproteinase-2 (TIMP-2) were measured by ELISA methods. Clinical data and biological parameters were assessed for all participants. Blood pressure and urine albumin-to-creatinine ratio data were the average results of three measurements.

We compared the ratios of six biomarkers to creatinine in urine between the two groups. The ratios of five biomarkers to creatinine were greater in the urine of diabetic patients with normoalbuminuria than in controls ($P < 0.001$ in podocalyxin, nephrin, and KIM-1; $P < 0.010$ in NGAL; and $P < 0.050$ in podocin). However, the ratio of TIMP-2 to creatinine in urine was not significantly different between the two groups ($P = 0.130$). We performed receiver operating characteristic (ROC) analyses of the five biomarkers that had statistically significantly higher concentrations in the group of diabetic patients with normoalbuminuria than those in the control group [Figure 1]. Four biomarkers had an area under the curve (AUC) >0.7; ranked from the best to the worst, these biomarkers were podocalyxin (0.879, 95% confidence interval [*CI*]: 0.806–0.952), nephrin (0.845, 95% *CI*: 0.762–0.929), KIM-1 (0.800, 95% *CI*: 0.700–0.900), and NGAL (0.727, 95% *CI*: 0.614–0.840). However, the AUC of podocin was low (0.658, 95% *CI*: 0.537–0.779).

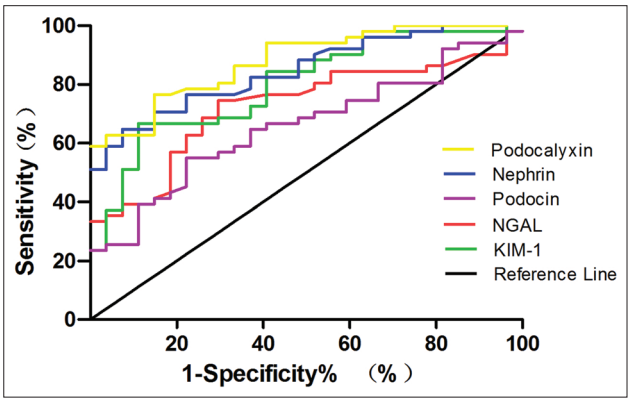

Figure 1: ROC curve analysis of podocalyxin, nephrin, podocin, NGAL, and KIM-1. The AUC values for these markers were 0.8791, 0.8453, 0.6576, 0.7269, and 0.7996, respectively. NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; AUC: Area under the ROC curve; ROC: Receiver operating characteristic.

Early identification of kidney damage in diabetic patients is crucial because it provides an opportunity to prevent the incidence of DKD or even slow the progression of ESRD attributed to

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diabetes. Currently, microalbuminuria is generally thought to be an early marker of DKD in clinical practice.[4] However, a previous study revealed changes in the structure and decreased densities of podocytes in DKD patients with normoalbuminuria by using morphometric analysis based on kidney biopsy.[5] Although biopsy is the gold standard for diagnosing DKD, it is not needed in most cases due to its invasive nature. In addition, biopsy has not been widely applied for routine use in clinical laboratories worldwide due to its technical complexity and need for experienced personnel. The ELISA method we used in the current study is safe, easy, quick, and sensitive. Our results clearly demonstrated that podocyte injury is present before the appearance of microalbuminuria in patients with diabetes. Elevated levels of podocyte-specific proteins, including podocalyxin, nephrin, and podocin, were observed in the diabetic normoalbuminuric patient group, and our results were consistent with those of previous studies. However, the mechanism underlying this phenomenon has not yet been fully elucidated. Some studies demonstrated that vesicles from the apical cell surface are shed into urine with podocyte biomarkers and provided evidence for this phenomenon in diabetic patients by demonstrating the presence of vesicles in urine using immunoelectron microscopy and immunofluorescence.[6] Recent studies have demonstrated that the roles of both glomerular and tubular damages in the early stage of DKD are very important and our research supported this hypothesis.[3] In addition to the markers of glomeruli, we also found some tubular markers, such as NGAL and KIM-1, whose levels were increased in diabetic patients with normoalbuminuria compared to those in the controls. Considering these data together, inflammatory stimuli and the hyperglycemic state likely cause glomerular as well as tubular damage in the early stage of DKD. ROC analysis showed that the AUC values for podocalyxin, nephrin, NGAL, and KIM-1 were >0.7. Thus, our findings indicate that these markers may have potential clinical applications in the early identification of high-risk individuals, thereby allowing the initiation of early treatment and the prevention or delay of DKD.

As a corollary, we have shown that a high percentage of DKD patients exhibit increased levels of these markers without microalbuminuria, suggesting their potential utility as early biomarkers. Further confirming these proteins to be biomarkers of preclinical DKD would promote our understanding of DKD pathogenesis and potentially provide new and earlier therapeutic targets. We recommend strict blood sugar control, more frequent follow-up, and appropriate drug applications to protect the kidneys of diabetic patients with early signs of DKD.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the forms, the patients/patients' guardians have given their consent for their images and other clinical information to be reported in the journal. The patients/patients' guardians understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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