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Chronic Kidney Disease in Diabetes: Biomarkers for Early Detection and Management

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Abstract: Proteinuria and serum creatinine are commonly employed biomarkers for monitoring the advancement of diabetic nephropathy, but they may not comprehensively capture all types of lesions and are indicative of declining glomerular filtration rate only in the later stages. Recent research has identified biomarkers reflecting different aspects of diabetic kidney disease (DKD) that can provide information on the risk of DKD progression and associated poor prognosis. This review outlines several biomarkers, such as renal injury molecule-1, Neutrophil gelatinase-associated lipocalin, heat shock protein 72, soluble urokinase plasminogen activator receptor, angiopoetin-Like protein-4, monocyte chemoattractant protein-1, and liver-type fatty acid-binding protein. The research revealed that, following adjustingments for serum creatinine or estimated glomerular filtration rate and proteinuria, the concentrations of plasma and urine biomarkers remained elevated in individuals with diabetic kidney disease and were additionally correlated with the progression of DKD. These new biomarkers represent different biological pathways such as tubular injury, glomerular injury, tubular dysfunction, and inflammation, and can be used as liquid biopsies to better characterize the disease of diabetic nephropathy. Novel blood and urine biomarkers improve clinicians' ability to assess the prognosis of DKD progression and may improve understanding of the pathophysiology of DKD. However, for preclinical biomarkers associated with DKD outcomes, there was considerable heterogeneity between study cohorts and designs, limiting the comparison of prognostic performance between different studies. Larger clinical studies are needed to determine how these biomarkers could be used in the clinic to improve the clinical management of DKD.

Keywords: Diabetic Kidney Disease; Biomarker; Early Detection; Diagnosis.

1. Introduction

Chronic kidney disease (CKD) is a general term for chronic kidney structure and function changes caused by various causes. Diabetes cause kidney damage called DKD, the definition of chronic kidney disease (CKD) is limited to the late, > 50% of the function has been damaged by the kidney damage, mainly contains UACR acuity 30 mg/g and/or estimated glomerular filtration rate (estimated glomerular filtration rate, eGFR) < 60ml·min-1·1.73m^{-2} and lasting more than 3 months. Lesions can accumulate in the whole kidney (glomeruli, renal tubules, renal interstitial and renal vessels, etc.), and 20%-40% of diabetic patients will eventually develop diabetic nephropathy and may develop end-stage renal disease (ESRD). Progression of DKD to ESKD significantly increases the risk of adverse events including death, and requires dialysis or kidney transplantation to further maintain quality of life [1]. In addition, in patients with type 1 or type 2 diabetes, the presence of chronic kidney disease significantly increases cardiovascular and cerebrovascular risk and medical costs [2]. Now, our understanding of DKD is not just limited to "kidney failure," it is inherently multifactorial and goes through a number of different processes, starting with stimulators that cause cell damage, triggering inflammatory responses due to cell damage, oxidative stress, and mitochondrial dysfunction. Macrophage and lymphocyte aggregation and cytokine secretion lead to inappropriate tissue repair, and as this process continues, healthy glomeruli experience compensatory hyperfiltration, leading to increased glomerular permeability and activation of the renin-angiotensin system, ultimately leading to renal interstitial fibrosis[3]. In clinical practice, the presence of renal interstitial fibrosis can usually only be determined by kidney biopsy, but due to its invasive nature and cost, clinicians and patients rarely choose it as a method for further assessment of kidney function.

2. Current Biomarkers in Clinical Practice

The treatment of DKD focuses on early intervention and integrated management to reduce proteinuria, delay eGFR decline, and improve adverse renal outcomes (such as ESRD, nephro-related death, etc.). Currently, according to the Fondation Kidney Disease Improving Global Outcomes (KIDIGO) guidelines, proteinuria and eGFR are used as laboratory biomarkers to assess the risk of progression to DKD, in addition to clinical variables such as patient demographic data and comorbiditis[4].

Proteinuria is the earliest and most classic biomarker clinically used to detect kidney function; however, only extensive assessment of proteinuria can identify patients with early DKD and enable rapid implementation of interventions. Given this evidence, it is particularly important to diagnose DKD early, before irreversible kidney damage occurs. Treatment based on angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may prevent the onset and progression of kidney disease in patients with T2DM and/or DKD. Yet, in spite of all this medications are widely used and the incidence of DKD continues to rise, reflecting the need to consider other strategies to prevent its development and progression before irreversible changes occur[5].With regard to eGFR, although serum creatinine

values are often measured during blood biochemical tests, DKD cannot be definitively diagnosed when eGFR > 60mL/min/1.73m^2, in more than 50,000 patients who meet the diagnostic criteria for DKD (eGFR<60mL/min/1.73m^2) , at least three months before and after the two tests, only 23% were diagnosed with DKD[6].

These two indicators, especially proteinuria, are not often evaluated in daily clinical practice even in patients with diabetes (less frequently than recommended by clinical guidelines), and their occurrence often indicates the presence of kidney damage, in addition to the fact that nephrotoxic drug use is more common in patients with DKD who have not been diagnosed with DKD. Therefore, it is inaccurate to assert albuminuria as a surrogate marker for kidney disease progression, as it still has uncertainties in predicting disease[7].

3. Novel Biomarkers

Urinary protein, eGFR, and serum creatinine, which are the most commonly used indicators of kidney function and injury, have some limitations. Although known risk factors for the progression of DKD, such as serum creatinine levels in proteinuria, have been taken into account, these indicators still vary widely in assessing clinical risk in patients with DKD. Therefore, more sensitive and specific biological indicators are needed to gain a more detailed understanding of the risk in the development and progression of DKD. The new DKD biomarker can help identify diabetic patients with normal urinary protein, eGFR and serum creatinine, but are developing structural and functional damage to the kidney, inflammation, early treatment, effectively delay the progression of kidney disease, and effectively reduce unnecessary invasive tests, such as kidney biopsy.

3.1. Kidney Injury Molecule-1 (KIM-1)

Kidney injury molecule-1(KIM-1) is a transmembrane glycoprotein expressed on the apical membrane of proximal renal tubule cells and mediates the absorption of apoptotic cells and oxidized lipids. KIM-1 is expressed at a low level in normal kidneys and other organs, but its expression in proximal tubule cells after renal ischemia/reperfusion injury is significantly up-regulated[8]. In rodent models, conditioned expression of KIM-1 in renal epithelial cells leads to progressive interstitial kidney inflammation and fibrosis and is therefore thought to have an adverse effect on DKD [9]. Studies have shown that KIM-1-mediated palmitic acid (PA) binding to the proximal tubular albumin uptake leads to DNA damage, proximal tubular cell cycle arrest, interstitial inflammation and fibrosis, and secondary glomerulosclerosis that enhances tubular damage. In addition, they performed kidney biopsies on 7 DKD patients and 5 non-DKD patients, and found tubulointerstitial inflammation and fibrosis and glomerulosclerosis in DKD patients. KIM-1 was expressed in proximal renal tubules in DKD patients through immunocytochemistry. It is specifically expressed in proximal renal tubules surrounded by α-smooth muscle actin + (αSMA+) myoblasts and CD3+ lymphocytes [10]. Schrauben et al. [11] conducted a multicenter, prospective, observational cohort study on 894 diabetic patients with chronic renal dysfunction. Even after adjusting for possible influencing factors such as age, gender, BP, BMI, and smoking, found that the higher the plasma KIM-1 concentration, the greater the risk of DKD progression. Hazard ratio (HR) was 1.26 (95%CI: 1.14-1.40;) And higher plasma KIM-1 concentration was significantly associated with the annual eGFR decline rate (ml/min/1.73m^2). GulnazBano et al. [12] conducted a cross-sectional study on 135 patients with type 2 diabetes, who were divided into normal albuminuria group (UACR < 30mg/g), microalbuminuria group (UACR 30-300mg/g) and macroalbuminuria group (UACR > 300mg/g). The study also observed that, after adjusting for age, BMI, duration of T2DM, glycosylated hemoglobin, and serum creatinine, the prevalence of DKD (PR) increased on a logarithmic scale with an increase in KIM-1 per unit (PR: 1.25; 95% CI: 1.14-1.38; $p < 0.001$).Therefore, KIM-1 is likely to play an important role in detecting early tubular injury and assessing the progression of chronic kidney disease in diabetic patients.

3.2. Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil-gelatase-associated lipocalin (NGAL) is a 25kda protein that mediates epidermal growth factor receptor (EGFR) signaling in chronic kidney disease. Its activation stimulates hypoxia-inducing factor (HIF-1α), which ultimately promotes proliferation, cytogenesis, kidney damage, and CKD progression [13]. Concentrations of NGAL can be measured in plasma, urine, and biological fluids such as peritoneal effluents. NGAL has been proven to be an effective biomarker for the diagnosis of acute kidney injury (AKI) [14]. Because elevated NGAL levels in diabetic nephropathy occur in the early stages of kidney injury, NGAL can be a predictor of renal failure even in patients with normal proteinuria [15].In addition, NGAL is also a circulatory biomarker strongly associated with hyperglycemia, insulin resistance, and obesity[16].In a cross-sectional study of 209 T2DM patients with normal urinary albumin and a diabetes course of more than 5 years, NGAL levels were elevated and weakly but significantly associated with eGFR ($r = -0.291$, $p = 0.001$) in patients with renal insufficiency with normal urinary albumin. This may be due to tubular damage caused by inflammation and oxidative stress responses, and urinary NGAL has sufficient diagnostic accuracy for eGFR in T2DM patients under the ROC curve (sensitivity: 0.591; Specificity: 0.545; AUC: 0.723) [17]. In a case-control study, 84 patients with type 2 diabetes and 21 healthy, non-obese patients were selected to assess the correlation between plasma NGAL and urine NGAL for DKD using appropriate covariate-adjusted linear regression analysis and receiver operating characteristics (ROC) curves. Both plasma NGAL and urine NGAL were found to be good differentiators between patients with DKD (whether early or late) and those with good renal function, and it appears that plasma NGAL is more reliable than urine NGAL in identifying T2D-induced kidney injury [18]. Tang et al. [19] conducted a stratified analysis and regression analysis, which included 14 studies involving 1561 individuals, including 1204 cross-sectional participants and 357 cohort participants. In cross-sectional studies, the combined sensitivity and specificity of NGAL for the diagnosis of DKD were 0.82(95% confidence interval (CI):0.75-0.87) and 0.81(95%CI:0.68-0.90), respectively. Urinary NGAL has an acceptable diagnostic accuracy in patients with diabetes, with an AUC of 0.88(95%CI:0.84-0.90) in cross-sectional studies. In the cohort study, the AUC was 0.98, which is of high diagnostic value. However, it has also been noted that urinary NGAL as a simple biomarker of tubular injury may not be able to detect chronic kidney disease with low levels of active tubulointerstitial injury or

inflammation in the glomeruli due to its limitations.

3.3. Heat Shock Protein 72(HSP72)

The primary role of the heat shock protein family is to protect cells and promote the restoration of damaged metabolic pathways. Some heat shock proteins are known to be constitutively expressed and have important roles in cellular functions, including controlling protein transport in the cytoplasm and across cell membranes, more recently facilitating the folding of synthetic proteins, and assisting in the assembly of large protein complexes. In contrast to the classical anti-inflammatory and protective effects of intracellular heat shock protein (iHSP), eHSP is thought to have pro-inflammatory effects, especially in age-related chronic diseases [20].Heat shock protein 72 (HSP72) is released in response to stressor exposure and effectively activates the inflammasome [21]. El-Horany et al. [22] conducted a study on 45 T2DM patients and found that uHSP72 level was significantly increased in the diabetic group compared with the healthy control group, with the highest average value in the macroproteinuria group. In addition, under the ROC curve, uHSP72 had a sensitivity of 90% and specificity of 87% for distinguishing between T2DM and DN patients, and the optimal cut-off point was 24.9pg/mL. A cross-sectional study of 160 patients with type 2 diabetes showed that plasma eHSP72 levels were elevated in patients with DKD, and, in the presence of proteinuria, eHSP72 increased dramatically. In addition, plasma eHSP72 level was significantly positively correlated with IL-1β level and negatively correlated with eGFR [23]. Therefore, HSP72 can be used as a noninvasive diagnostic biomarker for early DKD without microalbuminuria, and may be a good indicator of the progression of diabetic kidney injury. However, the current research on HSP72 in the direction of diabetic nephropathy is not perfect, in order to improve the accuracy of its diagnosis, further randomized and larger sample prospective studies are needed to support.

3.4. Soluble Urokinase Plasminogen Activator Receptor(suPAR)

Soluble urokinase plasminogen activator receptor (suPAR) is a signaling glycoprotein expressed in podocytes, endothelial cells and immature bone marrow cells. After activation, it is broken down into uPAR (urokinase plasminogen activator receptor), which is released into the blood circulation in a soluble state and acts as a circulatory factor. Activation of capsular β3 integrin leads to glomerular podocyte shedding and proteinuria [24]. Experiments have shown that blocking uPAR pathway can improve diabetic kidney lesions in rats [25]. One study showed that higher suPAR levels were an important and independent risk marker for eGFR decline, cardiovascular events, and mortality in patients with diabetes, and that the incidence of ESRD was significantly associated in participants with the highest quartile of plasma suPAR [26]. And suPAR is closely related to inflammation in different disease states [27]. Schrauben et al. [11] conducted a cohort study and found that a higher plasma suPAR level was associated with an increased risk of progression to DKD, even after adjusting for possible influencing factors such as age, sex, blood pressure, BMI, baseline eGFR, UPCR, etc. Hazard ratio (HR) was 1.40 (95%CI: 1.14-1.72;) . One study showed that serum suPAR levels were elevated in patients with DKD, and that suPAR levels were higher but comparable in patients with microalbuminuria or macroalbuminuria DKD compared to patients with normal albuminuria and diabetes. In addition, DKD patients with elevated suPAR levels compared to normal albumin at baseline also had normal GFR, and suPAR levels were significantly associated with annual changes in GFR($r2=-0.439, p<0.01$)[28].

3.5. Angiopoetin-Like Protein-4 (ANGPTL-4)

Angiopoietin-like proteins are a class of secreted proteins that play an important role in energy metabolism. ANGPTL-4 is a 50kDa protein. An in vitro study found that ANGPTL-4 expression was significantly increased in glomerular cells stimulated by high glucose [29],and AngPTL-4 expression in podocytes was associated with increased proteinuria, suggesting that angPTL-4 plays a role in dominant proteinuria in glomerular disease [30]. The current study found that patients with DKD with macroalbuminuria had significantly higher urine ANGPTL-4 levels than those with microalbuminuria. A cross-sectional study of 135 patients with type 2 diabetes found that urine angTPL-4 concentrations were elevated in patients with heavy proteinuria and increased with worsening kidney function, even after adjustment for potential confounders including T2DM duration, HbA1c, BUN, and eGFR. It was still found that ANGPTL-4 was significantly and generally elevated in diabetic nephropathy, and this study also showed that AUC from microalbuminuria group to macroalbuminuria group was also increased, and the best performance was in macroalbuminuria group, with a specificity of 97.8% [31].In addition, some studies have found that uANGPTL-4 is also correlated with HbA1c, FPG and BUN [32]. Therefore, ANGPTL-4 may be a new marker for the diagnosis and treatment of DKD as a marker of glomerular podocellular injury.

3.6. Monocyte Chemoattractant Protein-1(MCP-1)

Monocyte chemotactic protein-1 (MCP-1), also known as chemokine ligand 2 (CCL2), belongs to a broad family of chemokines and is an important mediator of innate immunity and tissue inflammation. After binding to its receptor, MCP-1 can induce homing, migration, activation, differentiation, and development of lymphocytes and NK cells, while promoting infiltration of monocytes and macrophages, thereby promoting inflammation associated with kidney disease [33]. ZZTo date, studies of MCP-1 in kidney disease have focused on urine, rather than plasma or serum MCP-1 concentrations. Chen et al. [34] found that in the fully corrected model, the risk of renal function decline was 2.18 times higher in the highest quartile of MCP-1 than in the lowest quartile, and in addition, patients in the highest quartile of uMCP-1 had the highest mortality. In a cross-sectional study of 185 diabetic patients, MCP-1 was independently associated with eGFR (MCP-1/Cr, $p=0.023$) and albuminuria (MCP-1/Cr, $p<0.001$) in diabetic patients, even after adjusting for risk factors for DKD [35]. In the early stages of diabetes, a study of 75 patients with type 2 diabetes showed that compared with diabetic patients and control subjects with normal albuminuria, Significantly elevated uMCP-1 levels were found in diabetic patients with macroalbuminuria ($p<0.001$) and microalbuminuria ($p<0.001$), and uMCP-1 was positively correlated with urinary albumin/creatinine ratio ($r=0.968$, $p<0.001$). In addition, ROC curves showed that, The critical value of uMCP-1 was 110pg/mg, the sensitivity

was 92%, and the specificity was 100%[36]. In addition, other studies have detected MCP-1 mrna in peripheral blood of diabetic nephropathy patients, and found that the prediction accuracy of MCP-1 mrna for dominant DN was 0.66 (95%CI: 0.55-0.77), while the prediction accuracy of McP-1 mrna for early DN was 0.61 (95%CI: 0.51-0.71) [37]. Therefore, MCP-1 may be considered as a new potential diagnostic biomarker for the early detection of diabetic nephropathy.

3.7. Liver-Type Fatty Acid-Binding Protein(L-FABP)

Liver fatty acid binding protein (L-FABP) is an intracellular fatty acid carrier protein, which is expressed in liver, small intestine, kidney and other tissues. Under normal circumstances, albumin is absorbed by proximal tubules after glomerular filtration and attached to free fatty acids (FFAs). After reabsorption, the albumin in the cytoplasm transfers FA to L-FABP and enters the lysosome, and the L-FABP attached to the FA is transferred to the peroxidase in the cytoplasm to realize the role of protecting the kidney [38]. In patients with DKD, fatty acids overload proximal tubules and a large amount of proteinuria is found. It has been reported that urinary L-FABP is a potential biomarker of acute kidney injury caused by various factors [39]. Urinary L-FABP level can accurately reflect the degree of renal tubulointerstitial injury and is significantly correlated with the prognosis and progression of CKD. This study also found that urinary L-FABP level in patients with normal albuminuria is higher than that in patients with microalbuminuria regardless of the type of diabetes [40]. Yang[41] et al. found that L-FABP expression was significantly increased after intermittent fasting in diabetic nephropathy patients diagnosed by kidney biopsy. In a cross-section study of 106 patients with type 2 diabetes and 30 patients without diabetes, latex-enhanced immunoturbidimetry was used to measure L-FABP and found a significant negative correlation between urinary L-FABP levels and eGFR ($r=-0.606$, $p<0.001$). Urinary L-FABP level in the normoalbuminuria diabetes group was significantly higher than that in the non-diabetic control group ($P<0.001$). In addition, ROC curve analysis showed that AUCL-FABP was significantly higher in both diabetic and normoalbuminuria diabetes patients than AUCACR ($p<0.001$) [42]. Therefore, hyperuria L-FABP can act as a strong and independent predictor of DKD progression.

4. Conclusion

The limitations of urinary protein and eGFR as predictors of further renal function development in diabetic patients have been widely described. The discovery of novel biomarkers holds considerable promise, which also provides an opportunity to assess kidney function more comprehensively. However, there are some challenges. First, although a large number of current studies have reported the existence of potential novel biomarkers to further predict renal outcomes in patients with diabetes, they have only slightly improved the accuracy of diagnosis. In addition, this will increase the financial burden on patients, although the detection of DKD can theoretically prevent kidney failure, it may not change the management of patients' disease. Second, although these new biomarkers have been discovered, in addition to the clear diagnosis may require the combination of multiple markers analysis, there are still difficulties in sampling, insufficient data, and difficult to further implement in clinical practice. Finally, the "labeling" of DKD patients may have an impact on health insurance and jobs.

While waiting for further development in this field, clinicians should also focus on blood pressure, blood sugar control, and measures to treat dyslipidemia to further slow the rate of disease progression, as they can improve the accuracy of DKD predictions. In addition, consensus on sample collection, processing, and analysis methods is needed to overcome the difficulty of copying samples. The broader practice will provide new insights into disease patterns, which will also facilitate diagnostic assessment on the clinical front line and improve patient outcomes. While further research and improvements are needed, new biomarkers for the kidney are critical for chronic diseases such as diabetes.

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