REVIEW

Early detection of diabetic kidney disease: Present limitations and future perspectives

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Abstract

Diabetic kidney disease (DKD) is one of the most common diabetic complications, as well as the leading cause of chronic kidney disease and end-stage renal disease around the world. To prevent the dreadful consequence, development of new assays for diagnostic of DKD has always been the priority in the research field of diabetic complications. At present, urinary albumin-to-creatinine ratio and estimated glomerular filtration rate (eGFR) are the standard methods for assessing glomerular damage and renal function changes in clinical practice. However, due to diverse tissue involvement in different individuals, the so-called "non-albuminuric renal impairment" is not uncommon, especially in patients with type 2 diabetes. On the other hand, the precision of creatinine-based GFR estimates is limited in hyperfiltration status. These facts make albuminuria and eGFR less reliable indicators for early-stage DKD. In recent years, considerable progress has been made in the understanding of the pathogenesis of DKD, along with the elucidation of its genetic profiles and phenotypic expression of different molecules. With the help of ever-evolving technologies, it has gradually become plausible to apply the thriving information in clinical practice. The strength and weakness of several novel biomarkers, genomic, proteomic and metabolomic signatures in assisting the early diagnosis of DKD will be discussed in this article.

Key words: Diabetic kidney disease; Early diagnosis; Genomics; Biomarkers

Core tip: Estimated glomerular filtration rate (eGFR) and albuminuria are currently the standard method for

detecting diabetic kidney disease (DKD). Creatinine-based GFR estimates are affected by muscle mass and diet pattern, as well as the formula chosen. Albuminuria majorly reflects glomerular dysfunction, and is less sensitive to tubulointerstitial and vascular damages. These facts limit the application of eGFR and albuminuria in the early diagnosis of DKD, especially in heterogeneous type 2 diabetic patients. Through the assistance of genetic information for screening of susceptible patients, together with novel biomarkers to reflect diverse renal tissue damage, early diagnosis of DKD could be facilitated.

INTRODUCTION

Diabetes mellitus is currently one of the most rapidly-growing "epidemics" around the world. According to the International Diabetes Federation, 415 million people are currently affected by this disease worldwide[1]. By the year 2040, the patient number is expected to rise up to 642 million, reaching a global prevalence of 10%[1]. This increasing number of patients, mostly with type 2 diabetes mellitus (T2DM), has influenced the rate of diabetic complications, including diabetic kidney disease (DKD). In developed countries, DKD is one of the most common complications of both type 1 diabetes mellitus (T1DM) and T2DM[2], and is also the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD)[3-5]. The costs of care for patients with DKD are extremely high, especially after they enter ESRD. In the United States, for the patients covered by Medicare, the average cost per person per year was USD 20000, whereas it was USD 40000 in the younger group (below 65 years of age)[2]. This leads to an increasing burden on the finance and health care systems. Therefore, different methods for identification and management of patients with DKD, especially in the early stages, have always been the priority in the research field of diabetic complications. At present, diagnosis of DKD in clinical settings relies upon the assessment of kidney function, usually by calculating estimated glomerular filtration rate (eGFR), and the assessment of kidney damage, usually by checking urinary albumin-to-creatinine ratio [UACR, urine albumin (mg/L)/urine creatinine (mmol/L)] in random spot urine samples[6]. Although these tests can be performed easily, they have certain limitations. Therefore, understanding these limitations is important to both clinical applications and the future quest for better diagnostic methods.

NATURAL HISTORY OF DKD

The first clinical sign suggestive of DKD is glomerular hyperfiltration, which is observed in about 70% and 50% of the patients with T1DM and T2DM, respectively[7]. Due to the increased intraglomerular pressure, the elevation in GFR may exceed 120 mL/min per 1.73 m^2[8]. In some patients, hyperfiltration is followed by the development of albuminuria. Most patients with T1DM have a normal UACR (< 3.4 mg/mmol) during the first 5 years after the disease onset. In the subsequent 10-15 years, albuminuria develops in some patients, and progresses gradually if no intervention is taken. Once UACR is over 34 mg/mmol, the GFR decreases progressively at a variable rate. Approximately 50% of the patients with UACR > 34 mg/mmol progress to ESRD over a period of 10 years and approximately 75% of the patients over a period of 20 years[6]. In patients with T2DM, however, the natural course of DKD is less understood, as the diagnosis is usually delayed by many years. Some patients already display various degrees of albuminuria at the time of diagnosis; however, only 20% of the patients with UACR > 34 mg/mmol progress to ESRD over a period of 20 years[9,10].

LIMITATIONS OF EGFR

In terms of renal excretory functions, GFR is considered the best overall index. However, due to its time-consuming nature, the measurement of 24-h creatinine clearance to assess GFR is not always easily performed in clinical settings. Instead, to assess renal function, calculating eGFR using serum creatinine level and formulae such as the modification of diet in renal disease [MDRD, eGFR = 175 × standardized Scr$^{-1.154}$ × age$^{-0.203}$ × 1.212 (if black) × 0.742 (if female), where Scr is serum creatinine][11] or the chronic kidney disease epidemiology collaboration [CKD-EPI, eGFR = 141 × min (Scr/k, 1)$^{\alpha}$ × max (Scr/k, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 (if female) × 1.159 (if black), where k is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/k or 1, and max indicates the maximum of Scr/k or 1][12] equations has become a routine practice. The National Kidney Foundation uses eGFR to classify stages of CKD[13]. Nonetheless, there are some potential flaws in using eGFR as a marker for the early diagnosis of DKD. First, serum creatinine levels are affected by the muscle mass and diet pattern (especially meat intake)[14,15], and therefore may interfere with the eGFR calculation. Second, the formula used may also cause imprecision in certain conditions. The MDRD equations become less reliable in patients with GFR > 60 mL/min per 1.73 m^2[16,17]. This would cause a considerable problem in the early diagnosis of DKD, as glomerular hyperfiltration appears early in the course of the disease. The CKD-EPI equation, on the other hand, is more accurate in patients whose GFR is > 90 mL/min per 1.73 m^2[18] and is, therefore, preferred when applying

it in patients with diabetes[6]. However, Camargo *et al*[19] reported a marked underestimation of GFR calculated with the CKD-EPI equation in diabetic patients compared to healthy individuals. Moreover, the MDRD and CKD-EPI equations have a P30 value between 80% and 90%, which means that the eGFR generated from these equations has, at best, a 90% chance of being within ± 30% of the measured GFR[2]. To sum up, caution should be exercised when using eGFR as the sole marker for diagnosis of DKD.

LIMITATIONS OF ALBUMINURIA

Albuminuria is considered a marker of kidney damage, especially with glomerular dysfunction. An assay for detecting low concentration of urinary albumin was first described in the 1960s[20]. When compared with semi-quantitative method, it is more sensitive and specific for disease survey and monitoring. Similar to GFR, measurement of 24-h urine albumin is time-consuming, and adds little to prediction or accuracy[13,21]. Therefore, calculating UACR by checking albumin and creatinine levels in random spot urine samples is currently the standard of clinical practice. However, urinary albumin excretion may also increase for reasons other than DKD, such as physical activity, diet pattern, infection, fever, congestive heart failure, marked hyperglycemia, menstruation, and marked hypertension[22]. Therefore, the diagnosis of persistent albuminuria is based on abnormal UACR in two out of three specimens collected within a period of 3-6 mo[6].

A crucial point of clinical significance is the discordance between the presence of albuminuria and the decline in renal function. Perkins *et al*[23] reported the development of advanced CKD (GFR < 60 mL/min per 1.73 m^2) without concomitant progression of albuminuria in patients with T1DM enrolled in the Joslin Kidney Study. In the Third National Health and Nutrition Examination Survey (NHANES Ⅲ), a normal urinary albumin level was identified in 36% of the 1197 patients with T2DM who had advanced CKD[24,25]. In the United Kingdom Prospective Diabetes Study 74, only 49% of the patients with renal impairment had preceding albuminuria[26]. In the Developing Education on Microalbuminuria for Awareness of Renal and Cardiovascular Risk in Diabetes study, advanced CKD was noticed in 17% of those with normal UACR[27]. This discordance might be caused by the heterogeneous nature of renal injury, especially in T2DM. As mentioned above, albuminuria is a marker of glomerular dysfunction, which is characteristic of DKD in T1DM[28,29]. However, glomerulopathy is a less common pathogenesis in DKD of T2DM. In fact, tubulointerstitial and/or vascular lesions are sometimes the major histological changes[30-32]. Penno *et al*[33] described a strong association between prevalence of cardiovascular diseases and "non-albuminuric renal impairment", suggesting a predominance of macroangiopathy as the underlying renal pathology. Further studies are required to clarify this assumption.

ALTERNATIVE BIOMARKERS

Due to the limitations of eGFR and albuminuria in the early diagnosis of DKD, enormous efforts have been made to investigate and validate alternative biomarkers in recent decades. A tremendous amount of biomarkers have been evaluated for the diagnosis of DKD, and many studies have shown promising preliminary results (Table 1). However, large-scale studies are still required to validate the value of these biomarkers over and above that of eGFR and UACR.

Cystatin C (CysC) is a 13.3 kDa plasma protein freely filtered through the glomerulus. It does not re-enter the bloodstream in an intact form after being re-absorbed and catabolized by tubular cells[34]. Validation studies have showed that serum CysC levels are not affected by muscle mass, which is a major defect of creatinine, and are well-correlated with GFR[35-37]. In addition, CysC-based GFR estimation is more accurate than creatinine-based estimation when GFR remains > 60 mL/min per 1.73 m^2[38,39], suggesting that CysC might serve as a better marker of glomerular function in the early stages of DKD. However, a greater intra-individual variability compared to serum creatinine[37], together with a higher cost, should be considered before its clinical application.

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa molecule which belongs to the lipocalin superfamily. It serves as a binder and transporter of small hydrophobic molecules, and a factor of innate antibacterial responses[40]. Urinary NGAL is closely related to the severity of renal impairment in various kidney disease. It is considered to play a protective role in such harmful conditions, as it is capable of promoting the proliferation and differentiation of renal cells[41]. Yang *et al*[42] reported that urinary NGAL correlated positively with serum CysC and creatinine levels, and inversely with GFR, whereas serum NGAL correlated negatively with serum CysC, in patients with T2DM. Furthermore, urinary NGAL has been shown to correlate positively with the severity of albuminuria in both T1DM[43] and T2DM[42] patients. In patients with short duration (less than 5 years) of T2DM, Fu *et al*[44] described a positive correlation between urinary NGAL and glomerular hyperfiltration. Such compelling evidences suggest the potential of NGAL as a novel biomarker for the early detection of DKD.

Kidney injury molecule 1 (KIM1) is a transmembrane protein with immunoglobulin-like and mucin domains in its ectodomain. Upregulated expression of KIM1 in renal tubules has been observed in ischemic, toxic, and proteinuric kidney diseases, suggesting its potential role as a marker of renal damage[45]. Similar to NGAL, elevated urinary KIM1 concentrations were identified in T2DM patients with glomerular hyperfiltration[44]. Nielsen *et al*[43] reported higher urinary KIM1 excretion in patients with T1DM than in healthy controls. Vaidya *et al*[46] showed that lower baseline concentration of urinary KIM1 was predictive of subsequent regression of albuminuria. These results indicate that the role of KIM1 in the early diagnosis of DKD is worth further investigation.

Table 1 Advantages of novel biomarkers in the early diagnosis of diabetic kidney disease

Biomarker	Validation study design	Sample size	Type of diabetes	Specimen	Advantages	Ref.
CysC	CO	52[38]	2	Serum	Not affected by lean body mass	[35-39]
		30[39]			Estimates more accurate than creatinine-based ones when GFR > 60 mL/min per 1.73 m^2	
NGAL	CC	112	2	Urine	Indicator of glomerular hyperfiltration	[44]
KIM1	CC	112	2	Urine	Indicator of glomerular hyperfiltration	[44]
NAG	CC	434	1	Urine	Baseline level predicts development of DKD	[51]
	CC	946	2			[52]
8-oxodG	PC	396	2	Urine	Baseline level predicts development of DKD	[59]
Pentosidine	CC	434	1	Urine	Baseline level predicts progression of albuminuria	[51]
TNFR1/2	RC	628	1	Serum	Baseline level predicts development of advanced CKD	[65]
	RC	410	2			[66]

CysC: Cystatin C; NGAL: Neutrophil gelatinase-associated lipocalin; KIM1: Kidney injury molecule 1; NAG: N-acetyl-β-(D)-glucosaminidase; 8-oxodG: 8-oxo-7,8-dihydro-2′-deoxyguanosine; TNFR: Tumor necrosis factor receptor; CO: Case-only; CC: Case-control; PC: Prospective cohort; RC: Retrospective cohort; GFR: Glomerular filtration rate; DKD: Diabetic kidney disease; CKD: Chronic kidney disease.

N-acetyl-β-(D)-glucosaminidase (NAG) is a 130 kDa lysosomal enzyme located in the brush border of proximal renal tubular cells. Under normal conditions, NAG is excreted in low amounts in urine during the process of exocytosis. Elevated urinary NAG has been observed in various kidney diseases, suggesting a reflection of renal damage[47,48]. In patients with diabetes, increased excretion of NAG in urine has been identified to associate with the severity of albuminuria[49-51]. Despite inconsistency has been observed in the correlation between urinary NAG and glomerular hyperfiltration[44], results from the studies of Kern *et al*[51] and Hong *et al*[52] have indicated that higher baseline concentrations of urinary NAG were predictive of future development of DKD. On the other hand, lower baseline urinary concentration of urinary NAG was associated with the subsequent regression of albuminuria[46]. In addition to DKD, increased excretion of NAG in urine has also been reported to predict macrovascular complications in patients with T2DM[52-54].

Oxidative stress has been considered to play an important part in the pathogenesis of diabetic complications[55]. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is an oxidized nucleoside - one of the major product of oxidative damage in nuclear and mitochondrial DNA[56]. Upon DNA repair, 8-oxodG is directly excreted into urine without further metabolization, so its urine concentration may serve as a generalized index of oxidative stress[57]. The study conducted by Hinokio *et al*[58] demonstrated a close correlation between urinary 8-oxodG excretion and the severity of microsvascular diabetic complications. In a 5-year cohort study of 532 Japanese patients with T2DM, baseline concentration of urinary 8-oxodG predicted subsequent development of DKD[59], indicating its potential as a predictive marker.

Hyperglycemia irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs), which cause qualitative and quantitative changes of the components of extracellular matrix. By affecting cell adhesion, growth, and matrix accumulation, AGE-induced changes are associated with the pathogenesis of diabetes complications[60]. One of the best chemically characterized AGEs found in human is pentosidine, which has been considered as a marker of formation and accumulation of AGEs[61]. Elevated urinary and plasma pentosidine levels were identified in T2DM patients with DKD[62]. Both urinary[51] and plasma[63] pentosidine levels have been demonstrated to correlate positively with the severity of albuminuria in patients with diabetes. In the study conducted by Kern *et al*[51], baseline urinary pentosidine excretion in patients with T1DM predicted the progression of albuminuria, with a seven-fold increase in risk for every 50% increase in urinary pentosidine.

Tumor necrosis factor (TNF)-α is a key mediator of inflammation and apoptosis. The signal transduction of TNF-α is commenced *via* two distinct receptors, TNF receptor (TNFR) 1 and TNFR2, which are presented in both membrane-bound form and soluble form in serum[64]. Serum levels of TNFR1 and TNFR2 were shown to correlate with GFR in patients with diabetes, and was independent of the status of albuminuria[64]. Recent studies in both T1DM[65] and T2DM[66] patients have indicated that plasma TNFR levels were capable of predicting the development of advanced CKD independently over 12 years of follow-up. These evidences suggest that serum concentrations of TNFR1 and TNFR2 may be utilized as predictors of DKD progression.

GENETIC SUSCEPTIBILITY

Genetic studies provide a powerful tool in the understanding of disease mechanisms. Emerging evidences have suggested that DKD is heritable[67-69]. Prior to the deployment of modern high-throughput technologies such as single nucleotide polymorphism microarray analysis and next-generation sequencing, linkage analysis had revealed variants on different chromosomal regions associated with DKD. For instance, variants on chromosome 18q have been identified to be associated with albuminuria and decreased renal function in different ethnic groups[70,71]. With the application of genome-wide association studies (GWASs) over the past decade, considerable progress has been made in the

understanding of genetic background of DKD. Genes such as engulfment and cell motility 1[72-77], FERM domain containing 3[78-81], cysteinyl-tRNA synthase[78,79,81], apolipoprotein L3-non-muscle myosin heavy chain 9[82,83] have been identified to be associated with the phenotypic presentations of DKD. Other risk loci have also been reported, yet data from different GWASs are not consistent[84]. Several fundamental problems remain to be solved before applying these results in clinical practice. First, genetic heterogeneity is always a major consideration when assessing the genetic background of any disease. Replication studies are essential for patients with DKD in different populations. Second, in most GWASs, DKD was defined as the co-existence of hyperglycemia and proteinuria; therefore, it is likely that these results are confounded by patients with renal damage due to causes other than diabetes. Last but not least, the actual functions of many genes which contain loci of risk are still unknown. Further studies are required to elucidate their roles in the pathogenesis of DKD.

EPIGENETIC MODIFICATIONS

Epigenetic modifications refer to DNA methylation, histone methylation, and histone acetylation, which alter the expression of a gene by changing its accessibility rather than nucleotide sequence[85]. In patients with diabetes, multiple factors, such as hyperglycemia, reactive oxygen species, and inflammation, can trigger epigenetic modifications[86]. Knowledge about the role of epigenetic modifications in the pathogenesis of DKD is currently very limited; however, since epigenetics is very sensitive to environmental factors, it is plausible that epigenetic imprints are responsible for the "metabolic memory" linked to diabetic complications[87]. Hasegawa *et al*[88] demonstrated that differentially methylated genes correlated with fibrogenesis in microdissected tubules obtained from patients with DKD. In a case-control study of 192 Irish patients with T1DM, Bell *et al*[89] reported that methylation at 19 CpG cites in several genes, including *UNC13B*, was associated with the time to development of DKD. Sapienza *et al*[90] identified 187 genes that were differentially methylated on at least two CpG sites among African American and Hispanic diabetic patients with ESRD. Intriguingly, many of these genes have been recognized previously through genome association or transcription profiling studies, and are associated with inflammation, oxidative stress, ubiquitination, fibrosis, drug metabolism, and development of DKD. These results suggest a very close connection between epigenetic modifications and genetic dysregulartions in the pathogenesis of DKD.

MICRORNA PROFILES

MicroRNAs (miRNAs) are small non-coding RNAs composed of 21-25 nucleotides that are produced by genes. By binding to target mRNAs, miRNAs induce degradation of RNAs or, more frequently, repression of protein translation[91]. Being packed within exosomes, miRNAs are stable in serum, plasma, and urine[92]. The stability makes miRNAs as potential candidate biomarkers for the non-invasive diagnosis of many diseases[93].

In vitro and *in vivo* studies have revealed the potential roles of miRNAs in the pathogenesis of DKD, especially in the early mesangial expansion stage. Changes in the expression of many miRNAs, such as miR-192[94-97], miR-216a[98], miR-377[99], miR-29c[100], miR-200b/c[101], miR-21[102], miR-1207-5p[103], miR-200a[104], and miR-23b[105], have been identified to be involved in the process of extracellular matrix expansion and fibrosis, interaction with transforming growth factor β and other pro-fibrotic genes. Long *et al*[106] identified miR-93 as a novel regulator of vascular endothelial growth factor in *in vitro* and *in vivo* experimental models under hyperglycemic conditions. Fu *et al*[107] described a significant reduction of endogenous miR-25 in rat mesangial cells treated with high glucose concentrations and in the kidneys of diabetic rats associated with increased nicotinamide adenine dinucleotide phosphate hydrogen oxidase (NOX) activity characterized by high NOX4 expression levels. Zhang *et al*[108] reported that over-expression of miR-451, which targets tyrosine3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta and p38 mitogen-activated protein kinase signaling pathways, resulted in reduced glomerular mesangial cell proliferation *in vitro* and *in vivo*. These experimental findings are summarized in Table 2.

The urinary and serum miRNA in patients with DKD have also been profiled. In T1DM patients with albuminuria, Argyropoulos *et al*[109] showed underexpression of urinary miR-323b-5p, miR-221-3p, miR-524-5p, and miR-188-3p, whereas miR-214-3p, miR-92b-5p, hsa-miR-765, hsa-miR-429, miR-373-5p, miR-1913, and miR-638 were overexpressed. On the other hand, an elevation in urinary miR-130a and miR-145 levels, with a reduction in miR-155 and miR-424, were reported by Barutta *et al*[110] in a similar setting. In patients with T2DM, Peng *et al*[111] described a positive correlation between urinary miR-29 levels and the severity of albuminuria.

Expression of miRNAs was also measured in venous blood from Chinese T2DM patients with and without DKD. Using a microarray-based approach, Zhou *et al*[112] confirmed the downregulation of miR-let-7a in the patients with DKD. Intriguingly, the authors also observed that the distribution of a specific variant within *let-7a* (rs1143770) was significantly higher in patients with diabetes than in healthy controls. These results are summarized in Table 3.

PROTEOMIC SIGNATURES

Proteomics is defined as "the knowledge of the structure, function, and expression of all proteins in the biochemical or biological context of organisms"[113]. The most attractive feature of proteomics is that it allows the monitoring of patterns of multiple urine and plasma proteins simultaneously. Considering the sophisticated

Table 2 *In vitro* and *in vivo* renal cell models demonstrating the potential involvement of miRNAs in development of diabetic kidney disease

miRNA	Species	Specimen	miRNA expression	Mechanism of action	Ref.
miR-192	Mice/Rat	M, Te, KT	Inconsistent results	Interaction with TGFβ-associated and other pro-fibrotic genes	[94-96]
	Human	Te, KT	Reduced		[97]
miR-216a	Mice	M, KT	Elevated		[98]
miR-377	Mice	M, KT	Elevated		[99]
	Human	M			
miR-29c	Mice	P, KT	Elevated		[100]
miR-200b/c	Mice	M, KT	Elevated		[101]
miR-21	Mice	KT	Elevated		[102]
	Human	Te			
miR-1207-5p	Human	P, M, Te	Elevated		[103]
miR-200a	Rat	Te	Reduced		[104]
	Mice	KT			
miR-23b	Mice	KT	Reduced		[105]
	Human	Te, HEK-293A			
miR-93	Mice	P, En, KT	Reduced	Regulation of VEGF expression	[106]
miR-25	Rat	M, KT	Reduced	Regulation of NOX4 expression	[107]
miR-451	Mice	M, KT	Reduced	Targeting YwhaZ and p38 MAPK signaling pathways	[108]

M: Mesangial cells; Te: Tubular epithelial cells; KT: Kidney tissue; P: Podocytes; En: Endothelial cells; TGFβ: Transforming growth factor β; VEGF: Vascular endothelial growth factor; NOX4: Nicotinamide adenine dinucleotide phosphate hydrogen oxidase 4; YwhaZ: Tyrosine3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta; MAPK: Mitogen-activated protein kinase; HEK-293A: Human Embryonic Kidney-293A cells.

nature of DKD, especially in patients with T2DM, it is plausible that early diagnosis of this disease, which relies only on a single biomarker, might eventually fail to reach optimal sensitivity and specificity[114]. The role of proteomics in the early diagnosis of DKD, therefore, is worthy of further evaluation.

DN65 is a panel composed of 65 urinary biomarkers, many of which are fragments of type I collagen. In the study conducted by Rossing *et al*[115], DN65 was capable of distinguishing between diabetic patients without albuminuria from those with DKD. It was also proved to be sensitive and specific in distinguishing DKD from CKD of other etiologies, as well as predicting the progression toward overt DKD in patients with diabetes who had albuminuria over 3 years. First described by Good *et al*[116] in 2010, CKD273 is another panel of 273 urinary peptides and proteins capable of identifying CKD of any cause with excellent sensitivity and specificity. In a cohort of 35 patients with diabetes, Zürbig *et al*[117] showed that the CKD273 classifier was capable of detectingthose who were at risk of DKD progression up to 5 years prior to development of overt albuminuria. In the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort, Roscioni *et al*[118] showed that the baseline CKD273 classifier score was independently associated with the progression of albuminuria. In urine samples obtained from 165 patients with T2DM at 9 different centers, Siwy *et al*[119] demonstrated that the classifier could identify DKD patients with high consistency.

METABOLOMIC SIGNATURES

Metabolomics refers to the identification of low molecular weight intermediate and end-products of cellular functions in a biological sample with nuclear magnetic resonance and mass spectrometry-based profiling

techniques[120,121]. As metabolome represents the complete collection of metabolites in an organism, understanding the perturbations in human metabolome might help with early unveiling of the pathological changes in disease processes.

Several studies have assessed the potential of metabolomics in diagnosis of DKD (Table 4). Han *et al*[122] described the diverse profiles of plasma fatty acids in different stages of DKD. In 82 patients with T2DM, Zhu *et al*[123] demonstrated that a panel of six plasma phospholipids was capable of distinguishing between patients with and without DKD. In 78 patients with diabetes, Hirayama *et al*[124] identified a panel of 19 serum metabolites correlated significantly with UACR. A multiple logistic regression model composed of the five best performing markers (including γ-butyrobetaine, symmetric dimethylarginine, azelaic acid, and two unknowns) yielded remarkable sensitivity and specificity for the diagnosis of DKD. Sharma *et al*[125] quantified 94 metabolites in urine obtained from healthy control, diabetic patients with and without DKD. A decrease in the urine levels of 13 metabolites, many potentially related to mitochondrial function, was found to be associated with DKD. Pena *et al*[126] described the different metabolomic profiles in the urine and plasma samples from the T2DM cohort of the PREVEND study. Differences were observed in the levels of plasma histidine, butenoylcarnitine, as well as urine hexose, glutamine, and tyrosine, between those who with and without albuminuria. Adding these metabolites to a predictive model composed of baseline urinary albumin excretion and eGFR improve risk estimation for the progression of albuminuria. In the T2DM cohort of the Joslin Kidney Study, Niewczas *et al*[127] identified a panel of 5 plasma metabolites capable of predicting progression toward ESRD, which was independent of UACR, eGFR, and hemoglobin A1c. Although

Table 3 Urinary and serum miRNA profiles in patients with diabetic kidney disease

Type of diabetes	Specimen	miRNA expression		Ref.
1	Urine	Decreased miR-323b-5p, miR-221-3p, miR-524-5p, miR-188-3p	Increased miR-214-3p, miR-92b-5p, hsa-miR-765, hsa-miR-429, miR-373-5p, miR-1913, miR-638	[109]
1	Urine	Decreased miR-155, miR-424	Increased miR-130a, miR-145	[110]
2	Urine	miR-29 expression positively correlated to the severity of albuminuria		[111]
2	Blood	Reduced expression of miR-let-7a		[112]

Table 4 Applications of metabolomics in the diagnosis of diabetic kidney disease

Specimen	Panel	Application	Ref.
Plasma	Fatty acids C10:0, C12:0, C14:0, C16:1n-9, C16:0, C18:2, C18:1n-9, C18:1n-11, C18:0, C20:4, C20:5, C20:3, C20:2, C20:0, C22:6	Diverse profiles in different stages of DKD	[122]
Plasma	Phospholipids C18:2-LPC, C16:0/18:1-PE, pC18:0/20:4-PE, C18:0/22:6-PI, C18:0/18:0-PS, dC18:0/20:2-SM	Diagnosis of DKD	[123]
Serum	γ-butyrobetaine, SDMA, azelaic acid, MID 114, MID 127	Diagnosis of DKD	[124]
Urine	3-hydroxy isovalerate, aconitic acid, citric acid, 2-ethyl 3-OH propionate, glycolic acid, homovanillic acid, 3-hydroxy isobutyrate, 2-methyl acetoacetate, 3-methyl adipic acid, 3-methyl crotonyl glycine, 3-hydroxy propionate, tiglylglycine, uracil	Reduced expression in DKD patients	[125]
Plasma and urine	Plasma: Histidine, butenoylcarnitine Urine: Hexose, glutamine, tyrosine	Addition to the original predictive model improved risk estimation for albuminuria progression	[126]
Plasma	P-cresol sulfate, phenylacetylglutamine, myoinositol, pseudouridine, urate	Predicting progression toward ESRD	[127]

LPC: Lysophosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PS: Phosphatidylserine; SM: Sphingomyelin; SDMA: Symmetric dimethylarginine; MID: Metabolite ID; DKD: Diabetic kidney disease; ESRD: End-stage renal disease.

these results seems promising, the complexity of the analysis techniques and the incomplete coverage of the human metabolome at present are problems than may need to be addressed before the application of metabolomics in everyday practice.

CONCLUSION

The development of DKD involves the dysfunction and damage of different renal tissues in multiple stages. Due to the complex nature of this disease, whether there is a "universal" biomarker is questionable. With extensive validations, albuminuria and eGFR are currently the standard diagnostic criteria for DKD. Nonetheless, the abilities of these markers to detect tissue damage and functional change in the early stage are limited. With the increasing understanding of pathogenesis and promising preliminary data, applying the information generated from the studies of novel biomarkers, genomic, and proteomic profiles to assist in the early diagnosis of DKD has gradually become plausible. An integration of the "traditional" and "next-generation" markers might be more practical in everyday settings, considering the financial and technical requirements of these novel assays. To sum up, large longitudinal cohort studies are still required to validate the abilities of the aforementioned novel early diagnosis and prediction techniques.

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