Molecular Pathways of Diabetic Kidney Disease Inferred from Proteomics

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Abstract: Diabetic kidney disease (DKD) affects an estimated 20–40% of type 2 diabetes patients and is among the most prevalent microvascular complications in this patient population, contributing to high morbidity and mortality rates. Currently, changes in albuminuria status are thought to be a primary indicator of the onset or progression of DKD, yet progressive nephropathy and renal impairment can occur in certain diabetic individuals who exhibit normal urinary albumin levels, emphasizing the lack of sensitivity and specificity associated with the use of albuminuria as a biomarker for detecting diabetic kidney disease and predicting DKD risk. According to the study, a non-invasive method for early detection or prediction of DKD may involve combining proteomic analytical techniques such second generation sequencing, mass spectrometry, two-dimensional gel electrophoresis, and other advanced system biology algorithms. Another category of proteins of relevance may now be provided by renal tissue biomarkers. The establishment of reliable proteomic biomarkers of DKD represents a novel approach to improving the diagnosis, prognostic evaluation, and treatment of affected patients. In the present review, a series of protein biomarkers that have been characterized to date are discussed, offering a theoretical foundation for future efforts to aid patients suffering from this debilitating microvascular complication.

Keywords: diabetic kidney disease, proteomics, diagnostic biomarkers, therapeutic targets

Introduction

Diabetic kidney disease (DKD) is a common microvascular complication that impacts 20–40% of individuals diagnosed with type 2 diabetes, resulting in high rates of morbidity and mortality. DKD is diagnosed based on the detection of persistent proteinuria, an albumin creatinine ratio greater than 30 mg/g creatinine, a reduction in the estimated glomerular filtration rate (eGFR), and the progressive deterioration of kidney function.[1,2] Persistent hyperglycemia in individuals with diabetes can drive the increased synthesis of extracellular matrix (ECM) proteins, enhanced cell proliferation, and the dysfunction of endothelial cells together with tubular atrophy, interstitial fibrosis, and the thickening of the glomerular and tubular basement membrane as a consequent of inflammation, oxidative stress, and advanced glycation end product (AGE) production.[3] DKD development is further driven by the increased accumulation of protein within the renal extracellular matrix, the expansion of the mesangial matrix, glomerular hyperfiltration, tubulointerstitial fibrosis, and the aggravated thickening of the glomerular basement membrane. Injury to glomerular podocytes can also contribute to DKD incidence.[1]

Both a reduction in eGFR and albuminuria are considered relatively non-specific biomarkers of DKD given that they are also altered in the context of many other chronic glomerular diseases, with 60% of nephron function already being absent with eGFR values falling to the threshold of 60 mL/min/1.73 m^2.[4] Given that DKD is a prevalent cause of end-stage renal disease (ESRD), it is critical that reliable biomarkers that can guide the diagnosis and treatment of this condition be defined. Both urinary albumin (UALB) and β2-microglobulin (Uβ2m) have been leveraged as biomarkers of glomerular and tubular injury,[5] yet these levels and eGFR changes are only evident after substantial glomerular damage has taken place. However, many patients exhibit histopathological alterations in renal structures prior to the onset of microalbuminuria.[6] While these changes can be detected through renal biopsy, enabling the more robust diagnosis and

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monitoring of affected patients, the utility of this approach is limited by the inherent invasivity of biopsy procedures. As such, there is a pressing need for the establishment of new, non-invasive biomarkers that can gauge the risk of future DKD onset or detect this disease while in its earliest stages.

Certain urinary and plasma protein biomarkers have been linked to the pathophysiology of many forms of renal disease, and some of these biomarkers have even been successfully implemented in clinical settings. The kidneys directly produce urine. It is relatively simple and non-invasive to collect urine protein because it is so stable. It can be a useful biomarker and is difficult to degrade.[7,8] Blood samples are also easy to collect, serving as an optimal sample type for efforts to diagnose early-stage DKD.[8–10] Proteins found in renal tissue allow for the monitoring of biomolecules' effects on tissues. As proteomic indicators of DKD, they are currently becoming more and more well-known.[11] Indeed, the proteome has long been regarded as an ideal focus for efforts to understand the molecular etiology of disease and to define prognostic or diagnostic biomarkers associated with particular disease states. The use of blood, urine, and renal tissue proteomic analyses to non-invasively detect and monitor DKD has emerged as an increasingly important field that can clarify the pathophysiological mechanisms underlying DKD while identifying key targets for future efforts to prevent subsequent disease progression.[12] The systematic examination of these proteomic targets offers more comprehensive insight into DKD and other complex metabolic diseases while also providing a foundation to design prognostic tools and targeted therapies. DKD biomarkers have been the subject of numerous transcriptomics and metabonomics investigations in the past. Lei et al conducted work on transcriptomics,[13] and Mu et al conducted experiments on metabonomics.[14] Proteomics is the topic of this essay. This article primarily examines and discusses the most recent developments in the proteome analysis of urine, blood, and kidney tissue samples for the purpose of identifying potential specific biomarkers for the diagnosis of DKD.

Proteins and Proteomics

The proteome is a catch-all term that refers to all proteins produced by a given tissue, cell, or organism. A range of proteomic techniques have been leveraged to date to characterize the proteome or the expression of particular protein subsets, including low molecular mass polypeptides. Protein-based biomarkers of DKD have been explored through enzyme-linked immuno-sorbent assay (ELISAs), 2-way gel electrophoresis (2-DE),[15] equivalent labeling for relative and absolute quantification (iTRAQ),[16,17] tandem mass labeling (TMT) and mass spectrometry, unlabeled approaches, and biochip analyses.[18] Proteomic analyses can enable the establishment of DKD-specific biomarkers through analyses of patient urine, blood, or renal tissue samples,[19,20] with advances in the underlying techniques having made the robust and routine screening of biofluid samples increasingly feasible, potentially enabling the detection of novel targets for the treatment of DKD.[21] These biomarkers also have the potential to advance current understanding of the mechanistic basis for DKD-related injury and to provide new diagnostic, monitoring, and classification options to support the individualized treatment of affected patients.[22] Labeled and unlabeled mass spectrometry methods, which may simultaneously screen thousands of target proteins, are currently the most widely used proteomic tools for locating biomarkers relevant to DKD diagnosis and prognosis.

Urinary Protein Biomarkers of DKD

In addition to being easy to collect in a non-invasive manner, urine can remain stable for years in storage such that even archived urine samples can be used to screen for DKD-related biomarkers.[23] Urinary proteomics analyses thus offer an opportunity to define small proteins and peptides characteristic of pathophysiological shifts associated with early-stage DKD. In one study, a proteomic biosignature consisting of 273 different urinary protein fragments known as the CKD273 classified was shown to be capable of differentiating between diabetic kidney disease patients and individuals with other forms of chronic renal disease.[24] The CKD273 classifier is a more accurate predictor of the incidence rate of DKD and can aid in the diagnosis, monitoring, and prognosis assessment of DKD patients. As it can detect individuals at risk of developing DKD, it can also help protect these patients from adverse renal outcomes.[25] Limonte et al determined that high urinary levels of the lysosomal proteolytic enzyme cathepsin D are linked to intracellular protein degradation, autophagy, and apoptotic cell death.[26] Elevated urinary cathepsin D levels are closely associated with rapid eGFR declines, tubular atrophy, tubulointerstitial inflammation, and fibrosis.[27] Ahn et al determined that urinary proteomic analyses are more reliable than measurement of albuminuria alone as a tool to predict DKD patient prognosis, with a proteomic biosignature comprised of ACP2, CTSA, GM2A, MUC1, and SPARCL1 offering substantial clinical

utility.[28] Chen et al determined that β2-microglobulin and Clara-cell protein are protein biomarkers associated with DKD that can aid in predicting the incidence of this condition and associated risks.

ITRAQ, TMT, etc. are technologies that can detect proteins in a specific way.[29] Through the use of a 2D-DIGE-MALDIQ-TOF strategy and related techniques, Patel et al were able to detect novel DKD-related protein biomarkers in at-risk individuals corresponding to the processes of renal tubulointerstitial fibrosis and tubulointerstitial fibrosis, including apolipoprotein A1, α-1microglobulin, and zinc α-2 glycoprotein.[30,31] Guo et al discovered that - 1-antitrypsin - 1-acid glycoprotein, ceruloplasmin, prostate stem cell antigen, APOA4 and other apolipoprotein AIV are prospective protein biomarkers of DKD in 23 diabetic patients and 16 healthy volunteers.[32] Liao et al also observed a significant increase in urinary haptoglobin (Hp) levels in DKD patients, allowing for the more accurate diagnosis and prognostic assessment of individuals with this disease.[33] Urinary retinol-binding protein 4 (uRBP4) and SH3 domain-binding glutamic acid-rich-like protein 3 (uSH3BGRL3) protein levels are closely associated with eGFR.[34] Through an SDS-PAGE approach, Araumi et al determined that urinary afamin (uAFM), uRBP4, and uSH3BGRL3 levels were elevated in DKD patients relative to individuals without renal damage, underscoring their potential utility as diagnostic biomarkers in this context.[20,35,36] Fan et al have also demonstrated that haptoglobin (HPT) and α-1-microglobulin / bicunin precursor (AMBP) are two protein biomarkers that can effectively differentiate between healthy individuals and diabetic patients who are or are not affected by DKD. Urinary proteomic techniques can aid in the non-invasive diagnosis of DKD and the identification of patients considered at high risk.[37]

The low molecular weight protein RBP is filtered by the glomeruli and ultimately reabsorbed via the proximal tubules, and has been found to offer substantial diagnostic utility in DKD patients who exhibit pronounced albuminuria. Al-Rubeaan et al reported the increased excretion of urinary transferrin, RBP, and serum osteopontin with diabetic kidney disease development, emphasizing the value of these biomarkers when diagnosing type 2 diabetic kidney disease.[38] Urinary L-FABP is a recently detected urinary protein biomarker used for early DKD detection, and Panduru et al reported higher urinary L-FABP levels in DKD patients relative to controls in a manner associated with disease severity. Urinary L-FABP thus represents a specific, sensitive protein biomarker capable of guiding early DKD diagnosis.[39] Nucleated cells produce the low molecular mass protein cystatin C (CST3), and cysteine aminotransferase activity can be used as a metric for kidney function in diabetic individuals with higher CST3 levels in urine being indicative of renal tubular damage. Through a CE-MS approach, Garg et al determined that urinary CST3 levels were significantly elevated in DKD patients relative to controls (P < 0.05), supporting the diagnostic utility of this protein (P < 0.05), indicating that urinary CST3 has diagnostic value.[40] A DKD-related proteomic classifier consisting of collagen fragments, α-microglobulin, β 2-microglobulin (β 2MG), A1AT, uromodulin, and other peptides has also shown promise as a tool for identifying type 2 diabetes patients at a high risk of DKD.[41] For further details regarding these urinary protein biomarkers, see Table 1.

Blood-Based Protein Biomarkers of DKD

Tubular cells are activated in response to high glucose levels, resulting in the secretion of inflammatory mediators and ECM components that contribute to tubular thinning and cell damage. Glomerular injury-associated proteinuria can act as a stressor that adversely impacts proximal renal tubular cells, driving inflammatory mediator secretion and renal damage. Blood can be kept stable in storage for a number of years and can be used to check for DKD-related biomarkers. Circulating biomarkers can offer insight into kidney damage in patients diagnosed with diabetes,[42] offering new potential means of guiding patient diagnosis, treatment, and prognostic assessment.[43] Plasma u/PC3 levels in the serum of DKD patients have been shown to be elevated as compared to diabetic individuals free of renal damage, emphasizing the potential diagnostic utility of this serum biomarker.[35] Chiang et al determined that SH3BGRL3 can function as an epidermal growth factor receptor conjugate, and that there is a significant positive correlation between uSH3BGRL3 levels and eGFR such that it can serve as a DKD-related biomarker.[34] Caseiro et al further detected increases in MMP-9, neutrophil gelatinase-associated lipocalin (NGAL), aminopeptidase N, azurocidin, and kallikrein 1 activity in type 1 DKD patients as compared to healthy individuals, suggesting that this protein biosignature may offer value in this type of DKD.[44] Moresco et al also confirmed that circulating NGAL levels correspond to renal tubular function, and a positive correlation has been reported between serum NGAL concentrations and proteinuria whereas these levels are negatively correlated with eGFR.[45] The lipocalin protein family member β-trace protein (β-TP), also known as lipoprotein

Table 1 Biomarkers of Urine Proteomics in DKD

PMID	Types (The Main Biomarkers)	Population	Authors	Methods	Disease	Main Findings
35377940	Urinary cathepsin D	Human and mouse models	Limonte et al[26]	LC-MS	Type 1 DKD	Elevated urinary cathepsin D was associated with rapid decline in EGFR, tubulointerstitial inflammation, fibrosis, and tubular atrophy.
32545899	ACP2, CTSA, GM2A, MUC1和SPARCL1	Human	Ahn et al[28]	Nano-LC-ESI-MS/MS Analysis	Type 2 DKD	A biomarker composed of five proteins, ACP2, CTSA, GM2A, MUC1 and SPARCL1, is important in the diagnosis of DKD
35327145	Glutathione S-transferase Mu 1, Zinc finger C2HC domain-containing protein 1A, Haptoglobin, Glutaredoxin-3, Zinc finger protein 2	Animal (dog)	Winiarczyk et al[70]	MALDI-TOF MS	DKD	All five proteins were up-regulated in diabetic canine urine, and the only protein up-regulated in microalbuminuric dogs was zinc finger protein 2.
29729954	U/PC3	Human	Li et al[22]	Sodium dodecyl sulfate (SDS)- polyacrylamide gel electrophoresis	DKD	The level of urinary C3 in diabetic nephropathy group was significantly higher than that in diabetic group without renal damage.
27293888	uRBP4	Human	Fiseha et al[36]	Sodium dodecyl sulfate (SDS)- polyacrylamide gel electrophoresis	DKD	uRBP4 is a urinary marker of early tubular damage in DKD, and the glomerular filtration rate of DKD is negatively correlated with uRBP4.
30486327	Haptoglobin and α-1-microglobulin/bikunin precursor	Human	Liao et al[33]	iTRAQ	Type 2 DKD	Haptoglobin and α-1-microglobulin/bikunin precursor are early indicators of DKD and markers associated with ERFD in diabetic patients.
30024955	β2-microglobulin and clara-cell protein	Human	Chen et al[29]	C18 plate–MALDI-TOF	Type 2 DKD	MALDI-TOF MS has high specificity in detecting protein markers by measuring protein quality, β2-microglobulin and clara-cell protein are DKD-related biomarkers.
32856461	408 N-linked glycoproteins, A1AT, and ceruloplasmin	Human	Jin et al[71]	iTRAQ	DKD	408 N-linked glycoproteins, A1AT, and ceruloplasmin as common or specific biomarkers for DKD.
31131043	Apolipoprotein A1, α-1microglobulin, zinc α-2 glycoprotein	Human	Patel et al[30]	2D-DIGE-MALDI Q-TOF	DKD	Proteomic analysis may be a useful tool to enable early detection of new markers in patients susceptible to diabetic nephropathy, such as apolipoprotein A1, α-1microglobulin, zinc α-2 glycoprotein, etc.
26608305	Alpha-1-antitrypsin, alpha-1-acid glycoprotein 1, ceruloplasmin, prostate stem cell antigen and apolipoprotein AIV	Human	Guo et al[32]	2D-LC-MS/MS and iTRAQ quantification	Type 2 DKD	Using 2D-LC-MS/MS and iTRAQ quantification technology, it was found that α-1-antitrypsin, α-1-acid glycoprotein, ceruloplasmin, prostate stem cell antigen and apolipoprotein AIV in urine are candidate protein biomarkers for DKD.
34458592	uAFM, uSH3BGRL3, uRBP4	Human	Araumi et al[35]	Sodium dodecyl sulfate (SDS)- polyacrylamide gel electrophoresis	DKD	uAFM, uRBP4 and uSH3BGRL3 are promising diagnostic markers for DKD.
34963468	HPT, AMBP	Human	Fan et al[37]	Nano HPLC–MS	DKD	HPT and AMBP are the two protein biomarkers with the highest capacity for diagnosing DKD patients.

30574040	Transthyretin precursor, Ig k chain C region, carbonicanhydrase 1, retinol binding protein, beta-2-microglobulin precursor and beta-2-glycoprotein 1.	Human	Bellei et al[72]	2-DE, Q-ToF-LC/MS, Agilent-Technologies, Western blot, ELISA	Type 2 DKD	In a proteome-related study, significantly differentially expressed proteins were detected in DKD patients compared with healthy control subjects.
29854824	Protein S100A9, Kallikrein-1, Pepsin A-3, Uteroglobin, Prostatic acid phosphatase, Ganglioside GM2, VCAM-1.	Human	Guillén et al[73]	TMT labeling, LC-MS, ELISA.	Type 2 DKD	Detection of these proteins in urine could serve as a potential new tool for biomarkers of DKD progression.
28577020	Urinary transferrin, urinary Retinol binding protein (RBP) and serum osteopontin	Human	Al-Rubeaan et al[38]	Biochip assay, ELISA	Type 2 DKD	Urinary transferrin, RBP and serum osteopontin have great diagnostic value in different stages of type 2 diabetic nephropathy.
23378622	L-FABP	Human	Panduru et al[39]	CE-MS, CKD273 classifier analysis	Type 2 DKD	The concentration of L-FABP in the urine of DKD patients was significantly increased, and it was closely related to the development and severity of the disease.
25634253	CST3	Human	Garg et al[40]	CE-MS analysis	DKD	Cystatin C (CST3) is a low-molecular-weight protein produced by nucleated cells in vivo. CST3 can be used to assess renal function. Urinary CST3 indicates renal tubular damage.
22872235	Collagen fragment, α-microglobulin, β2-microglobulin (β2MG), A1AT, uromodulin and other peptides	Human	Zürbig et al[41]	iTRAQ labeling, Nano-LC-MS/MS, Label-Free Quantitative Proteomics, ELISA	DKD	Collagen fragments, α-microglobulin, β2-microglobulin (β2MG), A1AT, uromodulin and other peptides have been shown to be promising protein biomarkers.
28396115	TNFR1, TNFR2	Human	Yamanouchi et al[50]	LC-MS	T2D, T1D	The median serum concentrations of TNFR1 and TNFR2 at baseline were almost twice that of T1D patients with normal proteinuria and normal renal function.
28851702	apoA4, CD5Land IBP3	Human	Peters et al[52]	Mass spectrometry	T2D, T1D	apoA4, CD5L, and C1QB independently predicted rapid decline (odds ratio 2.40 [95% CI 1.24–4.61], 0.52 [0.29–0.93], and 2.41 [1.14–5.11], respectively) and improved model performance and fit in 35 participants (P < 0.001).
23780945	AGEs, OPs	Human	Beisswenger et al[54]	LC-MS/MS	DKD	Blood samples from 103 normal albuminuria subjects demonstrated that the increase of advanced glycation end products (AGEs) and oxidation products (OPs) was considered to lead to the progress of DKD.

prostaglandin D2 synthase (L-PGDS), is reportedly an independent and reliable serum protein biomarker that responds to eGFR levels more reliably than do anhydride or cystatin.[46] Lu et al employed Q-Exactive mass spectrometry and proteomic iTRAQ techniques which ultimately revealed that DKD patient serum exhibited higher concentrations of gelatin, collectin-11, protein tyrosine phosphatase type J receptor (PTPRJ), and protein kinase anchoring protein-7 (AKAP-7), supporting their possible diagnostic value.[43] The circulating glycoprotein Hp is primarily produced in the liver, and plasma Hp levels are significantly higher in individuals with DKD. Plasma gelatin protein (PGSN) is primarily produced by skeletal muscle, smooth muscle, and the myocardium. Piktel et al determined that PGSN can promote PKC activation, thereby contributing to renal reactive oxygen species (ROS) production, inflammation, and DKD development.[47] DKD patients exhibit higher PGSN levels than do controls, emphasizing the potential value of this protein as a diagnostic biomarker in this pathological context.[48] Yang et al reported that serum levels of the inflammatory cytokine tumor necrosis factor-α (TNFα) are significantly correlated with early glomerular dysfunction and early-stage DKD.[49] Yamanouchi et al discovered that 221 individuals with type 2 diabetes and 279 patients with type 1 diabetes had higher serum levels of the tumor necrosis factor receptors (TNFR) 1 and 2. End-stage renal disease(ESRD), T2D, and T1D were all highly correlated with TNFR. According to the study, this indicator had an 85% predictive value and a 72% sensitivity.[50,51] In Phase II of the diabetes study, Peters et al used mass spectroscopy on 35 community patients. They discovered that the participants' plasma levels of apolipoprotein (apo) A-IV (apoA4), CD5 antigen like (CD5L), and insulin-like growth factor binding protein 3 (IBP3) independently predicted rapid decline (odds ratio 2.40 [95% CI 1.24–4.61], 0.52 [0.29–0.93], and 2.41 [1.14–5 (P < 0.001). New plasma biomarkers (apoA4, CD5L, and IBP3) have been discovered by recent study that may help predict the rapid loss in renal function in type 2 diabetic patients. Among these, the increase in ApoA4 level is a precursor to mild to moderate DKD, the increase in IBP3 level is connected to low baseline EGFR, and the inhibitor of apoptosis of CD5L or macrophage protein is related to immunological and inflammatory response.[52,53] Beisswenger et al performed an LC-MS/MS investigation on blood samples from 103 people with normal albuminuria diabetes and discovered that the progression of DKD was thought to be accompanied by an increase in advanced glycation end products (AGEs) and oxidation products (OPs).[54] El-Asrar et al found soluble CD40 ligand (CD40L) to represent a promising plasma protein biomarker associated with progressive DKD that can participate in endothelial injury and inflammatory activity.[55] In a separate analysis, Kobayashi et al detected an independent association between the LAYN, DLL1, MAPK11, MATN2, Endostatin, and ROR1 proteins and the risk of chronic renal failure owing to their role in the progression of DKD, emphasizing their promise as targets for renoprotective therapeutic interventions.[27] Niewczas et al additionally determined that plasma TNF-R1 and TNF-R2 play roles in renal inflammation and can predict the decline in renal function that is associated with DKD incidence.[56] For further details regarding blood-based protein biomarkers of DKD characterized to date, see Table 2.

Renal Tissue Protein Biomarkers of DKD

Recent advances in proteomic techniques have enabled the routine assessment of proteins in urine, plasma, and kidney tissue samples, allowing for the more in-depth interrogation of the mechanistic basis for DKD progression.[57] Renal protein levels may reflect the actual pathogenesis of tubular atrophy, glomerular injury, and tubulointerstitial fibrosis. To aid in the characterization of renal proteomic profiles, high-throughput matrix-assisted laser desorption/ionization imaging MS has been leveraged to analyze the proteins present in tissue sections.[58] Renal tissue proteomic analyses can detect biomarkers of glomerular injury such as albumin, as well as renal tubular injury-related proteins including α1MG, β2MG, RBP4, N acetyl β D glucosaminidase (NAG), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), liver type fatty acid binding protein (LFABP), NGAL, and renal injury molecule 1 (KIM1).[59] Moresco et al determined that the transmembrane glycoprotein Kim-1 is specifically expressed on proximal tubular cells on the apical membrane, serving as a reliable biomarker of proximal tubule injury that is closely linked to the extent of renal tubulointerstitial injury and fibrosis, offering a tool to diagnose early-stage DKD.[45] Vaidy et al observed elevated NAG and Kim-1 levels in type 1 diabetes patients with microalbuminuria, with these levels being even higher in patients with albuminuria, emphasizing the link between these protein biomarkers and type 1 diabetes-related DKD.[60] The 25 kDa lipocalin family member NGAL is often studied as a sensitive, specific biomarker for renal tubular injury that can predict DKD development.[61] Regucalcin is a mediator of cellular Ca21 homeostasis, oxidative stress regulation, and ascorbic acid biosynthesis. Using a 2D-DGE and MS-based

Table 2 Biomarkers of Blood Proteomics in DKD

PMID	Types (The Main Biomarkers)	Population	Authors	Methods	Disease	Main Findings
34458592	Plasma U/PC3	Human	Araumi et al[35]	Q-Exactive, Thermo Scientific	DKD	Plasma U/PC3 was highest in diabetic nephropathy group, which was significantly higher than that in non-diabetic nephropathy group (P<0.05).
26286913	SH3BGRL3	Human	Chiang CY et al[34]	Q-Exactive, Thermo Scientific	DKD	SH3BGRL3 is a conjugate of epidermal growth factor receptor, and uSH3BGRL3 in DKD patients was significantly positively correlated with estimated glomerular filtration rate (P>0.05).
22975642	MMP-9/neutrophil gelatinase-associated lipocalin, aminopeptidase N, azurocidin and kal-likrein 1	Human	Caseiro et al[44]	LC-MS/MS	Type 1 DKD	Fifteen patients with type 1 DKD had higher MMP-9/neutrophil gelatinase-associated lipoprotein, aminopeptidase N, azurocidin, and kallikrein 1 activities compared with healthy controls.
23485645	NGAL	Human	Moresco et al[45]	LC-MS/MS	DKD	Serum NGAL is positively correlated with proteinuria and negatively correlated with glomerular filtration rate, and is a promising biomarker for the diagnosis of DKD.
26124640	βTP	Human	Bacci et al[46]	LC-MS/MS	Type 1 DKD	β-Trace protein (βTP), a member of the Lipocalin protein family, is a new independent and reliable serum protein marker, a better GFR marker than creatinine and cystatin.
31384238	Gelsolin, collectin-11, PTPRJ, and AKAP-7 proteins	Human	Lu et al[43]	iTRAQ, LC-MS/MS	Type 2 DKD	The contents of gelatin, collectin-11, protein tyrosine phosphatase J-type receptor (PTPRJ) and protein kinase-anchored protein-7 (AKAP-7) were higher in DKD patients.
30149613	PGSN	Human	Piktel et al[47]	iTRAQ, LC-MS/MS	DKD	PGSN content is higher in DKD patients than in controls, so PGSN is also a promising protein diagnostic marker for DKD.
25986995	TNFRs	Human	Yang et al[49]	Surface-enhanced Laser desorption/ionization time-of-flight Massenspektrometrie (SELDI-TOF-MS)	Type 2 DKD	The levels of TNFRs were significantly correlated with early glomerular dysfunction and were protein biomarkers associated with early stages of DKD.
26975878	Orosomucoid	Human	El-Beblawy et al[74]	TMT labeling, LC-MS, ELISA	Type 1 DKD	Elevated Orosomucoid in the blood of DKD patients is an independent risk factor for kidney damage.
20205888	Transthyretin, Apolipoprotein A1, Apolipoprotein C1 and Cystatin C	Human	Overgaard et al[75]	CE-ESI-MS LC-MS, MALDI-MS	Type 1 DKD	Transthyretin, apolipoprotein A1, apolipoprotein C1 and cystatin C are potential plasma protein biomarkers.
22702645	sCD40L	Human	El-Asrar et al[55]	LC-MS	Type 1 DKD	Soluble CD40 ligands involved in inflammatory processes and markers of endothelial damage have emerged as potential markers of progressive DKD.
35618095	LAYN, ESAM, DLL1, MAPK11and endostatin	Human	Kobayashi et al[27]	LC-MS, MALDI-MS	DKD	LAYN, DLL1, MAPK11, MATN2, Endostatin and ROR1 proteins are involved in the progression of DKD disease.
31011203	TNF-R1, TNF-R2, KRIS proteins	Human	Niewczas et al[56]		DKD	Plasma levels of tumor necrosis factor-R1 or tumor necrosis factor-R2 are involved in renal inflammatory responses and are predictors of DKD.

Table 3 Biomarkers of Renal Proteomics in DKD

PMID	Types (The Main Biomarkers)	Population	Authors	Methods	Disease	Main Findings
23485645	KIM 1	Human	Moresco et al[45]	2-DE, CE-ESI-MS	Type 1 DKD	Kim-1 is closely related to the degree of tubulointerstitial injury and fibrosis, which provides the possibility of a new marker for early diagnosis of DKD.
20980978	NAG	Human	Vaidya et al[60]	Spectrophotometrically	Type 1 DKD	NAG and Kim-1 levels were elevated in type 1 diabetic patients with microalbuminuria, and NAG and Kim-1 levels were highest in diabetic patients with proteinuria.
21779943	NGAL	Human	Fu et al[61]	Sandwich ELISA kits	Type 2 DKD	NGAL is a sensitive and specific protein biomarker that reflects renal tubular injury, and has become a sensitive and specific early biomarker for predicting DKD.
26072307	L-FABP Regucalin	Animal.	Zubiri et al[62]	2-dimensional differential gel electrophoresis, mass spectrometry, Matrix Assisted Laser Desorption/Ionization MS	DKD	Differential analysis was performed on the renal tissue proteome of the early DKD rat model, and it was found that the Regucalin content in the renal tissue of DKD rats was significantly reduced compared with the control tissue.
29134286	Metalloproteinase TIMP3	Animal.	Rossi et al[65]	Nano-liquid chromatog-raphy–electrospray ionization–tandem mass spectrometry (nLC–ESI–MSE) analyses	DKD	
25884625	Periostin	Animal.	Satirapoj et al[66]	ELISA	Type 2 DKD	Glomerular periostin levels are elevated in patients with type 2 diabetes before overt albuminuria in diabetic patients.
22172726	Nephronectin, apolipo-protein E	Human	Nakatani et al[68]	QSTAR Elite liquid chromatogram-phy with tandem mass spectrometry, LC–MS/MS and iTRAQ	DKD	Nephronectin and apolipo-protein E play key roles in the development of renal extracellular matrix and are related to the development of diabetic glomerulosclerosis.
27457269	Ribosomal proteins, cytoskeletal proteins	Pig	Malagrino et al[76]	LC-ESI-MS/MS	DKD	Kidney tissue proteins can be used as non-invasive biomarkers for DKD.
21544065	Vanin-1, Ennp6 protein, alpha-1-acid glycoprotein, uromodulin	Rat	Fugmann et al[69]	MALDI-TOF, MALDI-TOF/TOF MS	Type 1 DKD	The up-regulated proteins in DKD were Vanin-1 and cystatin C, while the expression of uromodulin was down-regulated in diabetic rat samples.
28938946	PKM2	Rat	Chun et al[77]	LC–MS/MS	DKD	Pyruvate kinase M2 (PKM2) is one of the highly upregulated proteins in DKD glomeruli.
27356525	(MDR1/P-gp, BCRP/Bcrp and MRPs/Mrps 2 and 3	Human	Fallon et al[78]	NanoLC-MS/MS	DKD	(MDR1/P-gp, BCRP/Bcrpand MRPs/Mrps 2 and 3 are elevated in DKD.
23846697	Heme oxygenase-1 and other AngII-regulated proteins	Mice	Konvalinka et al[79]	LC-MS/MS	DKD	Heme oxygenase-1 and other AngII- regulated proteins represented novel markers of AngII activity.

approach, Zubiri et al compared the proteomic profiles of renal tissue samples from rats with early-stage DKD and observed significant decreases in regucalin levels in tissues from DKD model rats relative to controls.[62,63] The matrix protein TIMP3 (tissue inhibitor of metalloproteinases) can inhibit proteolytic enzyme activity and receptor activation in the ECM. DKD has been shown to be associated with TIMP3 downregulation.[64] Rossi et al employed differential proteomic analyses of renal tissues from 6-week-old wild-type (WT) and Timp3$^{-/-}$ mice using a nano-liquid chromatography-electrospray ionization-tandem mass spectrometry (nLC-ESI-MSE) approach, observing significant TIMP3 downregulation in DKD mice consistent with its possible relevance as a therapeutic target in this disease.[65] The cell adhesion molecule periostin (POSTN) has also been posited to serve as a protein-based marker of diabetes-related renal damage, with elevated glomerular POSTN concentrations in patients with type 2 diabetes even before the onset of significant proteinuria such that it may hold value as a protein marker of early-stage renal injury.[66] Increased glomerular ECM protein deposition is a primary mechanism governing the pathogenesis of DKD, contributing to higher glomerular membrane ECM accumulation that ultimately contributes to glomerular membrane dilatation and glomerulosclerosis.[67] LC-MS/MS and iTRAQ techniques have both been used to compare renal tissues from DKD and nondiabetic patients. Nakatani et al determined that both nephronectin and apolipoprotein E are important mediators of renal extracellular matrix development, while renal connexin is an integrin α8β1 ligand associated with diabetic glomerulosclerosis onset and progression. Further studies of these and other targets in patient renal tissues may aid in further understanding of DKD.[68] Fugmann et al further determined that significant renal Vanin-1 and cystatin C upregulation were evident in their model system, corresponding to proinflammatory and cytoprotective effects, while uromodulin was downregulated in diabetic model rates such that these proteins may offer value as therapeutic targets in DKD.[69] For further details regarding known renal tissue protein biomarkers of DKD, see Table 3.

Conclusions and Outlook

As the studies discussed herein emphasize, there is growing interest in the use of alternative biomarkers other than urinary albumin and creatinine when detecting early-stage DKD, including protein-based biomarkers present in urine, blood, and renal tissue samples. Proteomic technologies offer an increasingly robust means of detecting low-abundance proteins related to the progressive pathogenesis of kidney injury.

Numerous biomarkers discovered thus far have demonstrated strong diagnostic potential, including urine-based CKD273 proteomic biomarkers, which have demonstrated excellent potential. In the clinic, more and more diagnostics are now available, allowing for the independent and accurate prediction of microalbuminuria associated with DKD. These proteomic biomarker analyses are often simpler and less invasive than standard diagnostic techniques, potentially reducing the need for renal biopsy procedures as the primary means of diagnosing DKD. These strategies also offer a theoretical foundation for more reliably treating this disease and preventing its progression. Despite such promise, however, proteomics-based biomarker panels are not routinely applied in most clinical chemistry laboratories. Further research focused on these proteomic biomarkers will guide the early and noninvasive diagnosis of DKD, allow for more reliable patient monitoring, and support better patient outcomes at many levels. We anticipate that further research efforts centered on the role of targeted proteomics in diabetic kidney disease will yield novel breakthroughs in this field with clear clinical value.

Ethics Statement

This review does not involve animal or human trials, therefore it does not require ethical review.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this review.

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