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Review

Association of urinary biomarkers of chronic kidney disease with type 2 diabetes

Farhana Begum [1], Janani Ramesh [2], Karpagavel Lakshmanan [1] *

[1] Department of Biochemistry, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India
[2] Centre for Regenerative Medicine, Simches Research Centre, Massachusetts General Hospital, Massachusetts, Boston, United States of America

* Correspondence to: Karpagavel Lakshmanan, Department of Biochemistry, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India. E-mail: karpag@yahoo.com

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Abstract

This extensive review can assist the researcher in assembling and constricting the study's hypothesis on the molecular mechanism of CKD, which is more prevalent in type 1 (T1D) and type 2 diabetes (T2D) patients. It targets the role of urinary biomarkers and their importance compared to serum markers involved in CKD-associated T2D patients. This review highlights the significance of urinary exosomal RNAs, including expressions of miRNA and ncRNA, involved in the molecular diagnosis of renal injury. Next-generation sequencing and ncRNASeqScan serve as an emerging bioinformatic tool to predict the ncRNAs in urinary exosomes and to pattern transcriptomes extensively. Urinary biomarkers of CKD provide a primary target source in the prognosis of the disease, whereas their levels in serum are significantly high after the progression of CKD. Hence, biomarkers are heterogeneous groups of factors executing several molecular mechanisms involved in various signal transduction pathways occurring on the cell surface.

Keywords: chronic kidney disease, type 2 diabetes, biomarkers, urinary exosomes, miRNA, ncRNA.

Introduction

Chronic kidney disease (CKD) is well-delineated by the progression of hyperglycemia and high blood pressure, which are associated with major complications [1]. This review's main purpose is to elaborate on the importance of key factors, genes, and other components involved in the initiation and progression of CKD associated with T2D. Recent advancements in treating hyperglycemia with pancreas transplantation, insulin therapy, and specific biomarker identification can show beneficial effects on the early stage of CKD [2]. Identifying the protein-based markers and genes associated with the leading cause of disease can be obliging for the prognosis of the disease. Marker proteins in the blood, urine, and tissues can be analyzed to determine the severity and halt the progression. CKD causes a gradual decline in renal function and can lead to toxins deposition in the kidney that results in uremia. Uremic toxins have been recently analyzed based on renal toxicity, which can play an essential role in therapeutic targets against CKD [3]. Epigenetic regulators play a protective role in the CKD population; for instance, studies reported sirtuin 6 (SIRT6) was observed to downregulate in peripheral mononuclear cells and in radial tissues of CKD patients [4]. Certain studies have shown promising results on CKD repair mechanism using regenerative pluripotent embryonic stem cell (iPSC) therapy, macrophages resolving inflammation and remodeling of renal tissue [5]. Perhaps insight findings on the molecular mechanism of CKD are highly necessary for therapeutic interventions.

Insights of CKD in the T2D population

CKD associated with T2D is the initial origin of end-stage renal disease (ESRD) with global dejection up

to 40%. The pathogenesis of CKD with T2D was not well characterized earlier, whereas the targeted hyperlipidemia in renal damage was indicated through the induction and triggering of various signal transduction pathways [6]. CKD is believed to have been acquired due to diabetes-associated nephropathy (DN), which is a crucial cause of ESRD. DN is a severely complicated condition common in both T1D and T2D [7]. DN is distinguished by diligent albuminuria predicted at least twice in 6-month intervals and gradually increasing glomerular filtration rate (GFR) with eloquent blood pressure, which tends to cause ESRD [8]. In 40% of cases with diabetes, DN is developed after 10 years of T2D diagnosis. Few studies reported 50% of the T2D population is globally affected by CKD. Additionally, the prevalence of CKD associated with T2D was predominantly increased in low-to-middle-income countries [9]. Poor glycemic control, detrimental lifestyle, and elevated levels of HbA1c are provoking targets in patients with T2D and its comorbidities. Besides poor lifestyle management, obesity is identified as an individualistic risk factor for the progression of CKD, which is denoted to cause obesity-related glomerulopathy (ORG). Hence, glycemic index, HbA1c levels, obesity, GFR, glomerular hypertrophy, hemodynamic changes, creatinine clearance, and serum creatinine play a major role in early CKD diagnosis, whereas proteinuria indicates ESRD [10, 11].

Biomarkers of CKD

Urine, serum and plasma biomarkers concerning CKD plays important role in prognosis of CKD. Urinary albumin excretion and GFR are quite employed for evaluating CKD. Microalbuminuria has typically been scrutinized as the primary indicator of CKD progression and is frequently analogous with traditional remarkable glomerular destruction. CKD has acquired changes associated with biomarkers, which is accordant with explicit endothelial deterioration. Albuminuria screening can be well destined using the albumin-creatinine ratio (ACR) by collecting a contingent urine spot, where the defined values of ACR are greater than 30 mg/g with elevated levels of urinary albumin. Microalbuminuria is accompanied by inclined HbA1c levels, dyslipidemia, hypertension, T2D, and at early age diagnosis. Traditionally, microalbuminuria was examined as a predictor on behalf of indication of renal pathology. It actually served as the potent marker for CKD diagnosis, which is generally associated with the remarkable initiation of glomerular disruption. Therefore, it was originally believed to be the most prevalent and perceptive dislocation in CKD [12].

Urinary non-albumin protein (uNAP)

Urinary NAP may contribute beneficial characteristics and prognostic information. Urinary NAP consists of microglobulins of α2- and β2-subunits corresponding to tubulointerstitial pathology, and a decreased albumin to total urinary albumin ratio exhibits a secure relationship with renal biopsy of tubulointerstitial dysfunction [13]. Some studies disclose that the total protein-creatinine ratio is the most delicate bioindicator for the identification of albuminuria in CKD. There are other findings illustrating the clinical manifestation of uNAP extent for low GFR proportion in T2D. The increased uNAP makes obsolete the predicted report of tubular albuminuria, and it is crucially correlated with enormous bioindicators of tubular destruction. Most of the earlier findings did not contemplate total albumin levels in their assays and further defined that uNAP is a predominant indicator of kidney dysfunction compared to other urinary biomarkers. The statistical analysis by multivariate regression has revealed the association of non-albumin – creatinine ratio with all the markers in spite of perplexing factors adjustment. Additionally, it was reported that there is no significant association of nAPCR with transferrin to creatinine ratio, even behind the modification of perplexing facets of age, gender, disease onset, and blood pressure. Preceding studies imply that, amidst all the markers of tubular damage, non-albumin – creatinine ratio is extremely pertinent for the assessment of initiation and advancement of kidney disorders [14].

Urinary monocyte chemo-attractant protein-1 (uMCP-1) and kidney injury marker-1 (KIM-1)

Urinary monocyte chemo-attractant protein-1 is the influencing factor undergoing chemotaxis for WBC-engaged renal tubular injury. It is detained to be up-regulating factor in inflammatory renal injury and CKD associated with T2D. Other significant elements correlate with the up-regulation of uMCP-1, such as an increase in glycemic index, tubular proteins, advanced glycation end products (AGEs), and angiotensin II (AT-II). KIM-1 is a membranous protein mainly exhibited in the proximal tubular epithelium and sustains solidity following detachment of ectodomain

and delivers into the lumen of tubules, which results in urine detection. Urinary KIM-1 (uKIM-1) is considered a specific and delicate marker for proximal-tubular injury. It is up-regulated in proximal tubules during different conditions and is related to the tubulointerstitial damage and fibrosis range. Additionally, uKIM-1 is increased in T2D patients, especially in those with microalbuminuria, with sympathetic levels of insulin inversely associated with uKIM-1 concentrations. It is significantly manifested that levels of KIM-1 and MCP-1 are modified in CKD, the extent to which these markers are modified in T2D patients coinciding with both proteinuric and non-proteinuric categorization. Moreover, few studies have demonstrated the correlation between levels of KIM-1 and MCP-1 with threat factors related to CKD. Hence, MCP-1/Cr level was seriously associated with HbA1c, inflammatory markers, and diversion extent. The existence of KIM-1 in urine is a novel biomarker for kidney injury and an effective biomarker for proximal tubular injury in the diagnosis of CKD. Various mechanisms are involved in the up-regulation of uKIM-1 levels in proximal tubular cells; this also includes stimulation of the same by a tubule toxic ultra filtrate followed by excess protein stock. This change results in the induced production of KIM-1 in urine with the association of chronic tubular damage [15].

Urinary advanced glycation end-products (uAGEs)

Chronic hyperglycemia is a significant cause of diabetes-associated microvascular diseases, and that can rapidly lead to atherosclerosis. Studies have found that hyperglycemia can progress to long-term complications in people with diabetes, and this is because of the occurrence of glycation (a process where sugar molecules attach to proteins) reaction. Glucose-enhanced protein-linked moieties have led to the emergence of AGEs. Recent research has shown that apart from glucose as the main precursor to AGEs, there are many other metabolites that play a vital role in this process. Aldehydes are also one of the key components affiliated with the synthesis of AGEs. The intermediates are produced through the energy generation phase (methylglyoxal) or along the process of polyol synthesis. Studies have shown that over time, the body accumulates age-related damage in many different parts of the body, which can be the effect of long-term complications. CKD is a condition in which deposits of material called matrix build up in the glomerulus, which can be the supreme cause of sclerosis in the glomeruli. The aggregation of collagen-related proteins in the glomerular extravascular matrix can cause progressive capillary occlusion. Microscopic examinations depict that AGEs accumulated in both the glomeruli and the tubules in CKD. Another study found that the ligand-receptor synergy might be significant in the development of CKD. Over time, the kidneys become impaired and may not be able to filter blood effectively. This can lead to increased levels of blood sugar and the formation of albumin in the urine. Before the induction of proteinuria, the kidneys may become oppressed, and their ability to filter the impurities may increase. This can allow large amounts of proteins and other molecules to flow through the kidneys. People with normal renal function have different rates of aging than people who have renal failure. There is very little research on urinary AGE levels in people with diabetes, and the results are still inconclusive. Some studies revealed the level of AGEs in people with distinct phases of albuminuria and compared it with disparately important renal specifications. Other studies found that the clearance measure of creatinine was the best speculator of AGE evacuation, so measuring urinary AGEs would only provide limited information in patients with renal function impairment [16].

The AGEs, produced from enzyme-independent glycation reaction with the inception of hyperglycemia, gathered beneath the glomerular membrane, which advances the succession of CKD. Spontaneously, it is contrary that the extended AGE accumulation in the vasculum and the correlation between RAGE and AGEs may enhance the filtration of inflammatory cells and the release of proinflammatory molecules, like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), IL-6, tumor necrosis factor – α (TNF-α), MCP-1 [17].

Transforming growth factor-beta 1 (TGF-β1)

TGF-β1 is a versatile cytokine that mediates to emulate a crucial role in fibrosis. Parenchymal and inflammatory cells release TGF-β1, which leads to the assembly of extracellular matrix proteins and tubular epithelial cell differentiation into myofibroblasts. Inter-relationship between urinary levels of TGF-β1 (uTGF-β1) and renal parenchymal TGF-β1 and the extent of interstitial fibrosis in humans have been reported [18]. In CKD-associated T2D, the over-expression of TGF-β1 exists in high glucose concentrations of mesangial and proximal

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tubular cells of the kidney. Other studies have found the differential association of higher levels of urinary TGF-β1 creatinine (uTGF-β1Cr) in HIV infection-associated CKD patients with HIV-infected CKD-negative patients, where adjustment of other confounding factors is controlled. This has reported the remarkable positive correlation between uTGF-β1Cr and urinary protein-creatinine ratio (uPCR). Additionally, uPCR-dependent levels of uTGF-β1Cr have shown progressive decline across stages 1 to 5 of CKD in HIV-infected CKD-positive patients. Some researchers have predicted the role of interstitial density measurement for TGF-β1 in HIV-associated critically ill CKD patients [19].

Tumor necrosis factor – alpha (TNF-α)

TNF-α is generally a pleiotropic cytokine with receptors such as TNFR1 and TNFR2, belonging to the members of TNFR superfamily. These receptors play a significant role as mediators of chronic inflammation. The enhanced development of glomerulosclerosis and tubulointerstitial fibrosis is provoked by the assembly of cytokines and expression of adhesion molecules in the kidney triggered by signal transduction pathways and transcription factors. There are few studies evaluating the progression of T2D-associated CKD due to the activation of TNF-α in chronic inflammatory reactions occurring in the kidney. CKD-associated urinary biomarker investigations have revealed the extent of albumin-creatinine ratio (ACR) and the extremity of impaired renal function was crucially inter-related with urinary TNF-α (uTNF-α), which was comparatively not with serum TNF-α levels. Therefore, many available literature intended the significance of activated TNF-α signaling pathway in T2D-associated CKD and reported its prognostic value for ESRD [20].

Neutrophil gelatinase-associated lipocalin (NGAL)

Urinary NGAL can constitute congenital biomarkers of normoalbuminuric renal inadequacy in T2D. Additionally, its level was increased in patients with renal inadequacy and significantly correlated to eGFR in T2D patients. In such patients, changes in interstitial or vascular tissues were more often observed than the structural change of renal organization, which indicates tubulointerstitial damage involved in the microalbuminuria condition of CKD. This low molecular weight tubular damage biomarker reduces reabsorption and thus elevates urinary excretion in T2D patients. Some studies have depicted the elevated NGAL expression in T1D patients before the prognosis of microalbuminuria and rose gradually from uACR <10 mg/g to 10–30 mg/g to >30mg/g in T2D patients. This can be due to the progressive cause of inflammation and oxidative stress, which leads to tubular damage. Hence, tubulointerstitial damage is predominant in older age, and there are corresponding studies suggesting elevated NGAL with declined renal function and a significant correlation with eGFR in most elderly individuals [21].

Urinary exosomes, miRNAs, ncRNAs

Exocytosis-mediated exosomal cells are those enclosed by a double-walled membrane transmitting a wide range of molecules involved in receptor-ligand interactions via endocytosis. This signal transmission mechanism still needs to be studied to understand the role of exosomes in renal diseases. Some researchers have demonstrated that the role of exosomes originating from tubular cells induces *in vitro* activation of fibroblasts, which were exterminated through siege of exosome biogenesis. Similarly, exosome preventive obstruction of renal fibrosis indicates that exosomes act as distinctive and potent carriers for signal transduction between tubular and fibroblast cells [22].

Urinary exosomes secreted from all segments of nephrons mostly carry protein, mRNA, and miRNA markers of renal injury. The challenging tool known as urinary-exosomal miRNA analysis can discover the molecular biomarkers of CKD. Recent studies have shown that urinary-exosomal miRNA profiles for CKD are analyzed based on the mixed population of isolated DN, non-diabetic renal disease, and combined nephropathies. With this regard, the miRNA sorted were miRNA-192, miRNA-193a, miRNA-362-3p, miRNA-877-3p, miRNA-150-5p, and miRNA-15a-5p, which are informative but play an insignificant role in the pathogenesis of CKD. Another study investigated the miRNA signature in nephrotic and isolated DN groups, and a significant set of miRNAs was found. Hence, they have concluded that miRNA-188-5p, miRNA-150-3p, miRNA-760, miRNA-3677-3p, miRNA-548ah-3p, miRNA-548p, miRNA-320e, and miRNA-23c have shown significant up-regulation and down-regulation of miRNA-133a-3p and miRNA-153-3p in nephrotic DN patients. Therefore, further investigations on the functional aspects of newly identified target genes of each

miRNA are necessary in the pathogenesis of T2D-associated CKD [23].

Abundant studies on ncRNAs in urinary exosomes have proven to be a novel biomarker in CKD diagnosis, reflected by next-generation sequencing. A plethora of studies have depicted that the proximal tubules are the major source of targeted kidney injury and progression of the same. Novel ncRNAs are identified based on their contigs and secondary structure similarities using bioinformatics tools such as ncRNASeqScan, which authorize the complete pattern of comprehensive transcriptomes. Using ncRNASeqScan, 30 ncRNAs consisting of miRNAs, tRNA fragments, antisense RNAs, and mt-tRNAs, being distinctively enormous in exosomes of early stages of CKD compared to healthy individuals, have been identified. Hence, future studies can focus on miRNA-181a and identify ncRNAs as biomarkers for early CKD detection. To this end, this analysis has depicted the application of the qPCR panel, confirming the identification of significantly expressed urinary exosomal RNAs to a huge patient cohort for CKD-applied biomarkers authentication [24].

Conclusion

In conclusion, there are auspicious biomarkers diversified based on the pathophysiology of renal injury, such as microalbuminuria for glomerular dysfunction, KIM-1, NGAL and others that are responsible for tubulointerstitial disturbances. In contrast, significant levels of urinary exosomal miRNA and ncRNA are procured by proximal tubules in the progression of renal injury. Therefore, urinary biomarkers show highly significant and challenging results at the early stages of T2D-associated CKD, whereas their levels in serum are detected mostly during ESRD.

Conflict of interest

The authors declare no conflict of interest.

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