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Biomarkers of Diabetic Nephropathy Progression

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Abstract

Diabetic nephropathy is a leading cause of morbidity and mortality and leads to an end stage renal disease (ESRD). Standard biomarkers including serum creatinine, estimated glomerular filtration rate, and albuminuria do not directly measure renal tissue injury and they are relatively insensitive to small changes in renal function. Therefore, research focuses on discovering and validating additional biomarkers that improve risk stratification for future renal function decline and end-stage renal disease in patients with diabetes, along with already established biomarkers. In view of this, the utility of urinary biomarkers reported in the literature is discussed in this brief review.

Keywords: diabetic nephropathy, end stage renal disease, biomarkers.

1. Introduction

In 2010, worldwide adult population with diabetes mellitus was estimated to be about 285 millions, and by 2030 it is predicted to have an increase by 54 % totaling to about 439 millions (Tramonti, 2013). Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease (ESRD) in developed countries and is becoming more prevalent globally due to the rise in the incidence of obesity and type 2 diabetes (International Diabetes Federation, 2017). Diabetic nephropathy develops along with generalized microvascular disease, most often concomitant with macrovascular disease including cardiovascular, cerebrovascular, and peripheral arterial disease (Satirapoj, 2014; Satirapoj, 2015). Patients with DN have a higher risk of mortality, (mostly cardiovascular complications) than diabetic patients without nephropathy (Afkarian, 2013). DN is a progressive kidney disease caused by alterations in the glomerular capillary and tubular structure and function induced by the disturbed glucose homeostasis (Berkman, 1973). It affects all renal cellular elements: glomerular endothelia, mesangial cells, podocytes and tubular epithelia. Glomerular damage results in proteinuria, due to both increased permeability of plasma proteins, such as albumin and transferrin, that are normally not freely filtered through the glomerulus and increased synthesis of extracellular matrix (ECM) proteins (Tisher, 1976). DN severity is accessed by measuring urine albumin levels (albumin-to –creatinine ratio). Persistent microalbuminuria

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(between 30-300 mg/24hr) or macroalbuminuria (levels >300mg /24hr) is considered a marker and predictor of DN and its progression to ESRD (Zdler, 2003; Palmer, 2007). However, due to the inability of microalbuminuria to adequately predict diabetic kidney disease, especially in young patients or in non-albuminuric diabetic nephropathy, additional biomarkers of glomerular and/or tubular injury have been proposed to identify early renal dysfunction and structural lesions, even before microalbuminuria occurs.

The aim of this article is to update, through review of the relevant medical literature, the most promising biomarkers for early DKD detection. In recent years there has been an active growing interest in alternative biomarkers that might provide a more sensitive and rapid mean of detecting progression of diabetic nephropathy.

2. Results

Current biomarkers

In clinical practice, most commonly used markers of renal disease and progression of DN include serum creatinine, estimated glomerural filtration rate (eGFR), blood urea and proteinuria, or albuminuria. GFR measures the rate at which the glomeruli filter the plasma and remove waste products from it. If the kidney is injured the GFR gradually declines and the glomerular function can be estimated by measuring the GFR. GFR estimation is still largely creatinine based. It is the best index available to assess kidney function; yet, GFR reflects late functional changes in the kidney (Currie, 2014).

Clinically, microalbuminuria is considered the earliest manifestation for the onset of diabetic nephropathy (Mogensen, 1985). However, a large proportion of renal impairment occurs in a nonalbuminuric state or before the onset of microalbuminuria. Several studies have shown that diabetic patients can still develop DN without any change in their urinary albumin levels. Recent studies have raised growing concerns about the value of microalbuminuria as a very predictable marker of progression to ESRD. These data suggest that microalbuminuria may represent an initial reversible phase of kidney damage rather than the inevitability of progression to ESRD (Perkins, 2010). In patients with type 1 diabetes and new onset microalbuminuria the development of advanced chronic kidney disease may not display progressive proteinuria (Perkins, 2007).

Creatinine has been found to be a fairly reliable indicator of kidney function because a high creatinine level in the blood is associated with poor clearance of creatinine by the kidneys. The use of serum creatinine as an indirect filtration marker is limited by its biological variability because several factors influence serum creatinine level other than renal factors, including age, race, gender, pregnancy, muscle mass, drug metabolism, protein intake, hydration medications(corticosteroids),drugs. These intraindividual variabilities compromise the generalizability of the eGFR equations.

The next section links important aspects of DN pathogenesis including the processes of oxidative stress, tubular damage, and renal inflammation with some of the promising new biomarkers in serum and urine.

Oxidative stress markers

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. A significant correlation between the content of 8-oxo-7, 8-dihydro-2 ' -deoxyguanosine (8-OHdG), a product of oxidative DNA damage, in urine or leucocytes and the severity of diabetic nephropathy and retinopathy has been reported (Hinokio, 2002). Several studies showed that urinary 8-OHdG is increased in the urine of diabetic patients with nephropathy and tends to increase with the severity of the glomerular lesions. Naito et al demonstrated that the urinary albumin levels increased in diabetic mice in parallel with the increase in urinary 8-OHdG levels (Naito, 2004). Because the increased oxidative stress has a primary role in the pathogenesis of DN the 8-OHdG in urine may be a useful clinical biomarker to predict the development and progression of DN in diabetic patients.

Pentosidine is one of the best chemically characterized AGE compounds. The intracellular formation of advanced glycation end products (AGEs) which accumulate in the kidney and are excreted in the urine, is another pathogenetic aspect of diabetes. In patients with both type 1 and 2 diabetes, a significant association between the degree of albuminuria and urinary AGE-modified

proteins was found (Coughlan, 2011). Plasma pentosidine level was significantly influenced by the quality of glycemic control and renal function (Sugiyama, 1988).

There is evidence that uric acid (UA) is involved in varius stages of DN onset and progression. There is a paradox concerning UA function. Studies have shown that UA is one of the major antioxidants of the plasma. On the other hand, once UA enters the cell, it can induce oxidative stress, endothelial dysfunction and cytokine activation. An elevated serum UA level predicts the development of DN. Randomized controlled trials have demonstrated that chronic kidney disease progression can be decreased by lowering serum UA levels in diabetic patients (Jalal, 2013). Uric acid is a potential target for therapeutic intervention in diabetes.

Biomarkers of tubular/glomerular damage

Recently, certain biomarkers which were initially identified in acute kidney injury (AKI) also have been reported to confer value in evaluating patients with CKD. Biomarkers such as cystatin C, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), angiotensinogen, periostin and monocyte chemoattractant protein-1 (MCP-1) reflect tubular injury.

Cystatin C

Cystatin C is a low-molecular weight protease inhibitor, produced by all nucleated cells in the body and is reabsorbed and catabolized by the proximal tubule. A prospective observational study showed that urine cystatin is an independent predictor of CKD progression in type 2 diabetes (Kim, 2013). Elevated cystatin C is a better early predictor compared with serum creatinine-based formulae. Numerous studies have validated cystatin C as a marker of renal function. In addition, cystatin C levels not only correlate with progression of nephropathy but also show a more sensitive marker of early DN when eGFR remains>60mL/min.

Neutrophil – gelatinase associated lipocalin (NGAL)

Neutrophil –gelatinase associated lipocalin (NGAL) also known as oncogene 24p3 is a secreted glycoprotein. It is a member of lipocalin family of proteins that transport small hydrophobic ligands. NGAL is expressed in renal tubular epithelium and a rise in urinary concentrations may provide an indication of acute renal injury. It is a prognostic biomarker in numerous diseases such as malignant neoplasms and provides protection against bacterial infection (Bolignano, 2008). NGAL is also considered to be a sensitive and more accurate early predictor of acute renal damage before the rise in serum creatinine concentration. Higher urinary NGAL has been associated with the decline in GFR in type 2 diabetic patients with micro- or macroalbuminuria (Bolignano, 2008; Nauta, 2011). Models of acute kidney injury (AKI) to chronic kidney disease (CKD) transition have implicated its role as a potential biomarker of chronically injured kidney. In a related study carried out among patients with diabetic nephropathy, elevated urine NGAL level was reported to be associated with the progressive course of the disease leading to ESRD (Yang, 2009).

Kidney Injury Molecule-1 (KIM-1)

KIM-1 is a membrane protein expressed on the apical membrane of renal proximal tubule cells and reflects tubular damage in the most advanced stages of the renal disease in diabetic patients (Han, 2002).This biomarker is undetected when the kidneys are normal. Therefore, KIM-1 is considered a potential novel urinary biomarker in the early detection of AKI. From a cross-sectional descriptive study, urine KIM-1 increased in type 2 diabetes mellitus with normoalbuminuria and midly increased albuminuria. Serum and urine KIM-1 predicted the rapid decline of GFR (de Carvahlo, 2016; Nielsen, 2012). In a kidney biopsy study of 74 patients with CKD having various etiologies, KIM-1 was primarly expressed at the luminal side of dedifferentiated proximal tubules in areas with fibrosis and iflamation (van TImmeren, 2007). This ectodomain protein segment has been suggested to be a quantitative marker of AKI.

Liver-fatty acid-binding protein (L-FABP)

Liver-fatty acid-binding protein (L-FABP) is expressed in the cytoplasm of human renal proximal tubules. Renal L-FABP expression is up-regulated and urinary excretion of renal L-FABP is increased by various stressors, such as urinary protein, hyperglycemia, tubular ischemia, toxins and salt-sensitive hypertension, which lead to the progression of kidney disease. Urinary L-FABP

levels reflect the degree of tubulointerstitial damage and are strongly correlated with the prognosis of CKD patients in clinical studies. In patients with type 1 or type 2 diabetes, urinary L-FABP levels seem to be higher in patients with normal levels of urinary albumin than in those with microalbuminuria. Urinary L-FABP may be useful for the early detection of diabetic nephropathy (Kamijo,2004; Kamijo, 2011).

Angiotensinogen

Renal angiotensinogen is formed primarly in proximal tubular cells and is secreted in tubular fluid. The intrarenal angiotensin aldosterone system (RAS) was recently proposed to be involved in the progression of renal injury in models of hypertension and in kidney diseases (Suzaki, 2007). Recent studies revealed that high urinary level of angiotensinogen/creatinine was associated with lower eGFR and hypertension (Hayne, 2015). Angiotensinogen might be useful as an early biomarker of the activation of the RAAS in diabetic nephropathy.

Pigment Epithelium-Derived Factor (PEDF), Fibroblast Growth Factor 21 (FGF-21)

FGFs are multifunctional proteins with a wide variety of effects. Today FGFs are classified as intracrine, paracrine and endocrine FGFs by their action mechanisms (Itoh, 2015). Endocrine FGFs comprise FGF-19, FGF-21 and FGF-23. FGF-21 is a hepatoadipokine with pleiotropic metabolic regulatory actions.

Pigment epithelium-derived factor (PEDF) and fibroblast growth factor 21 (FGF-21) are two potential biomarkers of progression in diabetic nephropathy (Hui, 2014). PEDF is a secreted circulating glycoprotein with anti-oxidative, anti-inflamatory and anti-angiogenic properties, whereas FGF-21 is a hormone predominantly secreted from the liver and possess multiple metabolic regulatory properties. In subgroups of diabetic patients with relatively well-preserved kidney function, with an eGFR>60 mL/min/1,73m2 and normoalbuminuria, serum PEDF and FGF-21 levels were idependently associated with the progression to micro- or macroalbuminuria and eGFR decline respectively, even after adjusted for baseline eGFR levels. The elevation in both serum PEDF and FGF-21 levels reflect on the severity of the underlying renal inflammation and injury in type 2 diabetes which would contribute to the development and progression of diabetic nephropathy (Lee, 2015).

Fibroblast Growth Factor 23 (FGF-23)

Several tissues express FGF-23, such as bone tissue, bone marrow vessels ventrolateral thalamic nucleus, thymus and lymph nodes[35]. Its principal target is kidney, where it regulates phosphate reabsorption and production of 1,25(OH)2D3 (Feldman, 2003). Data from Chronic Renal Insufficiency Cohort (CRIC) study suggested that FGF-23 is superior to existing markers as a sensitive screening test to identify which patients are developing disordered mineral metabolism in early CKD (Zhang, 2011). Several other studies identified FGF-23 as a risk factor for CKD progression. Even though there was however no association between FGF-23 levels and the severity of AKI, in some AKI models FGF-23 rised more quickly than phosphate levels or NGAL (Zhang, 2011; Christov, 2013).

Biomarkers of renal inflammation

TNF-a is an important cytokine produced under high glucose conditions by macrophages, renal tubular cells and glomerular mesangial cells. Apart from being a major participant in promoting inflammation, TNF-a is known to induce apoptosis and accumulation of ECM in glomerular and tubular regions leading to alteration of glomerular filtration, tubular permeability and reabsorption (Donate-Correa,2015; Navarro, 2006). Clinical studies have reported higher serum and urinary levels of TNF-a in diabetic patients with renal dysfunction, which further increase with progression of the disease (Navarro, 2006). In terms of excretion of inflammatory cytokines, urinary concentrations of 27 cytokines in type 2 diabetic patients with normo- and micro-albuminuria have been evaluated. The inflammatory cytokines act as pleiotropic polypeptides that regulate inflammatory and immune responses, thus providing important signals in varius pathologic and physiologic processes, including diabetic nephropathy (Chen, 2008). Urinary levels of IL-6, IL-8, IP-10, MCP-1, G-CSF, EOTAXIN, RANTES in microalbuminuric patients were significantly increased compared to those in normo-albuminuric patients or controls.

Monocyte Chemoattractant Protein-1 (MCP-1)

MCP-1 is a cytokine and as a member of the CC chemokine family is a major factor influencing macrophage accumulation in animal and human models with renal impairment (Segere, 2000). MCP-1 is upregulated and expressed in the diabetic glomerular and renal tubular epithelium and a rise in urinary MCP-1 levels correlates with the extent of interstitial inflammatory infiltrate. A number of studies indicated that urinary detection of MCP-1 is a reliable early marker of DN over other conventional markers.

3. Conclusion

Diabetic nephropathy is the leading cause of CKD. Thus, estimation of renal functions is a crucial task in the management of patients with diabetes.

Over the past few years, a better understanding of DN pathogenesis has revolutionized and improved the approaches used for treating the patients with diabetes and its associated renal complications. The identification of biomarkers of early stages of DN, and progression toward ESRD, is of critical importance. In this review, we have summarized the novel biomarkers, based on the pathogenesis of the DN and presented a list of several putative prognostic biomarkers.

4. Conflict of interest

None.

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