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Tubular Biomarkers as Diagnostic Tools in Diabetic Kidney Disease: A Review of Published Evidence

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Abstract

Microalbuminuria predicts the onset and progression of diabetic nephropathy. Despite its use as the conventional glomerular biomarker for early detection of diabetic kidney disease, its predictive accuracy is not optimal because of some disadvantages. Since tubular injury occurs early in the course of diabetic nephropathy, tubular biomarkers should be more sensitive than microalbuminuria as early predictors of the disease. The present review aims to discuss the tubular biomarkers currently used as diagnostic tools in diabetic nephropathy. Using a combination of terms such as 'diabetic nephropathy and pathogenesis', 'biomarkers and diabetic nephropathy', 'tubular biomarkers and diabetic nephropathy' and 'diabetic nephropathy risk and predictors', the Pubmed data base was searched for articles which met the objective of the review.

Tubular biomarkers reported as predictors of diabetic kidney disease consist of cystatin C, kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, alpha 1-microglobulin, N-acetyl-β-D-glucosaminidase, and liver-type fatty-acid binding protein. Several studies show that these markers are not only more sensitive, but are much earlier predictors of diabetic nephropathy than microalbuminuria. Although their advantages over microalbuminuria are evidence-based, majority still need to be validated for diagnostic purposes.

Keywords: Tubular Biomarkers; Diabetes Mellitus; Diabetic Nephropathy; Microalbuminuria; Diagnosis

Introduction

Insulin-dependent diabetes mellitus (IDDM) occurs more frequently than non-insulin-dependent diabetes mellitus (NIDDM) in childhood: the latter being the predominant form of the disease in adulthood [1]. However, there has been a global rise in reported cases of NIDDM among children as well [2]. One of the end-organ micro vascular complications of both types of diabetes is diabetic nephropathy: which is characterized by three main features namely, macro albuminuria observed twice at an interval of three to six months, raised blood pressure and an on-going drop in the glomerular filtration rate (GFR) [3]. It takes about ten to twenty years for the disease to fully develop from microalbuminuria and terminate in stage 5 chronic kidney disease (CKD) [1]. This probably explains why overt diabetic nephropathy is rare in children [4], even though its occurrence among them has also been reported in medical literature [5-7].

In IDDM and NIDDM, microalbuminuria potentially predicts the development of diabetic nephropathy, as well as future risk of cardiovascular disease [8]. Despite its use as the conventional biomarker for early prediction of diabetic kidney disease, its predictive accuracy is not optimal because of some disadvantages. Firstly, not all macroalbuminuric diabetic patients will end up with end-stage renal disease [9]. Besides, 30% of them may actually present with normoalbuminuria (urine albumin excretion of < 30 mg/day) [10], while several glomerular and tubular biomarkers do appear in the urine before microalbuminuria: which means that the latter only occurs when there is significant renal injury [11].Furthermore, recent evidence shows that many macroalbuminuric diabetic patients can become normoalbuminuric even with a decline in GFR (the concept of 'non-albuminuric' diabetic nephropathy) [12].

Consequently, glomerular or tubular biomarkers are presently accepted as dependable diagnostic tools for diabetic nephropathy. Given that tubular damage occurs early in the trajectory of diabetic nephropathy, tubular biomarkers should be more sensitive than glomerular biomarkers as early predictors of disease [13]. Although most of these biomarkers

still need to be validated for routine clinical use, reports however indicate their usefulness in disease evaluation [14,15].

The present review aims to discuss the tubular biomarkers currently used as diagnostic tools in diabetic nephropathy. Using a combination of terms such as 'diabetic nephropathy and pathogenesis', 'biomarkers and diabetic nephropathy', 'tubular biomarkers' and 'diabetic nephropathy' and 'diabetic nephropathy risk and predictors', the Pubmed data base was searched for articles which met the objective of the review.

Diabetic nephropathy: Pathophysiologic pathways

Several pathways are activated in IDDM and NIDDM. Each of these pathways singly or holistically regulates the evolution of diabetic nephropathy [16]. In a sequence of complex molecular events, these pathways interact resulting in the main components of diabetic nephropathy, namely fibrotic changes in the kidney; mesangial and glomerular enlargement; oxidative stress; as well as inflammation of the tubules [13]. It is believed that the disease actually occurs following a synergistic influence between metabolic and hemodynamic factors, which activate mutual pathways leading to kidney injury [17]. During the course of diabetic nephropathy, hyperglycaemia-induced functional impairment of the kidneys and remodeling of the renal architecture, are related to several evolving intracellular reactions and activated signaling pathways [18]. Three main pathways characterized by derangement of intracellular metabolism include the activated polyol and protein kinase C pathways; the elaboration of advanced glycation end-products (AGEs), which represents a glomerular biomarker; and hypertension within the glomeruli triggered by hyper filtration [19]. At the opposing end of these pathways, hyperglycaemia appears to be the key propelling factor behind the evolution of diabetic nephropathy to end-stage renal disease, whereas in tandem with the pathways, micro inflammation and mesangial enlargement constitute the trajectories for development of diabetic kidney disease [19]. Thus, urine tubular marker-to-creatinine ratio and inflammatory marker-to-creatinine ratio have been shown as early indicators of kidney damage seen in diabetic nephropathy, despite the observation of normoalbuminuria [20].

Biomarkers of diabetic nephropathy: classification

Several markers of diabetic nephropathy have been identified, leading to different methods of classification [13]. They have been categorized based on their source and the major pathogenic events which result in nephropathy: markers of kidney dysfunction, markers of inflammation, and markers of oxidative stress [11]. Other authors have suggested a classification which placed the biomarkers into three major classes: glomerular markers, tubular markers, and miscellaneous proteins [21]. Nevertheless, there is an overlap in these proposed classifications as different categories of biomarkers are interchangeably represented; for instance, some inflammatory markers can be represented as glomerular markers as well.

Tubular biomarkers: predictive role in diabetic nephropathy

As previously mentioned, tubular biomarkers can serve as much earlier predictors of diabetic nephropathy than glomerular biomarkers because tubulo-interstitial lesions are associated with and may actually precede glomerular injury in the disease [22]. Several studies have provided evidence on the predictive role of this category of biomarkers (Table 1).

Neutrophil gelatinase-associated lipocalin (NGAL) or lipocalin-2

It is a universal iron-transporter macromolecule, expressed in the epithelium of the renal tubule which appears in the blood and urine following tubular injury. This biomarker was ab-initio identified as a 25 kDa protein in neutrophilic granules which is released into the circulation in response to bacterial infection; in innate immunity, it is involved in iron sequestration which ultimately interferes with bacterial growth [23]. In normoalbuminuric diabetic patients as well as in the assessment of tubular lesions in diabetic nephropathy, elevated urine NGAL has been demonstrated [24], and has also

Table 1: Tubular biomarkers as predictors of diabetic nephropathy: some study findings

Tubular biomarkers	Study (authors, year)	Predicts diabetic nephropathy	Precedes microalbuminuria	Insulin-dependent diabetes mellitus	Non-insulin-dependent diabetes mellitus
NGAL	-Yürük Yıldırım Z, et al, 2015	Yes	Yes	+	
	- Lacquaniti A, et al, 2013	Yes	Yes	+	
	- Zeng XF, et al, 2017	Yes	Yes		+
A1M	- Shore N, et al, 2010	Yes	Yes		+
	- Hong CY, et al, 2003	Yes	Yes		+
KIM-1	- Petrica L, et al, 2014	Yes	Yes		+
NAG	- Patel DN & Kalia K, 2015	Yes	Yes		+
	- Assal HS, et al, 2013	Yes	Yes		+
	- Ambade V, et al, 2006†	No	No		
	- Jones AP, et al, 1995	Yes	Yes	+	
Cystatin C	- Jeon YK, et al, 2011	Yes	Yes		+
L-FABP	- Nielsen SE, et al, 2010	Yes	Yes	+	
	- Kamijo-Ikemori A, et al, 2011	Yes	Yes		+

NGAL=Neutrophil gelatinase-associated lipocalin, A1M= Alpha 1-microglobulin, KIM-1= Kidney injury molecule-1, NAG= N-acetyl-β-D glucosaminidase, L-FABP= Liver-type fatty acid binding protein

†The authors did not specify the type of diabetes mellitus in their study subjects

been shown to precede microalbuminuria in type 1 diabetes mellitus [25,26]. Furthermore, high values of this biomarker were observed in normoalbuminuric NIDDM patients; the values increased progressively in those patients who had microalbuminuria and macro albuminuria [27]. Another report has also confirmed that urine NGAL predicted the evolution of diabetic nephropathy in NIDDM patients following a prospective study [28]. In fact, a recent consecutive cohort study showed that urine NGAL and cystatin C were elevated prior to microalbuminuria in NIDDM patients [29]. Other group of investigators reported that pediatric patients with IDDM, including subjects with normoalbuminuria, showed elevated urine NGAL: a finding which is thought to confirm a pre-existent tubular damage before overt diabetic nephropathy [30]. Thus, some of these reports not only underscore the predictive accuracy of this biomarker but also its advantage over microalbuminuria as a diagnostic tool.

Alpha 1-microglobulin (A1M)

This glycoprotein biomarker usually undergoes glomerular filtration and proximal tubular reabsorption; thus, tubular lesions interfere with its re-absorptive process leading to its excretion in the urine [13]. First, some researchers have reported that urine alpha 1-microglobulin could be an alternative biomarker for early diagnosis of tubulopathy in diabetic nephropathy [31]. The finding was based on a one-year observational study of adult patients with NIDDM and their normal controls. A relatively inexpensive electrophoretic technique was used to detect this biomarker in the urine samples of the study subjects, making the authors to conclude that alpha 1-microglobulin is a cheap diagnostic tool for diabetic nephropathy [31].

In another report, the authors observed that normoalbuminuric NIDDM patients had elevated urine alpha 1-microglobulin, which again underscores the fact that tubulopathy occurs before glomerulopathy in diabetic nephropathy [32]. It was thus concluded that this tubular biomarker is useful for the early diagnosis of diabetic kidney disease [32].

Kidney injury molecule 1 (KIM-1)

This transmembrane tubular glycoprotein, up-regulated about 50-100 fold in the kidney, is excreted in the urine after an injury to the proximal tubules [33]. It is well recognized as a sensitive biomarker for acute kidney injury (AKI) with a good predictive value [34,35]. Besides, a drug-safety study shows that KIM-1 significantly performed better than serum urea and creatinine in predicting tubular damage in murine models [36]. Interestingly, elevated urine levels of this biomarker are seen in normoalbuminuric a NIDDM patient, which again suggests that a tubulopathy does occur early in diabetic nephropathy [37]. In other words, diabetic patients with microalbuminuria present with more elevated urine KIM-1 levels than their normoalbuminuric counterparts [37].

N-acetyl-β-D-glucosaminidase (NAG)

NAG is an enzyme derived from the lysosomes and found in several human cells including the renal tubules [38]. The large size (>130 kDa) of this biomarker makes glomerular filtration difficult so that its presence in urine is presumed to be of tubular origin. Thus, elevated urine NAG indicates tubular injury, but may also result from increased lysosomal activity without cell injury [33]. Regarding its sensitivity as an early predictor of diabetic nephropathy, there appears to be no unanimity in some study findings [39-42].

Some investigators report that NAG could represent an early and the most sensitive marker of tubulopathy as seen in NIDDM patients [39,40]. Conversely, another study could not demonstrate its utility as an early predictive tool for diabetic nephropathy [41]. However, increased urine NAG has also been reported as a sensitive biomarker which can precede microalbuminuria in IDDM patients [42].

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

L-FABP is primarily seen in the liver where it plays a major part in the linkage, transport and metabolism of long-chain fatty acids; its altered expression has been linked to obesity and insulin resistance [43]. Elevated urine-FABP levels occur in normoalbuminuric IDDM patients, and can predict the onset of microalbuminuria, and progression of microalbuminuria towards macro albuminuria [44]. Furthermore, other authors have observed that normoalbuminuric NIDDM subjects also had elevated urine levels of this biomarker, which was seen as a reliable and early predictor of the onset, and evolution of diabetic nephropathy [45,46].

Cystatin C

This biomarker is a low-molecular-weight protein synthesized by nucleated cells in the body at a constant rate, with glomerular filtration and complete tubular reabsorption and catabolism [47]. Thus, elevated urine cystatin C levels are seen in tubulopathies because of impaired tubular reabsorption: making it a non-specific biomarker of AKI [48].

However, in diabetes and diabetic kidney disease, its excretion (which suggests tubular injury) is increased early in conjunction with NGAL [49]. More importantly, some authors have reported its ability to predict the progression of diabetic nephropathy [50], while its serum and urine levels are also reported as dependable markers for evaluating early nephropathy in NIDDM [51].

Conclusion

Tubular biomarkers generally represent earlier predictors of diabetic nephropathy than microalbuminuria in both IDDM and NIDDM. This is because tubular injury occurs early in diabetic kidney disease. Although their advantages over microalbuminuria are essentially evidence-based, majority still need to be validated for clinical use in disease evaluation.

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