REVIEW

Diabetic Nephropathy:
New Risk Factors and Improvements in Diagnosis

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■ Abstract

Diabetic nephropathy is the leading cause of end-stage renal disease. Patients with diabetic nephropathy have a high cardiovascular risk, comparable to patients with coronary heart disease. Accordingly, identification and management of risk factors for diabetic nephropathy as well as timely diagnosis and prompt management of the condition are of paramount importance for effective treatment. A variety of risk factors promotes the development and progression of diabetic nephropathy, including elevated glucose levels, long duration of diabetes, high blood pressure, obesity, and dyslipidemia. Most of these risk factors are modifiable by antidiabetic, antihypertensive, or lipid-lowering treatment and lifestyle changes. Others such as genetic factors or advanced age cannot be modified. Therefore, the rigorous management of the modifiable risk factors is essential for preventing and delaying the decline in renal function. Early diagnosis of diabetic nephropathy is another essential component in the management of diabetes and its complications such as nephropathy. New markers may allow earlier diagnosis of this common and serious complication, but further studies are needed to clarify their additive predictive value, and to define their cost-benefit ratio. This article reviews the most important risk factors in the development and progression of diabetic nephropathy and summarizes recent developments in the diagnosis of this disease.

Keywords: diabetic nephropathy · albuminuria · glomerular filtration rate · diagnosis · risk factors · diabetes

1. Introduction

The prevalence of type 2 diabetes (T2D) is increasing worldwide as a consequence of the rising number of obese patients [1]. Diabetic nephropathy affects approximately 25% of patients with T2D, and represents the leading cause of end-stage renal disease (ESRD) in high-income countries [2, 3]. Moreover, patients with diabetic nephropathy have very high cardiovascular risk, which is comparable with the cardiovascular risk of patients with coronary heart disease [4, 5]. Treatment with statins, angiotensin converting enzyme (ACE) inhibitors, or angiotensin receptor blockers delays renal disease progression and reduces cardiovascular morbidity in these patients [6-14], whereas antidiabetic treatment prevents diabetic nephropathy and delays the progression of diabetic nephropathy [15-21]. Accordingly, identification and management of risk factors for diabetic nephropathy as well as timely diagnosis and prompt management of the disease are of paramount importance.

In the present review, we discuss the most important risk factors for development and progression of diabetic nephropathy, and we summarize recent developments in the diagnosis of this disease.

Abbreviations:

8-oxodG – 8-oxo-7,8-dihydro-2'-deoxyguanosine
ACE – angiotensin-converting enzyme
ADA – American Diabetes Association
CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration
DCCT/EDIC – Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications
ESRD – end-stage renal disease
GFR – glomerular filtration rate
GWAS - genome-wide association studies
HbA1c – glycosylated hemoglobin
hsCRP - high-sensitivity C-reactive protein
KIM-1 – kidney injury molecule 1
LDL-C – low-density lipoprotein cholesterol
MDRD – Modified Diet in Renal Disease
NAG – N-acetyl-β-D-glucosaminidase
T1D – type 1 diabetes
T2D – type 2 diabetes
TC – total cholesterol
TG - triglyceride
UAE – urinary albumin excretion

2. Risk factors for diabetic nephropathy (Table 1)

2.1 Increased urinary albumin excretion

Increased urinary albumin excretion is a major risk factor for the progression of diabetic nephropathy in both type 1 diabetes (T1D) and T2D [22-24]. In most patients, the first sign of diabetic nephropathy is moderately increased urinary albumin excretion, i.e. 30-300 mg/g creatinine in a spot urine sample (also termed microalbuminuria) [25]. Patients who develop severely increased albuminuria, i.e. >300 mg albumin/g creatinine in a spot urine sample (also called macroalbuminuria or clinical albuminuria), are at particularly high risk for developing a decline in renal function [16, 25-27].

However, a substantial proportion (up to 40%) of patients with moderate albuminuria returns to normoalbuminuria [16, 28, 29]. Moreover, up to 50% of patients with T1D or T2D experience a decline in glomerular filtration rate (GFR), despite the presence of only moderate albuminuria or even normoalbuminuria [29-32]. Therefore, elevated urinary albumin excretion (UAE) is not a necessary prerequisite for the development of diabetic nephropathy. This finding has consequences for the diagnosis of the disease, namely that GFR should be assessed in addition to UAE. Moreover, elevated UAE is associated with increased cardiovascular risk, but it is controversial whether re-

ducing it translates to a lower incidence of cardiovascular events.

2.2 Elevated glucose levels

Inadequate glycemic control is a pivotal risk factor for the development and progression of diabetic nephropathy. In patients with both T1D and T2D, high HbA1c levels are associated with an increased risk for developing nephropathy [24, 28, 33, 34]. Observational studies reported a clear decrease in the incidence of diabetic nephropathy in both patients with T1D and T2D who achieved better glycemic control [16, 35, 36]. Indeed, in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study, patients with moderate albuminuria, but lower HbA1c levels, had a lower risk for progressing to severe albuminuria or ESRD [16]. Randomized controlled studies in patients with T1D and T2D reported similar findings [15-21]. In DCCT, intensive glycemic control reduced the risk of progression from moderate albuminuria to severe albuminuria or ESRD [16]. Moreover, strict glycemic control reduces the risk of progression from severe albuminuria to reduced GFR or ESRD [26]. However, it is unclear whether the different antidiabetic agents are all similarly effective in delaying the progression of diabetic nephropathy. This remains to be elucidated.

2.3 Other established risk factors for diabetic nephropathy

Patients with a longer duration of diabetes have a higher risk for developing nephropathy [33, 37]. Elevated blood pressure is another important independent risk factor for nephropathy [28, 33]. In the DCCT/EDIC study, lower blood pressure was associated with reduced risk for progression from moderate albuminuria to severe albuminuria or ESRD [16]. Moreover, in patients with T2D, lower blood pressure was associated with regression from moderate albuminuria to normoalbuminuria [38]. Inhibitors of the renin-angiotensin system appear to delay the progression of diabetic nephropathy more than other classes of antihypertensive agents, while the reduction in blood pressure is similar.

Dyslipidemia also appears to play a role in the pathogenesis of diabetic nephropathy. In the DCCT/EDIC study, lower low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels were associated with reduced risk for progression from moderate albuminuria to severe albuminuria

Table 1. Risk factors for diabetic nephropathy

Established/new risk factors	Modifiable risk factors	Non-modifiable risk factors
Established risk factors	• Increased urinary albumin excretion • Elevated glucose levels • Elevated blood pressure • Dyslipidemia • Obesity • Smoking	• Longer duration of diabetes • Advanced age • Female sex • Retinopathy
New risk factors	• Oxidative stress • Subclinical inflammation	• Genetic background • Ethnicity (African-Americans and Pima Indians) • Glomerular hyperfiltration

or ESRD [16]. In patients with T2D, elevated total cholesterol (TC) levels are also associated with increased risk for the development of both moderately and severely increased UAE [24]. In addition, low levels of TC and TG are associated with regression from moderate albuminuria to normoalbuminuria in T2D [29]. The interventional studies with statins mentioned above also provide evidence for a role of dyslipidemia as a risk factor for diabetic nephropathy by showing that lowering LDL-C levels is associated with delayed progression of the disease.

Obesity is also associated with increased risk of diabetic nephropathy [33, 37, 39]. In DCCT, abdominal obesity, evaluated by waist circumference, was associated with a higher incidence of albuminuria, but did not predict a decline in GFR [34]. On the other hand, weight loss reduces urinary albumin excretion and prevents the decline in GFR [40, 41].

Smoking is associated with increased albuminuria and with a decline in GFR in both patients with T1D and T2D [33, 34, 42].

Advanced age increases the risk for nephropathy in both T1D and T2D [24, 33, 34]. This association appears to be independent of diabetes duration [24, 33].

In the DCCT/EDIC study, female sex was associated with reduced risk for progression from moderate albuminuria to severe albuminuria or ESRD [16]. Other studies in patients with T1D and T2D reported similar findings [24, 28].

In patients with T1D, retinopathy almost invariably precedes the development of nephropathy [43, 44]. In the DCCT/EDIC study, the absence of retinopathy was associated with reduced risk for progression from moderate albuminuria to severe albuminuria or ESRD [16]. In contrast, in T2D, almost 50% of patients with nephropathy do not have retinopathy [31, 45, 46]. However, if nephropathy is due to diabetes, retinopathy is almost always present [31, 45, 46]. Moreover, retinopathy is associated with an increased incidence of albuminuria in patients with T2D [24].

2.4 New risk factors for diabetic nephropathy

Both oxidative stress and subclinical inflammation appear to contribute to the pathogenesis of diabetic nephropathy. Elevated urinary levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a marker of oxidative stress, predict the progression of diabetic nephropathy in patients with T2D [47]. Patients with T1D or T2D and with higher levels of proinflammatory cytokines and chemokines (interleukin 6, interleukin 18, and monocyte chemoattractant protein-1), high-sensitivity C-reactive protein (hsCRP, a marker of subclinical inflammation), or adhesion molecules (soluble vascular cellular adhesion molecule-1 and soluble E-selectin) are at higher risk for developing nephropathy and for advancing to more severe kidney disease [48-51]. In patients with T1D or T2D, elevated levels of tumor necrosis factor-α receptors are also independently associated with increased incidence of impaired kidney function [52, 53]. Unfortunately, studies that evaluated the effects of antioxidant or anti-inflammatory agents for delaying the progression of diabetic nephropathy yielded disappointing results, both regarding effectiveness and safety.

The development of diabetic nephropathy is also associated with the appearance of genetic factors. Patients with T1D, and those with T2D, who have a relative with diabetic nephropathy are at increased risk for developing nephropathy [54-56]. Several studies evaluated the association between angiotensin-converting enzyme (ACE) gene polymorphisms and the risk of diabetic nephropathy, but yielded conflicting results [57-59]. Other reports suggested that polymorphisms of angiotensin-2 receptor, aldose reductase, and protein kinase C might also modulate the development of diabetic nephropathy, but these associations have to be confirmed in larger studies and different ethnicities [60-62]. More recently, genome-wide association studies (GWAS) identified several loci associated with an increased risk for diabetic nephropathy in both T1D and T2D [63-65]. African-Americans and Pima Indians have higher in-

cidence of diabetic nephropathy than Caucasians [66, 67]. Genetic and socioeconomic factors as well as differences in glycemic and blood pressure control may contribute to these differences [66, 67].

In patients with T1D, glomerular hyperfiltration is associated with an increased risk for the development of diabetic nephropathy [68, 69]. This association is more pronounced when GFR is >125 ml/min [68]. Notably, almost half newly-diagnosed patients with T1D have glomerular hyperfiltration [70].

3. Diagnosis of diabetic nephropathy

Given that increased UAE is the most common feature of diabetic nephropathy, measuring albuminuria is an essential component in the diagnosis of diabetic nephropathy. According to the most recent guidelines issued by the American Diabetes Association (ADA), urinary albumin should be measured at least yearly in patients with T1D duration ≥5 years and in all patients with T2D [71]. UAE should be evaluated by measuring the urinary albumin/creatinine ratio in a spot urine sample, since it is as accurate as but more convenient than the measurement of UAE in a 24-h urine collection [71]. UAE varies over time, and therefore 2 or more urine specimens collected within a period of 3-6 months should show increased albumin excretion before making a diagnosis of nephropathy [71]. Moreover, urinary tract infections or other febrile infections, recent exercise, heart failure, and uncontrolled hyperglycemia or hypertension also increase UAE [71]. Therefore, UAE should not be measured in patients with febrile infections. The patients should avoid exercise before testing, and glucose levels and blood pressure should be controlled before evaluating UAE.

A substantial proportion of patients with T1D or T2D has decreased GFR, despite normal urinary albumin [29-32]. Therefore, creatinine levels should be measured, and GFR should be calculated at least annually in all patients with diabetes regardless of the presence of increased UAE [71]. Several equations have been developed for estimating GFR. The Modified Diet in Renal Disease (MDRD) Study equation underestimates GFR, particularly in patients with GFR >90 ml/min/1.73m² [72]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation appears to be more accurate than the MDRD equation in estimating GFR, particularly at higher GFR [73-75]. Accordingly, the ADA recommends the use of the CKD-EPI over the MDRD equation

for estimating GFR in patients with T1D or T2D [71].

Cystatin C is a 13.3 kDa protein that is freely filtered in the glomeruli before it is reabsorbed and catabolized in the renal tubular cells [76]. Its serum levels are independent of muscle mass [76]. Cystatin-C levels are a better marker of renal function than serum creatinine levels or the Cockcroft-Gault equation [77]. Changes in cystatin-C over time strongly correlate with changes in GFR [78]. However, cystatin-C-based equations for the calculation of GFR are more accurate than the MDRD or CKD-EPI equations [79, 80]. However, studies in this topic are few. More data are needed to clarify whether cystatin-C is more sensitive and specific for evaluating renal function in diabetic patients than creatinine-based equations.

4. Novel markers for the early diagnosis of diabetic nephropathy

In patients with T2D, smaller studies have suggested that elevated urinary transferrin excretion rates predict the development of moderate albuminuria [81, 82]. Markers of tubular damage, including urinary neutrophil gelatinase-associated lipocalin, kidney injury molecule 1 (KIM-1), liver-type fatty acid binding protein, and glycoprotein non-metastatic melanoma B, predict a decrease in GFR in both T1D and T2D more rapidly, but have no incremental predictive value over other established risk factors for the progression of diabetic nephropathy, particularly UAE [83-86]. On the other hand, a small study in patients with T1D suggested that low urinary levels of KIM-1 and N-acetyl-β-D-glucosaminidase (NAG) are independently associated with regression from moderate albuminuria to normoalbuminuria [87]. A nested case-control study in DCCT also reported that elevated urinary NAG levels independently predict the development of moderate and severe albuminuria [88].

Urinary proteome analysis also appears to be a promising new modality for the early diagnosis of diabetic nephropathy. It detects a variety of non-albumin urinary proteins. Small studies in patients with T1D or T2D reported that a variety of proteins detected with this method, including collagen fragments and tubular proteins, independently predicts the progression to moderate or severe albuminuria or GFR decline [89-91]. However, the high cost and limited availability of this method are important barriers for its wider implementation.

5. Conclusions

A variety of risk factors promotes the development and progression of diabetic nephropathy, including high glucose levels, obesity, dislipidemia, elevated blood pressure, oxidative stress, and others. Most of these risk factors are modifiable. Therefore, their intensive management is essential for preventing and delaying the decline in renal function. Many of these risk factors are also associated with a higher incidence of cardiovascular events, further supporting the importance of their management.

New (genetic) markers for diabetic nephropathy are being investigated. Their determination may contribute to an early and improved treatment and probably to an early identification of individuals at risk of developing the disease. The identification of novel mechanisms involved in the pathogenesis of diabetic nephropathy may also result in the development of new treatments for both micro- and macrovascular complications of diabetes. These advancements are subject of present and future research.

Early diagnosis of diabetic nephropathy is a pivotal component in the management of patients with both T1D and T2D. Glomerular filtration rate and UAE are easy to perform and inexpensive, but are considerably underused. This is a drawback in clinical practice, highlighting a major gap between necessity and reality in the management of diabetic nephropathy. Measurement of cystatin-C levels is more specific than creatinine for evaluating renal function, but it is more costly, and limited availability are substantial barriers to its wider implementation.

The existence of new markers may allow the earlier diagnosis of this frequent and high-risk complication, but further studies are needed to clarify their additive predictive value, and to define their cost-benefit ratio.

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