HORMONES 2017, 16(4):351-361

Review

Diabetic nephropathy: is it always there? Assumptions, weaknesses and pitfalls in the diagnosis

Kyriakos Ioannou

Department of Nephrology, American Medical Center, Nicosia, Cyprus; Department of Nephrology, Apollonion Private Hospital, Nicosia, Cyprus; European University School of Medicine, Nicosia, Cyprus

ABSTRACT

Diabetic nephropathy is defined as a microvascular complication of the kidneys induced by diabetes mellitus and is characterized by albuminuria and progressive loss of kidney function. However, neither albuminuria nor glomerular filtration rate decline are diabetic nephropathy-specific markers, thus the diagnosis of diabetic nephropathy greatly depends on assumptions. Several factors should be taken into account when urinary albumin levels are assessed before establishing the diagnosis of diabetic nephropathy, while newer more specific markers for diabetic nephropathy are urgently needed.

Key words: Diabetic nephropathy, Diabetic retinopathy, Microalbuminuria, Non-proteinuric diabetic kidney disease, Renal handling of albumin, SGLT1/SGLT2, Urine proteomics

INTRODUCTION

Diabetic nephropathy (DN) is defined as the microvascular complication of the kidneys induced by diabetes mellitus and is characterized by albuminuria and progressive loss of kidney function. It is considered as one of the microvascular complications of diabetes along with retinopathy and neuropathy.[1,2] DN is vitrually the leading cause of end-stage kidney disease (ESKD).[3-5] The prevalence of DN varies enormously between continents, countries and even between regions of the same country.[5-9]

However, the diagnosis of DN is an exclusion diagnosis depending on the presence of at least microalbuminuria in a patient with a history of diabetes of at least 5 years.[10] A kidney biopsy, which is the gold standard for definitive diagnosis, treatment guidance and prognosis for other types of nephropathies, is not indicated in diabetic patients, since the risk of such an intervention is not justified. This is mainly because there are no other treatment options available at present for DN beyond the current application of optimal control of diabetes, hypertension and dyslipidemia and lifestyle modification.[10,11]

Therefore, it could be postulated that the diagnosis of DN is highly subjective, depending on the doctor's judgment and experience when at least the above two criteria are fulfilled.

Address for correspondence:
Kyriakos Ioannou, 11 Halkis Str., Ap. 301, Nicosia 2107, Cyprus; Tel.: +357 97770699, Fax: +357 22206958, E-mail: kyr_ioannou@hotmail.com

Received: 03-05-2017, Accepted: 24-09-2017

NATURAL COURSE OF DN

The natural course of DN was first described by Mogensen et al.[12] Their description was in fact based

on patients with type 1 DM, where the oncet is more or less obvious, and not on patients with type 2 DM, where the oncet is less pronounced and diagnosis may be delayed for 3-5 years.

According to Mogensen et al there are 5 stages in the course of DN (Figure1):

Stage 1, the *stage at diagnosis*, is characterized by hyperfiltration-increased estimated glomerular filtration rate (eGFR) and hypertrophy (increased kidney size). Increase in urinary albumin excretion can be present, aggravated during physical exercise, but these changes are at least partly reversible by insulin treatment.

Stage 2, the *silent stage*, develops over many years, without signs of clinical disease but still with characteristic morphologic lesions on biopsy specimens (glomerular basement membrane thickening, mesangial expansion). Estimated GFR may still be increased and albuminuria is transient. If diabetes is well controlled, albumin excretion is normal; however, physical exercise leads to an increase in albuminuria. By contrast, poor diabetes control leads to increased albumin excretion both during exercise and at rest. A number of patients continue in stage 2 throughout their lives.

Stage 3, the *incipient diabetic nephropathy stage*, is characterized by abnormally elevated urinary albumin excretion, within the microalbuminuria range 30-300mg/24h. Estimated GFR is still high or at least normal. Blood pressure is rising and albumin excretion is higher in patients with increased blood pressure.

Stage 4, the *classic overt diabetic nephropathy stage*, is characterized by persistent proteinuria (>0.5 g/24h) and persistent high blood pressure and, if left untreated, eGFR declines at a mean rate of 1 ml/min/1.73m^2/month. Long-term antihypertensive treatment reduces this eGFR decline rate by about 60% and delays uremia.

Stage 5 is *end-stage renal failure* with uremia due to diabetic nephropathy.

RETINOPATHY AND DN

Diabetic nephropathy (DN) and diabetic retinopathy (DR) are considered as interrelated diabetic vascular complications since kidneys and retina share similar

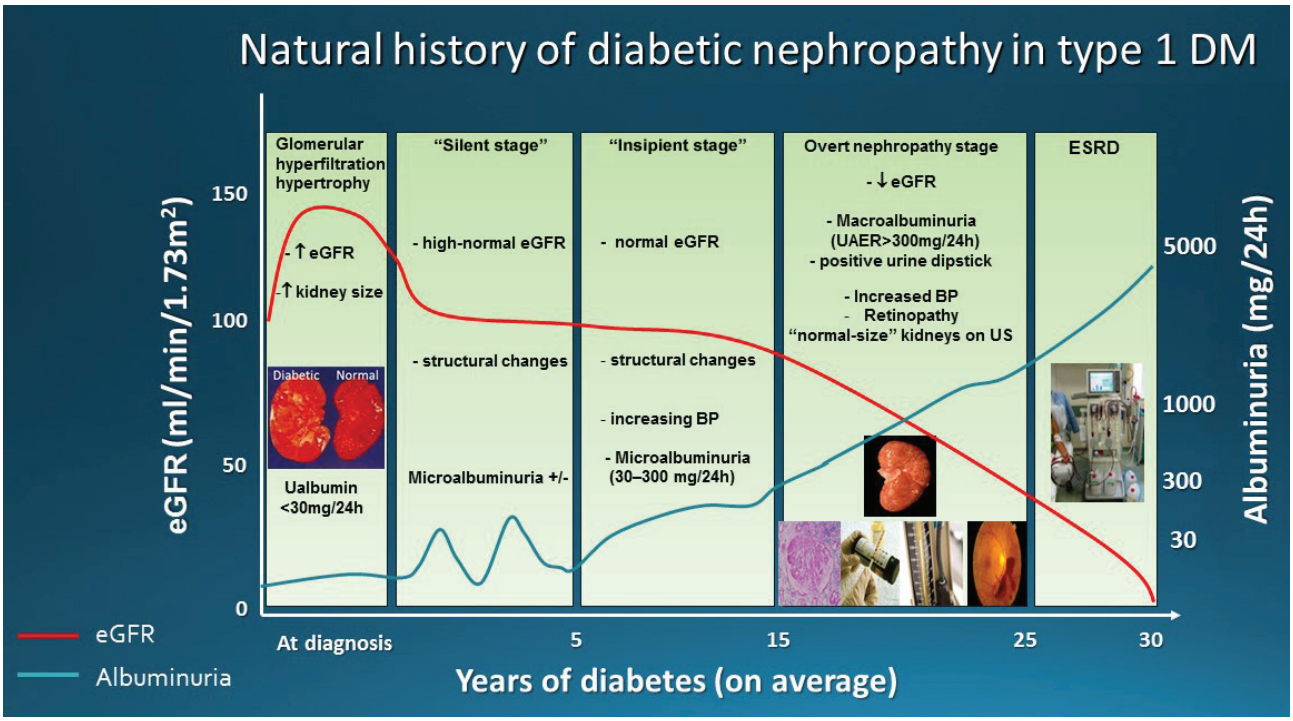

Figure 1. Natural history of diabetic nephropathy in type 1 DM, as described by Mogensen et al.

size arteries.[1,2] In clinical practice, the diagnosis of DR is used as the "*non-interventional kidney biopsy*" to diagnose DN. Therefore, in a diabetic patient with a history of DM for at least 5 years and albuminuria, with or without decrease in eGFR, the co-existence of DR strengthens the clinical suspicion that the patient also has DN. However, the absence of DR cannot exclude DN, since DR is present in approximately 60% of DN cases. Prakash et al reported an absence of diabetic retinopathy in 43% of the cases with biopsy-proven DN, while non-diabetic kidney disease was reported in 40% of the cases in the presence of DR.[13]

Diabetic retinopathy incidence varies between studies and this probably relates to the duration of the cohorts. For example, in the UK type 2 DM population studies, DR incidence was estimated to be 66% at 10 years and in the US population studies at 72.3% at 14 years.[14] Diabetic retinopathy seems to better correlate with DN in type 1 DM and much less in type 2. The prevalence of DR in type 1 DM in Europe and the USA ranges between 36.5-93.6%, while in type 2 DM in Western populations 28.5-40.3%.[14]

Pedro et al observed a prevalence of 36.47% DR in type 1 DM patients and 26.11% in type 2 DM patients.[15] In the same study, microalbuminuria was identified as a risk factor for DR in type 1 DM patients but not for type 2, whereas overt nephropathy was better correlated with DR.[15] Manaviat et al[16] showed that the prevalence of any stage of DR in the microalbuminuria stage is quite low (43%), while it increases in the overt proteinuria stage (79%), probably reflecting the more advanced diabetic vascular disease, while DR is even present in 28% of patients with normoalbuminuria.

Therefore, DR does not serve as a reliable indicator of DN in patients with type 2 DM.

MICROALBUMINURIA AS A MARKER OF ENDOTHELIAL DYSFUNCTION AND DN

Microalbuminuria (MA) can occur both in patients with DM without present or future DN as well as in patients without DM but with other types of progressive chronic kidney disease and therefore it does not serve as a specific marker for the presence of DN.[17] Mogensen et al showed that MA predicts early mortality in type 2 DM and identified MA a cardiovascular and renal risk factor in both diabetic and non-diabetic subjects.[18] Parving reported an increase in urinary albumin excretion rate in poorly controlled hypertensive patients[19] and Bigazzi et al showed that MA predicts cardiovascular events and renal insufficiency in hypertensive patients.[20] Endothelial dysfunction has been suggested as underlying the renal and/or cardiovascular organ damage observed in these diseases.[21]

The Steno hypothesis proposed that an increased permeability of the vascular endothelium constitutes a high risk for microangiopathy and a tendency to large vessel disease.[22] This systemic transvascular leakiness for albumin is associated with clinical atherosclerotic cardiovascular disease.[23] The initiating event of the atherogenesis is endothelium 'injury', e.g. by hemodynamic stress or due to dyslipidemia and, according to the 'response-to-injury' hypothesis, the increased transendothelial permeability to macromolecules is such a type of response.[24]

Therefore, while MA better predicts the development of DN in type 1 DM, MA in type 2 DM serves both as a marker of DN and of generalized endothelial dysfunction. Some type 2 DM patients with MA will not progress to the stage of overt proteinuria and these are probably the patients with hypertensive glomerulosclerosis compared to the patients that will progress and probably have diabetic glomerulosclerosis. It is obvious that in a type 2 DM patient with MA, with or without eGFR decline, but with also a long-standing history of hypertension and general atherosclerotic vascular findings, even if both of the criteria for DN are fulfilled, one could not suggest that this is DN. Longer follow-up of both eGFR and proteinuria may be needed before the diagnosis of DN is set.

REGRESSION OF MICROALBUMINURIA

Microalbuminuria may not always be a marker of an irreversible renal injury, but most likely of acute renal stress and, due to this, not rarely regression of MA is observed. In some small studies MA progression rate to overt proteinuria has been reported to be high, i.e. 85%[18] and 87%[25] risk within 6 and 14 years, respectively, although this rate may be over-

estimated. Perkins et al[26] found this rate to be at 19% in a 6-year follow-up, with approximately 60% of the patients showing regression to normal albumin excretion levels. This suggests a transient increase in albuminuria, especially in patients without optimal control of DM (HbA1c>8%) and/or of hypertension. In such patients, MA decreases or even normalizes with improvement of DM and hypertension control, but again this is not evident in all patients. Patients who do not seem to improve MA despite DM control could be individuals with more atherosclerotic disease, i.e. smokers, chronic hypertensives and patients with long-standing hyperlipidemia, where MA possibly reflects a generalized vascular endothelial disease.

Therefore, assessing MA as a marker of renal disease in type 2 diabetics should only be made after establishing good DM, blood pressure and lipids control for a reasonable time period so that the transient character of MA is excluded. According to the National Kidney Foundation recommendations for Diabetes and Chronic Kidney Disease,[27] patients with DM should be screened annually for DN. Initial screening should start 5 years after the diagnosis of type 1 DM or from a diagnosis of type 2 DM and should include measurements of urinary albumin to creatinine ratio (ACR) in a spot urine sample and serum creatinine and estimation of eGFR. Microalbumin must be measured in a first void urine sample and three samples are needed in a period of 3-6 months to establish the presence of MA.

MICROALBUMINURIA: IS WHAT IS MEASURED IN THE URINE WHAT IS LEAKING FROM THE GLOMERULUS? FACTORS INFLUENCING ALBUMINURIA LEVELS.

According to the current guidelines, for the establishment of a DN diagnosis detection of urinary albumin levels >30mg/24hours or ACR >30mg/g is crucial and mandatory. However, is the amount of albumin that we measure in the patient's urine what is really leaking from the damaged glomerulus (Figure 2)?

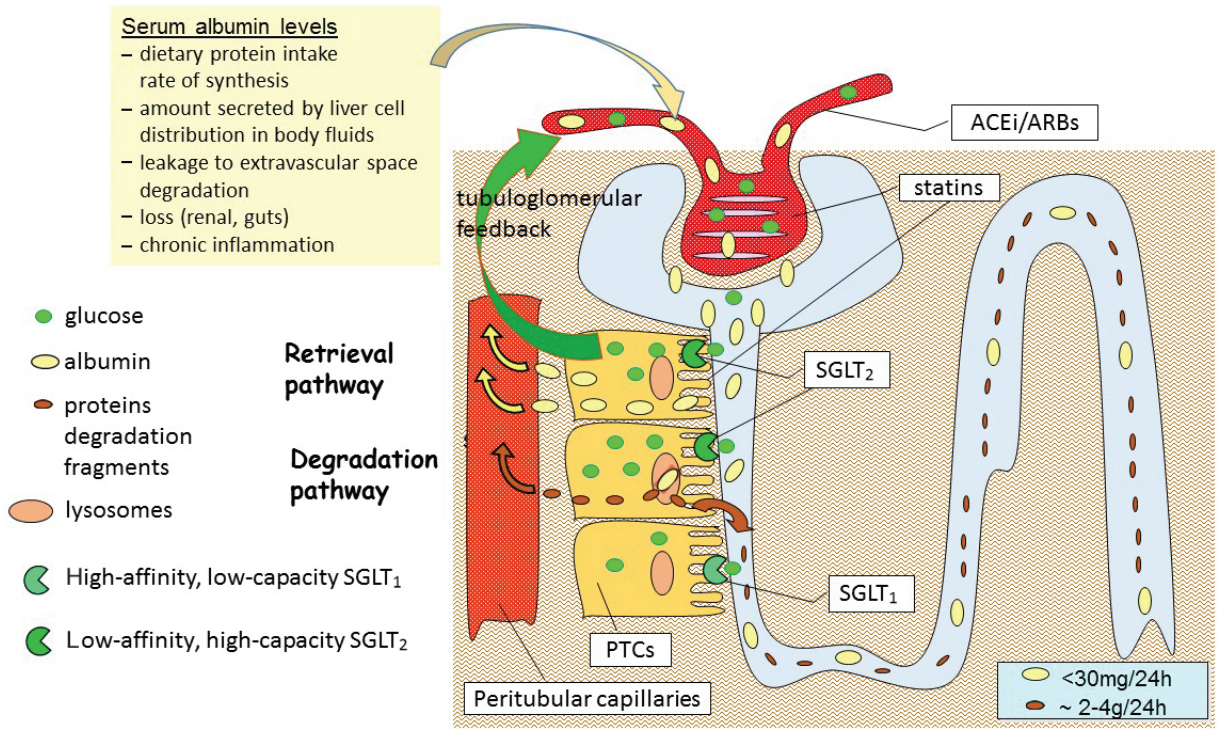

Figure 2. Factors influencing final urinary albumin levels. Albumin reabsorbed by the PTCs can return to the circulation either as intact albumin through a *retrieval transcytotic pathway* or as amino acids after being degraded in the lysosomes (*degradation pathway*). An amount of this degraded albumin is re-excreted in the urine as fractions of the initial molecule, not measured in urine samples when processed for MA measurement.

First, the amount of albumin filtered through the glomerulus depends on the serum concentration of albumin. However, not all people have the same serum albumin levels. Serum albumin levels are dependent on dietary protein intake, the rate of synthesis and the amount secreted from the liver cells, the distribution in body fluids, the level of degradation and loss.

Habitual dietary protein intake varies significantly in humans depending upon age, gender and lean body mass, well known factors that influence GFR.[28] Although there are reports suggesting that in healthy individuals dietary protein overload may not increase protein renal clearance,[29,30] animal models showed that protein overload increases proteinuria.[31,32] In humans also it was shown that consumption of excessive amounts of dietary protein promotes chronic renal disease through increased glomerular pressure and hyperfiltration[33] and might be harmful in patients with CKD.[34] Therefore, dietary differences in the amount of protein consumption may result in differences in GFR and albumin clearance and the amount of albumin measured in a urine sample.

On the other hand, hypoalbuminemia is a multifactorial process that results from a decrease in albumin synthesis as well as an increase in breakdown, leakage to the extravascular space and decreased protein intake.[35] Patients with obvious malnutrition, malabsorption syndrome (protein-losing enteropathy) or hepatic dysfunction and a chronic inflammatory state may have lower albumin serum levels due to lower intake, absorption or synthesis of albumin. Therefore, for the same degree of glomeruli damage, these patients are expected to have lower urine albumin levels due to the lower amount of plasma albumin filtered by the kidneys.

Second, for more than 30 years filtered albumin has been known to be reabsorbed by the proximal tubular cells (PTCs).[36] Despite the low, i.e. less than 30mg/day, amount of albumin found in final urine, this represents only the intact albumin that can be measured by available laboratory methods. It is known that filtered albumin is reabsorbed mainly by the proximal tubule and to a lesser extent by downstream parts of the nephron.[37] Albumin reabsorbed by the PTCs can return to the circulation either as intact albumin through a retrieval transcytotic pathway or as amino acids after being degraded in the lysosomes (degradation pathway).[38,39] An amount of this degraded albumin is re-excreted in the urine as fractions of the initial molecule, not measured in urine samples when they are processed for MA measurement (Figure 2). The protein content of the final urine has been estimated to be as high as 2 to 4 g/day[40,41] and the amount of filtered albumin has been estimated to be 50 times higher than previously assumed, suggesting that on a daily basis the normal kidney filters nephrotic levels of albumin, most of it retrieved by the proximal tubules.[42,43]

Furthermore, beyond the glomerulus, the proximal tubule also seems to contribute to DN pathology. Normally, under euglycemic conditions approximately 97% of filtered glucose is reabsorbed via the low-affinity-high-capacity Na^+-glucose cotransporter SGLT2, primarily in the early segments of the proximal tubule, and 3% is reabsorbed via the high-affinity-low-capacity SGLT1 in the late segments of the proximal tubule.[44] Hyperglycemia enhances the amounts of glucose filtered by the glomeruli and thus increases glucose delivery to both SGLT2 and SGLT1 enhancing glucose reabsorption in the proximal tubule. Glucose transporters GLUT2 and GLUT1 mediate glucose transport across the basolateral membrane, but GLUT2 may also translocate to the apical membrane in diabetes.[45]

Proximal tubular cells appear unable to decrease glucose transport rates adequately to prevent excessive changes in intracellular glucose when exposed to high glucose concentrations[46] and this leads to the notable growth phenotype of early diabetic proximal tubule hyperplasia followed by hypertrophy.[47] In the setting of normal tubuloglomerular feedback, this increased glucose tubular absorption leads to a strong tubular control of glomerular filtration in the early diabetic kidney, further enhancing glomerular hyperfiltration and proteinuria.[45,47]

Therapeutic agents, which have been developed to inhibit SGLT-2 or to effect dual inhibition of SGLT-2/SGLT-1 and are currently used for diabetes control in type 2 DM and type 1 DM, respectively, are also expected to intervene in these processes.[48,49]

SGLT2 inhibitors could potentially exert nephro-

protection not only through improved glycemic control but also through glucose-independent effects. Such effects are the blood pressure-lowering effect, since these reduce sodium reabsorption in the proximal tubule, but also afferent arteriole vasoconstriction through the tubuloglomerular feedback which leads to attenuation of diabetes-associated hyperfiltration and tubular hypertrophy and reduction in albuminuria, independent of their effects on blood pressure or glucose control.[50,51]

SGLT2 inhibition and the associated afferent vasoconstriction leads to an acute, dose-dependent reduction in eGFR by approximately 5 ml/min/1.73 m^2 and in albuminuria by approximately 30% to 40%.[52] Thus, the decrease in glomerular hyperfiltration following good diabetes control in general could in part explain the decrease in MA in clinical practice, while SGLT 2 inhibitors contribute to this through additional glucose-independent effects.

It is obvious that the anatomical and functional integrity of the proximal tubule is crucial for the final amount of albumin found in the urine and thus for the diagnosis of DN. Diseases other than diabetes that affect the PTCs,[53] but also such drugs as cisplatin, ifosfamide, tenofovir, sodium valproate and aminoglycoside antibiotics, may influence PTCs function and interfere with MA levels.[54]

Angiotensin-converting enzyme inhibitors (ACE-inh) and angiotensin II receptor antagonists (ARBs) are probably the most common classes of antihypertensive drugs prescribed in diabetic patients. Beyond their beneficial effects on cardiovascular risk factors and all-cause mortality, both categories have been shown in several studies also to have beneficial renal outcomes, including time to end-stage renal failure or doubling of creatinine, but also in preventing progression of micro- to macroalbuminuria or even remission of micro- to normoalbuminuria,[55-58] thus intervening in the result of albumin measurement in urine.

3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, referred to as "statins", widely used in diabetics for lipids control and cardiovascular protection,[59-61] have been shown also to increase albumin endocytosis by glomerular epithelial cells (podocytes), suggesting another pathway of retrieving albumin that leaks through the glomerular filtration barrier.[62-64] Conversely, statins have been shown to reduce albumin endocytosis by PTCs and may enhance albuminuria.[65,66]

Taking all the above into account, it could be postulated that the amount of albumin detected in a spot urine sample, which is the criterion in order to characterize a patient as having DN or not, is the net result of several parameters that are both patient related (degree of glomerular damage, proximal tubular integrity, serum albumin levels), but also health professionals related (use of therapeutic agents that influence glomerular filtration of albumin such as ACE-inh/ARBs or statins, or tubular processing of the filtered albumin like SGLT2 inhibitors, as well as the degree of hypertension, glycemic and lipids control in general). Therefore, for the same amount of MA detected, the degree of the actual diabetic glomerular damage may not be the same, therefore the diagnosis of DN should be individualized and carefully set after assessment of all possible parameters that may influence this process.

MICROALBUMINURIA: IS IT THE BEST WE CAN DO FOR EARLY DETECTION OF DN?

It has already been more than two decades since the first announcements of MA being an early marker of DN and, until lately, it has been regarded as the gold standard for the diagnosis and a predictor of progression to end-stage kidney disease in both type 1[67] and type 2 diabetes.[68]

However, as already analyzed above, though MA is an accessible and affordable screening marker for daily clinical practice, its predictive strength is not robust. On this account, scientists have put their efforts into identifying new markers that precede the microalbuminuria stage and are more predictive of the early stages, but also of progression of DN. In this effort, the development of new diagnostic methods and especially the omics and microRNAs technology has led to novel markers, mainly from the urine that could support this strategy.

A number of key biomarkers present in the urine that reflect the pathophysiologic processes taking place in the diabetic kidney along the nephron (glo-

merulus/podocytes, tubules) and also reflect the different mechanisms (tubular damage, oxidative stress, inflammation and activation of the intrarenal renin-angiotensin system) have been identified.[69-71] For example, podocytes injury and/or decrease in their number per glomerulus is an early finding in DN, even prior to proteinuria, and therefore podocyturia- and podocyte-specific markers in the urine, including nephrin or Wilm's tumor-1 protein, could serve as early biomarkers of DN.[72,73]

Nephrinuria was present in 100% of type 2 diabetic patients with microalbuminuria and macroalbuminuria, but also in 54% of type 2 diabetic patients with normoalbuminuria.[74] Positive urinary Wilms' Tumor-1 (WT1) protein was detected in 50% of diabetic patients without proteinuria, while in nondiabetic control subjects urinary WT1 was virtually absent.[73]

Vascular endothelial growth factor A (VEGF-A), a podocyte-derived biomarker, could also be a sensitive early marker of DN, but also a disease progression marker. VEGF urinary excretion was significantly higher in diabetics, even in the absence of albuminuria, compared to nondiabetic healthy controls, and urinary VEGF levels increased as DN advanced.[75]

Other podocytes markers could also be found early in the urine of diabetic patients, but their specificity might be an issue. For example, podocalyxin was found to increase in the urine of 53.8% of normoalbuminuric diabetic patients;[76] however, podocalyxin is also expressed in other renal and non-renal cells.[77]

Urinary transferrin may also be a sensitive marker of glomerular damage in patients with diabetes, even in the absence of albuminuria;[78] on the other hand, as with albumin it is not DN-specific, since other primary glomerular diseases also increase its excretion.[79]

Beyond podocytes (glomerular epithelium), glomerular endothelium has also been implicated in diabetic kidney disease pathophysiolgy and other microvascular complications in diabetes. Diabetic patients excrete in urine significantly more glycosaminoglycans (GAGs), part of the endothelial glycocalyx, than controls[80] and such an increase has been reported in patients with all stages of albuminuria, while urinary GACs positively correlated with disease progression.[81]

Moreover, several other proteins have been reported to increase in the urine of diabetic patients. Extracellular structural matrix proteins (collagen type IV, fibronectin, metalloproteinases), transforming growth factor (TGF)beta the potent inducer of extracellular matrix proteins, markers of tubular damage like the apical membrane receptors megalin and cubilin, the transmembrane protein of the apical membrane of PTCs-kidney injury molecule 1 (KIM-1), but also neutrophil gelatinase-associated lipocalin (NGAL) that is produced in the distal nephron are examples of such proteins. Furthermore, proteins that are normally freely filtered by the glomerulus and reabsorbed by the PCTs, including α1-microglobulin and retinol-binding protein, are also reported to increase in urine. Most of these proteins increase even in the normoalbuminuric stage,[69,70] with some of them being detected up to 5 years prior to the onset of macroalbuminuria.[71]

NON-DIABETIC NEPHROPATHY IN DIABETIC PATIENTS

When a diabetic patient develops clinical nephropathy with proteinuria, this could be due to progressive diabetic nephropathy, or another non-diabetic glomerulopathy, or both. It has been reported that another primary glomerulopathy may rarely (2-3%) implicate insulin dependent diabetes,[82] but from 10%[83] to as much as 25%[84] of non-insulin dependent diabetes cases. Among diabetic patients who clinically were suspected not to have diabetic nephropathy and underwent a renal biopsy, Soni et al[85] reported that the most common non-diabetic renal diseases were acute interstitial nephritis 18.1%, post infectious glomerulonephritis 17.24%, membranous nephropathy 11.20% and focal segmental glomerulosclerosis 7.75%.

Furthermore, even if diabetic changes such as diffuse and nodular glomerulosclerosis are found in the kidney biopsy, it is important to determine whether these are secondary changes due to diabetic nephropathy or are due to another renal disease, e.g. segmental glomerulosclerosis or idiopathic nodular glomerulosclerosis, in addition to diabetic nephropathy.[86] Other nephropathies that share similar nodular histological features with diabetic nephropathy such as membranoproliferative glomerulonephritis, mono-

clonal immunoglobulin deposition disease, amyloidosis, fibrillar glomerulopathy and idiopathic nodular glomerulosclerosis could be distinguished by detailed histopathological evaluation.[86,87]

On the other hand, many diabetic patients with chronic kidney disease may not have significant proteinuria or albuminuria even in the late CKD stages and, therefore, classic diabetic nephropathy does not appear to be the underlying renal lesion.[88] These subjects may represent almost 50% of diabetic patients with renal insufficiency and are more often older patients with a history of cardiovascular disease and usually treated with renin-angiotensin system blockers.[89,90] Disease progression in this subgroup is slower, although histological analyses may show surprisingly advanced glomerular lesions.[89] The latter histological findings are more frequently seen in type 1 DM, whereas in type 2 DM a substantial proportion of patients have more advanced tubulo-interstitial and vascular than glomerular lesions.[91,92] On this basis, Dalla Vestra et al[93] have proposed a different classification system for renal lesions in diabetic kidney disease, comprising three major groups: I) normal or near-normal renal structure (41%), II) typical diabetic nephropathology (26%) and III) atypical patterns of renal injury (33%) where there are only mild diabetic glomerular lesions and disproportionately more profound tubulo-interstitial lesions, advanced glomerular arteriolar hyalinosis and global glomerular sclerosis, in all possible combinations.

CONCLUSION

Microalbuminuria is currently used as the earliest marker for diabetic nephropathy. However, several limitations exist, since urinary albumin levels depend on several patient-related factors or health care professionals' interventions. Additionally, renal impairment could also occur even at the normoalbuminuric stage. Urinary biomarkers that are significantly elevated even in normoalbuminuric diabetic patients prior to the development of microalbuminuria could be promising biomarkers of DN at a very early stage. Nevertheless, larger multicenter prospective studies are needed to confirm their clinical utility and cost-effectiveness as a screening tool for daily practice. Until then, albuminuria must continue to be used as a marker of kidney damage in DM, but it must also be carefully assessed and monitored for a reasonable time period before setting the diagnosis of DN. If unexpectedly renal function deterioration occurs or overt proteinuria develops, nephrology consultation is advisable to exclude other primary renal pathology.

CONFLICT OF INTEREST

None.

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