Overview of pathophysiology, diagnosis, biomarkers, treatment and recent advances in the management of diabetic nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is the most common devastating complication of diabetes mellitus and prime etiology of end stage renal disease (ESRD). Age, genetic predisposition, poor glycemic control, uncontrolled blood pressure, dyslipidemia, obesity, smoking are major risk factors. Hyperglycemia is the primary pathogenic factor for activation and maintenance of molecular signaling pathways involving activation of renin-angiotensin-aldosterone system (RAAS), reactive oxygen species (ROS) and inflammatory cytokine production which play a key role in development of Diabetic kidney disease (DKD). Tubuloglomerular feedback (TGF) causes afferent arteriole dilatation, while RAAS activation leads to vasoconstriction of efferent arteriole which aggravates and sustains the glomerular hypertension may further worsen the nephropathy. Diagnosis is based on estimated glomerular filtration rate (eGFR) and albuminuria. Newer biomarkers are being investigated to detect DN in early stages even before Microalbuminuria (MA). Optimai glyemic control, blood pressure control and Renin Angiotensin System (RAS) blockers play a role in delaying the progression however newer therapies may address the unmet needs for prevention of DN.

Keywords: Diabetic nephropathy; T1DM; T2DM; Albuminuria; Estimated glomerular filtration rate; Chronic kidney disease; sodium glucose co-transporter 2 (SGLT2) inhibitors; Reactive oxygen species (ROS); angiotension converting enzyme inhibitors (ACEI); angiotensin II type 1 receptor blockers (ARB’s); Gluca-gon like peptide 1.

INTRODUCTION

Diabetic nephropathy (DN) is a long-term microvascular complication and an important cause of chronic kidney disease (CKD). It frequently leads to end stage renal disease (ESRD) which is devastating to the individual with enormous social and financial burden. Classic DN develops and progresses over many years with gradual increase in urinary albumin excretion (UA), blood pressure and decline in glomerular filtration rate (GFR). As nephropathy progresses the risk of other complications increases, in particular the risk of cardiovascular disease with significant mortality and morbidity. DN was reported in approximately 20% to 40% of type 2 diabetes mellitus (T2DM) and 30% of type 1 diabetes mellitus (T1DM)[1-3]. Occurrence of DN has been reported in patients with prediabetes also[4]. With the improvement in management of diabetes the prevalence of classic DN has come down in T1DM but not so in T2DM which is attributed to increased prevalence of obesity and ageing population[5]. Optimized glyemic, blood pressure, lipid control and the use of Renin-angiotensin-aldosterone system (RAAS) inhibitors are proven to be beneficial; still they remain suboptimal with an unmet need for effective therapies. Hence, early diagnosis is very essential to deliver appropriate therapy to prevent the occurrence and progression of the disease, thereby reducing morbidity and mortality.

Risk factors

DN is the result of interplay between genetic and environmental factors. Hyperglycemia, hypertension, dyslipidemia, anemia, life style factors such as obesity and smoking are considered as major risk factors. The extent of albuminuria is also an independent predictor at each stage of nephropathy[6]. T2DM patients with nonalcoholic fatty liver disease (NAFLD) are
more likely to have CKD[7]. Although several studies noted strong genetic predisposition to DN in T1DM and T2DM siblings concordant for diabetes, genetic factors related to DN are not clearly understood[8,9]. Studies for identification of genetic loci are ongoing with genome scanning and candidate gene approaches.

**Pathogenesis**

Though many factors play a role in the pathogenesis of DN, it is well known that hyperglycemia is a prime factor that activates and maintains the molecular signaling pathways in a genetically susceptible individual. It can trigger metabolic injury mediated by polyolpathway, increased advanced glycation end products (AGE) and protein kinase c (PKC) activation in both the glomerular and interstitial cells, with resultant increased intraglomerular pressure, which activate the diverse intracellular signaling pathways including RAAS (Figure 1). This results in enhanced production of reactive oxygen species, inflammatory mediators (NF-κβ, Toll like receptor (TLR)), growth factors (vascular endothelial growth factor (VEGF), connective tissue growth factor), TGF-β, pro-inflammatory and profibrotic cytokines causing structural and functional renal injury[5,10] (Figure 2). Hyperglycemia is also reported to upregulate SGLT2 channels in proximal tubule resulting in TGF and glomerular hypertension (Figure 3). The growing data suggest the role of elevated FGF 23 and deficient alpha klotho in the pathogenesis[11,12,13].

**Role of podocytes in diabetic nephropathy**

Glomerular hypertension increases the burden on podocytes to cover a larger area of glomerular basement membrane (GBM), resulting in foot process widening, decreased ability to bind to GBM leading to bare areas and consequent protein excretion, as evidenced in several studies[14-16]. Further studies on molecular structure of podocyte and slit diaphragm proteins expression are needed to recognize their role in the pathogenesis of DN.

**Pathology**

**Glomeruli** - Thickening of GBM, mesangial expansion due to accumulation of matrix, causing diffuse mesangial sclerosis, the hallmark lesion of DN. The distinctive nodular accumulations of mesangial matrix, known as Kimmelstiel-Wilson nodules.

**Capsular drop** - Hyaline is located between the basement membranes of Bowman’s capsule and the adjacent parietal epithelium.

**Fibrin cap** - Hyaline is found within capillary lumina and particularly when it is adherent to the capillary wall.

**Tubules** - Tubular basement membrane thickening, Armani Ebstein changes (accumulation of glycogen in cells of par’s recta) and tubular atrophy.

Interstitium - Fibrosis and infiltration with inflammatory cells.

**Blood vessels** - Afferent and efferent arteriolar hyalinosis.

In T1DM most important structural changes are seen in glomerulus, although tubular, interstitial and arteriolar lesions are present. In contrast Fioretto P et al, reported diverse structural changes in renal parenchyma in a large cohort of T2DM patients with albuminuria. Majority of the patients in that cohort had normal or near normal glomerular structure with or without tubulointerstitial and arteriolar abnormalities despite the presence of microalbuminuria[17-20].

**Natural history**

**T1DM**

The stages of diabetic kidney disease in T1DM can be divided into five stages

**Stage 1** - Renal hypertrophy and hyper function, which occur with the onset of diabetes. Some studies showed that glomerular hyper filtration is a risk factor for DN[21-25].

**Stage 2** - A stage of clinical quiescence associated with thickening of GBM and mesangial expansion. GFR is normal or may be higher. Patients with upper limit of normoalbuminuria and structural changes of diabetic glomerulosclerosis have a higher chance of developing MA[6,26]. Nocturnal non dipping on 24 hour ambulatory blood pressure monitoring may be an early sign of DN, which precedes the development of stage 3[27].

**Stage 3** – Incipient nephropathy stage, characterized by persistent MA. It starts 5-10 years after the onset of T1DM[28]. Approximately in one third of type 1 diabetics, MA can regress towards normoalbuminuria, it can persist as such, or it can progress towards macroalbuminuria[29,30]. GFR remains normal or may show decline. Persistent MA is an independent predictor of future cardiovascular risk[31, and progression to macroalbuminuria is associated with arterial hypertension and decrease in GFR[32].

**Stage 4** – It is characterized by overt proteinuria. This stage manifests 10-20 years after the onset of T1DM and if untreated, GFR decline progresses at mean annual rate of 10-12 ml/min/1.73m2[33,34]. At this stage diabetes associated chronic vascular complications are noted in most patients.

**Stage 5** - Progression to end stage renal disease (ESRD) occurs 5-15 years after the onset of proteinuria.

In T2DM the natural history of nephropathy is similar to T1DM with the exception that MA or proteinuria may be present at diagnosis, because onset of diabetes may go undetected for many years. Hence the relationship of MA to T2DM duration is not precisely known.
Diagnosis

DN diagnosis is based on clinical features, and assessment of estimated glomerular filtration rate (eGFR) and urine albumin secretion (UAE)\textsuperscript{[2,35]}. Non diabetic kidney is suspected in the following situations and needs appropriate evaluation, if required with renal biopsy except in contracted kidneys.

1. Proteinuria in patients with short duration of T1DM
2. Absence of retinopathy in type 1 diabetes, however absence in type 2 diabetes does not rule out nephropathy.
3. Presence of active urinary sediment.
4. Significant drop in eGFR than expected with angiotension converting enzyme inhibitors (ACEI)/angiotensin II type 1 receptor blockers (ARB’s).
5. Contracted kidneys on imaging.
6. Presence of other systemic features.

Albuminuria

First clinical indicator of DN is albuminuria. It’s measured in the following ways (Table 1)

1. 24 hour urine albumin estimation,
2. Timed overnight UAE rate,
3. Albumin–creatinine ratio (ACR).

Recent guidelines recommend use of ACR as it is performed easily on spot urine sample \textsuperscript{[36,37]}. A patient is considered to have persistent albuminuria when at least two of three measurements of urine ACR examined in 3-6 months are abnormal.

Estimated glomerular filtration rate (eGFR)

eGFR is considered as the better index of renal function\textsuperscript{[38]}. Regularly used equations for estimation of eGFR include Modification of Diet in Renal Disease (MDRD) study equation or the Chronic Kidney Disease Epidemiology (CKD-EPI) collaboration equation\textsuperscript{[39,40]}. Serum Creatinine is used to estimate these GFRs.

The CKD has been categorized into five stages according to eGFR\textsuperscript{[41]}

G1- GFR ≥90 mL/min/1.73 m2;
G2-60 to 89 mL/min/1.73 m2; G3a- 45 to 59 mL/min/1.73 m2; G3b-30 to 44 mL/min/1.73m2; G4- 15 to 29 mL/min/1.73 m2; G5- <15 mL/min/1.73 m2.

Cystatin C, a cysteine protease inhibitor is filtered freely by the glomerulus and absorbed in the proximal tubule, and considered to be a reliable marker of renal function\textsuperscript{[42,43]}. Cystatin C based estimation of GFR was demonstrated to be superior\textsuperscript{[44]}. As it is not widely available in routine practice, most guidelines use serum creatinine-based eGFR for diagnosing and monitoring diabetic kidney disease (DKD).

Risk assessment and monitoring

Annual monitoring of eGFR and albuminuria is recommended in stable DKD patients as per the latest KDIGO and ADA guidelines. As progression of kidney disease is more rapid in patients with low GFR and higher albuminuria, increased frequency of their measurement should be considered.

Early diagnosis with new biomarkers

Limitations in the measurement of UAE which led to identification of newer biomarkers are

1. Significant glomerular damage has already occurred by the time albuminuria is evident \textsuperscript{[45]}.
2. Decline in renal function is not always associated with increased albuminuria \textsuperscript{[46]}.

Several new biomarkers of glomerular, tubular injury, inflammation and oxidative stress are being investigated\textsuperscript{[47-51]}.

Albumin accounts for minor fraction of total urinary protein and becomes unreliable in normoalbuminuria\textsuperscript{[52]}. Hence, non-albumin proteinuria (NAP) may be considered as a marker for early detection of DN\textsuperscript{[53,54,55]}.

The source of Urinary NAP include\textsuperscript{[56-62]}

Glonerular biomarkers - Transferrin, immunoglobulin G, ceruloplasmin, type IV collagen, laminin, podocalyxin, VEGF.

Tubular biomarkers - Neutrophil Gelatinase Associated Lipocalin (NGAL), Alpha 1 microglobulin, Kidneyinjury molecule-1(KIM-1), N-Acetyl-β-Dglucosaminidase(NAG), cystatin C, angiotensinogen, liver-type fatty acid binding protein.

Inflammatory markers - TNF-alpha, orosomucoid.

Though small studies showed promise of multiple biomarkers in early diagnosis of DN, larger longitudinal trials are required to validate their clinical use in day to day practice\textsuperscript{[63]}.

Treatment

The progression of DN takes several years and several factors are known to modulate the course of nephropathy including glycemic control, hypertension, dyslipidemia, smoking, anemia, obesity and protein consumption. The “multifactorial” presence of treatable factors has led to the recommendation of “multifactorial approach” of therapeutic interventions for prevention at different stages of nephropathy. (Figure 4)

Smoking

Quitting smoking is mandatory as it is considered as important risk factor for DN which was recently confirmed by trial in T1DM patients which showed 12 year progressive risk of MA, macroalbuminuria and ESRD was higher in smokers compared to non-smokers\textsuperscript{[64,65]}.
Nutritional management

Significant reduction in BP was noted with dietary salt restriction to less than 5-6 g/d in patients with T1DM and T2DM [66]. Among individuals with T2DM on medical nutrition therapy or with impaired glucose tolerance restriction of salt intake resulted in reduced UAE and BP [67]. Salt intake independently affects the yearly decline of eGFR among T2DM patients with stage 4 CKD [68]. Salt paradox was reported in T1DM, suggesting that both higher and lower urinary sodium levels are associated with reduced survival and highest risk of progression of ESRD was reported with low urinary sodium levels [69].

The 2013 Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines update on diabetes and CKD supported the role of appropriate protein nutrition in DKD. For CKD stages 1 and 2, recommended daily protein intake is 0.8 g/kg and 0.6-0.8 g/kg is allowed in stages 3 and 4 [70].

Optimization of Glycemic control

Studies in T1DM and T2DM have shown favorable impact of blood glucose control on progression of DKD at every stage i.e. from normoalbuminuria to MA (primary prevention), micro to macroalbuminuria (secondary prevention) and macroalbuminuria to ESRD (tertiary prevention). The Diabetes Control and Complications Trial (DCCT) [71] in T1DM, UKPDS in T2DM [72], clearly demonstrated that intensive glycemic control delayed the development and progression of MA. The follow up studies of DCCT (EDIC trial) and UKPDS [72,73] also demonstrated that of initial strict glycemic control persisted far beyond, which is due to metabolic memory or legacy effect.

The more recent ADVANCE, ACCORD, and Veterans Affairs Diabetes trials also confirmed the same [74-76]. Fioretto P et al demonstrated reversal of established glomerular lesions in the native kidneys of T1DM patients with prolonged normalization of glucose levels after successful pancreas transplantation [77]. Though stricter glycemic control is essential, it is associated with risk of hypoglycemia in more advanced stages of DN with excess mortality as observed in ADVANCE and ACCORD. Hence glycemic targets should be individualized aiming to strike a balance between the risk of hypoglycemia and clear benefit of renoprotection. Antidiabetic agents including metformin, thiazolidinediones (TZD’s), glargue like peptide-1 (GLP-1) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors, and SGLT2 inhibitors were reported to have renoprotective benefits beyond glycemic control. Metformin activates adenosine monophosphate kinase (AMPK) pathway which leads to inhibition of mammalian target of rapamycin (mTOR) pathway [78,79]. AMPK activation also protects podocytes from hyperglycemia induced apoptosis [80].

In experimental models peroxisome proliferator activator receptor-gamma (PPAR-γ) agonists reported to have renoprotective benefits [81].

Though experimental studies showed renoprotective benefits of TZD’s, post-hoc analysis of the PROactive (Prospective Pioglitazone Clinical Trial in Macro-vascular Events) study results, did not show any significant change in eGFR compared to placebo [82].

A meta-analysis of 15 TZD trials (10 with pioglitazone and 5 with rosiglitazone) did not report a significant decrease in albuminuria [83]. Safety concerns of TZD’s makes them unlikely to find a position in the management of DN.

Glucagon like peptide 1 (GLP-1) is the incretin hormone derived from the gut showed to have direct renoprotective benefits. The GLP-1 agonists liraglutide in LEADER, semaglutide in SUSTAIN 6 and dulaglutide in AWARD 7 showed significantly better renal outcomes independent of glycemic control. The probable mechanisms involved are reduction in the levels of renal NADPH oxidase, reactive oxygen species (ROS) generation, UAE and mesangial expansion [84,85,86].

Small studies reported renal benefits of sitagliptin and vildagliptin, future trials adequately powered and designed are needed [87]. SAVOR-TIMI with saxagliptin comparing with placebo showed reduction in UAE without effect in eGFR independent of glycemic control [88]. A pooled analysis of four clinical studies in T2DM patients with linagliptin showed 32 % reduction in UAE in linagliptin compared to placebo [89].

SGLT2 inhibition promotes distal delivery of sodium and its absorption in distal tubule, resulting in generation of adenosine which leads to afferent arteriole vasoconstriction reducing glomerular hyperfiltration. SGLT2 inhibitors demonstrated favourable outcome in delaying the progression of DN.

Empagliflozin in EMPA-REG trial and canagliflozin in CANVAS program and CRESCENDO trial showed significantly better renal outcomes in T2DM [90,91,92].

Blood pressure control

Hypertension is an independent modifiable risk factor for the onset and progression of diabetic nephropathy. Glomerular hypertension plays a key role in the pathogenesis of DN even in normotensive diabetic animals indicating that effectively managing systemic blood pressure without reduction in intra glomerular pressure is may not be enough to prevent glomerular injury [93-95]. RAAS blockers are known to reduce both systemic and glomerular hypertension and have a dose dependent action in reducing proteinuria.

Pronounced RAAS blockade using ACEI and ARBs showed increased adverse events and hyperkalemia [96,97]. Similarly, usage of direct renin inhibitor aliskiren along with combination of ACE-I/ARB has been contraindicated following the adverse outcomes
Figure 1: Pathways showing the inflammatory mechanisms in the pathogenesis of diabetic nephropathy. NAD: Nicotinamide adenine dinucleotide; NADH: Redox form of NAD; TGF-β: Transforming growth factor-β; VEGF: Vascular endothelial growth factor

Figure 2: Simplified overview of ROS and its downstream signalling pathways. NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; RAS: Renin-angiotensin system

Figure 3: Hyperglycemia induced upregulation of SGLT2 and its role along RAAS in glomerular hypertension. SGLT2: sodium glucose co-transporter 2 (SGLT2) inhibitors; RAAS: renin-angiotensin-aldosterone-system; Na+: sodium
noted in ATTITUDE trial[99]. RAAS blockers did not prevent the occurrence of the earliest renal histologic lesions of DKD[99].

Though RAAS blockers are used as first-line therapy for hypertension in patients with diabetes, marked reduction in the development of DN is less likely.

National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines do not recommend RAAS blockers in normotensive normoalbuminuric patients with diabetes, although usage of ACEI and ARBs is not suggested in normotensive diabetic patients with microalbuminuria who are at risk for development of DKD in the future[100].

Non-dihydropyridine calcium channel blockers (diltiazem or verapamil) are a better choice, if albuminuria persists along with uncontrolled blood pressure on ACE-I/ARB monotherapy[101]. Dihydropyridine derivatives and diuretics may be used as add on agents[102]. Mineralocorticoid receptor antagonists (MRA) spironolactone, eplerenone, and finerenone are reported to have renoprotective effects but combination with RAS inhibition resulted in greater risk of hyperkalemia[103-105].

Stricter BP control is very important and must be continued long term if the benefits are to be maintained.

| Table 1: Definition of normal urine albumin excretion (UAE), MA, macro albuminuria |
|---------------------------------|-----------------|-----------------|
|                                   | Normal UAE      | MA              | Macro albuminuria |
| Albumin: creatine ratio (µg/mg)   | <30             | 30-300          | >300             |
| Urine albumin excretion          | <20             | 20-200          | >200             |
| 24hr urine albumin excretion (mg/24h) | <30             | 30-300          | >300             |
| KDIGO terminology for albuminuria based on albumin: creatinine ratio (mg/mmol) | A1 | A2 | A3 |

Figure 4: Approved and emerging treatments for diabetic nephropathy. TZD’s: thiazolidinediones; GLP1: Glucagon-like peptide-1; DPP-4: dipeptidyl peptidase-4; SGLT2: sodium-glucose cotransporter 2; CCB’s: calcium channel blockers; PKC: protein kinase C; AGE: advanced glycation end product; RAGE: receptor for advanced glycation endproduct; ACE-I: angiotensin converting enzyme-inhibitor; ARB: angiotensin II receptor blocker; AR inhibitors: aldose reductase inhibitors; MRA: mineralocorticoid receptor antagonists; ERA: endothelin receptor antagonists; Nrf2: nuclear factor-like 2; NF-κB:nuclear factor kappa-light-chain-enhancer of activated B cells; JAK/STAT: Janus kinase-signal transducer and activator transcription factor; IL17A : interleukin 17 agonist; CCR: C-C chemokine receptor; TGF-β1: transforming growth factor β1; VEGF: vascular endothelial growth factor; GBM: glomerular basement membrane; CCN2: connective tissue growth factor.
Lipid lowering therapy

The renoprotective effects of statins in T1DM and T2DM patients with micro and macroalbuminuria are variable and RCT’s with statistically significant results are lacking.

The statin therapies in DN showed reduction in the risk of major atherosclerotic events, in CKD stages 1-4 or post renal transplant patients, but not in patients on maintenance hemodialysis as evidenced in AU-RORA trial [109].

Due to concern about myopathy with higher doses of statins in CKD patients, concomitant usage of ezetimibe is also recommended to achieve better reduction of LDL-C with lower doses of statins [107]. For patients with ESRD both CAPD and hemodialysis have similar outcomes and kidney transplantation is an option provided if cardiovascular status is good.

Hyperuricemia: Serum UA is a strong and independent predictor for the increased UAE and overt proteinuria in T1DM patients [108]. A study reported that for every 1 mg/dL increase in serum UA the probability of development of albuminuria increased by 80% [109]. Another study in T1DM patients showed 2.4 folds increase in the unadjusted risk of eGFR loss with serum UA >6.6 mg/dL [110].

Allopurinol and febuxostat showed significant decrease in UAE and eGFR decline among T2DM patients [111,112].

Phosphate management

Hyperphosphatemia is due to impaired excretion of phosphate by the failing kidney. Renal elimination of phosphate is dependent of FGF23. It was reported that oral non calcium phosphate binder sevelamer carbonate has marked antiinflammatory properties and showed significant reduction in FGF23 levels, markers of inflammation and oxidative stress [113]. Dietary restriction of phosphate and sevelamer together significantly reduced the overall mortality and progression to renal replacement therapy among T2DM predialysis CKD patients [114].

Vitamin D receptor activators

Findings from phase III VITAL study [115] showed that paricalcitol seems effective only at a high dose (2 μg), and its effect in slowing the progression of CKD is still awaited.

Pentoxifylline

It showed renoprotective benefits in smaller trials which need to be validated in a larger population [116].

Newer therapies

The suboptimal preventive effect of current medication led to studies testing novel medications for DN, including inhibitors of advanced glycation end-product formation and agents to reduce oxidative stress and inflammation. Many newer agents like ruboxistaurin, bardoxolone methyl, sulodexide have shown negative outcomes in RCT’s and the role of aldose reductase inhibitors in DN is still unsatisfactory.

Endothelin receptor antagonists (ERA)

The ASCEND trial using Avosentan was terminated due to increased risk of fluid overload and congestive heart failure despite its favorable effects of reducing albuminuria [117]. A phase III trial (SONAR) with Atrasentan is ongoing which aims to clarify the cardiovascular and renal protective effects of ET receptor antagonists [118].

Exogenous klotho [119] showed renoprotective effects in animals models. Human data is yet to be available.

Many newer agents like AMPK activators, nuclear factor (erythroid-derived 2)-like factor 2 (NFE2L2) activators, inhibitors of renal leukocyte recruitment, exogenous idofo, low dose Interleukin 17A, ruboxistaurin, bardoxolone methyl, sulodexide and aldose reductase inhibitors need longer well powered RCT’s to identify their role in management of DN.

Stem cell therapy in animal DN models has not yet shown efficacy [120].

CONCLUSION

Although the recent advances in therapeutic strategy have meaningfully improved outcomes for diabetes complications, these improvements have not translated nearly as well to DN. The ongoing robust researches to explore the unanswered questions may provide new insights on the complex pathogenesis, to facilitate early diagnosis, prevention and tailored intervention to reduce the incidence and minimize progression, so as to relieve the huge burden of CKD.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial materials in the subject matter or material discussed in this manuscript.

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