Diabetic nephropathy (DN) is a major microvascular complication of diabetes and a leading cause of end-stage renal disease (ESRD) worldwide. DN is classically understood as a glomerular disease. However, this understanding is currently being contested with recent studies suggesting a pivotal role of the tubulointerstitium in the initiation and progression of DN. We performed a review of the current literature as detailed below. The literature suggests that chronic hyperglycemia may orchestrate several structural and functional abnormalities of the tubulointerstitium before significant glomerular changes. Abnormalities such as increased advanced glycation end products, oxidative stress, cytokine activation, inflammatory markers, and enhanced hypoxic milieu may result in progressive tubulointerstitial damage as reflected by dysfunction of tubular protein receptors such as cubulin and megalin, loss of charge-dependent tubular protein reabsorption, resulting in higher excretion of tubular injury markers such as N-acetyl-β-glucosaminidase (NAG) and retinol-binding protein (RBP), and overflow microalbuminuria in the presence of intact glomerulus and before the onset of microalbuminuria. In addition, people with diabetes may have higher excretion of NAG and RBP and low levels of tubular hormones such as erythropoietin and 1,25-dihydroxyvitamin D, before the onset of microalbuminuria, suggesting that tubulointerstitial changes are present before the onset of microalbuminuria. These abnormalities are highly suggestive of DN being of tubular, rather than glomerular origin.

**Introduction**

Diabetes mellitus (DM) is now pandemic and with an estimated 40 million people affected, India has acquired the dubious distinction of being labelled the diabetes capital of the world.[1] The treatment cost of diabetes and diabetes related complications is enormous and is a major burden on the limited resources of India and other developing countries. Diabetic nephropathy (DN) is a major microvascular complication of diabetes and a leading cause of end-stage renal disease (ESRD) worldwide; accounting for up to 40% of people requiring renal replacement therapy.[2] Epidemiological studies in the Indian diabetic population have estimated the prevalence of microalbuminuria and DN at 26.9 and 2.2%, respectively.[3]

DN is classically understood as a glomerular disease. Five different stages are recognized, beginning with glomerular hyperfiltration, and progressing through incipient nephropathy, microalbuminuria, overt proteinuria, finally to ESRD.[4] Persistent microalbuminuria is considered as incipient DN. Overtime, microalbuminuria may progress to overt (dipstick positive) proteinuria, which is the hallmark of established DN.[5] The collective risk of progression to renal failure after 5 years of persistent proteinuria is approximately 60% in both type 1 and type 2 diabetes.[6]

**Histological changes in diabetic nephropathy**

The histological changes in DN encompass structural changes in all the compartments of the kidney. The earliest morphological change in DN is glomerular basement membrane (GBM) thickening as a result of mesangial matrix deposition and hypertrophy of mesangial cells.[7] With disease progression, there is enhanced deposition of the normal extracellular matrix of type IV collagen, laminin, and fibronectin as a result of enhanced production and/or decreased degradation.[8]
This results in a diffuse mesangial expansion also termed as diabetic glomerulosclerosis, which can be associated with nodular lesions consisting of areas of marked mesangial expansion with a fencing of mesangial nuclei surrounding the nodule and compression of the associated glomerular capillaries (Kimmelstiel–Wilson nodules). These abnormalities are paralleled by tubulointerstitial changes including thickening of tubular basement membrane, suggesting that the glomerular hemodynamic disturbances are not a prerequisite for the tubular lesions. In contrast to the mesangium, initial tubulointerstitial expansion is primarily due to an increase in the cellular component of this renal compartment. The mesangial expansion encroaches upon the afferent and efferent arterioles leading to hyalinosis, which may contribute to glomerular sclerosis, through severe compromise of the glomerular blood flow. Efferent arteriolar hyalinisation is pathognomonic for DN. In the tubulointerstitium, thickening of the tubular basement membrane, tubular atrophy, interstitial fibrosis, and peritubular capillary rarefaction is seen. In addition, DN is characterized by the presence of fibrin caps, capsular drops, and capillary microaneurysms.

Although historically regarded as a primarily glomerular disease, due to the first clinical presentation as microalbuminuria, the characteristic findings of glomerulosclerosis are present in only one-third of patients with microalbuminuria, while another third has normal renal structure. The remaining third has lack of or has minimal glomerular changes, but disproportionately severe lesions in the tubulointerstitium. In addition, histological studies suggest that the decline of renal function in DN, as in other chronic renal diseases correlates better with tubulointerstitial changes than with glomerular changes.

**Early microalbuminuria: Glomerular or tubular?**

Microalbuminuria is an intermediary stage and one of the important predictors of progression of DN. An understanding of renal handling and excretion of various proteins in normal and diseased kidneys is vital for diagnosis and monitoring the progression of proteinuria in patients with kidney disease. Renal excretion of any substance is a balance between glomerular filtration, tubular reabsorption, and tubular secretion. Albumin with a molecular size of 65 kDa constitutes about 60% of the plasma proteins and is a major protein molecule excreted in urine. The amount of urinary albumin excretion is influenced by glomerular filtration rate, size, and electrical charge of the molecule, with more than 95% of the filtered albumin being reabsorbed by the tubules.

In healthy individuals, daily albumin excretion is about 25 mg/day or less. The endocytic uptake of albumin and other proteins is subjected to a complex hormonal and enzymatic regulation in the tubular milieu. Two major cellular pathways: the degradation pathway and the retrieval pathway are recognized in the handling of albumin. Most of the albumin is returned to the blood supply by the retrieval pathway, which works through the process of endocytosis. This process is carried out by two major protein-binding receptors such as megalin and cubulin along with other low molecular weight binding proteins on the tubular surface.

Megalin is a 600 kDa glycoprotein molecule, expressed on the apical membrane of the proximal tubules and is the major port for endocytosis of low molecular weight (LMW) proteins such as α-1 microglobulin, β-2 microglobulin, light chains, insulin, retinol binding protein (RBP), and vitamin D binding protein along with several other small molecules. Increased urinary excretion of these small proteins is an indicator of tubular dysfunction and damage. Cubulin is a 460kDa glycoprotein, which is the main receptor for large molecular weight proteins found in the glomerular filtrate such as lipoproteins (apo A, apo B, oxidized LDL, and HDL) and transferrin.

Along with the above-mentioned small proteins, one of the widely used tubular injury markers is N-acetyl-β-glucosaminidase (NAG). This is a large protein of the size between 130 and 150 kDa and is present in the serum in very low concentrations. Its presence in the urine invariably signifies tubular damage, as due to its large size, it is not filtered by the glomerulus, but is released in the tubular lumen as a result of proximal tubular damage. Urinary NAG levels reflect the activity of the lysosomal system, which is modulated by the process of endocytosis, in the tubular lumen. Hence, higher excretion of NAG is considered as a reflection of tubular damage and dysfunction.

In patients with DN, early proteinuria incorporates glomerular and tubular components. Tubular dysfunction in type 1 and type 2 DM with higher urinary excretion of tubular injury markers such as...
Elevated AGE as a result of chronic hyperglycemia may lead to megalin receptor damage and apoptosis of endothelial cells. Chronic hyperglycemia may also promote enhanced production of basement membrane components (collagen type IV and fibronectin), inflammatory markers (CRP, haptoglobin, and alpha-1 acid glycoprotein), cytokine system activation (IL-1β, IL-6, and TNF-α), and apoptosis of endothelial cells. In addition, chronic hyperglycemia may initially promote proximal tubular hypertrophy due to increased absorption of glucose, independent of hemodynamic, glomerular, or vascular pathology. However, in the long run, chronic hyperglycemia in conjunction with AGE may lead to tubular apoptosis as a result of chronic oxidative stress.

AGE and megalin dysfunction

AGES are a heterogenous group of end-product molecules produced as a result of nonenzymatic covalent bonding of glucose residues with free amino groups of proteins, lipids, and nucleic acids. Increased production of AGE has been implicated in the genesis of glomerulopathy and tubulopathy in DN. AGE is freely filtered by the glomerulus and is metabolized by the proximal tubule through megalin receptor. Binding of AGE with receptor for AGE (RAGE) on the tubular surface has been implicated in the pathogenesis of tubular cell injury. Elevated AGE as a result of chronic hyperglycemia may lead to megalin receptor damage and dysfunction resulting in higher excretion of tubular injury markers such as NAG, RBP, and several other LMW proteins, leading to functional microalbuminuria.

The tubulointerstitium in diabetes

Absorption of glucose by the tubular cells is independent of insulin action and hence makes the renal tubule vulnerable to glucotoxicity in periods of hyperglycemia. Chronic hyperglycemia in diabetes may promote tubulointerstitial damage and fibrosis by several direct and indirect mechanisms. Hyperglycaemia may modulate several aspects of the tubulointerstitial milieu through activation of glucose-dependent metabolic pathways (sorbitol, diacylglycerol/protein kinase C, and hexosamine), enhanced production of advanced glycation end products (AGE) and angiotensin II. Chronic hyperglycemia also promotes enhanced production of basement membrane components (collagen type IV and fibronectin), inflammatory markers (CRP, haptoglobin, and alpha-1 acid glycoprotein), cytokine system activation (IL-1β, IL-6, and TNF-α), and apoptosis of endothelial cells. In addition, chronic hyperglycemia may initially promote proximal tubular hypertrophy due to increased absorption of glucose, independent of hemodynamic, glomerular, or vascular pathology. However, in the long run, chronic hyperglycemia in conjunction with AGE may lead to tubular apoptosis as a result of chronic oxidative stress.

Functional vs. persistent microalbuminuria

Several studies have reported the association of increased excretion of tubular injury markers such as NAG and RBP with hyperglycemia and a decrease in these markers with improvement in glycemic control. As alluded earlier, early microalbuminuria in diabetes consists of glomerular and tubular components. There is a wide biological variation in microalbuminuria and it may be transient in several conditions such as poor glycemic control, exercise, urinary tract infection, isolated hypertension, and heart failure. To overcome this difficulty, it is more reliable to report the level of microalbuminuria as urinary albumin to creatinine ratio or ACR.

The prevalence of microalbuminuria in nondiabetic general population has been reported to be up to 13%. In view of the natural variability of albumin excretion, the diagnosis of persistent microalbuminuria requires ACR levels exceeding threshold values on two or more consecutive occasions, ideally within 1–3 months. There are a number of plausible mechanisms which might account for increased excretion of LMW proteinuria before the onset of persistent microalbuminuria. Hyperglycemia may increase tubular workload consequent to enhanced glucose absorption. Preferential absorption of larger proteins such as albumin in this setting may result in urinary leakage of the LMW proteins.

Chronic hyperglycemia in conjunction with higher levels of AGE products may impair the functional ability of megalin and cubulin receptors, resulting in reduced absorption of LMW protein. Chronic hyperglycemia has also been reported to cause early damage to charge-dependent tubular reabsorption before the loss of glomerular charge barrier. This may lead to inefficient tubular absorption of large and LMW proteins from the glomerular filtrate, contributing to early microproteinuria. In the long run, chronic hyperglycemia, through direct and indirect mechanisms, may institutionalize dysfunction of the tubules by causing permanent and progressive damage to the interstitium. This may result in the functional microalbuminuria becoming persistent. The process of structural alteration of the tubulointerstitium by chronic hyperglycemia may involve several mechanisms.

Tubulointerstitial injury and fibrosis

Proteinuria is an important predictor of progression of renal disease; and persistent protein load in the
tubular milieu as a result of impaired absorption may lead to injury and scarring of the tubular lumen.

This may lead to increased oxidative stress and stimulate activation of proinflammatory cytokines in the tubular cells. Chronic hyperglycemia adversely affects the tubulointerstitial milieu by facilitating several tissue injury mediators such as AGE, reactive oxygen species, inflammatory cytokines, and angiotensin II.

Diabetes is associated with decreased renal oxygen tension. Increased oxidative stress results in reduced nitric oxide, leading to an increased vascular tone and oxygen consumption.

In addition, chronic hyperglycemia may enhance the lactate concentration in the renal medulla and adversely modulate the intrarenal blood flow regulation. A reduction in the renal oxygen tension associated with inadequate nitric oxide may increase free radical damage in the tubulointerstitium. Tubulointerstitial fibrosis may lead to local ischemia by impairing diffusion from the capillary to the tubule.

Chronic hyperglycemia along with progressive microproteinuria and factors promoting increased oxidative stress may potentially facilitate endothelial and tubular cell apoptosis by promoting a hypoxic milieu in the tubulointerstitium, which if chronic, may lead to tubulointerstitial injury and fibrosis. The adverse functional and structural alterations in the tubulointerstitium may impact upon the production of important tubular hormones such as erythropoietin and 1,25-dihydroxyvitamin D.

Recently, we demonstrated the presence of low erythropoietin and 1,25-dihydroxyvitamin D along with higher excretion of tubular injury markers such as NAG and RBP in normoalbuminuric patients with type 1 and type 2 diabetes. The presence of tubular abnormalities associated with higher excretion of tubular injury markers along with reduced secretion of tubulointerstitial hormones, in the absence of glomerular proteinuria, favors the hypothesis that alterations first occur in the tubulointerstitium and this may initiate the process of DN.

Tubular hormones in DN

The renal tubulointerstitium is the primary site of synthesis of erythropoietin and hydroxylation of 25 hydroxyvitamin D to 1,25-dihydroxyvitamin D. Anaemia as a result of erythropoietin deficiency occurs early in both type 1 and type 2 DM, ever before significant loss in renal function. The low erythropoietin in these patients has been attributed to impaired functioning of the erythropoietin producing fibroblasts as a result of tubulointerstitial fibrosis.

EPO is a trophic hormone and its pleiotropic effects extend well beyond erythropoiesis. It has important cytoprotective effects, including protection from ischemic injury, inhibition of apoptotic death-related pathways, possess antioxidant and anti-inflammatory properties. EPORs have been demonstrated throughout the kidney, including both proximal and distal tubular cells. However, the affinity of EPOR to EPO is well below the normal plasma EPO concentration, suggesting that these EPORs respond to the circulating EPO in a paracrine fashion.

1,25-dihydroxyvitamin D is also a pleiotropic hormone. It manifests its action by binding to vitamin D nuclear receptor (VDR). The receptor for 1,25-dihydroxyvitamin D has been identified on several tissues including the intestines, bone, kidney (glomerular podocytes, proximal and distal tubules, collecting ducts), parathyroid gland, and pancreatic β-cells, among others. The presence of VDR on these tissues suggest the likely sites of trophic action of 1,25-dihydroxyvitamin D. Low levels of 1,25-dihydroxyvitamin D, and EPO, occurring before the onset of microalbuminuria, may result in inadequate protection against glomerulosclerosis and proteinuria, and may potentially, facilitate tubulointerstitial fibrosis and progression of DN.

Conclusion

This sequence of events occurring in the tubulointerstitium suggests that early DN is essentially a tubular disease, which is subsequently superseded by the glomerular component. Chronic hyperglycemia may adversely modulate the structural and functional integrity of tubulointerstitium by virtue of promoting several abnormalities such as increased AGE, oxidative stress, cytokine activation, and inflammatory markers, and enhanced hypoxic milieu. This may result in progressive tubulointerstitial damage as reflected by megalin dysfunction and loss of charge-dependent tubular protein reabsorption, resulting in higher excretion of tubular injury markers and overflow microproteinuria.

The functional damage once institutionalised may result in permanent structural damage and apoptosis of tubular cells, due to several factors as mentioned above. The early loss of tubular cells results in inadequate synthesis of erythropoietin and 1,25-dihydroxyvitamin D. Low levels
of these hormones mean relatively inadequate trophic effects of these hormones; which in turn, indirectly, may facilitate structural abnormalities in the tubules and glomerulus, leading to persistent microproteinuria and finally microalbuminuria.

Microalbuminuria is the first clinical indicator of presence of DN and reduction in levels of microalbuminuria is known to slow the progression of DN.

Current clinical strategies focus on maintaining optimum glycemic control and blood pressure and lifestyle measures (regular physical activity, dietary control, and avoidance of smoking). These therapies play an important role to slow the progression of DN, as suggested by major studies such as UKPDS and DCCT. However, despite these measures, the residual risk of these patients for progression of microalbuminuria and DN remains high. Low erythropoietin and 1,25-dihydroxyvitamin D, in the presence of higher tubular injury markers, may be good indicators of presence of structural and functional tubular dysfunction.

The period between diagnosis of diabetes and onset of persistent microalbuminuria may be regarded as a "golden opportunity" for diagnosis and intervention for tubular dysfunction and damage. The future of prevention of DN lies in exploration of other robust tubular injury markers for confirmatory diagnosis of tubular damage and therapeutic intervention to arrest the progression of this stage. Large-scale studies are warranted to confirm the findings from the smaller studies on tubular dysfunction; and to explore interventional opportunities, before the onset of microalbuminuria.

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References

Singh and Farrington: The tubulointerstitium in early diabetic nephropathy


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