[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

The tubulointerstitium in early diabetic nephropathy: Prime target or bystander?

Dhruv K. Singh, Ken Farrington[1]

Post Doctoral Fellow, Diabetes and Renal Medicine, Lister Hospital, Coreys Mill Lane, Stevenage, SG1 4AB, UK,
[1]Consultant Nephrologist Lister Hospital, Stevenage, UK

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 megalin and 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 microproteinuria 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.

KEY WORDS: Cubulin, diabetic nephropathy, erythropoietin, megalin, microalbuminuria, NAG, RBP, tubular dysfunction, 1,25-dihydroxyvitamin D

DOI: 10.4103/0973-3930.70857

Correspondence to: **Dr. Dhruv K. Singh**, Renal Unit, Lister Hospital Stevenage, UK. E-mail: dsingh4@nhs.net

Manuscript received: 25.02.2010; Revision accepted: 26.08.2010

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]

[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

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).[7]

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.[9] In contrast to the mesangium, initial tubulointerstitial expansion is primarily due to an increase in the cellular component of this renal compartment.[10] 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.[11] In the tubulointerstitium, thickening of the tubular basement membrane, tubular atrophy, interstitial fibrosis, and peritubular capillary rarefaction is seen.[12] In addition, DN is characterized by the presence of fibrin caps, capsular drops, and capillary microaneurysms.[11]

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.[13] 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.[14]

Early microalbuminuria: Glomerular or tubular?

Microalbuminuria is an intermediary stage and one of the important predictors of progression of DN.[15] 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.[16] 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.[17]

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.[18] 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.[19]

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.[20] 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.[19]

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.[21] 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.[22]

In patients with DN, early proteinuria incorporates glomerular and tubular components.[23] Tubular dysfunction in type 1[23] and type 2 DM,[21] with higher urinary excretion of tubular injury markers such as

[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

NAG and RBP, in the absence of microalbuminuria, has been previously reported and recently confirmed by our group.[24] These findings suggest early derangements in the tubulointerstitial milieu, before the onset of 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.[25] 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),[26] enhanced production of advanced glycation end products (AGE) and angiotensin II.[27]

Chronic hyperglycemia also promotes enhanced production of basement membrane components (collagen type IV and fibronectin),[28] inflammatory markers (CRP, haptoglobin, and alpha-1 acid glycoprotein),[29] cytokine system activation (IL-1β, IL-6, and TNF-α),[30] and apoptosis of endothelial cells.[31] In addition, chronic hyperglycemia may initially promote proximal tubular hypertrophy due to increased absorption of glucose,[25] 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.[32]

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.[33] Increased production of AGE has been implicated in the genesis of glomerulopathy[33] and tubulopathy[34] in DN. AGE is freely filtered by the glomerulus and is metabolized by the proximal tubule through megalin receptor.[35] Binding of AGE with receptor for AGE (RAGE) on the tubular surface has been implicated in the pathogenesis of tubular cell injury.[34] 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.

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.[23,36] As alluded earlier, early microalbuminuria in diabetes consists of glomerular and tubular components.[23] 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.[37] To overcome this difficulty, it is more reliable to report the level of microalbuminuria as urinary albumin to creatinine ratio or ACR.[38]

The prevalence of microalbuminuria in nondiabetic general population has been reported to be up to 13%.[37] 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.[38] 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.[22]

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.[39] 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

[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

tubular milieu as a result of impaired absorption may lead to injury and scarring of the tubular lumen.[22] 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.[40]

Diabetes is associated with decreased renal oxygen tension.[41] Increased oxidative stress results in reduced nitric oxide, leading to an increased vascular tone and oxygen consumption.[41] In addition, chronic hyperglycemia may enhance the lactate concentration in the renal medulla and adversely modulate the intrarenal blood flow regulation.[42] 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.[40] 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.[24] 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.[24] Anaemia as a result of erythropoietin deficiency occurs early in both type 1 and type 2 DM, ever before significant loss in renal function.[43] The low erythropoietin in these patients has been attributed to impaired functioning of the erythropoietin producing fibroblasts as a result of tubulointerstitial fibrosis.[44]

EPO is a trophic hormone and its pleiotropic effects extend well beyond erythropoiesis.[45] It has important cytoprotective effects, including protection from ischemic injury, inhibition of apoptotic death-related pathways, possess antioxidant and anti-inflammatory properties.[46] EPORs have been demonstrated throughout the kidney, including both proximal and distal tubular cells.[47] 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.[46]

1,25-dihydroxyvitamin D is also a pleiotropic hormone.[48] 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.[49] 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,[24] 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

[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

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.[50] 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[51] and DCCT.[52] 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.

Acknowledgments

We thank Carol Milton and Maureen Alexander, library staff at Lister Hospital, for helping us in obtaining some of the older references.

References

1. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 2007;125:217-30.
2. Ritz E, Rychlik I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. Am J Kidney Dis 1999;34:795-808.
3. Unnikrishnan RI, Rema M, Pradeepa R, Deepa M, Shanthirani CS, Deepa R, *et al*. Prevalence and Risk Factors of Diabetic Nephropathy in an Urban South Indian Population. Diabetes Care 2007;30:2019-24.
4. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes 1983;32:64-78.
5. Phillips AO, Steadman R. Diabetic nephropathy: The central role of renal proximal tubular cells in tubulointerstitial injury. Histol Histopathol 2002;17:247-52.
6. Hasslacher C, Ritz E, Wahl P, Michael C. Similar Risks of Nephropathy in Patients with Type-I Or Type-Ii Diabetes-Mellitus. Nephrol Dial Transplant 1989;4:859-63.
7. Wolf G, Reinking R, Zahner G, Stahl RA, Shankland SJ. Erk 1,2 phosphorylates p27(Kip1): Functional evidence for a role in high glucose-induced hypertrophy of mesangial cells. Diabetologia 2003;46:1090-9.
8. Kim Y, Kleppel MM, Butkowski R, Mauer SM, Wieslander J, Michael AF. Differential expression of basement membrane collagen chains in diabetic nephropathy. Am J Pathol 1991;138:413-20.
9. Brito PL, Fioretto P, Drummond K, Kim Y, Steffes MW, Basgen JM, *et al*. Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Kidney Int 1998;53:754-61.
10. Katz A, Caramori ML, Sisson-Ross S, Groppoli T, Basgen JM, Mauer M. An increase in the cell component of the cortical interstitium antedates interstitial fibrosis in type 1 diabetic patients. Kidney Int 2002;61:2058-66.
11. Alsaad KO, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: An update. J Clin Pathol 2007;60:18-26.
12. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM. Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. Kidney Int 1991;40:107-14.
13. Dalla VM, Saller A, Bortoloso E, Mauer M, Fioretto P. Structural involvement in type 1 and type 2 diabetic nephropathy. Diabetes Metab 2000;26:8-14.
14. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: More than an aftermath of glomerular injury? Kidney Int 1999;56:1627-37.
15. Rossing P, Hommel E, Smidt UM, Parving HH. Reduction in albuminuria predicts diminished progression in diabetic nephropathy. Kidney Int Suppl 1994;45:S145-9.
16. Gekle M. Renal Proximal Tubular Albumin Reabsorption: Daily Prevention of Albuminuria. News Physiol Sci 1998;13:5-11.
17. Bourdeau JE, Carone FA. Protein handling by the renal tubule. Nephron 1974;13:22-34.
18. Abbate M., Remuzzi G. Novel mechanism(s) implicated in tubular albumin reabsorption and handling. Am J Kidney Dis 2001;38:196-204.
19. Verroust PJ, Christensen EI. Megalin and cubilin--the story of two multipurpose receptors unfolds. Nephrol Dial Transplant 2002;17:1867-71.
20. Saito A, Nagai R, Tanuma A, Hama H, Cho K, Takeda T, *et al*. Role of megalin in endocytosis of advanced glycation end products: Implications for a novel protein binding to both megalin and advanced glycation end products. J Am Soc Nephrol 2003;14:1123-31.
21. Basturk T, Altuntas Y, Kurklu A, Aydin L, Eren N, Unsal A. Urinary N-acetyl B glucosaminidase as an earlier marker of diabetic nephropathy and influence of low-dose perindopril/indapamide combination. Renal Failure 2006;28:125-8.
22. Burton C, Harris KP. The role of proteinuria in the progression of chronic renal failure. Am J Kidney Dis 1996;27:765-75.
23. Gibb DM, Tomlinson PA, Dalton NR, Turner C, Shah V, Barratt TM. Renal tubular proteinuria and microalbuminuria in diabetic patients. Arch Dis Child 1989;64:129-34.
24. Singh DK, Winocour P, Summerhayes B, Viljoen A, Sivakumar G, Farrington K. Are low erythropoietin and 1,25-dihydroxyvitamin D levels indicative of tubulo-interstitial dysfunction in diabetes

[Downloaded free from http://www.ijddc.com on Monday, October 11, 2010, IP: 59.183.141.47]

without persistent microalbuminuria? Diabetes Res Clin Pract 2009;85:258-64.
25. Jones SC, Saunders HJ, Pollock CA. High glucose increases growth and collagen synthesis in cultured human tubulointerstitial cells. Diabet Med 1999;16:932-8.
26. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: The role of endothelial dysfunction. Clin Sci (Lond) 2005;109:143-59.
27. Koka V, Wang W, Huang XR, Kim-Mitsuyama S, Truong LD, Lan HY. Advanced Glycation End Products Activate a Chymase-Dependent Angiotensin II-Generating Pathway in Diabetic Complications. Circulation 2006;113:1353-60.
28. Cagliero E, Maiello M, Boeri D, Roy S, Lorenzi M. Increased expression of basement membrane components in human endothelial cells cultured in high glucose. J Clin Invest 1988;82:735-8.
29. McMillan DE. Increased levels of acute-phase serum proteins in diabetes. Metabolism 1989;38:1042-6.
30. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, *et al*. Inflammatory cytokines and the risk to develop type 2 diabetes: Results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52:812-7.
31. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhausl W. High-glucose--triggered apoptosis in cultured endothelial cells. Diabetes 1995;44:1323-7.
32. Yaqoob M, McClelland P, Patrick AW, Stevenson A, Mason H, White MC, *et al*. Evidence of Oxidant Injury and Tubular Damage in Early Diabetic Nephropathy. QJM 1994;87:601-7.
33. Bucala R, Vlassara H. Advanced glycosylation end products in diabetic renal and vascular disease. Am J Kidney Dis 1995;26:875-88.
34. Simm A, Münch G, Seif F, Schenk O, Heidland A, Richter H, *et al*. Advanced glycation endproducts stimulate the MAP-kinase pathway in tubulus cell line LLC-PK1. FEBS Lett 1997;410:481-4.
35. Gugliucci A, Bendayan M. Renal fate of circulating advanced glycated end products (AGE): Evidence for reabsorption and catabolism of AGE-peptides by renal proximal tubular cells. Diabetologia 1996;39:149-60.
36. Salem MA, El-Habashy SA, Saeid OM, El-Tawil MM, Tawfik PH. Urinary excretion of n-acetyl-beta-D-glucosaminidase and retinol binding protein as alternative indicators of nephropathy in patients with type 1 diabetes mellitus. Pediatr Diabetes 2002;3:37-41.
37. Haffner SM, Stern MP, Gruber MK, Hazuda HP, Mitchell BD, Patterson JK. Microalbuminuria. Potential marker for increased cardiovascular risk factors in nondiabetic subjects? Arteriosclerosis 1990;10:727-31.
38. Burden R, Tomson C. Identification, management and referral of adults with chronic kidney disease: Concise guidelines. Clin Med 2005;5:635-42.
39. Kunika K, Yamaoka T, Itakura M. Damage of charge-dependent renal tubular reabsorption causes diabetic microproteinuria. Diabetes Res Clin Pract 1997;36:1-9.
40. Singh DK, Winocour P, Farrington K. Mechanisms of disease: The hypoxic tubular hypothesis of diabetic nephropathy. Nat Clin Pract Nephrol 2008;4:216-26.
41. Palm F, Cederberg J, Hansell P, Liss P, Carlsson PO. Reactive oxygen species cause diabetes-induced decrease in renal oxygen tension. Diabetologia 2003;46:1153-60.
42. Palm F, Buerk DG, Carlsson PO, Hansell P, Liss P. Reduced nitric oxide concentration in the renal cortex of streptozotocin-induced diabetic rats - Effects on renal oxygenation and microcirculation. Diabetes 2005;54:3282-7.
43. Bosman DR, Winkler AS, Marsden JT, Macdougall IC, Watkins PJ. Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 2001;24:495-9.
44. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, *et al*. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996;39:1569-76.
45. Buemi M, Cavallaro E, Floccari F, Sturiale A, Aloisi C, Trimarchi M, *et al*. The pleiotropic effects of erythropoietin in the central nervous system. J Neuropathol Exp Neurol 2003;62:228-36.
46. Brines M., Cerami A. Discovering erythropoietin's extra-hematopoietic functions: Biology and clinical promise. Kidney Int 2006;70:246-50.
47. Westenfelder C, Biddle DL, Baranowski RL. Human, rat, and mouse kidney cells express functional erythropoietin receptors. Kidney Int 1999;55:808-20.
48. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest 2006;116:2062-72.
49. Kovesdy CP, Kalantar-Zadeh K. Vitamin D receptor activation and survival in chronic kidney disease. Kidney Int 2008;73:1355-63.
50. Rossing P, Hommel E, Smidt UM, Parving HH. Reduction in albuminuria predicts a beneficial effect on diminishing the progression of human diabetic nephropathy during antihypertensive treatment. Diabetologia 1994;37:511-6.
51. UK Prospective Diabetes Study Group Tight blood pressure control and risk of macrovascular and microvascular complications in type 2ádiabetes: UKPDS 38. BMJ 1998;317:703-13.
52. The Diabetes Control and Complications Trial Research Group The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. N Engl J Med 1993;329:977-86.

Source of Support: Nil, **Conflict of Interest:** None declared