



Sharma O.P et al.; International Journal of Pharmamedix India, 2013, 1(3), 435-59.

## **“Diabetic Nephropathy- Recent Trends and Future Prospective in its Management.”**

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Note- This article is property of International Journal of Pharmamedix India [ISSN: 2320-1304].

Published by: Pharmamedix India™ [www.pharmamedix.in]

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### **Abstract:**

Diabetic Nephropathy (DN) is a non-communicable metabolic disease which is characterized with reduction in glomerular filtration rate (GFR), protein- urea, and persistent albuminuria. Currently one-third patient with diabetes develop nephropathy making early diagnosis critical in preventing end stage renal failure. Persistent hyperglycemia and hyperlipidaemia involves in various inflammatory and oxidative pathways and protein deposition in extracellular matrix of the nephron with albumin leakage. No significant therapy is currently available in treatment of patients with nephropathy except for fewer agents like RAAS blockers, thiazolidinediones, statins, and fibric acid derivatives are being utilized. This review discussed various presently employed and recently developed therapeutic targets to treat diabetic nephropathy and to improve the function of diabetic kidney. The involvement of autophagy and Nrf2 activators in the pathogenesis of diabetic nephropathy has been currently under intense investigation.

**Keyword:** Diabetic Nephropathy, Therapeutic targets, Autophagy, Nrf2 Activators.

### **Introduction:**

Diabetic Nephropathy (DN) is a metabolic disorder with different etiologies, remains one of the most life threatening diseases worldwide <sup>[1,2]</sup>. WHO projects that diabetic deaths will increase by two thirds between 2008 and 2030 and the estimated diabetes prevalence worldwide for 2011 was 366 million and it is expected to affect 552 million people by 2030 <sup>[3-5]</sup>. The uncontrolled diabetes mellitus often leads to biochemical imbalances that can cause acute life threatening events such as ketoacidosis and hyperosmolar (non-ketotic) coma <sup>[6]</sup>. Serious complications of diabetes are

cataractogenesis, neuropathic pain, stroke, and lower limb amputations nephropathy and microangiopathy cardiomyopathy <sup>[7,8]</sup>. Even though there are strong genetic determinants in the risk of nephropathy, only 40–50% of patients with type1 or type2 diabetes will eventually end up in nephropathy <sup>[9]</sup>. Almost one in three people with type2 diabetes develops renal disease and diabetes is one of the most common causes of renal failure <sup>[10, 11]</sup>.

DN is a clinical syndrome associated with reduction in glomerular filtration rate (GFR), protein- urea, persistent albuminuria (>300 mg/d or >200 µg/min) and normally attributed to chronic hyperglycemia. Serum creatinine

urea or blood urea nitrogen (BUN) and microalbuminuria are earliest non-invasive marker for the development of DN <sup>[12, 13]</sup>. Several urinary markers for glomerular and tubulointerstitial injuries including KIM-1, NGAL and L-FABP, may have the potential to be clinical markers for identifying the development or progression of diabetic nephropathy <sup>[14,15]</sup>. Poor glycemic control as an independent predictor of development and progression of DN.

### Pathogenesis of Diabetic Nephropathy

Although the exact pathophysiology of DN remains mysterious and quite similar in both type 1 and type 2 DM patients <sup>[16]</sup>.

Various mechanisms that leads pathological changes in renal function, protein uria have been proposed and now actively being investigated for designing rationale approach for optimal therapy and prevention of DN <sup>[17, 18]</sup>. The novel target sites for DN have been depicted in Fig.1.

Hyperglycemia can leads to increase the production of reactive oxygen species (ROS) and proinflammatory transcription factor NF- $\kappa$ B (nucleus factor- $\kappa$ B) resulting in increased inflammatory gene expression, activation of protein kinase C (PKC), transforming growth factor- $\beta$  (TGF- $\beta$ ), and the renin-angiotensin-aldosterone system (RAAS), and

| Urinary biomarkers             | Serum biomarkers              | Multiple biomarkers              |
|--------------------------------|-------------------------------|----------------------------------|
| Micro albumin uria             | albumin, transferrin          | tubular injury                   |
| Protein uria                   | alpha-1-microglobulin,        | oxidative stress                 |
| Protein albumin                | Type IV collagen, fibronectin | Inflammation, endothelial damage |
| Blood uria nitrogen            | superoxide-dismutase (Cu-Zn)) | [Cu- mesangial cell expansion    |
| Urinary albumin excretion rate | alpha-2-glycoprotein-1        | glomerular filtration rate       |

increased formation of advanced glycation end-products (AGEs). These factors lead to cell injury and apoptosis of podocytes and accumulation of extracellular matrix proteins in the glomerulus and the tubulointerstitium [19,20]. Recently evidence indicates that altered insulin signaling play an important role in podocyte inflammation and microalbuminuria except hyperglycemia [21,22]. Additionally to in vivo studies cell culture studies in podocytes indicate that high glucose can activate several of these processes directly, including the RAAS, and result

in podocyte damage, which is a hallmark of diabetic nephropathy<sup>[23,24]</sup>. Cell culture studies involves PPPR- $\gamma$  activation can block the effect of glucose and RAAS in inducing podocytes apoptosis and similar finding also obtained in-vivo models of renal diseases associated with diabetes<sup>[25]</sup>. The increasing evidence for epigenetic mechanisms playing an important role in DN<sup>[26,27]</sup>. Epigenetic alterations result in regulation of genes that are involved in regulating the metabolism of diabetic complications including NF- $\kappa$ B and AGEs<sup>[28-30]</sup>.

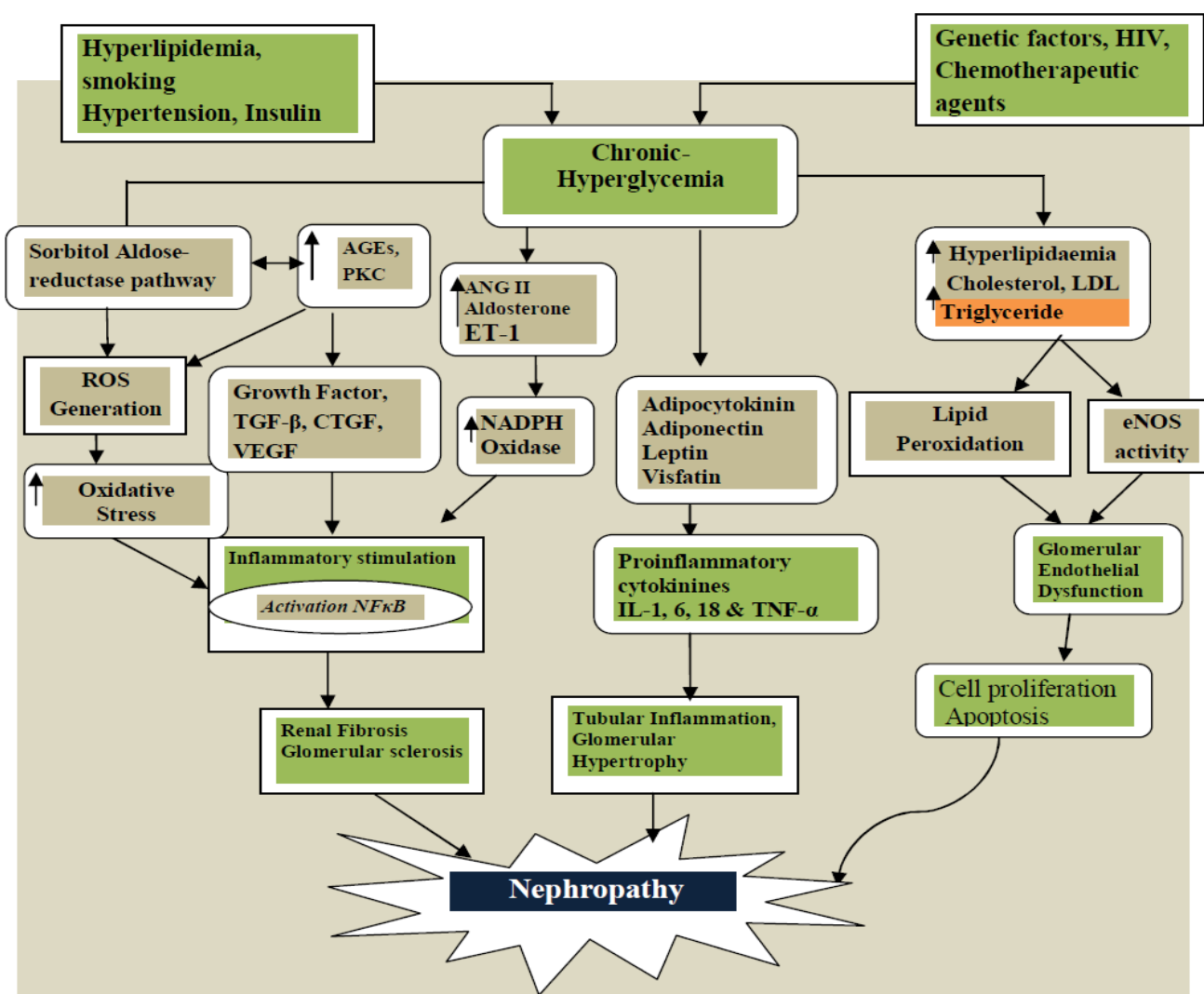


Figure-1: Pathophysiological mechanism of diabetic nephropathy.

## Therapeutic Agents for DN

### Agents that modulate rennin angiotensin system (RAS)

The RAS present in kidneys is comprised of angiotensinogen, renin, angiotensin I (Ang I), angiotensin converting enzyme related carboxypeptidase (ACE2), angiotensin converting enzyme angiotensin II (Ang II), aldosterone, Ang II type I receptor (AT1R) and the Ang II type II receptor (AT2R)<sup>[31]</sup>. Ang II is a major hormone of RAS and contributes to variety of renal and cardiovascular physiological and pathological mechanism<sup>[32]</sup>. During the diseases process the increased Ang II activity causes hypertrophy of mesangial cells and tubular epithelial cells. Moreover the Rho/Rho kinase pathway has been involved in the upregulation of TGF- $\beta$ , connective tissue growth factor (CTGF) and NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) in the kidney of diabetic rats<sup>[33]</sup>. Imidapril has been shown to decrease the urinary albumin excretion in type-1 diabetic nephropathy<sup>[34]</sup>. The renoprotective action of benazapril has been observed in diabetic rats that shown its inhibitory action on P42/44MAPK pathway<sup>[35]</sup>. The mechanism of renoprotection by irbesartan is complex process involving haemodynamic factors that lower the intra-glomerular pressure and expected to cause renal vasodilation<sup>[36]</sup>. Early treatment with olmesartan prevents juxtamedullary glomerular podocyte injury

and the onset of microalbuminuria in type 2 diabetic rats<sup>[37]</sup>.

Notably, the combination of calcium channel blockers with RAS inhibitors decreases the presence of drug-related side effects, particularly peripheral edema<sup>[38]</sup>. The combination of RAS inhibitors with thiazide diuretics reduces both electrolyte and metabolic disturbances<sup>[39]</sup>. The combination of telmisartan and hydrochlorothiazide appeared to be more efficacious than a monotherapy of high-dose ARBs in reducing BP and urinary protein excretion<sup>[40]</sup>. Newer calcium channel blockers such as manidipine block both L- and T-type receptors, inducing vasodilatation not only in the glomerular afferent arteriole but also in the efferent arteriole, resulting in a reduction of proteinuria<sup>[41]</sup>. The superior efficacy of benazepril plus amlodipine compared with benazepril plus hydrochlorothiazide reduces dizziness, hypokalemia, and hypotension<sup>[42]</sup>. Aliskiren is first known direct renin inhibitor which reduces the production of angiotensin II and aldosterone more than traditional RAS blockers<sup>[43]</sup>. The combination of aliskiren with ACE inhibitors or ARBs provide a complete blockade of the RAAS by suppressing residual angiotensin II production and counter-regulatory increase in plasma renin activity<sup>[44]</sup>. Recently, telmisartan a potent AT<sub>1</sub> receptor blocker has been shown its dual role as AT<sub>1</sub> receptor blocking action and peroxisome proliferator-

activated receptor gamma (PPAR- $\gamma$ ) partial agonistic property without producing cardiovascular adverse events that are associated with PPAR- $\gamma$  full agonists like rosiglitazone<sup>[45,46]</sup>.

### Inhibitors of Protein kinase C

Protein kinase is an enzyme with different isomer that phosphorylates various target proteins responsible for intracellular signal transduction involved in regulating vascular function and vascular permeability. The elevated glucose concentration primarily activates the  $\beta$  and  $\delta$  isoform of PKC. In diabetic retina hyperglycemia persistently activates PKC and p38 $\alpha$  MAPK (mitogen activated protein kinases) resulting increase the expression of SHP-1 (a protein tyrosine phosphatase) which is primarily unknown target of PKC signaling. The signaling cascade finally target in pericyte apoptosis<sup>[47]</sup>. In addition over activity of PKC has been implicated in decreased NO (nitric-oxide) production in smooth muscle. Ruboxistaurin has been shown to attenuate histological injury, functional decline, and expression of the profibrotic and proapoptotic growth factor TGF- $\beta$  in DN<sup>[48]</sup>. Macrophages and osteopontin accumulation play a role in the tubulointerstitial injury of DN. Ruboxistaurin has also been found to reduce macrophage infiltration and osteopontin expression in diabetic rats<sup>[49]</sup>. Recently in obstructive nephropathy PKCI, chelerythrine, is capable

of reducing renal tubular apoptosis and interstitial fibrosis associated with PKC $\alpha$  and PKC $\beta$  activation [50].

### Inhibitors of TGF- $\beta$

Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) is a pro-sclerotic cytokine widely associated with the development of fibrosis in diabetic nephropathy<sup>[51]</sup>. It is a heteromeric transmembrane complex consisting of both the TGF- $\beta$  receptor II (T  $\beta$  RII) and TGF- $\beta$  receptor type I (T  $\beta$  RI) also have activin-like kinase (ALK) receptors<sup>[52]</sup>. Phosphorylation and activation of the type TGF- $\beta$  I receptor within its cytoplasmic domain leads to binding of TGF- $\beta$  receptor II which results in the downstream phosphorylation and activation of its classic signaling mediators, Smad2 and Smad3<sup>[53]</sup>. It was shown that chronic diabetes mellitus activates TGF- $\beta$  mediated downstream signaling pathways in the renal cortex of experimental diabetic mouse<sup>[54]</sup>. The expression of GLUT-1 in mesangial cells has been upregulated by TGF- $\beta$  which increases the concentration of glucose intracellularly and accelerates metabolic abnormalities in the diabetic kidney<sup>[55]</sup>. Further, TGF- $\beta$  stimulates the expression of CTGF(connective tissue growth factor), which promotes the deposition of extra cellular matrix components like collagen I, collagen IV and fibronectin and thus enhances the hypertrophy of mesangial cells and

accumulation of extra cellular matrix protein<sup>[56,57]</sup>. Although TGF- $\beta$  1 is regarded as the major isoform involved in fibrosis, improved renal function coincides with reduced expression of both TGF- $\beta$  1 and TGF- $\beta$  2<sup>[58]</sup>. In the STZ-rat, renal expression of TGF- $\beta$  2 is markedly increased and parallels ECM deposition in early stages of the disease. By contrast, TGF- $\beta$  1 protein levels remain unchanged during this initial period despite increased mRNA levels<sup>[59]</sup>. The recombinant hepatocyte growth factor (HGF) has been reported to reduce the incidence of albuminuria, glomerulosclerosis, glomerular hypertrophy and tubulointerstitial fibrosis by suppressin<sup>[69,70]</sup>g the renal production of TGF- $\beta$  in diabetic mice with nephropathy<sup>[60]</sup>. The administration of murine (1D11), an anti-TGF- $\beta$  antibody in combination with lisinopril was noted to reduced the proteinuria in diabetic rats with nephropathy<sup>[61]</sup>. It was demonstrated that administration of soluble human TGF- $\beta$  type II receptor (sT-RII.Fc) produced renoprotective effect by reducing fibrosis and albuminuria through neutralizing TGF- $\beta$ <sup>[62]</sup>. An osteogenic factor, BMP-7 (Bone Morphogenic Protein-7) plays an important role in kidney development and regulation of nephrogenesis by decreasing TGF- $\beta$  -induced renal fibrosis<sup>[63]</sup> and antagonizes TGF- $\beta$  -induced Smad3-dependent EMT (epithelial-to-mesenchymal transition)<sup>[64]</sup>. The connecting-peptide(C-peptide) has been shown to neglects TGF- $\beta$  induced EMT in

cells of the proximal tubule and C-peptide could be used to reduces some renal complications of diabetes<sup>[65]</sup>. But this mechanism remains elusive since BMP-7 is unable to neglects the TGF- $\beta$  -induced EMT in human proximal tubule cells, (PTC) suggesting that the effects are region specific<sup>[66]</sup>. The extent by which BMP-7 blocks EMT therefore requires further clarification.

### Proinflammatory cytokines

Proinflammatory cytokines such as interleukin-1 (IL-1) & tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) playing an important role in inflammatory process which are involved in pathogenesis of DN<sup>[67]</sup>. The high level of IL-18 suggested as significant predictor of early renal dysfunction in type 2 diabetes<sup>[68]</sup>. A recent study has demonstrated that IL-18 is overexpressed in human tubular epithelial cells in diabetic nephropathy through the activation of MAPK pathways induced by TGF- $\beta$ 1 and mediates pro-apoptotic signaling in renal tubular cells through a Fas ligand-dependent mechanism. Experimental studies have shown that administration of infliximab (chimeric TNF- $\alpha$  antibody), etanercept (a soluble TNF- $\alpha$  receptor) and pentoxifylline inhibit the TNF- $\alpha$  mRNA accumulation to diabetic rat associated with reduction in urinary TNF- $\alpha$  excretion and albuminuria<sup>[71,72]</sup>. Pentoxifylline administration reduces the enhance renal expression of proinflammatory cytokines, leading to

decrease in urinary cytokines excretion and reduction in albuminuria<sup>[73]</sup>. The renoprotective effect of pentoxifylline has been determined by a reduction in urinary albumin excretion in DN patients. It reduces the serum C-reactive protein, IL-6, TNF- $\alpha$  and leptin levels<sup>[74]</sup>. A serine/threonine kinase, mTOR plays an important role in modulation of several forms of renal disease<sup>[75]</sup>. The over activation of m-TOR pathway leads to DN and systemic administration of rapamycin a potent inhibitor of mTOR ameliorates pathological changes and renal dysfunction in DN.<sup>[76]</sup> Yang et al concluded that rapamycin treatment can prevent the early renal structural changes of diabetes in experimental rats, and thus halt the early steps in the development of DN<sup>[77]</sup>. The anti-inflammatory, antifibrotic, and podocyte-protective effects of sirolimus seem to be a superior treatment for preventing DN in the rat<sup>[78]</sup>. Low-molecular-weight synthetic molecule, Pirfenidone (PFD) exerts its antifibrotic properties in cell culture and various animal models of fibrosis<sup>[79,80]</sup>. It also regulates the activity of eukaryotic initiation factor (eIF4E) which is mRNA cap structure-binding protein in mesangial cell culture<sup>[81]</sup>.

### Dual PPAR modulators

The dual PPAR agonists are fourth class of PPAR agonist which binds two or more PPAR isoforms. These agonists are currently under intensive investigation for treatment of

diabetes and its complication<sup>[82,83]</sup>. For the potential use of PPAR modulators in the treatment of metabolic diseases, the dosage regimen and the medicinal chemistry should be re-examined. PPARs contain 5 modular domains including an N-terminal region; DNA-binding domain which interacts with specific PPAR response elements (PPRE) in the promoter region of PPAR regulated target genes; ligand binding domain (LBD); flexible hinge region. LBD is the site at which the receptor is activated or inhibited. C-terminal region is the fifth domain<sup>[84]</sup>. The current known dual PPAR modulators including muraglitazar, tesaglitazar and aleglitazar have two methyl groups each with hydroxyl groups. The action of dual agonist is based on the combined action of PPAR- $\alpha$ , PPAR  $\delta/\beta$  and  $\beta$   $\gamma$  agonist. Muraglitazar have shown the preventive effect in diabetes and its complications in db/db mice<sup>[85]</sup>. In new study tesaglitazar can improve metabolic abnormalities and renal function or prevent the glomerular lesion in obese Zucker rats<sup>[86]</sup> and db/db mice<sup>[87]</sup>. The use of many dual agonists has been discontinued because of severe side effect such as renal failure urinary cancer, heart failure and anemia<sup>[88]</sup>.

### NADPH oxidase inhibitors

NADPH-oxidase as cytosolic enzyme complexes having ability to generating reactive oxygen species (ROS) such as super-

oxide and hydrogen peroxide where ROS play a crucial role in various physiological processes<sup>[89]</sup>. Recently it has been shown that excessive ROS production by an over active NADPH oxidase system disrupts redox circuits<sup>[90,91]</sup>. Oxidative stress mediated endothelial dysfunction play a central role in pathogenesis of DN<sup>[92]</sup>. NADPH oxidase enzyme complex is located in the plasma membrane of various renal cell types including mesangial and proximal tubules cells, fibroblast and vascular smooth cells<sup>[93]</sup>. In signaling mechanism activation of NADPH oxidase by Angiotensin-II or TGF- $\beta$  leads to excessive generation of free radicals which induce renal hypertrophy in rats<sup>[94]</sup>.

The above evidence provides a strong rationale for the use of pharmacological inhibitors of NADPH oxidase to combat oxidative stress and its associated vascular dysfunction in nephropathy. Most widely used NADPH oxidase inhibitors are apocynin and diphenyleneiodonium<sup>[95]</sup>. Although these agents proven invaluable pharmacological tool after some decades. The important NADPH oxidase inhibitor taurine significantly reduces the frequent albuminuria and glomerulopathy in diabetic rabbits. Recently apocynin restore endothelial dysfunction in diabetic rats through regulation of NO synthase and NADPH oxidase expression<sup>[96]</sup>.

## Nrf2 Activators

The transcription factor NFE2-related factor 2 (Nrf2) is a master regulator of cellular detoxification responses and redox status<sup>[97]</sup>. Physiologically Nrf2 locates in the cytoplasm and combines with its inhibitor kelch-like ECH-associated protein 1 (KEAP1) which mediate a rapid ubiquitination and subsequent degradation of Nrf2 by the proteasome. At the time of oxidative stress Nrf2 is free from KEAP1 and translocates into the nucleus to bind to antioxidant-responsive elements (AREs) in the genes encoding antioxidant enzymes such as NADPH quinone oxidoreductase (NQO1), glutathione S-transferase, hemeoxygenase-1 (HO1), and  $\gamma$ -glutamylcysteine synthetase, increasing their expression to play a role of detoxification, antioxidation, and anti-inflammation<sup>[98,99]</sup>. Recently it has shown that activation of cardiac Nrf2 by Dh404 in STZ-induced diabetic mice significantly prevented diabetes-induced cardiac oxidative damage and insulin resistance<sup>[100]</sup>. The conclusion from Zhang et al. study is that they used two activators of Nrf2: SNF and CA to treat STZ induce diabetic model in both Nrf2-KO and WT mice<sup>[101]</sup>. Recently sulforaphane as Nrf2 activators disrupt the Keap1-Nrf2 complex in mesangial cells of kidney in streptozotocin-induced diabetic nephropathy and glomerular injury in diabetic mice reduces with tert-butylhydroquinone (tBHQ) through nuclear

factor erythroid 2-related factor 2-dependent antioxidant gene activation<sup>[102,103]</sup>.

### **Vitamin D therapies**

Vitamin D compounds for renal diseases have been used from middle of the 20<sup>th</sup> century. Its past roles have been altering calcium and phosphorus metabolism to prevent bone disease and reduce PTH levels in dialysis patients and recently in pre-dialysis patients. In uremic individuals produce less vitamin D in their skin<sup>[104]</sup>. Vitamin D undergoes two hydroxylation steps to generate the final hormonal form that is first in the liver to produce 25(OH)D<sub>3</sub> and second in the kidney to create the hormonal form, 1,25(OH)<sub>2</sub>D<sub>3</sub> in presence of CYP27B1 enzyme. The reduction of this enzyme and 1, 25(OH)<sub>2</sub>D<sub>3</sub> increased FGF-23 observed in early stages of renal disease<sup>[105-108]</sup> Whereas on the heart vitamin D compound effects are likely through a secondary mechanism since the heart tissue itself does not harbor VDR protein<sup>[109]</sup>. Additionally vitamin D therapy to a standard regimen of glucose control and RAS inhibition is emerging as a strategy to manage diabetic nephropathy in patients<sup>[110]</sup>. VITAL was the first study in humans to investigate an active vitamin D analog paricalcitol exclusively in diabetic nephropathy patients<sup>[111]</sup>. Recently Kim and colleagues measured the effect of vitamin D repletion with cholecalciferol on urinary albumin, MCP-1 and TGF-β<sup>[112]</sup>.

Li and his colleagues also study the effect of vitamin D analog in RAS system outside of osteodystrophy and PTH suppression by using mo. This work has focused primarily on the RAS using a variety of mouse models, including severe model. Whereas Slatopolsky has conducted a number of investigations in this area using the 5/6th nephrectomy rat model<sup>[113-114]</sup>. Clinically Cholecalciferol, Paricalcitol are used in randomized, double-blind, placebo-controlled trials under investigation using vitamin D therapy in diabetic nephropathy patients. Cholecalciferol capsules, 400 IU/day or 4000 IU/day with calcium carbonate, 1 g/day shows change in Urinary albumin to creatinine ratio in T2DM, CKD stages 1–2 in American and African population. Paricalcitol capsules, 2 g/day for 1 month shows changes in serum 25(OH) D<sub>3</sub> and 1, 25(OH)<sub>2</sub>D<sub>3</sub> in T2DM, stable RAS inhibition population<sup>[115]</sup>.

These above studies support the idea that vitamin D compounds have therapeutic potential in slowing the progression of nephropathy as assessed by the surrogate endpoints of proteinuria, molecular markers such as renin, TGF-β etc. Failure to treat vitamin D deficiency in the diabetic nephropathy patient population prior to treatment with active vitamin D compounds complicates the interpretation of the results.

### Autophagy a new target

The term autophagy is derived from Greek which means self eating and it is a lysosomal protein degradation pathway that is involved in the clearance of long-lived proteins and organelles<sup>[116]</sup>. Major roles of autophagy in cells to recycle intracellular energy resources in response to nutrient-depleted conditions and to remove cytotoxic proteins, organelles and maintain cell homeostasis under stressful conditions<sup>[117]</sup>. Various types of autophagy such as macroautophagy, microautophagy and chaperone-mediated autophagy have been recognized in cells which differ in their mechanisms and functions<sup>[118,119]</sup>. Autophagy has been observed in proximal tubules, thick ascending limbs and proximal tubules in kidney and in several experimental animal models of glomerulonephritis it has been shown to play renoprotective and antiproteinuric roles in podocytes through the use of podocyte-specific Atg5 knockout mice<sup>[120]</sup>. It has been highlighted that autophagy in mammalian systems and in disease states (starvation, hypoxia, or ER stress) is advancing rapidly, and many investigators are entering this new and exciting field. It has been revealed that autophagy plays a crucial role in several organs, especially in metabolic organs, and that its alteration is involved in the pathogenesis of metabolic<sup>[121-123]</sup> and age related diseases<sup>[124,125]</sup>. The functional role of autophagy in the kidneys is currently under intense investigation (Figure 1), and it has

been revealed that autophagy has a renoprotective role in several animal models including those used for aging and acute kidney injury<sup>[126,127]</sup>.

Major nutrient-sensing pathway involves the mammalian target of rapamycin (mTOR)<sup>[128,129]</sup>, AMP-activated protein kinase (AMPK),<sup>[130,131]</sup> and oxidized NAD- (NAD<sup>+</sup>-) dependent histone deacetylase (Sirt1) which are also recognized as the regulatory factors of autophagy under nutrient-depleted condition<sup>[132,133]</sup>. The upregulation of autophagy to protect the kidneys was observed using 3-MA, chloroquine<sup>[134]</sup>, and proximal tubular epithelial cell-specific Atg5 knockout mice in renal ischemia-reperfusion injury models. In acute kidney injury models by cisplatin increased level of autophagosomes was observed by EM, LC3-II Western blotting and GFP-LC3 transgenic mice<sup>[123]</sup>. Hypoxia leads to renal tubular damage in aged kidney as previously shown that hypoxia induced autophagy activity declined with age, which led to accumulations of damaged mitochondria and mitochondrial ROS in the kidney<sup>[135]</sup>. Bnip3 expression is essential to induce autophagy under hypoxic condition and is positively regulated by a transcriptional factor Foxo3a where as Sirt1-knockout mice showed lower autophagy activity along with the decrease in Bnip3 expression<sup>[136]</sup>. The kidney of heterozygous was resistant to long-term calorie restriction (CR) restored CR-mediated antiaging effects

and existence of autophagy in other types of renal cells besides podocytes and proximal tubular cells is not known<sup>[123]</sup>.

### **Future Recommendations:**

From the previous research, tremendous efforts have been made in the identification of novel target sites and thus numerous agents have been used in treatment of DN. A recent study in using combination angiotensin converting enzyme angiotensin receptor blocker and statin normalized proteinuria and ameliorated diabetic glomerulosclerosis in experimental streptozotocin induced diabetic rats<sup>[137]</sup>. Active investigations in evaluating how various stem cells can be induced for beta cell and islet functions in order to enclose the problems of autoimmunity and immunosuppression are now underway and may offer future treatment options for long term glycemic control and prevention of DN<sup>[138]</sup>.

Another potential target to control blood glucose is to decrease proximal tubular glucose reabsorption by selectively blocking the renal apical proximal sodium- glucose cotransporter, SGLT2 with an SGLT2 inhibitor and induce glucosuria<sup>[139]</sup>. These inhibitors have no direct effect on insulin release and Phase 3 trials are currently underway to determine potential therapeutic viability of this agent for blood glucose control in DM<sup>[140]</sup>. Recently SGLT2 inhibitors

show improved blood glucose control without inducing insulin secretion and hypoglycemia or weight gain in experimental diabetic models<sup>[141]</sup>. A phase 2 study in diabetic patients with dapagliflozin was found to be improved glycemia and glucosuria but increased genital infections<sup>[142]</sup>.

Fenofibrates as antilipemic agents mediate its clinical effects via PPAR- $\alpha$  activation resulting in moderate reduction in total and LDL cholesterol and significant increases in high density lipoprotein cholesterol. Clinical studies support a significant decrease in microalbuminuria and progression to macroalbuminuria with fenofibrate use<sup>[143,144]</sup>. Vascular endothelial growth factor (VEGF) stimulates glomerular hypertrophy and has been associated with increased urinary protein excretion. VEGF inhibitors in experimental studies appear to decrease DN<sup>[145]</sup>. In earlier study low dose of sitagliptin ameliorates the development of renal dysfunction in diabetic ZDF rats<sup>[146]</sup>. Staurosporine is a uniquely potent inhibitor against PKC in in vitro studies was designed using SBDD (Structure-Based Drug Design approach). Induced Fit Docking studies in bisindolylmaleimide (BIS) and PKC  $\beta$ -II SBDD inhibitors may be potent in the in vitro and in vivo studies of diabetes complications and may help to design new potent inhibitors for PKC  $\beta$ -II in future<sup>[150]</sup>. Visfatin an adipocytokinin generally produced from visceral fats and promotes  $\beta$ -cell maturation

and inhibit neutrophil apoptosis. Experimentally in vitro & in vivo studies shown that visfatin is produced by renal cells in diabetic animals produce higher level of this adipocytokinin and further studies needed in renal failure <sup>[147,148]</sup>. Pancreas transplantation is generally preferred for DM1 patients with advanced DN requiring renal transplantation or for those that have already received renal transplantation. Recently some simultaneous kidney and pancreas transplantation have recommended for those patients with ESRD and DM <sup>[149]</sup>.

### Conclusion:

A significant cause for progressive renal disease, DN remains an area for active investigation of pathogenesis and possible treatments. The diversity of therapies currently in development reflects the pathogenic complexity. Numerous novel target sites such as TGF- $\beta$ , AGEs, PKC, Rho-kinase, NADPH oxidase, PARP and Nrf2, autophagy have been identified to play a pathogenic role in the progression of DN.

RAS has been proved to be the torch bearer in pathogenesis of diabetic nephropathy. Various trials have used for DN through blockade of RAS although none achieved complete blockade and further trials should be conducted at the molecular and genetic level. This review addressed some of the more promising therapeutic agents for delaying the progression of diabetic kidney disease.

Whereas taking the advantage of the potential use of PPAR modulators in the treatment of metabolic diseases, the dosage regimen and the medicinal chemistry should be re-examined. Recently, studies have indicated that Nrf2 play a critical role in preventing diabetes-induced oxidative stress, inflammation, and organ's dysfunction.

We look forward to testify successful management of diabetic nephropathy in the near future and further clinical studies are needed to illuminate their therapeutic potential in treating diabetic patients with nephropathy.

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