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REVIEW ARTICLE

Reactive Oxygen Species and Molecular Targets: Review on Diabetic Nephropathy

Viplove Kataria, Shamsher Singh*, Jatin Sharma, Avi Gupta and Sunil Bansal,
Department of Pharmacology I.S.F. College of Pharmacy, Moga Punjab, India -142001

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Corresponding Author

Shamsher Singh

Abstract

Diabetes mellitus is a metabolic disorder known to cause retinopathy, neuropathy and nephropathy. Diabetic nephropathy is a persisting major microvascular complication of uncontrolled hyperglycemia that affects a large number of people worldwide. Recent studies suggest that numerous pathways are activated during the course of diabetes mellitus and these pathways individually or collectively play a role in the induction and progression of diabetic nephropathy. However, clinical approaches targeting these pathways to manage diabetic nephropathy remain inadequate, as the number of diabetic patients with nephropathy is increasing yearly. For the development of new and effective therapeutic options to prevent the induction and progression of diabetic nephropathy, an ample understanding of the molecular mechanisms involved in the pathogenesis of the disease is obligatory. Thus, the purpose of this paper is to discuss the underlying mechanisms and downstream pathways involved in the pathogenesis of diabetic nephropathy.

INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Long term hyperglycemia often results in micro vascular and macro vascular complications such as nephropathy, neuropathy, retinopathy, cerebra vascular disease and peripheral vascular disease [1].

Diabetic nephropathy is a distinct phase clinical syndrome with partial or complete loss of kidney function. The structural changes in kidney including thickening of basement membrane, mesangial expansion, glomerular hypertrophy, fibroblast proliferation, matrix deposition glomerulosclerosis and tubular necrosis are generally observed in the patients of diabetic nephropathy (approx. in 30-40% of DM cases) [2]. Various functional abnormalities of kidney such as persistent elevated albuminuria, elevated arterial blood pressure, declined glomerular filtration rate (GFR) and fluid retention are also associated with diabetic nephropathy [3]. The down regulation of endothelial nitric oxide synthase (eNOS) [4], peroxisome proliferator-activated receptor- γ (PPAR-γ) has been noted to be involved in pathogenesis of diabetic nephropathy [5]. The elevated levels of several pathological substances such as vasoactive peptide like angiotensin-II [6] endothelin-1 and growth factors like vascular endothelial growth factor (VEGF), transforming growth factor- β1 (TGF-β) [7], advanced glycation end (AGE) products and lipid mediators such as 5-lipooxygenase derived substances like 12-hydroxyeicosatetraenoic acid (12-HETE) and 20-hydroxyeicosatetraenoic acid (20-HETE) [8] have been implicated in the pathogenesis of diabetic nephropathy.

Molecular pathways involved in Diabetic Nephropathy

***1. Role of RAAS in DN*:** Renin angiotensin aldosterone system has a crucial role in homeostatic control of tissue perfusion, arterial pressure and extracellular volume. Along with hemodynamic effects, RAAS is also involved in renal

tissue cell infiltration and inflammation [9]. It is evident that renin and its receptor [(Pro) renin receptor (PRR)] play an important role in the development and progression of kidney disease during diabetes by increasing the renal production of inflammatory cytokines i.e. tumor necrosis factor- α (TNF-α) and interleukin-1β (IL-1β), independent of the actions of renal angiotensin II (Ang II) [10] and renin is also involved in the expression of TGF-β1 in mesangial cells thereby stimulating plasminogen activator inhibitor-1 (PAI-1), fibronectin and collagen I [11], type IV collagen and VEGF, each of which was suppressed by aliskiren, a renin inhibitor, determining the role of renin in the induction and development of diabetic nephropathy [12,13].

Hyperglycemia increases renal aldosterone levels by inducing CYP11B2 expression [14] and found to be one of the key mediators in the pathogenesis of diabetic nephropathy. In the kidney, mesangial cells are recognized to produce aldosterone in response to Ang II, which leads to accumulation of extracellular matrix (ECM) [15]. In experimental diabetic nephropathy, treatment with spironolactone (an aldosterone receptor antagonist) has been proved to reduce albuminuria and alleviate glomerulosclerosis by downregulating the renal expression of matrix-regulating genes, such as TGF-β, matrix metalloproteinase (MMP), VEGF and insulin growth factor.[16] Further, spironolactone was illustrated in an experimental study to show renoprotective effects by decreasing oxidative stress and attenuating the overexpression of Monocyte Chemoattractant Protein-1 in patients with diabetic nephropathy [17]. Together, these studies demonstrate the direct detrimental role of RAAS in the pathogenesis of diabetic nephropathy.

Over-activation of intrarenal Ang II causes hypertension and renal injury and results in decreased renal function and structural changes in the kidney [18]. Also, Ang II directly induces podocyte injury via the activation of Ang II receptor type 1 (AT1) receptors, independent of hemodynamic changes [19]. Moreover, Ang II interacts with various local autocrine and paracrine factors i.e. NO, eicosanoids, adenosine, and superoxide, to affect the glomerular filtration rate [20]. It is important to note that glucose increases the expression of the angiotensinogen gene in proximal tubule cells and Ang II production in mesangial cells, suggesting that high glucose itself activates the renin–angiotensin system [21, 22].

Ang II and other elements of the RAAS have a vital role in the pathogenesis and succession of diabetic renal disease. A research in patients with type-1 diabetes and nephropathy proved that RAAS inhibition with ACE inhibitors was linked with a decreased risk of development to end-stage renal disease (ESRD) and mortality as compared to non-RAAS-inhibiting drugs. ACE inhibitors can also put a stop to microalbuminuria in type-2 diabetic patients who are hypertensive and normoalbuminuric. ARBs show renoprotective effects in patients with type-2 diabetes and microalbuminuria. Studies have found the renoprotective activity of other RAAS inhibitors, e.g. aldosterone antagonists and renin inhibitors, given either alone or in combination with ARBs or ACE inhibitors. An imperative job for the future will be determining the combination of drugs which achieves the best renoprotective activity at the lowest cost. These findings will have major significance, mainly in settings where resources like money and facilities are limited [120].

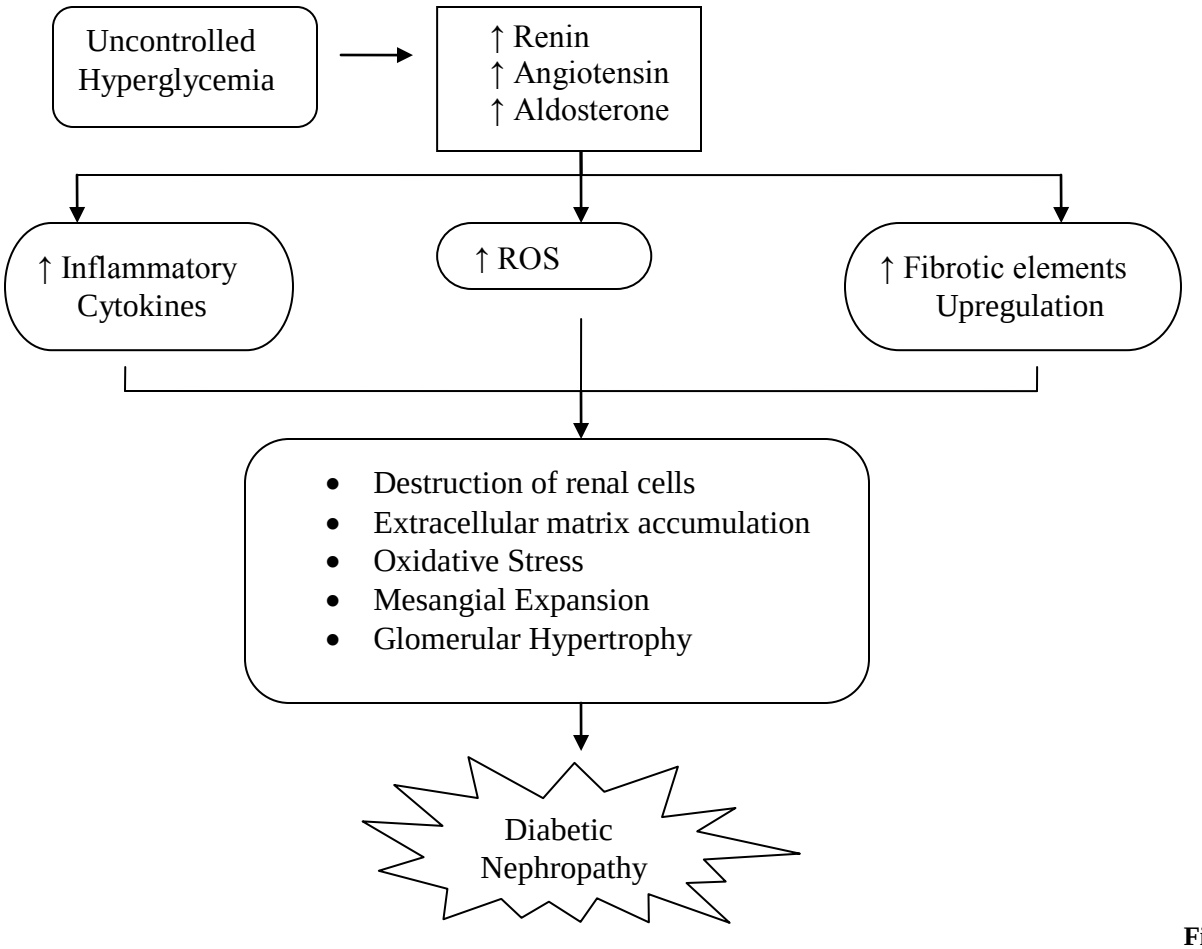

Fig.1. The signaling mechanism involved in RAAS-mediated initiation and development of diabetic nephropathy.

***2. Role of protein kinase C in Diabetic Nephropathy*:** PKC, a family of serine threonine kinases that consists of no less than 15 isoforms, including PKC-α, -β1, -β2, -δ and -ε, has been known to be activated in the glomeruli of diabetic rats and in mesangial cells exposed to high glucose [23,24,25]. Activated PKC regulates various vascular functions, such as contractility, cell proliferation and extracellular matrix protein synthesis [26, 27, 28]. The PKC-α isoform has been documented to be involved in the pathogenesis of diabetic nephropathy by upregulating VEGF expression [29]. Despite PKC-α, other isoforms of PKC, such as PKC-β and PKC-ε have also been implicated in mediating high glucose-induced VEGF expression in mesangial cells [30]. A study suggested that the diabetes-induced activated PKC-β isoform may induce renal fibrosis through the upregulation of TGF-β, type IV collagen, laminin and fibronectin in the glomeruli of diabetic rats resulting in the pathogenesis of diabetic nephropathy [31]. In a new study, the PKC-β isoform was found to be activated in the glomeruli of diabetic db/db mice, and treatment with a PKC-β-specific inhibitor i.e. ruboxistaurin mesylate inhibited glomerular PKC activation and ameliorated an increase in urinary albumin excretion. This treatment also helped to reduce the glomerular expression of TGF-β and ECM accumulation, thereby restoring the structural changes such as mesangial expansion [32]. Collectively, these studies express the mechanism involving these PKC isoforms in the induction and development of diabetic nephropathy.

***3. Role of AGE products in Diabetic Nephropathy*:** A number of studies have concentrated on the factors involved in the pathogenesis of diabetic complications; most looking for effective therapies, but none of them have successfully elucidated the exact cellular or molecular basis of these complications. Hyperglycemia is still regarded as the major cause of diabetes complications. Its harmful effects are determinable, along with other things, to the formation of sugar-derived substances known as advanced glycation end products (AGEs). The formation of AGEs takes place at a constant but slow rate in the normal body, initiating in early embryonic development and accumulating with time. But their production is markedly increased in diabetes due to high glucose levels. AGEs are a heterogeneous group of molecules formed from the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids (fatty material

etc.) and nucleic acids. The first product of this reaction is called a Schiff base, which suddenly rearranges itself into an Amadori product like in the case of the well-known hemoglobin A1c (A1C). These initial reactions are reversible depending on the concentration of the reactants. A lowered glucose concentration will detach the sugars from the amino groups to which they are attached; contrarily, high glucose concentrations will have the opposite effect, if persistent. As a result, a series of reactions, including successions of dehydration, oxidation-reduction reaction and other re-arrangements lead to the formation of AGEs [33]. Intracellularly, AGEs are derived from various dicarbonyls, mainly methylglyoxal, which is synthesized from Glyceraldehyde-3-Phosphate (G-3-P) or dihydroxyacetone following catalysis by G-3-P dehydrogenase (GAPDH) [34]. These AGEs can alter various intracellular events i.e. the activation of PKC, Mitogen-activated Protein Kinase (MAPK) and transcription factors such as nuclear factor κB (NF-κB) [35, 36]. These events, subsequently, regulate the expression of diverse growth factors and cytokines such as TGF-β, which influence the synthesis of different ECM proteins. Extracellular AGEs are formed by irreversible cross-linking of glucose with ECM structural proteins that are type IV collagen, fibronectin, laminin and proteoglycans [36]. These modified proteins are less susceptible to enzymatic hydrolysis by matrix metalloproteinases (MMPs), which would let them to accumulate in the extracellular space [37]. In addition, glycation of sulfated proteoglycans decreases their electronegativity and thus changes the charge-selective filtration properties of the basement membrane, leading to microalbuminuria [38, 39]. Moreover, extracellular AGEs can modulate cellular functions by binding to their associated receptor, RAGE, or with binding proteins, specifically OST-48, galectin-3, 80 K-H and type II macrophage scavenger receptor, which may also alter cell and matrix functions [34, 35]. Such alterations may cause interference with cell-matrix interactions and changes in neurite growth, adhesiveness and the hyperpermeability of capillaries [40]. A study suggests that changed functions related to the vascular complications of diabetes mellitus can be partially reversed (a) by the administration of aminoguanidine (an AGE inhibitor) and AGE cross-link breaker, or (b) by blocking RAGE [36]. High concentrations of intra- or extracellular AGEs in high-glucose atmosphere alter some other cellular events including the generation of ROS and quenching of NO; both ROS and NO are known to modulate PKC and MAPK activities and to activate transcription factors such as NF-κB and activator protein 1 (AP-1), consequently increasing the expression of ECM proteins [34, 36]. Interestingly, AGEs themselves can also covalently bind with ECM or cellular proteins, which further show their harmful effects in various tissues [34, 36]. In this way, AGEs can play detrimental role in the pathogenesis of diabetic nephropathy and various other complications.

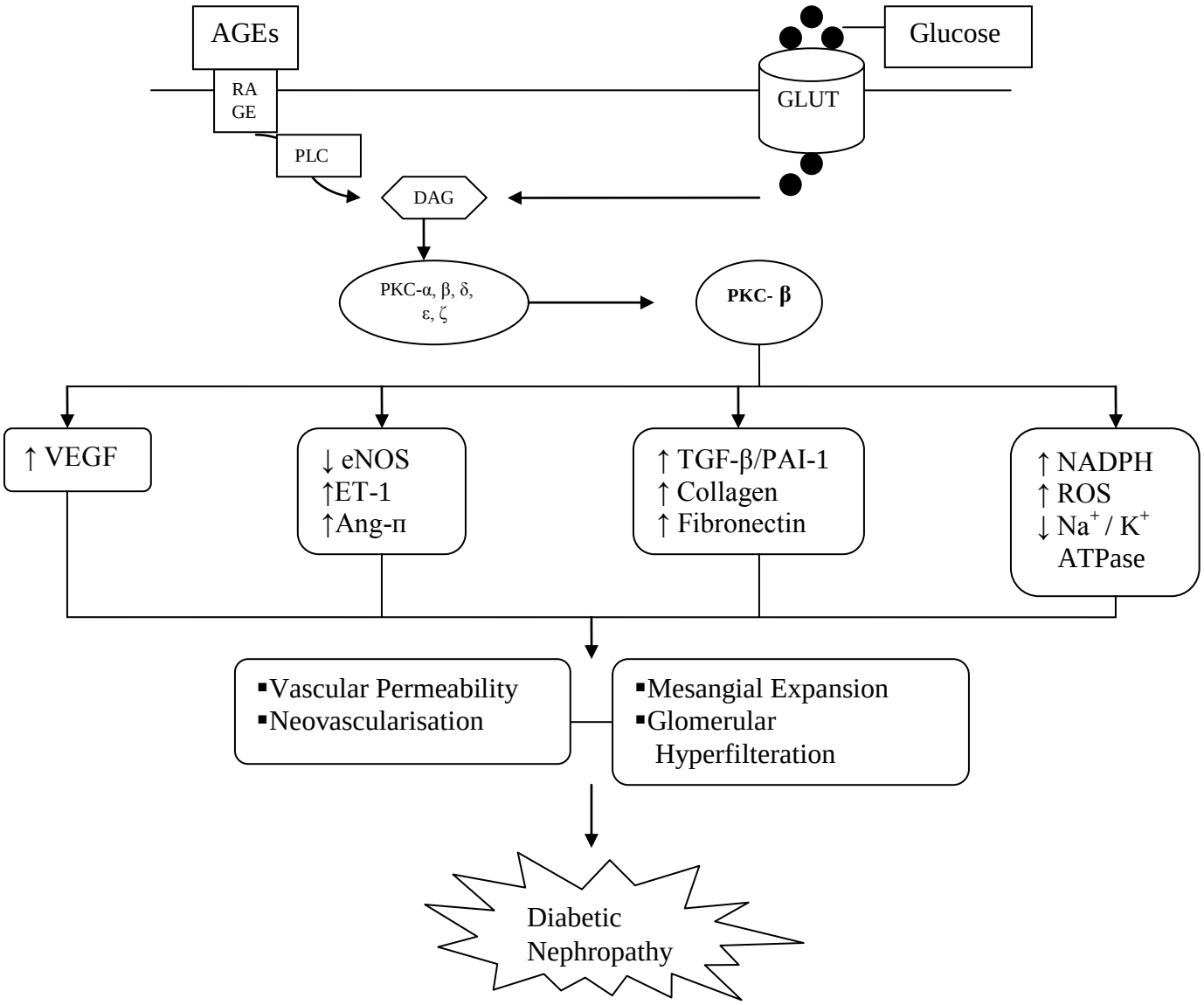

Fig.2. Sequence of events following AGE: RAGE (advanced glycation end products: receptor for advanced glycation end products) and activated protein kinase C (PKC), which modulate the expression of a wide variety of genes leading to increased vascular permeability, mesangial expansion, hyperfiltration, and proteinuria. Abbreviations: Ang II, angiotensin II; e-NOS, endothelial nitric oxide synthase; ET-1, endothelin 1; IP3, inositol trisphosphate; PAI-1, plasminogen activator inhibitor 1; PIP2, phosphatidylinositol 4,5-bisphosphate; ROS, reactive oxygen species; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

***4. Role of ROS (Reactive Oxygen Species) in Diabetic Nephropathy*:** Many studies suggest that the activities of Advanced Glycation End Products , Protein Kinase C and Reactive Oxygen Species (ROS) are interrelated and that the ROS may serve as reciprocal inducers and amplifiers of the signaling cellular events that occur in high-glucose environment [41, 38, 42, 43, 44]. Usually, ROS are formed in small amounts that are essential to maintain cellular homeostasis, but in case of hyperglycemia, their concentrations rise significantly, damaging various target organs [42]. Superoxide anion $O_2^{\cdot-}$, H_2O_2, hydroxyl radical, and peroxynitrite can induce renal injury [44]. Cytoplasmic CuZn superoxide dismutase (CuZnSOD), mitochondrial manganese superoxide dismutase (MnSOD), and heme oxygenase 1 are the enzymes that can scavenge ROS [45, 46]. Intriguingly, the latter undergoes an incredible adaptive response (> 10-15 fold increase) in case of hyperglycemia, apparently to reduce ROS mediated oxidant stress [46]. ROS are formed via two systems: (1) primarily via mitochondrial oxidative phosphorylation and (2) in minute amounts via the NADPH-

oxidase system [47, 48, 49, 50]. ROS are the by-products of oxidative phosphorylation, in which electron donors e.g. NADH and FADH2 produce a high membrane potential by pumping H^+ ions across the mitochondrial inner membrane [38, 51]. Consequently, electron transport is blocked, the half-life of free-radical intermediates of coenzyme Q increases, and molecular O2 is reduced to $O_2^{\cdot-}$ with ensuing oxidant stress.

High glucose concentrations enhance the expression of the NADPH oxidase subunits-p22phox and p47phox in mesangial cells in vitro and in vivo in a PKC-dependent manner [52, 53, 54]. Further, high glucose induces intracellular ROS in mesangial cells and tubular epithelial cells, which can be successfully blocked by the inhibition of NADPH oxidase; this suggests the crucial role of NADPH oxidase in high glucose-induced ROS generation [55, 56]. It is also to be noted that NADPH oxidase-mediated ROS further activates additional enzyme systems, e.g., ROS-dependent NOS uncoupling, predominantly of eNOS, whereby the enzyme no longer produces NO but rather becomes a source of superoxides [57, 58]. NADPH oxidase-mediated renal oxidative stress causes mesangial expansion and albuminuria by increasing the expression of fibronectin and collagen-1 in the kidney [59]. The harmful role of NADPH oxidase in the progression of diabetic nephropathy has been further confirmed by several studies in which treatment with NADPH oxidase inhibitors noticeably attenuated the progression of nephropathy by reducing the occurrence of albuminuria and preventing the development of glomerulosclerosis through a reduction of renal oxidative stress [36, 60, 61, 62]. All these key interactions between ROS, NADPH oxidase and the important pathways implicated in the progression of diabetic nephropathy are useful to understand the molecular basis of the complication.

***5. Role of Transforming Growth Factor β in Diabetic Nephropathy*:** TGF-β1 is extensively considered to be the most pertinent cytokine to the ECM glomerular pathology normally observed in patients with chronic progressive diabetic nephropathy.TGF-β1, a prototype of the TGF-β family, exerts pleotropic effects i.e. it inhibits proliferation in certain cells and apoptosis in others—but it induces hyperplasia and hypertrophy of mesangial cells [63, 64, 65, 66]. In the ECM, TGF-β1 occurs as an inactive, dormant form of propeptide complexed with TGF-β1-binding proteins and both are cross-linked with matrix proteins by transglutaminase [67, 68, 69, 70]. Plasmin, MMP-2 and −9 or thrombospondin 1 cleave these complexes and generate an active form of TGF-β1. The activated TGF-β1 binds first to a type II serine/threonine kinase receptor, which transphosphorylates and activates a type I receptor consequently. Modulation of the downstream-signaling SMAD protein, MAPK, and perhaps Protein Kinase-A cellular pathways and various nuclear events take place after this process. The activated TGF-β1 receptor combines with SMAD2 and −3 to form a heterodimeric complex with common-mediator co- SMAD4, which translocates into the nucleus and regulates transcription of TGF-β1 target genes like collagen α1(I), PAI-1,Jun B, c-Jun, and fibronectin [67, 68, 69, 70]. In addition to SMADs, the extracellular signal-regulated kinases 1 and 2 (ERK1 and −2), the p44/p42 MAPKs, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38 MAPK regulate the TGF-β1 signaling cascade in mesangial cells [63]. These kinases modulate the transcriptional regulation of proα1 (I) procollagen and fibronectin via AP-1 which is a heterodimer of the c-Fos and c-Jun family members as well [71]. TGF-β1 signaling is triggered by a number of mediators developed under high-glucose atmosphere, such as the AGEs, PKc, ROS, DAG, and the hexosamines; other mediators that accelerate renal injury include vasoactive substances, (such as endothelins, angiotensin II, and thromboxane etc.) as well as the physical cyclical stretching and relaxation of mesangial cells (which imitate intraglomerular hypertension) [73, 72]. The final effect is an elevated synthesis of various matrix proteins and accumulation of ECM [74, 75]. One study shows that in vivo studies justifies the in vitro effects of TGF-β1 in which upregulation of TGF-β messenger RNA (mRNA) and protein expression plus its type II receptor and TGF-β1 bioactivity, were observed in the kidneys of different murine of diabetes [76, 77]. The treatment with neutralizing anti-TGF-β1 antibodies inhibits mesangial matrix expansion, renal hypertrophy, increase in α1 (IV) collagen and fibronectin expression, and failing in renal function in mice with STZ-induced diabetes suggests a potential role of TGF-β1 in the Pathogenesis of diabetic nephropathy [78].

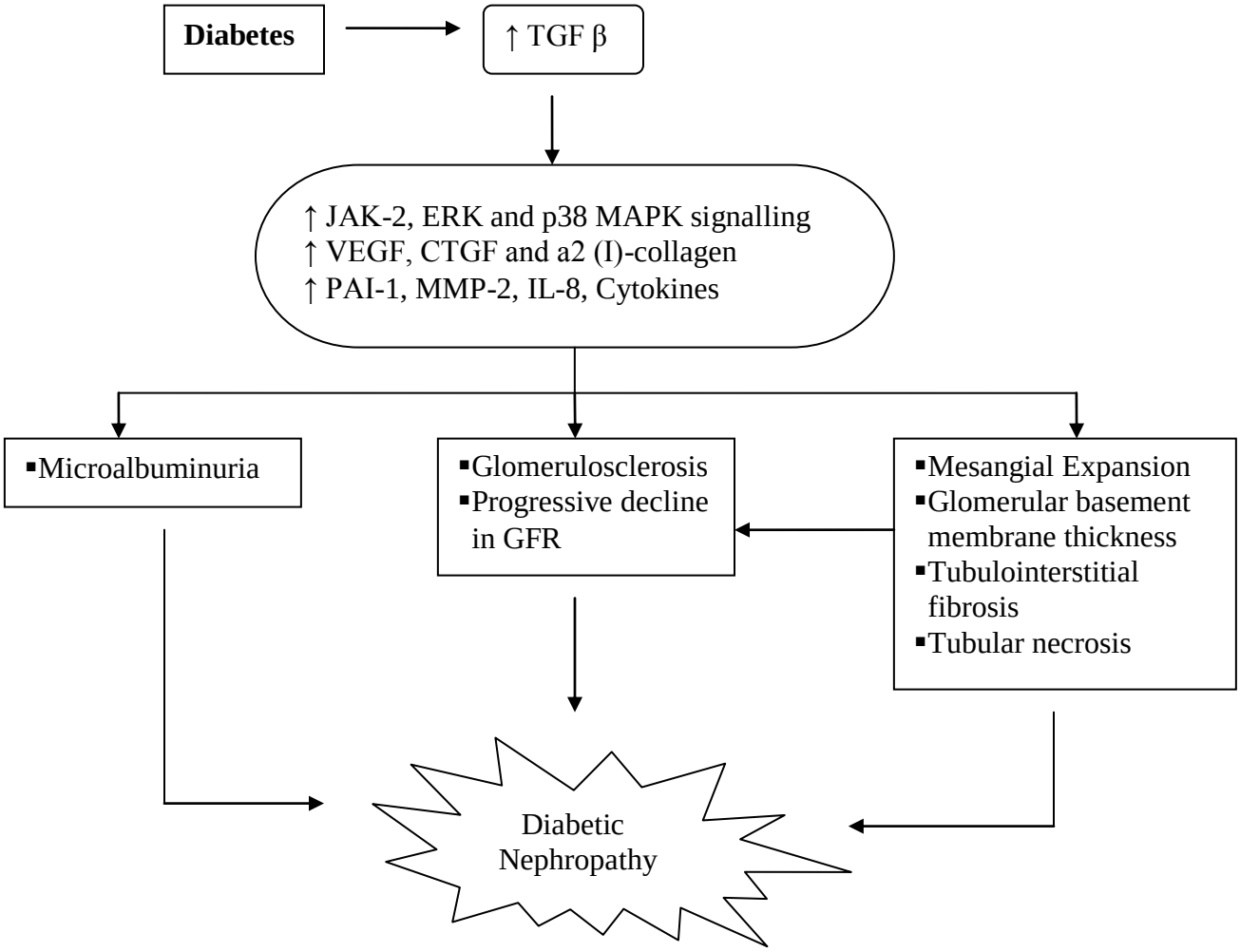

Fig.3. Showing role of TGF-β in induction and development of diabetic nephropathy. JAK-2 indicates Janus kinase-2; ERK indicates extracellular regulating kinase; p38 MAPK indicates mitogen-activated protein kinase; VEGF indicates vascular endothelial growth factor; CTGF indicates connective tissue growth factor; PAI-1 indicates plasminogen activator inhibitor-1; MMP-2 indicates matrix metalloproteinase-2; IL-8 indicates interleukin-8; GFR indicates glomerular filtration rate.

***6. Role of Tumor Necrosis Factor α in Diabetic Nephropathy*:** TNF-α is a pleiotropic cytokine formed mainly in macrophages and monocytes and is implicated in systemic inflammation [79, 80]. TNF-α generates a local inflammatory response by triggering a cascade of cytokines and increasing vascular permeability, thus recruiting macrophage and neutrophils to the site of infection/inflammation [79, 80]. TNF-α stimulates NF-κB signaling mediating the transcription of different cytokines required for cell survival and multiplication, inflammatory effects and cell adhesion, and anti-apoptotic factors [81-85]. Due to the cytotoxic activity of TNF-α to glomerular, mesangial, and epithelial cells, it can induce renal damage [86] and it has been reported to play a pathophysiological role in numerous experimental models of renal disease including crescentic glomerulonephritis, lupus nephritis, mesangial proliferative glomerulonephritis, hypertension, diabetes and the remnant kidney model of nephropathy [86-89].

Several studies have reported that there is a significant correlation between urinary albumin excretion and renal TNF-α level. Furthermore, urinary TNF-α excretion is also observed in Streptozotocin-induced rats. These findings suggest the role of TNF-α in Diabetic Nephropathy [90, 91]. Moreover, the elevated renal TNF-α level and excretion precede the increase in albuminuria in diabetes. Urinary TNF-α level are also high in type 2 diabetic patients and TNF-α levels continuously rise with the progression of diabetic nephropathy, suggesting that elevated TNF-α level lead to the

development of renal injury [91, 92]. TNF-α also is also responsible for sodium retention and renal hypertrophy (early characteristic signs of Streptozotocin-induced diabetic nephropathy) [93].
The relationship between oxidative stress and TNF-α is complex. They have been shown to increase the levels of each other [94, 95, 96]. In the streptozotocin-induced diabetic rat kidney, rise in TNF-α level increase oxidative stress resulting in increased albumin permeability and urinary albumin excretion, a common indicator of renal injury [96]. Moreover, increased peroxynitrite levels are associated with elevated TNF-α levels and increased glomerular lesion in streptozotocin-induced diabetic rats [97]. This data propose that TNF-α is upstream of oxidative stress in diabetic nephropathy. In comparison, the treatment with SOD mimetic decreases renal TNF-α levels and albuminuria in type 2 diabetic Zucker rats [98], and the antioxidant tocotrienol decreases oxidative stress and modulates TNF-α and TGF-β–induced inflammation, thereby provides reno-protection to streptozotocin-induced diabetic rats [96]. These studies imply that oxidative stress is upstream of TNF-α activation in diabetic nephropathy and they have potential role in the pathogenesis of DN.

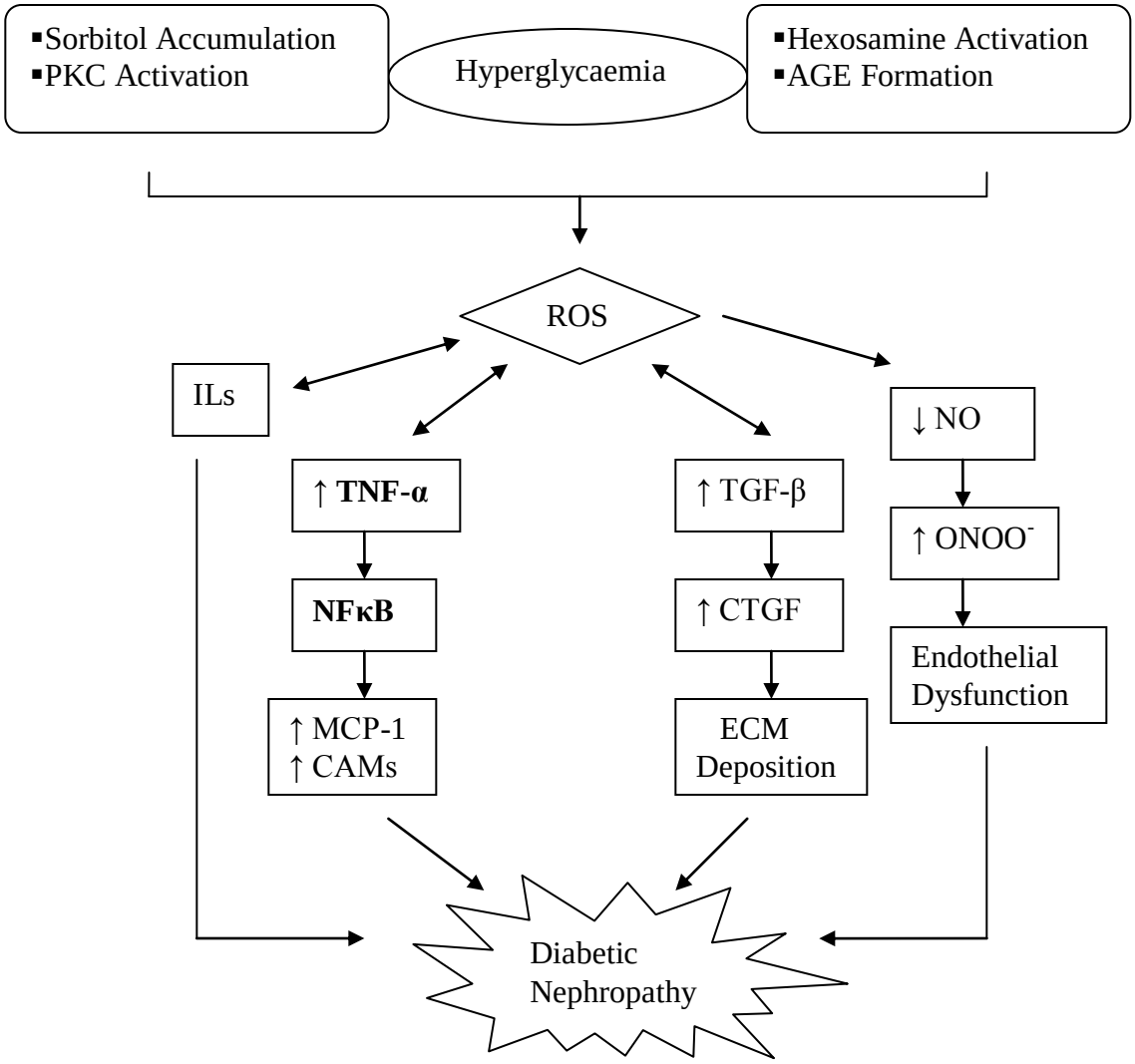

Fig.4. Schematic diagram showing the role of tumor necrosis factor-α (due to hyperglycemia), oxidative stress and inflammatory cytokines in the pathogenesis of diabetic nephropathy. ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; NFκB, nuclear factor kappa B; MCP-1, monocyte chemoattractant protein-1; CAMs, cellular adhesion molecules; NO, nitric oxide; ONOO–, peroxinitrite; TGF-ß, transforming growth factor-ß; CTGF, connective tissue growth factor; ILs, interleukins.

***7. Role of cannabinoid receptors in diabetic nephropathy*:** Two types of cannabinoid receptors are known i.e. CB1 and CB2, which are coupled to G-proteins and play a vital role in controlling the peripheral energy metabolism. Although these receptors are expressed mainly in the central nervous system but mRNA for both CB1 and CB2 receptors have also been found in mesangial cells [99]. Increased levels of renal CB1 receptors were observed to be involved in renal injury by the initiation of renal hypertrophy, together with glomerular and tubulointerstitial lesions, finally resulting in increased proteinuria, plasma creatinine, and urea nitrogen levels in obesity-induced nephropathy [100]. The potential role of CB1 receptors in renal injury was further verified in another study, which showed that the inhibition of CB1 receptors with AM281/SR141716, CB1 receptor antagonists, inhibits cisplatin-induced increased p38 MAPK activation, oxidative or nitrosative stress, cell destruction and interrelated inflammatory cell infiltration in the kidney, ensuing decreased renal tubular cell death and a prominent improvement in renal function [101, 102]. Furthermore, inhibiting CB1 receptor with AM251, a selective CB1 receptor antagonist, was noted to improve albuminuria by repressing the downregulation of nephrin and podocin in diabetic mice; this confirms the harmful role of CB1 receptor activation in the pathogenesis of diabetic nephropathy [103]. On the other hand, activated CB2 receptor minimizes the inflammation and inter-related oxidative/nitrosative stress, cell death linked to cisplatin-induced nephropathy [101]. These studies help to understand the detrimental role of cannabinoid receptors in the pathogenesis of diabetic nephropathy.

***8. Role of GTP Binding Proteins and cell cycle proteins in Diabetic Nephropathy*:** So far, some reports in the literature have illustrated the role of GTP-binding proteins in the pathogenesis of diabetic nephropathy. The main GTPases studied until now are the Ras, Ras-related, and Rho families of GTP-binding monomeric proteins, which vary from 20 to 40 kDa in size [104]. They alter a variety of cellular processes, such as cell hypertrophy, morphogenesis (development of shape by an organism), motility, axonal guidance, cytokinesis, and intracellular trafficking [105,104,106]. They serve as molecular switches in these processes; they change from inactive (GDP-bound) to active (GTP-bound) states [104,106]. In the members of GTPase family, the Ras, Ras-proximate 1 (Rap1), and Rho families are significant because they are essential for many transduction pathways. For example, in the Ras/Raf/MEK (MAPK/ERK) signaling pathway, Ras acts as an intermediary between the activated/phosphorylated growth factor receptor and MEK (Mitogen Activated Protein Kinase), moreover the JNK/SAPK and P38/MAPK pathways may be directly activated by Rho and Rho-related Rac, respectively [108, 105, 107]. Activation of the Rap1/Raf/MAPK pathway can occur through PKC and ROS that are generated following AGE: RAGE interaction [104, 106]. These signaling pathways have been elucidated both in vitro and in vivo through the upregulation of Rap1b in high-glucose atmosphere, which consequently increased ECM-fibronectin synthesis [109, 110]. Recent studies also suggest that the Rho GTPases play an important role in the pathobiology of ECM proteins, such as fibronectin, that are regulated by TGF-β-induced upregulation of CTGF, a powerful profibrogenic cytokine expressed in renal glomerular and tubulointerstitial cells [111, 112]. Likewise, Rho-dependent pathways are stimulated in the kidney by other profibrogenic molecules, including angiotensin II, platelet-derived growth factor, and endothelin 1 [111, 113]. These factors alter the expression of various ECM proteins and therefore are implicated in the pathogenesis of diabetic nephropathy.

9. Role of other novel targets in diabetic nephropathy: Some studies have found that reduced expression of lipoic acid synthase accelerate diabetic nephropathy by increasing microalbuminuria, glomerular basement thickening, and the mesangial matrix in diabetic mice [114], suggesting the direct renoprotective effect of lipoic acid synthase during diabetic conditions.

Osteopontin (OPN) is a phosphoprotein produced by the kidney that regulates cell adhesion and migration. OPN has been found to play a significant role in the development of interstitial fibrosis by the recruitment and activation of interstitial fibroblasts in the kidney [115]. Moreover, OPN was found to be a promoter of aldosterone-induced inflammation, interstitial fibrosis and oxidative stress in the kidney [116]. It is of interest that renal mRNA expression of OPN was elevated in diabetic conditions and has been shown to initiate interstitial fibrosis in the diabetic kidney, signifying a potential role for OPN in the pathogenesis of diabetic nephropathy [117].

Urotensin II (UII), an 11–amino acid vasoactive peptide that has been recognized as the ligand for a novel G protein-coupled receptor. Despite its basic vasoconstrictive actions, UII also shows profibrotic properties. Intriguingly, UII and its receptors are known to be over-expressed in the kidneys of diabetic patients, telling a role for UII and its receptor in the pathogenesis of diabetic nephropathy [118]. The mammalian target of the rapamycin (mTOR) signaling cascade, a chief component of two multiprotein complexes known as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), regulates cellular growth, survival and metabolism. Some studies reported that there is increased p- Akt and mTOR expression in Diabetic conditions [119] and this over-activation of mTOR signaling has been linked to increased levels of renal mRNA expression of the proliferating cell nuclear

antigens TGF-β1, VEGF, and MCP-1. This involvement resulted in noticeable renal structural and functional alterations, including increased albuminuria, glomerular hypertrophy, and glomerular basement membrane thickening [119].

***Conclusion*:** In this article, we have concluded that the activation of different cellular pathways in pathological conditions are responsible for the generation of reactive oxygen species. Excessive formation of ROS leading to increased lipid peroxidation oxidative damage of DNA, inhibition of mitochondrial electron transport chain, glutathione (GSH) depletion, enhanced superoxide activity and subsequent cellular apoptosis. This leads to the diabetic nephropathic manifestations of renomegaly, Kimmelstiel-Wilson lesions, mesangial matrix expansion, podocytopenia, TBM thickening, GBM thickening, interstitial fibrosis, and arteriolar hyalinization. However, these conditions characterize only a fraction of the complexities of the renal cellular machinery. Therefore, this cannot be regarded as complete explanations of the cause of diabetic nephropathy further studies are needed to explore the pathogenesis of diabetic nephropathy. Research goals for the future may include the finding of new links between the metabolic and hemodynamic events and the elucidation of how all these numerous events interact to produce the clinical features of proteinuria, hypertension, and chronic kidney failure. More research on the potential targets and molecular mechanisms of diabetic nephropathy is being done to discover effective therapy for it.

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