Diabetic nephropathy: prevalence, pathogenesis and signalling pathways

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One of the main causes of death and morbidity in people with diabetes mellitus is diabetic nephropathy (DN). Diabetes and DN have a complicated pathophysiology that includes the connection between metabolic and hemodynamic pathways, oxidative stress, development of cytokines and growth factors, eventually resulting in renal impairment. Three key lesions, viz. diffuse mesangial enlargement, thickened glomerular basement membrane, and arteriole hyalinosis, represent the pathological hallmarks of the illness. The mesangial cells, glomerular capillary membrane, tubulo-interstitium and vasculature as well as other functional structures are all dysfunctional in the pathogenesis. In low- to middle-income countries, the prevalence of diabetic kidney disease is gradually rising but irregularly. Also, it is underappreciated as a major global health concern. DN is caused by a number of anomalies in the signalling pathways. This article aims to clarify the nature of DN risk factors, prevalence, phases, aetiology and signal transduction pathways.

Keywords: Diabetic nephropathy, oxidative stress, pathogenesis, renal impairment, signalling pathways.

DIABETES mellitus (DM) is the complete lack of insulin or decreased insulin activity, which leads to anomalies in the metabolism of carbohydrates, proteins and fats. It is characterized by hyperglycemia, glucosuria, hyperlipidemia, polyuria, polyphagia, polydipsia and sometimes ketonemia. In accordance with recommendations from the Indian Council of Medical Research (ICMR), New Delhi, and the World Health Organization (WHO), Geneva, Switzerland, criteria for the diabetic condition are fasting blood glucose level ≥126 mg/dl, random plasma glucose level ≥200 mg/dl, oral glucose tolerance test (OGTT) 2 h post 75 g glucose level ≥200 mg/dl and glycated haemoglobin (HbA1c) level ≥6.5%. Some of the initial characteristic features of diabetes include increased thirst, hunger, frequent urination, blurred vision and weight loss. Diabetes is a global public health problem now emerging as a pandemic[1]. Diabetes complications are categorized as microvascular (diabetic nephropathy (DN), diabetic neuropathy and diabetic retinopathy) and macrovascular (cardiovascular disease and cerebrovascular disease)[2]. These are the leading causes of early death and disability worldwide[3].

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DN is now recognized as a major contributor to end-stage renal disease (ESRD). Kidney issues affect 30–40% of the people suffering from type 1 DM (T1DM) for 10–15 years[4]. Craig *et al.*[5] reported that 30–50% of people with type 2 DM (T2DM) developed DN after 20 years of the disease, and 15% had ESRD.

DN is a condition characterized by the development of proteinuria with a subsequent decline in glomerular filtration rate (GFR) and an increase in arterial blood pressure[6]. The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial matrix accumulation, nodular glomerulo sclerosis (Kimmelsteil–Wilson bodies), hyperfiltration and other changes due to long-standing DM[7].

The metabolic and haemodynamic mechanisms play an important role for the pathogenesis of diabetic nephropathy. These mechanisms result in the accumulation of mesangial matrix, endothelial dysfunction, thickening of glomerular basement membrane (GBM), renal tubule injury, fibrosis and inflammation in the tubulointerstitial space[8].

Risk factors

Table 1 lists the risk factors associated with DN[9–18].

Prevalance of diabetic nephropathy

DM has become a major global public health issue. Its incidence and prevalence have increased worldwide. According to the International Diabetes Federation (IDF), approximately 537 million adults (20–79 years) are living with diabetes (Figure 1)[19]. This represents 10.5% of the world's population in this age group. The total number of people with diabetes is projected to rise to 643 million (11.3%) by 2030 and 783 million (12.2%) by 2045. Three in four adults with diabetes are from low- and middle-income countries. Almost one in two (240 million) adults with diabetes are undiagnosed. More than 1.2 million children and adolescents (0–19 years) have T1DM. One in six live births (21 million) is affected by diabetes during pregnancy. Also, 541 million adults are at increased risk of developing T2DM[19].

Approximately 20–50% of patients with T2DM will ultimately develop diabetic kidney disease (DKD). Worldwide, DKD is the leading cause of chronic kidney disease (CKD) and ESRD, accounting for 50% of the cases[20].

Table 1. Diabetic nephropathy risk is significantly influenced by both environmental and genetic variables[9]

Factors	Comments	Drug treatment	Reference
Genetic susceptibility	Genes encoding angiotensin converting enzyme, angiotensin-II receptor, cytokines, proteins involved in glucose or lipid metabolism and extracellular matrix proteins[9]. Diabetes-related nephropathy is linked to chromosomes 3, 7, 18 and 20.	Gene therapy	10
Race	The chance of getting diabetic nephropathy is higher among Asian Indian, African American and Mexican American ethnic groups.	Anti-diabetics	11
Age	People with type 2 diabetes who were diagnosed before the age of 20 years, have a greater risk of fatal renal failure.	Anti-diabetic drugs	12
Raised albuminuria	Increased elimination of albumin in the urine, i.e. micro albuminuria (30–300 mg/g) or macro albuminuria (>300 mg/g).	Glycemic control, endothelin receptor antagonist	13
Duration of diabetes	People with a longer duration of diabetes have the highest risk factor for developing nephropathy, which may lead to increased glycated haemoglobin levels (HbA1c), activation of polyol pathway and advanced glycated end-products (AGEs).	Anti-diabetics–insulin, glipizide, gliclazide, GLP analogs, dipeptidyl peptidase inhibitors	14
Hypertension	People with diabetes could get chronic kidney disease due to other co-existing risk factors like hypertensive nephropathy.	Inhibitors of the renin–angiotensin system	15
Dyslipidemia	The 'lipid nephrotoxicity hypothesis' explains how dyslipidemia can lead to a reduction in renal function[19]. It indicates macrophage infiltration, excessive extracellular matrix synthesis and triggering podocyte death which contribute significantly to the development of diabetic kidney disease.	Statins	16
Obesity	Exact mechanism by which obesity causes diabetic kidney disease is unclear. It is assumed that obesity causes ORG (obesity related glomerulopathy) independent of the effect of hyperglycemia, proteinuria, glomerular damage and glomerular hypertrophy.	Renin-angiotensin-aldosterone system inhibitors, GLP-1 analogs	17
Smoking	Smoking plays a multi-factorial pathogenic role in the progression of diabetic nephropathy, including oxidative stress, build-up of AGEs, hyperlipidemia and glomerulo-sclerosis.	Antioxidants – resveratrol AGEs inhibitors – aminoguanidine	18

The prevalence of DKD was estimated to be around 62.3% in a multi-centre study in India (Figure 2)[21,22]. In 15,856 patients with diabetes, a cross-sectional study from a risk assessment management programme in China reported a 38.8% prevalence of CKD[23]. The cumulative incidence of CKD was reported to be 11.4% in a population-based study from the United Arab Emirates following a nine-year follow-up period[24]. The older population in the region of the eastern Mediterranean had the greatest prevalence rates of DM and DKD[25]. About 15.3% of T2DM patients with decreased GFR were reported in Japan[26]. Results of a prospective diabetic study conducted in the United Kingdom with 4006 T2DM patients showed that 28% of them experienced kidney impairment after a median follow-up of 15 years[27]. According to the cross-sectional analysis of the Third National Health and Nutrition Examination Survey, the prevalence of DKD in the US population was 2.2%. Additionally, from 1988 to 2008, the prevalence of DKD in the US increased proportionally to that of diabetes[28].

Stages of development in diabetic nephropathy

There are five phases in the development of DN.

Stage I: This is a hypertrophic, hyperfiltration stage. In this stage, GFR is neither normal nor elevated. Stage 1 lasts roughly five years after the beginning of the disease. Kidney size increases by around 20%, and renal blood flow increases by 10–15%, although increased excretion of albumin and arterial pressure are within the range.

Stage II: This is a quiet stage (normo-albuminuria). It begins about two years after the disease, and is characterized by renal destruction along with the thickening of the basement membrane and proliferation of mesangial cells. Clinical indications of the illness are still absent. GFR returns to normal values.

Stage III: This is the microalbuminuria stage (albumin range: 30–300 mg/dU), also known as initial nephropathy. It is the initial indicator of glomerular injury that can be observed clinically. It often occurs 5–10 years following the disease. Arterial pressure could be elevated or normal. About 40% of the patients typically progress to this level.

Stage IV: This is the chronic kidney failure (CKF) stage or irreversible stage. Blood pressure increases above normal, GFR falls below 60 ml/min, and proteinuria (albumin >300 mg/dU) occurs.

Stage V: This is the terminal renal failure stage. GFR is less than 15 ml/min at this stage. Kidney replacement therapy is necessary for about 50% of terminal renal failure patients (peritoneal dialysis, hemodialysis, kidney transplantation)[29].

Pathogenesis

DN has a complex pathogenesis due to the combination of metabolic and hemodynamic factors.

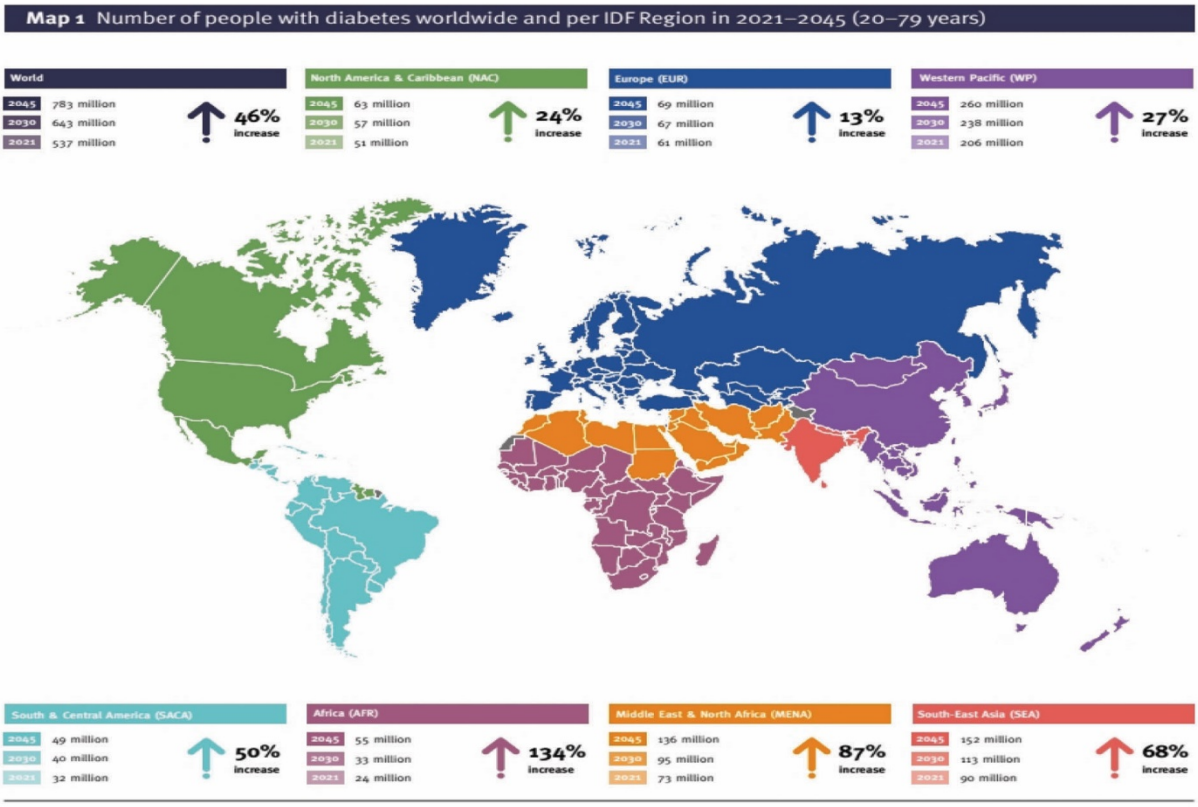

Figure 1. The number of people with diabetes worldwide in 2021–45 (ref. 19).

	Prevalence of diabetes (%)			Prevalence of pre-diabetes (%)		
States/Union Territory	Uraban	Rural	Overall	Uraban	Rural	Overall
Andhra Pradesh	12.6	6.3	8.4	11.1	9.6	10.1
Arunachal Pradesh	5.8	4.9	5.10	14.2	12.3	12.8
Assam	12.4	4.4	5.5	13.6	11.6	11.9
Bihar	10.5	3.5	4.3	15.5	9.3	10.0
Chandigarh	14.2	8.3	13.6	14.5	14.7	14.6
Gujarat	9.5	5.1	7.1	8.4	11.5	10.2
Jharkhand	13.5	3.0	5.3	10.7	7.4	8.1
Karnataka	11.1	5.6	7.7	14.1	10.2	11.7
Maharashtra	10.9	6.5	8.4	15.2	11.1	12.8
Manipur	7.1	4.4	5.1	7.2	7.5	7.5
Meghalaya	8.9	3.5	4.5	7.4	10.6	10.0
Mizoram	7.9	3.6	5.8	6.2	5.8	6.0
Punjab	12.0	8.7	10.0	8.6	7.9	8.2
Tamil Nadu	13.7	7.8	10.4	9.8	7.1	8.3
Tripura	15.5	7.2	9.4	16.2	14.2	14.7

Figure 2. Prevalence of diabetes and pre-diabetes in India[21].

The pathways for polyol mechanism, advanced glycated end-products (AGEs), activation of protein kinase-C (PKC), hexosamine pathway, the activity of xanthine oxidase, insufficient mitochondrial respiratory chain and NAD(P)H oxidase are a few hyperglycemia-mediated metabolic mechanisms that cause DN[30]. In addition, these mechanisms are involved in increased systemic pressure, glomerular pressure and activation of various vasoactive hormones such as the renin–angiotensin system (RAS), nitric oxide, endothelin, vascular endothelium growth factors (VEGFs), urotensin, etc.[31]. By activating inflammatory agents, pro-oxidants, and fibrosis-related pathways, they cause mesangial matrix build-up, thickening of the glomerular basement membrane, dysfunction of the endothelium, podocyte effacement, tubular atrophy, fibrosis, tubule interstitial inflammation, and renal arteriolar hyalinosis (Figure 3).

Metabolic mechanisms

Oxidative stress: It has been implicated in the development of several glomerular or tubulo-interstitial diseases because reactive oxygen species (ROS) exert numerous toxic effects on the kidney. In general, the metabolic activity of nephrons generates oxidative stress, which is then countered by antioxidant enzymes and free-radical scavenging mechanisms. Many harmful biological effects, such as protein oxidation, vasoconstriction of kidney blood vessels and DNA destruction, are mediated by ROS. DN is significantly influenced by the harmful glucose metabolic pathways, such as the polyol pathway, AGEs and PKC activation[32].

Polyol pathway: It includes two enzymes. Aldose reductase (AR) is the first and rate-limiting enzyme. It uses cofactor NADPH to convert glucose to sorbitol, while the second enzyme sorbitol dehydrogenase (SDH), uses cofactor NAD+ to convert sorbitol to fructose. This process increases the NADH/NAD ratio, causes oxidative stress and activates protein kinase C[33]. The utilisation of NADPH by AR results in the depletion of NADPH levels. NADPH also acts as a cofactor for glutathione reductase, which reduces oxidized glutathione (GSSG) into reduced glutathione (GSH) which is required for glutathione peroxidase (Gx). This ultimately results in decreased antioxidant status of the cell (Figure 4).

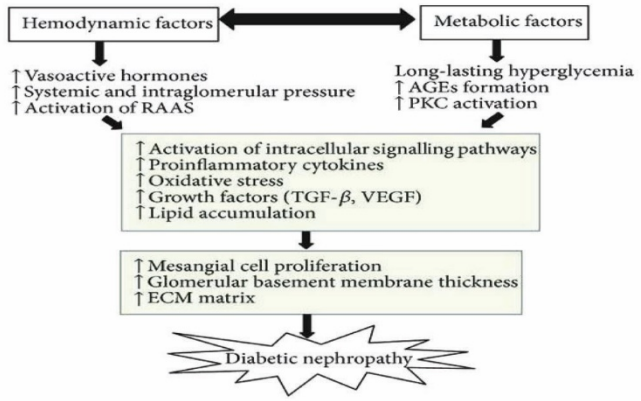

Figure 3. Pathogenesis of diabetic nephropathy involves interaction between hemodynamic and metabolic systems[31].

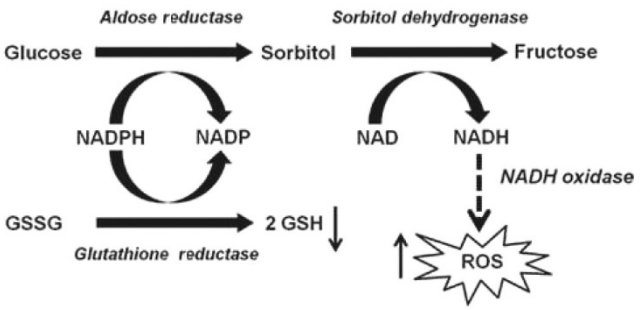

Figure 4. Polyol pathway[33].

AGEs: Excess glucose due to chronic hyperglycemia attaches to the free amino acids in the blood or tissue proteins. Reversible early glycated products and subsequently irreversible AGEs build up in the tissues, which lead to the emergence of microvascular problems related to diabetes, are the results of this non-enzymatic process. Upon stimulation of receptor (R)-dependent and R-independent signal pathways, AGEs affect cellular activation, signal transduction and the production of cytokine growth factors which bind to the podocyte receptors (RAGE). TGF-β (transforming growth factor-β) and the connective tissue growth factor play an important role in the onset of DN that may be induced by AGEs[34].

PKC activation: It is another theory on how hyperglycemia encourages the onset of DN. The specific mechanism by which this enzyme is activated is to promote the release of prostanoid vasodilators, which aid in glomerular hyperfiltration. PKC also increases mesangial cell synthesis of the extracellular matrix by activating TGF-1. *De novo* production of ROS and diaceyl glycerol is involved in the process by which hyperglycemia causes PK-C activation. This in turn triggers the activity of mitogen-activated protein kinases (MAPK) in response to extracellular stimuli. In the presence of high glucose concentration, the co-activation of PKC and MAPK suggests that these two families of enzymes are necessary for the progression of DKD[35].

Hexosamine pathway: As part of the glycolytic process, glucose is converted into glucose-6-phosphate in the presence of hexokinase enzyme and then converted into fructose-6-phosphate. The subsequent conversion of fructose-6-phosphate from glycolysis serves as a substrate for the synthesis of O-linked glycoproteins and proteoglycans. Using glutamine as an amino donor, this occurs under the supervision of the rate-limiting enzyme glutamine fructose-6-phosphate amido-transferase (GFAT). Thereafter, several further reactions take place, eventually producing other glucosamines that act as building blocks for the amino sugars needed to produce glycolipids, glycoproteins and proteoglycans. Higher PKC activation and elevated TGF-1 expression are necessary for increased flow through the hexosamine pathway[36].

Mitochondria dysfunction: This is associated with the complications of DM. With increased ROS production, hyperglycemia stimulates the overload of the electron transport chain, which in turn leads to DNA damage and decreased glyceraldehyde-3-phosphate dehydrogenase (GAPDH) function. Hence, the production of mitochondrial superoxide indicates healthy mitochondria and physiologic oxidative phosphorylation. The ability of the kidney to produce mitochondrial superoxide, oxidative phosphorylation and ATP is decreased in response to high glucose intake or stress. Lower levels of mitochondrial superoxides result in lower levels of adenosine monophosphate-activated protein kinase

(AMPK) activity and endothelial nitric oxide synthase (eNOS) activation, which triggers inflammation due to vascular dysfunction and nuclear factor-kB[37].

Hemodynamic pathways

Many factors are involved in DN, which include nitric oxide, prostanoids, vascular endothelial growth factor (VEGF), TGF-β1 and RAS, especially angiotensin II, etc.[38].

Renin–angiotensin physiology: RAS is made up of renin, angiotensinogen, angiotensin I (Ang I), angiotensin converting enzyme (ACE), angiotensin II (Ang II), aldosterone, Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R). The juxtaglomerular cells of the kidney produce the proteolytic enzyme renin. ACE, which is found in numerous tissues, particularly the pulmonary vascular endothelium, reacts with angiotensinogen to produce Ang I, which is subsequently transformed into Ang II by ACE. As a vasopressor, Ang II directly impacts the smooth muscle of the arteries to increase blood pressure. Additionally, it stimulates the zona glomerulosa of the adrenal cortex to produce more aldosterone, which helps the kidneys reabsorb sodium. Angiotensin causes the blood vessels to constrict when the blood volume is low. This increases blood pressure and releases aldosterone. Efferent arterioles are more tightly closed in the kidneys than the afferent arterioles, causing blood to accumulate in the glomerulus and increasing the glomerular pressure. Researchers have analysed the potential function of RAS in relation to variations in intraglomerular hemodynamics and structural alterations in the diabetic kidney at the glomerulus and tubulointerstitial spaces[39].

Candidate genes of the RAAS cascade

Each component of the renin-angiotensin-aldosterone system (RAAS) controls and maintains blood pressure and the normal function of the kidneys. The variants of the RAAS pathway genes are important in disease progression. Some of the best-studied candidate genes and the role of their variants are as follows.

Renin (REN) gene: This encodes the renin protein, which is the first and the rate-limiting enzyme of the RAAS cascade. It catalyses the initial stage of converting angiotensinogen into Ang I. It is primarily expressed in the kidneys but is also found in other organs, including the brain, where it regulates various activities. Its gene mutations are associated with plasma renin levels, blood pressure and susceptibility to DM[40].

Angiotensinogen gene (AGT): This encodes angiotensinogen, the precursor of Ang 1 and is secreted by the liver into the circulating bloodstream. Mutations in *AGT* are associated with pre-eclampsia, plasma angiotensinogen levels and susceptibility to DM. It contains two common missense polymorphisms, i.e. T702C (M268T, earlier known as M235T) and C521T (T207M, earlier known as T174M) in exon 2. Both these polymorphisms may affect the expression of *AGT* and were found to be associated with the plasma *AGT* concentration[41].

Angiotensin converting enzyme gene (ACE): This gene encodes the angiotensin converting enzyme, which hydrolyses Ang I into Ang II, a powerful vasoconstrictor mainly produced by the lungs. It also inactivates bradykinin which is required to synthesise nitric oxide (a vasodilator). *ACE* genetic variants have been associated with elevated serum and tissue *ACE* levels. One of the best-studied *ACE* gene polymorphisms involves the insertion/deletion (I/D) of 287 bp AluI element in intron 16 and is responsible for the development of hypertension, T2DM and its complications[42].

Angiotensin-II type-I receptor gene (AT1R): This gene encodes the AT1R protein, which mediates all the known actions of Ang II, including increased arterial blood pressure, increased myocardial contractility, sodium and water retention. It comprises the *AT1R* A1166C polymorphism that is involved in the post-transcription modification of the AT1R mRNA. This polymorphism was reported to be associated with the occurrence of T2DM, its complications and hypertension[42].

Aldosterone synthase (CYP11B2) gene: This gene provides instructions for the synthesis of aldosterone synthase enzyme, a steroid 11/18-β-hydroxylase, that is only expressed in the zona glomerulosa of the adrenal cortex to synthesize the mineralocorticoid aldosterone. The polymorphism of the *CYP11B2* gene is T-344C, which is located in the promoter region and have been found to be linked with increased aldosterone to renin level and with the increased risk of kidney disease and T2DM[42].

Vasoactive hormones

Nitric oxide: It is recognized as an endothelium-derived relaxing factor, a signalling molecule that controls numerous cellular and organ processes, comprised of renal hemodynamics and salt water balance. Three nitric oxide synthase (NOS isoforms), including neuronal (NOS1/nNOS), endothelial (NOS2/eNOS) and inducible (NOS3/iNOS), produce nitric oxide enzymatically from L-arginine. These three NOS isoforms exist in the mammalian kidneys; however, NOS1 is particularly abundant in macula densa, parietal epithelium of the glomerulus, medulla, thin ascending limb of henle and collecting ducts. NOS2 is present in the glomerular capillaries of the endothelium, afferent arterioles, efferent arterioles, renal blood vessels, descending vasa recta, proximal tubules and medullary thick ascending limb of henle.

NOS3 is present in the proximal tubules of S3 segments, collecting duct and medullary thick ascending limb[43].

Endothelium: This is the inner lining of blood arteries. It performs a variety of biological tasks, including controlling vascular constriction and maintaining free blood flow in the arteries. The endothelium releases several vasoactive hormones by the endothelium, by interfering with endothelin ETA and ETB receptors. ETB receptors on endothelial cells facilitate hypotension by producing nitric oxide and prostacyclin. Vascular smooth muscle cells mostly contain ETA receptors, which facilitate the constriction of blood arteries. The advancement of DN is due to DM, which causes renal over-expression of endothelin 1 (ET1) in the glomeruli and epithelial cells of renal tubules[43].

Urotensin: Urotensin-II (U-II) is a peptide ligand. It is an agonist for the U-II receptor which is a G protein-coupled receptor. It is a vasoactive peptide of 11 amino acids and plays a significant role in the pathophysiology of heart failure due to its powerful vasoconstrictive, trophic and profibrotic activities. High plasma levels of urotensin lead to renal insufficiency, diabetes and DN. The over-expression of urotensin in the endothelial cells leads to vascular endothelial dysfunction and the formation of ROS[43].

Signal transduction pathways in diabetic nephropathy

One of the main causes of ESRD, DN is a fatal diabetic complication characterized pathologically by a thick tubular basal membrane of a glomerulus, progressive mesangial hypertrophy and accumulating extracellular matrix.

MAPK signalling pathway

A group of Ser/Thr protein kinases are involved in the signalling pathway for MAPKs. They induce an amplifying cascade to be activated, regulating the conversion of signal transduction into cellular transduction, which results in various impacts on the cells. The three kinases that make up the core of MAPK pathway are MAP3K, MAPKK and MAPK. MAP3K undergoes phosphorylation and activates MAPKK, which further activates MAPK. The four sub-families are p38 mitogen-activated kinase (p38MAPK), c-Jun N-terminal kinase (JNK1/2/3), extracellular signal-regulated kinase (ERK1/2) and ERK5. Recent studies have shown that DN formation and progression are influenced by alterations in the MAPK pathway. High glucose levels have been associated with the phosphorylation and activation of the p38MAPK signalling pathway, which stimulates the production of fibronectin in mesangial cells that halts the progression of DN and increases the glomerular basement membrane thickness. According to reports, renal damage in diabetic patients is caused by high glucose-mediated activation of the RAAS via the p38MAPK pathway (Figure 5)[44].

In T1DM and T2DM, selonsertib, an inhibitor of MAP-3K5, and sulodexide, a sulphated glycosaminoglycan, reduce renal damage by targeting p38/MAPK[45].

JAK–STAT signalling cascade

Numerous growth factors and agonists, such as Ang II, function via signalling pathways involving Janus kinase (JAK)/signal transducers and activation of transcription (STAT). In mouse models of diabetes and high blood glucose, tyrosine phosphatases and JAK are activated. Tyrosine and serine phosphorylation then activate other downstream transcription factors, such as the STAT family (STAT1, STAT3 and STAT5A/B), once the STATs have bound to the receptor. It leads to the production of the cytokine TGF-β and extracellular matrix proteins such as collagen IV and fibronectin, which result in reduced kidney function, glomerular mesangial expansion and renal tubulointerstitial fibrosis, which is associated with the development and progression of DN. By inhibition of JAK-STAT pathway, plays a key role in treating DM and DN[46].

Albuminuria, inflammatory infiltration, kidney injury (mesangial enlargement, oxidative stress, tubular atrophy, and fibrosis) and serum amyloid protein are all decreased by drugs that selectively target JAK2 (tyrphostin), JAK1/2 (baricitinib), STAT1 (fludarabine) and STAT3 (nifuroxazide). JAK inhibitors prevent STAT phosphorylation as a potential therapeutic target for autoimmune and inflammatory diseases[47].

Wnt signalling pathway

This consists of a group of cysteine-rich glycoproteins. Two genes, viz. 'wingless' and 'int-1', which produce homologous

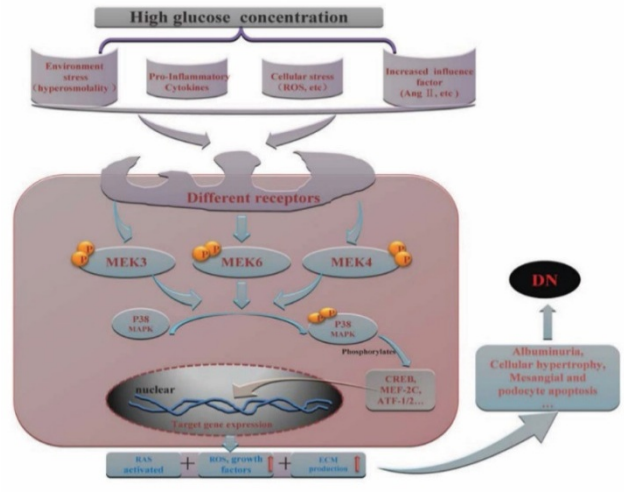

Figure 5. Diabetic nephropathy signalling pathway regulated by the p38 mitogen activated protein kinase (p38MAPK)[44].

proteins, are combined to word 'Wnt'. Signalling transduction of the Wnt pathway plays a crucial role in oncogenesis, embryogenesis and also acts as an important regulator in many diseases, like T2DM, breast cancers, prostate cancer and glioblastoma. The Wnt signalling pathway is divided into two pathways: (i) canonical β-catenin-dependent pathway and (ii) non-canonical β-catenin-independent pathway. Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8a, Wnt8b, Wnt10a and Wnt10b are frequent canonical pathway activators. Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt7b and Wnt11 are non-canonical pathway activators[48].

The canonical Wnt signalling pathway exhibits significant levels of inter-species mechanism conservation. More than 18 types of Wnt-associated proteins are identified in the mouse and human genes. Inhibiting the expression of Wnt4 and Wnt5a mRNA in glomerular mesenchymal cells under high glucose stress causes Wnt to be down-regulated and intracellular signalling to be activated, which in turn increases the activity of the glycogen synthase kinase-3 (GSK-3) enzyme. The *N*-terminal phosphorylation of catenin is promoted by forming a β-catenin degradation complex, including axin, adenomatosis polyposis coli (APC), casein kinase-1 (CK1), PERK and GSK3. This results in the degradation of β-catenin by proteasomes and maintains low amounts of it in the cells. Nuclear β-catenin that is down-regulated inhibits the formation of transcription factor β-cat/TCF, enhancing TGF and fibronectin gene expression. This causes an increase in extracellular matrix (ECM) gene expression, and excessive ECM deposition results in the development of glomerular sclerosis, diabetic renal progressive fibrosis, and glomerular mesangial enlargement and fibrosis (Figure 6)[49].

ERK signalling pathway

The primary signalling system that controls cell growth, change, differentiation and apoptosis is known as the ERK pathway. Research has shown that ERK is essential for integrating the transcription of genes involved in a wide range of cellular responses in DN[50]. The ERK pathway can be triggered in various renal cells, including podocytes, mesangial cells and growth factors.

Under the influence of hemodynamic and metabolic factors, i.e. activated Raf-1 kinase undergoes phosphorylation, further activating MAPK/ERK kinase (MEK1/2), a dual-specificity kinase which phosphorylates the threonine and tyrosine residues of distinct isoforms of MAPK, activating ERK-1/2 and subsequently activating RAAS. Ang II is particularly known to have the potential to damage kidney cells when the RAAS components are activated. This is due to a number of mechanisms: including pressure induction that results in renal injury, activation of renal fibroblasts that transform into myofibroblasts, stimulating the production of osteopontin and chemokines, sensitization of the profibrotic cytokine transforming growth factor-β and ROS, which results in localized inflammation and stimulate proliferation of mesangial cells, renal excretion of albumin, hyperfiltration and glomerular hypertrophy in diabetic rats (Figure 7)[50]. It is a crucial signalling pathway in DN. Understanding the ERK processes and identifying specific target proteins and drugs to treat DN require further research.

NOX oxidase signalling pathway

Nox isozymes are expressed in a variety of renal cells, including endothelial cells (Nox4), smooth muscle cells (Nox1), glomeruli (Nox1, Nox2, Nox4 and Nox5) and tubule cells (Nox4 and Nox1) (Figure 8).

The beginning and development of DN are characterized by activation of the RAAS pathway, activation of PKC, formation of AGEs and TGF-induced fibrosis. AGEs, which act as ligands for the cellular receptor RAGE and transmit signals for inflammation, are produced in larger

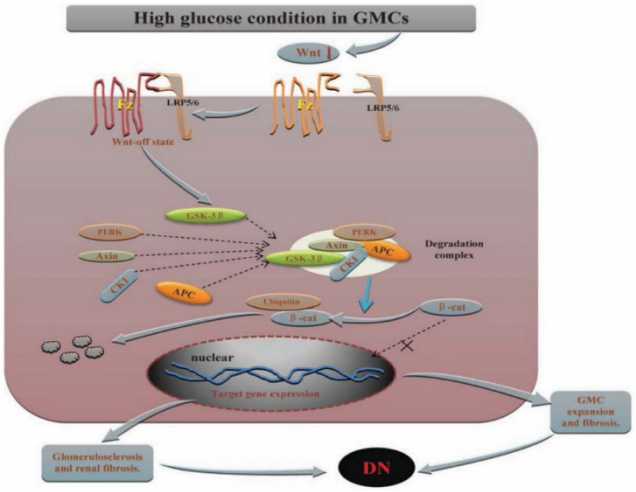

Figure 6. Canonical pathway for Wnt signalling in diabetic nephropathy[49].

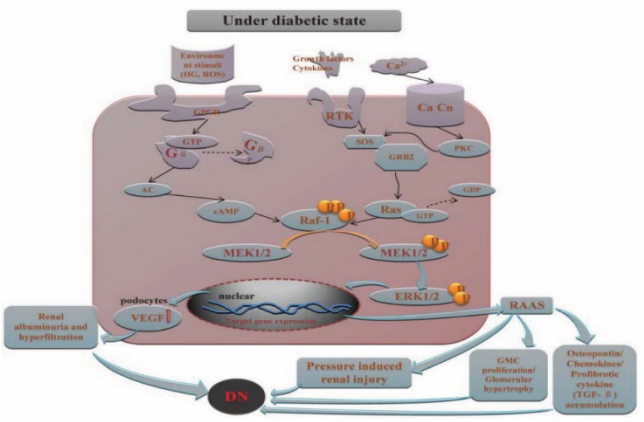

Figure 7. Diabetic nephropathy extracellular signal-regulated kinase (ERK) signalling pathway[50].

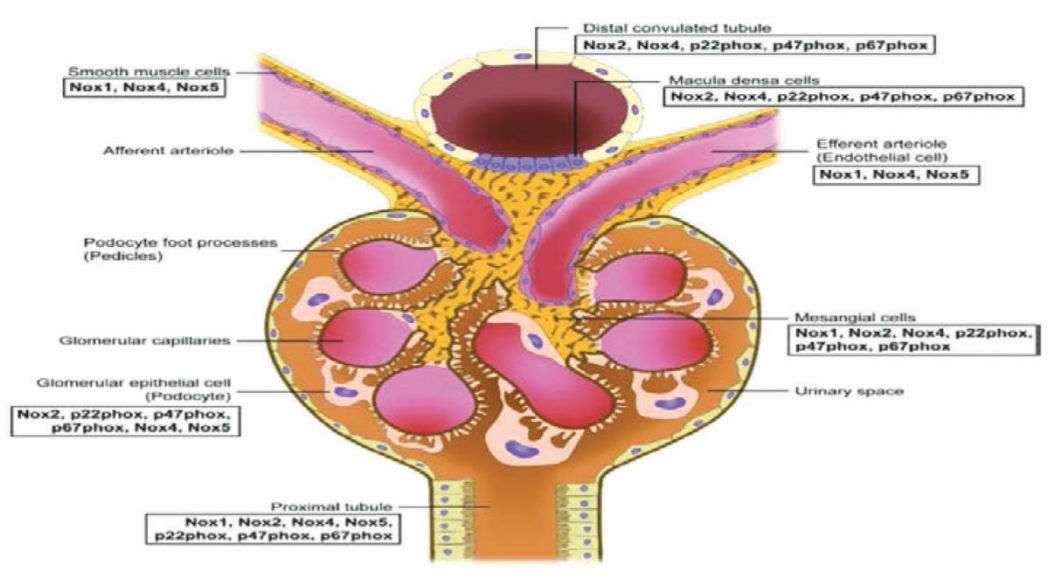

Figure 8. Distribution of Nox isozymes and their components in the kidney[51].

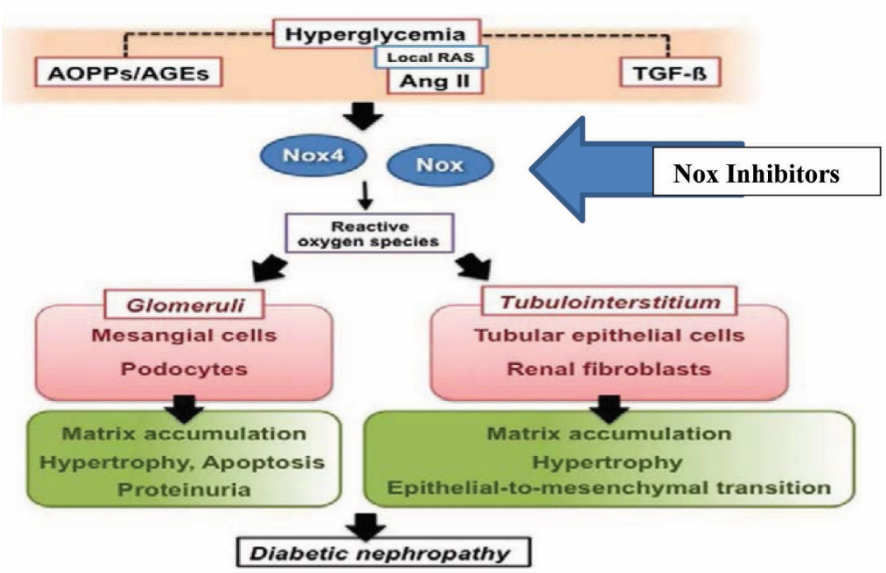

Figure 9. Nox oxidase pathway in diabetic nephropathy[51,52].

amounts as a result of hyperglycemia. Activation of signalling networks by AGEs stimulates nuclear factor-kB (NF-kB) activity, which induces the generation of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). AGEs activates the PKC and VEGF expression which activates Nox isoenzymes causing mesangial cell hypertrophy that contributes to ECM accumulation, hypertrophy and glomerular basement membrane thickening (Figure 9)[51].

Two Nox inhibitors, GKT136901 Genkyotex and APX-115 (Aptabio), were the lead compounds identified from high-throughput screening. These compounds protect against DN[52].

Role of oxidative stress in diabetic nephropathy

Oxidative stress plays an important role in diabetic renal injury. High glucose generates intracellular ROS and superoxide anions in the mesangial and tubular epithelial cells through mitochondrial metabolism. ROS indirectly activates aldose reductase activity and the polyol pathway, which in turn activates AGEs and PKC through *de novo* synthesis of diacylglycerol (DAG). Activation of PKC in the glomeruli has been associated with processes increasing mesangial expansion, thickening basement membrane, endothelial dysfunction, smooth muscle cell contraction, and activation of cytokines and TGF-β. ROS produced by the

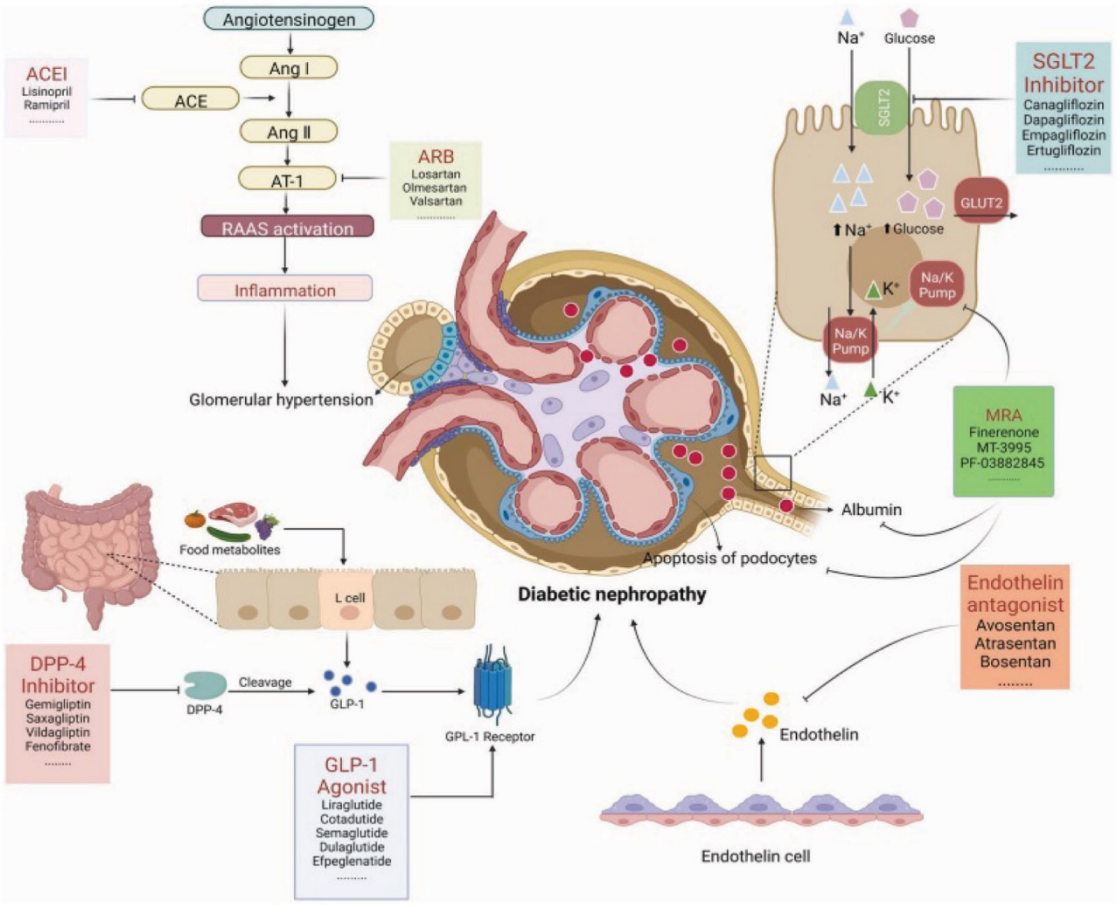

Figure 10. Underlining mechanisms of representative pharmacotherapy on diabetic nephropathy[55].

mitochondria plays a critical role in DN. Antioxidants inhibit high glucose-induced TGF-1 and ECM expression in the glomerular mesangial and tubular epithelial cells and ameliorate features of DN[53].

Current pharmacotherapy against diabetic nephropathy

Activation of RAAS promotes the development of renal inflammation by converting Ang I to Ang II through ACE. Accordingly, the blockade of RAAS via angiotensin converting enzyme inhibitors (ACEIs) and angiotension II receptor blockers (ARBs) shows reno-protective effects in DN patients. The mineralocorticoid receptor antagonist finerenone is a new therapeutic approach for treating DN, which reduces the CKD progression, cardiovascular diseases and risk of hyperkalaemia[54].

Inhibitors of the sodium–glucose co-transporter-2 lower the reabsorption of glucose in the renal tubule. Metformin and canagliflozin treatment for T2DM patients decreased TNFR1, IL-6, metalloproteinase-7 and fibronectin-1 levels, suggesting that canagliflozin helps to revert molecular processes related to inflammation, extracellular matrix and renal fibrosis. The treatment of persistent proteinuria in DN can be done with dipeptidyl peptidase-4 inhibitors. GLP-1 analogues reduce albuminuria, histological renal damage, down-regulate genes related to inflammation (NF-κB, TNF-α), oxidative stress (Nox4 and sub-units), *de novo* lipogenesis/lipotoxicity and fibrosis (Figure 10)[55].

Renin inhibitors (aliskiren), endothelin-A receptor inhibitors (atrasentan or avosentan), PKC inhibitors (ruboxistaurin), aldose reductase inhibitors (epalrestat and tolrestat), AGEs inhibitors (aminoguanidine or pimagedine) are lowers elevated albuminuria, glomerulosclerosis, interstitial renal fibrosis, macrophage accumulation, mesangial expansion, glomerular hypertrophy, hyperfiltration and extracellular matrix deposition in the kidneys[56].

Conclusion

In conclusion, diagnosing micro-albuminuria early will help quickly identify people with DN. Some of the risk factors for DN described in the literature include poor control of blood sugar level, prolonged duration of DM, uncontrolled arterial pressure, smoking and physical inactivity. The signalling pathways, associated proteins and their interactions in

these pathways that are important for revealing the aetiology of DN should be given more focus in future studies.

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