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Molecular Mechanism of Pathogenesis and Management of Diabetic Nephropathy

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Review Article

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Abstract

Kidney disease is continuously rising in the society, which may be acute or chronic. The earlier one is attributed to chemical pollution, side effects of drugs/food. The later is mainly due to life style related metabolic disorders, resulting to systemic low grade inflammation, oxidative stress, immunological hyperactivity and cellular apoptosis in kidney. Diabetes and high blood pressure are the main factors to induce chronic kidney disease (CKD). At molecular level it simultaneously activates several pathways, which collectively induce cell apoptosis/necrosis in various types of kidney cells, such as activation of protein kinase C (PKC) and transcription factors like NFkB and HIF-alpha. Their respective target genes have been identified as objective parameters to assess the severity of the disease and also to develop newer molecular therapeutics. This review emphasizes the prominent pathways and their signal transduction, which would be helpful in understanding the target molecules for development of newer drugs, to manage kidney diseases. It also covers the possible therapeutic modalities which are under development.

Introduction

As per epidemiological studies, kidney about 382 million people are diabetic worldwide and approximately1/3 of them may develop chronic kidney disease, which needs dialysis or kidney replacement, resulting a great financial burden and also beyond the reach of common men [1,2]. The kidney disease is continuously rising and today about 10% of world population is under its ambit. As per prediction statistics, it is estimated that by 2030, there will be at least 400 million individuals with type 2 diabetes mellitus worldwide, with predominance of relatively younger population of low or middle-income countries [3]. Some ethnic groups such as Asian population are pre-disposed to metabolic syndrome (MS) and recently WHO has projected the highest number of diabetics in India by 2030. Recently, International society of Nephrology has launched a campaign called '0-25' to eliminate preventable death from kidney disease by 2025 (The lancet March 2016).

The complications of CKD include myocardial infarction (MI), stroke, blindness and end-stage kidney disease (ESRD). The High Blood pressure (BP) and diabetes are well-established risk factor for CKD and its complications [4,5]. Because of better medical interventions, the longevity of diabetic persons is increasing rapidly, but number of people living with morbidity due to these diseases is also on proportional

rise. This is creating a huge financial burden on the society along with reduction of work efficiency of an individual. So, early diagnosis and timely intervention is the only way to reduce this number.

Diabetic nephropathy, also called diabetic chronic kidney disease (DCKD), is a progressive disease with abnormal kidney function. It is usually asymptomatic and frequently remains un-diagnosed in its early stage of pathogenesis. As per an epidemiological study, 1/3 of diabetic patients revert to normal, 1/3 remains stable and only 1/3 of them proceed to end stage renal disease (ESRD) and needs dialysis and renal transplant. Even this number is very high and many people fail to afford this mode of therapy, because of high expenses and also shortage of kidney donors. Thus its prevention could be achieved only by retarding the rate of progress of pathogenesis with proper medical intervention, especially at early stage [6-8].

The diabetes and hypertension are the primary causes, but factors like adulterated food, environmental pollutions, drug's side effects, and acute kidney injury are also significant contributors for its pathogenesis. Since DN pathogenesis brings irreversible changes in kidney tissue, so its early detection and prevention is the best approach. Diabetes is characterized by high blood glucose, with low insulin level, systemic low grade inflammation, compromised immune system, beta-cell apoptosis, amyloid deposits and fibrosis in pancreas [9]. The glycosylated proteins, angiotensin II, TGF-β, and high proteinuria play important roles in stimulating the fibrosis in pancreas and kidney.

The DN is characterized in terms of proteinuria, which is associated with increased glomerular permeability for plasma proteins and its reuptake by proximal tubular cells, initiates an inflammatory process. Clinically, DN is defined as urine albumin >200 mg/24 hr or total protein >300/24 hr urine along with reduced estimated glomerular filtration rate (eGFR). The urinary albumin excretion rate between 30 to 199 mg/24 hours is considered *microalbuminuria* [10]. In CKD, there is significant increase in urine protein, serum urea and creatinine. There is increase in protein/creatinine ratio and reduced eGFR in these patients. The patients of diabetic nephropathy (DN) with serum creatinine (Scr) >106 μmol/L (>1.8 mg/dL), urine protein >300mg/24 Hrs, creatinine clearance (Ccr) <80 mL/min, eGFR <90 ml/min/1.73 M^2, will be registered. Usually the diabetic patients with urine protein >300mg/24hr are considered as patients of diabetic nephropathy and referred to the nephrology clinic, but early detection of micro-albuminaria would be essential. eGFR, if it is <60 then it is considered as ESRD, but >60 to <90, then it comes under CKD. The severity of DN is classified in 4 stages, on basis of eGFR (normal= 90 ml/min/1.73 M^2).

(1) Diminished renal reserve eGFR <90)
(2) Diminished renal reserve when eGFR remains up to 80% of normal value (>60);
(3) Renal insufficiency when eGFR remains 20-50% of normal value;
(4) Chronic renal failure when eGFR is less than 20% of normal value and
(5) ESRD, when eGFR is less than 5% of normal value.

At molecular level DN is associated with low activity of MMP-9 in the kidney, resulting increase in ECM accumulation and basement thickening. There is high rate of apoptosis of kidney cells, especially the podocytes, cells of glomerulur vessels and tubular cells.

Regarding, ESRD, it is reported that more than 40% of such patients are linked to the long-standing diabetes mellitus [11]. Though the onset of ESRD is irreversible but its progress can be retarded in early stage [12-14]. The progress of DN is defined as the doubling of serum creatinine and 50% reduction in eGFR from normal values.

Proteinuria is useful for the screening and diagnosis of overt diabetic nephropathy [15,16]. It is an independent risk factor for decline in GFR in CKD and also for cardiovascular disease. The persistent proteinuria induces local inflammation in the kidney, resulting damage to glomerular and tubule-interstitial damage, through release of prostaglandins [15]. Thus, anti-inflammatory drugs like COX-2 inhibitors and those agents which inhibit proteinuria may be effective in preventing the progress of DN. (UK Guidelines for Identification, Management and Referral for Chronic Kidney Disease in Adults: Royal College of Physicians, London, 2006: http://www.renal.org/CKDguide/full/CKDprintedfullguide.pdf).

The rise in urine protein may be due to (1) defects in glomerular filtration barrier called Glomerular proteinuria (eg, glomerulonephritis or nephrotic syndrome) or (2) due to incomplete tubular re-absorption of proteins (called tubular proteinuria-interstitial nephritis) or (3) due to increased plasma concentration of proteins-Overflow proteinuria. Since proteinuria could be due to other reasons also so albuminaria is considered more specific to kidney disease. Albumin in urine is the principal component of proteinuria in glomerular disease.

High albumin indicates towards tubular proteinuria, which is due to decreased tubular re-absorption of filtered albumin. Many reports indicate that only albumin concentration is not very reliable in diagnosis of kidney damage, so albumin/creatinine ratio (ACR) is being considered as better option. High similarity between albumin concentrations in 24 hr and albumin/creatinine ratio in spot urine test has shown great similarity. However, it is expensive and also needs sex- and age-specific discriminator values [17-20].

The normal range of ACR is < 3.0 mg/mmol creatinine, but urine ACR between 3.0 - 30.0 mg/mmol is considered moderate nephropathy and >30.0 mg/mmol creatinine is considered severe nephropathy. The urine protein/creatinine (PCR) ratio is also in practice in several clinics, because of cost factor. The normal value of PCR is considered as normal if it is <15 mg/mmol Creatinine. The ratio ranging between 15 – 45 mg/mmol Creatinine will be considered Trace Proteinuria; 50 – 99 mg/mmol Creatinine = Significant proteinuria, 100 – 300 mg/mmol Creatinine = High proteinuria., >300 mg/mmol Creatinine = "Nephrotic range" proteinuria. In terms of mg unit, the normal protein-to-creatinine ratio for males is <0.11 mg/mg creatinine and for females it is <0.16 mg/mg creatinine.

When we talk of rate of protein excretion/day, then 24 h urine collection is the best option, because the urine protein concentration is significantly affected by status of dehydration at different time point of the day, which may give false reading in spot test. However it is not patient friendly, difficult to collect and it has poor compliance. Thus random urine samples are being considered by most professional organizations for microalbuminuria screening. The spot urine collection or morning-time urine collection for a fixed time will be considered.

Pathogenesis

Acute nephrotoxicity is usually linked to high pollution, impurity and adulterants in food & drinks and also due to adverse effect of several life saving drugs such as chemotherapeutic drugs and antibiotics, immunosuppressive drug e.g. Cyclosporin A (CsA). Most of the time, they are auto-healed after the removal of the toxin. However in some cases medicine is required for early healing. Cisplatin induced acute nephro-toxicity is of great concern as it is linked to chemotherapy of cancer patients. In most of the cases, such nephrotoxicity are linked to oxidative stress due to sudden increase in free radicals in the system [21].

In CKD, persistent-hyperglycemia and high blood pressure are the basic causative factors for pathogenesis of chronic kidney disease (CKD) covering diabetic nephropathy (DN). However, other factors like Anemia, Nephrotoxins, drugs, intravenous contrast media, decreased perfusion due to severe dehydration, persistent proteinuria, Hyperlipidemia, recurrence of stone formation. Hyperphosphatemia with calcium phosphate deposition, Smoking, recurrence of acute kidney injury (AKI), systemic inflammation, vascular disease, Glomerular disease (primary or secondary), Cystic kidney diseases, Tubulointerstitial disease, Urinary tract obstruction or dysfunction and metabolic acidosis are also responsible. This is due to high synthesis of acids, especially from sulpher containing proteins and increased loss of bicarbonate. Higher action of Endothelin-1, mainly due to metabolic acidosis, is also responsible of renal functional decline.

Collectively these factors cause hemodynamic disturbance, vasoconstriction and high rate of nutrient filtration through glomerulus, Hyper-filtration and hypertrophy of residual nephrons, glomerular capillary pressure, capillaries damage and glomerulosclerosis. On biochemical basis, there is decline in the activity of renal antioxidant enzymes, high metabolic rate in podocytes induces hypoxia, high free radical (FR) generation, endoplasmic reticulum stress (ER stress), inflammation and finally apoptosis. The Podocyte-loss and tubular injury raises proteinuria (9). Both genetic and epigenetic factors are also involved in its pathogenesis [22].

In case of diabetes, hyperglycemia and hypoxia activates glycolysis and associated rise in glucosamine pathway and polyol pathway. They generate more AGEs and activate PKC isoenzymes. This combined cellular milieu, induces VEGF, reduces MMP-9, depletes nephrin and podocin in podocytes and also induces apoptosis in podocytes [23,24]. Tubulo-interstitial fibrosis is predominantly found in the progression of chronic kidney disease. The accumulation of connective tissue and infiltration of inflammatory cells and myo-fibroblasts into the renal parenchyma result in irreversible organ damage.

More specifically at cellular level in the kidney, the proximal tubular-cells get converted to fibroblast-like cells and migrate into the interstitium to produce collagen and fibronectin. The histo-pathological parameters indicate changes in Bowmann's capsule dilation, lymphocyte and macrophage infiltration, and disappearance of the PAS-positive glycocalyx from under the brush border, interstitial collagenes of types I, III, and VI, in PAS–haematoxylin stained tissue sections [25].

Another factor is the Oxidative stress. The FRs and their peroxidation-products along with advanced glycosylated end products (AGEs), get accumulated within the cell and affect its normal metabolism. They also alter the structure and function of ECM [26, 27]. It interacts with specific cell surface receptors, resulting to raised level of inflammatory cytokines, platelet-derived growth factor, fibronectin TGF-β, vascular endothelial growth factor (VEGF) and protein kinase C (PKC) [28].

The free radicals also induce hypoxic condition within the cell resulting induction of HIF-1α, a transcription factor for many target genes including VEGF. This condition simultaneously increases the expression of various PKC iso-enzymes in the kidney tissue, finally resulting cell death. Protein kinase-C (PKC) has been implicated in a big way. It is a family of diacylglycerol (DAG) responsive enzymes that are recruited to cellular membranes as a consequence of DAG production, where they phosphorylate specific target proteins responsible for regulating cell growth. The PKC hyper activation has been associated with vascular alterations such as increases in permeability, contractility, extracellular matrix synthesis, cell growth, apoptosis, angiogenesis, leukocyte adhesion, and cytokine activation. The PKC has a large family with 10 iso-enzymes, with different functional properties. Mainly PKC-α, -β-1/2, and PKC-δ are linked to the development blood vessel abnormalities. It mainly affects macro and micro-blood vessels resulting atherosclerosis, cardiomyopathy, retinopathy, nephropathy and neuropathy etc. Primarily, the activation of PKC-β PKCα, PKC β-I, β-II, and epsilon mRNA have been reported in high proteinuria and cell damage [15]. PKC-ß induces the mesangial proliferative glomerulonephritis (L). It also activates NADPH oxidase, which significantly enhances reactive oxygen species (ROS) generation in glomeruli.

Hypoxia, is another important factor and sometimes results due to ischemia/reperfusion injury. It induces HIF-1 as an adaptive mechanism, which is a transcription factor for many nephroprotective genes and also for erythropoietin [29,30]. However under sustained hypoxia and induction of HIF-1 is pathogenic. They have 2 isoenzymes i.e. hypoxia inducible factors (HIF)-1α and HIF-2α. The HIF-1a is the master regulator of angiogenesis and mainly expressed in tubular cells. On the contrary, HIF-2α is expressed in peri-tubular interstitial, endothelial, and glomerular cells.

Therapeutic Modalities

Interestingly, no drug is available for direct targeting the signaling molecules, involved in its pathogenesis. Only hypoglycemic and antihypertensive drugs are clinically used. Agents, which directly inhibit or degrade TGF-β or inhibit AGEs or inhibit the raised-PKC activity, are being developed as drug for DN. Besides, the drugs with cyto-protective action are also effective e.g. (1) thiol-disulfide redox protectors, glutathione, metallothionein, *N*-acetyl-L-cysteine, and other low-molecular-weight thiols. The modulation of signaling pathways, involving epidermal growth factor, the mitogen-activated protein kinase family, protein kinase C and B (Akt), heat shock proteins, p53 and p21, peroxisome proliferator activator receptor-γ coactivator-1α (PGC-1α), intracellular pH and glycine level, chloride channels and metabolic acidosis are significant in developing nephroprotective drugs.

The specific inhibitors of PKCs have also been developed as a new class of therapeutic strategy. Use of LY333531 (a PKC β inhibitor) and Ruboxistaurin (LY333531) mesylate (a bis-indolyl-maleimide) a PKC inhibitor, have shown clinical significance in reduction of albuminuria, protection of structural injury to kidney tissue and excess fibronectin production.

The accumulation of AGEs is directly involved in damage of proximal tubular cells. The ACEs activation increases proteinuria, decreases glomerular filtration rate (GFR), resulting to end-stage renal disease (ESRD). Its use is associated with angio-edema and enhancement of kinine production via the stimulation of angiotensin receptor type II. It enhances (1) lipid peroxides and 8-hydroxy --deoxyguanosine, resulting to tissue injury and albuminuria. Thus, by breaking the cross-links in AGE complexes is another strategy for drug development, e.g. ALT-711. On the contrary, inhibition of its synthesis by reducing hyperglycemic and oxidative stress may be also useful. Pyridoxamine, an AGE inhibitor has shown protection against diabetic nephropathy.

The PGI_2 analogs also show nephroprotection. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide, a well-known cytoprotective agent and affects such renal toxicity. A mitochondria-targeted compound, which is a conjugate of a positively charged rhodamine molecule with plastoquinone (SkQR1) are also nephroprotective as its injection decreases the level of cytochrome c in the blood [30,31].

In current clinical practice, major medical interventions include anti-hypertensive and anti-diabetic drugs. In the 1st group, the inhibitors of renin-angiotensin-aldosterone system (RAAS) and angiotensin *receptor blockers* (*ARBs*) are more in use. The angeotensin receptor blockers (ARBs) like irbesartan, Eplerenone valsartan, and

aldosterone receptor blocker like Eplerenone, endothelin [32]; calcium channel blockers, beta-blockers (metoprolol, hydralazine), diuretics are effective drugs. The agents for neuro-hormonal activation (neprilysin inhibition) and renal inflammation/ fibrosis are also in use [33,34].

In the 2nd group, of hypoglycemic agents [35], drugs include tubuloglomerular feedback mechanisms (sodium glucose co-transporter 2 inhibition and incretin-based therapy sulphonylureas, biguanides, Alpha glucosidase Inhibitors, Thiazolidinediones, Incretin based therapies, dipeptidyl peptidase4 (DPP4) inhibitors, ETA-selective antagonist, and heparinase, known to modulate the signaling of pro-fibrotic factors such as FGF-2 and TGF-β [36,37].

In addition, the agents, who modulate the production of cytokine and nitric oxide, responsible for various actions including inflammation and vasoconstriction are in trial. The anti-fibrotic therapy; vaso-peptidase inhibition; antioxidant therapy; PKC inhibitors and 3-Hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, statin therapy; glycosaminoglycan therapy; anemia management are in practice [38]. Further, agents that directly inhibit or degrade TGF-β or inhibit AGEs are also important [39].

Some synthetic drugs, like sulphoraphane (AGEs inhibitors) [40], Sevelamer (anti-inflammatory), Sulodexide, (antioxidant) [41], Nicorandil (reduced glomerular injury), Tolrestat and Finerenone, a Steroidal mineralo-corticoid receptor antagonists [42], patiromer (potassium-binding polymer) [43], Endothelin Receptor Antagonists [44] are being developed, but still they are not approved or clinical use [45].

The herbal medicines from Indian systems of medicine like Ayurveda and Chinese medicine have also been tried for management of CKD. Several of them has been claimed to have reno-protective potentials. In lack of scientific evidences, these medicines, though with high therapeutic potentials, are not being used by doctors of allopathic medicine, even when they have limited medicines for DN. Some of them includes *Punarnava* plant (Spreading Hogweed/Boerhavia diffusa), *Gokshura* (Tribulus Terrestris), *Lal Chandan* (Red Sandalwood-Pterocarpus Santalinus), *Apamarg* (Achyranthes aspera), siris (*Albizia lebbeck), Shigru*' (Moringa Oleifera), *Varuna* (Crataeva nurvala), makoi (Solanum nigrum),kakri (Cucumis sativus), Palash (Butea monosperma) [46], Shenzhuo-formula (TCM), mulberry leaf, *Punica granatum* [47].

In our laboratory, we have found an active fraction of tubers of *Pueraria tuberose* DC, (PT) Leguminosae (Fabaceae), Indian kudzu, *Vidarikand* as an effective therapy for DN. Our earlier experiments with animals and cell culture have shown its antioxidant [48,49], anti-inflammatory and nephro-protective potentials [50-52]. It has also shown DPP-4 inhibitory property, which indirectly linked to nephroprotection [53,54]. Recently, we have tested in the streptozotocin (STZ) induced diabetic nephropathy in rat model. It significantly reduced the extra cellular matrix (ECM) accumulation by activation of MMP-9 [55]. Recently we have reported that it also acts as antioxidant as it ingibits the expression of HIF-alpha and it target gene VEGF.

Since, DN is multi-etiological disease, so multi-targeted drugs would be more useful for its treatment. The herbal medicines could be more suitable, because it is a natural cocktail of many phytochemicals, which might be acting simultaneously on different pathogenic targets in its signaling pathway. This has been recently seen in case of total extract of *Artemesia anua*, which showed better response against resistant malarial parasites, in comparison to its pure active compound (award of 2015 Noble prize). However, proper pre-clinical toxicity, clinical trials and herb-drug interaction with ongoing allopathic drugs are needed for their better clinical use.

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