

Molecular Mechanisms of Diabetic Nephropathy, General Preventive Measures and Novel Therapeutic Strategies

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Abstract: Diabetic nephropathy (DN) is a major micro-vascular complication in diabetes mellitus (DM). One third of type 1 DM and 1/6th of type 2 DM develop DN account for more than 30% of total end stage renal disorders (ESRD), the main cause of renal replacement therapy. It is characterized by mesangial expansion, glomerulosclerosis and increased intracellular matrix accumulation. Injury of podocytes and reduced cellular density are considered to be root of the disease. Molecular mechanisms that leads diabetic patients toward nephropathy is hyperglycemia induced production of reactive oxygen species (ROS), advanced glycation end products (AGEs), activation of polyol pathway, increased expression of TGF- β , angiotensin II and aldosterone induced oxidative injury. DN can be prevented by controlling the glycemic levels, blood pressure, body weight and consumption of low protein diets along with high potassium supplementation. Therapeutic strategies including intensive glycemic control, blockage of the renin-angiotensin system and anti-hypertensive therapies are proved to be beneficial but still failed to gain complete response. Limited researches are focused toward invent and formulate novel therapeutic agents that would be able to target at a molecular level so as to be more beneficial even if the glycemic level is not well controlled. Therapeutic strategies may include AGEs inhibitors, blockers of receptors for advanced glycation end products, PKC targeted agents, tyrosine kinase inhibitors and mitochondria targeted anti-oxidants.

Keywords: Diabetic nephropathy, ESRD, ROS, AGE, PKC

1. Introduction

DN: Characteristics and etiology

DN is the most serious secondary complication of DM with high rate of mortality which is the main cause of ESRD in adults¹. About 30% of diabetic patients develop DN², in the case of poor control of blood pressure and urinary albumin excretion, they lead to ESRD³.

DN develops in following characteristic phases.

- 1) Elevation of kidney plasma flow and increase in GFR, hypertrophy of kidney and renal hyper-filtration
- 2) Renal parenchymal changes and normo-albuminuria, mesangial expansion and thickening of basement membrane
- 3) Early hypertension and micro-albuminuria
- 4) Observable albuminuria
- 5) End stage renal disorder⁴

These disorders contribute in generating of cell injury and therefore apoptosis of podocytes, extracellular proteins accumulate in tubular interstitial and in glomerular region⁵.

Mechanisms of development of DN: Persistent hyperglycemia has a strong relationship with development of DN. Various mechanisms of involvement of hyperglycemia in the development of DN are proposed.

- 1) Activation of oxidative stress by high concentration of glucose and production of ROS
- 2) Production AGEs (advanced glycated end products)
- 3) Activation of PKC (protein kinase C), proinflammatory transcription factor NF- κ B, transforming growth factor (TGF) and RAS (renin angiotensin system)⁶.

ROS: Mechanism of production in DM and their role in development of DN

Free radical production due to hyperglycemia has important role in induction of cellular oxidative damage⁷, its role in production of diabetes associated macro vascular complication is also approved. During high glucose concentration, there is overproduction of mitochondrial superoxide which is blamed to be responsible for the hyperglycemia induced apoptosis⁸.

Pathways responsible for production of secondary complication of DM like AGEs formation, RAGE ligand binding, specific inhibitors of aldose activity, activation of protein kinase C and hexose amine flux are correlated to the production of high level of superoxide induction in mitochondrial electron transport chain due to hyperglycemia⁹. Superoxide converted into different other free radicals which may be more reactive and causes cellular damage by various mechanisms¹⁰. In ETC transfer of electrons take place through complex I, II and IV which expel protons to intermembrane space thus the proton gradient generates which activates ATP synthase or complex V to bring back protons to matrix via inner membrane. In the case of higher concentration of glucose or in diabetic cells there is high level of pyruvate generated from glucose and oxidized in TCA cycle providing more NADH and FADH₂ as electron donors into the ETC, increasing the voltage gradient of mitochondrial membrane till reaching of critical threshold. This is the point where complex III is blocked and become unable to transfer electrons thus electrons go back to coenzyme Q. As this coenzyme just donates one electron to molecular oxygen therefore it generates superoxide. Figure (3) The mitochondrial superoxide oxide catalyzes superoxide which yields H₂O₂ and O₂ by other enzymes. The ex vivo

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studies of arterial endothelial cells showed that hyperglycemic conditions increase the electron gradient up to the threshold level and thus increase the production of ROS which then can produce dynamic changes in mitochondrial morphology. The fluctuation in ROS is prevented by inhibition of mitochondrial fission¹¹.

In diabetics patients decrease in the activity seen of GAPDH is seen where mitochondrial superoxide leads to increase the level of upstream glycolytic intermediates¹³.

- 1) Methylglyoxal generated non-enzymatically from glyceraldehyde 3P, activates AGEs pathway, increases the expression of RAGE and also activates the ligand S100 calgranuline and HMG B1¹⁴
- 2) High level of glyceraldehyde 3P also activates PKC pathway due to production of DAG a potent physiologic activator of PK-C pathway
- 3) Blockage of GAPDH further increases the level of fructose 6P which then undergo in hexose amine

pathway and produces UDP- N acetyl glucose amine by the help of GFAT.

- 4) Inhibition of GAPD causes increased concentration of glucose inside the cell which is then consumed through polyol pathway by using NADPH as reducing equivalent.

Advanced glycated end products:

AGEs are the products of non-enzymatic reaction of reducing sugars and amino group of proteins, lipids and nucleic acids. AGE produced via a series of reactions in which Amadori products and Schiff bases produced prior to AGE⁶. The production of AGEs require couple of weeks therefore glycation affects long-lived proteins for example structural components of basement membrane in which collagen is the most affecting protein but myaline, complement C₃, fibrinogen, tubulin and plasminogen activator factor could be affected^{16, 17}.

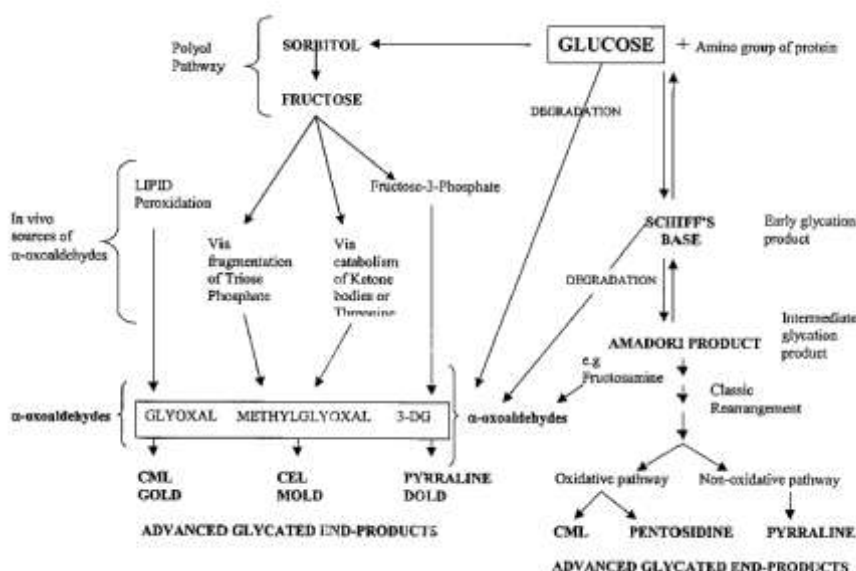


Figure 1: Mechism of formation of advanced glycated end products from glucose and incorporated polyol pathway

In early stages of Millard reaction the rate of reaction is concentration dependent therefore the rate is improved in DM^{18, 19}. The process of glycation by glucose is slower but the rate is faster in the case of Glucose-6-P and fructose²⁰ the rate is accelerated by the presence of transitional metals while it is inhibited by reducing agents such as vit C²¹ and green tea²². Glyoxidation is another phenomena which is used when glycation occurs along with oxidation, pentosidine and N^e-[Carboxy methyl lysine] (CML) are examples of such reaction. In Millard reaction the production of dicarbonyls or oxoaldehydes is of clinical importance. These compounds produce during Amadori rearrangement as reaction intermediates. Methylglyoxal (MGO) and 3-deoxyglucose (3DG) are good examples of such intermediates²³.

Methyl glyoxal with a strong electrophilic nature so it reacts with neucleophilic centers of macromolecule for example DNA, RNA and proteins therefor is considered as a toxic compound in high concentrations, and can cause

cell death²⁴. It is constituted by non-oxidative mechanisms, non-enzymatically in an aerobic glycolysis, it is also produced from poly unsaturated fatty acids²⁵, during the fragmentation of triose phosphate, catabolism of threonine and ketone bodies²⁶. It is related to the dihydroxy acetone phosphate an intermediate of glycolytic sequence. After detoxification it is converted to the lactate (figure)²⁷. Furthermore, it reacts with the side chain of amino acids lysine, cysteine and arginine; it also binds with guanine base and to a lesser extends with adenine and cytosine²⁸. It is suggested that the cytotoxic characteristic of methylglyoxal is due to its inhibitory action of DNA replication²⁹. Mutagenic characteristic of methylglyoxal was proposed by Marnet et al.; when he observed mutagenic changes in *Salmonella typhimurium* cells by converting arabinose sensitive strains to arabinose resistant strains³⁰.

3-Deoxyglucosone:

3-Deoxyglucosone is another important advanced glycated end product, presence and also involvement of which is reported in diabetic microangiopathies such as retinopathy, nephropathy and neuropathy³¹.

3-Deoxyglucosone forms of two distinct pathways.

1-Polyol pathway: 3-deoxy glucosone is generated after the hydrolysis of fructose 3-phosphate.

2-Maillard reactions: In this pathway first the enzymatic glycation of amino group of proteins is occurred and Amadori products are yielded and there after a multiple dehydration reactions and subsequent rearrangements, the 3-Deoxyglucosone and other highly reactive carbonyl compounds are produced. 3DG is able to react with free amino group and AGEs such as pyrraline and CML is generated³².

By controlling the level of blood glucose with anti-hyperglycemic agents the reduction was seen in 3DG level, the level of CML was also decreased but the level of pyrralysin was not affected. Which indicate more involvement of 3DG in the production of CML rather than in pyrraline³³.

Apart from the potential of 3-DG in producing of advanced glycation end products, it has some unique biological activities³⁴ it is accepted that the level of plasma 3DG increases in DM and also it is consider that plasma 3DG plays role in producing the complication of DM³⁵. Moreover it has been revealed that 3-DG is associated with the pathology and bioactivities of senile disease³⁶.

Accumulation of carbonyl precursor of glyoxidation product such as pentosidine and CML or dicarbonyl precursor (precursors of methylglyoxal and deoxyglucosone) and lipoxidation products are known carbonyl stress³⁷. The condition of carbonyl stress is observed in DM and uremia and is considered as an important factor of producing and accelerating vascular damage in both conditions³⁸.

Cross linking induced by AGE

Though the AGEs are differ chemically from each other but they have some consequences of covalent cross linking of proteins. Usually stable and long lived proteins such as collagen undergo cross linking process. The main mechanism of cross linking is not fully understood but according to researchers the lysine residue of the protein is likely to be involved in³⁹. The mechanism of physiological cross linking of proteins such as collagen in which enzyme lysyl oxidase is involved, is well understood. But there is not any evidence to prove such mechanism in accelerated cross linking situation like in DM³⁹. Pathological crosslinking of proteins causes stiffness of proteins as well the process of removal by proteolytic mechanism becomes slower and thus the tissue remodeling process is also affected. All of these changes occur in aging and DM accelerates their rate⁴⁰. The evidence of these process are delivered by the observation of crosslinks between AGE pyrraline and pentosidine by immunostaining in immunohistological studies of diabetic

nephropathy and also by the correlation between accumulation of AGE and stiffness of aorta in the postmortem reports of humans⁴¹.

Cross linking leads to atherosclerosis, thickening of basement membrane capillaries and sclerosis of renal glomeruli. The mechanism of developing atherosclerosis is not only by cross linking but they also trap lipoproteins and thus hampers the efflux of cholesterol from vessel walls and causes macro-vascular disease⁶.

Interaction of AGE with their receptors:

Many receptors of AGE are known which include oligosaccharyl transferase 48(AGE-R1), Macrophage scavenger receptor type I and II, 80 K-H phosphoprotein (AGE-R2), receptor for AGE (RAGE) and galectin (AGE-R3)⁴². The receptors are present in different cells such as microglia, macrophages, podocytes, endothelial cells, monocytes, astrocytes and smooth muscle cells⁴³. In DM the expression of some receptors increased for example expression of RAGE is increased in endothelial cells of kidney. Galactine is increased in kidney of diabetic patients. The RAGE receptor which is present in endothelial cells is the well characterized, the member of immunoglobulin superfamily, multiligand in nature and plays the role of scavenger and also as a mediator of cellular signaling⁴⁴. According to in-vitro observation it is revealed out that when AGE-RAGE complex is produced on macrophage and microglia, it creates oxidative stress and causes activation of a free radical sensitive transcription factor NF- κ B then modulates transcription factor for VCAM-1, thrombodeline, tissue factor and indotheline⁶.

As the non-enzymatic glycation in non-diabetic individual also occur but the harmful complication are mostly seen in the case of DM because for producing of complication the rate of accumulation of AGE is more important than the concentration of AGE, this was proved by comparative study of young diabetic patient with micro-vascular complication who had less concentration of AGE accumulation with older age group having more AGE accumulation but without any complication⁴⁴.

Activation of polyol pathway:

The first enzyme in this pathway is aldose reductase that is present in cytosol and is in monomeric form. It catalyzes the NADPH dependent reduction of glucose and other carbonyl compounds⁴⁵. Affinity of aldose reductase is low (high km value). So in normal glucose concentration, very small level of glucose is catalyzed by this pathway but in diabetes the intracellular glucose increases which undergo catalyzed by polyol pathway in higher rate concomitant with depletion of NADPH reservoirs. In the second step of polyol pathway fructose is produced from the oxidation of sorbitol and NADH is used as coenzyme in this reaction⁴⁶.

Different mechanisms are proposed to explain the harmful effects of hyperglycemic induced flux of polyol pathway include the hyper osmolarity induced by sorbitol, decrease in cellular NADPH level, decreased activity of Na⁺/K⁺

ATPase and an increase in NADH/NAD⁺ ratio. As the diffusion of sorbitol across the cell membrane is not easy so it can cause osmotic damage to micro-vascular cells. Although concentration of sorbitol in diabetic cells is too low to cause damage, the other suggested mechanism is a decrease in activity of Na/K ATPase which occurs as a result of Protein Kinase C (PKC) activation. As oxidation of sorbitol increases the ratio of NADH/NAD which further causes inactivation of GADP and thus production of AGEs including DAG take place and it is previously described the latter is a potent activator of PKC. Though hyperglycemia causes increase in NADH⁺/NAD ratio, but the absolute concentration of NAD⁺ is decreased because of its utilization in the synthesis of PARP (poly ADP ribose polymerase) that is activated by ROS⁴⁷.

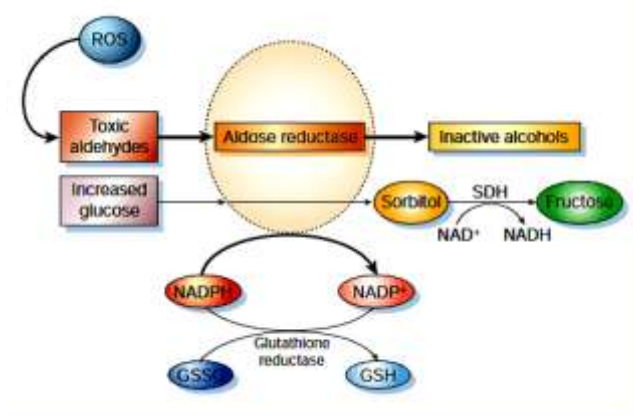


Figure 2: Production of ROS

The most deleterious mechanism is the consumption of NADPH⁺ for the formation of sorbitol that causes oxidative stress and the process of reduction of oxidized glutathione which is ADP dependent hampers⁴⁷.

Activation of protein kinase C:

Protein kinase C subfamily consists of eleven isoforms. Nine of which are activated by DAG. Studies in cultured micro-vascular cells and retinal and renal cells of diabetic mice have shown that the high level of glucose in the intracellular environment causes production of DAG at a higher rate. This mechanism was revealed by de novo production of DAG from reaction of dihydroxy acetone phosphate to Glycerol-3 P and its subsequent acylation⁴⁸. In vascular cultured cells, retinal and glomerular cells of diabetic mice DAG were seen to activate β and δ isoforms of PKC. Other isoforms were also activated for example α and β isoforms were reported in glomerular cells and ϵ and β isoforms were activated in retinal cells^{48, 49}. Protein kinase C can also indirectly be activated by hyperglycemia induced ROS through activation of polyol pathway and ligation of AGE receptor⁴⁷. In the early stages of experimental diabetes the renal and retinal blood flow abnormalities were mediated by PKC- β isoforms may be due to stimulating the activity of endothelin -1 or depressing of nitric oxide. The decrease in nitric oxide synthesis in diabetic animal models occurs in glomerular cells. Furthermore PKC inhibit mRNA for the synthesis of endothelial nitric oxide (eNO). And in glomerular cells of hyperglycemia induced PKC also increase endothelin-1 stimulated MAP kinase activity⁵⁰.

Apart from induction of abnormalities in blood flow and its permeability, PKC contributes to expression of TGF β 1, type IV collagen and fibronectin in both glomeruli of diabetic rats and cultured mesangial cells⁵¹. Furthermore, PKC is also responsible for fibrinolytic inhibitor PAI-1, activation of NF- κ B in the regulation of membrane associated NAD (P) H dependent oxidases⁵².

Activation of Hexose amine pathway and its effects

Activation of hexose amine pathway can also be contributed to the production of diabetic complications⁵³ and induction of diabetic nephropathy. In this pathway fructose 6-P undergo catalysis by GFAT to provide Glucose amine 6-P a substrate for the synthesis of UDP-Glc NAc which is then utilized for the formation and synthesis of proteoglycan and O-linked glycoproteins.

HSP is considered as a part of the glycolytic pathway normally about 3% of glucose is utilized via this pathway⁵⁴. First step in HSP pathway is rate limiting and is catalyzed by a Glutamine:

Fructose-6Phosphate amidotransferase in result the fructose-6P and glutamine are converted to Glucose amine 6P and glutamate, subsequently GlcN-6P is metabolized to CMP-syalic acid, N-acetyl galactose amine (UDP-GalNAC) and N-acetyl glucose amine, glycolipids, essential building blocks of the glycosyl side chains of glycoproteins, gangliosides and proteoglycans. Among these metabolites, UDP-GlcNAC has attracted more interest because:

1. Its quantity is higher as compare to other metabolites of HSP
2. It regulates the entry of glucose into HSP by a feedback mechanism by binding to GFAT allosterically.
3. It plays a role of obligatory substrate for muscular and cytosolic enzyme O-Glc NAC transferase, an enzyme responsible for post translational modification of proteins by transferring of N-acetyl gluseamine to O-linkage of serine or threonine residue of specific proteins⁵⁵

GlcNAC modification has a regulatory function and usually they are found adjacent to phosphorylation site⁵⁶. This type of acylation have functional significance for different proteins including transcription factors c-myc, Sp1, CMP responsive element binding protein, pancreatic duodenal home box-1, enzymes of cytosol and nucleus, RNA polymerase II and glycogen synthase, IRS 1 & 2 and Glu 4⁵⁷.

Studies have showed that HSP can cause insulin resistance and glucose amine that too enters HSP after the catalyzation by GFAT also causes insulin resistance but in lower concentration⁵⁸. The role of HSP in the development and pathogenesis of renal and vascular complication in diabetic patients is proved by remarkable evidences. As in diabetic nephropathy the initial stage is the accumulation of extracellular matrix in glomerular region which is promoted by persistent hyperglycemia in diabetic experimental models and diabetic patients⁵⁷. McClain et al

suggested that in hyperglycemic conditions HSP affects vascular smooth muscles genes in smooth muscle cells⁵⁹. After that it become clear that for the effect of high glucose concentration, synthesis of transforming growth factor β is compulsory⁶⁰. Recently the mechanism of stimulation of TGF- β 1 is suggested as, the sequence of promoter region of TGF- β 1 homologs the glucose response elements in the gene of proteins contribute in glucose metabolism and regulated by glucose for example pyruvate kinase. GREs than by binding with stimulatory factors USF-1 and 2 enhance the expression of TFG- β 1. In hyperglycemic state over expression of TGF- β 1 take place which then stimulate the expression of USF-1 and 2, thus upregulate TGF- β 1's promoter activity⁶¹. Apart from GRE there are two other protein binding sites in promoter region which are activated by MAP kinase and PKC, that are also dependent to a high glucose concentration⁶¹.

Suggested therapeutic and preventive mechanisms

Table 1: Mechanisms for therapy of DN

Mechanism	Treatment
Metabolic	
✓ Hyperglycemia	Insulin
✓ Increase glucose derived protein	Aminoguanidine ,AGE cross link breakers
✓ Polyol	Aldose reductase inhibitors
Mechanical/Hormonal	
✓ Elevated systemic blood pressure	Anti- hypertensive drug ACE inhibition, lower protein diet
✓ Increased intraglomerular pressure	ACE inhibition, Angiotensin VI antagonist
✓ Increased vasoactive hormones	ET receptor antagonist
Intramediate pathways	
✓ Growth factors eg: TGFβ, TGF	Antibodies PKC β inhibitors
✓ Protein kinase C dependent	

Glycemic control

Hyperglycemia is considered as a major cause of development of diabetic nephropathy in both type 1 and type 2 diabetic patients⁶². According to several research groups including the Diabetic Control and Complication Trail, intensified glycemic control can prevent the incidence and development of microalbuminuria and also overt proteinuria in type 1 diabetic patients⁶³. The 6 year study done by Ohkubo et al 1995 in Japanese patient with type 2 DM, multiple insulin therapy showed marked decrease in development of diabetic nephropathy⁶². Diabetic Control and Complication Trail (DCCT) has suggested that by controlling intensively glycemic level (goal HbA1c < 6.5% and mean achieved Hb \approx 7%) in both type 1 and type 2 diabetic patients marked reduction in the development of micro-vascular complications like retinopathy, nephropathy and neuropathy is seen⁶⁴. In UK, a 10 year study was done on the newly diagnosed patients with type 2 DM, in whom the intensified glycemic control, showed a 25% decrease in the rate of progression and development of secondary diabetic complications as compare to standard therapy. Study which was done by Vijan S et al 1997 showed that glycemic control in type 2 DM is more beneficial in prevention of development of secondary complications than in type 1 DM⁶². However

some controversial studies are also present like according to the research done by DCCT and Micro albuminuria study group, intensified blood glucose control was not able to decrease the rate of progression from microalbuminuria to macroalbuminuria in type 1 diabetic patients^{63, 65}. But glycemic control along with blood pressure control in type 1 diabetic patients was reported to prevent the worsening of renal function⁶⁶.

In type 2 diabetic patient, the role of strict glycemic control is less studied but there are reports on some hypoglycemic agents for example Rosiglitazone is reported beneficial in decreasing the UAE rate as compare to the Glyburide⁶⁷. Use of metformin due to the risk of lactic acidosis is inhibited in patients with high level of creatinine⁶⁴ in these patients use of drugs independent from renal excretion are safe, for example Repaglinide and nateglinide but sulfonuria and its derivatives will worsen the condition (Young BA, 2003). However in the study for type 2 patients with exogenous insulin should be administered because of low production of endogenous insulin in response to insulin secretagogues⁶⁴.

Intensive blood pressure control

Hypertension is a common problem of diabetic patients, about 40% of type 1 and 70% of type 2 diabetic patients are with normo-albuminuria⁶⁹ thus the hypotensive agents are reported to significantly decrease the risk of development of micro and macro vascular complications⁶⁴. The study in UKPDs has showed that a decrease of 10mmHg (from 154 to 144 mmHg) reduces the risk of development of DN to 29%. As hypertension is consider critical to renal function so control of blood pressure with any of hypotensive agent may be beneficial⁷⁰but RAS blockers either ACE inhibitors or ARBs despite of their anti-hypertensive characteristics are preferred due to the role of this system in the pathogenesis of diabetic nephropathy and their effect in decreasing intraglomerular pressure that results little passage of proteins to proximal tubules⁷¹.

Though the preventive effects of ACE inhibitors has not been defined yet but a 3 year study in normotensive, normo-albuminuric type 1 diabetes showed delay in progression of DN about 24% in type 2 diabetic patients and also Ramipril was reported to decrease the urinary albumin excretion rate, thus ACE inhibitors can be beneficial agents in prevention of developing DN⁶⁴. The meta analysis of evaluation of 12 trails containing 698 non-hypertensive type 1 diabetic patients showed ACE inhibitors are not only beneficial in decreasing the chance of progression from micro-albuminuria to macro-albuminuria but they are also beneficial in increasing the chances of regression from micro-albuminuria to normo-albuminuria⁷². Furthermore, ARBs are proved to be efficient in prevention of the development of micro-albuminuria to macro- albuminuria in type 2 diabetic patients, treated with Irbastan 300mg/dl which showed a 70% decrease in development of diabetic nephropathy⁷³.

Novel Therapy

1. Strategies to block AGE formation: The AGE can be blocked by several pathways. Most of therapeutic strategies till now, work on the inhibition of synthesis of AGEs. Aminoguanidine is the most studied AGE blocker; it is a nucleophilic compound by interacting with the intermediates of AGEs inhibits the process of cross linking⁷⁴. The studies done in diabetic animal model have shown the efficacy of aminoguanidine in the attenuation of the signal transduction, over expression of growth factor, structural and functional alteration of diabetic nephropathy⁷⁵. The study done by Ateon a pharmaceutical company responsible for this research has concluded the significant reduction in the albuminuria following the administrating of aminoguanidine⁷⁶. But there was no statistical significant change in GFR level. Some other AGE inhibitors that require further studies for clinical use are ALT 486, NNC 39-0028 and OPB 9195
2. Cross link breakers: other proposed strategy of therapy is the breaking of cross linking, the idea was developed when phenacylthiazolium (PBT) a cross link breakers was discovered⁷⁷. But the study conducted on diabetic rats did not proved the efficacy of PBT in the treatment of DN. Another cross link breaker is ALT 711 which is able to inhibit and also to improve age related stiffness of myocardium and has also been shown to significantly beneficial to reduce blood pressure, UAE and renal lesions^{78, 79}.
3. Receptor blocker: Schmidt and colleagues suggested another therapeutic strategy by administration of soluble, extracellular domain of RAGE (sRAGE) that was able to bind with AGE and thus inhibiting its receptor, subsequent gene activation and underlying pathophysiology⁸⁰.
4. Protein kinase C inhibitors: As the number of pathogenic pathways toward DN is activated by PKC the inhibition of PKC can be efficient therapeutic strategy in the management of DN on other side there are several isoforms of enzyme performing different function, therefore while inhibiting the specific isozyme related induction of DN (PKC- β) should be targeted⁸¹. Inhibitors of PKC β ameliorate glomerular lesions thus normalize GFR and inhibit protein excretion in diabetic rat models⁸². Till now the known inhibitor of PKC is LY333531 that was able to reduce UAE and GFR in diabetic rat models. That also resulted in attenuation of mesangial, reduction of collagen and expression TGF- β expression in Ren-2 diabetic rats, even in the presence of hyperglycemia.
5. Inhibition of vasopeptidase: The important vasopeptidases that contribute to the control of blood pressure are RAS, Kalikarin-Kinin system and natriuretic peptide system. All of these systems together play role in modulating the vascular tone, water and salt balance and have growth factor like activity. Interrelations of these systems are also important in the development of hypertension and renal complication. ACE and neutral endopeptidase have structural similarities and both are zinc containing cell surface peptidases therefore can be inhibited by single inhibitor. The inhibition of both systems can lead to better control of blood pressure. Furthermore, other vasoactive peptidases are also reported to be affected by changes in these systems. For example the degradation of bradykinin is inhibited by ACE inhibitors and NEP inhibitors reduces the endothelium and potentiate the natriuretic effect of adrenomedullin (JC, 1999). Several preclinical and clinical studies are going on dual ACE/NEP vaso-peptide inhibitors like Omapatrilate the phase II and III studies for which are completed⁸⁴. SA7060, MLD100240, MLD100173, Fasidodril, Sampatrilat, Alanopril, CGS30440 and S21402⁸⁵. Number of studies are done on animal models to evaluate the comparative action of dual VPIs and ACE inhibitors. In one study the S21402 a dual VPIs was compared with ACE inhibitors and in result the S21402 was found more efficient in controlling of blood pressure while the effect on AER was similar⁸⁶. The other study was done of seminephrectomized mice with some characteristics of DN, to study the effects of CGS 30440 and Omapatrilate, both of these VPIs were able to significantly reduce proteinuria⁸⁷.
6. Miscellaneous therapeutic strategies: Overdose of thiamin and its derivative benfothiamin due to decrease oxidative stress, PKC and protein glycation is reported to slow the development of microalbuminuria in diabetic nephropathy⁸⁸. The administration of heparin glycosaminoglycan apart from the beneficial effects of PKC inhibitors it also decrease the accumulation of tubular and glomerular matrix and inhibit the synthesis of PKC mRNA⁸⁹. Pimagedine a second generation of AGE inhibitor and suldexide a glycosaminoglycan also have beneficial effects in the decreasing of the urinary albumin excretion and in normalization of GFR in diabetic rat models. Taniguchi K et al 2013 suggest the role of Src kinase in collagen accumulation and PP2 by inhibition of Src kinase that leads to the inhibition of collagen IV accumulation, high glucose induced phosphorylation of proteins and the pathological mechanisms of diabetic nephropathy thus Src inhibitors were suggested as a novel therapeutic targets for diabetic nephropathy⁹⁰. Role of PG in the pathogenesis and development of DN is not clear but there is higher amount of PG in the kidney of patient and also diabetic animal models with diabetic nephropathy. Makino et al 2002 showed that administration of selective antagonists of PGE receptor EP-1 subtype was able to selectively prevent development of diabetic nephropathy in STZ induced diabetic rats, Which was able to decrease mesangial expansion, ameliorate glomerular hypertrophy, inhibit up regulation of fibronectin and transcriptional growth factor β 1 (TGF- β 1) in mesangial cells cultured in high glucose concentration. According to this study the role of PG-EP1 system in the development of diabetic nephropathy become clear. Makino et al 2002 also explained that aspirin a non-selective prostaglandin synthase inhibitor and EP-1 antagonist both decreases mesangial expansion but aspirin is not able to inhibit glomerular hypertrophy and proteinuria while EP-1 inhibitor is able to produce these changes suggesting that the mode of action of these drugs may

be different and suggests the novel therapeutic strategy⁹¹. Studies conducted on STZ induced diabetic rat models have suggested that in renal mitochondria high level of SO is produced along with the post transcriptional modification of mitochondrial complex III. Thus Chacko BK et al revealed out that in *Ins2^{+/-AKita}* mice targeted antioxidant therapy with mitochondria-targeted ubiquinone (Mito Q) was able to prevent and treat diabetic nephropathy⁹². However the studies in human being are still needed to approve the effect of these novel drugs.

2. Conclusion

Diabetic nephropathy is a serious secondary complication of DM. That has increased the health and financial problems of diabetic patients. It is an appeal for the development of cardiovascular and major cause of renal replacement therapy. DN develops approximately after 5-10 years of the incidence of DM in 5 characteristic phases, therefore providing of quality preventive and therapeutic measures not only help in prevention of the incidence and the progression of disease but can also ameliorate the tissue damage of the kidney. For getting such a successful restorative strategies the underlying mechanism should be understood.

Persistent hyperglycemia causes to alter normal metabolism of glucose or increases the level of production of compounds which normally produce in small concentrations. High concentration of glucose and its metabolic products causes more synthesis of reactive oxygen species, activation of polyol pathway, production of AGEs, cross linking of AGEs, more expression of RAGE, activation of PKC and hexose amine pathway all of which contributes in production of oxidative stress, activation of inflammatory cytokines, inflammation and that leads to injury and damage of endothelial cells, accumulation of extracellular matrix, mesangial expansion and glomerulosclerosis, strict glycemic control, maintaining of BP at normal level, protein restricted diet, exercise and prevention of obesity are proved beneficial but maintaining of these factors are difficult and still DN is the leading cause of ESRD. Thus struggle is going on for the development of therapeutic agent which would be able to act at molecular level to prevent the incidence of DN, the propose mechanisms are AGEs blockade, cross linking breakers, RAGE blockers, mitochondrial targeted antioxidant etc. although at animal models these agents have given remarkable results but still their efficiency should be checked at humans.

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