

# Pathophysiology of Diabetic Nephropathy

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## Abstract

Diabetes mellitus is one of such problems with multiple etiologies and serious life threatening co morbid complications. Diabetic nephropathy is the most serious secondary complication of DM with high rate of mortality and is the main cause of ESRD in adults. About 30% of diabetic patient (either type 1 or type 2) develops diabetic nephropathy and in the case of poor control of blood pressure and urinary albumin excretion, they lead to ESRD about 80% of which have hypertension which contributes in worsening of renal disease. In constant high glucose concentration, there is overproduction of mitochondrial superoxide which is blame to be responsible for the hyperglycemia induced apoptosis. Diabetic glomerulosclerosis is characterized by nodular lesions that consist of mesangial expansion that makes fibrillar mesangial zones, where the mesangial nuclei accumulate around nodules and compress the glomerular capillaries. The diabetic nephropathy pathology ultimately affect almost all the organs through different mechanism and hence it need to be understand and these information can be used for the development of advanced therapeutic strategy.

Keywords: *diabetes; nephropathy; free radicals; renal; etiology; capillaries*

## Introduction

By gaining advances in human life day by day the life of human being has become easy and comfortable but side by side with developments in science, technology and sanitary life style, the health problems especially chronic degenerative and metabolic disease is also getting increased.

Diabetes mellitus is one of such problems with multiple etiologies and serious life threatening co morbid complications. The most serious complication of diabetes mellitus is diabetic nephropathy, the main cause of end stage renal disorder (ESRD) and RRT. Thou there are multiple hypoglycemic medicine and also commercial forms of insulin are available but still 1/3<sup>rd</sup> of DM type 1 and 1/6<sup>th</sup> of DM type 2 develops diabetic nephropathy, account for about 1/3<sup>rd</sup> of total ESRD most of which undergo renal replacement therapy. That's why medical science is trying to develop new hypoglycemic agents to use them as medicine or to include them in the daily diet and consequently the control of glycaemia and prevention of diabetic nephropathy will be done more efficiently.

## Diabetic Nephropathy: Characteristics and etiology

The range of influence of metabolic disorder and other chronic degenerative disease is being wider day by day and the age of incidence and diagnosis of the disease is rapidly decreased [1]. Metabolic syndrome is characterized by a group of symptoms indicating an abnormality such as obesity, hypertension, dyslipidemia, glucose intolerance and insulin resistance. This syndrome is also considered as "diabesity" and is a call for the incidence of DM [2].

Metabolic disorders are interrelated connected with each other. Obesity is recognized as a major cause of hypertension and essential hypertension is usually followed by insulin resistance [3]. Insulin resistance as well as with high risk of hypertension occurs at early stages of type 2 and later on in type 1 DM. Furthermore, the risk of development of type 1 DM is greater in patients with uncontrolled blood pressure as compared to the controlled blood pressure [4]. Though there are multiple antihypertensive agents available but still maintaining of blood pressure at normal range is difficult especially in the case of hypertension associated with DM [5].

Patient with hypertension, DM or both are at high risk of developing a wide range of chronic complications of inclusive nephropathy and cardiovascular disease [6]. More ever different modifiable risk factors like obesity, blood glucose and lipid level, blood pressure and smoking are known for their association with bad image of poor renal and cardiovascular complications [7]. It also should be known that association of DM and hypertension worsens the situation of enhancing their secondary complication and thus puts salt to the wound of social dysfunction and premature death [8].

DM is a major health problem characterized by hyperglycemia with main etiology of insufficient insulin secretion, insulin action or both. About 6.6% of the world population suffers from this disease and it is estimated that the number will be increased up to 7.8% of global population in the next 20 years. As DM is known as an important risk factor for the development of chronic complications in vital and target organs for example cardiovascular disease, nephropathy, retinopathy and neuropathy, different research groups are working on comprehension of these mechanisms and also on treatment and prevention of these complications [9].

Diabetic nephropathy is the most serious secondary complication of DM with high rate of mortality and is the main cause of ESRD in adults [10]. About 30% of diabetic patient (either type 1 or type 2) develops

diabetic nephropathy [11] and in the case of poor control of blood pressure and urinary albumin excretion, they lead to ESRD about 80% of which have hypertension which contributes in worsening of renal disease.

In DM type 2 hypertension is the part of syndrome characterized by insulin resistance, hyperuremia, dyslipidemia, obesity and atherosclerosis [12] though the exact cause and explanation of condition is still unknown but according to many researchers. Insulin resistance plays important role in the development of syndrome [13], but in the case of DM type 1 hypertension develops secondary to the occurrence of DN [14]. The link between glycemia level and development of hypertension has been proved through landmark follow up by DCCT (Diabetes Control and Complication Trail), according to that on 8<sup>th</sup> year of follow up there was 10% decline in development of hypertension in intensified insulin regimen treatment group as compare to the conventionally treated group. Likewise there were beneficial effects on renal consequences of first group [5].

### **(a) Phases of development of DN**

DN develops in 5 following characteristic phases. (i) Plasma flow of kidneys increases along with an increase in GFR, hypertrophy of kidney and also renal hyper-filtration occurs. (ii) Renal parenchymal changes and normo-albuminuria, mesangial expansion and thickening of basement membrane occur. (iii) Early hypertension and micro-albuminuria (iv) Observable albuminuria (v) End stage renal disorder [15] All of the above disorder contributes in generating of cell injury and therefore apoptosis of podocytes, extracellular proteins accumulate in tubular interstitial and in glomerular region [16].

### **(b) Mechanism 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.

- i. Activation of oxidative stress by high concentration of glucose and production of ROS
- ii. Production AGEs (advanced glycated end products)
- iii. Activation of PKC (protein kinase C), proinflammatory transcription factor NF- $\kappa$ B, transforming growth factor (TGF) and RAS (renin angiotensin system)

### **Reactive Oxygen Species (ROS)**

Free radical production due to hyperglycemia and its important role in induction of cellular oxidative damage has been known [17]. Its role in production of diabetes associated macro vascular complication is also approved. In constant high glucose concentration, there is overproduction of mitochondrial superoxide which is blame to be responsible for the hyperglycemia induced apoptosis [18]. More interestingly the production of oxidative stress and also role of cellular apoptosis was high in intermittent high glucose level as compare to constant glucose [19]. Piconi L *et al.* (2006) by observing enhancement of apoptosis of cells by adding of 8 OHdG and nitrotyrosine and reversed in apoptosis due to SOD and MnTBAP the SOD mimetic and mitochondrial electron transporter complex II inhibitors, revealed that the major oxidative stress due to high glucose level is mitochondrial based [20].

All pathways responsible for production of secondary complication of DM like AGEs formation, RAGE ligand binding, specific inhibitors of aldose reductase 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 hyperglycemia. Superoxide then can be converted into different other free radicals which may be more reactive and causes cellular damage by various mechanisms. In ETC transfer of electrons take place through complex I, II and IV and 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 FADH<sub>2</sub> as electron donors into the electron transport chain, 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. Electrons go back to coenzyme Q which can donate just one electron to molecular oxygen therefore it generates superoxide. Mitochondrial superoxide dismutase catalyzes this superoxide and yields H<sub>2</sub>O<sub>2</sub> which then be converted to water and O<sub>2</sub> by other enzymes. The *ex-vivo* 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 was prevented by inhibition of mitochondrial fission [21]. As previously described hyperglycemia induce oxidative stress occurs in mitochondria and the pathway that are activated by SOD

and are responsible for hyperglycemia induced cellular damage are NADPH oxidases, uncoupled eNOS and redox changes [19-20].

Mitochondrial superoxide activates hyperglycemic induced damaging pathways. In diabetic patients, diabetic animal models and hyperglycemic cells the decrease is seen in the activity of GAPDH. Mitochondrial superoxide is considered as a main reason and the condition leads to increase the level of upstream glycolytic intermediates. As methylglyoxal is generated non-enzymatically from glyceraldehyde 3P, so it activates AGEs pathway, increases the expression of RAGE and also activates the ligand S100 calgranuline and HMG B1 [22]

- 1- High level of glyceraldehyde 3P also activates PKC pathway because glyceraldehyde 3P produces DAG which is the physiologic activator of PK-C pathway
- 2- 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.
- 3- At last inhibition of GAPD causes increased concentration of glucose inside the cell which then is consumed through polyol pathway by using NADPH as reducing equivalent.

#### ***Advanced glycated end products:***

Advanced glycated end products are the products of non-enzymatic reaction of reducing sugars and amino group of proteins, lipids and nucleic acids. AGE produces via a series of reactions in which Amadori products and Schiff bases produces prior to AGE [15]. This reaction was first described in early 1900 when the development of brown color was observed by heating of amino acids with reducing sugars, the reaction was known as Mailard reaction [18]. The production of advanced glycation end products require couple of weeks therefore glycation affects long-lived proteins for example structural components of basement membrane or connective tissue matrix in which collagen is the most affecting protein but myaline, complement C3, fibrinogen, tubulin and plasminogen activator factor could also be affected [20]. Exception is uremia where even short lived compounds like nucleic acids and lipids are also affected.

In early stages of Millard reaction the rate of reaction is concentration dependent therefore the rate is improved in DM. The process of glycation by glucose is slower but the rate is faster in the case of Glucose-6-P and fructose the rate is accelerated by the presence of transitional metals while it is inhibited by reducing agents such as vit C and green tea. Glycooxidation is another phenomena which is used when glycation

occurs along with oxidation, pentosidine and N<sup>ε</sup>-[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 intermediates [23].

Methyl glyoxal with a strong electrophilic nature is considered as a toxic compound in high concentrations, and can cause cell death. It is constituted by non-oxidative mechanisms, non-enzymatically in an aerobic glycolysis, it is also produced from poly unsaturated fatty acids methylglyoxal also produce during the fragmentation of triose phosphate, catabolism of threonine and ketone bodies. It is related to the dihydroxy acetone phosphate an intermediate of glycolytic sequence. After detoxification it is converted to the lactate. Methylglyoxal is electrophilic in nature so it results with nucleophilic centers of macromolecule for example DNA, RNA and proteins. Furthermore, it reacts with the side chain of amino acids lysine, cysteine and arginine; it also binds with guanine base and to a lesser extent with adenine and cytosine [24]. It is suggested that the cytotoxic characteristic of methylglyoxal is due to its inhibitory action of DNA replication. Mutagenic characteristic of methylglyoxal was proposed by Marnett et al.; when he observed mutagenic changes in *Salmonella typhimurium* cells by converting arabinose sensitive strains to arabinose resistant strains [25].

#### **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 demonstrated that the high level of glucose in the intracellular environment causes production of DAG at a higher rate. This mechanism was revealed by *denovo* production of DAG from reaction of dihydroxy acetone phosphate to Glycerol-3 P and its subsequent acylation [26].

DAG was seen to activate  $\beta$  and  $\delta$  isoforms of PKC *ex-vivo* in vascular cultured cells and *in-vivo* in retinal and glomerular cells of diabetic mice. Other isoforms were also activated for example  $\alpha$  and  $\beta$  isoforms were reported in glomerular cells and  $\epsilon$  and  $\beta$  isoforms were activated in retinal cells. Protein kinase C can also indirectly be activated by hyperglycemia induced ROS through activation of polyol pathway and ligation of AGE receptor [26].

In the early stages of experimental diabetes the renal and retinal blood flow abnormalities were mediated by PKC- $\beta$  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 is present in glomerular cells. Furthermore, PKC inhibit mRNA for the synthesis of endothelial nitric oxide (eNO) synthesis. And in glomerular cells of hyperglycemia induced PKC also increase endothelin-1 stimulated MAP kinase activity [26].

Apart from induction of abnormalities in blood flow and its permeability, PKC contributes to expression of TGF  $\beta$ 1, type IV collagen and fibronectin in both glomeruli of diabetic rats and cultured mesangial cells [18]. Furthermore, PKC is also responsible for fibrinolytic inhibitor PAI-1, activation of NF- $\kappa$ B in the regulation of membrane associated NAD(P)H dependent oxidases [24].

### ***Activation of Hexose amine pathway and its effects***

Activation of hexose amine pathway can also be contributed to the production of diabetic complications [26] 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 pathway [25]. 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-sialic 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 proteins [26]

GlcNAc modification has a regulatory function and usually they are found adjacent to phosphorylation site [24]. 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 4 [26].

Studies have shown 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 concentration [26]. 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 patients [25]. McClain et al suggested that in hyperglycemic conditions HSP affects vascular smooth muscles genes in smooth muscle cells [23]. After that it becomes clear that for the effect of high glucose concentration, synthesis of transforming growth factor  $\beta$  is compulsory [26].

Recently the mechanism of stimulation of TGF- $\beta$ 1 is suggested as, the sequence of promoter region of TGF- $\beta$ 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- $\beta$ 1. In hyperglycemic state over expression of TGF- $\beta$ 1 take place which then stimulate the expression of USF-1 and 2, thus upregulate TGF- $\beta$ 1's promoter activity [27]. 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 concentration [27].

### **Histopathological changes in DN**

In DN extracellular matrix and its normal components like collagen (type IV and VI), fibronectin and laminin accumulate in higher quantity that leads to thickening of GBM (the first sign of DN occur after 1.5-2.5 year of onset of DM) [26], thickening of tubular basement membrane and mesangial expansion. The latter is usually seen in all type 1DM patients with renal insufficiency which is also called diabetic glumerulosclerosis [27].

Diabetic glumerulosclerosis is characterized by nodular lesions that consist of mesangial expansion that makes fibrillar mesangial zones, where the mesangial nuclei accumulates around nodules and compress the

glomerular capillaries. Increased collagen contents in fibrillar regions are seen in the patients when GFR decrease [28].

After few years of onset of DM, hyalinosis occur in afferent and efferent arterioles. In these situations exudate lesions composed of complement, fibrinogen, immunoglobulins, albumin and other plasma proteins replaces endothelial smooth muscles. These types of vascular lesions further contribute in development and worsening of the glomerulosclerosis. Such lesions can also occur in sub endothelial cells of glomeruli and parietal surface of Bowman's capsule [23].

Patients with end stages of disease with prominent proteinuria show abnormality in glomerulotubular junction (GTJA). These abnormalities are classified according to the severity of disease as normal tubular glomeruli, atrophic tubular glomeruli and atubular glomeruli. Atrophic tubular glomeruli is further divided into short ATs and long ATs, in first one atrophy occur just in first few cells while in second one the longer part of PCT cells are atrophic. In atubular glomeruli there is open blood circulation and no tubular attachment, these glomerulus are nonfunctional [24].

Tip lesions are seen in all forms of GTJA, they are reported in all short ATs, 82% of ATs without observable opening, in 64% of long ATs and in 9% of normal tubular glomeruli while presence of such lesions are not reported in normal individuals. Furthermore

severity of GTJA are strongly associated directly with the excretion of protein in urine and inversely related to GFR [27].

In all renal disorder with proteinuria injury, detachment or effacement of podocyte is seen. In DN also defect of podocytes play important role in the progression and worsening of disease. In DN width of podocyte foot process increased which decreases the area of slit pore length and thus causes proteinuria. Changes in shape of podocytes are documented in all T1DM young patients with normoalbuminuria [28]. Increased albuminuria worsens detachment of podocytes that can lead to decrease in number of podocytes [26]. Different studies showed decreased number of podocytes in DN patient suggesting the role of podocytes in the development of overt DN [27].

## Conclusion

Oxidative stress is the well-known mechanism of diabetes and hyperglycemia for the development of microvascular complication including DN. In DM higher concentration of glucose produces more pyruvate and subsequently NADH and FADH in ETC. Which increases the voltage gradient of the mitochondrial membrane, blocks complex III of ETC and generates superoxide. Superoxide than can be converted into several more reactive free radicals [23]. Overproduction of ROS causes the alteration of the balance of pro-oxidants and anti-oxidants. Free radical interacts with nucleic acids, proteins and lipids, which produces marked changes in normal physiology and histology of the organ. All other pathways, for example production of DAG, PKC, Hexose amine pathway, and polyol pathway have link with ROS production. Thickening of glomerular basement membrane and expansion of mesangial extracellular matrix occurs. A change of basement membrane causes hyperfiltration and elevates glomerular hydrostatic pressure. All these mentioned changes lead to microalbuminuria. Expansion of mesangial matrix, encroach glomerular capillaries and narrowing of lumen occurs that decrease the surface for the filtration. Tubule interstitial fibrosis also occurs by the same mechanism. Mesangium expands due to accumulation of excess amount of matrix proteins either because of excess production or decrease in turnover of these proteins. Studies have shown that hyperglycemia, AGE and glycated albumin causes the upregulation of genes responsible for the mesangium expansion such as collagen IV, fibronectin and TGF- $\beta$ . Diabetic glomerulosclerosis is characterized by nodular lesions that consist of mesangial expansion that makes fibrillar mesangial zones, where the mesangial nuclei accumulate around nodules and compress the glomerular capillaries. The diabetic nephropathy pathology ultimately affects almost all the organs through different mechanism and hence it needs to be understood and this information can be used for the development of advanced therapeutic strategy.

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