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CROSS LINKAGE BETWEEN OXIDATIVE STRESSES IN DIABETIC NEPHROPATHY: AN UPDATED REVIEW

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Abstract

Diabetic nephropathy is a micro vascular complication of diabetes mellitus. The treatment of diabetes and its complications require identification of various factors involved in the pathogenesis and development of diabetic nephropathy. The last stage in chronic kidney disease known as end stage renal failure (ESRD) or various other kidney diseases are found to be caused by diabetic nephropathy (DN) and the instances of death due to ESRD are increasing at a very high rate. DN is marked by elevation in levels of blood sugar accompanied with elevated oxidative stress. Oxidant species or reactive oxygen species (ROS) are the key players in the development of DN. These ROS are generated as macromolecules or by defects in certain pathways in kidneys. The enzyme protein kinase C is initially activated by increase in the amount of oxidant species which leads to over expression of genes of extracellular matrix, development of fibrosis and eventually ESRD. A better understanding towards the role of oxidative stress in diabetic nephropathy and its various sources has led to the establishment of experimental studies on various new therapeutic agents for reducing oxidative stress. This review article describes various sources of ROS and their significant role in the pathogenesis of DN.

Key words: Diabetic nephropathy, Diabetes mellitus, Oxidative stress, Reactive oxygen species, End stage renal failure.

Introduction

Diabetes mellitus (DM) is characterised by alterations in the normal insulin metabolism affecting its secretion and action or both. Insulin which is secreted by special cells or β cells in pancreas is responsible for upholding blood sugar levels and its deficiency leads to hyperglycaemia. Prolonged elevation in levels of glucose in blood and intolerance to glucose are the major features of diabetes (Pires *et al*., 2018). Increased blood sugar or glucose levels are the major cause for recurrent urination, increased appetite or thirst which is the early symptoms for diabetic patients (Trott and Olson, 2010). The other major cause for diabetes is glucose intolerance wherein the cells become insensitive to insulin already produced by pancreas (Morris *et al*., 2009). If not treated, it can lead to various acute and long term difficulties (Trott and Olson, 2010). Nowadays, obesity has become the leading cause for diabetes therefore; altering the diet and lifestyle may act as a preventive measure. Diabetes mellitus can be fatal in several cases due to higher risk of cardiovascular problems including myocardial infarction and stroke. Diabetes is classified into type 1 DM (T1DM) and type 2 DM (T2DM) on the basis of aetiology which is the set of underlying causes by the Diabetes Association of America into the two most prominent types (Fig. 1) (Alberti and Zimmet, 1998).

Due to the degradation of insulin producing β-cells and its subsequent insufficient release in T1DM, it is generally treated with parenteral administration of insulin (Trott and Olson, 2010). T2DM caused due to obesity is treated by weight loss surgery when oral medications fail to respond effectively (Stamler *et al*., 1993). The frequency of cardiovascular problems in diabetic patients is twice or even four times than non-diabetic patients due to which mortality and morbidity rate is very high in such patients. Atherosclerosis is also responsible which occurs in young age and develops at an exponential rate (Chen *et al*., 2011). In addition to these macro-vascular difficulties; micro-vascular conditions also prevail in which eye and kidney are most commonly affected. The major reason for blindness and end stage renal failure (ESRD) is diabetes related kidney disease and retinopathy manifested micro-vascular injuries (Haffner *et al*., 1998; *Picot et al., 2009;* Ripsin *et al*., 2009).

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T1DM

It is an auto-immune disorder which occurs due to degradation of ß-cells of pancreas thus altering the levels of insulin. It is less prevalent than T2DM and comprises of only 5-10% cases called as "insulin-dependent diabetes mellitus" [2,5].

T2DM

It is due to insensitivity of cells to insulin thus affecting its secretion or action in the body. It is prevalent in 90% cases. Younger and obese patients are more prone. It is called as "non insulin-dependent diabetes mellitus" [2,5,6].

Fig. 1: Types of Diabetes mellitus.

Diabetic Nephropathy

Diabetic kidney disease is a persistent escalating clinical condition indicated by high levels of albumin protein in urine and diminishing glomerular filtration rate (GFR). It is the most fatal and dreaded micro-vascular complication in diabetes (Ritz *et al*., 1994). Diabetes leads to structural changes in glomerular capillaries signalled by increased thickness of glomerular basement, glomerulosclerosis and augmentation of mesangial cells which collectively causes kidney fibrosis. These changes make glomerular capillaries susceptible to albumin leakage leading to albuminuria (Bamashmus *et al*., 2014). But surprisingly, only one third of the diabetic patients develop nephropathy favoured by high cholesterol, activation of inflammatory mediators, poor glucose control and oxidative stress (Congdon *et al*., 2004; Hovind *et al*., 2001). Once the metabolic control is initially stabilised, the patient has to be screened for albuminuria annually at least. Diabetic nephropathy is detected or tested by observing the ratio of albumin and creatinine levels in the sample of urine collected first in the morning (Dronavalli *et al*., 2008). Diabetic nephropathy has five distinct stages characterised by higher levels of albumin in urine excretion and GFR (Table 1) (Balakumar *et al*., 2009).

Currently, the complications arising due to diabetes in kidney have been declared to be the major reason for ESRD. On improper functioning of kidneys, the patients necessitate the replacement of kidney to restore its function. Nearly 45% of patients have been confirmed with diabetes worldwide (Friedman and Gross, 1991).

Epidemelogy of Diabetic Nephropathy

In T1DM, the kidney damage is mainly due to DN. In the initial 5 years of persistent diabetes, chances of occurrence of diabetic nephropathy are certainly rare. But in the next 10 years the incidence increases rapidly and reaches to about 3% per year after a total of 15 years of persistent diabetes. In the patients suffering with T1DM for 40 years or more, the rate of developing diabetic nephropathy reduces to 1%. The T1DM patients either with a family record of diabetic nephropathy or parental record of hypertension or any CVS disease have more threat or chances of acquiring DN (Dronavalli *et al*., 2008). In T2DM, the chances of other kidney disease most importantly hypertensive ischaemic damage in older patients are more than the incidence of diabetic nephropathy. Younger patients are however more affected to it and are accompanied by retinopathy and a distinctive development of overt proteinuria from micro-albuminuria. In the older patients, retinopathy and proteinuria are either absent or minimal with atypical features of kidney disease (Mogensen *et al*., 1983). Fig. 2, illustrates the change in pattern of GFR and levels serum creatinine with duration of diabetes.

Pathogenesis of Diabetic Nephropathy

The mechanism involved in the progression of diabetic nephropathy is due to the interactivity of renal microcirculation, among various metabolic and haemodynamic elements or their interactions with some reactive oxygen species (ROS) dependent pathways (Fig. 3). These interactions are collectively responsible for altering gene expression and activating various transcription factors further influencing various functional and morphological modifications producing characteristic features of DN (Raptis and Viberti, 2001).

Table 1: Stages of diabetic nephropathy.

Stages of diabetic nephropathy	Characteristics
Stage 1: Glomerular hyper-filtration	Initial structural changes leading to hyper-function and enlargement of capillaries.
Stage 2: Silent stage	This stage is characterised by glomerular abrasions devoid of any clinical condition.
Stage 3: The developing stage or incipient nephropathy	Moderate increase in urine albumin levels up to 30-300 mg/day
Stage 4: The visible stage or Overt nephropathy	UAE: > 300 mg/day
Stage 5: End stage renal disease (ESRD)	Kidney loses function completely and may require dialysis.

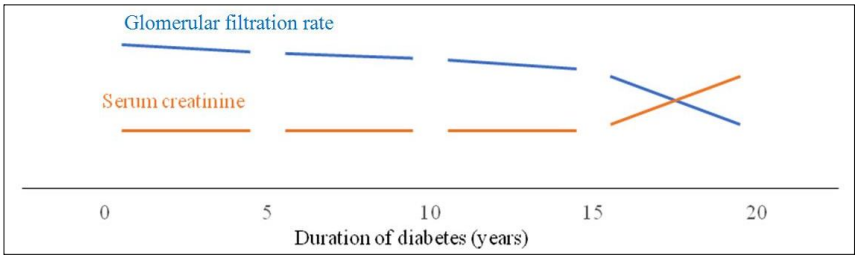

Fig. 2: Natural history of diabetic neuropathy.

The various pathophysiological mechanisms that are associated with the incident and escalation of the diabetic kidney disease have been explained below (Fig. 4):

• **Increased PKC activity:** Protein kinase C-β (PKC-β) affects a great number of cellular functions and is known to have increased activity during diabetes thus causing severe damage to kidney cells (Raptis and Viberti, 2001).

• **Elevated advanced glycation end products (AGEs):** Amino acids on long exposure to elevated levels of glucose produce AGEs. They alter the cellular proteins activity and functions of the cell membrane thus causing cellular damage (Raptis and Viberti, 2001).

• **Increased transforming growth factor-β (TGF-β):** It causes kidney fibrosis induced by an array of compounds capable of causing inflammation (Cao and Cooper, 2011).

• **Hyper-filtration and hypertension in the glomerulus:** There is a marked increase in the glomerular pressure which can be reduced or managed by blocking the actions of angiotensin II (Cao and Cooper, 2011).

The body tissues and kidneys of diabetic patient have been detected with increase in levels of oxidative stress (Schena and Gesualdo, 2005; Tuttle and Anderson, 2003; Williams and Tuttle, 2005). The mechanisms stated above are either found to increase the oxidative stress level or are triggered by its increased levels. Therefore, oxidative stress has been considered as the central problem in the progression of the diabetic kidney disease.

Oxidative Stress: A Major Factor for Diabetic Nephropathy

The normal metabolism of oxygen yields a number of oxidant species which have been proved to be important in cell signalling, degenerative disease and ageing of cells (Lee *et al*., 2003). Oxidant species are highly reactive molecules and interact with bio-molecules such as proteins, carbohydrates and lipids causing their abnormal changes (Koya *et al*., 2003). Normally, a complex balance is maintained between oxidant species which is considered important for the normal physiological cellular functions (Beisswenger *et al*., 2005). This balance also plays a major role in arresting the oxidation of cells to prevent the abnormal changes and potential damage. Alterations in this anti-oxidant equilibrium lead to oxidative stress. These alterations may be either due to increase in production of ROS or degraded action of anti-oxidants (Fig. 5) (Lee *et al*., 2003). It has been seen that the pathophysiological mechanisms playing role in DN are either the cause of oxidative stress or results from it. Thus, oxidative stress holds a major part in development of diabetic nephropathy (Brownlee, 2001). Hyperglycaemia and glomerular hypertension, metabolic and haemodynamic elements respectively are the main triggers of oxidative stress in DN. Kidney is highly sensitive to the increased levels of circulating glucose. The constitutive and the functional part of kidney include nephron which is independent of the actions of insulin so the amount of glucose entering the cells is controlled by the surrounding glucose levels and the expression of glucose transporters (GLUT1) (Remacle *et al.,* 1995). Glomerular hypertrophy occurs as a result of hyperglycaemia due to AGE generation along with abnormal increase in filtration rate up to 5-10% (Brownlee, 2005). Oxidative stress is also increased

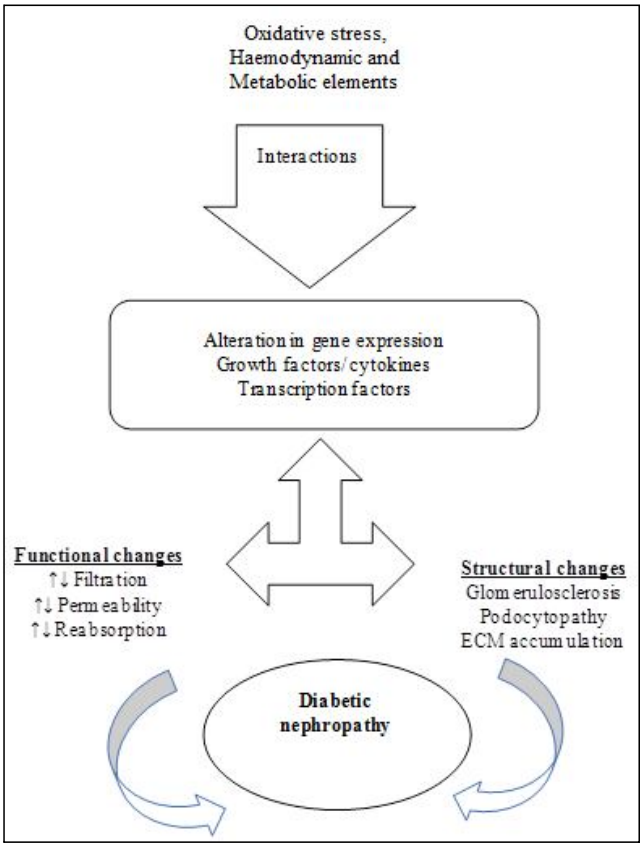

Fig. 3: Pathogenesis of diabetic neuropathy.

by the liberation of growth factors, cytokines and pro-inflammatory markers due to hyperglycaemia mediated increased tension and mechanical force in the glomeruli. Fig. 6, depicts the effect of oxidative stress on diabetic nephtropathy.

Sources of Oxidative Stress

Numerous sources of oxidative stress have been identified in diabetic nephropathy (Fig. 7).

• **Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase Pathway:** NADPH oxidase, first discovered in neutrophils, is a multi-subunit enzyme which accelerates the superoxide production by reducing molecular oxygen using either NADPH or NADH in electron transport. This superoxide is responsible for innate immunity and the nonspecific host-pathogen defence mechanism. It is a cytosolic enzyme (De Boer *et al*., 2011; Gnudi *et al*., 2007). The enzyme complex comprises of five subunits e.g. membrane associated $p22^{phox}$ and $gp91^{phox}$ (also known as NADH oxidase 2-NOX2), $p47^{phox}$, $p67^{phox}$, cytosolic subunits, $p40^{phox}$ and GTPase rac1 or rac2. When stimulated, $p47^{phox}$ phosphorylates and two of the four subunits from cytosol form a complex. This complex further translocate to plasmalemma where the active form of NADPH oxidase multi subunit complex is formed by the association with membrane subunit. This active complex is responsible for transfer of electrons to molecular oxygen and generating superoxide (De Boer *et al*., 2011). In addition to the neutrophils, this enzyme is also present in various kidney cells like mesangial cells, interstitial fibroblasts and smooth muscle cells of vascular tissue and epithelia of proximal convoluted tubule (El-Benna *et al*., 2009). Renal cells possess other analogues of NOX2 including NOX3, NOX4 and NOX5. These are non-phagocytic cells and the production of superoxide is relatively lower in these cells than phagocytic cells. Therefore, their function also differs in the two types of cells. NADPH oxidase in non-phagocytic cells when activated causes ROS generation in the intracellular compartment. These ROS act as second messengers thus facilitate various biological functions. NADPH oxidase is activated by interactions of ligand with the receptor with subsequent increased yield of ROS. Ligands can be growth factors, cytokines and G-PCR agonists. Certain metabolic factors like AGEs, hyperglycaemia and fatty acids modulate the activity of NADPH oxidase (Dale *et al*., 2008). NADPH derived ROS are capable of modulating various biophysiological (cell growth, migration, multiplication and differentiation) and pathobiological (fibrosis, inflammation, hoarding of extracellular matrix and endothelial dysfunction) processes (Griendling *et al*., 1994; Nauseef, 2007; Ushio-Fukai *et al*., 2009). Thus, NADPH oxidase acts as a potential mediator of ROS production mediated by hyperglycaemia.

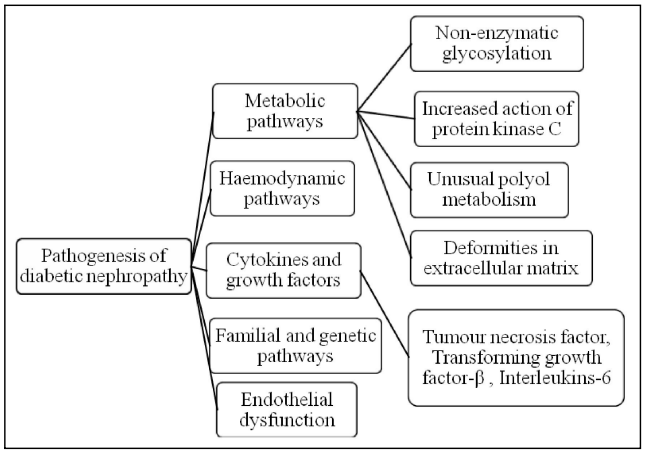

Fig. 4: Various pathophysiological mechanisms associated with diabetic nephropathy.

• **Mitochondrial Origin of Oxidative Stress:** Most of the glucose that we intake is used up by the body to generate ATP (energy) in the mitochondrial respiratory chain complex via oxidative phosphorylation. Majority of glucose after reaching the cells is transformed to pyruvate through glycolysis. The pyruvate is then utilised for the generation of ATP, NADH and FADH2 via Kerbs cycle. They donate electrons or are responsible for the transfer of electrons between species during oxidative phosphorylation when they are being transported from cytosol to mitochondria either through glycerol phosphate shuttle or malate-aspartate system. By acting as electron donor, the electrons from NADH and FADH2 are conducted to O_2 in the respiratory chain complex I to IV thus, generating ATP. In this process, less than 1% of the entire O_2 is changed to O_2- (superoxide anion) by partial reduction and the rest 99% generates water through complete reduction under normal physiological state. This does not happen in pathological conditions such as hyperglycaemia or mitochondrial dysfunction. In such conditions the electrons are leaked excessively at two major sites- complex I and the interface between complexes III and Co-Q (coenzyme Q). Thus, in diseased conditions, the major source for superoxide is mitochondrial respiratory chain (Forstermann, 2008; Sachse and Wolf, 2007; Ushio-Fukai, 2006).

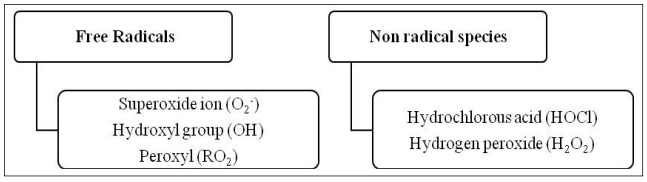

Fig. 5: Reasons for alteration in anti-oxidant equilibrium during oxidative stress.

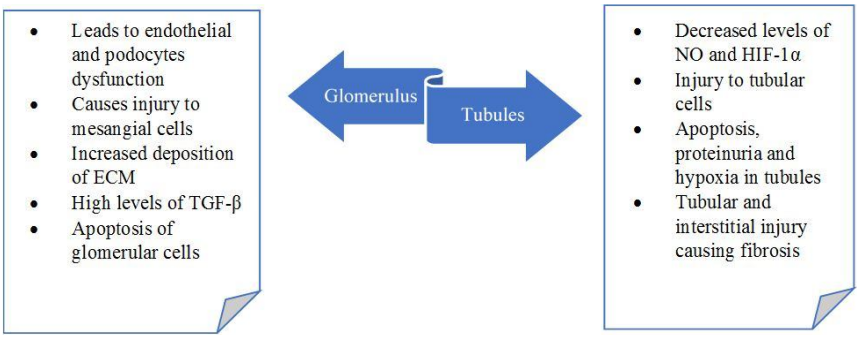

Fig. 6: Effects of oxidative stress on diabetic nephropathy. ECM: Extracellular matrix; TGF-β: Transforming growth factor-β; NO: Nitric oxide; HIF-1α: Hypoxia inducible factor 1-α.

The main target cells *e.g.* mesangial, neuronal and endothelial cells are sensitive to increased blood glucose levels. Due to which, on increased influx of glucose there is immense leakage of electrons at the leakage sites due to enhanced oxidative phosphorylation. Thus increasing the ROS mediated oxidative stress (Kowaltowski *et al*., 2009; Riemer *et al*., 2009). The accumulation of these anions and other free radicals are responsible for the causation of breaks in DNA strand, degrading the sugar moieties or histones (base) (Addabbo *et al*., 2009; Skulachev *et al*., 2009). Mitochondrial DNA is more easily degraded by oxidative damage than nuclear DNA because it is histone deficient. This oxidative DNA damage impairs the respiratory chain thus leading to further production of ROS. This cycle continues leading to excessive damage and eventually the cell undergoes apoptosis or necrosis (Esposito *et al*., 1999; Forbes *et al*., 2008).

• **Endothelial Nitric Oxide Synthase (Enos) Uncoupling:** In renal cells, nitric oxide synthase (NOS) exists as dimer mainly in its three isoforms *i.e.* inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) (Ide, *et al*., 2001; Lieber and Karanjawala, 2004; Williams, 2000). Cofactors such as bihydrobiopterin (BH4), flavin mononucleotide (FMN), flavin adenine nucleotide (FAD) and calmodulin are required by NOS to generate free NO radicals. NOS uncoupling during diabetes causes superoxide anion formation instead of NO radicals due to lesser accessibility of L-arginine, which is the substrate for NOS or its cofactor BH4 thus causing imbalance in oxidant species equilibrium (Ishii *et al*., 2001; Madamanchi and Runge, 2007). Satoh *et al.,* recommended that glomerular superoxide is mainly due to the uncoupling of NOS and NADPH oxidase. The same study showed that by elevating BH4 to its normal physiological level decreased the ROS production *via* uncoupling of NOS and improved the function of renal cells (Sullivan *et al*., 2010).

• **Xanthine Oxidase Pathway:** The enzyme xanthine oxidoreductase (XOR) is involved in generating high levels of ROS. XOR catalysed oxidation of xanthine and hypoxanthine leads to the formation of uric acid. XOR exist in its two exchangeable forms *i.e.* xanthine oxidase and xanthine dehydrogenase. The former has minimal activity in normal physiological conditions. Xanthine oxidase is responsible for the production of different kinds of ROS like O_2-, H_2O_2 and OH radicals by using O_2 as an electron acceptor (Channon, 2004; Cosentino and Katusic, 1995). During pathological conditions in DN, it has been shown to accelerate uric acid synthesis (Satoh and Fujimoto, 2005; Kosugi and Nakayama, 2009). Uric acid is known to play role in promoting cell death by increasing oxidative stress and modulating inflammatory processes. There are various studies which signal the risk factors of developing renal complication in diabetes due to hyperuricemia (Komers *et al*., 2016; Matsumotom and Koshiishi, 2009).

• **Cyclo-Oxygenase (COX) Pathway:** In kidneys, COX pathway occurs in arachidonic acid metabolism. The arachidonic acid which is substrate for COX is obtained by the cleavage of phospholipids present in the membrane by the enzyme phospholipase A2. COX catalyses the synthesis reaction of prostaglandins G2 and eventually of prostaglandin H2 from arachidonic acid. The further metabolism is catalysed by prostaglandin and thromboxane synthase resulting in the formation of various other prostaglandin and thromboxane A2 which mediates the tone and diameter of blood vessels (vascular tone) and maintain the normal physiological balance of water and salt in kidneys. COX is present in mammals in two

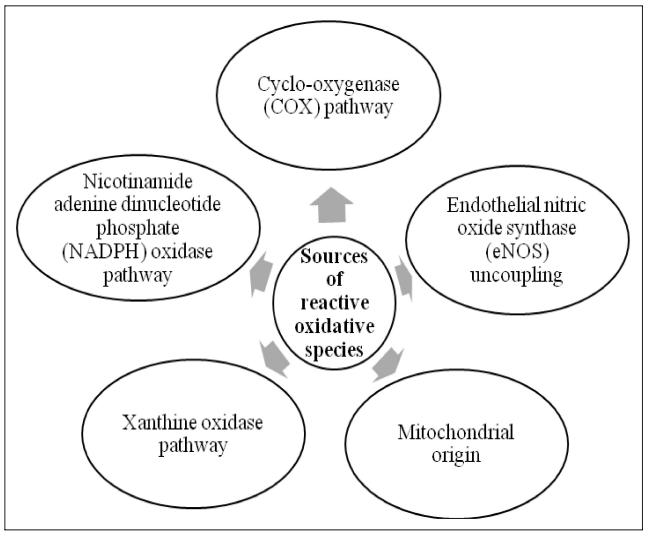

Fig. 7: Sources of reactive oxidative species in oxidative stress.

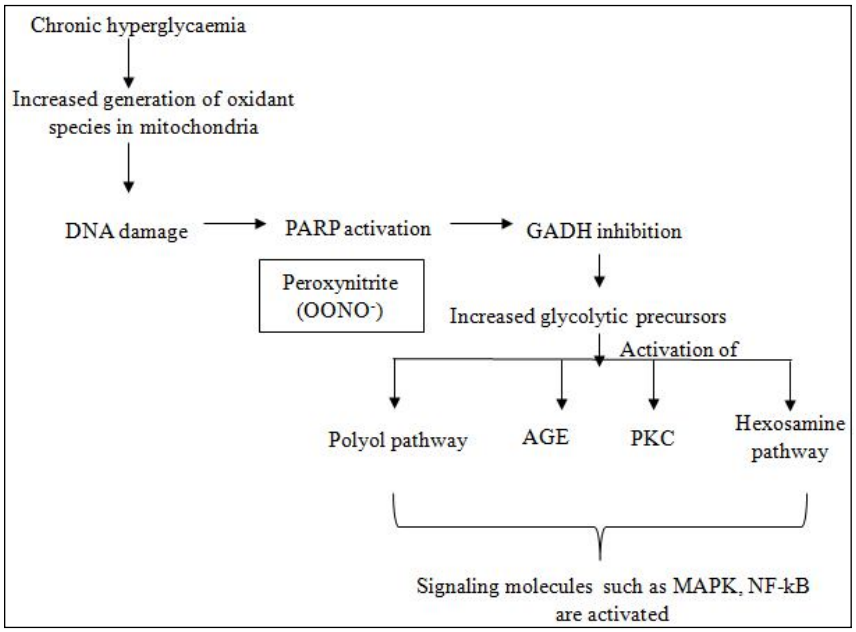

Fig. 8: Mechanism of complications in diabetic neuropathy. MAPK: Mitogen-activated protein kinase; NF-kB: Nuclear Factor kappa light-chain-enhancer of activated B cells.

different isoforms *i.e.* COX-1 isoform which is also called as constitutive COX because it regulates all the housekeeping functions. It is present in tubular compartment as well as in glomerulus in kidney and COX-2 isoform which is mediated through inflammatory processes and is present only at the level of macula densa (Dawson and Walters, 2006; Sautin and Johnson, 2008; Cirillo and Gersch, 2009). Free radicals are produced only through COX-2 and the increase in its expression is correlated with accelerated generation of ROS and ultimately leads to renal cell death or apoptosis (Harris and McKanna, 1994; Harris, 2006). Thromboxane and PGE2 produced by the reaction catalysed by COX-2 are capable of inducing the activity of NADPH oxidase, producing oxidant species which further increases the expression of COX-2. Approximately 20-30% of the total oxidant species produced in kidneys is due to the COX-derived oxidative stress. The activity of COX-2 increases with age, therefore, might find a part in ageing (Kiritoshi and Nishikawa, 2003; Rockwell *et al*., 2004; Xu *et al*., 2006).

Mechanism of Complications in Diabetic Neuropathy

Brownlee gave a hypothesis for the pathophysiology involved in diabetic complications and presented a theory wherein the single major cause for the initiation of the cascade of events leading to chronic complications in diabetes is persistent hyperglycaemia induced increase in generation of oxidant species (Wilcox, 2005). Diabetic nephropathy caused due to continual increased blood glucose levels is regulated by various signalling pathways (Jaimes and Tian, 2005). Increased oxidant species in mitochondria leads to an increase in rate of cellular apoptosis and is therefore considered as the major factor involved in mechanism of DN. Oxidant species also causes damage to the nucleic acids (DNA). To repair the damage caused an enzyme such as poly-ADP-ribose polymerase (PARP) is activated and this leads to the accumulation of ADP (ribose) and also inhibits the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme which plays a major role in glycolysis (Lee and Williams, 2005). As a result of which it leads to excess of precursors in the glycolytic process and activates various other metabolic pathways involved in progression and development of DN such as pentose, advanced glycation end products, glucosamine or hexosamine and protein kinase C pathways (Fig. 8).

• **Polyol Pathway:** Under normal physiological conditions, a small amount of glucose is converted into sorbitol through polyol pathway using a NADPH dependent enzyme such as aldose reductase. But during chronic hyperglycaemic conditions in diabetes, an excess amount of glucose enters the polyol pathway and leads to accumulation of sorbitol in cells. Increased sorbitol alters the osmolality in the cells and initiates the generation of NADP+ which a major contributor of oxidative stress (Wilcox, 2005). Furthermore, this sorbitol when oxidised to fructose with the help of an enzyme such as sorbitol dehydrogenase produces NADH which accelerates the inhibition of GAPDH (Brownlee, 2005).

• **Advanced Glycation End Products (AGEs):** They are heteropolymers made up of various biomolecules such as nucleic acids, lipids and proteins which are linked

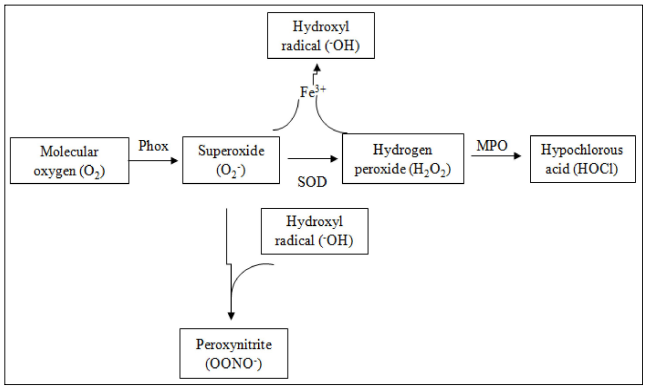

Fig. 9: Reactive oxygen and reactive nitrogen species.

to sugars containing a free aldehyde or ketone group. AGEs help in determination of extent of signalling molecules activated and associated with the increase in oxidative stress. Various AGEs predecessors- 3-deoxyglucosone, methylglyoxal and glyoxal are the products of autoxidation of fructose and glucose (Kersten *et al*., 2000; Wilcox, 2005). Therefore an increase in glucose levels during diabetes accelerates the generation of AGEs which is confirmed by the presence of elevated AGE levels in the glomeruli of patients suffering from diabetes. AGEs and their predecessors play a significant role in the cellular damage caused during pathophysiology of diabetic kidney disease via diverse range of mechanisms such as atypical changes in the ECM, generation of ROS via AGE receptors and disruption of intracellular proteins (Williamson and Chang, 1993).

• **The PKC Pathway:** The protein kinase C (PKC) enzyme exists in its eleven isoforms. A second messenger i.e. diacyl glycerol is responsible for the activation of most of these isoforms and DAG is present in heavy amounts in diabetic patients due to increased level of glucose. PKC can be activated either directly due to hyperglycaemia or indirectly through other pathways such as polyol pathway, or increase in levels of AGEs (Degenhardt *et al*., 1998; Yan and Schmidt, 1994). The over expression of PKC-β enzyme leads to various modification in the actions of intracellular proteins causing characteristic changes ranging from thickening of basement membrane, changes in vascular permeability and altered gene expression. PKC-β inhibitors are therefore involved in preventing the expansion of mesangial cells of glomerulus (Horie and Miyata, 1997).

• **Hexosamine Pathway:** Due to increased levels in hyperglycaemic patients, the excess glucose is metabolised through hexosamine pathway and high levels of fructose-6-phosphate are obtained which leads to increase in the expression of TGF-β1, plasminogen activator inhibitors (PAI-1) and TGF-α. Ultimately this results in the ECM accumulation and increased expression (Horie and Miyata, 1997; Yan and Schmidt, 1994).

Treatment for Oxidative Stress

• **Antioxidant Therapy:** Under normal physiological conditions an equilibrium state is achieved between factors promoting and inhibiting cellular injury due to oxidative stress. This balance is maintained by various antioxidant factors and certain enzymes. Biliverdin reductase, glutathione peroxidase, heme oxygenase and superoxide dismutase (SOD) are major antioxidant factors involved in cytoprotective action of kidney cells (Fig. 9) (Koya and King, 1998). SOD is involved in conversion of superoxide anion into hydrogen peroxide which is subsequently metabolised by catalase and glutathione peroxidase. Therefore, SOD is considered as the first-line factor reducing oxidative stress under normal conditions (Keogh *et al*., 1997; Koya *et al*., 2000). Clinical trials on administration of a SOD mimetic tempol have shown positive results by reducing TGF-β levels, oxidative stress, albuminuria and synthesis of collagen. Tempol is also known to have high membrane permeability, therefore can interact with both intracellular and extracellular reactive oxygen species (Koya *et al*., 2000; Wagener *et al*., 2009). Glutathione peroxidase which is responsible for reduction of hydrogen peroxide to water is also a potent antioxidant factor. This enzyme is also involved in the reduction of peroxynitrite thus reducing the levels of reactive nitrogen species also (Bagby, 2007). Ebselen, which has similar actions to glutathione peroxidase, has shown encouraging results under trial. Heme oxygenase accounts for the conversion of heme to biliverdin, iron and carbon monoxide. The biliverdin thus obtained is then reduced to a potent antioxidant- bilirubin by an enzyme biliverdin reductase (Fujita, 2009).

• **Current Therapies:** Due to inefficiency in measuring the levels of biomarkers of oxidative stress and dysfunction of endothelial cells, targeted antioxidant therapies are still not determined and used. Because the major factor inducing elevations in oxidative stress is hyperglycaemia, strict control of levels of glucose is an effective measure of antioxidant therapy. Other therapies which are used to curb oxidative stress either directly or indirectly are aldosterone blocking agents such as spironolactone, blockers of angiotensin receptor and ACE (angiotensin converting enzyme) inhibitors. These reactions activate eNOS, inhibit the synthesis of angiotensin 2 and TGF-β and also increase the bioavailability of nitric oxide ultimately reducing fibrosis of interstitial cells (Samuni and Graff, 2001; Yamaguchi, 1998). Pioglitazone is known to reduce oxidative stress by its antihyperglycemic action and reducing insulin resistance. It has been shown to decrease glomerular sclerosis and hypertrophy, expansion of mesangial cells, fibrosis of interstitial cells and albuminuria in patients of diabetic kidney disease (Abraham and Kappas, 2005; Ogawa, 2006). Agents involved in reducing lipid levels (statins) by inhibiting the enzyme HMG-CoA reductase also activates eNOS and also maintains GFR thus reducing the lesions formed during the progression of disease (Calkin, 2006; Takebayashi and Matsumoto, 2006). Benfotiamine is a drug used for treating diabetic neuropathy but has shown clinically proven results of reducing proteinuria and hyperfiltrations in patients suffering from diabetic nephropathy as well by reducing

oxidative stress (Okada, 2006).

• **Potential Therapies:** Future Perspective: An ideal antioxidant therapy should be targeted and influence all the mechanisms and processes involved in the production of oxidant species in mitochondria with minimal side effects. Various therapies have been derived but are still ion their experimental phase.

1. **Inhibition of AGE generated ROS:** Excessive advanced glycation in diabetic patients leads to elevation in production of ROS, therefore various studies and approaches are designed to inhibit the synthesis of AGEs. Alagebrium has shown positive results in preclinical and clinical studies. It is a prototypic compound and has been proven to reduce AGE levels in kidney (Usui, 2003). Experiments for aminoguanidine and pyridoxamine are still under test. They have been known to exert their effect by carbonyl intermediates trapping. Other anti-AGE agents are ascorbic acid and hydralazine (Babaei-Jadidi and Karachalias, 2003; Endres and Laufs, 2004). Role of ACE inhibitors and blockers of angiotensin receptors in decreasing production of oxidant species and AGE synthesis is well established and can be of potential use (Abbas *et al*., 2019).

2. **PKC and TGF-β inhibition:** Hyperglycaemia induced over expression of PKC and TGF-β is a potent contributor in the ROS generation. Ruboxistaurin- inhibits PKC and Pirfenidone- inhibits TGF-β have been shown to reduce oxidative stress in pre-clinical studies on rodents (Bakris, 2004; Menon, 2013). These studies are pre liminary and therefore evaluation is needed.

3. **Other approaches:** Various other agents having anti-oxidant properties are under test. L-prpionylcarnitine is an intracellular scavenger of superoxide thus reduces DNA damage and improves the functions of mitochondria. This is based on experimental study. Pentoxifylline which is a potent inhibitor of phosphodiesterase and platelet aggregation when administered alone or with ACE inhibitors have shown antioxidant activity. Vitamin C and E also exhibit anti-oxidant property. They exert their action by increasing the activity of eNOS along with their scavenging action of free radicals. Lipoic acid decreases endothelial cellular dysfunction and is known to reduce the production of ROS in mitochondria. They have been tested experimentally and clinically (Bolton, 2004; Krone and Ely, 2004).

Conclusion

Oxidative stress though under-rated but is undoubtedly present in all diabetic patients. It plays an important role in the development mechanism of micro-albuminuria and endothelial cellular dysfunction in the early stages. It can be suggested that prolonged elevation in blood glucose levels is the major and the foremost factor involved in the generation of continuous high levels of oxidative stress. Other pathways that lead to enhanced levels of oxidative stress due to hyperglycaemia include increased AGE production, the PKC pathway, xanthine oxidase pathway and decreased nitric oxide production. The characteristic feature of oxidative stress in diabetic patients is the reduced bioavailability of nitric oxide and increase in production of ROS. Reports and clinical trials provide proof for the part played by oxidative stress in pathophysiology of diabetic kidney disease. Prolonged elevation in glucose levels along with oxidative stress are key players in the development of structural changes characteristic in diabetic kidney disease such as endothelial dysfunction, changes in vascular permeability, accumulation of ECM, induction of cellular injury, apoptosis and ultimately fibrosis in the interstitials of tubules due to the stimulation of TGF-β.

Oxidant species being a major and important factor in the progression of diabetic kidney disease, antioxidants should provide a potential treatment, but the experimental results of trials for the use of antioxidants does not meet the theoretical expectations. The reason for this is still unknown and studies are still ongoing. Any molecular capable of altering the levels of oxidant species is of great importance for its use in clinical practice. Therefore different targets such as uric acid and other molecules involved in the pathogenesis are being targeted and trials are in proceeding. The NOX1 and NOX4 inhibitors have shown promising effects in phase 1 trial but were not able to lower the levels of albumin excreted in urine of the diabetic patients suffering with kidney disease. However, till the time antioxidants with definitive treatment are available with assured results and lesser adverse effects, regular surveillance of diabetic patients for various clinical and lifestyle factors should be the primary action for treatment of the disease.

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