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A DETAILED REVIEW ON MECHANISMS INVOLVED IN CHRONIC STAGES OF TYPE II DIABETIC COMPLICATIONS

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. In most instances, type 2 diabetes is a dual-defect disease characterized by insulin resistance and impaired β-cell function. High blood glucose levels can lead to disease-related long-term complications in case of diabetic cardiomyopathy. There are data indicating that ROS formation is a direct consequence of hyperglycaemia. More recent studies have suggested that increased (free fatty acids) FFA levels may also result in ROS formation. In turn ROS production and oxidative stress as well as activate stress-sensitive pathways. One of these major pathways is activation of NADPH oxidase complex which further worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes. NADPH oxidase activity is regulated by factors such as cytokines (TNF-α, IL-1), growth factors (EGF, VEGF) and hormones (e.g. insulin). In addition, shear stress, flow cessation, thrombin, serotonin, endothelin-1, angiotensin-II (Ang–II), histamine, bradykinin, lysophosphatidic acid, phorbol 12 myristate 13-acetate (PMA), prostaglandin $F_2\alpha$ (PGF2α) and sphingosine 1-phosphate are known to have an influence on NADPH oxidase activity . Since the development of diabetic nephropathy is faster in patients with bad metabolic control hyperglycemia has been suggested to cause the renal changes by metabolism of glucose and Non-enzymatic reaction of glucose. The present review focused on, molecular mechanisms which cause major Diabetic complications in type 2 diabetes.

Key words: EGF: Endothelial growth factor, IL: Interleukin, NADPH: Nicotinamide adenine dinucleotide phosphate, ROS: Reactive oxygen species, TNF: Tumor necrosis factor, VEGF: Vascular endothelial Growth factor.

INTRODUCTION

Each year, diabetes kills nearly four million people with the total number of diabetes-related deaths estimated to increase by more than 50 percent over the next 20 years. At least 50 percent of all people with diabetes are unaware of their condition and in some countries this figure may reach 80 percent. By the time they are diagnosed, a great many have already started to develop diabetes associated complications, such as visual Up to 80 percent of type 2 diabetes cases could be

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by adopting a healthy diet and increasing impairment, kidney impairment, heart disease, stroke and nerve damage. Cardiovascular disease is the major cause of death in diabetes. (IDF Report, April 2010).

Prevalence

➢ Diabetes is the fourth leading cause of global death by disease.
➢ Every ten seconds two people develop diabetes.
➢ Each year, nearly four million deaths are attributable to diabetes.
➢ Projected prevalence of diabetes is expected to rise from 285 million to 438 million by 2030.

(IDF Report, April 2010)

physical activity, yet every 10 seconds a person dies from diabetes-related causes. (IDF Report, April 2010). If high blood glucose (hyperglycemia) is left uncontrolled or is not controlled long-term, it can lead to serious medical complications in all parts of the body, especially where nerves and blood vessels play a vital role. (WHO Report, April 2010).On average, people with type 2 diabetes may die 5-10 years earlier than people without diabetes, mostly due to cardiovascular disease. (IDF Report, April 2010).

Diabetes

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American diabetes association, 2006). There are three main types of diabetes mellitus (DM). Type 1 diabetes mellitus results from the body's failure to produce insulin, and presently requires the person to inject insulin or wear an insulin pump. This form was previously referred to as "insulin-dependent diabetes mellitus" or "juvenile diabetes". Type 2 diabetes mellitus results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as non insulin-dependent diabetes mellitus or "adult-onset diabetes". The third main form, gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 diabetes mellitus.

Both type 1 and type 2 diabetes possess a significant genetic component. In the case of type 2 diabetes, additionally environmental factors, including hormones, increased caloric intake, decreased physical inactivity, and adiposity, have a marked influence on the disease. There evidences that elevated levels of metabolic substrates contribute to the diabetic phenotype. (DeFronzo RA, 1997; Kahn CR *et al.*, 1996; Unger RH *et al.*, 1998).

Type 2 diabetes

Type 2 diabetes is a chronic progressive disease and the most common form of diabetes, accounting for 85 to 95 percent of the total number of diabetes cases. Many people with type 2 diabetes show no symptoms at the early stages of the disease and may only be diagnosed when complications occur (IDF Report, April 2010) and (WHO Report, April 2010). In most instances, type 2 diabetes is a dual-defect disease characterized by insulin resistance and impaired β-cell function. Glucose derived from dietary carbohydrate or hepatic glucose production is tightly regulated by the hormone insulin. Type 2 diabetes results from an imbalance between insulin sensitivity and insulin secretion. Glucose production fails to be regulated adequately by insulin, leading to hepatic

glucose overproduction and diminished glucose uptake by muscle tissue. (Goldstein B., 2006) Over time, the impaired glucose metabolism leads to a loss of β-cells and the remaining β-cells eventually fail to maintain their high rate of insulin secretion, leading to glucose intolerance, insulin resistance, and overt type 2 diabetes. High blood glucose levels can lead to disease-related long-term complications (Goldstein B, 2006).

Diabetic complications: As with all diabetes patients, those with type 2 Diabetes are at risk of serious complications including:

Cardiovascular disease: People with type 2 diabetes are up to four times more likely to have a heart attack or stroke as people who do not have diabetes. Indeed, people with type 2 diabetes are as likely to suffer a heart attack as people without diabetes who have already had a heart attack, while 45 percent of those hospitalized for a heart attack have known or previously diagnosed diabetes (IDF Report, April 2010). These cardiac complications include diabetic cardiomyopathy.

Kidney disease: Type 2 diabetes is now the most frequent cause of kidney failure in countries of the Western world. The reported incidence varies between 30 percent and 40 percent in countries such as Germany and the USA (IDF Report, April 2010). These renal complications include diabetic nephropathy.

Renal failure and cardiovascular risk: The risk of developing cardiovascular disease is up to three times greater in patients with impaired renal function (IDF Report, 2003).

Eye complications: People with diabetes often develop diabetic retinopathy (changes in the retina of the eye) and have a higher risk of blindness. It is estimated that more than 2.5 million people worldwide are affected by diabetic retinopathy, the leading cause of vision loss in adults of working age (20 to 65 years) in industrialized countries IDF Report, April 2010; ADA , April 2010).

Foot complications: People with diabetes can develop different forms of foot problems. Foot problems commonly occur when there is nerve damage or poor circulation in the feet. These problems are especially severe when nerve damage and poor circulation coincide (WHO Report, April 2010).
There are data indicating that ROS formation is a direct consequence of hyperglycemia (Brownlee M *et al.*, 2001) more recent studies have suggested that increased (free fatty acids) FFA levels may also result in ROS formation. In turn ROS production and oxidative stress as well as activate stress-sensitive pathways. One of these major pathways is activation of NADPH oxidase complex which

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further worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes (Guzik and Harrison, 2006).

Oxidative stress in diabetes

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

Oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species and antioxidant defenses, leads to tissue damage. Oxidative stress results from increased content of reactive oxygen species (ROS). Examples of ROS include charged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide (Rosen P *et al.*, 2001). There are data indicating that ROS formation is a direct consequence of hyperglycemia (Brownlee M *et al.*, 2001); more recent studies have suggested that increased (free fatty acids) FFA levels may also result in ROS formation. Because of their ability to directly oxidize and damage DNA, protein, and lipid, ROS are believed to play a key direct role in the pathogenesis of late diabetic complications (Rosen P *et al.*, 2001) and (Nishikawa T *et al.*, 1995) (Fig 1 B).

Under normal physiologic conditions, ROS are produced at low levels and are controlled by endogenous antioxidant systems that include superoxide dismutase (SOD), glutathione peroxidase, catalase, and antioxidant vitamins (A). ROS lead to harmful effects, such as: damage of membrane lipids, peroxidation of docosahexaenoic acid, cleavage of DNA during the hydroxylation of guanine, and methylation of cytosine. ROS can also block mitochondrial respiration by inhibiting complex enzymes involved in the electron transport chain (Yamamoto T *et al.*, 2002; McCord JM *et al.*, 1969; Babior *et al.*, 1973) (fig 1).

An additional target of oxidative stress is the β-cell. β-Cells are responsible for sensing and secreting the appropriate amount of insulin in response to a glucose stimulus. Although this process is complex and dependent on many factors, the critical importance of mitochondrial glucose metabolism in linking stimulus to secretion is well. Therefore, the ability of oxidative stress (H_2O_2) to damage mitochondria and markedly blunt insulin secretion is not surprising (Meglasson MD *et al.*, 1986). In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules to activate a number of cellular stress-sensitive pathways that cause cellular damage, and are ultimately responsible for the late complications of diabetes. Furthermore, these same pathways are linked to insulin resistance and decreased insulin secretion. ROS and oxidative stress induced by elevations in glucose and possibly FFA levels play a key role in causing insulin resistance and β cell dysfunction by their ability to activate stress sensitive signaling pathways (Joseph L. Evans *et al.*, 2003).

Transfer of one electron to oxygen leads to multiple reactive oxygen species through the superoxide anion radical, $O_2^-\bullet$ .Superoxide dismutase to hydrogen peroxide ($O_2^-\bullet$) + $O_2^-\bullet$ Further addition of electrons requires cleavage of the bond between the oxygen atoms, a reaction catalyzed by iron (Fenton Reaction) to form the highly reactive hydroxyl radical ($OH\bullet$). $OH\bullet$ reacts instantaneously with any molecule from which it can abstract a hydrogen atom. A recently, discovered iron-independent pathway to $NO_2\bullet$ from Nitric oxide (NO) leads to nitrogen dioxide radicals ($NO_2\bullet$) as well Hypochlorous acid (HOCl), a strong oxidants. The toxicity of ROS can be farther increased by the reaction between the two ROS to form peroxynitrite (ONOO-) formed if Nitric oxide (NO) is in exces.

Diabetic cardiomyopathy

Greater number of people dies from cardiovascular disease made worse by diabetes-related lipid disorders and hypertension. Genetic and lifestyle factors, including increased caloric intake and reduced physical activity, contribute to the development of type 2 diabetes. This increases the production of reactive oxygen species (ROS) resulting in myocardial fibrosis, stimulation of apoptotic pathways, and altered contractile function. These changes in myocardial structure and function are the hallmarks of diabetic cardiomyopathy Strong experimental evidence indicates that increased oxidative stress and associated oxidative damage are mediators of renovascular injury and cardiovascular pathologies increased production of superoxide anion and hydrogen peroxide, reduced nitric oxide synthesis, and decreased bioavailability of antioxidants have been demonstrated in experimental and human hypertension (M Brownlee, 2001).

Pro-atherogenic mechanisms associated with hyperglycemia

A key feature common to all end-stage forms of diabetes ishyperglycemia, which may in part account for macrovascular complications even though it may not be the only mechanism as discussed below. Different molecular mechanisms associated with hyperglycemia have been identified including (1) increased glucose flux through the polyol pathway (M Brownlee, 2001), (2)

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formation of advanced glycation end products (AGE), (3) activation of protein kinase C (PKC), (4) increased glucose flux through the hexosamine pathway and activation of the 12/15-lipoxygenase (12/15-LO) pathway (R Natarajan et al., 2004). All these mechanisms finally lead to increased superoxide formation (M Brownlee, 2001).

Polyol pathway

The polyol pathway consists of the rate-limiting enzyme aldose reductase (AR) and sorbitol dehydrogenase (SDH). Under normoglycemic conditions, AR reduces toxic aldehydes to inactive alcohols, thereby protecting cells (SE Spycher *et al*., 1997). AR has a low affinity for glucose and normally does not metabolize significant amounts of glucose. However, under hyperglycemic conditions, AR catalysis of glucose results in sorbitol production, which is subsequently metabolized by sorbitol dehydrogenase to fructose (KM Bohren *et al*., 1989).

This may lead to increased osmotic stress because of intracellular sorbitol accumulation and increased oxidative stress because of consumption of NADPH which is needed to regenerate glutathione. Sorbitol is oxidized to fructose by sorbitol dehydrogenase, with NAD^+ reduced to NADH. This process is NADPH-consuming, and determines decreased regeneration of reduced glutathione. Hyperglycemia induces an overproduction of reactive oxygen species (ROS), which leads to increased conversion of glucose to sorbitol and its oxidation to fructose, with final exacerbation of ROS production, therefore inducing or exacerbating ROS production (Brownlee, 2001), in turn triggering deleterious vascular changes .

Advanced glycation end products (AGE)

Intracellular hyperglycemia leads to generation of reactive dicarbonyls, namely glyoxal, methylglyoxal and 3-deoxyglucosone, which react with amino groups of proteins and form AGE (Brownlee, 2001). Modification of extracellular and intracellular proteins because of AGE formation may alter their function either by cross-linking molecules of the extracellular matrix (ECM) or by binding to the receptor for AGE (RAGE) expressed on several cell types relevant in atherogenesis, for example endothelial cells, macrophages and vascular smooth muscle cells (A Goldin *et al*., 2006, AM Schmidt *et al*., 1999). Ligation of RAGE leads to translocation of NFκB into the nucleus and increased transcription of adhesion molecules like ICAM-1, E-selectin, VCAM-1, and pro-inflammatory factors like tissue factor, VEGF, interleukin (IL-)1α, IL-6, or tumor necrosis factor (TNF)-α (A Goldin *et al*., 2006). Furthermore, AGE induce endothelial hyperpermeability and have been shown to be chemotactic for monocytes, induction of scavenger

receptor class A and CD 36 in macrophages by AGE may represent another proatherogenic (A Goldin *et al*., 2006). Mechanism involves is, Hyperglycemia induces an overproduction of ROS, which in turn leads to increased autoxidation of glucose to glyoxal, decomposition of the Amadori products to 3-deoxyglucosone, and fragmentation of glyceraldehyde-3-phosphate to methylglyoxal. These reactive intracellular dicarbonyls react with amino groups of intracellular and extracellular proteins to form AGEs.

Protein kinase C (PKC)

Protein kinase C (PKC) phosphorylates proteins at serin and threonine residues. Of 11 known isoforms, eight are activated by diacylglycerol (DAG). Intracellular hyperglycemia induces PKC activity via increased levels of DAG resulting mainly from DAG de novo synthesis. Indirect PKC activation may be because of RAGE engagement or polyol pathway activation (M Brownlee, 2001) or activation of 12/15-LO (M.D. Williams *et al*., 2007, R Natarajan *et al*., 2004). Various effects of PKC activation have been described which may promote atherogenesis. High concentrations of intracellular glucose increases production of the second messenger diacylglycerol in endothelial cells, which activates PKC and causes the subsequent expression of an array of proinflammatory genes and growth factors, such as TGFβ, collagen, fibronectin, PAI-1, VEGF, possibly linked by the activation of NF-κB.

Hexosamine pathway

Intracellular hyperglycemia also activates the hexosamine pathway. The hexosamine pathway converts fructose-6-phosphate to glucosamine-6-phosphate through glutamine-fructose-6-phosphate amidotransferase (GFAT) activity, thereby providing substrates for synthesis of proteoglycans and glycoproteins. Here, fructose-6 phosphate, a glycolytic intermediate, is converted to glucosamine-6 phosphate by the rate-limiting enzyme GFAT. Hyperglycemia-induced activity of GFAT has been associated with transcription of different factors. Functional consequences of the activation of this pathway are represented by increased expression and activity and of plasminogen activator inhibitor-1 (PAI-1) (Buse *et al*., 2007) and transforming growth factor-β1 (TGF-β1) (Kolm-Litty *et al*., 1998). The major end-product in such reactions is uridine diphosphate N-acetylglucosamine, which is a substrate for the subsequent O-linked acetylglucosamine (O-GlcNAc) modification of target proteins and transcription factors at serine and threonine residues. Both PAI-1 and TGF-β1 effects mediated by O-GlcNAc modification of the transcription factor Specificity Protein. Only a small fraction of glucose (approximately 1–3%) is metabolized through this pathway, but such a fraction increases in conditions of

hyperglycemia. Thus, the hexosamine pathway may well be related to microvascular diabetic complications, but may not represent a strong link between diabetes and atherosclerosis. Hyperglycemia determines an increased synthesis of O-linked acetylglucosamine through the hexosamine pathway, with subsequent O-GlcNAc modification of target proteins and transcription factors that regulate activity and expression of PAI-1 and TGFβ.

12/15-lipoxygenase (12/15-LO) pathway

12- and 15-Lipoxygenase are enzymes that insert oxygen at the 12 or 15 carbon position into arachidonic acid leading to formation of 12(S)- and 15(S)-hydroxyeicosatetraeonic acid. 12/15-LO is expressed in endothelial cells, smooth muscle cells, monocytes and macrophages, its activity has been shown to be increased by hyperglycemia. Several effects of 12/15-LO activity are considered pro-atherogenic. 12/15-LO promotes oxidation of native to more atherogenic oxidized LDL. Reduced IL-12 secretion in 12/15-LO-deficient mice upon stimulation with LPS suggests a role in the inflammatory response. 12/15-LO seems to be involved in hyperglycemia- as well as mm LDL-mediated adhesion of monocytes to the endothelium and promotes vascular smooth muscle cell hypertrophy. Also, 12(S)-HETE, one of the products of 12-LO, promotes monocyte-adhesion to endothelial cells, probably in part by inducing the fibronectin splice variant CS-1 and VCAM-1 on endothelial cells (R Natarajan *et al.*, 2004).

A potential source of ROS are NADPH oxidases, the only known enzyme family that is only dedicated to ROS production. NADPH oxidases are activated and regulated by several factors, as mechanical forces, hormones and cytokines, considering the thrombin, the Platelet-Derived Growth Factor (PDGF), the a Tumor Necrosis Factor (TNF-α), the lactosylceramide, the Interleukin-1 and the oxidized LDL stand out among these factors (Griendling KK *et al.*, 1994). The kidney and vasculature are rich sources of NADPH Oxidase derived ROS, which under pathological conditions play an important role in renal dysfunction and vascular damage.

NADPH oxidase structure and activation

NADPH oxidases are comprised of membrane proteins (i.e. the catalytic flavin–heme protein); NOX, of which five isoforms exist (NOX_{1-5}); and the non-catalytic 22 kDa binding protein, p22phox (Lassegue and Clempus, 2003). Other components of the NADPH oxidase complex can include a cytosolic organizer (p47phox or NOX_{O1}), an activator (p67phox or NOX_{A1}) and other proteins (p40phox and Rac). These cytosolic subunits vary with the different NOX isoforms (Opitz *et al.*, 2007).

NADPH oxidase activity is regulated by factors such as cytokines (TNF-α, BNF-β, IL-1), growth factors

(EGF, FGF-β, IGF-1, PDGF, TGF-β1, VEGF) and hormones (e.g. insulin) (Guzik and Harrison, 2006). In addition, shear stress, flow cessation, thrombin, serotonin, endothelin-1, angiotensin-II (Ang – II), histamine, bradykinin, lysophosphatidic acid, phorbol 12 myristate 13-acetate (PMA), prostaglandin $F_2α$ (PGF2α) and sphingosine 1-phosphate are known to have an influence on NADPH oxidase activity (Rhee *et al.*, 2003). Recent studies emphasize the stimulating effect of glucose, AGE, non esterified fatty acids (NEFAs) and oxidized low density lipoproteins (ox-LDL) on NADPH oxidase activity as well (Ray and Shah, 2005). PKC is required to enable binding of this subunit with others and activate NADPH oxidase (Massenet *et al.*, 2005) (Fig 10). Of the many vasoactive factors that stimulate this process, Ang-II appears to be one of the most important in the vasculature (Griendling KK *et al.*, 2000; Touyz RM *et al.*, 2002; Berry C *et al.*, 2000). Activation of NADPH oxidases may result from the stimulation of a number of cell surface receptors, such as the angiotensin II receptor, which is particularly important in hypertension and heart failure (Morawietz *et al.*, 2001). AT1 receptor stimulation by angiotensin II may lead to phospholipase C and D (PLC and PLD) activation, both of which produce diacylglycerol (DAG). PLC also produces inositol triphosphate (IP3). DAG and IP3 mediated calcium release activate protein kinase C (PKC) (Lyle and Griendling, 2006).

NADPH oxidase localizations and functions

Upon phosphorylation, cytosolic subunits translocate to the NOX-containing membrane resulting in ROS formation. However, NOX_4 and NOX_5 may be independent of cytosolic subunits and constitutively active (Brandes and Kreuzer, 2005; Bedard and Krause, 2007; Opitz *et al.*, 2007). Activation of NAD(P)H oxidase is a multistep process, initiated by phosphorylation of p47phox, which triggers complex formation of cytoplasmic subunits followed by translocation to the membrane where, together with Rac, it associates with cytochrome b558 to assemble the active oxidase (Babior BM *et al.*, 2002).

Phosphorylation of the p47phox subunit by PKC is required to enable binding of this subunit with others and activate NADPH oxidase (Massenet *et al.*, 2005). NADPH oxidase may also be activated via the production of lipid second messengers, Rac (Ras superfamily of small GTPases) and guanine nucleotide exchange factors (GEF) such as Sos (son-of-sevenless). Sos may be stimulated by c-Abl (tyrosine kinase) and hypothetically by phosphatidylinositol 3,4,5-triphosphate (PIP3) (Lyle and Griendling, 2006). The last process is thought to bind Rac1 to the p67phox subunit and activate the oxidase (Ray and Shah, 2005). Activation of NAD(P)H oxidase by angiotensin II. In the unstimulated state, NADPH

oxidase subunits are disassociated. Angiotensin II, through processes that involve c-Src, phospholipase D (PLD), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3-K) and epidermal growth factor receptor (EGFR) transactivation, induces phosphorylation of p47phox, which triggers complex formation of cytoplasmic subunits followed by translocation to the membrane, where it associates with membrane-associated units to assemble the active Oxidase.

Complexity of regulation and signaling of NADPH oxidases:

Superoxide anion, a major product of NADPH oxidases, reacts with NO causing peroxynitrite generation, which is the major and best characterized mechanism of endothelial dysfunction (Guzik *et al*., 2002b). Superoxide anion also interacts with other enzymes and is spontaneously or enzymatically dismutated to a more stable H_2O_2 (Rhee *et al*., 2003). Hydrogen peroxide may then be converted into reactive nitrogen and reactive chlorine, which attack both LDL and HDL, contributing to plaque formation (Lyle and Griendling, 2006; Nicholls and Hazen, 2004).

Angiotensin II and other signalling pathways stimulate production of intracellular reactive oxygen species (ROS) primarily through activation of the multisubunit enzyme NADPH oxidase. Intracellular ROS modify the activity of tyrosine kinases, such as JAK2, phosphatidylinositol 3-kinase (PI3-K) and epidermal growth factor receptor (EGFR), as well as mitogen-activated protein kinases (MAPK), particularly p38 MAPK, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK).

ROS regulates transcription factors primarily via the formation of disulfide bonds at DNA binding domains in critical amino acid residues or indirectly by modulating redox signaling pathways (phosphorylation/dephosphorylation) (Kregel and Zhang, 2007). Both activator protein-1 (AP-1, via MAPKs pathway, especially ERK1/2 and c-Jun N-terminal protein kinases (JNKs), (Abe and Berk, 1998; Kunsch and Medford, 1999)) and NF-κB (Kunsch and Medford, 1999) have been found to be activated under oxidative stress conditions and lead to the expression of pro-inflammatory genes along with cytokine and chemokine production (Kregel and Zhang, 2007; Piette *et al*., 1997; Yao *et al*., 1994). Moreover NF-κB is involved in E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression causing monocyte adhesion to endothelial cells and their activation (Abe and Berk, 1998) (Fig: 10).

Reactive oxygen species may inhibit protein tyrosine phosphatase activity, further contributing to protein tyrosin kinase activation. Reactive oxygen species also affect gene and protein expression by activating transcription factors, such as nuclear factor NF-κB and activator protein-1 (AP-1). Reactive oxygen species stimulate ion channels, such as plasma membrane $Ca^{2}+$ and K+ channels, leading to changes in cation concentration. Activation of these redox-sensitive pathways results in numerous cellular responses, which, if uncontrolled, could contribute to hypertensive vascular damage.

Aside from modulating intracellular signaling pathways, ROS contributes to DNA, lipid and protein damage. ROS-induced DNA damage activates poly (ADP-ribose) polymerase (PARP), leading to PKC activation and a further increase in NF-κB activity (Brownlee, 2005). In the signaling pathways described above, it is very likely that other intermediate proteins such as intracellular reducing agents (i.e. glutathione, catalase, peroxidases and SOD) are involved (Guzik *et al*., 2005).

Through these molecular mechanisms, ROS influences platelet aggregation, monocyte migration, lipid peroxidation and redox sensitive gene expression. ROS is also involved in the mediation of endothelial cell dysfunction, vascular smooth muscle cell (VSMC) growth, proliferation, differentiation, apoptosis and migration, as well as immune responses (Griendling and FitzGerald, 2003; Lyle and Griendling, 2006).

Major targets of ROS include protein tyrosin phosphatases (PTP), protein tyrosine kinases (PTK), mitogen-activated protein kinases (MAPK), ion channels, phospholipases and transcription factors(Sauer H *et al*., 2001 and Droge W, 2001), all of which have been implicated to play a role in Ang-II mediated vascular changes in cardiac disease.

H_2O_2 influences key intracellular signaling components, affecting both protein kinases and phosphatases. H_2O_2 modulates protein phosphorylation by inactivating phosphatases and activating some protein kinases through active site cysteine oxidation (Rhee *et al*., 2000). An accepted paradigm is that an increase in intracellular ROS production may inhibit tyrosine phosphatases and tip the balance toward tyrosine kinase activation. This would lead to the phosphorylation of mitogen-activated protein kinases (MAPKs), also known as extracellular signal-regulated kinases (ERKs), serine-threonine kinases and stress-activated protein kinases (SAPKs). Akt kinase (downstream of IP 3-kinase) and caspase-3 activity are both involved in the apoptosis process, and are regulated by ROS (Irani, 2000).

Protein tyrosine phosphatases

Protein tyrosine phosphorylation is of major importance for cell proliferation, differentiation, migration and transformation. The regulation of tyrosine phosphorylation is mediated by the antagonistic activity of PTK and PTP. Because of their particular structure,

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PTP are susceptible to oxidation and inactivation by ROS. In addition, the inactivation of PTP is involved in oxidative stress-induced activation of several protein tyrosine kinases, such as the EGFR, insulin receptor. This is particularly important with respect to Ang-II, which mediates many of its signaling events in vascular cells through EGFR transactivation (Saito Y *et al*., 2002).

Protein tyrosine kinases

Receptor and non-receptor tyrosine kinases are also targets of oxidative stress (Droge W, 2001; Yang S *et al*., 2000). Oxygen intermediates, which are produced in response to tyrosine kinase receptor activation, are also involved in transactivation of PDGFR and EGFR by Ang-II. Under pathological conditions associated with oxidative stress, such as hypertension, ROS may activate cell surface receptors directly, thereby amplifying the process of superoxide generation. Non-receptor tyrosine kinases such as Src, JAK_2, STAT and Akt, all of which have been implicated in cardiovascular remodelling and vascular damage, are also regulated by ROS.

Mitogen-activated protein kinases

Mitogen-activated protein kinases are a family that participates in signal transduction classically associated with cell differentiation, cell growth and cell death. Enhanced activation of vascular MAPK has been demonstrated in Ang-II dependent hypertension and seems to be a major mechanism contributing to vascular damage in hypertension. The MAPK are regulated by phosphorylation cascades. In addition, these kinases are strongly activated by ROS (Pearson G *et al*., 2001; Xu Q *et al*., 1996; Touyz RM *et al*., 2002).

Gene expression

Oxygen intermediates regulate many cardiovascular-related genes, including adhesion molecules that control inflammatory cell recruitment, anti-oxidant enzymes that regulate ROS interactions with signalling systems, nitric oxide synthase and vasoactive agents. Modulation of gene expression by oxidative stress occurs primarily through the redox regulation of transcription factors, such as nuclear factor NF-κB, activator protein-1 (AP-1) and hypoxia-inducible factor-1 (Sauer H *et al*., 2001; Droge W, 2001). Increased activation of NF-κB and AP-1 by AngII has been demonstrated in VSMC from spontaneously hypertensive rats (SHR) (Ruiz-Ortega M *et al*., 2003). Accordingly, this may be another mechanism whereby oxidative stress contributes to hypertensive vascular damage.

Ion transport systems

In addition to influencing signalling pathways associated with cell growth and inflammation, ROS modulate intracellular Ca^2+ concentration [Ca^2+], a major determinant of vascular contraction. Superoxide and H_2O_2 increase [Ca^2+] in VSMC and endothelial cells (Lounsbury KM *et al*., 2000).

These mechanisms repeatedly occur chronically in type 2 diabetes which ultimately causes cardiovascular complications.

B) Diabetic nephropathy

The classical definition of diabetic nephropathy is a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining glomerular filtration and eventually end stage kidney failure (Enyioma N Obineche *et al*., 2005).

Diabetic nephropathy is the leading cause of renal failure in the United States. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or "microalbuminuria." Microalbuminuria is defined as albumin excretion of 30–299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes (Michael J flower, 2006). Diabetes is the leading cause of kidney failure in the developed world and accounts for approximately 35% to 40% of new cases each year. Over a lifetime about 50% of people with diabetes develop microalbuminuria. Approximately 20% of people with diabetes develop kidney failure. Kidney failure from diabetes happens so slowly that they may not feel sick at all for many years (Australian diabetes council, 2010).

Significant structural changes particularly thickening of glomerular basement membrane and mesangial expansion occur only after several years of diabetes. Electron microscopic studies show that the increase of glomerular extracellular matrix correlates well with the extent of microalbuminuria. This correlation is found in both type 1 and type 2 diabetic patients (Osterby R *et al*., 1992; Osterby R *et al*., 1993). Detailed immunohistochemical studies have shown that during the development of diabetic glomerulosclerosis there is an increase of the physiologically occurring collagen species. During the development of diabetic nephropathy new types of collagen appear gradually substituting the physiologically occurring collagen types (Nerlich A *et al*., 1994; Kim Y *et al*., 1991; Nerlich A *et al*., 1991).

Since the development of diabetic nephropathy is faster in patients with bad metabolic control hyperglycemia has been suggested to cause the renal changes. According to (Ayo SH *et al*., 1990; Schleicher E *et al*., 1996). Two different mechanisms are currently given (Table: 1) by which glucose exerts its toxic action:

I. Non-enzymatic reaction of glucose

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Glucose may react with proteins without enzymatic action by the chemical reactivity of its carbonyl group. This reaction known as non-enzymatic glucosylation or glycation leads to increased addition of glucose to proteins according to the increased glucose levels which is particularly significant in proteins with long life-time, e.g. collagens (Table:1). The glycations of proteins proceeds further resulting in the formation of advanced glycation end products (AGE products) (Brownlee M *et al.*, 1988).

It has been shown that AGEs accumulate in the renal cortex of diabetic rats (Mitsuhashi T *et al.*, 1993) and in sclerosing glomeruli of diabetic patients (Witztum JL *et al.*, 1997). Recent reports indicate that accumulation of AGE in tissues is associated with possibly toxic effects (Schmidt AM *et al.*, 1995). These include either cross-linking of long-lived matrix proteins or quench nitric oxide, both by chemical reactions. Furthermore, it has been shown that AGEs increase vascular permeability, promote the influx of mononuclear cells, and induce the production of growth factors and cytokines (Doi T *et al.*, 1992). AGE-mediate cellular effects have led to the detection of a receptor for AGE (RAGE) (Vlassara H *et al.*, 1986). This receptor is expressed on vascular cells which may be involved in the development of diabetic vasculopathies (Schmidt AM *et al.*m1999). The cells include macrophages, vascular endothelial and smooth muscle cells and mesangial cells (Doi T *et al.*, 1992; Bierhaus A *et al.*, 1998). Stimulation of this receptor by AGE-modified proteins results in increased oxidative stress of the cells (Yan S *et al.*, 1994). Furthermore, it has been shown that AGE–RAGE interactions lead to activation of the transcription factor NFκB (Bierhaus A *et al.*, 1997). Activation of this factor is involved in the expression of genes of the inflammatory response, such as cytokines, growth factors.

Glycation involves covalent reactions between free amino groups of amino acids, such as lysine, arginine, or protein terminal amino acids and sugars (glucose, fructose, ribose, etc), to create, first, the Schiff base and then Amadori products, of which the best known are Heamoglobin A1c and fructosamine (fructoselysine). Additional reactions occur successively AGE formation.

AGE formation from fructoselysine involves the nonoxidative dissociation of fructoselysine to form new reactive intermediates that again modify proteins to form AGEs of various different chemical structures. Alternatively, fructoselysine decays and releases its carbohydrate moiety either as glucose or as the more reactive hexoses, such as 3-deoxyglucosone, which themselves may modify proteins. In addition, it has recently been found that glucose can auto-oxidize to form reactive carbonyl compounds (glyoxal and methylglyoxal) which can react with proteins to form glycoxidation products .In addition to this, products of

oxidative stress, such as peroxynitrite, can also induce the formation of carboxymethyl lysine by oxidative cleavage of Amadori products and/or the generation of reactive dicarbonyl compounds from glucose (Goldin A *et al.*, 2006; Meerwaldt R *et al.*, 2008; Jakus V *et al.*, 2004).

Thus, AGEs can arise from glucose and lipids through several, partially intermingling reactions. Once formed, they may damage cellular structures via a number of mechanisms, including the formation of cross-links between key molecules in the basement membrane of the extracellular matrix (ECM) and the interaction of AGEs with RAGEs on cell surfaces, thus altering cellular functions (Yan SF *et al.*, 2006; Basta G, 2008; Yan SF *et al.*, 2007).

Accumulation of AGEs in the ECM occurs on proteins with a slow turnover rate, with the formation of cross-links that can trap other local macromolecules. In this way, AGEs alter the properties of the large matrix proteins collagen, vitronectin, and laminin. Once AGEs develop in the arterial wall and myocardium, they form stable and irreversible crosslinks with adjacent collagen polymers thereby decreasing the compliance of the blood vessels and myocardium. The AGEs and reactive oxygen species will also affect ion channel, calcium homeostasis, and mitochondrial function, as well as initiating apoptosis, leading to contractile dysfunction-glucotoxicity (Dike Bevis Ojji., 2011).

Cellular effects of the AGE-RAGE interaction

RAGE is expressed in many tissues and is most abundant in the heart, lung, kidney, skeletal muscle, and vessel wall. In addition, it is present in monocytes/macrophages and lymphocytes. In vessels, it is located in the endothelium and in smooth muscle cells. The most important pathological consequence of RAGE interaction with its ligands is the activation of several intracellular pathways, leading to the induction of oxidative stress and a broad spectrum of signaling mechanism. The interactions lead to prolonged inflammation, mainly as a result of the RAGE-dependent expression of proinflammatory cytokines and chemokines (Yan SF *et al.*, 2006; Basta G, 2008; Yan SF *et al.*, 2007). In the vasculature, the first pathological consequence of RAGE interaction with its ligands is the induction of increased intracellular reactive oxygen species (ROS), the generation of which is linked, at least in part, to the activation of the NADPH-Oxidase system. In addition, in endothelial cells, mitochondrial sources of ROS are also involved, following the AGE-RAGE interaction.

Experimental evidence demonstrates that RAGE dependent modulation of gene expression and cellular properties depends upon signal transduction. Based on the intensity and duration of stimulation, diverse signalling pathways may be triggered, including p21ras, erk1/2, mitogen-activated protein kinases (MAPKs), p38 and

SAPK/JNK MAPKs, PI3K, and the JAK/STAT pathway. The downstream consequence of these changes is the activation of key transcription factors (nuclear factor-κB [NFκB], in particular), which in turn cause induction of molecules with damaging actions on the cells.

In human endothelial cells, RAGE activation enhances the expression of adhesion molecules, including VCAM-1, ICAM-1, and E-selectin. AGE bound to RAGE on the endothelium also determines alterations to the surface antithrombotic properties of flowing blood, as shown by a reduction in thrombomodulin expression and the concomitant induction of tissue factor expression that confers procoagulant properties. The interaction of AGEs with RAGEs in monocytes induces their activation to macrophages, which manifests with the induction of platelet-derived growth factor, insulin-like growth factor 1, and proinflammatory cytokines, such as IL-1 and TNF-α

In addition to all this, AGE-RAGE interaction promotes monocyte chemotaxis and, at the level of smooth muscle cells, is associated with increased cellular proliferation. Viewed together, these findings indicate that the AGE-RAGE interaction elicits and potentiates inflammatory responses through the enhanced generation of reactive oxygen species, proinflammatory adhesion molecules, and cytokines, causing continued amplification of inflammatory events (Bierhaus A *et al.*, 2005; Yonekura H *et al.*, 2005).

II. Metabolism of glucose

Elevated glucose concentrations may also exert toxic effects by glucose transporter-mediated entrance into the cell and subsequent enzymatic conversion. Several pathways have been identified which are activated upon high ambient glucose concentration (Table 1). These include: activation of the sorbitol pathway, increase in oxidative stress (Du XL *et al.*, 1998), activation of protein kinase C (PKC) (Schleicher ED, 1999; Ayo SH *et al.*, 1991) and activation of the hexosamine pathway (Kolm-Litty V *et al.*, 1998) all of which may lead to increased cytokine and growth factor production.

In diabetic nephropathy**,** increased oxidative stress has been shown in patients with diabetes and has been implicated in the development and progression of diabetic microvascular complication, including diabetic nephropathy (Piwowar A *et al.*, 2009; Saklayen MG *et al.*, 2010). The high glucose significantly increases intracellular ROS and upregulates different pathways through NADPH oxidase like in diabetic cardiomyopathy. Diabetic nephropathy is characterized with abnormalities in the glomerular endothelium and mesangium as well as in podocytes or glomerular visceral epithelial cells. Podocytes cover the outer aspect of the glomerular basement membrane via foot processes, and modified

tight junctions between adjacent cells forms the slit diaphragm. This unique structure is specially designed to allow filtration and represents the final barrier to albumin entering the urinary space (Asanuma K *et al.*, 2003). The foot processes of podocytes, in diabetic nephropathy, broaden and efface, and there is a loss of podocyte-specific proteins such as nephrin and eventually loss of podocytes themselves. Such changes in podocytes may contribute to the development of albuminuria, a hallmark of diabetic nephropathy.

Diabetic Retinopathy

Diabetic Retinopathy is a condition occurring in persons with diabetes, which causes progressive damage to the retina, the light sensitive lining at the back of the eye. It is a serious sight-threatening complication of diabetes. Over time, diabetes affects the circulatory system of the retina. Diabetic retinopathy is the result of damage to the tiny blood vessels that nourish the retina. They leak blood and other fluids that cause swelling of retinal tissue and clouding of vision. The condition usually affects both eyes. The longer a person has diabetes, the more likely they will develop diabetic retinopathy. If left untreated, diabetic retinopathy can cause blindness.

Diabetic retinopathy is classified into two types:

➢ **Non-proliferative diabetic retinopathy** (NPDR) is the early state of the disease in which symptoms will be mild or non-existent. In NPDR, the blood vessels in the retina are weakened causing tiny bulges called microanuerysms to protrude from their walls. The microanuerysms may leak fluid into the retina, which may lead to swelling of the macula.

➢ **Proliferative diabetic retinopathy** (PDR) is the more advanced form of the disease. At this stage, circulation problems cause the retina to become oxygen deprived. As a result new fragile blood vessels can begin to grow in the retina and into the vitreous, the gel-like fluid that fills the back of the eye. The new blood vessel may leak blood into the vitreous, clouding vision. Other complications of PDR include detachment of the retina due to scar tissue formation and the development of glaucoma. Glaucoma is an eye disease defined as progressive damage to the optic nerve. In cases of proliferative diabetic retinopathy, the cause of this nerve damage is due to extremely high pressure in the eye. If left untreated, proliferative diabetic retinopathy can cause severe vision loss and even blindness (AOA, 2012)

Treatment for diabetic retinopathy depends on the stage of the disease and is directed at trying to slow or stop the progression of the disease. In the early stages of Non-proliferative Diabetic Retinopathy, treatment other than regular monitoring may not be required. If the disease advances, leakage of fluid from blood vessels can lead to macular edema. Laser treatment (photoco

agulation) is used to stop the leakage of blood and fluid into the retina. A laser beam of light can be used to create small burns in areas of the retina with abnormal blood vessels to try to seal the leaks. When blood vessel growth is more widespread throughout the retina, as in proliferative diabetic retinopathy, a pattern of scattered laser burns is created across the retina. This causes abnormal blood vessels to shrink and disappear. With this procedure, some side vision may be lost in order to safeguard central vision (AOA, 2012).

Persons with diabetic retinopathy can suffer significant vision loss. Special low vision devices such as telescopic and microscopic lenses, hand and stand magnifiers, and video magnification systems can be prescribed to make the most of remaining vision.

Diabetic foot Complications

People with diabetes can develop many different foot problems. Even ordinary problems can get worse and lead to serious complications. Foot problems most often happen when there is nerve damage, also called neuropathy, which results in loss of feeling in your feet. Poor blood flow or changes in the shape of your feet or toes may also cause problems. Diabetic nerve damage can also lessen your ability to feel pain, heat, and cold. Loss of feeling often means you may not feel a foot injury. Nerve damage can also lead to changes in the shape of your feet and toes.

➢Diabetes can cause changes in the skin of your foot. At times foot may become very dry. The skin may peel and crack. The problem is that the nerves that control the oil and moisture in your foot no longer work.

➢Calluses occur more often and build up faster on the feet of people with diabetes. This is because there are high-pressure areas under the foot.

➢Ulcers occur most often on the ball of the foot or on the bottom of the big toe, neglecting ulcers can result in infections, which in turn can lead to loss of a limb. Poor circulation (blood flow) can make your foot less able to fight infection and to heal.

➢People with diabetes are far more likely to have a foot or leg amputated than other people. Many people with diabetes have artery disease, which reduces blood flow to the feet. Also, many people with diabetes have nerve disease, which reduces sensation. Together, these problems make it easy to get ulcers and infections that may lead to amputation. Most amputations are preventable with regular care and proper footwear.

➢Diabetes causes blood vessels of the foot and leg to narrow and harden. One of the biggest threats to your feet is smoking. Smoking makes arteries harden faster which affects small blood vessels. It can cause decreased blood flow to the feet and make wounds heal slowly (ADA, 2012).

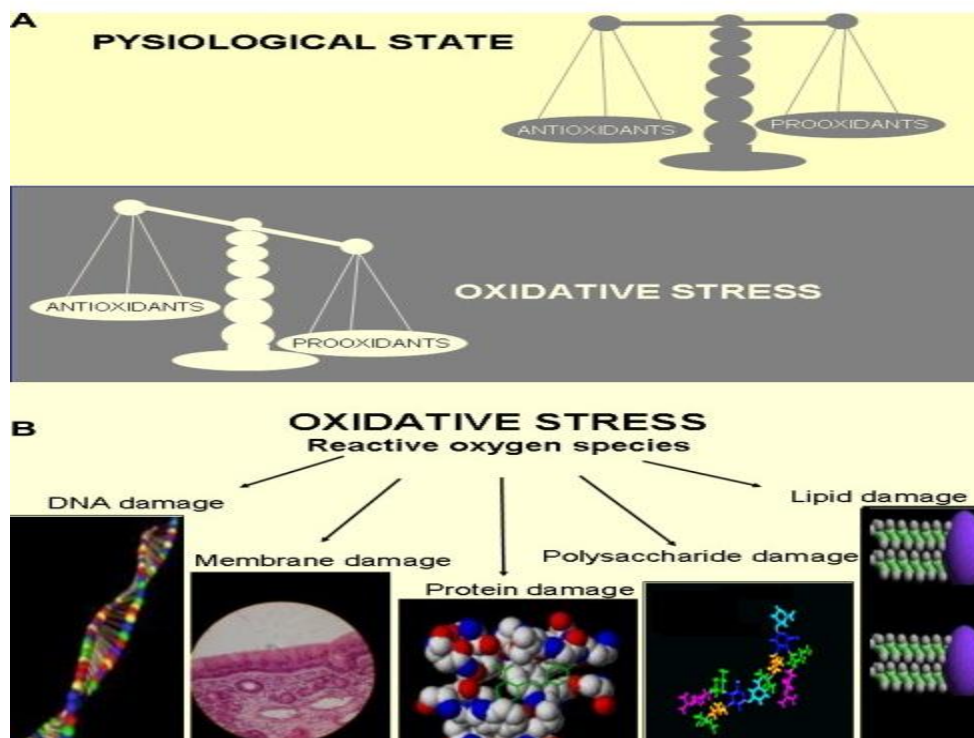**Figure 1**. **Role of ROS in Normal and Diabetic conditions**

Table 1. Pathogeneic pathways of glucose toxicity

(1) Non-enzymatic pathways	**Glycation of proteins**
	Formation of AGE
	Induction of oxidative stress via AGE/RAGE interaction
(2) Metabolic pathways of glucose	**Sorbitol pathway**
	Hexosamine pathway
	Increase in oxidative stress
	Activation of PKC

CONCLUSION

These findings have evoked considerable interest because of the possibilities that therapies targeted against free radicals by decreasing ROS generation or by increasing nitric oxide availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress diabetic end-organ damage. Many novel treatments are also available for diabetic eye and foot complications. Our review suggested that Antioxidants treatment may be a promising agents for treating the development and progression of diabetic cardiomyopathy and diabetic nephropathy. Administration of these agents to the patients diagonised in the early stages of diabetic cardiomyopathy and diabetic nephropathy would be very beneficial in preventing the pathological changes that occurs in diabetic condition.

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