Oxidative Stress in Diabetes Mellitus

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Abstract

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of cell is overwhelmed. The present review briefly summarized the underlying role of overwhelming levels of RONS in the pathophysiology of diabetes mellitus (DM). The review is based on using keywords to obtain information from publications in PubMed, ScienceDirect and Google Scholar from 1970-2015. The primary causative factor of oxidative stress in DM is hyperglycemia, which operates via several mechanisms. However, the individual contribution of other intermediary factors to hyperoxidative stress remains undefined, in terms of the dose response relationship between hyperglycemia and overall oxidative stress in DM. Intuitively, the inhibition and/or scavenging of intracellular free radical formation provide a therapeutic strategy to prevent oxidative stress and ensuing pathologic conditions. The integration of antioxidants formulations into conventional therapeutic interventions, either by ingestion of natural antioxidants or through dietary supplementation should be encouraged for a holistic approach to the management and prevention of DM and complications associated with the pathology.

Key words: Antioxidants, diabetes mellitus, hyperglycemia, oxidative stress, radicals

Introduction

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of cell is overwhelmed (Shin et al., 2001; Styskal et al., 2012; Sellamuthu et al., 2013; Poljsak and Fink, 2014). Ordinarily, the peculiar molecular configuration of oxygen (O₂) confers a very slow reactivity between O₂ and biomolecules. Two main factors make O₂ kinetically insert; the spin restriction imposed by its triplet state and the negative standard potential for one electron reduction of O₂ to superoxide radical (O₂⁻). However, O₂ possesses the attributes of free radicals in that it has two unpaired electrons with parallel spin in different -anti-bonding orbitals that is responsible for its paramagnetic properties and relative stability (Pollack and Leeuwenburgh, 2000; Poljsak and Fink, 2014). Spin restriction can be overcome by single electron exchange that converts O₂ to strong oxidizing agent (Rotilio et al., 2000; Thannickal and Fanburg, 2000). Therefore, the activation of O₂ by specific enzymes is achieved by the presence, at the active site, of either flavins or reduced transition metals such as iron (Fe²⁺) and copper (Cu²⁺), which donates single electron to O₂ (Rotilio et al., 2000). In the case of metalloproteins, a varying degree of electron transfer from the metallic moiety to O₂ is possible. On this basis, metalloproteins can behave either as O₂ carriers...
Int. J. Biol. Chem., 9 (3): 92-109, 2015

(hemoglobin, hemocyanin, hemerythrin, myoglobin), where reversible interaction with O₂ occurs, or as O₂ reductants. Studies showed that autoxidation of oxy-hemoglobin elicit the generation of free radicals (Moussa, 2008).

Electron transfer to O₂ is catalyzed by oxidases for production of chemical energy or oxidation of substrates. These enzymes, located in different subcellular compartments (mitochondria, endoplasmic reticulum, peroxisomes) are potential sources of partially reduced Cu²⁺ derivatives in biological milieu. Cytosolic enzymes (xanthine oxidase, NADPH oxidases, lipooxygenase, cyclooxygenase (COX), cytochrome P450 enzymes and aldehyde oxidase), uncoupled endothelial nitric oxide synthase (eNOS) and other hemoproteins also produce O₂⁻ during catalysis (Yung et al., 2006; Alfadda and Sallam, 2012; Styskal et al., 2012). The mitochondrial electron transport chain reduces O₂⁻ at ubiquinone and NADH dehydrogenase sites, whereas; microsomal cytochrome P450 and its reductases produce O₂⁻ during xenobiotic biotransformation (Desco et al., 2002; Wright et al., 2006; Sugatani et al., 2006; Bajaj and Khan, 2012). The “Leaky” inner mitochondrial membrane electron transport chain directly reacts with O₂ to generate O₂⁻, which dismutase to form hydrogen peroxide (H₂O₂), which can further react to form the hydroxyl radical (·OH) (Pollack and Leeuwenburgh, 2000; Alfadda and Sallam, 2012; Styskal et al., 2012). Additionally, the mitochondrial outer membrane enzyme, monoamine oxidase, catalyzes the oxidative deamination of biogenic amines and it’s a quantitatively large source of H₂O₂ that contributes to increase in steady state concentrations of reactive species within both the mitochondrial matrix and cytosol (Cadenas and Davies, 2000). Specifically, O₂⁻ is the primary radical formed by the reduction of O₂ leading to secondary radicals or reactive oxygen species (ROS) such as H₂O₂ and ·OH in the mitochondria (Pollack and Leeuwenburgh, 2000; Styskal et al., 2012).

Although, the cause-effect relationship remains tentative, there appears to be a strong association between mitochondrial dysfunction and chronic metabolic diseases such as Type II diabetes mellitus (T2DM) and obesity (Alfadda and Sallam, 2012). The origin, enzymatic pathways of ROS and their oxidized products, as well as their enzymatic inactivation pathways in T2DM have previously been summarized (Hayden and Tyagi, 2004).

The RONS have been implicated in the pathophysiology of various disease states, including diabetes mellitus (DM) and long-term development of associated complications (Hayden and Tyagi, 2004; Wright et al., 2006; Giacco et al., 2010; Alfadda and Sallam, 2012; Bajaj and Khan, 2012). Oxidative tissue damage is mediated by activating a number of cellular stress-sensitive pathways, which include nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase, NH₂-terminal Jun kinases/stress-activated protein kinases and hexosamines (Evans et al., 2003). Consequently, imbalance between cellular generation and scavenging capacity of free radicals elicits tissue damage associated with DM pathology (Betteridge, 2000; Bajaj and Khan, 2012; Styskal et al., 2012). Also, incidents of oxidative stress-induced neurological disorders mediated by inhibition of enzymatic activities connected with neurotransmission have been reported in experimental diabetic rats (Ashokkumar et al., 2006; Ghareeb and Hussen, 2008; Alipour et al., 2012). As a follow up to these findings, it is obvious that understanding the relationship between oxidative stress and DM pathology has the potentials to expand the therapeutic intervention options against the pathogenesis and progression of the disease. Therefore, the present review briefly summarized the underlying role of overwhelming levels of RONS in the pathophysiology of DM. The review is based on using keywords to obtained information from publications in PubMed, ScienceDirect and Google Scholar from 1970-2015.
Oxidative damage and modification of macromolecules: The radicals (O$_2$•$, \cdot$ OH, NO$^-$, $^1$O$_2$, RO$^-$, $^\cdot$ONOO) and pro-radicals (H$_2$O$_2$, HOCl, RS and O$_3$) are extremely reactive molecules. In biological systems, RONS cause substantial damage/modification to functional and structural macromolecules (lipids, nucleic acids and proteins), as well as modulation of activity of antioxidant enzymes (Poljsak and Fink, 2014). Oxidative attack of polyunsaturated fatty acids (PUFAs) gives rise to peroxided molecules, which subsequently breakdown to form reactive metabolites. For the fact that lipids are the major components of biological membranes, fluidity and permeability of these supra-molecules are severely affected, together with membrane protein functionality (Poljsak and Fink, 2014). The reactive aldehydes are cytotoxic products of lipid peroxidation. Specifically, 4-hydroxynonenal (HNE) causes long-lasting biological consequences by covalent modification of macromolecules, whereas at physiological levels, HNE is considered as second messengers of free radicals and signaling molecules. Report showed that HNE and related reactive aldehydes may play critical roles in the pathophysiology of DM, in terms of the pathogenesis, progression and complications of the disease (Jaganjac et al., 2013).

Base modification, scission of deoxyribose rings, strand breaks and ultimately, chromosomal aberration are outcomes of oxidative damage to nucleic acids. Oxidative challenge on proteins leads to the modification of amino acids side chains with the introduction of carbonyl groups, or oxidation of sulphydryl groups with consequent cross linking and aggregation of protein molecules. The presence of oxidative modifications ultimately results in increased susceptibility of modified proteins to specific proteases, enzyme deactivation, or conversely, unwarranted activation of enzymes (Desco et al., 2002; Poljsak and Fink, 2014).

There appears to be a direct mechanistic link between oxidative stress and the etiology of DM through the accumulation of oxidative damage to critical macromolecules. Several studies have established an association between increased carbonylation and nitrosylation of proteins in insulin-sensitive tissues and T2DM (Kaneki et al., 2007; Grimsrud et al., 2008; Muellenbach et al., 2008). In another study, evidence showed that oxidation of specific proteins compromised their function in vitro (Levine, 1983; Levine et al., 1999) and there is a correlation between increasing oxidative stress and diminished protein folding and function in different animal models (Pierce et al., 2008; Perez-Matute et al., 2009).

Oxidative stress is as a result of free radicals generated during autoxidation of glucose in DM (Aronson and Rayfield, 2002; Evans et al., 2003). Overall, DM is characterized by raised level of oxidative stress with associated increased generation of glycoxidation products, notably, HbA$_1c$ above the benchmark plasma value <7% (Hayden and Tyagi, 2004; Wright et al., 2006; El-Wassef et al., 2012). The presence of hyperglycemia promotes increase in intracellular levels of advanced glycation end products (AGEs) (Wolf and Ziyadeh, 2007; Di Naso et al., 2011; Musabayane, 2012). Furthermore, auto-oxidation of glucose generates ROS, such as O$_2$•$, \cdot$ OH (Bajaj and Khan, 2012; Moussa, 2008), which in turn, accelerate lipid peroxidation with corresponding accumulation of advanced lipoxidation end products (ALEs) and more free radicals (Rolo and Palmeira, 2006; Jaganjac et al., 2013). Increased levels of ROS in T2DM also contribute to a hypercoagulable state and evidence suggests that accumulation of oxidation products occur prior to the development of DM (Matteucci and Giampietro, 2000).

Antioxidants such as the flavonoids prevent the formation of AGEs by impeding the glucose dependent formation of Amadori, Schiff bases or Milliard products, which are intermediary products leading to the formation of AGEs (Keaney and Loscalzo, 1999; Musabayane, 2012). Likewise, disruptions of AGEs cross linkages by drugs such as alagebrium or inhibition of AGE
signal transduction pathway can substantially prevent the accumulation and formation of AGEs, respectively (Hartog et al., 2007). The option of shielding or obliteration of AGEs’ receptor (RAGE), expression of RAGE antisense cDNA or anti-RAGE ribozyme may reverse atherosclerosis in experimental animals (Ihara et al., 2007; Giacco et al., 2010). Also, notable inhibitors (amino guanidine and pyridoxamine) of AGEs formation exhibit reno-protective effects in diabetic animals (Lassila et al., 2004; Hartog et al., 2007).

Mechanisms of hyperglycemia induced production of oxygen free radicals: Hyperglycemia is known to cause elevation in plasma free radical concentrations (Hammes et al., 1997; Cimato et al., 2008). The production of free radicals is engendered by uncontrolled hyperglycemia, which may occur via several routes (Rolo and Palmeira, 2006; Giacco et al., 2010; Bajaj and Khan, 2012): (1) increased glycolysis (Vaag et al., 1992), (2) intercellular activation of sorbitol (polyol) pathway (Williamson et al., 1993; Di Naso et al., 2011), (3) autoxidation of glucose (Wolff et al., 1991), (4) protein kinase C (PKC) dependant activation of NAD(P)H oxidase (Inoguchi et al., 2003), (5) increased hexosamine pathway flux (Rolo and Palmeira, 2006), (6) increased intracellular formation of AGEs (Giacco et al., 2010), (7) increased expression of receptor for AGEs and its activating ligands (Giacco et al., 2010) and (8) non-enzymatic protein glycation (Ceriello et al., 1992). The overall rate of formation of oxidative products leading to oxidative tissue damage is summarized in Fig. 1. Hyperglycemia appears to enhance non-oxidative catabolism of glucose to lactate, which is associated with increase in NADH/NAD+ ratio (Vaag et al., 1992; Williamson et al., 1993). Under the condition of accelerated glycolysis, oxidation of glyceraldehyde 3-phosphate (GAP) to 1, 3-biphosphoglycerate (1, 3-DPG) by glyceraldehyde 3-phosphate dehydrogenase is coupled to reduction of NAD+ to NADH and appears to become the rate limiting step in glycolysis (Kobayashi and Neely, 1979). In the cytosol, NADH is oxidized to NAD+ by lactate dehydrogenase (LDH) with concomitant reduction of pyruvate to lactate.

Fig. 1: Relationship between rates of oxidant generation, antioxidant activity, oxidative stress and oxidative damage in diabetes, RAGE: Receptor for AGEs (Aronson and Rayfield, 2002)
Thus, increase in the ratio of NADH/NAD+ reflects increased lactate/pyruvate ratio (Williamson et al., 1993). The mechanism by which increased rate of glycolysis increases free cytosolic NADH/NAD+ ratio (redox imbalance) suggest disequilibrium between the rate of oxidation of GAP to 1, 3-DPG and the rate of reduction of pyruvate to lactate (Kobayashi and Neely, 1979). Thus, enhanced glycolysis as a result of hyperglycemia is associated with increase in NADH/NAD+ ratio due to impaired oxidation of NADH to NAD+.

The increase in glucose flux via sorbitol pathway (a pathway of a minor significant under normal glycemic condition) elicits one of the major metabolic disturbances associated with diabetic hyperglycemia (Ciuchi et al., 1996). In this pathway, glucose is reduced to sorbitol by aldose reductase (AR) coupled with oxidation of NADH/NAD+ (Dallak et al., 2008). Subsequently, sorbitol is oxidized to fructose by NADH dependent sorbitol dehydrogenase (SDH) (Cameron et al., 1997; Giacco et al., 2010). Previous studies have suggested several hypotheses for tissue injury engendered by increased sorbitol pathway activity, thus:

The decreased availability of NADPH, which is required for maintenance of reduced glutathione (GSH), is oxidized to NADP+ by the reduction of glucose to sorbitol by AR pathway (Tilton et al., 1995). Furthermore, the competition between AR and glutathione reductase (GSH-R) for NADPH cofactor further depletes intracellular GSH (Ciuchi et al., 1996). Attention has been focused on GSH depletion, because it dictates levels of cellular ROS production and accumulation, which in turn have a bearing on extent of oxidative tissue damage in DM (Brownlee, 1994). Increased ratio of NADH/NAD+ is connected with accelerated oxidation of sorbitol to fructose by NADH dependent SDH (Tesfamariam and Cohen, 1992; Brownlee, 2001). Consequently, NADH molecules generated in the cytosol by oxidation of sorbitol to fructose are eventually conveyed to the mitochondria and oxidized by respiratory chain reaction that result in production of O$_2^•-$ and other ROS (Williamson et al., 1993; Ceriello et al., 1996). Thus, an increase in the cytosolic NADH may be accompanied by increased load of mitochondrial NADH, which in turn, leads to increased ROS generation.

In a cell-free system under physiological conditions, glucose can be auto-oxidized to H$_2$O$_2$, through enediol tautomer formation, which elicits the accumulation of reactive intermediate such as •OH and O$_2^•-$ and ketoaldehydes (Brownlee et al., 1988; Packer, 1993). Transition metals such as Fe$^{2+}$ promote auto-oxidation of glucose and therefore, are crucial in these reaction cascades (Packer, 1993). Several studies have equally shown that auto-oxidation of glucose in this manner are responsible for increased levels of ROS in DM (Monnier, 1990; Santini et al., 1997).

Non-enzymatic glycation is a spontaneous reaction between glucose and amino groups of proteins in which reversible Schiff bases and more stable Amadori products are formed (Aronson and Rayfield, 2002). The AGEs are produced by auto-oxidation of Amadori product (Keaney and Loscalzo, 1999; Ahmed, 2005; Rolo and Palmeira, 2006). Glucotoxicity is elicited through the binding of AGEs to RAGEs, which have been identified in endothelial cells, monocots/macrophages, mesangial cells, neurons and smooth muscle cells (Aronson and Rayfield, 2002; Inoguchi et al., 2003; Hayden and Tyagi, 2004; Yonekura et al., 2005; Wright et al., 2006). The presence of AGEs elicits poor matrix protein flexibility as a result of formation of cross-links among extracellular matrix proteins, which leads to abnormal interactions with other matrix components (Yonekura et al., 2005). Additionally, the interaction of AGEs with endothelial surface RAGEs promote intracellular oxidative stress via the activation of AR of polyol-sorbitol pathways, activation of PKC isoforms and transforming growth factor-β (TGF-β) as well as activation of nuclear factor (NF-κB) (Aronson and Rayfield, 2002; Evans et al., 2003). The activation of NF-κB
promotes increase in expression of a variety of cytokines such as tumor necrosis factors (TNF-α and TNF-β), interleukins (IL) 1, 6, 8 and 18 and interferon-γ, even in the presence of intact antioxidant mechanisms, which may engender overt diabetic nephropathy with associated glomerulosclerosis (Esposito et al., 2002; Aronson and Rayfield, 2002; Inoguchi et al., 2003; Hayden and Tyagi, 2004; Basta et al., 2004; Wright et al., 2006; Styskal et al., 2012).

Also, increased cellular uptake of glucose stimulates PKC activity (Lee et al., 1989) which, amongst other effects, activates peroxidase enzymes and the COX pathway (Lee et al., 1989; Feener and King, 1997; Golbidi et al., 2012), with resultant overproduction of RONS. The process leading to this pathology is further enhanced and amplified, when antioxidant defense mechanisms are compromised (Bierhaus et al., 1997).

Mechanisms of hyperinsulinemia induced production of oxygen free radicals: Decline in physical fitness, increase in body fatness and upper body fat distribution are frequently associated with hyperinsulinemia and insulin resistance (DeFronzo and Ferrannini, 1991). Several lines of evidence indicated that hyperinsulinemia promoted the generation of free radicals by NADPH-dependent mechanism, which involved the activation of phosphatidylinositol 3'-kinase and stimulation of proliferative extracellular signal-regulated kinases (ERK-1 and ERK-2)-dependent pathways (Ceolotto et al., 2004). Furthermore, Krieger-Brauer and Kather (1992) reported that prolong exposure of human adipocytes to insulin caused a time- and dose-dependent accumulation of H$_2$O$_2$ in vitro. This effect, which has been linked to the presence of a membrane-bound NADPH oxidase, was observed to persist after cell disruption and devoid of ATP utilization; an indication that the receptor-kinase activity step was bypassed. In addition, increased insulin concentration in rats following intra-peritoneal injection of dextrose has been reported to be associated with increased free radical production (Habib et al., 1994).

Fasting hyperinsulinemia is considered to be a hallmark of insulin resistance (DeFronzo and Ferrannini, 1991) and there is a relationship between insulin resistance and plasma free radical concentration (Ceriello, 1995, 2000). Factors that contribute to the elevation of free radicals and pathogenesis of insulin resistant DM are as follows:

- Hyperinsulinemia overdrive of the sympathetic nervous system (Rowe et al., 1981). Specifically, catecholamine increases free radical production through induction of metabolic rate and auto-oxidation pathway in DM (Singal et al., 1983)
- Insulin resistance is associated with elevated fasting plasma non-esterified fatty acid (NEFA) concentration (DeFronzo and Ferrannini, 1991; Randle et al., 1994)

Toborek and Henning (1994) showed that NEFA caused raised levels of oxidative stress in cultured endothelial cells following initial decreased level of GSH after 6h of incubation. It is worthwhile to note that the complexity of these multitudes of findings suggests that the generation of free radicals may represent a potential mechanism by which chronic hyperinsulinemia activates proliferative events and down-regulates metabolic signals (Ceolotto et al., 2004).

Oxidative stress induced lipid peroxidation in diabetes mellitus: Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders (Armstrong et al., 1982) including naturally occurring and chemically induced DM (Rerup, 1970; Nishigaki et al., 1981; Higuchi, 1982). Lipid peroxidation is the primary cellular damage resulting from free radical reactivity of which cellular lipid structures are mostly affected (Toborek et al., 1992; Ahmed, 2005).
Oxidative deterioration of PUFAs of cellular membrane phospholipids, via intermediate radical reactions involves the production of hydroperoxides (Rungby et al., 1992; Cameron et al., 1994). The chain reactions are associated with the generation of highly toxic peroxyl radicals (RO$_2^-$) in a cycle of reactions that generate new lipid hydroperoxides (LHP) because of the proximity of PUFAs in biomembranes (Kajanachumpol et al., 1997; Betteridge, 2000).

Also, both radical and non-radical oxidants can induce lipid peroxidation in lipoproteins, particularly those that contain PUFAs. For instance, peroxynitrite (ONOO$^-$) is particularly a powerful oxidant of low-density lipoproteins (LDL) (Violi et al., 1999). Similarly, in vitro studies have revealed the presence of oxidized LDL (ox-LDL) fractions with identifiable auto-antibodies against ox-LDL in plasma of Type I DM (T1DM) patients, which suggest that the oxidation LDL can as well occurs in DM in vivo (Jain et al., 1998). Accordingly, Maejima et al. (2001) noted raised levels of ONOO$^-$ in T2DM patients. Additionally, LDL receptor does not recognize ox-LDL and are subsequently taken up by scavenger receptors in macrophages to form foam cells, which leads to atherosclerotic plaques (Boullier et al., 2001; Aronson and Rayfield, 2002).

Early evidence that suggested lipid peroxidation in DM was reported by Sato et al. (1979), in which they noted that the levels of lipid peroxides in plasma of DM patients were significantly higher than that of normal subjects. Likewise, levels of plasma lipid peroxides of DM patients with angiopathy were relatively higher than that of DM patients. They further inferred that raised level of lipid peroxides was among other several factors that initiates atherosclerosis in DM. In another study, Davison et al. (2002) used electron spin resonance (ESR) spectroscopy in conjunction with alpha-phenyl-tert-butylnitrone spin trapping to measure pre- and post-exercise free radical concentration in the venous blood of young male patients suffering from T1DM in order to ascertain their susceptibility to rest and exercise-induced oxidative stress. They suggested that greater concentration of oxidants and LHP were as a result of glucose auto-oxidation couple with lower rate of exercise-induced oxidation of major lipid soluble antioxidant; α-tocopherol in DM. Furthermore, they noted that ESR-detected radicals, in the course of the investigation, were secondary species derived from decomposition of LHP, which were major initial reaction products following free radical attack on biomembranes.

The underlying mechanisms of the formation of LHP and biologically active metabolites, together with their effect on cellular structure and function are becoming of increasing importance in understanding the pathogenesis and management of DM (Crabbe, 1987). For instance, lipoxygenase products, especially 12(S)-HETE and 15(S)-HETE, are involved in the pathogenesis of several diseases including DM (Bajaj and Khan, 2012). The LHPs are produced from a variety of PUFAs precursors via intermediate radical reactions involving O$_2$ and metal cations (Fe$^{2+}$ and Cu$^{2+}$). The reactions generate highly reactive and cytotoxic lipid radicals. Extracellular LHP are transported in the systemic circulation by low- and high-density lipoproteins (Nishigaki et al., 1981). When released locally, LHP elicits structural damage to variety of biomolecules. For instance, the formation of LHP and their metabolites are important in ophthalmic medicine in that the retinal portion of eye is particularly sensitive to oxidative stress. Additionally, a steady irreversible decline in electroretinogram is observed in streptozotocin (STZ)-induced diabetic rats (Pautler and Ennis, 1980), when synthetic LHP was injected into the vitreous chamber of experimental animals (Armstrong et al., 1982). Fortunately, LHP induced oxidative damage to biomolecules is ameliorated by lipid and water-soluble antioxidants, as well as by specific antioxidant enzymes.
Oxidative stress indicators in diabetes mellitus: The concept of raised level of oxidative stress (increased generation of free radicals) in DM was derived principally from in vitro experiments (Wolff, 1993; Schiekofer et al., 2003; Wright et al., 2006). One of such investigations involved the use of cultured human umbilical vein endothelial cells incubated in variable glucose concentrations followed by monitoring the generation of ROS by a measure of cellular level of nitrotyrosine (Quagliaro et al., 2003; Wright et al., 2006).

Early observations have focused attention in understanding underlying mechanisms that may be relevant to atherogenesis in patients suffering from T2DM and in obesity. Persons suffering from T2DM and/or obese individual exhibit raised level of oxidative stress and inflammatory response (Jorns et al., 1999; Alfadda and Sallam, 2012), which from reports have been linked to increased cellular levels of inflammatory cytokines, TGF-β and insulin-like growth factor binding protein (IGFBP)-3 (Jorns et al., 1999; Wright et al., 2006; Bajaj and Khan, 2012). Raised level of oxidative stress in T2DM is indicated by an increase in ROS generation by circulating mononuclear cells, increased lipid peroxidation (Nishigaki et al., 1981), protein carbonylation (Aljada et al., 1995), nitro-tyrosine formation (Aydin et al., 2001) and DNA damage (Dandona et al., 1996; Shin et al., 2001; El-Wassef et al., 2012; Styskal et al., 2012). Importantly, even pre-DM individuals showed elevated 8-hydroxyguanosine, which suggested that oxidative damage to DNA is present even before the clinical development of DM (Styskal et al., 2012). Recently, raised level of oxidative stress was also demonstrated in the obese as reflected in increased lipid peroxidation, protein carbonylation and ortho-tyrosine and meta-tyrosine formation in DM individuals (Keaney and Loscalzo, 1999; Cumaoglu et al., 2007; Cimato et al., 2008; Chis et al., 2009; Styskal et al., 2012). However, the levels of these oxidative stress indicators, as well as generation of ROS by leucocytes, were reversed following restriction to 1,000 calories/day for 4 weeks (Dandona et al., 2001).

The primary causative factor of oxidative stress in DM is hyperglycemia, which operates via, several mechanisms (Fig. 2). However, the individual contribution of other intermediary factors to hyperoxidative stress remains undefined, in terms of the dose response relationship between hyperglycemia and overall oxidative stress in DM.
In the presence of elevated calcium levels in endothelial cell, hyperglycemia stimulates the synthesis of NO$^-$ (Cohen, 1993; Poston and Taylor, 1995), in which in the presence of O$_2^•$, NO$^-$ is converted to highly potent oxidant ONOO$^-$ that promotes endothelial cell damage and endothelial dysfunction (Beckman et al., 1990; Landmesser et al., 2003). Hyperglycemia causes paradoxical increase in the generation of NO$^-$ but low availability of NO$^-$ (Santilli et al., 2004; Wright et al., 2006), which appears to activate NF-κB and thereby engendering increased expression of inducible nitric oxide synthase (iNOS) (Spitaler and Graier, 2002). However, Santilli et al. (2004) noted that low availability of NO$^-$ is attributable to uncoupling of receptor-mediated signal transduction (El-Missiry et al., 2004) and is the primary causative factor of endothelial dysfunction and diabetic angiopathy. In addition, overwhelming levels of O$_2^•$ directly, inactivates two critical anti-atherosclerotic enzymes (eNOS and prostacyclin synthase) and consequently, precipitate defective angiogenesis (Giacco et al., 2010).

Although, there are extreme difficulties in measuring free radicals in vivo, some evidence in support of the notion of raised level of oxidative stress in DM and its association with poor metabolic control and coronary heart disease has been derived from observations in patients with DM (Griffin et al., 1997). Raised level of oxidative stress may provide a plausible pathophysiologic basis for the direct link between hyperglycemia and increased cardiovascular risk in DM (Lehto et al., 1997). There is persuasive evidence and definitive clinical proof that oxidative stress is associated with the pathogenesis and progression of atherosclerosis in both diabetic and non-diabetic subjects (Aronson and Rayfield, 2002). Insulin resistance and raised level of oxidative stress have been observed in obese T2DM patients (Skrha et al., 1996).

There is a relationship between plasma malondialdehyde (MDA) concentration and hyperglycemia (Hayden and Tyagi, 2004; Chikezie and Uwakwe, 2014). Earlier reports by Sato et al. (1979) noted increased level of TBARS in blood samples of patients with poorly controlled DM and diabetic angiopathy. The elevation in TBARS concentration is considered to be an indicator of marked organ or tissue degeneration (El-Missiry et al., 2004). Also, elevation of TBARS concentration provides an indirect measurement of level of lipid peroxidation and alterations in erythrocyte antioxidant enzyme activities in diabetic patients (Arai et al., 1987; Sharma et al., 2000) as observed in heart, pancreas and blood of STZ induced diabetic rats (Kakkar et al., 1995). In another instance, TBARS is considered as an indicator of free radical production. An increase in TBARS level in liver may therefore be due to raised level of oxidative stress that might promote DNA and protein alterations (Wolff et al., 1991), including; changes in the enzyme activities implicated in lipid metabolism and free radicals scavenging process (Douillet et al., 1998).

Raised level of oxidative stress accounts for low erythrocytes count because of low levels of erythrocyte GSH coupled with increased utilization of GSH, in efforts to ameliorate oxidative stress associated with diabetic erythrocytes (Jain and McVie, 1994). Consequently, pathophysiology of DM promotes oxidative damages of phospholipids and associated biomolecules of erythrocyte membrane. This is supported by the fact that erythrocytes of diabetic patients are more susceptible to lipid peroxidation when treated with H$_2$O$_2$ in vitro (Matkovics et al., 1982; Uzel et al., 1987). In addition, low hematocrit (PCV) percentage may be attributed to the reduction in the total red blood cell count due to failure in blood osmoregulation and elevation of plasma osmolarity (Evan-Wong and Davidson, 1983).

**Diabetes mellitus induced alterations in antioxidant enzymes activities:** Several studies on tissue levels of activity of enzymatic antioxidant systems are characterized with divergent
results. For instance, studies using STZ-treated diabetic rats close to three decades ago showed that increase in pancreatic superoxide dismutase (SOD) activity might be an adaptive response to low pancreatic SOD level, whereas reduction in SOD activity in liver and kidney has direct linkage with the damaging effect of free radicals on the enzyme (Wohaieb and Godin, 1987). In another report, Pieper et al. (1995) demonstrated that in experimental DM, the activity of CAT was elevated in vascular tissues, whereas no significant alterations in the activity of other major antioxidant enzymes (SOD and glutathione peroxidase (GSH-Px)) were noted. Ojiako et al. (2015) reported that levels of renal and hepatocyte antioxidant enzymes (GPOx, SOD, CAT) and low molecular weight antioxidant (LMWA) (GSH/GSSG ratio) were altered in alloxan-induced hyperglycemic rats. In addition, Wohaieb and Godin (1987) reported increased CAT and SOD activities in pancreatic tissues of DM rats, whereas the hepatocytes showed generalized low CAT, SOD and GSH-Px activities. They noted that increase in both CAT and SOD activities occurred in tissues with the lowest antioxidant enzymatic activities (pancreas) before onset of DM. Thus, suggesting a compensatory response to an increase in endogenous oxidant radicals in the pancreas of DM rats. Decreased tissue concentrations of antioxidants, such as vitamin E, SOD and CAT, have also been demonstrated in vitro (Wohaieb and Godin, 1987).

Low levels of GSH in erythrocytes of DM subjects is as a result of low activities of the enzymes involved in GSH synthesis (γ-glutamylcysteine synthetase) and/or in the export of oxidized glutathione (GSSG) out of the cell (Murakami et al., 1989) as well as enhanced sorbitol pathway (Ciuchi et al., 1996). In addition, low activity of GSSG-R, which acts to reduce GSSG to GSH, has also been reported in DM (Tagami et al., 1992). Murakami et al. (1989) and Matkovics et al. (1998) reported low level of activity of GSSG-R in erythrocyte haemolysate of STZ-induced DM rats, which they attributed to be the effect of enzyme glycation in uncontrolled hyperglycemia (Jain and McVie, 1994). Also, earlier reports showed significant reduction in the level of activity of erythrocyte GSH-Px in diabetic children and adolescents when compared with that of the control subjects (Dominguez et al., 1998). These previous reports attributed low level of activity of erythrocyte GSH-Px to low blood GSH content in DM subjects, since GSH is a substrate and cofactor for GSH-Px activity. Therefore, low GSH content resulted in corresponding low GSH-Px activity and propensity to elicit oxidative stress. Accordingly, enzyme inactivation either through glycation process (Arai et al., 1987) or under conditions of increased oxidative stress also contribute to low GSH-Px activity (Lyons, 1991).

Antioxidant defenses mechanisms are often impaired in DM with corresponding hyperoxidative stress (Rolo and Palmeira, 2006; Bajaj and Khan, 2012). Furthermore, there is evidence to suggest that DM induces alterations in the activities of antioxidant enzymes in various tissues (Oberley, 1988; Ojiako et al., 2015). Theoretically, alterations in antioxidant enzyme activity are consequences of oxidative stress, glycation of antioxidant enzymes/proteins and disturbances in micronutrient status in DM (Szaleczky et al., 1999; Kang, 2003; Yuan et al., 2010).

CONCLUSION

The critical roles of overwhelming cellular levels of RONS play in the pathophysiology of DM have been incontrovertibly established. Intuitively, the inhibition and/or scavenging of intracellular free radical formation provide a therapeutic strategy to ameliorate oxidative stress and prevent ensuing pathologic complications associated with DM. Therefore, the integration of antioxidants formulations into conventional therapeutic interventions, both by ingestion of natural antioxidants or through dietary supplementation, should be encouraged for a holistic approach to the
management and prevention of DM and associated complications. However, despite the obvious usefulness and potential merit/advantages of antioxidant pharmacotherapy, there is still the need to investigate and evaluate the efficacy and safety scores of this therapeutic strategy. Moreover, previous studies on the effect of certain LMWAs on endothelial dysfunction in T2DM revealed contradictory results. Besides, the query of whether antioxidants could have beneficial effect by reducing the risks associated with DM, especially, cardiovascular disease has remained unresolved and inconclusive.

Finally, another novel approach to DM therapy is to provoke over-expression of antioxidant enzymes in a tissue-specific manner, as exemplified in genetic mutant mice model, to serve as control measure against the development of metabolic diseases associated with oxidative stress (Styskal et al., 2012). This proposed DM therapy shared similar concepts with the reports of Alfadda and Sallam (2012) in which they noted that activation of transcription nuclear factor, nuclear factor-erythroid 2-related factor 2 (Nrf2) induced several antioxidant and detoxification genes in patients with lung cancer. Unfortunately, the metabolic fallouts and effect of this proposed therapeutic approach on general haemostasis of DM individuals is yet to be elucidated.

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