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Review Article

OXIDATIVE STRESS AND DIABETES: AN OVERVIEW

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ABSTRACT

Oxidative stress is well known to be involved in the pathogenesis of lifestyle related diseases. Oxidative stress contributes to many pathological conditions including cancer, asthma, atherosclerosis, hypertension, and diabetes. It is a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of balance between them. Reactive oxygen species (ROS) are produced from molecular oxygen as a result of normal cellular metabolism and environmental factors such as air pollutants, cigarette smoke and sedentary lifestyle. ROS are highly reactive molecules that can damage carbohydrates, nucleic acids, lipids and proteins. There is considerable evidence that induction of oxidative stress is a key process in the onset of diabetes. Lipid peroxidation owing to free radical activity plays an important role in complications of diabetes. Increased levels of lipid peroxidation are a consequence of free radical activity in both Type 1 and Type 2 diabetes. The human body has several mechanisms to counter the effects of these reactive species by the production of antioxidant enzymes like glutathione and catalase. Antioxidants can also be taken exogenously through the diet. In this review article, we summarize the effect of oxidative stress in the development of diabetes.

Keywords: Diabetes, Oxidative stress, Lipid peroxidation, Reactive oxygen species.

INTRODUCTION

Diabetes is a chronic disease with serious metabolic disturbances in carbohydrate, protein and fat metabolism arising due to insulin deficiency or insulin action. It is not only common in older people but occurs frequently in younger generations as well. The most common symptoms are thirst, polyurea, blurring of vision and weight loss [1]. Some serious complications arise if the glucose level of the blood is not controlled in time. Some of the complications are cardiovascular disease including heart attack, severe neuropathy, retinopathy, nephropathy, and osteoporosis and foot damage.

Immune system of an individual works in a very well organized manner, cells communicate with each other for increasing the efficiency of this process [2]. Unfortunately, the immune system can be overprotective in some cases, leading to an increased emission of the oxidative molecules. This increased release of free radicals for neutralizing the stress in an individual is the key cause of the oxidative stress. These free radicals are responsible for diabetic complications and also a leading cause of lipid peroxidation [3]. Oxidative stress and resultant tissue damage and cell death are hallmarks of chronic disease like diabetes, hypertension, cancer, etc., Recent studies have been reported that the various mechanisms accompanying hyperglycemia cause more oxidative damage (increased oxidative stress) in the blood and tissue of diabetic patients, as compared with healthy individuals [4].

DIABETES AND ITS CAUSES

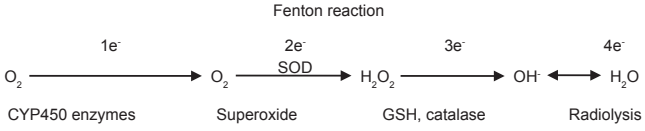
Diabetes mellitus is accompanied by vascular disorders, which is relatively specific to diabetes, and caused primarily by hyperglycemia. There are three types of diabetes depending upon its basic cause, Type 1, Type 2 and Type 3 diabetes. The differentiation between the different types of diabetes was first observed by Dupertius, which was later verified and confirmed by Lister *et al*. and Cudworth [5,6].

Type 1 diabetes is also frequently referred to insulin-dependent, juvenile or childhood diabetes. It is characterized by inefficient or no insulin production by the pancreas. It is common in children [7]. It is immune mediated diabetes, where the immune cells are responsible for the destruction of the islet of langerhans β cells. Type 2 diabetes is the most common and constitutes a total of 90% of all the cases. It is usually caused by the insulin resistance produced in the body. Although the pancreas produces insulin efficiently, the body fails to recognize and utilize it, leading to an increase in the glucose concentration in the blood. Type 3 diabetes is often referred to as gestational diabetes, occurs in pregnant and it is not very common [7]. Diabetes is generally caused by the controllable factors related to lifestyle, eating habits and insufficient sleep [8-10]. It may also be linked to uncontrollable factors like false action of the immune system and also microbial infection. Immune response of the body is directly linked with all the three types of the diabetes.

OXIDATIVE STRESS AND ORIGIN OF REACTIVE OXYGEN SPECIES (ROS)

Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage. ROS are produced by living organisms as a result of normal cellular metabolism. They sometime produce adverse modifications to cell components, such as lipids, proteins, and DNA [11]. ROS can be divided into two groups: Free radicals and non-radicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When two free radicals share their unpaired electrons, non-radical forms are created. The three major ROS are superoxide anion (O_2^-), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2). Superoxide anion is formed by the addition of 1 electron to the molecular oxygen [12]. The process is mediated by nicotine adenine dinucleotide phosphate (NADPH) oxidase or xanthine oxidase or by mitochondrial electron transport system. OH^- is formed by radiolysis of water and by reaction of H_2O_2 with ferrous (Fe^{++}) ions; the latter process is termed as Fenton reaction.

Formation of process of ROS by four-electron step: [11]



Normally, electrons are transferred through mitochondrial electron transport chain for reduction of oxygen to water. NADPH oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages. Upon phagocytosis process, these cells produce a burst of superoxide that lead to bactericidal activity. Superoxide is converted to hydrogen peroxide by the action of superoxide dismutase (SOD) [13]. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NADPH oxidase and in peroxisomes by consumption of molecular oxygen in metabolic reactions. H_2O_2 can breakdown to OH^- in the presence of transmission metals like Fe^{2+} or Cu^{2+}. O_2^- itself can also react with H_2O_2 and generate OH^-. Hydroxyl radical is the most reactive and can damage proteins, lipids, carbohydrates and DNA. It can also start lipid peroxidation by taking an electron from polyunsaturated fatty acids (Fig. 1).

Lipid peroxidation

Lipid peroxidation, owing to free-radical activity, plays an important role in the development of complications of diabetes. Lipids that contain phosphate groups (i.e. phospholipids) are essential components of the membranes that surround the cells and cell structures. Free radicals in the presence of oxygen may cause degradation (peroxidation) of lipids within plasma and organellar membranes. Oxidative damage is initiated when the double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen derived free radicals particularly by OH. The lipid free radical interactions yield peroxides, which are reactive and unstable. An autocatalytic chain reaction ensues which can result in extensive membrane, organellar, and cellular damage [14]. Oxidative destruction of polyunsaturated fatty acids by lipid peroxidation is caused damage as it may alter the integrity of cell membranes [15].

It has been reported that increased levels of lipid peroxidation in Type 1 and Type 2 diabetes, as a consequence of free radical activity. Lipid peroxide is a very important compound formed by a chain reaction. Here, the non-radical lipids are converted to radicals by species such as O_2^-, OH^-, NO and other ROS. This reaction causes damage to various molecules, finally leading to the cell damage [16]. Diabetes is linked with a high blood glucose level and the high lipid content of the adipose tissues during obesity. This leads to increase in the size of adipocytes and thus leading to the generation of phospholipase A_2. This activation of phospholipase A_2 finally leads to the process of lipid peroxidation [17]. Immune system plays an important role directly or indirectly in diabetes. Since, the immune system is responsible, and the immune reaction involves the ROS, which in turn causes lipid peroxidation. The ageing is generally linked with the lipid peroxidation by ROS and so the diabetic has a role in lipid peroxidation.

RELATION BETWEEN OXIDATIVE STRESS AND DIABETES

Diabetes is also caused by the false immune response of the body, i.e. response against self-cells and self-biomolecules. Immune response of the body is directly linked with all the three types of diabetes where β - islet of Langerhans is destroyed by the immune cells causing Type 1 diabetes. Some studies were able to prove the role of autoreactive CD8+ T cells in causing the Type 1 diabetes [18,19]. Similarly, Type 2 and Type 3 diabetes are also linked to the immune response. Many hypotheses have been proposed regarding the inflammatory cause of diabetes. It was also hypothesized that the diabetes may be an outcome of the non-specific response of the immune system. Many researchers found that the patients showed that there was an association of interleukin-6 (IL-6) and C-reactive proteins (CRP) with diabetes [20]. A review by pickup has clearly explained the effect of nutrition on the quantity of CRP and IL-6. An increase in the fat content in the meal leads to an increase in CRP and IL-6, thus increasing the risk of diabetes. In obese person, the adipose tissue becomes saturated with fat, adipocytes starts secreting low level of tumor necrosis factor-α, which in turn stimulates preadipocytes to the production of monocyte chemoattractant protein-1. This is responsible for attracting the macrophages towards the adipose tissues. The increased lipid content of the adipose tissue also triggers the oxidative damage [21].

Diabetic patients have also shown faster ageing as the ageing process is linked with the lipid peroxidation by ROS [7]. Free radical peroxidation of lipids causes local injury to cell membranes and impairment of the activity of enzymes and receptors bound to the membrane, finally leads to damage of organs. Immune system is responsible for direct or indirect role in diabetes. Immune system is defective in diabetes and thus leads to lipid peroxidation.

It is possible; however, that tissue damage in early life may have more severe consequences because of the greater potential for cell growth and division. Human studies have shown that stress can stimulate hyperglycemia, hypoglycemia, or have no affect at all on glycemic status in diabetes. In contrast, more consistent evidence supports the role of stress in Type 2 diabetes [22]. Akkus *et al*. [23] have clearly shown that immune reactions involve ROS, which also causes lipid peroxidation.

Kalaivanam *et al*. studied on successive patients and revealed that excessive production of free radicals observed both in Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes and its

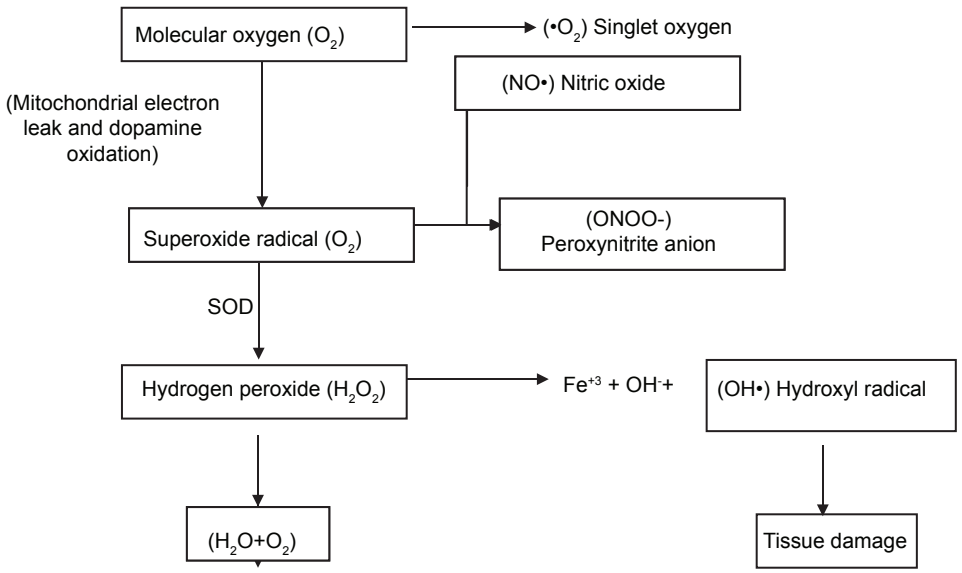


Fig. 1: Representation of generation of oxygen free-radical

insufficient removal results in damage to cellular proteins, membrane lipids, and nucleic acids [24].

It is reported that increased lipid peroxidation is seen due to elevated free radicals in both Type 1 and Type 2 diabetes [4]. The oxidative stress is enhanced in response to hyperglycemia in vascular tissues of patients with diabetes mellitus, leading to the peroxidation of cellular membrane lipids as well as the increased oxidative modification of amino acids and DNA [4]. Overproduction of active oxygen under hyperglycemic conditions is reported to be associated with factors like auto-oxidation of glucose, effects of protein glycation end products, activation of protein kinase C and active oxygen produced by mitochondria. It has been also reported that phospholipids in plasma lipoprotein undergo oxidative modification in patients with Type 2 diabetes, leading to an increase in peroxide lipids. It also explains the association of the intracellular metabolic dysfunction accompanying hyperglycemia, with the increased production of active oxygen and with impaired antioxidant defense [4]. Intracellular carbohydrate metabolism is impaired under hyperglycemic conditions, which is followed by the overproduction of active oxygen, which leads to specific vascular disorders.

Advanced glycation end products (AGEs)

High glucose produces ROS as a result of glucose auto-oxidation, metabolism and the development of AGEs. AGE formation is dependent on oxidative processes and can create ROS through the Maillard reaction [25]. AGEs can propagate oxidative stress in the cells and fluids in which it is produced. AGEs play an important role in the development of diabetic complications. The molecules, such as aminoguanidine and pyridoxamine, work to trap glycoxidation intermediates and impede crosslink formation [25]. AGEs combine with the receptors called RAGE and activate signal transduction pathways, which in turn activate NADPH oxidase that produces ROS and NFκB, a nuclear transcription factor. An imbalance between ROS (ROS) production and antioxidant scavenging has been implicated in Type 2 diabetes [25].

ROS are a byproduct in Type 2 diabetes, generated during protein glycation and as a consequence of RAGE binding; they impair insulin signaling pathways and induce cytotoxicity in pancreatic β cells [26].

In the review of Basta *et al*., the formation of AGEs is an important abnormality that accompanies diabetes mellitus and inflammation. AGEs potently modulate initiating steps in atherogenesis involving blood-vessel wall interactions, triggering an inflammatory-proliferative process and, furthermore, contribute to propagation of inflammation and vascular damage [27].

Recent evidence suggests ROS are also important as second messengers in the regulation of intracellular signaling pathways and, ultimately, gene expression [24].

Abnormal mitochondria and active oxygen production [4]

Mitochondrion plays an important role in active oxygen production particularly under hyperglycemic conditions. In a recent study, it has been reported that the production of active oxygen is increased when the oxidative phosphorylation in mitochondria is enhanced. Hyperglycemia-induced activation of protein kinase C, AGE production and sorbitol accumulation have been reported to be reversed after inhibiting active oxygen production caused by mitochondria in aortic endothelial cells, suggesting the importance of mitochondria in the production of active oxygen under high glucose conditions.

The study was conducted, and it was observed that chronic hyperglycemia increases mitochondrial stress in mesangial cells. Mitochondrial damage resulting from chronic hyperglycemia treatment inhibits basal mitochondrial respiration [28].

In the research of Jang *et al.*, increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue damage [29].

The effect of oxidative stress on DNA, lipids and proteins

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. Higher production of ROS in the body may change DNA structure, results in the modification of proteins and lipids, activation of several stress-induced transcription factors, and production of pro-inflammatory and anti-inflammatory cytokines. ROS can lead to DNA modifications that involve degradation of bases, single- or double- standard DNA breaks purine, pyrimidine or sugar-bound modifications, mutations, deletions and cross-linking with proteins. Most of these DNA modifications are highly relevant to carcinogenesis, aging, and neurodegenerative, cardiovascular and autoimmune diseases [30].

ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability.

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases [31].

Consequences of oxidative stress

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors and production of pro-inflammatory and anti-inflammatory cytokines [29].

ROS production overwhelms the antioxidant defenses of the cell, either because of copious production of oxidants or depletion of antioxidant defenses thereby damaging many cellular components. The key targets are lipids, protein, and DNA [26]. Dyer *et al*. [31] suggested that individuals with severe oxidative stress might still be at greater risk for development of complications, in same way as diabetic patients with hypertension or hyperlipidemia might be at greater risk for renal or vascular disease.

In a study of Ha and Lee, it was shown that oxidative stress is one of the important mediators of vascular complications in diabetes [32]. In a research by Brownlee [33] it was shown that the hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain by different mechanisms and has been implicated in glucose-mediated vascular damage. Martin-Gallan *et al*. [34] in a study explained the role of oxidative stress in the onset of disease-related pathophysiological complications in young Type 1 diabetes patients, and diabetics were observed with no enough antioxidant defenses. Oxidative stress damages the tissue or organ, caused by free radicals. ROS is reported to be the cause of diabetes induced by chemicals such as streptozotocin in experimental animals [34].

In Ceriello *et al*. [35], study, Ceriello examines facts that involve hyperglycemia-derived oxygen free radicals as mediators of diabetes-associated complications. Current studies have specified that a hyperglycemia-induced overproduction of superoxide is a major event in the development of complications of diabetes.

Hyperglycemia leads to some important complications through oxidative stress in many cells. Free radicals contribute to the development of diabetic complications such as changes in kidney, nerve, vascular tissue, etc. Hyperglycemia leads to increased oxidative stress, and monocyte and endothelial cell dysfunction [36]. In a study of Yung *et al*., it was found that ROS have physiological and pathophysiological contacts on vascular cells causing dysfunction of vascular system and causes oxidative damage by decreasing the bioavailability of NO, damaging endothelium, accelerating endothelial cell migration, and

initiating adhesion molecules and inflammatory reaction, directing to endothelial dysfunction.

Current studies have accentuated that ROS are very much related with the progress of diabetes-specific complications. In a study by Niiya *et al*., it was shown that there is the accumulation of AGEs in the formation of vascular complications in diabetes mellitus [37] (Fig. 2).

Antioxidants and its role

There are several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated *in situ* (endogenous) constituents or externally supplied through foods (exogenous). The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [39]. Antioxidants in our regular diet play an important role as endogenous antioxidants for the neutralization of oxidative stress. Each nutrient is unique in terms of its structure and antioxidant function. Kojda and Harrison have shown that the patients supplied with antioxidant probucol and/or SOD showed reduced diabetic complications thus showing a relation between the two [40].

CONCLUSION

Oxidative stress exacerbates the development and progress of diabetes and its complications. It can arise from overproduction of ROS. ROS is produced by cellular metabolic activities and environmental factors too. As ROS molecules are highly reactive molecules, they react with several biological macromolecules in the cell, such as carbohydrates, nucleic acids, lipids, proteins, and alter their function causing impairment of the immune system. These reasons lead to changes in the immune system and lipid peroxide formation that causes damage to various cell molecules. Further research in this field will benefit the diabetic patients' population world-wide.

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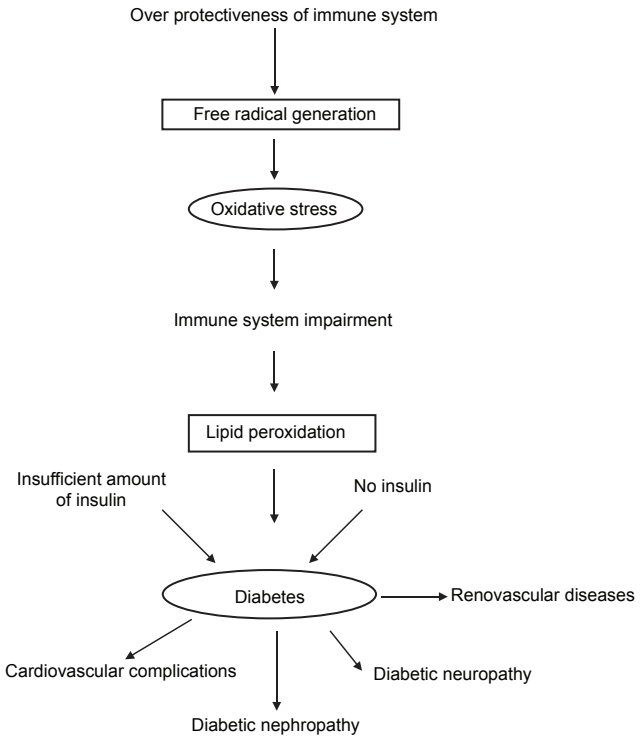

Fig. 2: Relation between oxidative stress and diabetes [38]

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