Review

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Diabetes, Oxidative Stress and Endothelial Dysfunction

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ABSTRACT

Cardiovascular diseases are the most common causes of morbidity and mortality in diabetic patients. Oxidative stress plays an important role in diabetic endothelial dysfunction. Under conditions of oxidative stress, free oxygen radicals may increase insulin resistance and affect pancreatic beta cells. Several experimental animal models to understand the pathogenesis of diabetes mellitus have been developed, and the model including high fat diet which leads to insulin resistance is the best animal model to mimic type 2 diabetes mellitus. In diabetes mellitus, it is well known that there is an increase in lipid peroxidation. This condition is also associated with oxidative stress. Endothelial dysfunction creates imbalance between vasoconstriction and vasodilatation. In large arteries, nitric oxide plays a main role in endothelium-dependent vasodilatation. Abnormal production or response of nitric oxide contributes vascular and endothelial dysfunction in diabetes mellitus. Oxidative stress reduces the levels of nitric oxide and diminishes endothelium-dependent vasodilatation. Therefore, in recent years, the studies to treat the diabetic complications have focused on antioxidant agents. The goal of the treatment is to decrease oxidative stress, as well as lipid and glucose levels. Thus, endothelial dysfunction may be ameliorated and diabetic vascular complications can be avoided.

Keywords: Antioxidant, diabetes, endothelial dysfunction, oxidative stress

Introduction

Diabetes mellitus (DM) is a common chronic metabolic disease that causes disturbances in the metabolism of carbohydrates, fats, proteins and electrolytes. It can also be defined as a syndrome that is associated with chronic hyperglycemia, resulting from lack of insulin secretion from the pancreas, or lack of insulin effect (1).

Factors such as decrease in the release of insulin into the blood, reduction in the use of blood glucose and increase in production of blood glucose may cause glucose levels to remain high in DM. Considering the blood glucose level is high in DM, there is a pathology that concerns all organs and systems, especially the heart and arteries. The main cause of renal failure, adult blindness and non-traumatic lower extremity amputations is DM, according to studies conducted in developed countries (2).

In all types of DM, the main finding is hyperglycemia, but the mechanism that causes hyperglycemia is different. DM can be divided into two types: type 1 and 2 (3). Type 1 DM is characterized by a deficiency in insulin secretion and develops due to viral, toxic or autoimmune damage to the B cells of pancreas. This type is also known as juvenile DM and accounts for about 10% of all diabetic patients, and the likelihood of developing it increases in the second decade of life (4). Type 2 DM is characterized by impaired insulin secretion and peripheral target tissue resistance to insulin, and usually occurs with the loss of beta cell function. A decrease in the number of insulin receptors present in target cells may result in no response to insulin (5,6).

The classification of DM is gradually evolving with the better understanding of etiology and pathogenesis of DM. In addition to the two main types of DM; the World Health Organization has

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started to classify special types of DM including malnutrition-related DM, DM accompanying certain conditions and syndromes, the type along with impaired glucose tolerance and gestational DM (7). The most common type among these special types is gestational DM. This particular type is similar to type 2 DM and is related to insulin resistance. Insulin resistance develops in pregnant women due to pregnancy hormones.

In patients with diabetes, structural, biochemical and functional changes occur in tissues and organs. Acute complications can be life threatening. Long-term vascular pathologies cause loss of function in the organs. In the early stage, control of blood glucose can prevent progression of vascular complications and coronary artery disease and diabetic nephropathy can be prevented (8).

Experimental Models of Diabetes Mellitus

Experimental animal models are the methods used to examine the mechanisms of the formation of diseases, to investigate the prevention and treatment possibilities of diseases. Some of the *in vivo* experiments in drug research are carried out in representative animal models, if possible. Some models are made by changing the animal's diet or by making a lesion with toxic substances in specific target organs. Experimental DM can be made by pancreotectomy or the destruction of pancreatic beta cells. Experimental animals such as mice, rats, rabbits, guinea pigs, hamsters, monkeys, pigs, dogs and cats can be used to create experimental DM (Figures 1 and 2). Experimental DM can be done with chemical agents (9,10), spontaneously (11,12) or through the virus (13). Although there are many experimental animal models defined today, none of these models can be fully equivalent to human DM.

There has been a significant increase in frequency of DM as frequency of obesity has increased throughout the world and type 2 DM constitutes 95% of DM worlwide. Therefore, there is a need to develop new approaches and to find better methods for the treatment of type 2 DM. In this way, the pathogenesis of vascular and neuronal lesions, and the mechanisms of action of therapeutic agents are tried to be clarified to prevent complications and risks associated with type 2 DM. Although there are various animal models on this subject, they generally do not match the course of type 2 DM and its clinical presentation in humans. For this reason, researchers are trying to create new animal models or combined models for type 2 DM. A successful model should include cheap, easy-to-apply and practically testable parameters aimed at the treatment of type 2 DM.

Diet with high fat content [high fat diet (HFD)] is thought to be quite a good way to produce insulin resistance, which is an important feature of type 2 DM. Although various studies on rats has reported that HFD does not cause hyperglisemia or DM, it causes insuline resistance (14-17). Streptozotocin (STZ) is the most widely used agent in animals to form experimental DM. STZ causes death in pancreatic β cells. High-dose STZ severely impairs insulin secretion and creates a pathology similiar to type 1 DM. It is known that low doses of STZ cause a moderate deterioration in insulin secretion, which is similar to the late phase of type 2 DM (18-20).

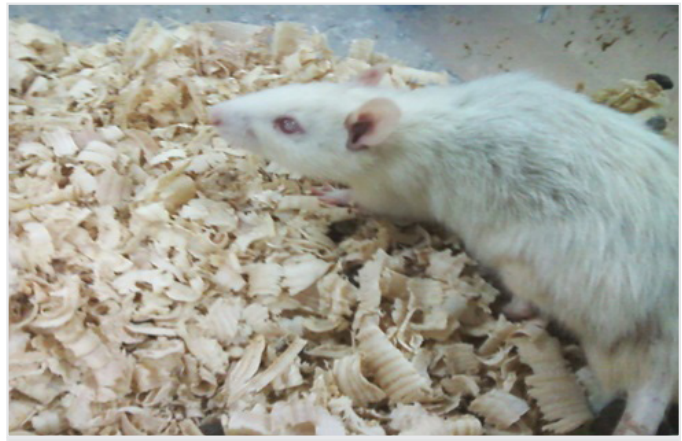

Figure 1. Non-treated diabetic rat appearance, significant weight loss and cataract formation in the eye

Figure 2. Normal rat appearance

A new rat model with low dose STZ administration following HFD, which is in line with the metabolic characteristics and the natural course of type 2 DM has been tried to be established. Studies on DM with this model are quite high in recent years (14,18-21).

In addition to this combination, there are studies that indicate that repeated low-dose STZ administration can be performed. It is reported that repeated low-dose STZ administration, instead of one single high-dose STZ administration, may lead to gradual and autoimmune destruction of beta cells in rats (21-23).

Diabetes Mellitus and Oxidative Stress

Although oxygen is very important and necessary for human life, some exogenous and endogenous reactive oxygen species have the potential to harm the organism (24). Many of them are free radicals and they are reactive oxygen species with high chemical reactivity. Free oxygen radicals (FOR) are formed by adding one or more unpaired electrons to the outer orbit of oxygen. Because these compounds contain unpaired electron in their final orbits, they form compounds that can easily react with other molecules and destroy them and cause very effective damage to the organism (25). They can damage many biological materials,

Table 1. Oxidant sources and antioxidant defence systems

Oxidants	Antioxidant defence
Cigarette smoke	Superoxide dismutase
Environmental pollutants	Catalase
Radiation	Glutathione peroxidase
Carsinogens	Glutathione
Pesticides	Selenium
Exercise	Vitamin E
Febrile diseases	Vitamin C
Ischemia	Ubiquinol
A diet rich in polyunsaturated fatty acids	Uric acide
	β-carotene ve diğer carotenoids

including proteins, lipids, DNA, and nucleotide coenzymes. This damage accelerates aging and also may cause many diseases such as cardiovascular diseases, various types of cancer, cataract, attenuation of immune system and degenerative diseases of the nervous system (26).

Oxygen metabolism in living cells and many factors such as environmental pollutants, radiation and pesticides inevitably lead to the formation of oxygen free radicals. The main ones of these radicals are single oxygen, superoxide anion, hydroxy, peroxy and alkoxy radicals. In response to the damage of reactive oxygen species, different natural defense systems in the body control free radicals (Table 1).

Substances that prevents oxidation events due to free radicals and that have the ability to stabilize free radicals are referred as antioxidants (27). Antioxidants are divided into two types according to the mechanisms: primary and secondary antioxidants. Primary antioxidants are compounds that react with radicals to prevent them from becoming more harmful and forming new free radicals. Enzyme systems such as superoxide dismutase, glutathione peroxidase and catalase are primary antioxidants and are capable of destroying free radicals. In general, these enzymes can prevent the passage of free radicals from one cell to another by limiting the damage to cellular components such as DNA, proteins and lipids (24). Secondary antioxidants are compounds such as vitamin E, vitamin C, bilirubin, uric acid, and polyphenols that capture oxygen radicals and break radical chain reactions.

Oxidative stress is described as an important mechanism in the emergence of diabetic complications (28). The rate at which free radicals are formed and the rate at which they are eliminated are in a balance and this balance is called oxidative stability. In cases where oxidative stability is impaired, the organism is affected by free radicals. A decrease in the rate of elimination or increase in the rate of formation of free radicals can cause this. Oxidative stress reflects an important imbalance between antioxidant defense mechanism and free radical formation, resulting in tissue damage.

Mechanisms that increase oxidative stress in DM include; non-enzymatic glycosylation, autooxidative glycosylation, activity of sorbitol path, metabolic stress resulting from changes in energy metabolism, levels of inflammatory mediators, tissue damage that occurs as a result of changes in antioxidant defense system (29).

In hyperglycemia, free radicals are produced inside the cell. Pancreatic beta cells are one of the most sensitive structures to oxidative stress and the damage to them is thought to be due to a toxic effect of hyperglycemia. The expression rate of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase in pancreatic islet cells is the lowest compared to other tissues (30). It has been reported that the levels of malondialdehyde (MDA) and glutathione pexidase, commonly used as an oxidative damage index, increase in liver, kidney and mitochondria in rats with DM produced by STZ. Diabetic rats have been shown to have increased MDA levels in their kidneys.

Hydrogen peroxide converts into hydroxyl radicals, a product of reactive oxygen species with high reactivity. It is believed that hydroxyl radicals have an important effect on insulin receptor signal pathway and signal transduction (31).

In various cell culture studies, free radical formation was observed when endothelial and smooth muscle cells were incubated in an environment containing high concentration of glucose (30). There is evidence that STZ, which is used to create experimental model of DM, also causes oxidative stress when damaging the pancreas and disrupts the responses of nitric oxide (NO) and leads to DM (31).

It is believed that not only increased level of blood sugar but also increased level of triglycerides in blood is a risk factor for development of diabetic complications (32). There is a consensus that lipid peroxidation is important in this relationship. There is evidence that lipid peroxidation increases in various tissues in DM. Lipid peroxidation may emerge enzymatically via lipooxygenase pathway from prostaglandins or nonenzymatically via effect of free radicals from lipids found in membranes of endothelial and phagocytic cells.

In addition, increase in low-density lipoprotein cholesterol (LDL) oxidation due to hyperglicemia has been shown in patients with DM with vascular complications. In addition, protein oxidation has been shown, especially in myelin, elastin and collagen which may result in diabetic complications such as atherosclerosis, cataract, angiopathy and nephropathy (33).

Diabetes Mellitus and Endothelial Dysfunction

Cardiovascular disease is the most important cause of morbidity and mortality in diabetic patients. These changes in the vessels due to DM are called micro or macrojaniopathy, according to the size of the vessel involved. The involvement of kidney vessels is called renal microangiopathy and it plays an important role in diabetic nephropathy. In the same way retinal microangiopathy causes diabetic retinopathy and microangiopathy of vaso nervorum causes diabetic neuropathy. Macroangiopathy is a

severe form of atherosclerosis in diabetic patients which affects coronary, carotid and peripheral arteries and increases the risk of myocardial infarction and leads to the development of stroke and diabetic foot (34).

The most important factor in the development of microvascular angiopathies in DM is high blood sugar (35). In addition, factors such as obesity, high blood pressure, smoking, hypercholesterolemia and dyslipidemia can cause microangiopathy. In macroangiopathy, other risk factors that may cause microangiopathy come to the fore rather than high blood sugar. These risk factors lead to inflammation and endothelial dysfunction and, as a result, progressive damage to the vessel wall. Studies after the determination of the importance of endothelial dysfunction in micro and macroangiopathy formation have focused on this subject (36).

Endothelium is simply the inner layer of the vessel. Endothelial function is a key factor in regulation of vascular functions. Dysfunction of endothelium due to any damage is called endothelial dysfunction (37). Factors such as hypercholesterolemia, dyslipidemia, smoking and DM are among the risk factors that may lead to endothelial dysfunction. The development of retinopathy, nephropathy and atherosclerosis in diabetic patients is associated with endothelial dysfunction.

HFD can cause hypercholesterolemia and storage of cholesterol on artery walls. Atherosclerosis occurs as a result of the association of fatty degeneration (atherosis) and narrowing of the arteries (sclerosis). Atherosclerosis has been shown to impair endothelial vasodilation in experimental animal models (38) and in humans (39), and may lead to vasoconstriction and vascular spasm. Many studies have shown that the vascular relaxation response is caused by the relaxation factor (EDRF) originating from the endothelium (40). Later, EDRF was reported to be the same substance with NO (41).

Pathophysiology of endothelial dysfunction is complex and it can develop through various mechanisms. The most important of these mechanisms is reduction in the release of NO from endothelium. NO is the most important vasodilator substance released from endothelium. Besides vasodilatation, it inhibits the growth of smooth muscle cells and inflammation and also has antiaggregant effects. Many studies have reported that reduction in NO levels is associated with endothelial dysfunction. This may be due to a decrease in the activity of endothelial NO synthetase (eNOS) or a decrease in the biological activity of NO. It is known that FORs react with NO and cause formation of peroxynitrite and this cytotoxic oxidant disrupts the function of cellular proteins via the nitration of proteins and leads to endothelial dysfunction. Peroxynitrite plays a proatherogenic role leading to oxidation of LDL. It also reduces eNOS activity by interacting with tetrahydrobiopterin, a cofactor of eNOS. Increase in oxidative stress causes more production of FORs by activating the reductase function of eNOS enzyme. FORs also initiate proinflammatory events on the vessel wall. FORs increase adhesion (vascular cell adhesion molecule and intersellular adhesion molecule) and production of chemotactic molecules (macrophage chemotactic peptide) increase. The onset of inflammation decreases the activity of NO. C-reactive protein has been shown to reduce eNOS activity (42).

Oxidative stress reduces NO levels and leads to deterioration of endothelium-dependent vasodilation. In patients with chronic renal failure, oxidative stress markers have been shown to increase with deterioration of endothelial functions. FORs are also thought to induce endothelial damage by causing apoptosis (42).

Another mechanism leading to NO reduction is increase in asymmetric dimethyl arginine (ADMA) level which is endogenous compensatory inhibitory of eNOS enzyme. ADMA is a product that is produced during protein catabolism and is eliminated via kidneys or via metabolizing to citrulline. It has been shown that eNOS inhibition is associated with increased plasma ADMA levels in patients with chronic renal failure. A negative correlation was found between the increase in ADMA levels and endothelium-associated vasodilation in hypercholesterolemic individuals, and the infusion of L-arginine, which is the substrat of eNOS enzyme and competative inhibitor of ADMA, has been shown to improve endothelial functions (42).

Endothelial dysfunction in DM manifests itself with imbalance between vasoconstriction and vasodilation. In large arteries, NO plays a major role in endothelium-dependent relaxation. The abnormal production or response of NO contributes to vascular and endothelial dysfunction seen in DM.

NO is produced as a result of oxidation of guanido nitrogen of L-arginine amino acid by NO synthase (NOS). It is an unstable substance and is reduced to nitrite and nitrate. Synthesis of nicotinamide adenine dinucleotide phosphate, calmodulin, oxygen and as cofactors; hem, flavin, mononucleotide, flavin adenine dinucleotide and tetrahydrobiopterin are required. The NOS enzyme, which is a tool for NO synthesis, has three different isoforms: neuronal, endothelial and immunological. Neuronal and endothelial isoforms are named as structural NOS (43).

Hyperglycemia affects NO formation and function; it also increases the formation of superoxide. It is thought that the imbalance between the production of superoxide and NO leads to endothelial dysfunction in DM (44). The superoxide inactivates NO by converting it into peroxynitrite, thereby reducing NO formation and bioavailability. In the treatment of vascular complications of DM, the goal should be to increase NO formation and bioactivity and reduce the formation of reactive oxygen radicals.

In coronary arteries of diabetic rats, the decrease in acetylcoline-induced vascular relaxation was associated with a decrease in plasma NO levels and expression of eNOS protein (45). A similar result was obtained in thoracic aorta of rats which were made diabetic with STZ (20). Endothelial NOS synthesizes NO in endothelium. Endothelial NO synthesis and vasodilation were significantly impaired in diabetic rats with insulin resistance. Expression of eNOS in diabetic rats was shown to decrease.

Conclusion

Endothelial dysfunction is responsible for the development of vascular complications of DM. The development of endothelial dysfunction is the result of oxidative stress and a pathological process associated with it. Reducing oxidative stress sources and antioxidant treatments can help reduce and prevent serious complications by preventing the development of endothelial dysfunction in DM. It is important to know the pathophysiological process in order to create new treatment alternatives and there is much need for both experimental and epidemiological studies.

Ethics

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