

Role of Superoxide Dismutase 2 Gene Ala16Val Polymorphism and Total Antioxidant Capacity in Diabetes and its Complications

Katayoun Pourvali¹, Mehrnaz Abbasi,¹ and Azadeh Mottaghi^{2*}

1. Department of Basic Sciences and Cellular and Molecular Nutrition, Faculty of Nutrition Sciences and Food Technology and National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Obesity Research Center, Nutrition and Endocrine Research Center, Research Institute of Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Diabetes Mellitus (DM) is a chronic heterogeneous disorder and oxidative stress is a key participant in the development and progression of it and its complications. Antioxidant status can affect vulnerability to oxidative damage, onset and progression of diabetes and diabetes complications. Superoxide dismutase 2 (SOD2) is one of the major antioxidant defense systems against free radicals. SOD2 is encoded by the nuclear SOD2 gene located on the human chromosome 6q25 and the Ala16Val polymorphism has been identified in exon 2 of the human SOD2 gene. Ala16Val (rs4880) is the most commonly studied SOD2 single nucleotide polymorphism (SNP) in SOD2 gene. This SNP changes the amino acid at position 16 from valine (Val) to alanine (Ala), which has been shown to cause a conformational change in the target sequence of manganese superoxide dismutase (MnSOD) and also affects MnSOD activity in mitochondria. Ala16Val SNP and changes in the activity of the SOD2 antioxidant enzyme have been associated with altered progression and risk of different diseases. Association of this SNP with diabetes and some of its complications have been studied in numerous studies. This review evaluated how rs4880, oxidative stress and antioxidant status are associated with diabetes and its complications although some aspects of this line still remain unclear.

Avicenna J Med Biotech 2016; 8(2): 48-56

Keywords: Diabetes complications, Diabetes mellitus, Polymorphism, Superoxide dismutase 2

Introduction

Diabetes Mellitus (DM) is a chronic disorder that affects different people of all ages, race and sex. There are several acute and chronic complications related to this disorder¹⁻³. Cardiovascular diseases, diabetic nephropathy, neuropathy and retinopathy are major and common complications of diabetes. These complications are affecting the vascular system, kidney, retina and peripheral nerves in diabetic patients and will also bring high cost for both individuals and society⁴⁻⁶. Recent evidence introduces oxidative stress as a key participant in development and progression of diabetes and its micro and macro vascular complications^{1,6,7}. Antioxidant defense system works against free radicals^{4,6}. Each antioxidant reduces the action of free radicals by different mechanisms⁸, including enzymes that degrade Reactive Oxygen Species (ROS)^{4,9}. Superoxide dismutase 2 (SOD2) is a key component of antioxidant defense system against mitochondrial superoxide radicals. Mutations or variations in antioxidant genes happen in diabetes and progress in diabetic patients^{1,10}.

The valine-to-alanine substitution in MnSOD Ala-16Val SNP decreases the transport efficiency of the enzyme into the mitochondria and modifies the antioxidant defense against ROS. Production of a β -sheet secondary structure instead of the expected α -helix structure results in a reduced MnSOD activity, which in turn increases oxidative stress¹¹. This process is an important pathophysiological mechanism in development and progression of diabetes and its complication^{1,10,12,13}. Total antioxidant status decreases in diabetic patients⁸. Total Antioxidant Capacity (TAC) modification in plasma during oxidative stress and dietary modulation of plasma redox status can ameliorate oxidative stress and may delay or prevent progression and onset of the disease^{3,6,8,14}.

In agreement with these opinions, some studies showed association between SOD2 gene polymorphisms with diabetes mellitus and some of its complications^{1,10,13,15,16}. In diabetes type 2 patients, diminishing of total antioxidant capacity and depletion of plas-

* Corresponding author:
Azadeh Mottaghi, Ph.D., Obesity
Research Center, Nutrition and
Endocrine Research Center,
Research Institute of Endocrine
Sciences, Shahid Beheshti
University of Medical Sciences,
Tehran, Iran
Tel: +98 21 22432500
E-mail:
amottaghi@sbm.ac.ir
Received: 22 Sept 2015
Accepted: 23 Dec 2015

ma antioxidants could be related to the complications of diabetes¹⁷. Lower plasma TAC and higher serum levels of malondialdehyde (MDA), which indicate increased oxidative stress and compromised antioxidant defenses are reported in type 2 diabetic patients with severe Diabetic Nephropathy (DN) and Chronic Kidney Disease (CKD)^{18,19}. In this article, the importance of antioxidant enzyme gene polymorphisms and total antioxidant capacity in diabetes and the complications were reviewed.

Diabetes mellitus and its complications

DM is recognized as a heterogeneous condition and also a multifactorial syndrome^{1,2}. Chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism due to deficiencies in insulin secretion and/or insulin action are characteristics of diabetes mellitus^{1,8}. DM is certainly a strong contributor to deaths from other causes such as ischemic heart disease, cerebrovascular disease and CKD^{1,2,17}.

Type-1 diabetes mellitus (T1DM) is characterized by a near absolute deficiency of insulin secretion. Classical type-2 diabetes mellitus (T2DM) is attributed to insulin resistance and deficient beta-cell function. It is the major form of diabetes worldwide and accounts for nearly 90-95% of those with diabetes. Some risk factors for T2DM are obesity, physical inactivity, hypertension, certain ethnicities (e.g. Middle Eastern, South Asian and Hispanic) and dyslipidemia. Furthermore, family history of T2DM and several genetic risk markers have been shown to be important in relation to diabetes^{17,20}.

Based on a recent report by the International Diabetes Federation (IDF), cases are increasing everywhere. There are an estimated 387 million adults having diabetes and by 2035 this will rise to 592 million. In addition, IDF reported the prevalence of diabetes in Iran as 8.6% in 2014 in the Iranian population aged 20 to 79 years²¹. From 2005 to 2011, Iranian trend analysis revealed 35.1% increase in DM prevalence²².

DM chronic complications affect different organs and tissues, including eyes, kidneys, heart, blood vessels and peripheral nerves. Diabetic patients are at high risk for micro vascular complications (e.g., nephropathy, retinopathy and neuropathy) and macro vascular complications (e.g., peripheral vascular disease, cerebrovascular disease and cardiovascular disease)^{1,3,20}. Diabetes is the most common cause of CKD, which is a worldwide problem with high and rising prevalence affecting 7.2% of the global adult population. The prevalence of some degree of CKD among adults with type 2 diabetes is 40%. CKD and diabetes are considered as important risk factors for cardiovascular disease and all three conditions are key components of multiple morbidities²³⁻²⁵.

Antioxidant system, oxidative stress and diabetes

Humans have evolved highly complex antioxidant systems (enzymatic and non-enzymatic). Antioxidants

can be produced endogenously or be obtained from exogenous sources e.g., as a part of a diet or dietary supplement. Common antioxidants include the vitamins A, C, E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase^{6,8}. Antioxidants counter the action of free radicals by several mechanisms. These mechanisms include: 1. enzymes that degrade free radicals, 2. proteins such as transferrin that can bind metals which stimulate the production of free radicals, and 3. antioxidants such as vitamins C and E that act as free radical scavengers⁴. Antioxidant defense systems work in synergy with each other to eliminate excess Reactive Oxygen Species (ROS) and maintain balance between oxidation and antioxidation in normal conditions in order to protect cells and organ systems against free radical induced damage^{6,13}.

Oxidative stress is defined as excess formation and/or insufficient removal of highly reactive molecules such as ROS¹. Excessive generation of ROS is a deleterious factor that leads to pathological consequences including damages to proteins, lipids and DNA^{6,8}. Oxidative stress is induced by elevations in glucose and Free Fatty Acid (FFA) levels and has a key role in the pathogenesis of both types of diabetes mellitus. Disruption of antioxidant defense in diabetic subjects (types 1 and 2) as well as increased formation of free radicals reported in many studies lead to oxidative damage of cell components in several tissues, including the kidney, eye, and nervous system^{4,26,27}.

Insulin Resistance (IR), β -cell dysfunction, Impaired Glucose Tolerance (IGT) and ultimately T2DM, all occur by oxidative stress through activating stress pathways (e.g., increased production of Advanced Glycosylated End (AGE) products, sorbitol, cytokines, activation of nuclear factor kappa-light-chain-enhancer of activated cells (NF- κ B), and p38 class of mitogen activated protein kinases (P38MAPK))^{1,8}. Activation of these stress-related signaling pathways by reactive metabolites results in Vascular Smooth Muscle Cell (VSMC) migration and proliferation, a decrease in endothelial production of nitric oxide, insulin action alteration in glucose uptake and insulin clearance or insulin secretion, which ultimately cause insulin resistance²⁸⁻³⁰. On the other hand, oxidative stress results in activation of multiple stress-sensitive kinase signaling cascades such as inhibitor kinase beta (IKK- β) and some isozymes of protein kinase C (PKC). Once activated, these kinases are able to phosphorylate multiple targets, such as the insulin receptor substrate (IRS) proteins (including IRS-1 and IRS-2). Increased phosphorylation of them decreases downstream signaling molecules (e.g., phosphatidylinositol 3-kinase [PI3K]), resulting in reduced insulin action³¹.

Furthermore, many studies demonstrated that increased oxidative stress can cause insulin resistance by inhibition of insulin signals and adipokines deregulation^{1,6,8,20}. In pathology of T1DM, oxidative stress has

also been suggested to play a role as a destructive factor of pancreatic β -cells in the Islets of Langerhans³².

Oxidative stress can also participate in development and progression of diabetes complications^{6,20}. Preventing cardiomyopathy, retinopathy, nephropathy and neuropathy in patients with DM through neutralization of reactive molecules has been demonstrated³³. Mechanisms of oxidative stress in diabetes complications are partly known and they include activation of transcription factors, AGEs products and protein kinase C⁶. Oxidative stress is an important participant in pathogenesis and progression of CKD as well. Oxidative stress is increased in these patients as a result of mitochondrial respiratory system impairment. The increased production of ROS and impaired antioxidant defense mechanisms in patients with CKD can inhibit normal cell function by damaging cells biomolecules. A significant increase in the generation of ROS causes progression of CKD to advanced stages^{34,35}. Increasing generation of ROS has been identified as a potentially major contributor to pathogenesis of diabetic kidney disease. Responsible pathways are still under investigation³⁶⁻³⁸. ROS overproduction and increased oxidative stress can also cause vascular endothelial and smooth muscle dysfunction. This can lead to pathogenesis of diabetic vascular diseases including diabetic neuropathy, retinopathy and nephropathy³⁹⁻⁴¹. There are extensive investigations on evaluation of the ability of antioxidants in management of diabetes and amelioration of its complications. Animal studies have shown that primary antioxidants or genetic manipulation of antioxidant defenses can at least partially ameliorate oxidative stress and consequentially, reduce severity of diabetes complications but data from humans is less clear and more studies are required^{9,26,27}.

Antioxidant capacity

The concept of TAC was introduced to consider the cumulative antioxidant capacity of all the antioxidants present in foods (dietary total antioxidant capacity), plasma/serum and other body fluids^{15,42,43}. Total antioxidant capacity provides an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance *in vivo* between oxidants and antioxidants¹⁴. Coexistence of decreased antioxidant status with diabetic oxidative stress can further increase the deleterious effects of free radicals. Reduction in antioxidant potential of plasma increases complications of diabetes including cardiovascular disease, nerve damage, blindness and nephropathy. Thus, the increasing incidence of diabetes is a significant health concern beyond the disease itself^{5,44}.

Some studies have reported dietary TAC as a good indicator of diet quality and plasma antioxidant status in different populations^{42,45}. Dietary TAC can be calculated by using TAC food databases that have been constructed by using a number of assays for measuring

TAC in commonly consumed foods, including the Oxygen Radical Absorbance Capacity (ORAC), the Total Radical-trapping Antioxidant Parameters (TRAP), and the Ferric-Reducing Antioxidant Power (FRAP) assays. The ORAC, TRAP, and FRAP assays can also be used for analyzing TAC in blood plasma^{42,46,47}.

Total antioxidant capacity and diabetes

Dietary total antioxidant capacity has been shown to be inversely associated with risks of chronic diseases⁴³. Several studies have shown that the total plasma antioxidant status is significantly lower in diabetic patients rather than normal subjects. A lower risk of type 2 diabetes mellitus in individuals with higher levels of serum antioxidants was also reported^{4,8,48}. Decreased aqueous humor TAC level in diabetic patients and its correlation with retinopathy progression was also reported⁴⁹. The blood TAC was significantly depleted in the diabetic group but wasn't correlated with the serum glucose level, HbA1c and the duration of DM. Furthermore, there was no significant correlation between TAC and peripheral nerve conduction parameters⁵⁰. Diminishing of the total antioxidant capacity noted in diabetes type 2 patients, especially inadequately metabolically controlled may constitute the essential pathogenic factor of vascular complication in diabetes. Low TAC level accompanied by high blood glucose in poor metabolically controlled condition can increase and exacerbate oxidative stress in diabetic patients. Activation of stress pathways through oxidative stress and hyperglycemia in these patients and also elevation in radical superoxide under this condition all can cause vascular complication progression by increasing lipid oxidation, accelerating atherogenesis and decreasing Nitric Oxide (NO) bioavailability⁵¹. Presence of Coronary Artery Calcification (CAC) in type 1 diabetes is significantly associated with serum Total Antioxidant Status (TAS) reduction. The reduction in Total Antioxidant Status (TAS) in type 1 diabetic subjects was associated with increasing HbA1c and duration of diabetes in Valabhji *et al*'s study, although it suggested that the effect and contribution of oxidative stress to the higher coronary risk in diabetic patients isn't mediated solely by hyperglycemia⁵². This decrease in TAC of diabetic patients reflects the action of antioxidants against oxidative stress in order to control its related damages⁸. In contrast to most studies in this area, some studies demonstrated that the total plasma antioxidant status was significantly higher in patients with diabetes compared with the control subjects. These studies concluded that plasma TAC increased in order to provide greater protection against free radical aggression. Because the results are controversial and the exact mechanisms underlying this effect are not clear yet, further studies are required⁵³⁻⁵⁵.

Superoxide dismutase (SOD)

Regarding superoxide dismutase [SOD catalyzes the dismutation of the superoxide (O_2^-) radical into either

oxygen molecule (O_2) or hydrogen peroxide (H_2O_2)] it plays important protective roles against cellular and histological damages that are caused by ROS. In comparison with other antioxidant enzymes, SOD has a high turnover (speed of reaction with its substrate) and the reaction is limited only by the frequency of collision between it and O_2^- . Therefore, SOD catalyzes dangerous reaction of O_2^- , protecting cells from the toxicity of this anion. SOD family of antioxidant enzymes includes intracellular (Cu Zn-SOD), mitochondrial (MnSOD), and extracellular (EC-SOD) enzymes also referred to as SOD types 1, 2 and 3, respectively^{1,3,5}. Serious illnesses in mice lacking these enzymes evidence the physiological significance of SODs³. Many studies have shown SOD levels in diabetic tissue and blood decline^{5,56,57}. There is compelling evidence that superoxide excess induced by diabetic hyperglycemia plays a central role in tissue damage and diabetic vascular complications. Alteration of SOD activity in diabetic animals in various tissues, red blood cells and plasma are surveyed in many studies^{6,58-60}. SOD activity in peripheral blood cells is reduced in diabetic patients with DN as compared with those without diabetes complication⁶¹. Death of mice lacking SOD2 due to the strong oxidative stress, cardiomyopathy and lipid accumulation in the liver and skeletal muscles has also been shown³.

SOD2 gene polymorphisms

MnSOD (SOD2) gene is located on chromosome 6q25. It is the only known antioxidant enzyme present within the mitochondria. Considering the relevance of MnSOD as the first line of defense to ROS production, structural and/or functional SNP of the MnSOD encoding gene are of high importance in the maintenance of cellular ROS levels. In humans, at least 190 SNPs have been identified for MnSOD¹¹. Ala16Val (rs4880) is a functional and most studied MnSOD SNP polymorphism in exon 2 of SOD2 gene. A functional polymorphism in exon 2 of SOD2 gene Ala16Val (rs4880) was identified that resulted in structural alterations in the mitochondrial targeting domain, implicating its decreased antioxidant potential to limited post-transcriptional transport¹. This substitution of C to T (GCT to GTT), that is alanine to valine, results in structural alterations in the mitochondrial targeting domain from β -sheet to α -helix, which induces a 30-40% increase in MnSOD activity in mitochondria^{1,11,62}.

SOD2 Ala16Val SNP and antioxidant status

Conflicting interaction between Ala16Val SNP and antioxidant status was first found in Ala homozygous women who had lower antioxidant intake that showed higher risk for breast cancer development¹¹. Shanghai Breast Cancer Study provided some evidence that genetic polymorphism in the MnSOD gene may be associated with increased risk of breast cancer among Chinese women with high levels of oxidative stress or low intake of antioxidants⁶³. Greatest risk of breast cancer

among women who consumed lower amounts of dietary antioxidants rather than high consumers indicates that a diet rich in sources of antioxidants may minimize the deleterious effects of the MnSOD polymorphism⁶⁴. Modulating effects of serum antioxidant nutrient status (beta-carotene, lycopene, zeaxanthin/lutein, retinol, alpha-tocopherol and gamma-tocopherol) on interaction between MnSOD rs4880 polymorphism and cervical carcinogenesis risk was suggested according to findings of Tong *et al*'s study⁶⁵. These conflicting results in investigation of Ala16Val SNP, antioxidant status and cancer may be due to the complex cancer etiology, methodological limitations and differences in study designs, including the nature and duration of intervention, age, sex, health status and lifestyle characteristics of the study populations¹¹. Despite inconsistencies, the overall results suggest that the MnSOD Ala16Val SNP can be modulated by dietary factors. However, future studies are needed to be performed to clarify the nature of this association¹¹.

SOD2 Ala16Val SNP and diabetes

Impact of SNPs in genes encoding for antioxidant enzymes on oxidative stress modulation and preventing subsequent disease development in recent studies has raised a growing interest. Protecting effect of MnSOD against diabetes has been also shown by numerous studies^{12,66,67}. Protective role of "CC" (Ala/Ala) genotype was first reported in T1DM cases and control subjects with A16V polymorphism (rs4880)⁶². Mutations or variations in antioxidantase (Ala16Val polymorphism) can decrease its activity, which in turn increases oxidative stress. This process is an important pathophysiological mechanism in the development and progression of diabetes and its vascular complication. As Ala16Val polymorphism can decrease MnSOD activity, it makes Val carriers less resistance to oxidative stress because of limited antioxidant potential and consequently stressful conditions like hyperglycemia itself and activation of other stress-sensitive signaling pathways by Reactive Metabolites (RMs) resulting in further damages rather than Ala/Ala and Ala/Val carriers. Through such hypothesis that presented in relation to A16V SNP, some studies investigated whether this polymorphism is related to etiology of type 2 diabetes or diabetes complication in a sample of population^{1,13}. The Ala16Val polymorphism of SOD2 might be a risk factor for diabetes among Japanese Americans. Results of this study suggest that an insufficiency of ROS scavenging associated with the lack of Ala allele may lead to glucose intolerance⁶⁸. Another study showed that TT genotype (Val/Val) was most common in both T1DM and T2DM patients; CT genotype was common in healthy subjects whereas the CC genotype (Ala/Ala) was rare in all groups⁶⁹.

SOD2 SNP and diabetes complications

Renal complications: DN is one of the important microvascular complications of DM, which is the lead-

ing cause of CKD^{33,70,71}. Risk of cardiovascular diseases, progression to End-Stage Renal Disease (ESRD) and all-cause mortality increased in these patients^{70,71}. Association of functional impairment of the MnSOD gene with an increased risk of DN was studied and results showed that V allele of the SOD2 rs4880 polymorphism in patients with T1DM increased the risk of DN¹⁰. This association was also studied in Chinese patients with type 2 diabetes which indicated protective effects of Ala allele for the development of DN¹³. The VV type showed a significantly higher frequency in Japanese diabetic patients with nephropathy than AA or VA¹⁶. In agreement with a role for SOD2 in the protection against oxidative stress and kidney disease in type 1 diabetes, the results of Mohammedi *et al*'s study showed that V-allele of rs4880 (V16A) was associated with the incidence and the progression of diabetic nephropathy, with a faster decline in estimated Glomerular Filtration Rate (eGFR)⁷⁰. Results of another study on Korean type 2 diabetic patients suggested that V16A polymorphism of MnSOD gene is not related to pathogenesis of diabetes but is associated with stages of albuminuria. Also, patients with nephropathy, micro and macro groups, had significantly lower A allele frequency than those patients without nephropathy⁷². Homozygosity for the MnSOD Val allele contributed to development of DN in Finnish and Swedish patients with type 1 diabetes⁷³. Results from 8 years of type 1 Danish diabetic patients follow up, showed that VV genotype patients had double risk for DN compared with those with AA genotype⁷⁴. The frequency of the V allele was higher in the Mexican type 2 diabetes patients with macro albuminuria than in the norm albuminuria group (41.6 vs. 32.9%). Data of this study showed that the SOD2 Ala16Val A allele, which codes for the amino acid alanine, confers a protective effect against macro albuminuria¹⁰. It is now clear that susceptibility to DN could be affected by genetic factors⁷⁰. The role of MnSOD genetic variants in protection against diabetes kidney complication is supported by previous studies. These studies are important in order to help us clarify the effect of oxidative stress related genes SNPs on diabetes complication^{10,70}.

Neuropathy: Increased oxidative stress, which is induced by DM, has been implicated in the etiology and development of diabetic neuropathy^{33,75,76}. Oxidative stress can cause damage in neurons via nerve lipid peroxidation, the breakdown of mitochondrial DNA and inhibition of the respiratory chain, and the cross-linking of the neurofilament protein⁷⁵. Rapid changes of glia cells may also occur through oxidative disorders³³. Severe pain, suffering, disability, cardiac death and silent myocardial ischemia are some of the important consequences of diabetic neuropathy⁴⁰. Antioxidant enzymes may protect against the rapid onset and progression of diabetic neuropathy. Chistyakov *et al* showed that the frequencies of the Val allele (49.4 vs. 31.5%) of the MnSOD gene and the Val/Val genotype

(15.9 vs. 2.4%) were significantly more in the DN patients than in the control group⁷⁵. Significantly higher frequencies of the Val allele and the homozygous Val/Val genotype in patients with diabetic neuropathy than diabetics without neuropathy were also shown in Egyptian type 1 diabetic patients. But alanine to valine substitution in MnSOD gene was not a significant factor in Egyptian diabetic patients with nephropathy⁷⁶. According to the research findings, oxidative stress is an important linkage between hyperglycemia and development and progression of diabetic neuropathy. Much more studies are needed in order to investigate the role of genetic factors directly related to oxidative stress in DN formation and progression and to compare their results with each other^{40,75}.

Retinopathy: The other complication of DM is retinopathy³³. The significant role of oxidative stress in the development of diabetic retinopathy has been indicated in some studies^{77,78}. Oxidative stress can cause damage to the vascular endothelium in diabetic retinopathy⁷⁸. High consumption of oxygen, high proportion of polyunsaturated fatty acids, and exposure to visible light are some reasons for high susceptibility of retina to oxidative stress⁷⁷. Study on Slovene population (Caucasians) with type 2 diabetes, provides evidence that the VV genotype of the V16A polymorphism of the MnSOD gene might be a risk factor for diabetic retinopathy⁷⁷. Contribution of the V allele to the development of diabetic retinopathy was shown in Chinese type 2 diabetic patients, too⁷⁹. Study on the results of Korean type 2 diabetic patients suggests that V16A polymorphism of the MnSOD gene is not related to the development of diabetes and progression of Diabetic Retinopathy (DR), but is associated with Diabetic Macular Edema (DME)⁸⁰. Further studies, with larger sample size from different populations are needed to better understand the genes associated with diabetic retinopathy. In this way, the biochemical mechanisms of the disease can be identified that help to develop new tools for identification of patients at risk^{77,78}.

Cardiovascular disease: Cardiovascular disease is one of the major diabetes complications³⁴. Oxidative stress is implicated in the onset and progression of cardiovascular disease by its possible important role in atherogenesis and LDL oxidation^{33,81}. Genotypic distribution or allelic frequency of the MnSOD Ala16Val polymorphisms do not individually contribute to the etiology of diabetic Cardio Vascular Disease (CVD) in Chinese type 2 diabetes patients but may contribute to hypertriglyceridemia⁸¹. A meta-analysis of the Ala16Val polymorphism in SOD2 gene suggested that A allele of it has reduced the risk of diabetes mellitus, including type 1 and type 2 diabetes, and Diabetic Micro vascular Complications (DMI) including diabetic nephropathy, diabetic retinopathy and diabetic polyneuropathy⁸². A cohort study in Denmark concluded that MnSOD V16A polymorphism is the independent predictable

risk factor of CVD. Increased risk of developing CVD in VV and AV genotypes compared with AA genotype was also shown in this study⁷⁴. Because of inconsistency between results of these studies, further study will be required to elucidate the association between MnSOD Ala16Val genotype and CVD in diabetic patients⁸¹.

Conclusion

Oxidative stress, largely caused by excess generation of highly reactive free radicals due to hyperglycemia, not only has a potential role in diabetogenesis and development of diabetic complications but also can exacerbate progression of them. Antioxidant enzymes play crucial role in regulating oxidative status, which is affecting diabetes and its related complications. MnSOD antioxidant enzyme plays a major role against ROS within the mitochondria of aerobic organisms. SNP in gene encoding for MnSOD antioxidant enzyme may directly impact on the oxidative stress modulation and protection against diabetes and its induced abnormalities. MnSOD Ala16Val SNP as the most studied structural SNP within the MnSOD encoding gene has been shown to alter the enzyme localization and mitochondrial transportation, affecting the redox status balance. Patients with mutant MnSOD demonstrate increased susceptibility to oxidative stress and severe mitochondrial dysfunction resulting from elevated ROS production. Insufficient ROS scavenging related to the MnSOD gene genotype may be associated with susceptibility to glucose intolerance. Many studies have investigated the association of genetic variation of rs4880 SNP with diabetes and some of its complications. Significant association between this SNP and diabetes complications like cardiovascular disease, nephropathy, neuropathy and retinopathy were also shown in different studies. Because of inconsistencies between studies, many environmental factors such as dietary and plasma antioxidant capacity as significant modifying factors have been studied in relation to diabetes and some of its complications. Study results have shown that these antioxidant statuses can mediate oxidative stress consequences in patients^{4,83,84}. The interaction of antioxidant status in association with MnSOD A16V SNP and some chronic diseases like cancers have been studied in different population. However, these probable dietary and plasma antioxidant capacity interactions with rs4880 SNP in relation to diabetes and its complications have not been investigated yet. Association of antioxidant status, as an environmental factor, with the Ala16Val genotypes in diabetes and its related diseases still needs to be clarified.

Acknowledgement

We are thankful to the collaborating members in Research Institute of Endocrine Sciences. The authors also thank Ms. N. Shiva for critical editing of English grammar of this manuscript.

Conflict of Interest

All authors have seen and agreed with the contents of the manuscript and there is no conflict of interest to report.

References

1. Banerjee M, Vats P. Reactive metabolites and antioxidant gene polymorphisms in type 2 diabetes mellitus. *Redox Biol* 2013;2C:170-177.
2. Bhutani J, Bhutani S. Worldwide burden of diabetes. *Indian J Endocrinol Metab* 2014;18(6):868-870.
3. Marrazzo G, Barbagallo I, Galvano F, Malaguarnera M, Gazzolo D, Frigiola A, et al. Role of dietary and endogenous antioxidants in diabetes. *Crit Rev Food Sci Nutr* 2014;54(12):1599-1616.
4. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005;59(7):365-373.
5. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. *J Biomark* 2013;2013:378790.
6. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003;17(1):24-38.
7. Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro- and macrovascular complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev* 2011;7(5):313-324.
8. Rani AJ, Mythili SV. Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. *J Clin Diagn Res* 2014;8(3):108-110.
9. Houldsworth A, Hodgkinson A, Shaw S, Millward A, Demaine AG. Polymorphic differences in the SOD-2 gene may affect the pathogenesis of nephropathy in patients with diabetes and diabetic complications. *Gene* 2015;569(1):41-45.
10. Ascencio-Montiel Ide J, Parra EJ, Valladares-Salgado A, Gomez-Zamudio JH, Kumate-Rodriguez J, Escobedo-de-la-Pena J, et al. SOD2 gene Val16Ala polymorphism is associated with macroalbuminuria in Mexican type 2 diabetes patients: a comparative study and meta-analysis. *BMC Med Genet* 2013;14:110.
11. Bresciani G, Cruz IB, de Paz JA, Cuevas MJ, Gonzalez-Gallego J. The MnSOD Ala16Val SNP: relevance to human diseases and interaction with environmental factors. *Free Radic Res* 2013;47(10):781-792.
12. Crawford A, Fassett RG, Coombes JS, Kunde DA, Ahuja KD, Robertson IK, et al. Glutathione peroxidase, superoxide dismutase and catalase genotypes and activities and the progression of chronic kidney disease. *Nephrol Dial Transplant* 2011;26(9):2806-2813.
13. Liu L, Zheng T, Wang N, Wang F, Li M, Jiang J, et al. The manganese superoxide dismutase Val16Ala polymorphism is associated with decreased risk of diabetic nephropathy in Chinese patients with type 2 diabetes. *Mol Cell Biochem* 2009;322(1-2):87-91.

14. Serafini M, Del Rio D. Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool? *Redox Rep* 2004;9(3):145-152.
15. Nomiya T, Tanaka Y, Piao L, Nagasaka K, Sakai K, Ogihara T, et al. The polymorphism of manganese superoxide dismutase is associated with diabetic nephropathy in Japanese type 2 diabetic patients. *J Hum Genet* 2003; 48(3):138-141.
16. Mohammedi K, Maimaitiming S, Emery N, Bellili-Munoz N, Roussel R, Fumeron F, et al. Allelic variations in superoxide dismutase-1 (SOD1) gene are associated with increased risk of diabetic nephropathy in type 1 diabetic subjects. *Mol Genet Metab* 2011;104(4):654-660.
17. Karalliedde J, Gnudi L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. *Nephrol Dial Transplant* 2014 Dec 30.
18. Calderon-Salinas JV, Munoz-Reyes EG, Guerrero-Romero JF, Rodriguez-Moran M, Bracho-Riquelme RL, Carrera-Gracia MA, et al. Eryptosis and oxidative damage in type 2 diabetic mellitus patients with chronic kidney disease. *Mol Cell Biochem* 2011;357(1-2):171-179.
19. Bhatia S, Shukla R, Venkata Madhu S, Kaur Gambhir J, Madhava Prabhu K. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. *Clin Biochem* 2003; 36(7):557-562.
20. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J Diabetes* 2015;6(3):456-480.
21. International Diabetes Federation. *IDF Diabetes Atlas*. 6th ed. Brussels, Belgium: International Diabetes Federation, 2014. 160 p.
22. Esteghamati A, Etemad K, Koohpayehzadeh J, Abbasi M, Meysamie A, Noshad S, et al. Trends in the prevalence of diabetes and impaired fasting glucose in association with obesity in Iran: 2005-2011. *Diabetes Res Clin Pract* 2014;103(2):319-327.
23. Pyram R, Kansara A, Banerji MA, Loney-Hutchinson L. Chronic kidney disease and diabetes. *Maturitas* 2012; 71(2):94-103.
24. Suckling R, Gallagher H. Chronic kidney disease, diabetes mellitus and cardiovascular disease: risks and commonalities. *J Ren Care* 2012;38 Suppl 1:4-11.
25. Detournay B, Simon D, Guillausseau PJ, Joly D, Verges B, Attali C, et al. Chronic kidney disease in type 2 diabetes patients in France: prevalence, influence of glycaemic control and implications for the pharmacological management of diabetes. *Diabetes Metab* 2012;38(2): 102-112.
26. Pazdro R, Burgess JR. The role of vitamin E and oxidative stress in diabetes complications. *Mech Ageing Dev* 2010;131(4):276-286.
27. Golbidi S, Ebadi SA, Laher I. Antioxidants in the treatment of diabetes. *Curr Diabetes Rev* 2011;7(2):106-125.
28. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010;107(9):1058-1070.
29. Wu G, Meininger CJ. Nitric oxide and vascular insulin resistance. *Biofactors* 2009;35(1):21-27.
30. Nowotny K, Jung T, Hohn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules* 2015;5(1):194-222.
31. Banerjee M, Vats P. Reactive metabolites and antioxidant gene polymorphisms in type 2 diabetes mellitus. *Indian J Hum Genet* 2014;20(1):10-19.
32. Delmastro MM, Piganelli JD. Oxidative stress and redox modulation potential in type 1 diabetes. *Clin Dev Immunol* 2011;2011:593863.
33. Zatalia SR, Sanusi H. The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. *Acta Med Indones* 2013;45(2):141-147.
34. Sung CC, Hsu YC, Chen CC, Lin YF, Wu CC. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. *Oxid Med Cell Longev* 2013;2013: 301982.
35. Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. *Iran J Kidney Dis* 2015;9(3): 165-179.
36. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 2008;57(6):1446-1454.
37. Kennedy DJ, Tang WH, Fan Y, Wu Y, Mann S, Pepoy M, et al. Diminished antioxidant activity of high-density lipoprotein-associated proteins in chronic kidney disease. *J Am Heart Assoc* 2013;2(2):e000104.
38. Oberg BP, Mcmenamin E, Lucas FL, McMonagle E, Morrow J, Ikinzler TA, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004;65 (3):1009-1016.
39. Yorek MA. The role of oxidative stress in diabetic vascular and neural disease. *Free Radic Res* 2003;37(5):471-480.
40. Pop-Busui R, Sima A, Stevens M. Diabetic neuropathy and oxidative stress. *Diabetes Metab Res Rev* 2006;22 (4):257-273.
41. Pitocco D, Tesaro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: implications for vascular and other complications. *Int J Mol Sci* 2013;14 (11):21525-21550.
42. Wang Y, Yang M, Lee SG, Davis CG, Koo SI, Chun OK. Dietary total antioxidant capacity is associated with diet and plasma antioxidant status in healthy young adults. *J Acad Nutr Diet* 2012;112(10):1626-1635.
43. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* 2000; 29(11):1106-1114.
44. Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models? *Free Radic Biol Med* 2012;52(1):46-58.
45. Puchau B, Zulet MA, de Echavarri AG, Hermsdorff HH, Martinez JA. Dietary total antioxidant capacity: a novel

- indicator of diet quality in healthy young adults. *J Am Coll Nutr* 2009;28(6):648-656.
46. Wang CC, Chu CY, Chu KO, Choy KW, Khaw KS, Rogers MS, et al. Trolox-equivalent antioxidant capacity assay versus oxygen radical absorbance capacity assay in plasma. *Clin Chem* 2004;50(5):952-954.
 47. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996;239(1):70-76.
 48. Tupe RS, Diwan AG, Mittal VD, Narayanam PS, Mahajan KB. Association of plasma proteins at multiple stages of glycation and antioxidant status with erythrocyte oxidative stress in patients with type 2 diabetes. *Br J Biomed Sci* 2014;71(3):93-99.
 49. Beyazyildiz E, Cankaya AB, Ergan E, Anayol MA, Ozdamar Y, Sezer S, et al. Changes of total antioxidant capacity and total oxidant status of aqueous humor in diabetes patients and correlations with diabetic retinopathy. *Int J Ophthalmol* 2013;6(4):531-536.
 50. Dordevic G, Duric S, Apostolskit S, Dordevic V, Zivkovic M. [Total antioxidant blood capacity in patients with type 2 diabetes mellitus and distal symmetrical polyneuropathy]. *Vojnosanit Pregl* 2008;65(9):663-669. Serbian.
 51. Blaszczak R, Kujawski K, Kedziora-Kornatowska K, Kornatowski T, Kedziora J, Szadujkis-Szadurski L, et al. [The total antioxidant capacity and low-molecular antioxidant concentration in plasma of type-2 diabetes patients with different stage of metabolic compensation and concomitant diabetic nephropathy]. *Pol Merkur Lekarski* 2005;18(103):29-32. Polish.
 52. Valabhji J, McColl AJ, Richmond W, Schachter M, Rubens MB, Elkeles RS. Total antioxidant status and coronary artery calcification in type 1 diabetes. *Diabetes Care* 2001;24(9):1608-1613.
 53. Korkmaz GG, Konukoglu D, Kurtulus EM, Irmak H, Bolayirli M, Uzun H. Total antioxidant status and markers of oxidative stress in subjects with normal or impaired glucose regulation (IFG, IGT) in diabetic patients. *Scand J Clin Lab Invest* 2013;73(8):641-649.
 54. Al-Shebl MM, Mansour MA. Evaluation of oxidative stress and antioxidant status in diabetic and hypertensive women during labor. *Oxid Med Cell Longev* 2012;2012:329743.
 55. Savu O, Ionescu-Tirgoviste C, Atanasiu V, Gaman L, Papacoea R, Stoian I. Increase in total antioxidant capacity of plasma despite high levels of oxidative stress in uncomplicated type 2 diabetes mellitus. *J Int Med Res* 2012;40(2):709-716.
 56. Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? *Metabolism* 1995;44(3):363-368.
 57. He K, Li X, Chen X, Ye X, Huang J, Jin Y, et al. Evaluation of antidiabetic potential of selected traditional Chinese medicines in STZ-induced diabetic mice. *J Ethnopharmacol* 2011;137(3):1135-1142.
 58. Kaul N, Siveski-Iliskovic N, Thomas TP, Hill M, Khaper N, Singal PK. Probuocol improves antioxidant activity and modulates development of diabetic cardiomyopathy. *Nutrition* 1995;11(5 Suppl):551-554.
 59. Kedziora-Kornatowska KZ, Luciak M, Blaszczyk J, Pawlak W. Effect of aminoguanidine on erythrocyte lipid peroxidation and activities of antioxidant enzymes in experimental diabetes. *Clin Chem Lab Med* 1998;36(10):771-775.
 60. Sailaja Devi MM, Suresh Y, Das. Preservation of the antioxidant status in chemically-induced diabetes mellitus by melatonin. *J Pineal Res* 2000;29(2):108-115.
 61. Fujita H, Fujishima H, Chida S, Takahashi K, Qi Z, Kanetsuna Y, et al. Reduction of renal superoxide dismutase in progressive diabetic nephropathy. *J Am Soc Nephrol* 2009;20(6):1303-1313.
 62. Vats P, Sagar N, Singh TP, Banerjee M. Association of Superoxide dismutases (SOD1 and SOD2) and glutathione peroxidase 1 (GPx1) gene polymorphisms with type 2 diabetes mellitus. *Free Radic Res* 2015;49(1):17-24.
 63. Cai Q, Shu XO, Wen W, Cheng JR, Dai Q, Gao YT, et al. Genetic polymorphism in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. *Breast Cancer Res* 2004;6(6):R647-655.
 64. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, et al. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res* 1999;59(3):602-606.
 65. Tong SY, Lee JM, Song ES, Lee KB, Kim MK, Lee JK, et al. Functional polymorphism in manganese superoxide dismutase and antioxidant status: their interactions on the risk of cervical intraepithelial neoplasia and cervical cancer. *Gynecol Oncol* 2009;115(2):272-276.
 66. Kanwar M, Chan PS, Kern TS, Kowluru RA. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci* 2007;48(8):3805-3811.
 67. Goto H, Nishikawa T, Sonoda K, Kondo T, Kukidome D, Fujisawa K, et al. Endothelial MnSOD overexpression prevents retinal VEGF expression in diabetic mice. *Biochem Biophys Res Commun* 2008;366(3):814-820.
 68. Nakanishi S, Yamane K, Ohishi W, Nakashima R, Yoneda M, Nojima H, et al. Manganese superoxide dismutase Ala16Val polymorphism is associated with the development of type 2 diabetes in Japanese-Americans. *Diabetes Res Clin Pract* 2008;81(3):381-385.
 69. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. *BMC Med Genet* 2008;9:30.
 70. Mohammedi K, Bellili-Munoz N, Driss F, Roussel R, Seta N, Fumeron F, et al. Manganese superoxide dismutase (SOD2) polymorphisms, plasma advanced oxidation protein products (AOPP) concentration and risk of kidney complications in subjects with type 1 diabetes. *PLoS One* 2014;9(5):e96916.
 71. Min TZ, Stephens MW, Kumar P, Chudleigh RA. Renal complications of diabetes. *Br Med Bull* 2012;104(1):113-127.
 72. Lee SJ, Choi MG, Kim DS, Kim TW. Manganese superoxide dismutase gene polymorphism (V16A) is associ-

- ated with stages of albuminuria in Korean type 2 diabetic patients. *Metabolism* 2006;55(1):1-7.
73. Mollsten A, Marklund SL, Wessman M, Svensson M, Forsblom C, Parkkonen M, et al. A functional polymorphism in the manganese superoxide dismutase gene and diabetic nephropathy. *Diabetes* 2007;56(1):265-269.
 74. Mollsten A, Jorsal A, Lajer M, Vionnet N, Tarnow L. The V16A polymorphism in SOD2 is associated with increased risk of diabetic nephropathy and cardiovascular disease in type 1 diabetes. *Diabetologia* 2009;52(12):2590-2593.
 75. Chistyakov DA, Savost'anov KV, Zotova EV, Nosikov VV. Polymorphisms in the Mn-SOD and EC-SOD genes and their relationship to diabetic neuropathy in type 1 diabetes mellitus. *BMC Med Genet* 2001;2:4.
 76. el-Masry TM, Zahra MA, el-Tawil MM, Khalifa RA. Manganese superoxide dismutase alanine to valine polymorphism and risk of neuropathy and nephropathy in Egyptian type 1 diabetic patients. *Rev Diabet Stud* 2005;2(2):70-74.
 77. Petrovic MG, Cilensek I, Petrovic D. Manganese superoxide dismutase gene polymorphism (V16A) is associated with diabetic retinopathy in Slovene (Caucasians) type 2 diabetes patients. *Dis Markers* 2008;24(1):59-64.
 78. Kangas-Kontio T, Vavuli S, Kakko S, Penna J, Savolainen E-R, Savolainen M, et al. Polymorphism of the manganese superoxide dismutase gene but not of vascular endothelial growth factor gene is a risk factor for diabetic retinopathy. *Br J Ophthalmol* 2009;93(10):1401-1406.
 79. Ye LX, Yang MP, Qiu H, Guo KQ, Yan JS. [Association of the polymorphism in manganese superoxide dismutase gene with diabetic retinopathy in Chinese type 2 diabetic patients]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2008;25(4):452-454. Chinese.
 80. Lee SJ, Choi MG. Association of manganese superoxide dismutase gene polymorphism (V16A) with diabetic macular edema in Korean type 2 diabetic patients. *Metabolism* 2006;55(12):1681-1688.
 81. Chen H, Yu M, Li M, Zhao R, Zhu Q, Zhou W, et al. Polymorphic variations in manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPX1), and catalase (CAT) contribute to elevated plasma triglyceride levels in Chinese patients with type 2 diabetes or diabetic cardiovascular disease. *Mol Cell Biochem* 2012;363(1-2):85-91.
 82. Tian C, Fang S, Du X, Jia C. Association of the C47T polymorphism in SOD2 with diabetes mellitus and diabetic microvascular complications: a meta-analysis. *Diabetologia* 2011;54(4):803-811.
 83. Opara EC. Oxidative stress, micronutrients, diabetes mellitus and its complications. *J R Soc Promot Health* 2002;122(1):28-34.
 84. Martin-Gallan P, Carrascosa A, Gussinye M, Dominguez C. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic Biol Med* 2003;34(12):1563-1574.