Review Article

Reactive metabolites and antioxidant gene polymorphisms in type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM), by definition is a heterogeneous, multifactorial, polygenic syndrome which results from insulin receptor (IR) dysfunction. It is an outcome of oxidative stress caused by interactions of reactive metabolites (RMs) with lipids, proteins and other molecules of the human body. Production of RMs mainly superoxides ($\bullet O_2^-$) has been found in a variety of predominating cellular enzyme systems including nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, cyclooxygenase, endothelial nitric oxide synthase (eNOS) and myeloperoxidase. The four main RM related molecular mechanisms are: increased polyol pathway flux; increased advanced glycation end-product formation; activation of protein kinase C isoforms and increased hexosamine pathway flux which have been implicated in glucose-mediated vascular damage. Superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and NOS are antioxidant enzymes involved in scavenging RMs in normal individuals. Functional polymorphisms of these antioxidant enzymes have been reported to be involved in the pathogenesis of T2DM. The low levels of antioxidant enzymes or their non-functionality results in excessive RMs which initiates stress related pathways thereby leading to IR and T2DM. An attempt has been made to review the role of RMs and antioxidant enzymes in oxidative stress resulting in T2DM.

Key words: Antioxidants, oxidative stress, polymorphisms, reactive metabolites, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a chronic disorder characterized by impaired metabolism of glucose and lipids due to defect in insulin secretion (beta cell dysfunction) or action (insulin resistance [IR]). The characteristic properties of DM are hyperglycemia, microvascular (e.g., retina, renal glomerulus and peripheral nerve) as well as macrovascular (e.g., atherosclerosis, coronary artery disease [CAD], stroke) pathologies with more than 17.5 million deaths world-wide.[1] Oxidative stress has been implicated as the underlying cause of both macrovascular and microvascular complications associated with type 2 diabetes mellitus (T2DM). It is believed that therapies aimed at reducing oxidative stress would benefit patients with T2DM and also those at risk. The accumulation of glucose and fatty acids within muscles adipose tissue and pancreatic cells combined with a sedentary life-style lead to the generation of excess reactive metabolites (RMs). Oxidative stress and RMs are interrelated terms defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive thiyl species (reactive thiyl and tyrosyl radicals [RTR]). According to International Diabetes Federation Diabetes Atlas 5th Edition-2012 update, 371 million people have been reported with DM and the number is expected to rise to > 552 million by 2030. The 2012 Indian statistics showed 63.0 million diabetic cases and prevalence of 8.37% in the adult population.[2] Moreover, a 4.0% prevalence of T2DM was reported in North Indian population.[3] This short review aims to explain the role of oxidative stress leading to IR, β-cell dysfunction, impaired glucose tolerance and ultimately T2DM and also the association of antioxidant gene polymorphisms.

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Types of RMs [Table 1]

ROS

Oxygen derived free radicals (ODFR) and oxygen derived non-radicals (ODNR) are generated in metabolic pathways of biological systems. ODFR include superoxide ($\bullet O_2^-$), hydroxyl ($\bullet OH$), peroxyl ($\bullet RO_2$), hydroperoxyl ($\bullet HRO_2^-$) while ODNR include hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl). These metabolites are responsible for lipid and protein modifications in case of oxidative stress. Basal oxidative cellular metabolism generates a number of ODFR species through the activation of enzymes that produce superoxide anions and/or byproducts of mitochondrial respiration.[4]

RNS

Like ROS, RNS can be classified into radical and non-radical species, nitrogen derived free radicals (NDFR) and nitrogen derived non-radicals (NDNR). NDFR include nitric oxide ($\bullet NO$), nitrogen dioxide ($\bullet NO_2^-$) while NDNR include alkyl peroxynitrates ($RONOO^-$), nitrous oxide (HNO_2) and peroxynitrite ($ONOO^-$). $\bullet O_2^-$, $\bullet NO$ and $ONOO^-$ are the most widely studied species and play important roles in cardiovascular complications.[4] Nitric oxide ($\bullet NO$) is responsible for the formation of many end-products involved in oxidative stress directly or indirectly after reaction with oxygen. $\bullet NO$-derived RNS react with aromatic amino acids, lipids and thiols resulting in lipid and protein modifications. Leukocyte peroxidases are involved in the formation of $\bullet NO_2$ after utilization of H_2O_2 and NO_2^- as substrates. $\bullet NO_2$, $\bullet OH$ and ONOOH are responsible for damages related to oxidative stress e.g., oxidation, nitrosation and nitration reactions.[4,5]

RTR

Thiyl radicals (TR) may be formed by $\bullet OH$, $ONOO^-$ and/or Fe^{3+} mediated oxidation of thiols. TR may also be derived from sulfur containing moieties including disulfide, thioester or thioether functionalities under conditions of oxidative stress. Once formed, TR not only reacts with themselves and oxygen but also oxidize biological electron donors including ascorbic acid, nicotinamide adenine dinucleotide and ferricytochrome C.

Production of RMs

Production of RMs mainly superoxides ($\bullet O_2^-$) have been found in a variety of predominating cellular enzyme systems including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase (XO), cyclooxygenase (COX), uncoupled endothelial nitric oxide synthase (eNOS) and myeloperoxidase (MPO) [Table 1].[6] The various sources of ROS and action of antioxidant enzymes have been represented in Figure 1. NADPH oxidase uses NADPH as a substrate and is considered as an important source of ROS generation in vascular cells. The lipoxygenase (LPO) and COX generate ROS indirectly by promoting the formation of inflammatory mediators. RM production may result from action of arachidonic acid (AA) metabolizing enzymes including cytochrome P-450, LPO, COX and those in the mitochondrial respiratory chain.[7] AA is cleaved from the membrane by phospholipase A2 and is then metabolized by 5-LPO in the presence of its accessory protein 5-LPO activating protein to form leukotrienes. AA is also metabolized by COX to form members of another family of inflammatory mediators, the prostaglandins. Mitochondria also generate superoxides as electrons are transferred from complexes I to IV during normal cellular respiration. XO, which converts hypoxanthine and xanthine to uric acid, is an additional source of ROS. Finally, eNOS uncouples to generate superoxide in preference to NO.[8]

RMs and T2DM

ROS, $\bullet O_2^-$ leads to several damaging pathways resulting in micro and macrovascular complications in diabetes. There are four main molecular mechanisms implicated in glucose-mediated vascular damage viz., increased polyol pathway flux; increased production of advanced glycation end-product; activation of protein kinase C (PKC) isoforms, sorbitol, cytokines and prostanoids along with increased hexosamine pathway flux [Figure 2]. $\bullet O_2^-$ and H_2O_2 stimulate stress-related signaling mechanisms such as nuclear factor kappa light chain enhancer of activated B cells (NF-κB), p38 mitogen activated protein kinase and Janus

kinase signal transducer and activator of transcription resulting in vascular smooth muscle cell (VSMC) migration and proliferation. H_2O_2 also mediates apoptosis and pathological angiogenesis in endothelial cells.[5] Pathway-selective IR also results in decreased endothelial production of the anti-atherogenic molecule, nitric oxide. Additional stress-sensitive kinases that are reported to be involved in IR substrate (IRS)-mediated IR include several isozymes of PKC such as PKCβ, PKCγ and inhibitor kinase beta NF-κB.[9] Once activated, these kinases are able to phosphorylate multiple targets including IR and IRS proteins such as IRS-1 and IRS-2. Oxidative stress on IRS serine phosphorylation can lead to IR.[10] This reflects decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors such as angiotensin II and endothelin-1

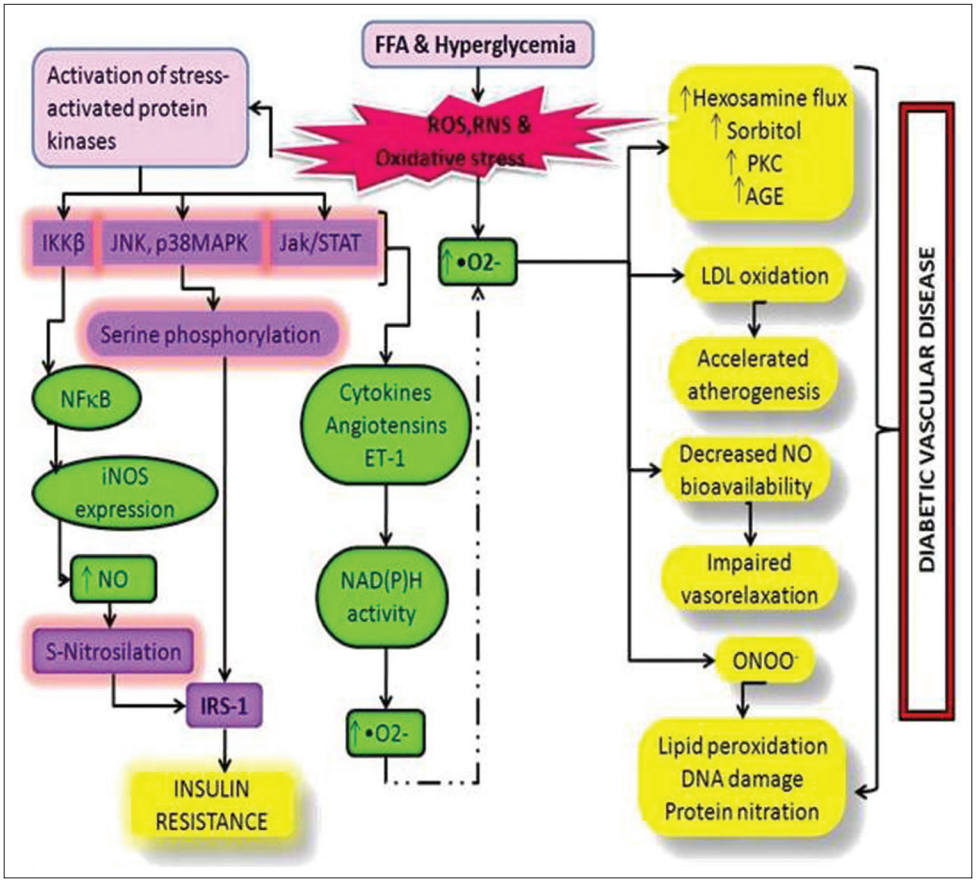

Figure 1: Schematic representation of oxidative stress and the pathways leading to type 2 diabetes mellitus and its complications

Table 1: Enzymatic pathways: Origin, ROS, RNS and their products[48,49]

Origin-location of enzymatic pathway	ROS and RNS potent oxidants	Products-oxidized lipids and proteins
Mitochondrial respiratory chain	$•O_2–$, •OH	Oxidized lipids, proteins, nucleic acids and autoxidation by products
Inflammatory macrophage	$•O_2–$,	ALE products Ortho o-tyrosine, meta m-tyrosine
Membranous NAD (P) H oxidase	•OH, H_2O_2	
Granular MPO	HOCl	3-chlorotyrosine dityrosine
	Tyr $•NO_2–$	$• NO_2$ -Tyrosine (nitrotyrosine)
Macrophage NOS	ONOO–	$•NO_2$ -Tyrosine (nitrotyrosine)
NOS and cNOS	eNOS → •NO	$•NO_2$
	nNOS → •NO	-Tyrosine (nitrotyrosine)
	$•NO+ •O_2→ONOO$	

ROS: Reactive oxygen species, RNS: Reactive nitrogen species, NOS: Nitric oxide synthase, cNOS: Constitutive nitric oxide synthase, eNOS: Endothelial nitric oxide synthase, nNOS: Neural endothelial nitric oxide synthase, MPO: Myeloperoxidase, HOCl: Hypochlorous acid, ALE: Advanced lipoxidation end

and elaboration of permeability factors such as vascular endothelial growth factor. Quantitative and qualitative abnormalities of extracellular (EC) matrix contribute to an irreversible increase in vascular permeability. Microvascular cell loss occurs with time as a result of programmed cell death and progressive capillary occlusion. Both occur due to extracellular matrix and overproduction induced by growth factors such as transforming growth factor-β and deposition of plasma proteins.[8] A causative link among hyperglycemia, mitochondrial ROS generation, oxidative stress and development of complications has been suggested which plays a key role in the pathogenesis of diabetes. Damage due to RMs is associated with complex metabolic and structural changes in the body for example, oxidation of low-density lipoproteins (Ox-LDL) which are taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques.[11,12] ROS-induced peroxidation of membrane lipids alters the structure and fluidity of biological membranes which ultimately affects cellular function.[13]

In T2DM, such activation of stress-sensitive pathways and elevation in glucose and free fatty acid levels lead both to IR and impaired insulin secretion. Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increases vascular permeability. In diabetic arteries, endothelial dysfunction seems to involve both IR specific to the phosphatidylinositol-3-OH kinase pathway and hyperglycemia. Both IR and hyperglycemia have also been implicated in the pathogenesis of diabetic dyslipidemia. Hyperglycemia seems to cause raised levels of atherogenic cholesterol-enriched apolipoprotein B-containing remnant particles by reducing expression of heparan sulphate proteoglycan and perlecan on hepatocytes. Hyperglycemia may also decrease production of trophic factors for endothelial and neuronal cells. Together, these changes lead to edema, ischemia and hypoxia-induced neovascularization in the retina, proteinuria, mesanglial matrix expansion and glomerulosclerosis in the kidney and multifocal axonal degeneration in peripheral nerves. Therefore, as a consequence of its microvascular pathology, diabetes is the leading cause of blindness, end stage renal disease and a variety of debilitating neuropathies. The pathogenesis of atherosclerosis also begins with endothelial dysfunction. Postprandial hyperglycemia may be more predictive of atherosclerosis than fasting plasma glucose level or hemoglobin A1c. Atherosclerotic

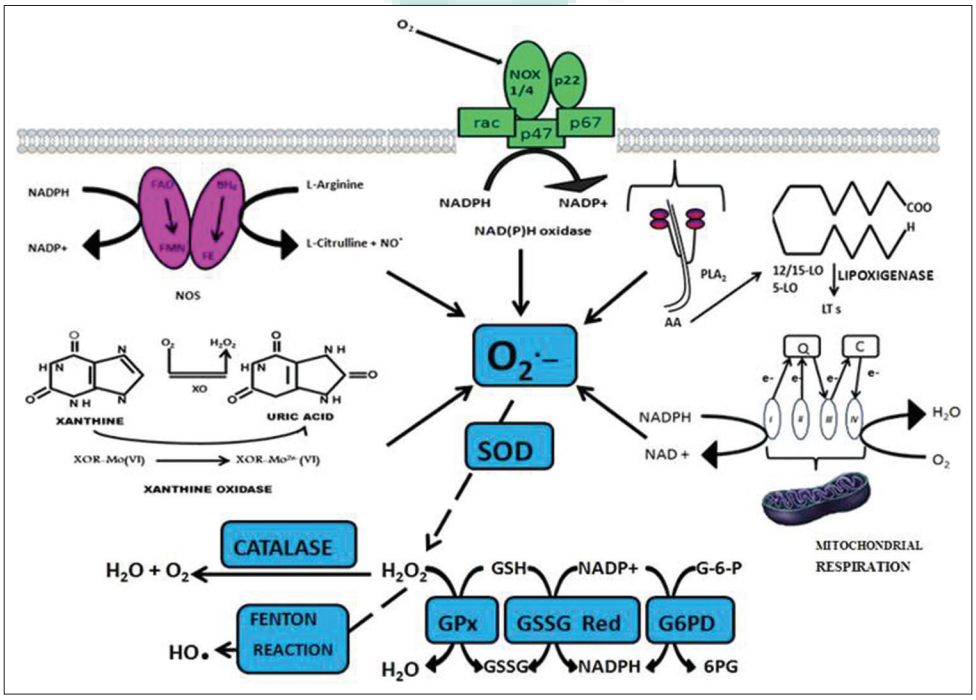

Figure 2: Outline of various sources of reactive oxygen species and action of antioxidant enzymes. Q: Indicates coenzyme Q; C: Cytochrome C; FAD: Flavin adenine dinucleotide; FMN: Flavin mononucleotide; FE: Heme iron; BH4: Tetrahydrobiopterin

macrovascular disease affects arteries that supply the heart, brain and lower extremities. As a result, patients with diabetes have a much higher risk of myocardial infarction, stroke and limb amputation.[14]

In healthy individuals both enzymatic and non-enzymatic antioxidant defense play important roles in scavenging ROS and RNS [Table 2]. Impaired antioxidant defense increases oxidative stress and contributes to the development of T2DM and diabetic cardiovascular disease (CVD). $\bullet O_2^-$ produces H_2O_2 on its dismutation by copper superoxide dismutase (Cu-SOD) and manganese-SOD (Mn-SOD). H_2O_2 produces hydroxyl radical (•OH) by reaction with reduced transition metals (Fe or Cu) i.e. fenton reaction and can be metabolized to HOCl by MPO.[6] H_2O_2 is converted to H_2O and O_2 by glutathione peroxidase (GSH-Px) or catalase (CAT) in the mitochondria and lysosomes respectively. The inner mitochondrial membrane also contains vitamin E which is a powerful antioxidant as it can accept unpaired electrons to produce a stable product.[14]

Antioxidant Gene Polymorphisms [Table 2 and 3]

SOD

As mentioned earlier, once formed the $\bullet O_2^-$ is dismutated enzymatically to H_2O_2 and oxygen by the SOD family of antioxidant enzymes which include intracellular (Cu Zn-SOD), mitochondrial (Mn-SOD) and extracellular (EC-SOD) enzymes also referred to as SOD types 1, 2 and 3 respectively.[8] An increase in Cu Zn-SOD (SOD1) expression projects human smooth muscle cells against oxidative injury. Ox-LDL causes an increase in the DNA binding activity of activator protein-1 and NF-κB which is inhibited by Cu Zn-SOD overexpression. *Cu Zn-SOD* (*SOD1*) gene is located on chromosome 4p15.1-15.3 while *Mn-SOD* (*SOD2*) gene is located on 6q25. A functional polymorphism in exon 2 of *SOD2* gene A16V (C/T) (rs4880) was identified that resulted in structural alterations in the mitochondrial targeting domain, implicating its decreased antioxidant potential to limited post-transcriptional transport. The substitution from valine to alanine was shown to induce a 30-40% increase in Mn-SOD activity in mitochondria.

Individuals harboring this variant had an increased carotid intima-to-media thickness (IMT) and were at increased risk for CAD and acute myocardial infarction.[15] Increased vascular oxidative stress also led to decreased SOD activity through post-translational modification of the enzyme and *Mn-SOD* polymorphism affected Ox-LDL induced apoptosis and CAD.[16] Polymorphic conditions of *SOD1* 35 A/C (rs2234694) and *SOD2* A16V (C/T)

Table 2: Antioxidants in catalytic/enzymatic inactivation of free radicals and non-enzymatic antioxidants[48,49]

Enzymatic antioxidants	Location	Enzymatic action
SOD types EC-SOD (extracellular) Mn-SOD (mitochondrial) Cu-Zn SOD (intracellular)	Mitochondria	$\bullet O_2^- + SOD \rightarrow H_2O_2 + O_2$
Catalase	Peroxisome	$2H_2O_2 + catalase \rightarrow 2H_2O + O_2$
Glutathione peroxidase and interrelated actions Glutamyl-cysteinyl-glycine tripeptide GPx Glutathione reductase	Mitochondria/cytosol	Glutathione reduced-SH to the oxidized disulfide GSSG $GSH + 2H_2O_2 \rightarrow GSSG + H_2O + O_2$ (GSSG→GSH) at the expense of (NADH→NAD+) and/or (NAD (P) H→NAD (P)+)
NOS types eNOS nNOS iNOS	Membrane isoforms	Endothelial •NO is a scavenger of ROS and acts as a chain breaking antioxidant for scavenging ROS Good •NO •NO good in host defense and bad in chronic inflammation, ischemia-ischemia reperfusion injury, acute and chronic as in autoimmunity-T1DM
Non-enzymatic antioxidants	Location	Enzymatic action
Uric acid	Bind copper and iron in forms participate in the fenton reaction which cannot	
Vitamin A, vitamin C, vitamin E		
Thiols		
Apoproteins types: Ceruloplasmin and transferring		

SOD: Super oxide dismutase, Mn-SOD: Manganese-superoxide dismutase, NOS: Nitric oxide synthase, eNOS: Endothelial nitric oxide synthase, nNOS: Neural endothelial nitric oxide synthase, iNOS: Inducible nitric oxide synthase, ROS: Reactive oxygen species, GPx: Glutathione peroxidase, GSH: Glutathione, T1DM: Type 1 diabetes mellitus, NAD (P) H: Nicotinamide adenine dinucleotide phosphate

Table 3: Antioxidant gene polymorphisms in T2DM

Enzyme	Location	Locus	Polymorphisms	Disease risk	Post-translational modification (s)	References
SODs Cu, Zn-SOD (SOD1)	Cytosol, nuclear, and lysosomes	4p15.3-p15.1	None associated with ↑ disease risk	N/A	Tryptophan nitration of Trp32	Flekac *et al.* 2008
Mn-SOD (SOD 2)	Mitochondria	6q25.3	Ala16→Val	↑ carotid intima-media thickness	Tyrosine nitration of Tyr34, Tyr45, Tyr193	Flekac *et al.* 2008
EC-SOD (SOD 3)	Bound to matrix and EC proteoglycans	21q 22.11	None associated with ↑ disease risk	N/A	None reported	Yamashita *et al.* 2007; Liao *et al.* 2008
Catalase	Peroxisomes	11p 13	GA insertion in exon 2 G insertion in exon 2 T → G substitution in intron 7-262C → T substitution in exon 9	↑ diabetes mellitus ↑ homocysteine ↑vascular oxidant stress	Tyrosine nitration Cys377 Chlorination Carbonylation	Ghosh *et al.* 2006; Chen *et al.* 2012
GPx's GPx-1	Intracellular, membrane bound	3p21.3	Pro198 → Leu	↑ carotid intima-media thickness ↑ peripheral arterial disease ↑ coronary artery disease ↑ thoracic aortic aneurysm	None reported	Chen *et al.* 2012
GPx-3	Extracellular	5q23	Plasma7-SNP promoter haplotyope	↑ stroke ↑ cerebral Venous thrombosis	None reported	Voetsch *et al.* 2007
eNOS	Extracellular	7q36	−786 T/C, Glu298Asp (rs1799983), 27 bp VNTR (intron4)	↑ coronary artery disease ↑ hypertension	None reported	Thameem *et al.* 2008
iNOS	Intracellular	17p13.1	Ser608 Leu (rs2297518)	-	No association	Makuc and Petrovic 2012
GST GST M1 GST T1 GST P1	Intracellular, cytosolic	 1p13.3 22q 1.23 11q 13	M1*0 and T1*1 alleles	↑ coronary artery disease ↑ peripheral arterial disease	Tyrosine nitration Carbonylation	Cilenšek *et al.* 2012

SOD: Superoxide dismutase, T2DM: Type 2 diabetes mellitus, Mn-SOD: Manganese-superoxide dismutase, GPx: Glutathione peroxidase, eNOS: Endothelial nitric oxide synthase, VNTR: Variable number of tandem repeats, iNOS: Inducible nitric oxide synthase, GST: Glutathione-S-transferase

showed that serum SOD activity was higher in individuals with CC genotype than TT genotype of *SOD2* gene and higher in AA when compared to CC genotype of *SOD1* gene. Better diabetes control was found in patients with CC genotype of *SOD2* gene. Significantly different allele and genotype frequencies of *SOD2* gene polymorphism were found among T2DM patients with and without macroangiopathy, diabetic retinopathy (DR) in Chinese, diabetic macular edema and albuminuria in Koreans.[17-19]

EC-SOD (SOD3) is bound to matrix and EC proteoglycans. *EC-SOD* (*SOD3*) gene is located on chromosome 21q22.11. Clinical studies have shown a decrease in EC-SOD activity in aged, African-Americans with hypertension, patients with vasospastic angina, thoracic aortic aneurysm and calcified aortic stenosis.[20,21] Serum SOD activity was significantly decreased in T2DM subjects compared to control subjects. The variant R213G in the heparin-binding domain of EC-SOD has been linked to CVD risk. This polymorphism was linked to increased risk of ischemic heart disease (IHD) in a Danish case control study as well.[22]

CAT

CAT is present in peroxisomes and exists as a dumbbell-shaped tetramer of four identical subunits. It rapidly catalyzes the decomposition of H_2O_2 into less reactive oxygen and water molecules. Deficiency of this enzyme was known to lead to T2DM development.[23] Exon 2 and neighboring introns of the *CAT* gene located on chromosome 11p13 were thought to be mutation hot spots for T2DM susceptibility.[24,25] Under conditions of oxidative stress, modification of cystein to cysteic acid leads to tyrosyl nitration of CAT and its decreased activity.[26,27] The – 262C/T promoter polymorphism in *CAT* gene was examined in types 1, 2 and gestational diabetes and complications such as DR, diabetic nephropathy (DN), IHD and CVD.[28,29] The C111T functional polymorphism in exon 9 of *CAT* gene contributed to the CAT activity in different types of T2DM.[29,30]

GPx

GPx are selenocysteine-containing enzymes that catalyze the reduction of H_2O_2, lipid hydroperoxides to H_2O

and lipid alcohols respectively in a reaction that utilizes GSH as a reducing co-substrate.[28] There are 5 known forms of GPx: cellular (GPx-1), gastrointestinal (GPx-2), plasma (GPx-3), phospholipid (GPx-4) and sperm (snGPx). The importance of GPx family of antioxidant enzymes limits the oxidative risk for atherothrombosis. GPx-1 is an ubiquitous antioxidant enzyme whose deficiency has been shown to promote endothelial dysfunction, heart failure and abnormal structural changes in vasculature and myocardium.[31] Interestingly, hyperhomocystinemia appears to enhance vascular oxidative stress and atherothrombosis, in part by suppressing expression of the *GPx-1* gene which is located on chromosome 3p21.3. Erythrocyte GPx-1 activity and association of *GPx-1* genotypes was shown as independent determinants of cardiovascular risk and CAD.[32] A polyalanine sequence polymorphism in exon 1 of *GPx-1* gene produces 3 alleles with 5, 6, or 7 alanine repeats. Men with at least one 6-alanine repeat had significantly increased risk of CAD.

The Pro198 Leu C/T polymorphism in *GPx-1* gene increased carotid IMT, prevalence of cardiovascular and peripheral vascular disease in Japanese patients with T2DM.[28,33] Out of a large number of factors mediating atherosclerotic risk in plasma, focus lays on GPx-3, the essential extracellular peroxidase and its role in modulating oxidative stress. Deficiency of GPx-3 was associated with decreased nitric oxide bioavailability and increased platelet dependent thrombosis. There was a reduction in plasma GPx-3 activity with increased platelet activation and cerebrovascular arterial thrombosis.[34,35] *GPx-3* promoter revealed seven polymorphisms that are tightly linked and form two novel haplotypes, out of which one was associated with hypoxic conditions, arterial thrombotic stroke and cerebral venous thrombosis.[36] Over expression of *GPx-4* reduces oxidized phospholipids, cholesterol hydroperoxides as well as proinflammatory lipid peroxides generated by LPO and COX thereby decreasing vascular oxidative stress and progression of atherosclerosis.[37]

Glutathione-S-transferases (GST)

The GSTs are dimeric cytosolic xenobiotic-metabolizing enzymes that catalyze the conjugation of an active xenobiotic to GSH, an endogenous water-soluble substrate and detoxify reactive electrophiles such as those contained in tobacco smoke. In addition to their catalytic role in detoxification, GSTs were also found to possess selenium-independent peroxidase activity with hydroperoxides, steroid isomerization capacity, binding and transport of bilirubin, heme, bile salts and steroids in a process that is associated with a decrease in enzymatic activity.[38] Several studies have found an association between GST polymorphisms and decreased enzymatic activity and atherosclerosis. Elevated levels of plaque DNA damage as well as levels of inflammatory markers such as C-reactive protein, fibrinogen and adhesion molecules were detected in individuals with *GSTM1**0 null allele.

GST M1, T1 and *P1* have been reported to be involved in T2DM development and various diabetes related complications.[39-43] Microsomal *GST3* encoded by *MGST3* gene, which maps to chromosome 1q23 is a potential susceptibility gene linked to T2DM in Pima Indians, Caucasian and Chinese populations.[44]

NOS

Nitric oxide (NO) plays a fundamental role in the regulation of endothelial function and vascular tone in many organs including kidney. It inhibits platelet aggregation, leukocyte adhesion to vascular endothelium and Ox-LDL.[45] Upon release, NO diffuses rapidly through the cell membrane and relaxes neighboring vascular smooth cells through the production of cyclic guanine 3'5'- monophosphate (cGMP). cGMP then activates the protein kinase G family, leading to a cascade of responses at the levels of transcription and translation. Inducible nitric oxide synthase (NOSI/iNOS), neural NOS (NOS II/nNOS) and NOSIII/eNOS are the three isoforms of NOS. Clinically, eNOS uncoupling has been associated with hypertension, DM, hypercholesterolemia and atherosclerosis.[46] Impairment of NO production causes endothelial dysfunction which contributes to the development of IR, T2DM, chronic renal failure and cardiovascular complications including hypertension and hypercholesterolemia.[47]

eNOS or *NOSIII* gene, mapped to chromosome 7q36 is highly polymorphic and several studies have been undertaken to investigate the potential association of polymorphisms and risk of atherothrombotic vascular disease in Caucasian and Asian populations. SNPs in the promoter region (−786 T/C), G/T substitution at nucleotide 894 in exon 7 leading to an amino acid

change (Glu298Asp) and a 27 bp variable number of tandem repeats in intron 4 have received much attention because of their functional relevance to eNOS activity and association with cardiovascular and renal diseases.[45] The Glu298Asp gene polymorphism is responsible for a decrease in basal NO production and increased frequency of hypertension.[48] Additionally, eNOS Glu298Asp can interact with gene polymorphisms of other endogenous antioxidant enzymes in T2DM patients.[48-51]

In atherosclerosis, VSMC, monocytes, macrophages and dendritic cells all express iNOS. Induction of iNOS may occur following exposure to inflammatory cytokines, including interleukin-1 β, interferon-γ and tumor necrosis factor-α. In contrast to eNOS, iNOS binds Ca^{2+}/calmodulin tightly and does not require an increase in intracellular Ca^{2+} for activation.[52] The presence of iNOS localized to macrophages and VSMC was found to colocalize with oxidized lipid and protein derivatives found in atherosclerotic plaques.[53] However, no association of eNOS Glu298Asp (rs1799983), eNOS 4a/b and *iNOS* Ser608 Leu (rs2297518) polymorphisms was found in T2DM patients with DN.[54]

Conclusions

T2DM and oxidative stress have both clinical and genetic correlation required to be established to a greater extent. The overall play of the RMs has shown to be a leading cause of late onset IR. These RMs are generated inside the human body in a scheduled manner in normal individuals and have a feedback control with antioxidant system. Glycemic load and RMs are highly interrelated leading to various harmful molecular mechanisms implicated in glucose-mediated vascular damage. The various pathways lead to increased expression of other signals which result in IR and T2DM. This short review undertaken to understand the role of RMs in T2DM and IR will provide a lead for future research in identifying genes of oxidative stress control pathways. Furthermore, the proper understanding of antioxidant gene polymorphisms and their association with T2DM may lead to the development of prognostic markers in the near future.

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