Advances in Biochemistry & Applications in Medicine

Chapter 1

Advanced glycation end products (AGEs)-mediated diabetic vascular complications

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1. Introduction

Diabetes mellitus is a group of metabolic disorders leading to defects in insulin secretion and action of insulin or both. Diabetes is caused by a combination of hereditary and environmental factors. In the human body, blood glucose levels are controlled by a complex interaction of multiple chemicals and hormones, including insulin and glucagon. Insulin is a peptide hormone produced in the beta cells of the pancreas that allows blood glucose to enter various cells of the body where it is oxidized to yield energy needed by the muscles and tissues to function [1]. Glucagon is also a peptide hormone, produced by the alpha cells of the pancreas, which causes a rise in the concentration of glucose in the blood. The effect of glucagon is opposite to that of insulin, which lowers the glucose concentration.

There are three main types of diabetes, namely: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM) and gestational diabetes (GDM). T1DM is a chronic autoimmune disorder that occurs in genetically susceptible individuals by environmental factors. In this condition, the body's own immune system attacks the pancreatic β-cells, and destroy or dam-

age these cells to an extent where they are not able to meet the body's insulin requirements. T2DM is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin. GDM is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (especially during their third trimester).

The global prevalence of diabetes, especially T2DM, is increasing at an alarming rate. According to the recent update by the International Diabetes Federation (IDF) more than 382 million adults aged 20-79 years had diabetes in 2013 [2].The prevalence is increasing in every country, and major economic, social and health care impacts will be seen in developing countries, as these countries are home to as much as 80% of people with diabetes. If these trends continue, by 2035, some 592 million people, or one adult in 10 will have diabetes, according to data of IDF (Fig 1) [IDF, sixth edition. 2013]. This equates to approximately three new cases in every 10 seconds or almost 10 million per year. Diabetes caused 5.1 million deaths in 2013 and every six seconds a person dies from diabetes. Diabetes is rampant in Indian subcontinent. India is the 2nd topmost country having the highest number of people with diabetes. In India alone, it is estimated that the total number of people with diabetes in 2013 was around 65.1 million, rising to 109 million by 2035.

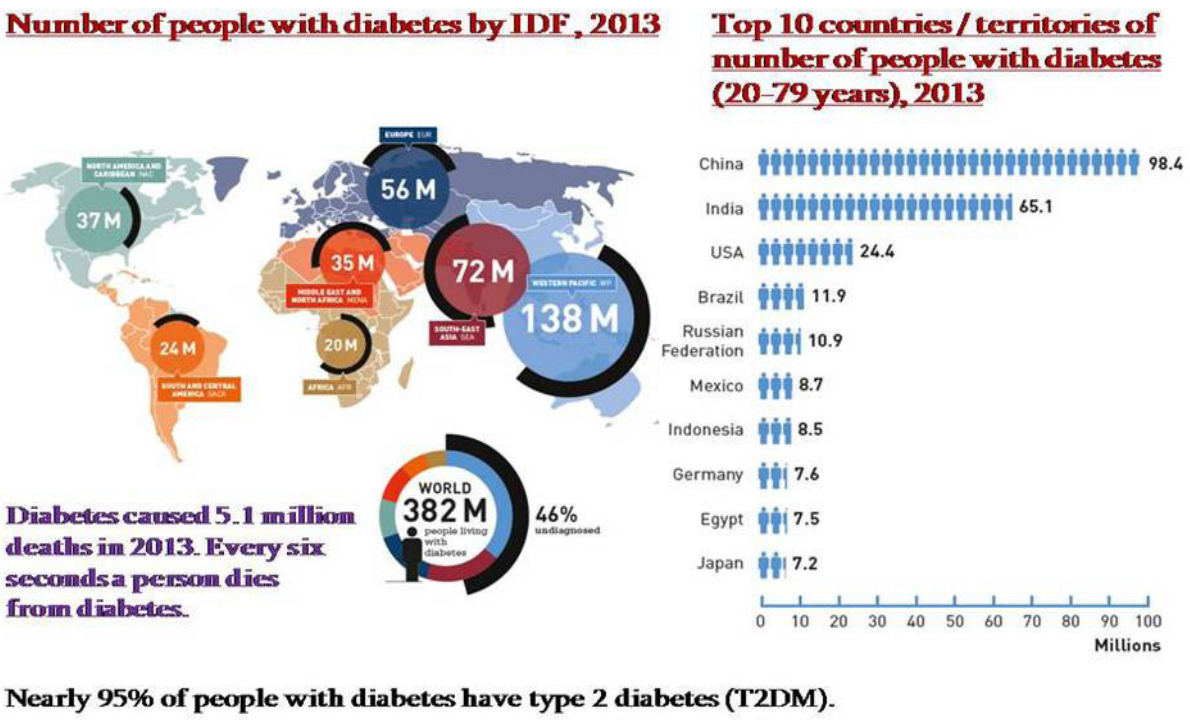


Figure 1: Global prevalence of diabetes mellitus (Adapted from Guariguata et al [2])

1.1. Vascular complications of diabetes

Microvascular and macrovascular complications manifesting as chronic vascular complications of diabetes, which are the major causes of morbidity and mortality (Fig 2). Diabetes especially type 2 diabetes (due to increased prevalence) has become the principal cause

of blindness and end stage renal disease. About 30-45% of all diabetic subjects suffer from microvascular complications. Patients with diabetes are at two to four times increased risk of coronary heart disease, cardiovascular disease and related deaths than those in the general population. Patients with diabetes are at four times higher risk of developing peripheral vascular disease (PVD) [3].

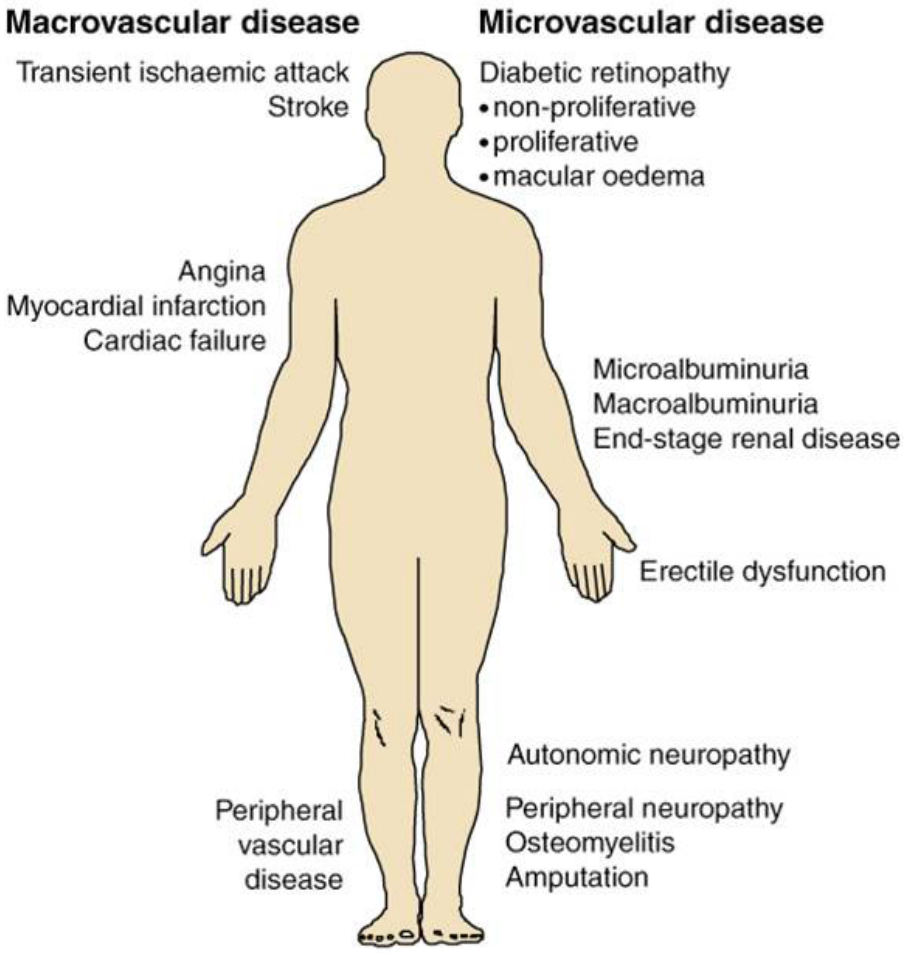


Figure 2: The major diabetic complications (Adapted from Bate et al [3])

The Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study Trial (UKPDS) have clearly demonstrated the vital importance of intensive glycemic control in preventing the progression of diabetic complications. Hyperglycemia induces a variety of metabolic changes, which includes activation of polyol pathway, activation of the diacylglycerol protein kinase, and increased oxidative stress (Fig 3). Hyperglycemia inflicts cumulative long-term structural and functional changes in important macromolecules through advanced glycation end products (AGEs). Endothelial dysfunction in diabetes is most often described in the context of oxidative stress. This chapter summarizes the role of AGE-RAGE-oxidative stress system in diabetic vascular complications and its therapeutic interventions.

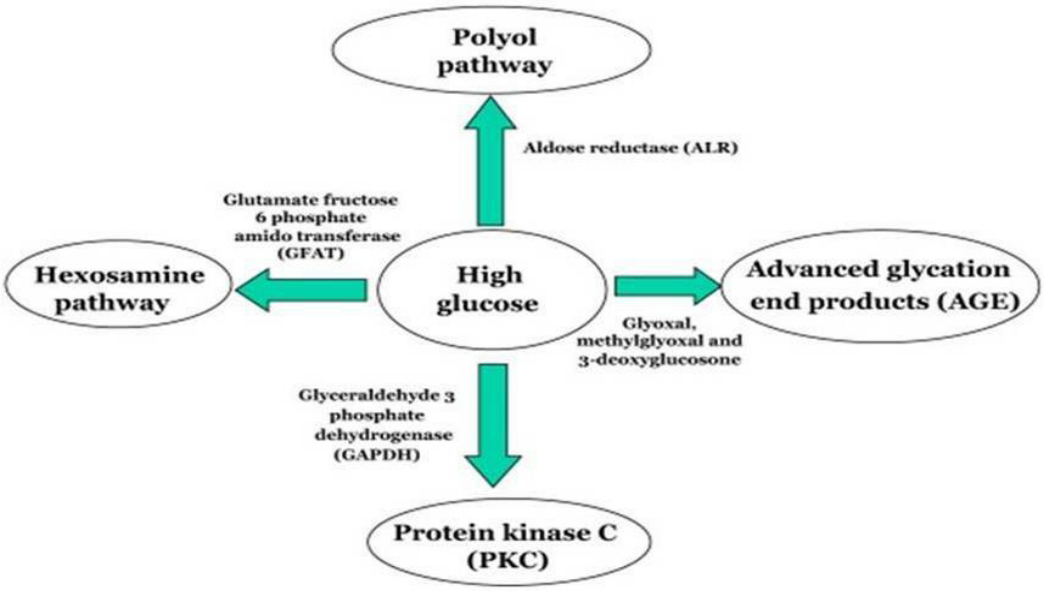


Figure 3: Hyperglycemia and its mechanism (Adapted from Brownlee M [8])

1.2. Hyperglycemia and oxidative stress

Oxidative stress (OS) is an imbalance between the production of free radicals and the body's antioxidant defense system. Free radicals are atoms or molecules that contain one or more unpaired electrons. Oxygen alone or either associated with hydrogen ion or nitrogen, can be converted into very highly reactive molecules, including OH radicals, superoxide, NO, H_2O_2 etc., which rapidly interact with proteins, lipids and carbohydrates [4]. These highly reactive molecules attach to the normal cellular components, changing them into abnormal ones. Thus a critical cell membrane protein can become very rigid instead of being flexible, which impairs cell functions and may lead to cell death giving rise to various complications (Fig 4) [5].

Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and subsequent oxidative degradation of glycated proteins [6]. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. Therefore OS is considered a common endpoint of chronic disease like diabetes [7] and is often characterized by an increase in superoxide and hypochlorous acid. OS can modulate a wide variety of biological processes by coupling signals at the cell surface with changes in gene expression, suggesting the multiple signaling pathways are involved [8]. Indeed, reactive oxygen species (ROS) may be defined as true second messenger molecules that regulate various signal transduction cascades upstream of nuclear transcription factors, including modulation for Ca^{2+} signaling protein kinase and protein phosphate pathways [8].

At physiologic levels, oxidants are important signaling molecules that are involved in processes such as cell growth and regulation of transcription factors like nuclear factor-kappa B (NF-kB). Normally the cell regulates the amount of oxidants very closely. The amount of oxidants in the cell is determined by the balance of production of oxidants versus the destruction of oxidants (called reduction) by antioxidants. Thus, increased oxidant production and/or decreased antioxidant function can lead to increased oxidant stress. Increased production of oxidants in diabetes occurs due to high glucose. Brownlee et al, have shown that high glucose activates superoxides production (a form of oxidant) in the mitochondria [9]. According to their hypothesis, the superoxides produced by the mitochondria lead to inhibition of a particular glycolytic enzyme glyceraldehyde 3 phosphate dehydrogenase (GAPDH) causing a build-up of molecules upstream of this enzyme. These molecules are then shunted to other pathways and can activate PKC β, aldose reductase and other pathways.

Increased hyperglycemia derived electron donors from the TCA cycle (NADH and FADH) generates a high mitochondrial membrane potential by pumping protons across the mitochondrial inner membrane. This inhibits electron transport at complex III, increasing the half life of free radical intermediates of coenzyme Q (ubiquinone), which reduce O_2 to superoxide. Another well described source of increased oxidant production is via activation of an enzyme called reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling these to molecular oxygen to produce superoxide anion, a reactive free radical [10].

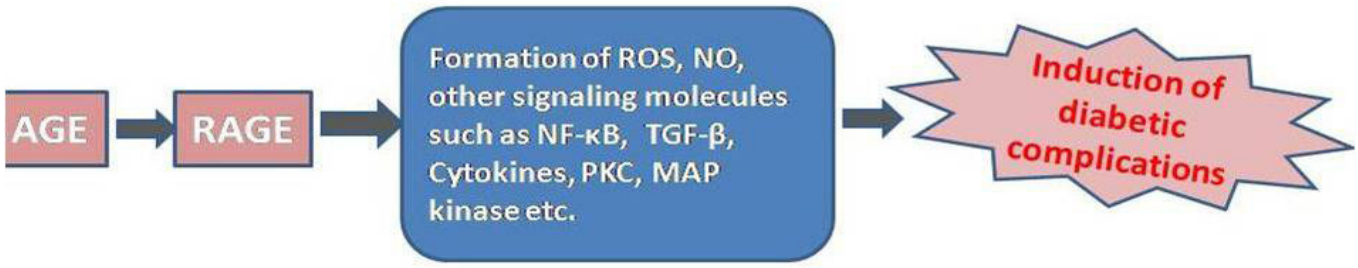


Figure 4: Hyperglycemia and oxidative stress.

The mechanisms described above are the causal factor in the development and progression of diabetic complications by increased OS development. Under hyperglycemic conditions, the factors that stimulate the above mentioned enzyme remained to be defined. AGEs may be one of an important factor which leads to increased NADPH oxidase activity and thereby enhance the OS. NADPH oxidase is a critical component of macrophages and neutrophils and is also found in many other cell types. NADPH oxidases are multi-subunit, membrane associated proteins that catalyze electrons reduction of oxygen using NADPH as an electron donor [11]. Diabetic patients exposed to high level of OS, which play an important role in pathogenesis

of diabetes associated complications. Increasing evidence in both experimental and clinical studies suggested that OS plays an important role in the pathogenesis of diabetes mellitus [12, 13].Oxidative stress has also been strongly implicated in the development and progression of vascular complications [14]. Chronic exposure of biomolecules like lipids, proteins and DNA to higher level of ROS leads to peroxidation and oxidation reaction that results in protein carbonyl (PCO) formation, oxidants of thiol (T-SH) groups, advanced oxidation protein products (AOPP) generation, lipid peroxidation and DNA damage. These OS markers have been shown to be enhanced significantly in diabetic patients [13].

Serum malondialdehyde (MDA) level is a sensitive marker of lipid peroxidation that is a useful measure of OS status. MDA is a decomposition product of peroxidised polyunsaturated fatty acid. Lipid peroxidation was estimated by measuring the level of MDA through thiobarbituric acid reaction [15]. In our previous report, we observed higher serum MDA level in patients with diabetes mellitus compared with healthy controls and significantly more elevated in patients having vascular complications compared with T2DM patients without vascular complications showing enhanced OS in diabetic patients [16]. Cakatay et al, has reported that plasma level of MDA in diabetic patients with poor glycemic control may contribute to the development of diabetic complications [17]. Various studies have also reported high levels of lipid peroxidation in diabetic patients [16-19]. Lipid peroxidation of several structures, a consequence of increased oxygen free radicals, is thought to play an important role in the development of vascular complications in diabetes [20]. MDA is a major player in low density lipoprotein (LDL) modification and is a product of the peroxidation of arachidonic, eicosapentaenoic and docosahexaneoic acids [21]. Oxidised-LDL (ox-LDL) results from the interaction between aldehydes such as MDA and lysine residue in apoB-100 of LDL [22]. The pathologic effects of ox-LDL plays an important role in the induction of diabetic complications.

Another important marker of protein oxidation AOPP has begun to attract the attention of various investigations. Advanced oxidation protein products have been described by Witko-Sarsat et al for the first time [23]. They are formed during OS by the action of chlorinated oxidants, mainly hypochlorous acid and chloramines (produced by yellow peroxidaes in activated neutrophils). AOPP are defined as dityrosine-containing cross-linked protein products and are considered as a reliable marker to estimate the degree of protein oxidation [23]. Protein oxidation may represent an important mechanism in the onset of complications in patients with diabetes. Oxidative modification of proteins that give rise to dityrosine and carbonyl groups generally cause loss of catalytic or structural function in the affected proteins. It is likely the level of oxidized proteins observed during diabetes may have serious deleterious effects in the development of vascular complications. In our previous study, we reported an elevated level of PCO and AOPP in diabetic patients having vascular complications [16]. Various studies also observed elevated levels of these protein oxidation markers in diabetic subjects [24-26].

The mechanism how AGEs induce OS in diabetes is not clearly understood. AGEs can mediate their effect via specific receptors, such as receptors for AGEs (RAGE). AGE-RAGE interaction activates multiple signals such as NADPH oxidase, p21RAS, NF-kB, MAP kinase, TGF-β, vascular adhesion molecules, etc. This transcribes number of pro-inflammatory genes and subsequently elicits vascular inflammation, over expression of endothelial growth factor, impaired fibrinolytic affinity, platelet aggregation, angiogenesis and thrombosis, thereby playing a central role in the pathogenesis of vascular complications in diabetes by enhancing the OS development [27,28].

2. Biochemistry of Formation of Advanced Glycation End Products

Hyperglycemia associated with diabetes mellitus stimulates non-enzymatic reaction between the free amino groups of proteins and carbonyl groups of reducing sugars or other carbonyl compounds leading to enhanced formation of AGEs, also known as the Maillard reaction [29-31]. Advanced glycation end product formation is a complicated molecular process involving multistep reaction. At an early stage, glucose (or other reducing sugars such as fructose, pentose, galactose, mannose, xylulose) reacts with a free amino group of biological amines to form an unstable compound, the Schiff base which undergoes a rearrangement to a more stable product known as Amadori product [32]. In an intermediate stage, the Amadori product degrades to a variety of reactive dicarbonyl compounds such as glyoxal, methylglyoxal, and deoxyglucosones via dehydration, oxidation and other chemical reactions. In the late stage of glycation, non-reversible compounds called AGEs are formed through oxidation, dehydration and cyclization reactions (Fig 5). The AGEs are yellow-brown, fluorescent and insoluble adducts that accumulate on long-lived proteins, thus impair their physiological functions [33]. AGEs-modified proteins lose their specific functions and undergo accelerated degradation to free AGEs such as 2-(2-Furoyl)-4(5)-(2-furanyl)-1H-imidazole (FFI), imidazolone, N-ε-carboxy-methyl-lysine (CML), N-ε-carboxy-ethyl-lysine (CEL), glyoxal-lysine dimmer (GOLD), methyl-glyoxal-lysine dimer (MOLD), and others. In addition, AGEs can also act as cross-linkers between proteins, resulting in the production of proteins-resistant aggregates.

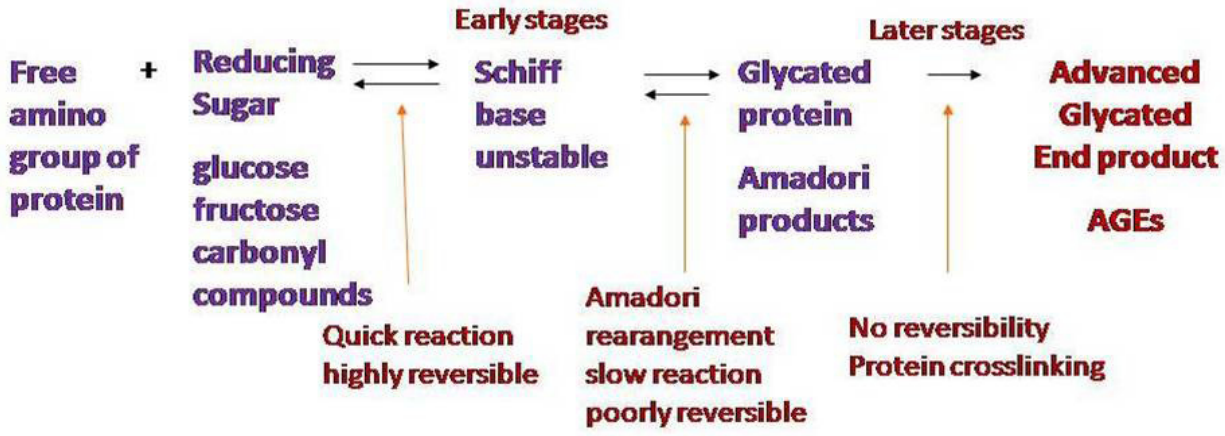


Figure 5: Formation of advanced glycation end products.

In the Maillard reaction, formation of reactive intermediate products during Amadori rearrangement is very important. These compounds are known as α-dicarbonyls or oxoaldehydes such as 3-deoxyglucosone, methylglyoxal (an intermediate product of Maillard reaction) [7]. Furthermore, humans are also exposed to exogenous AGEs, which are ingested with food. Over a dozen AGEs have been detected in tissues and can be divided into three categories: 1. Fluorescent cross-linking AGEs such as pentosidine and crossline. 2. Non-fluorescent cross-linking AGEs such as imidazolium dilysine cross-links, alkyl formyl glycosyl pyrrole (AFGP) cross-links and arginine-lysine imidazole (ALI) cross-links. 3. Non-cross-linking AGEs such as pyrraline and N-carboxymethyllysine (CML) [34].

The other well studied mechanism for the formation of AGEs is the polyol pathway, where glucose is converted into sorbitol by the enzyme aldolase reductase and then to fructose by the action of sorbitol dehydrogenase [35, 36]. Fructose metabolism as fructose 3-phosphate, then is converted into α-oxaldehydes and interacts with monoacids to form AGEs. Thus, at least three pathways are responsible for AGEs formation including Malliard reaction, oxidation of glucose and peroxidation of lipids and finally through polyol pathway. The serum AGEs level was determined spectrofluorometrically at emission maximum (440 nm) upon excitation at 350 nm [26]. Briefly, serum was diluted 1:50 with phosphate buffer saline (PBS) (pH=7.4) and fluorescence intensity was expressed in arbitrary units (AU). Total serum AGEs were also determined by ELISA using commercial kits. Previously, we reported higher levels of circulating AGEs in patients with micro and/or macro-vascular complications indicating that higher the serum AGEs level, higher the likelihood of development of vascular complication of diabetes [16,37,38]. Earlier gradual increase in serum AGEs level have been reported with the severity of atherosclerosis in diabetic patients [39,40]. Kalusova et al determined AGEs spectrofluorometrically and found AGEs were about 23% higher in diabetic patients compared to healthy individuals [26]. In recent studies, AGEs level has been suggested to act as a predictor of CVD mortality and diabetic nephropathy [39-43].

2.1. Metabolism of advanced glycation end products

Once the AGEs are formed, they interact with their receptors namely: AGE-R1, AGE-R2, AGE-R3 and RAGE.

2.1.1. AGE-R1 (oligosaccharyltransferase-48)

AGE-R1 is a cell surface associated receptor that opposes excessive ROS generation by AGEs. AGE-R1 is linked to the endocytosis and removal of AGEs [44] and to the suppression of MAPK and NF-kB activity, via inhibition of AGE-induced ROS generation [45]. Thus, AGE-R1 appears to control the activation of distinct cellular pathways and protects against

vascular disease promoted by oxidants [46]. Interruption of AGE-R1 dependent uptake of AGEs and subsequent degradation is associated with accelerated glomerular renal pathology in the spontaneous non-obese diabetic strains of mice [47]. AGE-R1 may be suppressed or saturated in circumstances of sustained AGE-induced OS when RAGE is up-regulated and AGE-R1 to RAGE ratio is negative. Diminished expression of AGE-R1 in circulating mononuclear cells and a corresponding elevation in serum AGE levels are seen in human subjects with severe diabetic complications [47].

2.1.2. AGE-R2 (80K-H phosphoprotein)

AGE-R2 is an 80 to 90kD protein involved in the intracellular signaling of various receptors like fibroblast growth factor receptor. AGE-R2 contains a tyrosine phosphorylated section in the plasma membrane of the cell [48].

2.1.3. AGE-R3 (galectin-3)

AGE-R3 belongs to lectin family of carbohydrate binding protein. AGE-R3 is up-regulated in hyperglycemia after exposure to AGEs. Galectin-3 knockout mice have developed accelerated glomerulopathy in response to diabetes, with increased renal glomerular AGE accumulation and diminished scavenger receptor expression [49].

2.1.4. Receptor for advanced glycation end products (RAGE)

RAGE is the best characterized and the most studied receptor for AGEs [50]. The main receptor which is responsible for AGEs related diabetic complications is RAGE which appears to activate a stress response leading to inflammation and cellular dysfunction. AGEs bind to specific receptor RAGE which is expressed in many of the cell types which includes endothelium, monocytes/macrophages, T-lymphocytes, neuronal cells and glomerular epithelial (podocyte) cells [51-54]. RAGE is highly expressed at the mRNA and protein levels in early developmental stages under normal physiological conditions [55]. RAGE expression occurs in most tissues, including the heart, liver, brain and kidney [56-58]. Since its isolation in 1992, a growing body of scientific evidence has demonstrated a role for RAGE in the pathogenesis of diabetes and its vascular complications [59].

2.1.5. Molecular structure of RAGE

RAGE is a 45kD transmembrane receptor of immunoglobin super family and is composed of 404 amino acid [50]. RAGE consists of three distinct domains as shown in Fig.6

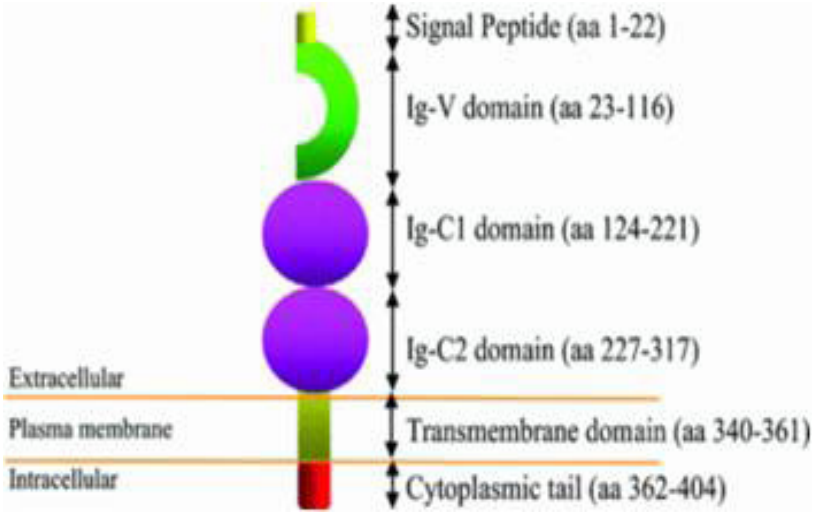


Figure 6: Molecular structure of RAGE (Adapted from kalea et al. [53])

1. Extracellular domain (amino acids 1-339): This extracellular component has three immunoglobulin-like (Ig) domains. The N-terminal Ig domain is assigned to the V-set of Ig-like molecules and is known as the V domain of RAGE. The other two Ig domains are part of the C1 and C2 set. The N-terminal V domain is located far away from the plasma membrane, but the C2 domain lies close to the membrane. V and C1 domains can be joined together to form an elongated structure. V and C1 domains can be fixed and become the VC1 domain, which can connect to the C2 domain by several amino acids that have no secondary structure, which allows the VC1 and C2 domains to link. NMR spectroscopy studies have shown that VC1 moves as a single unit and can combine with the C2 domain.

The V domain consist a large amount of arginine and lysine, which carry positive charge at neutral pH. RAGE V domain has more arginine and lysine than the V-set of Ig domains. Arginine and lysine residues form large positively charged patches on the surface of the V and C1 domains. Meanwhile, C2 domain has mainly acidic residues on its surface and is negatively charged. Because the two domains are oppositely charged, the extracellular component of RAGE is subdivided. This subdivision is reflected in the ligand binding properties of the different domains since ligands do not bind to C2 due to charge repulsion. Most ligands tend to bind to the V domain or the VC1 domain since ligands are negatively charged. There is only one case where a ligand has bound to the C2 domain. Nonetheless, charge-charge interactions are important for the formation of the receptor-ligand complex and suggested that the positively charged ligand-binding domain of the RAGE molecule can recognize certain arrangements of negative charges of ligands and can recognize these as common features for the ligands.

2. Single transmembrane domain (amino acid 340-361): Transmembrane domain is a single hydrophobic helix.

3. Short intracellular cytoplasmic tail (amino acids 362-404): An intracellular domain consists of highly acidic short 42 amino acid cytoplasmic tail, which is essential for RAGE-mediated signaling and overall RAGE function.

Apart from the full length, RAGE also available as soluble circulating isoform including sRAGE1/2/3, esRAGE (endogeneous soluble RAGE) and hRAGEsec (human RAGE secreted). A number of mechanisms have been reported that lead to the production of soluble proteins, alternative splicing of the mRNA to remove the transmembrane domain and the proteolytical cleavage from the cell surface. Various studies of RAGE have shown that sRAGE can be formed by both alternative splicing and proteolytic cleavage [60-62].

3. Role of RAGE Genetic Variants And Its Expression In Diabetes And Its Complications

Type 2 diabetes mellitus is genetically heterogenous disease, caused by interaction between genetic and environmental factors [63]. As described earlier, prevalence of T2DM varies with geographic regions and ethnicity of the population and AGE-RAGE interaction play a significant role. The up-regulation and pathogenic effects of RAGE in diabetic vascular complication as well as multiple genetic variants identified for RAGE, suggests a significant role of RAGE as an important contributing mechanism in diabetes and its complications. The gene encoding RAGE is located on chromosome 6 in major histocompatibility complex, a region of a genome containing number of inflammatory genes [64]. Genetic studies have identified approximately 30 polymorphisms in the RAGE gene at exons, introns and in the 5' flanking region as shown in Fig 7 [65]. Sequence variation in the RAGE gene has been studied and a relatively large number of single nucleotide polymorphisms (SNPs) in the coding and non-coding region of the RAGE gene have been identified recently. The functional impact of several of them on the transcriptional activity, ligand binding or intermediate phenotype has been described.

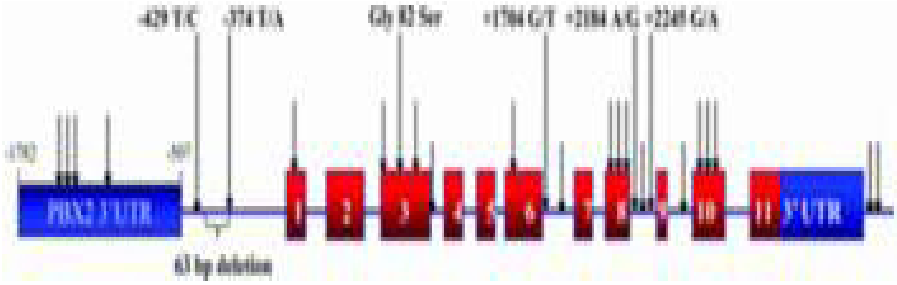


Figure 7: SNP map for RAGE gene. (Adapted from Hudson et al. [65])

3.1. Promoter region polymorphism of RAGE gene

The up-regulation of RAGE gene expression is a hallmark of vascular disease and therefore genetic variation affecting RAGE m-RNA or protein levels may therefore an important disease marker. The RAGE gene is regulated by a promoter region (1.5 KB) up-stream of the transcription start site and within this region, numerous polymorphisms have been identified including -374T/A and -429T/C substitution [65]. Recent studies have revealed that -374 T/A (rs 1800624) and -429T/C (rs 1800625) polymorphisms in the promoter region considered

as an important genetic variation as targets for association studies due to its marked effect on transcriptional activity which may alter AGE-RAGE interaction leading to an altered signaling cascade [66-68].

Falcone et al has reported that conversion of T to A substitution of -374 T/A polymorphism results in repression of receptor expression inefficiency in RAGE cellular signaling. In our previous study, we reported protective nature of -374A mutant allele towards macrovascular complications in diabetic subjects [69, 70]. Similar observations were found in case control studies which revealed a strong link between an individual's possessions of the -374A allele of AA genotype and protection against vascular disease. This includes decreasing risk of cardiocerebral vascular disease [71-74]. A decrease restenosis of the angioplasty [75], decrease in the number of affected vessels [76,77], a decrease mortality after myocardial infarction [78]. Reduce risk of coronary artery disease for -374A allele has reported in African – Brazilian and Caucasian-Brazilian population independent of the risk factor associated with this complication [73,77]. In Caucasian populations Zee et al has reported that patients carry -374A variant has reduced risk of ischemic stroke [72]. However, no association has been reported with diabetic retinopathy [79,80]. A meta analysis report has also reaffirmed the protective nature of -374A allele towards development of macrovascular complications in DM patients [81].

Proximal to the −374T/A polymorphism, −429T/C is another polymorphic variant. Although this variant was shown to increase transcriptional levels in various studies, unlike the −374T/A SNP. In our recent study, we reported -429C mutant allele was associated with the development of macrovascular complications in T2DM subjects in a highly significant manner ($p < 0.001$) [70]. However, in Chinese and Slovene population, no association has been reported [82,83]. In contrast, another study reported increased risk of diabetic retinopathy among patients with the TC or CC genotypes [68]. Not many studies are available on -429T/C promoter polymorphism in Indian population.

The dissimilar association of two promoter polymorphisms i.e. -374T/A and -429T/C reveals an interesting outcome. The protective nature of -374T/A polymorphism towards macrovascular complication, while -429T/C showed significant association towards the development of macrovascular complication in diabetic patients. This opposite phenotypic characteristics of these two promoter region polymorphisms may be attributed to their dissimilarity in association to diabetic complications and also due to strong linkage disequilibrium. -374T/A polymorphism has been shown to cause repression of RAGE gene expression [68]. The lesser the RAGE gene expression, lesser will be its binding with ligand and consequent lesser signal transduction for pro-coagulant or pro-thrombotic gene and thereby imparting protection towards vasculature. On the other hand -429T/C promoter polymorphism has been shown to increase RAGE gene transcript [76]. The enhanced availability of RAGE augments AGE binding and induce downstream signaling resulting in release of proinflammatory cytokines and

adhesion protein that favors thrombosis and eventually capillary leakage and occlusion.

3.2. Coding change polymorphisms in the RAGE gene

The G82S polymorphism was one of important identified naturally occurring polymorphism of the RAGE gene at position 82 proximal to an N-glycosylation site (position 81) is involved in ligand binding and downstream signaling has also attracted considerable interest and therefore strongly suggests that this variant may affect RAGE function [65,84]. This polymorphism is the only frequent coding-change polymorphism in the RAGE gene, with all other polymorphisms identified to change the amino acid sequence occurring in less than 1% of subjects [65]. Most recently, Xie et al. [85] investigated the protein structural changes of this variant and revealed that the Ser82 substitution lead to local structural changes in the V-domain, and also it imparted more widespread overall tertiary structural alterations in the extracellular domain. In our previous study, we observed a significant association of Ser82 mutant allele with microvascular complications in T2DM patients [70]. Similar to our finding, the mutant allele (Ser82) has been shown to be associated with the development of microvascular complications in Chinese, Caucasian and North Indian population [86-89]. A meta analysis report has also reaffirmed the significant association of Gly82Ser polymorphism towards development of diabetic retinopathy among Asian populations [90]. Although, various studies found no association between Gly82Ser polymorphism and diabetic complications in various other populations such as Malaysian, Brazilian and Japanese [91-93]. Mutant allele (Ser82) of G82S RAGE displays enhanced ligand binding and downstream signaling which leads to microvascular complications. How RAGE signaling induces microvascular complications in T2DM subjects has not been fully elucidated. It was reported that NF-κB mediated enhanced expression of pro-inflammatory and pro-fibrotic cytokine TGF-β is responsible for retinal and renal damage, characteristics of microvascular complications [94,95].

The other functional variant identified in the RAGE gene was a 63 bp deletion which spans from −407 to −345 of the promoter (transcriptional) region. Due to its incidence being <1% in populations the use of this variant as a genetic marker for the disease is limited to date [67]. Studies of other RAGE gene promoter variants in the 5' flanking region make difficult due to the overlapping 3′-UTR of the PBX2 gene on chromosome 3 [94]. In essence, this means any variant amplified within this region is in fact amplifying both chromosome 6 and 3 and therefore renders it very difficult to genotype specific variants. The other possible genetic variation in the RAGE gene occurs within the intronic regions, which may affect RAGE mRNA splicing and therefore alter the ratio of splice variant and hence the levels of full-length and sRAGE. Although no variants exist proximal to or within exon/intron boundaries, a number of variants have been identified within introns 7 and 8 [94]. These include the +1704G>T, +2184A>G and +2245G>A which were first identified in a Czech population and demonstrat-

ed to be associated with diabetic microvascular dermatitis [94]. Subsequent studies by these researchers demonstrated an association of the +1704G>T and +2184A>G with antioxidant status in Type 2 diabetes, but not with proliferative retinopathy [89]. However, limited studies of only the +1704G>T have been performed in other populations [89,93]. Further studies in large numbers of subjects are required to thoroughly investigate these variants.

4. Therapeutic Intervention of AGEs

Inhibition of AGEs formation and attenuating the AGEs-mediated effects may be considered as ideal candidates for pharmaceutical intervention in the amelioration of diabetic vascular complications. Therapies against the AGEs mediated effect can through diverse pathways, like inhibiting the production of Amadori products, decreasing AGE-RAGE interaction, detoxifying dicarbonyl intermediates and interrupting biochemical pathways that impact on AGEs level. Since OS plays an important role in the development of vascular complications, antioxidants shows beneficial effects on AGE- mediated vascular complications through suppression of intracellular ROS generation.

4.1. Aminoguanidine

Aminoguanidine (Pimagedine,) is a pharmacological inhibitor of the advanced glcosylation pathway and it also reacts with many biological molecules, including pyridoxal phosphate, pyruvate, glucose, MDA, and others [30, 96]. Aminoguanidine is a therapeutic agent for prevention for AGEs formation. It reacts rapidly with alpha, beta-dicarbonyl compounds such as methylglyoxal, glyoxal and 3-deoxyglucosone to prevent the formation of AGEs [97]. Inhibition of disease mechanisms, particularly vascular complications in diabetes, by aminoguanidine has provided evidence that accumulation of AGEs is a risk factor for disease progression [30]. Aminoguanidine has other pharmacological activities, inhibition of nitric oxide synthase and semicarbazide-sensitive amine oxidase, at pharmacological concentration achieved in vivo for which controls are required in anti-glycation studies [98]. Use of the high concentration of aminoguanidine in-vitro brings these reactions and related effects into play. Aminoguanidine prevents the formation of fluorescent advanced non-enzymatic glycosylation products and of glucose derived collagen cross links in-vitro. It appears that the primary mechanism by which aminoguanidine inhibits the formation of advanced glycosylation end products is by reacting with Amadori derived fragmentation products such as 3-deoxyglucosone [99].

4.2. N-acetylcysteine

N-acetyl-L-cysteine (NAC), a thiol containing acetylated form of the amino acid L-cysteine that functions as a precursor of glutathione synthesis [100]. Glutathione (GSH) is an

important thiol involved in cellular detoxification. GSH plays a physiological role in maintaining the body homeostasis and in protecting cells against oxidants, toxicants, DNA damaging agents, and carcinogens of either exogenous or endogenous source [101]. The presence of sulfhydryl groups in NAC also enables the neutralization of free radicals. It is a powerful scavenger of HOCl and is capable of reducing HO and H_2O_2 and act as a powerful antioxidant [102]. NAC occurs in two forms L and D, only L-NAC is active; L-NAC is metabolized to cysteine and then GSH, but D-NAC is not. NAC acts outside the cell to reduce cystine to cysteine, which can be transported into the cell 10 times faster than cystine and further used in the biosynthesis of GSH. By facilitating GSH biosynthesis, NAC serves an indirect anti-oxidant role where it can enhance glutathione-S-transferase activity; supply GSH for glutathione peroxidase catalyzed detoxification of peroxides [103]. This compound is sold as a dietary supplement commonly claiming anti-oxidant and liver protecting effects. It is used as a cough medicine because it breaks disulfide bonds in mucus and liquefies it, making it easier to cough up. It is useful in thinning the abnormally thick mucus in cystic and pulmonary fibrosis patients [104].

NAC has found to protect the beta cells in culture and in vivo from “glucose toxicity” preserving insulin synthesis and secretion [105]. Gibson et al suggested that NAC reduce thrombotic propensity in T2DM patients by increasing platelet anti-oxidant status and GSH synthesis, thereby lowering platelet derived ROS [106]. NAC helps to increase nitroglycerin activity, thereby, potentiates the coronary dilating and anti-platelet effects of nitroglycerin and limits the development of hemodynamic tolerance to nitroglycerin. AGE-RAGE mediated ROS generation induces mesangial cell hypertrophy, and fibronectin synthesis has been reported to be inhibited by NAC [107].

4.3. Resveratrol

Resveratrol (3,5,4 trihydroxy-trans-stillbene) is a stilbenoid, a type of natural phenol, and phytoalexin produce naturally by several plants. Resveratrol is a member of a group of plant compounds called polyphenols. These compounds are thought to have anti-oxidant properties, protecting the body against the kind of damage linked to increased risk for conditions such as cancer and heart disease. Beneficial effects of resveratrol including telomere lengthening, telomerase activity enhancement, anti-inflammatory and blood sugar lowering have been reported in mouse and rat model. Reseveratrol gets extensively metabolized in the body. Liver and gut is the major site of metabolism. [108]

Resveratrol has been shown to inhibit platelet activation and aggregation, suppress TNF-induced activation of nuclear transcription factors [109], inhibit the generation of reactive oxygen species by human polymorphonuclear leukocytes [110]. Resveratrol has also been reported to reduce the risk of cardiovascular and tumoral disease by acting on the mechanisms

that regulate the expression of growth factors and cytokines such as transcription factor NF-kB [111]. Resveratrol has been reported to afford cardiovascular protection and to reduce atherosclerosis by various mechanisms. These include modulation of lipid turnover, production of eicosanoids, oxidation of lipoproteins and reduction of platelet adhesion [112]. Thus, resveratrol may be considered as an effective therapeutic agent for the treatment of diabetes mellitus and its associated complications.

4.4. Curcumin

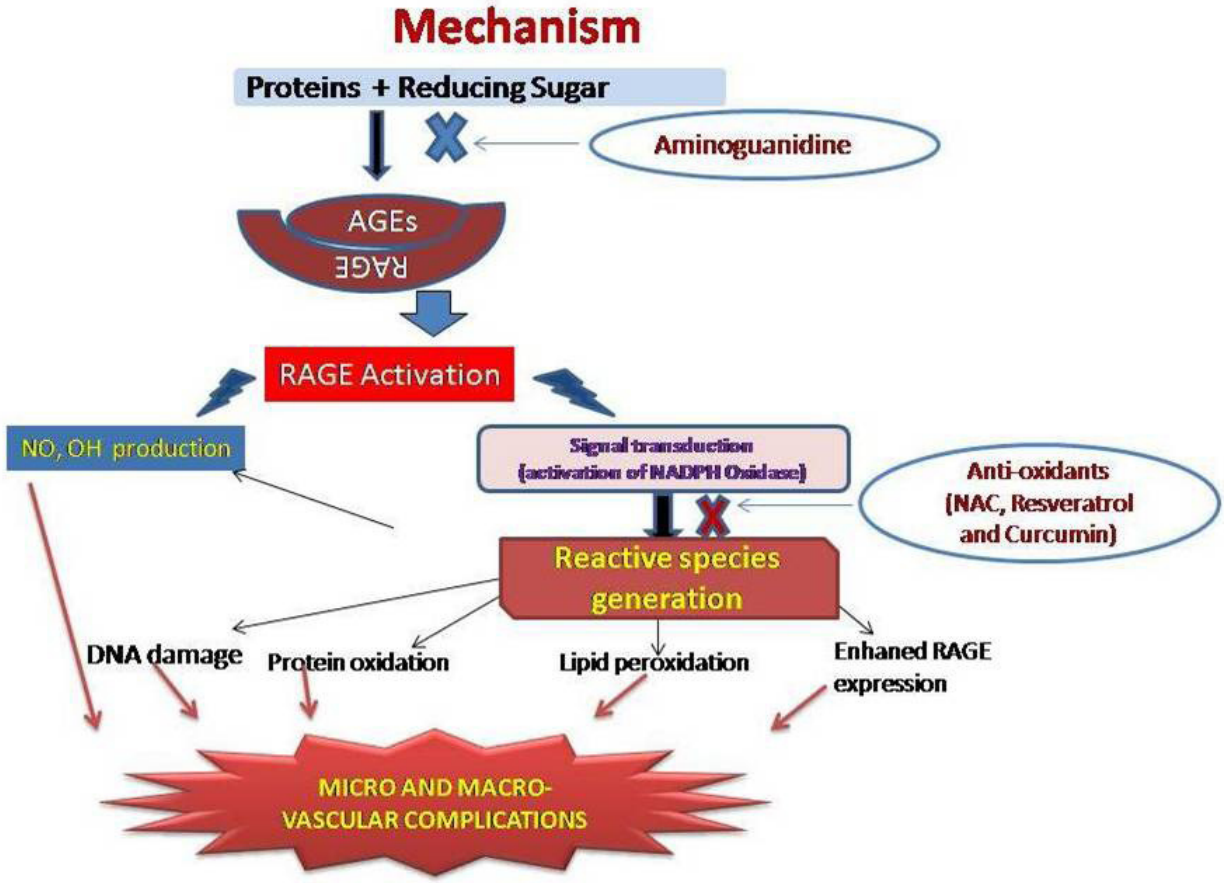
Curcumin is the principal curcuminoid extracted from the rhizomes of the plant Curcuma longa (popular Indian spice turmeric), a member of ginger family found in south and southeast tropical Asia [113]. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and Enol. The Enol form is more energetically stable in the solid phase and in solution. Curcumin incorporates several functional groups having molecular formula $C_{21}H_{20}O_6$. Potential factors that limit the bioavailability of curcumin include poor absorption, rapid metabolism and rapid systemic elimination. Numerous approaches to increasing curcumin bioavailability have been explored, including the use of adjuvants [114].

Curcumin has shown diverse and versatile beneficial effects, including anti-inflammatory, anti-oxidative, anti-viral, anti-hypercholestermic, anti-infective and anti-carcinogenic effects [115]. This polyphenol has been shown to reduce risk of cancer, heart disease, Alzheimer's disease, and diabetes mellitus [116]. Curcumin supplementation improved diabetes-induced endothelial dysfunction through lowering plasma glucose levels, decreasing superoxide production and inhibiting protein kinase C activation [117]. The preventive effect of curcumin on the level of AGEs and cross-linking of collagen in diabetic rats has been reported with respect to prevention of AGE-induced complications in diabetes mellitus [118]. Xu et al. has reported that curcumin inhibited the proliferation of activated hepatic stellate cells and this has been mediated by curcumin induction of gene expression of PPARg and PPARg activation [119]. Okamoto et al has also been reported the preventive effects of curcumin on AGE-induced increase in NF-kB and AP-1 activity, vascular endothelial growth factor m-RNA up-regulation and the resultant increase in DNA synthesis in microvascular endothelial cells [120].

5. Summary

This chapter summarizes that AGEs formation appears to be enhanced under hyperglycemic condition. Increased glycation of plasma protein and its accumulation plays an important role in the pathogenesis of diabetic vascular complications. Up-regulation and pathogenic effect of AGEs receptor, i.e. RAGE, expression and its genetic variant plays an important role in the pathogenesis of diabetes and its vascular complications, the molecular mechanism of

activation of RAGE needs to be investigated. The possibility of reducing glycation of protein or circulating AGEs or blocking RAGE is an approachable target of delaying or preventing the onset of diabetic complications. Various compounds are under investigation for their possible therapeutic intervention. Finally, the use of AGEs as biomarkers/predictors of diabetic complications may be helpful to reduce health problems in diabetic patients.



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7. References

1. Harris M, Zimmet P. Classification of diabetes mellitus and other categories of glucose intolerance. Alberti K, Zimmet P, Defronzo R, editors. International Textbook of Diabetes Mellitus. Second Edition. Chichester: John Wiley and Sons Ltd; 1997: p9-23.

2. Guariguata L, Whiting DR, Beagley J, Linnenkamp U, Hambleton I, Cho NH, et al. Global estimates of diabetes prevalence in adults for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014; 103: 137-49.

3. Bate KL, Jerums G. 3: Preventing complications of diabetes. Med J Aust. 2003; 179:498-503.

4. Pitocco D, Zaccardi F, Stasio ED, Romitelli F, Santini SA, Zuppi C, et al. Oxidative Stress, Nitric Oxide, and Diabetes. Rev Diabet Stud. 2010; 7: 15-25D.

5. Wu J, Jin Z, Yan LJ. Redox imbalance and mitochondrial abnormalities in the diabetic lung. Redox Biol. 2016 ;11:51-59.

6. Chang YC, Chuang LM. The role of oxidative stress in the pathogenesis of type 2 diabetes: from molecular mechanism to clinical implication. Am J Transl Res. 2010; 23: 316-31.

7. Bayne JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1-9.

8. Zhang C. Effects of interventions on oxidative stress and inflammation of cardiovascular diseases. World J Cardiol 2011; 3: 18-24.

9. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813-20.
10. Lee H, Yu MR, Yang Y, Jiang Z, Hunjoo H. Reactive oxygen species-regulated signalling pathways in diabetic nephropathy. J Am Soc Nephrol. 2003; 14: S221-S6.

11. Arora S, Vaishya R, Dabla PK, Singh B. NAD(P)H oxidases in coronary artery disease. Adv Clin Chem. 2010;50:65-86.

12. Vichova T, Motovska Z. Oxidative stress: Predictive marker for coronary artery disease. Exp Clin Cardiol. 2013; 18:e88-91.

13. Piwowar A. [Advanced oxidation protein products. Part II. The significance of oxidation protein products in the pathomechanism of diabetes and its complications]. Pol Merkur Lekarski. 2010 ;28:227-30.

14. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Cirulation J. 2009; 73: 411-8.

15. Girotti MJ, Khan N, Mclellan BA. Early measurement of systemic lipid peroxidation products in the plasma of major blunt trauma patients. Journal of Trauma-Injury Infection & Critical Care. 1991; 31: 32-5.

16. Chawla D, Bansal S, Banerjee BD, Madhu SV, Kalra OP, Tripathi AK. Role of advanced glycation end product (AGE)-induced receptor (RAGE) expression in diabetic vascular complications. Microvasc Res. 2014; 95:1-6.

17. Cakatay U. Protein oxidation parameters in type 2 diabetic patients with good and poor glycemic control. Diabetes Metab 2005; 31:551-7.

18. Nakhjavani M, Esteghamati A, Nowroozi S, Asgarani F, Rashidi A, Khalilzadeh O. Type 2 diabetes mellitus duration: an independent predictor of serum malondialdehyde levels Singapore Med J. 2010; 51: 582-5.

19. Pasaoglu H, Sancak B, Burkan N. Lipid peroxidation and resistance to oxidation in patients with type 2 diabetes mellitus. Tohuku J Exp Med. 2004; 203: 211-8.

20. Likidlilid A, Patchanans N, Peerapatdit T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activities in erythrocytes of type 2 diabetic patients. J Med Assoc Thai. 2010; 93:682-93.

21. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. Arterioscler Thromb. 1994; 14: 605-16.

22. Ganjifrockwala F, Joseph J, George G. Serum Oxidized LDL Levels in Type 2 Diabetic Patients with Retinopathy in Mthatha Region of the Eastern Cape Province of South Africa. Oxid Med Cell Longev. 2016;2016:2063103.

23. Witko-Sarsat V, Friedlander M, Nguyen-Khoa T, Capeillere-Blandin C, Nguyen AT, Canteloup S, et al, Advanced oxidation protein products as novel mediator of inflammation and monocyte activation in chronic renal failure, J. Immunol. 1998; 161: 2524 32.

24. Piwowar A, Knapik-Krdecka M, Szczecinska J, Warwas M. Plasma glycooxidation protein products in type 2 diabetic patients with nephropathy. Diabetes. Metab. Res. Rev. 2008; 24: 549-53.

25. Sarkar P, Kaushik K, Mondal MC, Chakraborty I, Kar M. Elevated level of carbonyl compounds correlates with insulin resistance in type 2 diabetes, Anna. Acad. Med. Singapore. 2010; 39: 909-12.

26. Kalousova M, Krha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiol Res. 2002; 51: 597-04.

27. Yamagishi SI, Matsui T. Advanced glycation end products, oxidative stress and diabetic nephropathy, Oxidative Medicine and Cellular Longevity. 2010; 3: 101-8.

28. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: Sparking the development of diabetic vascular injury, Circulation. 2006; 114: 597-605.

29. Semba RD, Sun K, Schwartz AV, Varadhan R, Harris TB, Satterfield S, et al. Serum carboxymethyl-lysine, an advanced glycation end product, is associated with arterial stiffness in older adults. J Hypertens. 2015;33:797-803.

30. Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Can. 1992; 15: 1835-43.

31. John WG, Lamb EJ. The Maillard or browning reaction in diabetes. Eye. 1993; 7: 230-7.

32. Monnier VM. Nonenzymatic glycosylation, the Maillard reaction and the aging process. J Gerontol. 1990; 45: B105-B11.

33. Lapolla A, Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, et al. Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. Diabetes care. 2007; 30: 670-6.

34. Ahmed N. Advanced glycation end products-role in pathology of diabetic complications. Diabetes Res. Clin. Pract. 2005; 67: 3-21.

35. Kaneko M, Bucciarelli L, Hwang YC, Lee L, Yan SF, Schmidt AM, et al. Aldose reductase and AGE-RAGE pathways: key players in myocardial ischemic injury. Ann N Y Acad Sci. 2005; 1043: 702- 9.

36. Lorenzi M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive and resilient. Exp. Diabetes Res. 2007; 61038.

37. Bansal S, Chawla D, Banerjee BD, Madhu SV, Tripathi AK. Association of RAGE gene polymorphism with circulating AGEs level and paraoxonase activity in relation to macro-vascular complications in Indian type 2 diabetes mellitus patients.Gene. 2013; 526:325-30.

38. Bansal S, Chawla D, Siddarth M, Banerjee BD, Madhu SV, Tripathi AK. A study on serum advanced glycation end products and its association with oxidative stress and paraoxonase activity in type 2 diabetic patients with vascular complications.Clin Biochem. 2013; 46:109-14.

39. Nin JW, Jorsal A, Ferreira I, Schalkwijk CG, Prins MH, Parving HH, et al. Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes. Diabetes Care. 2011; 34: 442-7.

40. Yoshida N, Okumura K, Aso Y. High serum serum pentosidine concentrations are associated with increased arterial stiffness and thickness in patients with type 2 diabetes. Metabolism. 2005; 54: 345-50.

41. Kerkeni M, Saïdi A, Bouzidi H, Letaief A, Ben Yahia S, Hammami M. Pentosidine as a biomarker for microvascular complications in type 2 diabetic patients..Diab Vasc Dis Res. 2013; 10: 239-245.

42. Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Hanssen KF, et al. Increased serum levels of advanced glycation endproducts predict total, cardiovascular and coronary mortality in women with type 2 diabetes: a population-based 18 year follow-up study. Diabetologia. 2007; 50: 1409-17.

43. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes, Diabetes. 2005; 54: 3103–3111.

44. Lu C, He JC, Cai W, Liu H, Zhu L, Vlassara H. Advanced glycation endproduct (AGE) receptor 1 is a negative regulator of the inflammatory response to AGE in mesangial cells. Proc Natl Acad Sci USA. 2004; 101: 11767-72.

45. Cai W, He JC, Zhu L, Chen X, Striker GE, Vlassara H. AGEreceptor- 1 counteracts cellular oxidant stress induced by AGEs via negative regulation of p66shc-dependent FKHRL1 phosphorylation. Am J Physiol Cell Physiol 2008; 294: C145-52.

46. Cai W, Torreggiani M, Zhu L, Chen X, He JC, Striker GE, et al. AGER1 regulates endothelial cell NADPH oxidasedependent oxidant stress via PKC-delta: implications for vascular disease. Am J Physiol Cell Physiol. 2010; 298: C624-34.

47. He CJ, Koschinsky T, Buenting C, Vlassara H. Presence of diabetic complications in type 1 diabetic patients correlates with low expression of mononuclear cell AGE-receptor-1 and elevated serum AGE. Mol Med. 2001; 7: 159-68.

48. Li YM, Mitsuhashi T, Wojciechowicz D, Shimizu N, Li J, Stitt A, et al. Molecular identity and cellular distribution of advanced glycation end product receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc Natl Acad Sci U S A. 1996; 93: 11047 52.

49. Pugliese G, Pricci F, Iacobini C, Leto G, Amadio L, Barsotti P, et al. Accelerated diabetic glomerulopathy in galectin-3/AGE receptor 3 knockout mice. Faseb J. 2001; 15: 2471-79.

50. Yan SF, Ramasamy R, Schmidt AM. The RAGE axis: a fundamental mechanism signalling danger to the vulnerable vasculature. Circ Res. 2010; 106: 842-53.

51. Yan SF, Ramasamy R, Schmidt AM. Mechanisms of Disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. Nat Clin Pract Endocrinol Metab. 2008; 4:285-293.

52. Herold K, Moser B, Chen Y, Zeng S, Yan SF, Ramasamy R, et al. Receptor for advanced glycation end products (RAGE) in a dash to the rescue: inflammatory signals gone awry in the primal response to stress. J Leukoc Biol. 2007; 82:204-212.

53. Kalea AZ, Schmidt AM, Hudson BI. RAGE: a novel biological and genetic marker for vascular disease. Clinical Science. 2009; 116: 621-37.

54. Hudson BI, Kalea AZ, Del Mar Arriero M, Harja E, Boulanger E, D'Agati V, et al. Interaction of the RAGE cytoplasmic domain with diaphanous-1 is required for ligand-stimulated cellular migration through activation of Rac1 and Cdc42. J Biol Chem. 2008; 283:34457-68.

55. Srikrishna G, Huttunen HJ, Johansson L, Weigle B, Yamaguchi Y, Rauvala H, et al. N-Glycans on the receptor for advanced glycation end products influence amphoterin binding and neurite outgrowth. J Neurochem. 2002; 80:998-1008.

56. Al-Mesallamy HO, Hammad LN, El-Mamoun TA, Khalil BM. Role of advanced glycation end product receptors in the pathogenesis of diabetic retinopathy. J Diab Compl. 2011; 25:168-174.

57. Huebschmann AG, Regensteiner JG, Vlassara H, Reusch JE. Diabetes and advanced glycation end products. Diabetes Care. 2006; 29:1420-1429.

58. Wada R, Yagihashi S. Role of advanced glycation end products and their receptors in development of diabetic neuropathy. Ann N Y Acad Sci. 2005; 1043:598–604.

59. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YCP, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. J. Biol. Chem. 1992; 267: 14998-15004.

60. Schlueter C, Hauke S, Flohr AM, Rogalla P, Bullerdie J. Tissue-specific expression patterns of the RAGE receptor and its soluble forms: a result of regulated alternative splicing? Biochim. Biophys. Acta. 2003; 1630: 1-6.

61. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem. J. 2003; 370: 1097-109.

62. Park IH, Yeon SI, Youn JH, Choi JE, Sasaki N, Choi IH et al. Expression of a novel secreted splice variant of the receptor for advanced glycation end products (RAGE) in human brain astrocytes and peripheral blood mononuclear cells. Mol. Immunol. 2004; 40: 1203-11.

63. Ali O. Genetics of type 2 diabetes. World J Diabetes. 2013;4:114-23.

64. Traherne JA. Human MHC architecture and evolution: implications for disease association studies. Int. J. Immunogenet. 2008; 35: 179-92.

65. Hudson BI, Stickland MH, Grant PJ. Identification of polymorphisms in the receptor for advanced glycation end-products (RAGE) gene: prevalence in type II diabetes mellitus and ethnic groups. Diabetes. 1998; 47: 1155-57.

66. Hudson BI, Carter AM, Harja E, Kalea AZ, Arriero M, Yang H, et al. Identification, classification, and expression of RAGE gene splice variants. FASEB J. 2007; 22: 1572-80.

67. Hudson BI, Stickland MH, Futers TS, Grant PJ. Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. Diabetes. 2001; 50: 1505-11.

68. Hudson BI, Stickland MH, Grant PJ, Futers TS. Characterization of allelic and nucleotide variation between the RAGE gene on chromosome 6 and a homologous pseudogene sequence to its 5_ regulatory region on chromosome 3: implications for polymorphic studies in diabetes. Diabetes. 2001; 50: 2646-51.

69. Falcone C, Geroldi D, Buzzi MP, Emanuele E, Yilmaz Y, Fontana JM, et al. The -374T/A RAGE polymorphism protects against future cardiac events in nondiabetic patients with coronary artery disease. Arch. Med. Res. 2008; 39: 320-5.

70. Tripathi AK, Chawla D, Bansal S, Banerjee BD, Madhu SV, Kalra OP. Association of RAGE gene polymorphism with vascular complications in Indian type 2 diabetes mellitus patients.Diabetes Res Clin Pract. 2014; 103:474-81.

71. Pettersson-Fernholm K, Forsblom C, Hudson BI, Perola M, Grant PJ, Groop PH. The functional -374 T/A RAGE gene polymorphism is associated with proteinuria and cardiovascular disease in Type 1 diabetic patients. Diabetes. 2003; 52: 891-94.

72. Zee RY, Romero JR, Gould JL, Ricupero DA, Ridker PM. Polymorphisms in the advanced glycosylation end product-specific receptor gene and risk of incident myocardial infarction or ischemic stroke. Stroke. 2006; 37: 1686-90.

73. dos Santos KG, Canani LH, Gross JL, Tschiedel B, Pires Souto KE, Roisenberg I. The -374A allele of the receptor for advanced glycation end products gene is associated with a decreased risk of ischemic heart disease in African-Brazilians with type 2 diabetes. Mol. Genet. Metab. 2005; 85: 149-56.

74. Falcone C, Campo I, Emanuele E, Buzzi MP, Zorzetto M, Sbarsi I, et al. Relationship between the -374T/A RAGE gene polymorphism and angiographic coronary artery disease. Int. J. Mol. Med. 2004; 14: 1061-64.

75. Falcone C, Emanuele E, Buzzi, MP, Ballerini L, Repetto A, Canosi U, et al. The -374T/A variant of the rage gene promoter is associated with clinical restenosis after coronary stent placement. Int. J. Immunopathol. Pharmacol. 2007; 20: 771-7.

76. Falcone C, Campo I, Emanuele E, Buzzi MP, Geroldi D, Belvito C, et al. -374T/A polymorphism of the RAGE gene promoter in relation to severity of coronary atherosclerosis. Clin. Chim. Acta 2005; 354: 111-16.

77. Picheth G, Costantini CO, Pedrosa FO, Leme da Rocha Martinez T, Maltempi de Souza E. The -374A allele of the receptor for advanced glycation end products (RAGE) gene promoter is a protective factor against cardiovascular lesions in type 2 diabetes mellitus patients. Clin. Chem. Lab. Med. 2007; 45: 1268-72.

78. Falcone C, Geroldi D, Buzzi MP, Emanuele E, Yilmaz Y, Fontana JM, et al. The -374T/A RAGE polymorphism protects against future cardiac events in nondiabetic patients with coronary artery disease. Arch. Med. Res. 2008; 39: 320-5.

79. Globocnik PM, Steblovnik K, Peterlin B, Petrovic D. The -429 T/C and -374 T/A gene polymorphisms of the receptor of advanced glycation end products gene are not risk factors for diabetic retinopathy in Caucasians with type 2 diabetes. Klin. Monatsbl. Augenheilkd. 2003; 220: 873-6.

80. JiXiong X, BiLin X, MingGong Y, ShuQin L. -429T/C and -374T/A polymorphisms of RAGE gene promoter are not associated with diabetic retinopathy in Chinese patients with type 2 diabetes. Diabetes Care. 2003; 26: 2696-97.

81. Lu W, Feng B. The -374A allele of the RAGE gene as a potential protective factor for vascular complications in type 2 diabetes: a meta-analysis. Tohoku J Exp Med. 2010; 220:291-297.

82. Peng WH, Lu L, Wang LJ, Yan XX, Chen QJ, Zhang Q, et al. RAGE gene polymorphisms are associated with circulating levels of endogenous secretory RAGE but not with coronary artery disease in Chinese patients with type 2 diabetes mellitus. Arch Med Res. 2009; 40:393-398.

83. Kirbis J, Milutinović A, Steblovnik K, Teran N, Terzić R, Zorc M.. The -429 T/C and -374 T/A gene polymorphisms of the receptor of advanced glycation end products gene (RAGE) are not risk factors for coronary artery disease in Slovene population with type 2 diabetes. Coll Antropol. 2004; 28:611-616.

84. Hofmann MA, Drury S, Hudson BI, Gleason MR, Qu W, Lu Y, et al. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. Genes Immun. 2003; 3: 123-35.

85. Xie J, Reverdatto S, Frolov A, Hoffmann R, Burz DS, Shekhtman A. Structural basis for pattern recognition by the receptor for advanced glycation end products (RAGE). J. Biol. Chem. 2008; 283: 27255-69.

86. Prevost G, Fajardy I, Besmond C, Balkau B, Tichet J, Fontaine P, et al. Polymorphisms of the receptor of advanced glycation end products (RAGE) and the development of nephropathy in type 1 diabetic patients. Diabetes Metab. 2005; 31: 35-39.

87. Zhang HM, Chen LL, Wang L, Liao YF, Wu ZH, Ye F, et al. Association of 1704G/T and G82S polymorphisms in the receptor for advanced glycation end products gene with diabetic retinopathy in Chinese population. J Endocrinol Invest. 2009; 32: 258-62.

88. Vanita V. Association of RAGE (p.Gly82Ser) and MnSOD (p.Val16Ala) polymorphisms with diabetic retinopathy in T2DM patients from north India. Diabetes Res Clin Pract. 2014; 104:155-62.

89. Yang L, Wu Q, Li Y, Fan X, Hao Y, Sun H, et al. Association of the receptor for advanced glycation end products

gene polymorphisms and circulating RAGE levels with diabetic retinopathy in the Chinese population. J Diabetes Res. 2013; 2013:264579.

90. Yuan D, Yuan D, Liu Q. Association of the receptor for advanced glycation end products gene polymorphisms with diabetic retinopathy in type 2 diabetes: a meta-analysis. Ophthalmologica. 2012; 227:223-232.

91. Ng ZX, Kuppusamy UR, Poh R, Tajunisah I, Koay AC, Fong KC, et al. Lack of association between Gly82Ser, 1704G/T and 2184A/G of RAGE gene polymorphisms and retinopathy susceptibility in Malaysian diabetic patients. Genet Mol Res., 2012; 11:455-461.

92. Naka CL, Picheth G, Alcântera VM, Réa RR, Chautard-Freire-Maia EA, Pedrosa Fde O, et al. The Gly82Ser Polymorphism of the Receptor of Advanced Glycation End Product (RAGE) Gene Is Not Associated With Type 1 or Type 2 Diabetes in a Brazilian Population. Diabetes care. 2006; 29:712-713.

93. Yoshioka K, Yoshida T, Takakura Y, Umekawa T, Kogure A, Toda H, et al. Relation between Polymorphisms G1704T and G82S of RAGE Gene and Diabetic Retinopathy in Japanese Type 2 Diabetic Patients. Intern Med. 2005; 44:417-21.

94. Kankova K., Zahejsky J., Marova I., Muzik J., Kuhrova V., Blazkova M., et al. Polymorphisms in the RAGE gene influence susceptibility to diabetes-associated microvascular dermatoses in NIDDM. J. Diabetes Complications. 2001; 15:185–192.

95. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. J Am Soc Nephrol. 2003; 14: S254-S258.

96. Edelstein D, Brownlee M. Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. Diabetes. 1992; 41: 26-9.

97. Thornalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys. 2003; 419: 31-40.

98. Zhao W, Tilton RG, Corbett JA, McDaniel ML, Misko TP, Williamson JR, et al. Experimental allergic encephalomyelitis in the rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase. J Neuroimmunol. 1996; 64: 123-33.

99. Fu MX, Wells-Knecht KJ, Blackledge JA, Lyons TJ, Thorpe SR, Baynes JW. Glycation, glycoxidation, and cross-linking of collagen by glucose. Kinetics, mechanism, and inhibition of late stages of the Maillard reaction. Diabetes. 1994; 43: 676-83.

100. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci. 2003; 60: 6-20.

101. Bakker J, Zhang H, Depierreux M, van Asbeck S, Vincent JL. Effects of N-acetylcysteine in endotoxic shock. J Crit Care. 1994; 9: 236-43.

102. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic Biol Med. 1989; 6: 593-7.

103. Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv Pharmacol. 1997; 38: 205-27.

104. Dekhuijzen PNR. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. Eur Respir J. 2004; 23: 629-36.

105. Haber CA, Lam TK, Yu Z, Gupta N, Goh T, Bogdanovic E. N-acetylcysteine and taurine prevent hyperglycemia-induced insulin resistance in vivo: Possible role of oxidative stress. Am. J. Physiol. Endocrinol. Metab. 2003; 285:

E744-E53.

106. Gibson KR, Winterburn TJ, Barrett F. herapeutic potential of N-acetylcysteine as an antiplatelet agent in patients with type-2 diabetes. Cardiovasc Diabetol. 2011; 10: 43.

107. Zhou ZH, Jiang JL, Peng J, Deng HW, Li Y J. Reversal of tolerance to nitroglycerin with N acetylcysteine or captopril: A role of calcitonin gene related peptide. Eur. J. Pharmacol. 2002; 439: 129-34.

108. Fremont L. Biological effect of resveratrol. Life Sci. 2000; 66: 663-73.

109. Bertelli AA, Baccalini R, Battaglia E, Falchi M, Ferrero ME. Resveratrol inhibits TNF-alpha induced endothelial cell activation. Therapie. 2001; 56: 613-6.

110. Bertelli AA, Giovannini L, Giannessi D, Migliori M, Bernini W, Fregoni M. Antiplatelet activity of synthetic and natural resveratrol in red wine. Int J Tissue React. 1995; 17: 1-3.

111. Ignatowicz E, Baer-Dubowska W. Resveratrol, a natural chemopreventive agent against degenerative diseases. Pol J Pharmacol. 2001; 53: 557-69.

112. Olas B, Wachowicz B, Saluk-Juszczak J, Zielinski T. Effect of resveratrol, a natutal polyphenolic compound, on platlet activation induced by endotoxin or thrombin. Thromb Res. 2002; 107: 141-5.

113. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems andpromises. Molecular Pharmaceutics. 2007; 4: 807-18.

114. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Advan Exp Med Biol. 2007; 595: 1-75.

115. Rogers N, Stephenson M, Kitching A, Horowitz J, Coates P. Amelioration of renal ischaemia-reperfusion injury by liposomal delivery of curcumin to renal tubular epithelial and antigen presenting cells. Br J Pharmacol. 2012; 166: 194-209.

116. Basnet P, Skalko-Basnet N. Curcumin: an anti-inflammatory molecule from a curry spice on the path to cancer treatment. Molecules. 2011; 16: 4567-98.

117. Zhang DW, Fu M, Gao SH, Liu JL. Curcumin and diabetes: a systematic review. Evid Based Complement Alternat Med. 2013; 2013:636053.

118. Karimian MS, Pirro M, Majeed M, Sahebkar A. Curcumin as a natural regulator of monocyte chemoattractant protein-1. Cytokine Growth Factor Rev. 2016 Oct 8. pii: S1359-6101(16)30131-9. doi: 10.1016/j.cytogfr.2016.10.001

119. Xu J, Fu Y, Chen A. Activation of peroxisome proliferator- activated receptor-(gamma) contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth. Am J Physiol Gastrointest Liver Physiol. 2003; 285:G20-30.

120. Okamoto T, Yamagishi S, Inagaki Y, Amano S, Koga K, Abe R, et al. Angiogenesis induced by advanced glycation end products and its prevention by cerivastin; FASEB J. 2002; 16:1928-30.