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Chapter 4

Oxidative Stress Biomarkers for Diabetic Retinopathy and Medical Management Affecting Oxidative Stress

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Additional information is available at the end of the chapter

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Abstract

Changes in dietary habits and lifestyles associated with rapid economic growth have dramatically increased the incidence of diabetes and related vascular complications. Diabetic retinopathy (DR), a microvascular complication of diabetes, is associated with both environmental and genetic factors. Several metabolic abnormalities are implicated in its pathogenesis; however, the exact mechanism remains to be determined. Among them, oxidative stress is expected to play an important role.

Environmental, genetic, and epigenetic factors affecting the oxidative stress responsible for DR are reviewed in this paper. The knowledge about genetic biomarkers of DR is quite extensive, whereas the awareness about epigenetics and epigenetic markers is only beginning to be understood.

Modulation of epigenetic changes by pharmaceutical means may provide a potential strategy to retard the progression of DR. In addition to the intense medical management, these strategies include dietary measures (antioxidants) and the introduction of epigenetic drugs, such as inhibitors of DNA methylation and histone demethylases.

Keywords: oxidative stress, diabetic retinopathy, gene polymorphisms, epigenetics, medical management

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The prevalence of diabetes has

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reached global epidemic proportions. According to the Internal Diabetes Federal (IDF) data, there were 382 million people living with diabetes in 2013, whereas a further 316 million with impaired glucose tolerance are at high risk of the disease—an alarming number that is set to reach 471 million by 2035. Type 2 diabetes (T2DM) is the most prevalent type of diabetes. It is by far the most common form of diabetes in elderly people, but is increasingly seen in children and adolescents IDF, 2013 as well. The causes of the T2DM epidemic are embedded in a very complex group of genetic and epigenetic systems interacting within an equally complex societal framework that determines behavior and environmental influences [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

2. Pathogenesis of DR

Chronic elevation in circulating blood glucose damages blood vessels, which results in many micro- and macrovascular complications. DR is one of the major microvascular complications affecting the vision and is the leading cause of blindness in working-age adults [3]. It progresses from mild nonproliferative abnormalities, characterized by increased vascular permeability, to nonproliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels in the retina and the posterior surface of the vitreous [4] (**Figure 1**).

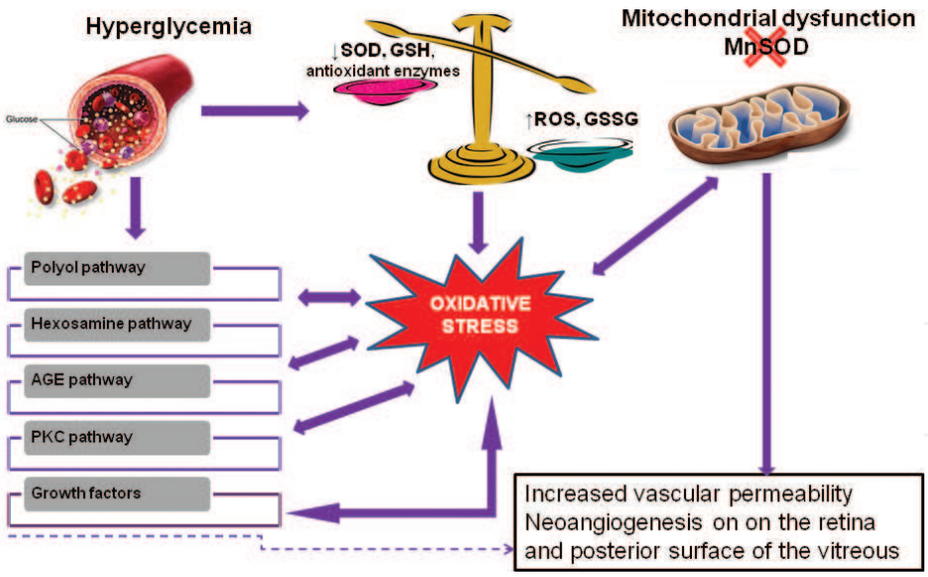


Figure 1. Major pathways implicated in the development of diabetic retinopathy.

It is a multifactorial condition for which the pathophysiology is incompletely understood [5]. There are many pathophysiological mechanisms through which diabetes might affect the initiation and promotion of the many underlying pathologies associated with DR [6]. The strong impact of hyperglycemia on DR incidence was confirmed by the Diabetes Control and

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Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) clinical trials [7,8]. Hyperglycemia activates several well-characterized biochemical pathways that play a significant role in the development of DR [9]. Major pathways implicated in the development of DR are the polyol pathway, protein kinase C (PKC) activation, accumulation of advanced glycation end products (AGEs), oxidative stress, activation of the hexosamine biosynthesis pathway, and growth factors (**Figure 1**) [3,6,9–11]. The activation of these pathways, in turn, leads to the secondary production of reactive oxygen species (ROS) and the consequent increase in oxidative stress that affects carbohydrates, lipids, proteins, and nucleic acids [9]. The oxidative stress plays a pivotal role in cellular injury from hyperglycemia.

3. Biomarkers

A biomarker is a measurable indicator of a specific biological state, usually one relevant to the risk, presence, severity, prognosis, or predicted therapeutic response of the disease. In medicine, biomarkers are often compounds isolated from serum, urine, or other fluids that can be used as an indicator of the presence or severity of a particular disease state. Molecular biomarkers can themselves take many forms, and as a consequence there are many strategies available for their discovery and validation. Transcriptional profiling, DNA methylation studies, and kinase sequencing have shown a strong potential for biomarker discovery in several disorders; metabolomics approaches are beginning to show promise for metabolic diseases, such as DR. Molecular biomarkers (DNA gene polymorphisms, RNA gene polymorphisms, proteins) hold special promise for a wide range of clinical and biomedical applications in several disorders, including DR [12].

4. Oxidative stress and its role in the development of DR

Oxidative stress may be defined as an imbalance between the level of ROS or oxygen radicals and the antioxidant defenses in a biological system [10]. The term "ROS" includes all unstable metabolites of molecular oxygen (O_2) that have a higher reactivity than O_2, such as the superoxide radical ($O_2'^{\bullet}$) and the hydroxyl radical ($HO^{\bullet}$), and nonradical molecules, such as hydrogen peroxide (H_2O_2) [13]. To counteract the harmful effects of ROS, the cell has developed antioxidant defense mechanisms. Antioxidants may be classified according to their structure (enzymes or small nonenzymatic protein molecules) and antioxidants according to their source (endogenous or exogenous). There are many enzymes with an antioxidant role in the organism, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferases (GSTs), and the thioredoxin (Trx) system [14,15].

Increasing data indicate that oxidative stress is involved in the development of DR [16–19]. The retina has a high content of polyunsaturated fatty acids and has the highest oxygen uptake and glucose oxidation relative to any other tissue. This phenomenon renders the retina more susceptible to oxidative stress [20]. Oxidative stress-induced biochemical changes contribute to either functional or structural changes in the microvasculature in the retina [6]. Structural

changes range from basement membrane thickening and microvascular cell loss to capillary closure and acellular capillary formation [6]. ROS mediate these changes by both direct and indirect mechanisms. Structural changes may both contribute to and result from functional changes, such as altered blood flow, loss of intercellular junctions, and increased vessel permeability. Thus, oxidative stress-induced structural and functional changes appear to be highly interrelated in the pathogenesis of diabetic retinopathy (DR) [6].

Since long-term exposure to oxidative stress is strongly implicated in the pathogenesis of diabetic complications, polymorphic genes of detoxifying enzymes may be involved in the development of DR.

5. Polymorphisms in oxidative stress genes and risk for DR

Gene	Polymorphism		Relation to DR (significance level)	Population	Number of patients	Author	Year
MnSOD	rs4880	VV genotype (V16A)	Positive association ($p = 0.006$)	Slovenian	426	Petrovič et al.	2008
		AA genotype (V16A)	Positive association ($p = 0.03$)	Finnish	755	Kangas-Kontio et al.	2009
		C allele (C47T)	No significant association	Different ethnic origins	(17 articles) meta-analysis	Tian et al.	2011
		(V16A)	No significant association	North Indian	758	Vanita	2014
		AV genotype (V16A)	Positive association ($p < 0.0001$)	Northern Iranian	280	Haghighi et al.	2015
CAT	rs1001179	−262C/T	No significant association	Brazilian	520	Dos Santos et al.	2006
GPx	rs1050450	Pro197Leu	No study on DR	/	/	/	/
GSTM1, T1	Null genotype	GSTT1null	Positive association ($p = 0.01$)	Scottish	2015	Doney et al.	2005
		GSTT1null GSTM1null	Positive association ($p < 0.0001$) Positive association	Slovenian	604	Cilenšek et al.	2012

Gene	Polymorphism		Relation to DR (significance level)	Population	Number of patients	Author	Year
			(p = 0.01)				
		GSTM1null	Positive association (p = 0.04)	Iranian	115	Dadbinpour et al.	2013
		GSTM1null GSTT1null	No significant association	Iranian	404	Moasser et al.	2014
		GSTT1null	Positive association	Caucasian	3563 (meta-analysis)	Sun et al.	2015
GSTP1	rs947894	Ile105Val	No significant association	Slovenian	604	Cilenšek et al.	2012
		A313G	No significant association	Egyptian	105	Zaki et al.	2015
Trx	rs4485648	CT genotype (T9921C) TT genotype (T9921C)	Positive association (p = 0.028) Positive association (p = 0.026)	Slovenian	953	Mankoč Ramuš et al.	2015

Table 1. Genes affecting oxidative stress and diabetic retinopathy.

5.1. Manganese superoxide dismutase (MnSOD)

SOD catalyzes the breakdown of superoxide into H_2O_2 scavenging superoxide, and, because of its mitochondrial localization, MnSOD is considered as the first line of defense against oxidative stress [19]. A number of polymorphisms in the mitochondrial targeting sequence of *MnSOD* have been described, but only the A16V (C47T; rs4880) evokes functional consequences [21–24]. In fact, the alanine variant of MnSOD is thought to have an α-helical mitochondrial-targeting domain, whereas the valine variant of MnSOD appears to have a β-pleated sheet conformation. This conformational difference is thought to result in a more efficient transport of the alanine variant of MnSOD into mitochondria than the valine variant [25]. Thus, the valine variant has been associated with a 30–40% lower activity and an increased susceptibility to oxidative stress [22].

There are only a few studies indicating the association of the V16A polymorphism of the MnSOD gene with DR (**Table 1**). A meta-analysis comprising 17 studies, including type 1 and type 2 diabetic patients from different ethnic origins, implied that the C (Ala) allele of the C47T polymorphism in the MnSOD gene had a significant protective effect against microvascular

complications (DR an diabetic nephropathy), although the aforementioned C allele had no significant effect on the risk for DR alone [26]. Petrovič et al. have reported that the ValVal genotype of the Val16Ala polymorphism of the MnSOD might be a risk factor for DR [27]. In contrast, Kangas-Kontio et al. [24] could not confirm such association in their study, as they found a significantly higher frequency of the AlaAla genotype in diabetics (type 1 or type 2) with DR [24]. Furthermore, a study of northern Iranian T2DM patients revealed that the heterozygosity in codon 16 of the MnSOD is considered as a risk factor for DR in T2DM [28]. However, another study from north India did not confirm an association between this SNP and DR in T2DM patients [29].

These conflicting results may be due to the ethnical differences, different genetic backgrounds, and sizes of the study populations.

5.2. Catalase (CAT)

CAT is a potent scavenger of H_2O_2 and provides a powerful antioxidant defense in the retina. It prevents the formation of the more toxic hydroxyl radical ($HO^{\bullet}$) resulting from the reaction of H_2O_2 and ferrous ions [30]. In structures like the eye, a significant contribution of CAT to H_2O_2 detoxification was reported. The inhibition of catalase activity in the rabbit eyes increased the H_2O_2 concentration 2.5-fold, which was not compensated for by GPx activity [31].

It has been shown that genetic variations in the *CAT* gene and its promoter may play a role in a number of diseases associated with oxidative stress (e.g., atherosclerosis, hyperlipidemia, diabetes mellitus, hypertension, and neurodegenerative diseases) [32]. Although catalase is broadly studied, to the best of our knowledge, there is only one report in which no association was observed between the −262C/T polymorphism in the promoter region of the *CAT* gene and DR in Caucasian-Brazilian T2DM patients (**Table 1**) [33].

5.3. Glutathione peroxidases (GPxs)

GPxs are selenocysteine-containing enzymes that catalyze the reduction of H_2O_2 and lipid hydroperoxides to H_2O and lipid alcohols, respectively, in a reaction that utilizes reduced glutathione (GSH) as a reducing co-substrate. There are five known forms of GPx: cellular (GPx-1), gastrointestinal (GPx-2), plasma (GPx-3), phospholipid (GPx-4), and sperm (snGPx) [34]. The most abundant intracellular isoform is GPx-1; it is known as the classical or cytosolic antioxidant enzyme and is ubiquitously expressed. GPx-1 deficiency has been shown to promote endothelial dysfunction, heart failure, and abnormal structural changes in vasculature and myocardium [2,34].

GPx-1 has four SNPs that change the amino acid produced, but only one has been studied extensively in human disease [35]. This missense polymorphism changes the amino acid from proline (Pro) to leucine (Leu) at position 197 (rs1050450) and was associated with a reduction in transcription and enzyme activity of GPx-1 [36].

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The GPx-1 Pro/Leu genotype has been linked to lung cancer, bladder cancer, and complications in T2DM. Studies assessing the association between GPx-1 Pro197Leu SNP genotypes and diabetes, stroke, brain tumors, and prostate cancer are inconclusive [35].

An abundance of GPx has been localized in the rabbit retina through immunohistochemistry [37]. However, to date, there has been no study to show the association between *GPx* gene polymorphisms and DR in T2DM patients (**Table 1**).

5.4. Glutathione S-transferases (GSTs)

The human glutathione S-transferases (GSTs) are a family of enzymes known to act in the body as a defense system for neutralizing free radicals. They play an important role in the detoxification of electrophiles by glutathione conjugation [38]. GST enzymes are coded by at least eight distinct loci: α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), σ (GSTS), k (GSTK), o (GSTO), and τ (GSTZ), each containing one or more homodimeric or heterodimeric isoforms. Three loci in particular, GSTM1, GSTT1, and GSTP1, have received most of the attention. The GSTM1 locus has been mapped on chromosome 1p13.3, while the GSTT1 and GSTP1 loci can be found on chromosomes 22q11.2 and 11q13. Persons with homozygous deletions of either the GSTM1 or GSTT1 loci have no enzymatic functional activity of the respective enzyme [39,40]. A GSTP1 variant with a substitution in the active site of valine for isoleucine at codon 105 (Ile105Val) has a reduced ability to conjugate reactive electrophiles with glutathione and may therefore sensitize cells to free radical-mediated damage. The Val105 variant has been associated with susceptibility to smoking-related cancer and cardiovascular disease [41].

Numerous GST polymorphisms have been associated with an increased or decreased susceptibility to several diseases [39,42–45], but only a few studies examined the association of GST polymorphisms and DR in T2DM patients (**Table 1**). Cilenšek et al. proposed a protective effect for the GSTM1-null genotype against retinopathy [46], explained by an up-regulation of other antioxidant enzymes, such as MnSOD [47]. On the contrary, the result of the aforementioned study is inconsistent with the study that showed a significant correlation between the GSTM1-null genotype and DR [38]. The study carried out by Doney et al. demonstrated that GSTT1-null individuals have a more generalized vasculopathy with an increased risk of progression of both retinopathy and nephropathy [41]. These findings are in agreement with the reports by Cilenšek et al., who recently reported that individuals homozygous for the deletion of GSTT1 are at an ≈ 2-fold-greater risk of DR [46]. There is only one report suggesting that GST allelic variants are not associated with individual susceptibility to DR [48].

Since the results of studies were conflicting and inconclusive, Sun et al. [49] performed a meta-analysis. A total of five studies were included, all of which were conducted in Caucasians; one study used T1DM patients, while other studies used T2DM patients. They reported that an increased risk of DR was associated with the null genotype of GSTT1 and GSTT1 polymorphisms, respectively [49].

As regards the GSTP1 gene polymorphism (rs947894), the domination of the G allele results in the reduction of GSTP1 enzyme activity. Consequently, the cell becomes more susceptible

to mutation and damage from exposure to electrophiles and ROS [50]. Despite the significance noted in the G allele in the GSTP1 gene polymorphism among diabetic cases [51,52], two studies failed to demonstrate any significant association between the GSTP1 polymorphism and DR in T2DM patients [46,50].

5.5. Trx system

The Trx system is one of the central antioxidant systems in mammalian cells, maintaining a reducing environment by catalyzing electron flux from NADPH through Trx reductase to Trx, which reduces its target proteins using highly conserved thiol groups [53]. In mammals, both Trx and TrxR are expressed as dedicated isoforms for either predominantly cytosolic (Trx1 and TrxR1) or mitochondrial (Trx2 and TrxR2) localization [54].

Up-regulation of thioredoxin-interacting protein (TXNIP), an endogenous inhibitor of Trx, compromises cellular antioxidant and antiapoptotic defenses and stimulates pro-inflammatory cytokines expression [55]. Moreover, it is highly induced in the diabetic retina and plays a critical role in DR pathogenesis [56–59]. Mankoč Ramuš et al. searched for a connection between genetic variants within the mitochondrial Trx antioxidant defense system and DR (**Table 1**). The aforementioned study was the first to explore the association between seven single nucleotide polymorphisms (SNPs), including rs8140110, rs7211, rs7212, rs4755, rs1548357, rs4485648, and rs5748469, in the Trx2/TXNIP and TrxR2 genes, and the risk of DR in a case–control study of Slovenian patients with T2DM. They found an association between the rs4485648 polymorphism of the TrxR2 gene and DR in Caucasians with T2DM [60].

6. The role of epigenetics in the pathogenesis of diabetic retinopathy

The heritable, yet reversible changes in the gene expression that are independent of the order of the nucleotides within a gene are called epigenetic modifications. An organism's genome can be modified by naturally occurring ROS which are regularly produced as an inevitable by-product of the normal oxygen metabolism. Oxidative stress is defined as a condition associated with an aberrant increase in ROS generation in a cell.

In diabetes, oxidative stress is increased in the retina and its capillary cells and is considered as one of the major metabolic abnormalities associated with the development of DR [61–63]. Arguably, the resulting hyperglycemia-induced ROS production may also promote epigenetic alterations in DR. Fundamental epigenetic mechanisms include DNA cytosine methylation, histone post-translational modifications (PTMs) in the chromatin, and noncoding RNAs (ncRNAs), all of which can affect gene expression individually or cooperatively and modulate disease states [64].

DNA methylation is considered to be one of the most important modifications leading to disease [65]. In general, DNA methylation at 5′ cytosine of the CpG dinucleotides forms 5-methylated cytosine (5mC). The formation of 5mC in the promoter regions leads to gene

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repression, whereas in genes bodies it might regulate transcription elongation and alternative splicing [66]. DNA methylation is brought about by DNA methyltransferases (Dnmts), and these enzymes use S-adenosyl methionine (SAM) as the methyl donor [67,68]. It is noteworthy that some studies have begun to uncover the role of DNA methylation in diabetes and its complications. In animal models, epigenetic silencing due to increased promoter DNA methylation has been linked to islet dysfunction and development of diabetes [69,70]. In a case-controlled study of 168 patients with type 2 DM, the global DNA methylation status was shown to be associated with DR. Additionally, the DNA methylation status exhibited a strong correlation with the progression of DR [71,72]. Apart from the increased activity of Dnmts in the retina and its capillary cells [73], histone-modifying machinery is also affected in diabetes.

In mammalian cells, chromosomal DNA is packed into chromatin, and chromatin is made up of subunits called nucleosomes. Each nucleosome consists of an octamer protein complex, containing two copies each of core histone proteins H2A, H2B, H3, and H4 with 147 bp of chromosomal DNA wrapped around it [74]. Despite such sophisticated DNA packaging, the N-terminal of histones remains vulnerable for PTMs and can be acetylated, methylated, and phosphorylated. Such epigenetic modifications alter the chromatin structure which subsequently affects the binding of transcription factors and can regulate the selective expression of genes in a particular tissue by acting as switches to control gene activity [75–79]. Acetylation, the most common histone modification, which is generally associated with gene activation, is regulated [80] by fine-tune between histone-acetylating and histone-deacetylating enzymes; histone acetyltransferases (HATs) add the acetyl group, while histone deacetylase (HDAC) removes the acetyl group. Histone K acetylation (Kac) is enzymatically mediated by HATs, such as p300, the CREB-binding protein (CBP), and the Tat-interactive protein 60 kDa (Tip60). In general, histone Kac (such as H3K9ac, H3K14ac, and H4K5ac) at gene promoters correlates with transcriptional activation, whereas its removal is associated with gene repression [81]. Experimental evidence using *in vitro* and *in vivo* models of DR has shown increased HDACs and decreased HAT activities and global acetylation [82]. However, Kadiyala et al. have discovered an increased histone acetylation [83]. The reason for the divergence of the published data is not yet known.

Histone methylation is the most complex modification, since its function depends on the precise methylation site and the degree of modification. Lysine residues can have up to three methylation sites, whereas arginines (R) can have up to two methylation sites [84]. Lysine methylation (Kme) is mediated by histone K methyltransferases (HMTs) and removed by K demethylases (KDMs) [85,86]. H3K4me1/2/3 and H3K36me2/3 are generally associated with transcriptionally active genome regions, whereas H3K9me3, H3K27me3, and H4K20me3 are related with repressed domains [81]. In the development of DR, superoxide levels are elevated in the retina, antioxidant defense system is compromised, MnSOD is inhibited, and mitochondria are swollen and dysfunctional [77,87–90]. Overexpression of MnSOD protects diabetes-induced mitochondrial damage and the development of DR [19,91]. Furthermore, *SOD2* is epigenetically modified with increased H4K20me3, H3K9ac, and p65 subunit of NF-kB at its promoter/enhancer [77]. Besides, Zhong and Kowluru revealed that the exposure of retinal capillary cells to high glucose decreases H3K4me at *SOD2* promoter and enhancer regions,

suggesting the role of H3K4 methylation in *SOD2* repression [78]. The possible mechanism for such decrease of methylation is the activation of lysine-specific demethylase-1 (LSD1).

Apart from histone epigenetic modifications, the role of ncRNA has evoked great interest because gene expression can vary due to the function of RNA molecules themselves as well as their interactions with DNA and/or proteins [92]. NcRNAs with less than 200 nucleotides are generally classified as short (i.e., microRNAs), while all larger transcripts are regarded as long ncRNA (lncRNA). There are several subtypes of long and short ncRNA species, many of which are involved in the regulation of gene expression, and these can be further grouped according to their genomic origins and biogenic processes [92]. Specifically, increasing emphasis is being placed on the ability of miRNAs and lncRNAs to regulate gene expression and modulate the actions of growth and inflammatory factors related to diabetic complications [64]. MicroRNAs are a class of highly conserved 19–25 nucleotide single-stranded ncRNAs that regulate gene expression at the posttranscriptional level [93,94]. They block gene translation via binding to complementary regions of the mRNA. Micro-RNAs are also able to initiate the degradation of mRNA strands to which they are bound [95]. Various recent reports have demonstrated alterations in miRNA expression in diabetic eyes. Subjects with proliferative and nonproliferative DR have different serum levels of miR-21, miR-181c, and miR-1179 [96]. Downregulation of miR-200b was observed in the retina in diabetes. In parallel, VEGF (target of miR-200b) mRNA and protein were elevated [97]. It is now becoming clear that oxidative stress causes the activation of the redox-sensitive transcription factors and altered expression of a number of genes, including *VEGF*. Under diabetic conditions, it acts to increase vascular permeability in the early stages of DR and fluid accumulates in the retinal tissue, causing macular oedema and exudate [98]. Up-regulation of miR-195 is shown to downregulate deacetylase Sirtuin 1(Sirt 1) [99], and in DR, the inhibition of Sirt 1 in the retina activates NF-kB, a redox-sensitive proapoptotic factor [100]. On the other hand, up-regulation of miR-29b exerted an antiapoptotic function in the retinal ganglion cells [101]. In addition, miRNAs are stable in biological fluids, such as urine and serum [64], in this view; miRNAs appear to represent valuable noninvasive biomarkers and a promising tool of new approaches for the treatment of DR.

LncRNAs participate in a variety of biological processes, such as chromosome imprinting, epigenetic regulation, cell-cycle control, cell apoptosis, and reprogramming of induced pluripotent stem cells [102,103]. Recently, a human β-cell transcriptome analysis had indicated that lncRNAs are dynamically regulated and abnormally expressed in type 2 diabetes [104]. The field of lncRNA research in ocular diseases is expanding rapidly. Notably, lncRNAs are involved in the pathogenesis of DR through the modulation of multiple pathogenetic pathways. Metastasis-associated lung adenocarcinoma transcript 1, a conserved lncRNA, may become a potential therapeutic target for the prognosis, diagnosis, and treatment of DR [105]. The following year, Yan et al. revealed a regulatory role of the lncRNA myocardial infarction-associated transcript (MIAT) in diabetes mellitus-induced microvascular dysfunction [106].

In the pathogenesis of DR, retinal mitochondria become dysfunctional, and capillary cell apoptosis precedes the development of retinal histopathology associated with DR [107–109]. Mitochondrial homeostasis is maintained by a close cooperation between nuclear DNA and

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mitochondrial DNA (mtDNA) [110]. Due to the lack of supporting histones, and the close proximity to the superoxide-generating electron transport chain, mtDNA is prone to oxidative damage [111,112]. In diabetes, the activity of retinal Dnmts is increased, and the mtDNA replication enzyme, the polymerase γ-1 (*POLG1*) gene, is hypermethylated and its binding at the *D-loop* is impaired, resulting in decreased mtDNA biogenesis [73].

Lately, it is becoming apparent that small interfering RNAs ameliorated the hyperglycemia-induced decrease in mtDNA transcription and the increase in apoptosis. In fact, Mishra and Kowluru have recently discovered that modulation of Dnmt1 by pharmaceutical or molecular means could help maintain mitochondrial integrity and serve as a potential strategy to inhibit/halt the development of DR [113].

The "new antioxidant" concept represents the benefit of the consumption of fresh fruit and vegetables in diabetic patients. Research on foods of plant origin shows that they contain many non-nutritional compounds with an oxidative stress-protective effect (green tea, α-lipoic acid, carnitine, glucosinolates, carotenoids, epigallocatechin, flavonoids, resveratrol, etc.) [100,114–118]. It has been suggested that these compounds regulate free radical over-generation at the mitochondrial level, increase intracellular defenses, and secrete and activate detoxifying enzymes [100,118]. The same principles work for the aforementioned novel compounds.

7. Antioxidants and diabetic retinopathy

So far, oxidative stress has been demonstrated to play an important role in the development and progression of DR; therefore antioxidants are expected to be helpful in preventing DR and its progression [100,114–118]. Lipid peroxidation (LPO) is considered to be a major harmful consequence of ROS formation, as it reflects irreversible oxidative changes of membranes. Moreover, it must be emphasized that retinal cells are highly sensitive to oxidative damage caused by the constant photochemical reactions, and the high concentrations of polyunsaturated fatty acids that constitute their membranes are directly affected by LPO.

Several antioxidants have so far been considered helpful in terms of prevention of DR and its progression; however, only a few research groups demonstrated an important effect of antioxidants in a few ocular disorders, such as in macular degeneration and in DR [119,120].

The administration of combined antioxidant therapy is helpful by improving antioxidant capacity against ROS and protecting photoreceptors against radiation. Vitamins C and E act by normalizing numerous chemical reactions to diminish aging and degeneration caused by ROS. CAT is recommended in macular degeneration [119]. Just recently an interesting study has been reported to demonstrate the effect of an adjunctive antioxidant treatment in subjects with DR [120]. Either coenzyme Q10 (400 mg/day) or combined antioxidant therapy (composed of 10 mg of lutein, 4 mg of astaxanthin, 1 mg of zeaxanthin, 180 mg of vitamin C, 30 mg of vitamin E, 20 mg of zinc, and 1 mg of copper) proved to be effective and safe for improving the oxidative stress in DR [120]. Moreover, ingestion of coenzyme Q10 and combined antiox-

idant therapy was significantly superior for decreasing the LPO levels, to values closer to normal, an outcome similar to that reported recently in vitreous humor [120,121].

8. Pespectives

Changes in dietary habits and lifestyles associated with rapid economic growth have dramatically increased the incidence of diabetes and related chronic microvascular complications, i.e., DR. So far, several studies have demonstrated the importance of several environmental, genetic, and epigenetic factors. Modulation of epigenetic changes by pharmaceutical means may provide a potential strategy to retard the progression of DR. Besides intense medical management, these strategies include dietary measures and the introduction of epigenetic drugs, such as inhibitors of DNA methylation and histone demethylases. We presume that intense medical management may be especially helpful with subjects having increased genetic risk according to the findings of genetic studies.

To conclude, the impact of nutritional factors is still insufficiently understood for patients with DR and well-designed prospective randomized clinical trials are needed to address the role of nutritional factors, including antioxidants. Genetic biomarkers (DNA and RNA gene polymorphisms) may be especially helpful in risk prediction, prognosis, or prediction of response of DR on drugs or nutritional factors. Finally, personalized medicine will most probably have an important part in managing subjects at increased risk for DR according to clinical, genetic, and epigenetic information providing that genetic tests (i.e., cost) become more widely available and that the genetic markers will be confirmed in prospective studies.

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