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Potential molecular biomarkers of oxidative stress in age-related macular degeneration

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Abstract

Molecular biomarkers are of utmost importance for the diagnosis, prognosis, and treatment monitoring of several diseases. In age-related macular degeneration (AMD), the major cause of irreversible blindness in the elderly, the diagnosis, prognosis, and therapeutic assessment are performed by means of imaging exams such as color fundus photography, fundus autofluorescence, optical coherence tomography, fluorescein angiography and indocyanine green. The use of molecular biomarkers to warn physicians and patients about a favorable or unfavorable progression of AMD is not usual. The current review aims to describe the participation of oxidative molecules in triggering AMD and correlate them with the respective serum, plasma, and urine measurements, non-invasive and low-cost methods, which provide a personalized pre-clinical, and consequently a pre-symptomatic, approach for AMD screening, control, and prevention. The data sources used in this review study include Pubmed, MedlinePlus Health Information, and Elsevier Science. Articles cited in the reference list obtained through this search were also reviewed, whenever relevant.

Introduction

Age-related macular degeneration (AMD) is the main cause of irreversible blindness in the elderly. Factors related to its genesis such as aging, genetic, environmental, dietetic, and cardiovascular, become the prognosis of this disease unfavorable. Biological markers (biomarkers) in AMD are hardly used; rather, imaging exams such as color fundus photography (CFP), fundus autofluorescence (FAF), optical coherence tomography (OCT), fluorescein angiography and ICG are the selected options for its diagnosis, prognosis, and assessment of the therapeutic efficacy. A biomarker is an indicator of normal biological and pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. Hence, the analysis of oxidative stress biomarkers in AMD may be valuable considering that oxidative stress is the state where there is an imbalance between the antioxidant defense system and the generation of reactive species [of oxygen (ROS) or nitrogen (RNS)], with the involvement of multiple molecules in the progression of the disease.

It is first necessary to point out that oxidizing and antioxidant substances are generated in a scenario of oxide-reduction reactions, where oxidation implies electron gain and reduction, its loss. Many authors have adopted the term redox system imbalance to refer to oxidative stress [2,3], the main AMD triggering factor. The retina is a tissue exposed to oxidative stress due to its high metabolism, large concentrations of polyunsaturated fatty acid content, exposure to visible light (between 400 - 700 nm) and the presence of photosensitive molecules such as rhodopsin and lipofuscin [4]. The chronic oxidative stress induced by UV-light exposure, along with high ocular oxygen levels, generate ROS and RNS, such as superoxide ($O2^{-}$ •), hydrogen peroxide (H2O2), singlet oxygen (1O2), and peroxynitrite (ONOO-) and trigger permanent peroxidation of polyunsaturated lipids in the membrane system of ocular photoreceptor membranes leading to lipid peroxidation–derived protein modifications, which induce damage to retinal pigment epithelial (RPE) cells [5-8]. Nevertheless, the body synthesizes antioxidant enzymes by means of complex mechanisms such as the nuclear factor e2-related factor 2 (Nrf2) activated. The Nrf-2 activation induced by the reactive oxygen species promotes an increase in the expression of antioxidant enzymes, responsible for maintaining the retinal homeostasis and consequent visual function [9]. With aging, the increase of ROS/RNS and the decrease in the expression of antioxidant enzymes are observed, making the macula more susceptible to AMD [10]. The redox imbalance can potentially increase the expression of toxic molecules such as malondialdehyde (MDA) and advanced glycation end products (AGEs), which induce the accumulation of lipofuscin inside the RPE cells [11-13]. The accumulation of intracellular lipofuscin causes dysfunction of RPE cells [14-15], leading to an anomalous deposition of lipids and cholesterol esters in the Bruch's membrane (BM) [16-17], forming druses and other extracellular deposits [18-19], clinically characterizing the onset of AMD. Lack of cure has encouraged the discovery of new diagnostic, prognostic and therapeutic strategies that may help to prevent or slow the onset and/or progression of AMD. From the onset of AMD (characterized by redox imbalance) to the advanced stage of the disease (characterized by the increase of cytokines, enzymes, and growth factors) several molecules come into play. Hence, this review aims to describe the role of oxidative molecules responsible for triggering AMD and correlate them with their serum, plasma, and urine levels.

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It is meant to serve as a practical guide for ophthalmologists in their daily practices, with an attempt to assess the possible AMD biomarkers by means of non-invasive, safe, and inexpensive exams, and limited to nongenetic compounds.

Methods of literature search

The data sources used in this review study include Pubmed, MedlinePlus Health Information, and Elsevier Science. We used the following keywords and their synonyms in various combinations: age-related macular degeneration; biomarker; free radicals; aging; antioxidant/pro-oxidant balance; oxidative stress; macromolecular damage. When a specific molecule was identified, the specific risk factor was also used as a keyword in a second PubMed search to identify additional publications. Articles cited in the reference list obtained through this search were also reviewed, whenever relevant.

Oxidative stress of molecules

The reactive species, generated by enzymatic and non-enzymatic systems, have been associated with a large number of physiologic and pathological processes (20). Although they are essential for a variety of cellular defense mechanisms [21,22], ROS (as well as RNS) can cause oxidative damage in biomolecules [lipids, proteins, carbohydrates, and deoxyribonucleic acid (DNA)] when present in larger quantity than their system-mediated neutralization antioxidant defense. Under these conditions, ROS/RNS become unstable, very reactive, and short-lived, preventing the detection of these reactive species in biological samples, such as tissues, fluids, and complex biological systems [23,24]. The suggested option to measure ROS is the use of a species with stable, detectable, and measurable characteristics that can reveal the pathways that may trigger some pathologies [25]. The cellular products resultant from the oxidative damage into lipids, proteins, carbohydrates, and DNA are used as biomarkers of oxidative stress, providing indirect detection of the ROS activity in several diseases [23], including AMD [4, 26-27].

Lipid peroxidation biomarkers

The lipids are organic compounds with essential functions for the human body which include energy storage, membrane integration and structure (phospholipid bilayer), in the biosynthesis process of important substances such as prostaglandins, and as an enzyme cofactor [28,29]. The lipids represent the main target for the ROS [30], especially the glycolipids, phospholipids, and cholesterol [31]. The lipid peroxidation resultant from the oxidation of polyunsaturated fatty acids (PUFAs) produces lipid hydroperoxides and several aldehydes [29,32], with a negative impact in the body, triggering several cellular damages which may lead to multiple diseases [31,33]. The retina is one of the body tissues with the highest concentration of PUFAs, essential for the maintenance of the physiological retinal function and development [34,35]. The photoreceptor outer segments (POS), with high PUFAs (up to 70%), are easily peroxidized due to the presence of high concentrations of oxygen and UV irradiation. These POS are continuously phagocyted and degraded by the RPE cells. Under oxidative stress and inflammation, these lipids become dysfunctional and generate a mix of lipid oxidation products (LOP) which will further form malondialdehyde (MDA) or 4-hydroxy-trans-2-nonenal (HNE), constituting a material source resistant to lysosomal degradation named lipofuscin [8,14,36-39]. The accumulation of non-degradable material in the lysosomes causes intracellular accumulation of phagosomes and autophagosomes, with subsequent reduced phagocytosis and autophagic sequestration. This sequence of events has been demonstrated in an experiment in which the lysosomal dysfunction induced by Bis-retinoid N-retinyl-N-retinylidene ethanolamine (A2E), the major component of lipofuscin, significantly reduced the capacity of cultivated RPE cells to phagocyte the POS and sequester cytoplasmic material [40]. Extracellular deposits of non-degradable material, resultant from the reduced phagocytic capacity, may trigger additional damage, such as apoptosis and formation of drusen [41-44]. Many studies demonstrate the presence of esterified and unesterified cholesterol, triglyceride, and lipoproteins in drusen [19,45-47], which induce the inflammatory response [48]. In this regard, the accumulation of apolipoprotein B100 (apoB100) lipoprotein particles in the BM and RPE cell apoptosis are considered critical events for the triggering of AMD [4,6,19,49-50]. Regarding the systemic biomarkers, several studies have shown that AMD patients have higher lipid peroxidation levels in their plasma [51-52].

Low-density lipoproteins

Oxidized Low-Density Lipoproteins (OxLDL) originate from low density lipoprotein (LDL) in pro-oxidant tissue environment in which lipids and proteins become oxidatively modified causing diseases such as atherosclerosis [53,54]. The retina, particularly the photoreceptor layer rich in unsaturated lipoproteins, is readily susceptible to oxidation by ROS. Lipid oxidation increases with the age and presence of oxidized low-density lipoproteins are particularly relevant to retinal para-inflammation [55]. Studies suggest that OxLDL may also be involved in pathobiological alterations of RPE cells [56-59]. It is important to point out that RPE cells express functional receptors for both LDL (LDL-R) and CD36 [60,61], which take up LDL and OxLDL in large quantities, both in vitro and in vivo [44]. The accumulation of oxidized lipid-protein complexes in the RPE prevents phagosome maturation by blocking phosphatidylinositol 3-kinase (PI3K) recruitment to the phagosomal membrane, leading to delayed processing of internalized POS [62]. Consequently, the oxysterols, products of autoxidation or cholesterol enzymatic oxidation, in OxLDL become cytotoxic to RPE cells [44,62]. Both the alterations of the sensory retina as well as those of RPE, induced by OxLDL, contribute to AMD pathogenesis [44,58]. From these alterations, formation of subretinal deposits may be formed [63] since accumulation of oxidized lipids and lipoproteins has been found in BM and is thought to be an early event in the development of AMD [59,64]. Another study revealed that besides OxLDL, native LDL can potentially up-regulate the expression of vascular endothelial growth factor (VEGF), a major angiogenic and inflammatory factor in RPE cells (56). Nevertheless, it has been demonstrated that the OxLDL treatment decreased human retinal pigment epithelial (ARPE-19) cells viability in a dose-dependent manner, whereas native LDL had no effect. Incubation of ARPE-19 cells with 10 mg/mL OxLDL induced marked apoptosis, compared with untreated control cells. OxLDL also increased VEGF expression and decreased Pigment Epithelium-Derived Factor (PEDF) expression, whereas native LDL had no significant effect. The VEGF-to-PEDF ratio was elevated after OxLDL treatment, suggesting that OxLDL treatment induced cellular changes in ARPE-19 cells that seemed to reflect pathogenic events in neovascular AMD [65]. It was also demonstrated that OxLDL induces apoptosis of human retinal pigment epithelium through activation of ERK-Bax/Bcl-2 signaling pathways [66], and promotes NLRP3 inflammasome activation [67], playing a role in the pathogenesis of AMD, also through this pathway. A study corroborated the reported findings that oxidized LDL increases the expression of inflammatory factors as well as the production of ROS, which could be regulated by the activation of the canonical pathway [68]. It was suggested that OxLDL could promote senescence of RPE cells, inducing outer blood-retinal barrier dysfunction as an early

pathogenesis of AMD [69]. Additionally, free-radical oxidation of lipids in LDL produces protein-bound (carboxyalkyl) pyrroles [70], which bind to cellular proteins forming advanced lipoxidation end products (ALEs) and induce an inflammatory response, contributing to the progression of AMD [71,72]. Corroborating this theory, studies report that LDL and OxLDL treatments increase the expression of fibronectin, laminin alpha 1, collagen type IV alpha 2, and transforming growth factor beta-2 (TGF-b2) in RPE cells in vitro, similar to the pathogenic events identified in AMD [58]. It has been shown that OxLDLs are immunohistochemically detected in surgically excised choroidal neovascular (CNV) membranes, revealing those macrophages and RPE in the CNV membranes express cell surface scavenger receptors for oxidized lipoproteins. This study suggested that macrophages may accumulate to take up oxidized lipoproteins in AMD [57]. Experimental studies in vivo and in vitro, inducing RPE to a lipid oxidative damage and chronic inflammation by means of exposure to OxLDL, have been performed to assess the antioxidant and anti-inflammatory effects of several substances [73-77].

Another theory correlates the origin of the lipoproteins in BM with plasma LDL particles in the choriocapillaris. Circulating human plasma LDL may enter the RPE through fenestrated junctions in the choriocapillaris endothelium, crossing BM to reach the RPE [44,78]. Considering this hypothesis, the serum results of OxLDL become relevant. The increased LDL in serum was related to the increased risk of AMD whereas increased high-density lipoprotein (HDL) was related to decreased risk [79,80]. A study with 45 patients affected by exudative age-related macular degeneration (E-AMD), compared with 45 sex- and age-matched healthy controls, reported a positive and significant correlation between plasma OxLDL concentration and homocysteine in patients with E-AMD [81]. Conversely, an observational prospective cohort study assessed the relationship between serum OxLDL cholesterol and the incidence of AMD over a 25-year period. A cohort of 2468 participants was selected for OxLDL measurements, revealing that OxLDL was associated with neither a worsening condition along the AMD severity scale, nor with the incidence of late AMD [82]. Another study with fewer participants also did not correlate the serum levels of OxLDL with AMD [83].

Malondialdehyde

Malondialdehyde (MDA), a highly reactive three-carbon dialdehyde produced as a byproduct of PUFA peroxidation, is catalyzed by free radicals [11,84] and has been detected in high levels in several diseases [85]. Its molecule is small, with polar characteristics and highly soluble in water [86]. MDA is one of the most studied biomarkers of oxidative stress, used in several experimental human, animal and even plant models. This biomarker presents mutagenic and cytotoxic effects in the body, important in the assessment of lipid peroxidation [87].

A study reported that the aerobic illumination of human lipofuscin isolated from aged donors leads to formation of hydroperoxides and MDA [88]. Another study confirmed the presence of MDA in RPE [26]. In fact, some studies have also shown that MDA induces RPE dysfunction and VEGF expression in RPE [89-93]. Similarly, MDA was found in the drusen [26,93]. From the epidemiologic perspective, clinical trials have shown the risk of high dietary intake of linoleic acid (LA) for AMD [94,95]. Experimentally, higher dietary intake of LA promoted progression of the CNV membrane in mice with increased MDA levels [92]. Many studies have suggested that MDA levels are higher in the blood of AMD patients than in that of healthy subjects [96-100]. A systematic review and meta-analysis, involving twelve case-control studies with a total of 634 AMD patients and 656 controls without AMD, concluded that there is some evidence of higher levels of MDA in AMD patients compared with healthy controls; however, this result should be interpreted with caution because of extreme between-study heterogeneity and the possible effect of publication bias [101]. It is important to point out that age is an important confounder when assessing the potential role of blood MDA in oxidative stress. Indeed, advanced age is associated with increasing MDA. Although most studies that report MDA as a risk factor for AMD claim that patients and controls were age-matched, the precision of matching and the statistical test of the outcome of matching are seldom shown [101].

Isoprostanes

A great advancement in the assessment of lipid peroxidation was made with the discovery of F2 isoprostanes (F2-IPs). They comprise a class of compounds structurally similar to prostaglandin-F2 (PGF2), which are produced by the peroxidation of the arachidonic acid (AA) and of other PUFAs, such as the linolenic, the eicosapentaenoic (EPA) and the docosahexaenoic acid (DHA) [102-104]. F2-IPs are considered the most sensitive and most stable marker of lipid peroxidation and oxidative stress [105-106]. High levels of F2-IPs in human fluids and tissues were found in atherosclerosis, chronic inflammation, diabetes, pulmonary diseases, Alzheimer's, and other neurodegenerative disorders [107-109].

At first, it is important to emphasize that the retinal photoreceptors contain the highest concentrations of AA and DHA in relation to any other known membrane [110]. They are highly peroxidizable [111]. Additionally, the formation of F2-IPs is regulated by the oxygen tension, so that the production of isofurans (IsoF), oxidation products generated from the nonenzymatic oxidation of AA (formed instead of F2-IPs in settings of increased oxygen tension), is favored as the oxygen concentration increases [105], which makes the retina an appropriate tissue for the formation of F2-IPs [4]. High levels of this biomarker were related to risk factors for diseases associated with AMD [112], such as smoking [113], and multiple systemic diseases [106,114-115]. It is known that the lipid peroxidation has been widely associated with inflammation [116], one of the main pillars of AMD genesis. While one of the studies did not report any association between early AMD and serum levels of 8-Iso-Prostaglandin F2 [117], another one suggested a significant association of mean plasma levels of IsoFs with AMD [118]. Nevertheless, a more relevant association was observed in a study that included 238 adults with AMD and 390 age- and sex-matched controls without AMD, reporting that higher levels of urinary F2-IPs were associated with AMD [119] and have been used as biomarkers of AMD in several studies [120-123].

4-Hydroxynonenal

4-Hydroxy-2,3-trans-nonenal (4-hydroxynonenal, HNE) or 4-hydroxyhexenal (4-HNE) is highly reactive α,β-unsaturated hydroxyalkenal [123]. 4-HNE is one of the main end products of the lipid peroxidation of the AA and has been widely accepted as an inducer and mediator of the oxidative stress, being involved in the pathogenesis of several degenerative diseases such as Alzheimer's, atherosclerosis, cataract, and cancer [11,124-125]. In the retina, a large number of proteins contain HNE adducts, suggesting that HNE is the main oxidant of the retina. It has been shown that 4-HNE binds to cellular proteins forming ALEs [71-72], which may induce an inflammatory response, believed to play a role in the pathogenesis of AMD. A significant number of proteins modified by the HNE have been identified in damages induced by light [126-127], age [128-129] and in a model of retinitis pigmentosa in pigs [130]. As an important

mediator of oxidative stress, 4-HNE induces lysosome dysregulation, lipofuscin generation and apoptosis of RPE cells [131-133]. It is known that proteins modified by the HNE in the photoreceptors exert their toxic effects on RPE for also inducing angiogenic cytokines [134], such as interleukin-6 (IL-6), interleukin-1-β (IL-1β) and tumor necrosis factor alpha (TNF-α) [135]. In a study, high levels of 4-HNE generated superoxide anion which leads to the apoptotic degeneration of RPE through the activation of NADPH oxidase 4 (NOX4) [136]. However, despite the confirmed participation of HNE in the AMD pathogenesis [39], it was observed that the proteins modified this biomarker and did not accumulate in the neurosensory retina during the progression of the disease, suggesting that the pathways involved in the HNE detoxification or in the removal of the proteins modified by HNE are adequate to prevent its accumulation. Hence, HNE has been considered a little sensitive retinal biomarker for AMD [137]. Conversely, due to the toxicity in ARPE-19) cells, HNE has been used in experimental studies that search for information about antioxidant and anti-proliferative properties of several substances such as the quercetin [138], N-acetyl-cysteine [139], and edaravone [140]. HNE has also been used in experiments that seek a better understanding of AMD mechanism [26,90,139,141-142]. For example, a study investigated the molecular and cellular effects of cigarette smoke exposure on human RPE cells. Exposure of ARPE-19 or primary human RPE cells to cigarette smoke extract (CSE) or hydroquinone (HQ) caused oxidative damage and apoptosis. Evidence of oxidative damage also included increased lipid peroxidation (4-HNE) and mitochondrial superoxide production, as well as a decrease in intracellular glutathione (GSH) [143]. Therefore, HNE plasma levels have been used to assess the effect of oxidant and antioxidant substances in AMD epidemiologic studies [120].

Carboxyethylpyrrole

Carboxyethylpyrrole (CEP) protein adducts belong to a family of 2-(ω-carboxyalkyl) pyrrole adducts generated from the oxidation of PUFA [70], exclusively from DHA compounds [144]. DHA is present in small quantities in most tissues, but it is a major structural lipid of the retina presenting particularly high levels in this neural tissue [145-146]. Although rare in most human tissues, o DHA is the most oxidizable fatty acid in humans and is present in ~80 mol % of the polyunsaturated lipids in POS [110]. DHA may be involved in the permeability, thickness, fluidity, and other properties of the membrane of photoreceptors (146), and its insufficiency is linked to changes in the function of the retina [146-147]. Immunocytochemical analysis showed that CEP was present in photoreceptor rod outer segments and RPE cells in both mouse and human retinas [13,144]. Additionally, it has been reported that CEP protein adducts found in AMD are immunogenic, inducing autoantibody production and inflammation in the retina [13,144,148]. The immunization of mice with CEP-modified mouse serum albumin (CEP-MSA) induced antibodies against CEP and led to inflammatory responses such as the deposit of complement component-3 in BM and macrophage infiltration [149]. CEP is involved in the pathogenesis of both angiogenesis in the retina (wet AMD) [150] and geographic retinal atrophy (dry AMD) [149]. Regarding wet AMD, it has been shown that CEP adducts stimulate angiogenesis in the chick embryo chorioallantoic membrane (CAM) and corneal micropocket assays and exacerbate CNV in a mouse model. These results, coupled with the elevated levels of CEP adducts in AMD tissues, strongly suggest that CEP may play a role in the development of the wet (exudative) form of AMD. Overall, these results suggest that CEP-induced angiogenesis utilizes VEGF-independent pathways and that anti-CEP therapeutic modalities might be of value in limiting CNV in AMD [150]. Confirming the inflammatory and angiogenic condition of CEP, studies revealed that it induces PYD domains-containing protein 3 (NLRP3) inflammasome priming, via Toll-like receptor 2 (TLR2) in macrophages, promoting IL-1β release [151]. Similarly, other studies reported that CEP-adducts cooperate in a highly specific manner to amplify low-grade inflammation mediated by TLR2/TLR1-activating pathogen associated molecular patterns (PAMPs) that produce TNF-α, IL-12 polarization [152]. Hence, the presence of CEP protein adducts in the outer retina is considered an early marker of high risk for AMD development [13,149,153]. An immunocytochemical analysis of BM/choroid tissue demonstrated higher CEP and carboxymethyllysine immunoreactivity in BM/RPE/choroid tissues of donors with AMD than of donors with normal eyes [13,144]. A study confirmed these findings revealing that CEP protein adducts are more abundant in the tissue of donors with AMD than in those with normal eyes [154]. Consequently, DHA oxidation products may be used as biomarkers to identify susceptibility to AMD [144]. Specifically, CEP markers increase the predictive accuracy of AMD [155], as well as its prognosis [156].

A study with 19 donors revealed that the mean level of anti-CEP immunoreactivity in human plasma with AMD was 1.5-fold higher than in age-paired controls. The serum levels of AMD patients presented a mean of anti-CEP antibody 2.3-fold higher than those of controls. Out of the patients (n = 13) who presented higher levels of antigen and autoantibodies than the control patients without AMD, 92% had AMD [144]. Another study that used a significantly larger sample size (916 AMD and 488 control donors), determined a 1.6-fold increase in mean plasma anti-CEP immunoreactivity and a 1.3-fold increase in mean plasma anti-CEP autoantibody titer in AMD donors with respect to control donors [155]. The plasma analysis of the 58 AMD and 32 control donors also revealed that CEP increased ~86% in the AMD cohort and that in combination with N-ε-carboxymethyllysine (CML) and pentosidine increased the potential use of the biomarker to assess risks of and susceptibility to AMD [156]. Interestingly, proteomic CEP markers alone can distinguish between AMD and normal donors with approximately 76% accuracy and when analyzed together with genomic markers, the discriminatory accuracy increased to about 80% [157].

Protein oxidation biomarkers protein carbonyl

Protein carbonyl content is an index of the amount of oxidative damage to proteins due to the direct attack of free radicals or modification of proteins by the carbohydrate oxidation products or PUFA. The carbonylated proteins are composed of a carbon atom double-bonded to an oxygen atom, commonly found in determined functional groups, named aldehydes and ketones [158-160]. The binding of carbonyl groups in residues of protein amino acids is a major hallmark for oxidative modification [161]. Carbonyl protein, considered the most widely studied marker of protein oxidation, has been used in several publications as a parameter of oxidative stress [162-164], due to the chemical stability of the carbonyl group, its oxidation-irreversible products and irreparable changes induced by the group, although the cells display native enzymatic systems that eliminate the changed proteins and maintain homeostasis and cell survival [165]. When these enzymatic systems fail, the carbonylated proteins accumulate in the cells, interrupting their functions [166]. Concentration increase, and consequent accumulation in the body, is associated to smoking and aging, as well as to the development of several pathologies such as Alzheimer's, Parkinson, Huntington, and respiratory syndromes [160,167-168]. The plasma biological samples are the most used analytical methods for the detection of the carbonyl groups [158]. Values of protein carbonyl groups in plasma significantly higher than in CG have been observed in patients with wet AMD [169].

Other studies have shown similar results, reporting significant increase of protein carbonyl groups in AMD when compared to control group [170-171]. It has also been demonstrated that aging significantly affects the antioxidant status and oxidative damage in AMD patients when compared to controls, and an increase of protein carbonyl groups was identified in both early- and late-AMD patients [99]. Conversely, a study that sought to examine the relationship between inflammation, oxidative stress, and endothelial dysfunction markers with a 20-year cumulative incidence of early AMD did not find a relationship between total carbonyl content (TCC) and early AMD [117].

Biomarkers of carbohydrate oxidation

AGEs comprise a heterogeneous group of compounds derived from a non-enzymatic reaction named glycation, Maillard reaction, a spontaneous post-translational modification in which a carbonyl group of reducing sugars is covalently bound to proteins, lipids and nucleic acids [171-172] The α-dicarbonyls can react with lysine and arginine functional groups on proteins, leading to the formation of stable AGE compounds, such as CML, a lysine modification, and pentosidine, a fluorescent lysine-arginine cross-link, generated by oxidation of carbohydrate [173]. Their physiological serum level is in the range of 2–10 Lg/mL and increases with age [174]. AGEs, generated from oxidized carbohydrate products, promote inflammation in many general age-related diseases such as Alzheimer's disease, atherosclerosis, diabetes, osteoarthritis [175-177], and represent a risk factor for AMD development [178]. CML, a major circulating advanced glycation end-product, was the first AGE to be found in AMD BM and drusen [179]. Further studies reported that CML increases with age in BM [180-181]. Other studies have identified glycoxidation products in subretinal membranes of patients with AMD [178,182], whereas the study that assessed the macula of human donor retinas from normal eyes and eyes with early AMD and advanced AMD with GA demonstrated that RAGE and AGE were elevated on RPE and photoreceptor cells in early and advanced dry AMD [183], especially in RPE overlying drusen-like deposits on BM [184]. Other studies confirm the presence of AGEs also in the drusen, as well as in the BM, RPE, and choroidal extracellular matrix of elderly people [13,185]. AGEs influence the profiles of the ARPE-19 expression and may contribute to the decrease in the degrative capacity of the lysosomal enzyme and increase in the lipofuscin accumulation. AGEs formation in the BM may have important consequences for RPE dysfunction related to aging and may damage the outer retina [186]. AGEs stimulate RPE cells to secrete different anti/proinflammatory factors, which trigger the para-inflammation state in RPE cells, that is, short-term adaptive RPE cell reaction on AGE stimulation [187]; however chronic RPE exposure to AGE favors deregulation of RPE function and leads to photoreceptors and RPE cells degeneration and atrophy [181]. Oxidative protein modifications like CML, elevated in AMD BM, stimulate neovascularization in vivo, suggesting possible roles in CNV [188]. Corroborating the findings of this study, it has been demonstrated that AGEs can stimulate the proliferation of choroid endothelial cells, the expression of matrix metalloproteinase type 2, and growth factors such as VEGF [189]. AGEs undergo endocytosis and are removed by macrophages [190]. The failure in macrophage recruitment may lead to increased RPE exposure with AGEs and damage retinal tissue in the pathological angiogenesis process [187]. Several different receptors for AGEs have been discovered, one of which, RAGE, initiates the intracellular signaling that disrupts cellular function through its recognition and binding of AGEs. RAGE is a member of the immunoglobulin superfamily of receptors [191-192]. A study with 300 early AMD patients, 300 patients with exudative AMD, and 800 healthy controls revealed a significant association between RAGE gene rs1800624 and rs1800625 polymorphisms and AMD risk [193]. AGE-R3, also known as galectin-3, is elevated in AMD BM [194]. In vitro studies have shown that AGE-RAGE binding on macrophages and microglia leads to oxidant stress and activation of the nuclear factor kappa β (NF-κβ) [170,195-196]. Activation of NF-κβ induces an increase in the expression of genes associated with inflammatory cytokines, enzymes, and adhesion molecules, which, in turn, are closely related to AMD [197]. NF-κβ sites control cellular expression of RAGE, linking RAGE to the inflammatory response [198]. A study performed in vitro in ARPE-19 cells revealed that AGEs increased the NF-κβ activation resulting in pro-apoptotic changes in ARPE-19 cells and that, along with OxLDL, homocysteine (Hcy), homocysteine thiolactone (HCTL), they act as pro-oxidant metabolites in RPE that promote AMD through oxidative stress, inflammation, chemotaxis, and neovascularization [72].

The plasma analysis of 58 AMD and 32 control donors demonstrated that CEP was elevated ~86% in the AMD cohort and that, in combination with CML and pentosidine, it increases the potential use of the biomarker in the risk evaluation of and susceptibility to AMD [199]. Another study revealed that the mean level of anti-CEP immunoreactivity in AMD human plasma (n = 19 donors) was 1.5-fold higher than in the age-matched controls (n = 19 donors). Similarly, the serum from AMD patients demonstrated mean titers of anti-CEP autoantibody 2.3-fold higher than controls. It has also been observed that out of individuals (n=13) presenting both antigen and autoantibody levels above the mean for non-AMD controls, 92% had AMD. These results suggest that together CEP immunoreactivity and autoantibody titer may have a diagnostic role in predicting AMD susceptibility (144). Another analysis called skin autofluorescence (AF), a non-invasive marker for AGE in tissues, identified an increase in patients with neovascular AMD, suggesting that AMD is accompanied by enhanced systemic AGE accumulation, which may indicate a role in the pathophysiology of AMD [200]. However, a large population-based cohort involving 4,907 older adults revealed that the higher serum CML concentration had no significant cross-sectional association with prevalent AMD) [201].

Biomarkers of nucleic acid oxidation

Aging is associated with slowing down of the efficiency repair of DNA, and consequent accumulation of DNA damage [202-203], which leads to dysregulation of the cell function, therefore, to aging [206]. This oxidative damage to DNA may induce harmful genetic alterations [205]. It is known that the oxidative damage to DNA is processed by means of the cellular DNA repair systems, whose efficiency may reduce with age [205]. These concepts are valid for all body cells, including the cells of the retina [206]. It has been shown that AMD patients present a significantly higher level of endogenous DNA damage in their peripheral blood lymphocytes (PBL) than the control patients and that oxidative damage to DNA has contributed to this increase. Simultaneously, a higher sensitivity to hydrogen peroxide and to ultraviolet light, as well as a slower kinetics of DNA damage repair induced by these mutagenic were observed in AMD patients when compared to control patients. Additionally, AMD patients presented a larger extent to DNA damage than the mean of the general population [206].

8-Hydroxy-29-Deoxyguanosine

Oxidation of the nucleic acids, by means of the reaction of the hydroxyl radical on the 8-carbon of guanine results in the formation of 8-OHdG. This compound, which presents the molecular formula of

C10H13O5, is a highly mutagenic molecule, with stable characteristics. 8-OHdG is one of the most representative products of oxidative damage to DNA [207]. It can cross the cell membranes and may be detected in urine, serum, plasma, saliva, and tissue samples [33,208]. The increase in its levels has been observed in many systemic diseases, as well as in several human organs in age-related processes [209-210]. An age-related increase in oxidative DNA damage product in the intraocular fluid has been reported, providing further support that oxidative DNA damage is associated with aging [211]. Corroborating this hypothesis, a higher level of 8-OHdG in RPE and choroid of adult rats was reported when compared with young control groups [212].

In AMD experimental models, the increase of 8-OHdG in the retinal tissue was induced by the sub-retinal injection of human lipid hydroperoxide (HpODE), a product derived from the sub-macular region of elderly and AMD patients [213]. It may also be induced genetically [214], using H2O2 in cultured ARPE19 cells [215], induced by light [216-217] or the exposure to chronic cigarette smoke [218]. In regard to smoking, one of the main modifiable AMD factors, it has been demonstrated that exposure of ARPE-19 cells to a cigarette smoke concentrate (CSC) not only enhanced ROS levels but also induced 8-OHdG DNA lesions [219].

8- OHdG has been used as a marker of the oxidative stress in experiments that aimed to assess the antioxidant effects of substances such as vitamin D, associated with diphlorethohydroxycarmalol [215], astaxanthin [217] and a nutritional complex [220], as well as to assess the neuroprotective effect of SUN N8075 [221]. With this evidence, it is possible to affirm that 8-OHdG, the major product of oxidative DNA damage [222], is associated with AMD [148,170]. This hypothesis is strengthened by findings of increased levels in the 8- OHdG aqueous humor of AMD patients when compared with those in control groups [223-234]. The same results were observed in the blood serum of AMD patients, who presented increased levels of 8-OHdG when compared with non-AMD cohorts [170]. The serum levels of 8-OHdG measured in early- and late-AMD patients *versus* healthy controls showed that both early- and late-AMD patients had higher 8-OHdG levels than healthy controls (99).

Total oxidant status (TOS)

Total oxidant status (TOS) and total antioxidant status (TAS) and oxidative stress index (OSI) are oxidative stress parameters used to evaluate the overall oxidative stress status in the body [225]. An imbalance between TOS and TAS has been proposed to be responsible for the increased lipid, protein and DNA damage observed in AMD patients [170]. Serum (or plasma) concentrations of different oxidant species can be measured in laboratories separately, but the measurements are time-consuming, labor-intensive, and costly and require complicated techniques [226]. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the TOS of a sample is measured and it is named total peroxide (TP), serum oxidation activity, reactive oxygen metabolites (ROM) or some other synonyms [227]. A study with 156 early-AMD patients, 80 wet-late AMD, 72 dry-late AMD and 207 healthy controls reported that a significantly increased oxidative damage was associated with AMD patients >60 years of age of both genders when compared with controls. Both early- and late-AMD patients presented higher TOS levels than healthy controls [225]. Corroborating these findings, other studies also observed a significant increase in TOS levels in the sera of AMD patients when compared to controls [170,228-229].

Conclusion

At molecular level, the cell products resultant from the oxidative damage in lipids, proteins, carbohydrates, and DNA induce an up-regulated expression of inflammatory cytokines, adhesion molecules, matrix metalloproteinase type 2, VEGF, decreased PEDF expression, NLRP3 inflammasome activation, and NF-κβ activation. These molecular alterations are associated with the increase in the inflammatory response, endothelial activation, macrophage infiltration and consequently stimulate the proliferation of choroid endothelial cells. At structural level, the deposit of complement component-3 in BM and macrophage infiltration, drusen formation, photoreceptors and RPE cells degeneration and atrophy, and the subretinal neovascular membrane are observed. Regarding the use of the oxidation products as biomarkers in serum, plasma, and urine samples, it has been observed that the molecules resultant from the oxidative damage of lipids such as MDA, F2-IPs and CEP, as well as the molecules derived from the DNA oxidative damage, 8-OHdG, and TOS revealed an important association with AMD, and may be considered biomarkers of this disease. It is important to point out that because of the presence of the blood-retinal barrier, biomarkers might be only locally dysregulated inside the eye with no measurable systemic effect. Additionally, aging is a relevant point of misunderstanding, as some molecules besides presenting up-regulation in AMD, are also naturally increased with aging.

Conflicts of interest

None

Criteria for inclusion in the authors/contributors list

Active participation in the research, article drafting and revision.

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