

Potential Role of Oxidative Stress in Ocular Surface Inflammation and Dry Eye Disease

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Reactive oxygen species (ROS) are produced as a by-product during the mitochondrial respiration of the oxygen and potentially able to damage the tissues. Oxidative stress occurs as a result of the disruption of the balance between the anti-oxidant system and the pro-oxidant system found in cells. It has been accepted that overexpression of ROS can be induced in the ocular surface as a result of many acute and chronic diseases and even in normal aging. Recent studies demonstrated that oxidative stress damages the ocular surface and plays an important role in the mechanism of dry eye disease. There is a need to investigate the therapeutic modalities employing topical/systemic use of antioxidants in dry eye disease. This review will summarize the recent studies showing the important relationship between oxidative stress and dry eye disease.

Keywords: oxidative stress, reactive oxygen species, dry eye

Oxygen is the primary oxidant that is utilized during mitochondrial oxidative metabolism of various organic molecules in metabolic reactions providing cellular energy. Reactive oxygen species (ROS) that might be potentially damaging to tissues are generated as by-products during oxidative metabolism.¹ Reactive species, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals, involve radical and nonradical oxygen derivatives, which are generated by partial reduction of oxygen.² Oxidative stress is generated by a disturbance in the balance between anti-oxidant and pro-oxidant system in the favor of pro-oxidant in cells. The main idea that is emphasized by the definition of oxidative stress is the presence of intact systems generating oxidants and causing detoxification continuously during aerobic metabolism in cells.³ Balance is disturbed in favor of the pro-oxidant system when additional oxidation stress occurs and potentially generated ROS might result in cellular death by damaging lipids, proteins, carbohydrates, and nucleic acids via oxidative stress (Fig. 1).

It has been accepted that the oxidative damage plays a role in the aging process.⁴ In addition, oxidative damage has a role in the etiology of hypoxic damage, cancer, and cardiovascular diseases associated with reperfusion, and neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis). In the field ophthalmology, oxidative stress is involved in the pathogenesis of several eye diseases, such as senile cataract,⁵ age-related macular degeneration,⁶ uveitis,⁷ premature retinopathy,⁸ keratitis,⁹ and ocular inflammation.¹⁰ It has been considered that oxidized protein levels are increased in accordance with the elevated inflammatory activity during these diseases.

Tear fluid contains several antioxidants to protect the ocular surface against certain radicals, such as ascorbic acid, lactoferrin, uric acid, and cysteine.¹¹ Studies performed in autoimmune disease patients such as systemic lupus erythematosus,¹² rheumatoid arthritis,¹³ and Sjögren's syndrome (SS)¹⁴ indicated

elevated levels of oxidative stress biomarkers involving 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-Gua) and 8-Oxo-2'-deoxyguanosine (8-oxodG) (markers of oxidative DNA damage), 4-hydroxy-2-nonenal (4-HNE) and hexanoyl-lysine (HEL) (markers of late and early-stage lipid peroxidation, respectively), thioredoxin and methyl glyoxal in the saliva, salivary gland, synovial fluid, and serum in comparison to the control groups.¹⁵⁻¹⁹ The overexpression of ROS production might be induced on the ocular surface in consequence of prolonged exposure to atmospheric oxygen and inadequate support of antioxidants due to the instability in tear film.

We carried out a PubMed query including the key words "oxidative stress," "dry eyes," and "antioxidants" searching the literature between 1990 and 2017. American Academy of Ophthalmology Preferred Practice Pattern guidelines for scientific evidence were adapted for the selection of the literature.

While in vitro, in vivo experimental, and animal studies were included in this review, on level three, studies including leader opinion, descriptive studies, expert committee reports, case reports, and case series reports were excluded.

The Role of Oxidative Stress in Sjögren and Non-Sjögren Related Dry Eye Disease

Research in recent years provide accumulating evidence for a possible role of oxidative stress in the pathogenesis of Sjögren or non-Sjögren syndrome related dry eye disease.

In 2007, Cejkova et al.²⁰ reported elevated expression of xanthine oxidoreductase/xanthine oxidase in conjunctival impression cytology samples of SS patients with dry eyes. Cejkova et al.²¹ also reported increased expression of superoxide dismutase (SOD), catalase, and glutathione peroxidase in SS patients with similar methodology. Recently, Choi et al.²² reported increased expression of late lipid peroxidation markers, 4-HNE, and malondialdehyde (MDA) in the tear film



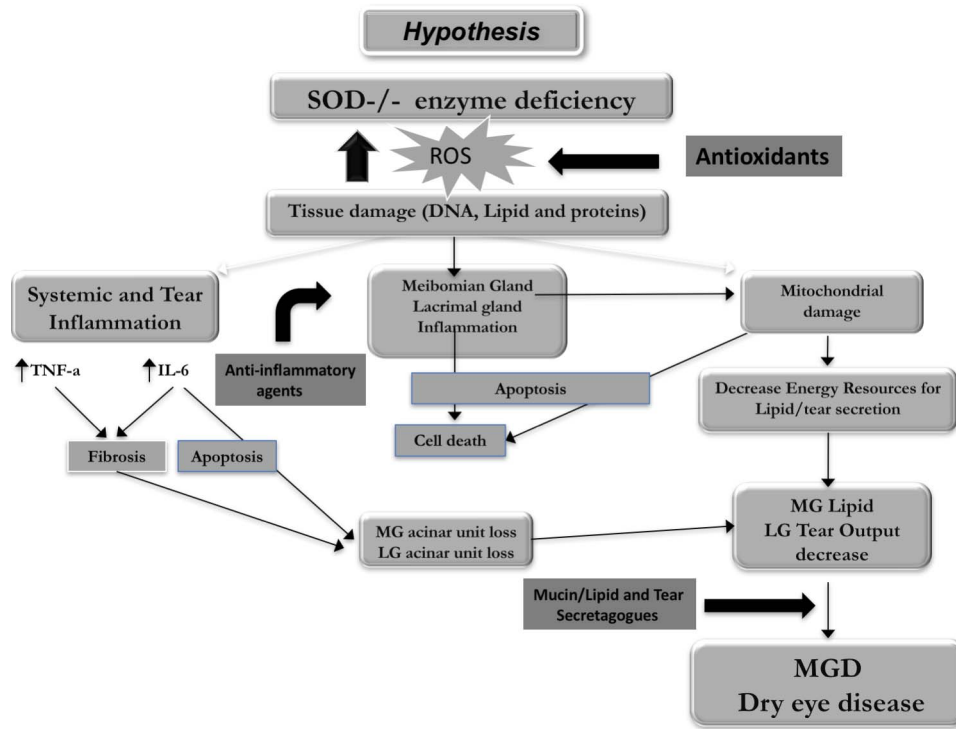


FIGURE 1. SOD enzyme family is a major antioxidant system in all tissues and responsible for the removal of ROS from cells. An imbalance between radical-scavenging systems and free radical generation in the tears, Meibomian gland, and mitochondria may result in tissue inflammation, damage, and accumulation of ROS. The effects of these reactive species are wide-ranging, but three reactions are particularly relevant to cell injury: lipid peroxidation of membranes, oxidative modification of proteins, oxidative damage to DNA. The accumulation of ROS due to SOD enzyme deficiency in ocular tissues may lead to Meibomian gland disease and dry eye disease.

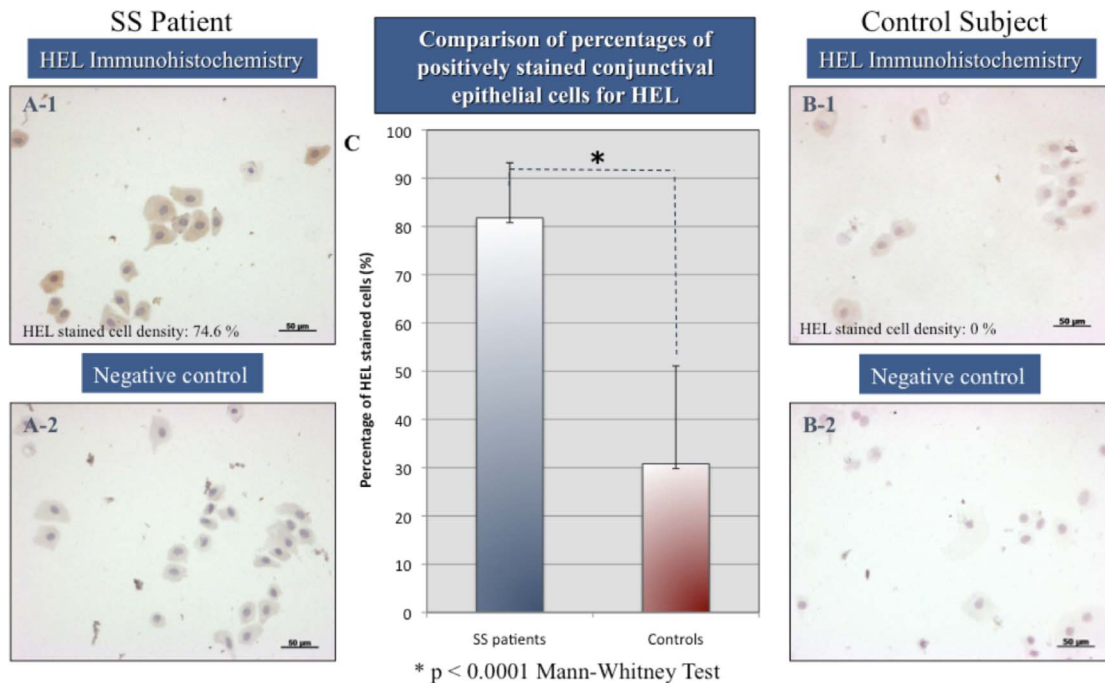


FIGURE 2. Immunohistochemistry staining analysis of the early lipid oxidation marker in brush cytology specimens from an SS patient and a control subject. Compared to the healthy control subject (B-1), immunohistochemistry reveals that diffuse lipid oxidative stress increases the positively HEL stained cell density in the SS patient (A-1). As can be seen from the graph, notable higher percentage of positively HEL stained cells in the SS patients (C). Images of the immunohistochemical negative controls (A-2, B-2).

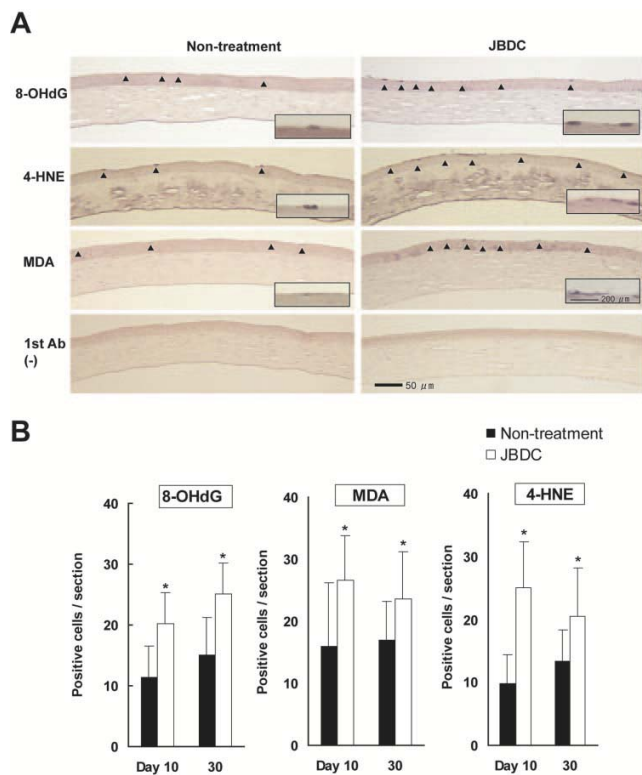


FIGURE 3. Immunohistochemical analysis of mice corneal epithelial tissue, using the oxidative stress-related markers. **(A)** Representative images from mice corneal epithelium on day 10. *Black arrowheads* indicate the positive stained cells. **(B)** Note the significant increase in number of oxidative stress markers positive stained cells in corneal epithelia after environmental dry eye stress at 10 and 30 days. Quantitative analysis in positive 8-OHdG (*left*), MDA (*center*), and 4-HNE (*right*) cells. Data represent the mean \pm SE of 16 corneas. $**P < 0.05$ versus the nontreatment group. JBDC, Jogging board dry eye condition group.

and ocular surface of patients with non-SS dry eyes. MDA levels were found to correlate with the tear film break up time, Schirmer scores, vital staining scores, conjunctival goblet cell density, and symptom scores. A previous study by Wakamatsu et al.²³ reported lipid oxidative stress-related alterations in the tear film and conjunctiva of patients with SS. They demonstrated elevated levels of HEL (Fig. 2) and 4-HNE lipid oxidative stress markers in the tear film and bulbar conjunctival impression cytology samples of patients with SS.²³ They also reported that ROS production was associated with cell membrane lipid peroxidation, and inflammatory cell infiltration in the ocular surface-lacrimal gland unit. Their study suggested a role for oxidative damage in the pathogenesis of SS.²³

Such observations appear to have paved the way to investigations focusing on elimination of oxidative stress in dry eye disease as well.

Cavet et al.²⁴ reported a significant dose dependent decrease in glucose oxidative induced ROS production after treatment of human corneal epithelial cells with green tea epigallocatechin gallate (EGCG). The authors concluded that EGCG had anti-inflammatory and antioxidant properties holding therapeutic potential in dry eye disease.

Macri et al.²⁵ looked into the changes of lipid peroxidation markers in conjunctival epithelial samples of dry eye patients and noted improvement upon 1 month of treatment with combination of preservative free hyaluronic acid 0.15% and

vitamin B12 eye drops, delivered before cataract surgery. In a recent study, Li et al.²⁶ suggested radical scavenging agent against hyperosmolarity primary human corneal epithelial cells. Chen et al.²⁷ later found that herbal extractions of ferulic acid, an anti-oxidant agent, and Kaempferol, an anti-inflammatory agent, were effective in dry eye treatment in rabbits.

The Role of Oxidative Stress in Aging Animal Models of Dry Eye Disease

Animal studies provide invaluable information into the role of oxidative stress in various forms of dry eye disease. Batista et al.²⁸ studied the effects of aging on lacrimal gland structure and secretory activity and changes in the expression of oxidative stress markers in male Wistar rats.

The authors noted accumulation of lipofuscin-like material in the cytoplasm of lacrimal gland epithelial cells with a decline in intracellular vitamin E from 2 to 24 weeks. Bucolo et al.²⁹ demonstrated that an ophthalmic formulation based on taurine and sodium hyaluronate had a relevant antioxidant effect on the corneal epithelium of rabbits in which dry eye was induced by atropine sulfate.²⁹

Nezzar et al.³⁰ characterized the expression profiles of isotypes of antioxidant enzymes such as glutathione peroxidases in human Meibomian and conjunctival tissues, which may help pave the way for the development of diagnostic procedures and have implications for the treatment of dry eye disease.

Nakamura et al.³¹ showed that superficial punctate keratopathy was associated with an elevation of oxidative stress markers including 8OHdG, MOA, and 4-HNE and antioxidant-related genes including metalloproteinase-9 (MMP-9) and TNF- α in the blink suppressed dry eye mouse model (Fig. 3). According to these findings, there is distinct relationship between the deposition of oxidative stress and corneal epithelial changes in the jogging-board dry eye mouse model. This study detected a strong correlation between accumulation of oxidative stress and corneal epithelial alterations in the dry eye due to reduction in blinking and inconsistency of differentiation capacity in the corneal epithelium exposed to desiccating stress.³¹ These findings demonstrated that elevated ROS production overcomes the antioxidant capacity in the corneal epithelium in the blink-suppressed dry eye mice model.

Additionally, Birkedal-Hansen et al.³² reported a significant increase in the expression levels of matrix MMP-9 gene. Matrix MMP enzymes dissolve the corneal epithelial basement membrane, play a role in the deterioration of extracellular matrix, and are involved in inflammatory cell trafficking and inflammation through the breakdown of type IV collagen. According to these results, chronic exposure to environmental stress that causes an elevation in the oxidative stress markers activates the cell regulatory molecules, which chronically impair the regenerative capacity of the corneal epithelial cell layer.³¹

Recent literature suggests an important role of SOD enzymes in the pathogenesis of dry eye disease. There are adequate levels of SOD, glutathione peroxidase, catalase, lactoferrin, and calcium inhibiting free radicals in the tear film and ocular surface-LG units.^{10,33} SOD enzyme family is one of the most effective antioxidant system and it consists of three isoenzymes (SOD1, SOD2, and SOD3). SOD1 is responsible for 90% of total SOD activity and it is present at a high concentration in all tissues. In various studies performed on *Sod1* knockout (KO) mice, damage associated with oxidative stress was shown in several ocular tissues. Kojima et al.³⁴ performed a study in the *Sod1* deficient mice and investigated the morphologic changes and the secretory function of the

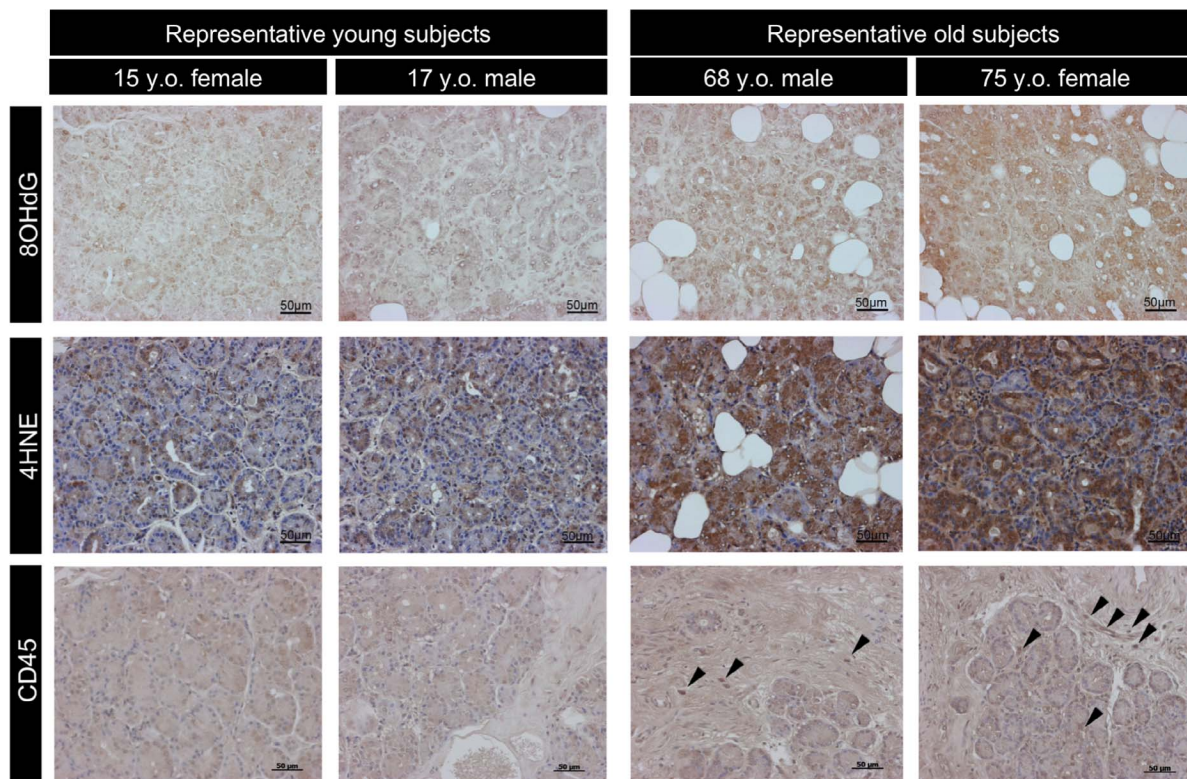


FIGURE 4. Representative immunohistochemistry staining (8-OHdG, 4-HNE, and CD45) images of human lacrimal glands samples from young (15-year-old girl and 17-year-old boy) and old individuals (68-year-old man and 75-year-old woman). Age-related oxidative stress and morphologic changes can be seen obviously in the human lacrimal gland. Compared to younger individuals, older individuals showing diffuse immunohistochemistry staining of oxidative stress markers (4-HNE and 8-OHdG).

lacrimal glands. Their study showed a loss of lacrimal gland function as a result of atrophy of acinar units; elevated CD4⁺ T cells, neutrophil, and monocyte cell inflammation with increased lipid and DNA damage related to oxidative stress. Additionally, the existence of apoptotic cell death, epithelial-mesenchymal transition, and existence of swollen and degenerated mitochondria were also evidenced by the electron microscopy in the same study. These changes were believed to cause a reduction of tear quantity, the deposition of secretory vesicles in the acinar epithelial cells, and a decrease in protein excretion from the lacrimal glands. Immunohistochemistry for 8OHdG, 4-HNE, and CD45 in human lacrimal gland biopsy samples confirmed increased oxidative stress with aging (Fig. 4).

In another study by Kojima et al.,³⁵ age-related alterations in the conjunctival epithelium in response to elevated levels of oxidative stress were shown. In that study, they also demonstrated a marked reduction in goblet cell density, a decline in the intensity of immunohistochemistry stainings of Muc1 and Muc5ac accompanied by a reduction in mRNA expression levels of Muc1 and Muc5ac in the aged *Sod1* mice. Moreover, the PAS staining of conjunctiva showed a decrease of goblet cell density and thickening of the conjunctival epithelium in the aged *Sod1* mice. They also investigated the mRNA expression levels of Spdef, transglutaminase 1, and involucrin in the same mouse model. As a result, aged *Sod1* deficient mice demonstrated a notable reduction in the Spdef expression and a significant increase in the transglutaminase 1 and involucrin mRNA expression in the conjunctival tissues compared with the aged wild type (WT) mice. These results also indicated that conjunctival epithelial phenotype and conjunctival differentiation alterations occur due to increased oxidative stress condition.³⁵ Ibrahim et al.³⁶ investigated age-

dependent Meibomian gland alterations due to oxidative stress in the *Sod1* KO mice. They examined anterior segment vital staining scores, tear, and serum IL-6 and TNF- α levels; oil red O staining scores; immunohistochemistry stainings for oxidative stress markers, including CD45 as well as TUNEL immunofluorescence staining for apoptosis. Based on the alterations of these parameters, they reported morphological variations in the Meibomian glands, which resulted in dry eye and ocular surface disease that was associated with lipid and DNA damage due to elevated oxidative stress status.

Motohashi and Yamamoto³⁷ also looked into the relation between oxidative stress and dry eyes using a new mouse model, *Nrf2* KO mice. Nuclear factor erythroid-2-related factor 2 (*Nrf-2*) recognizes cellular oxidative stress relieves stress conditions by regulating transcriptional response and a substantial role in the cell protection against chemicals. *Nrf-2* regulates a number of response enzymes (such as catalase or SOD) and indirect response enzymes (such as heme oxygenase-1, glutathione, and thioredoxin generating enzymes and enzymes), which are all important in the anti-oxidant response.³⁸ Kojima et al.³⁹ showed elevated levels of *Nrf-2* expression in the cornea and conjunctival epithelial cells, the first structures encountering the external factors. In the same study, they showed a reduction in the tear instability and abnormalities on the ocular surface via the accumulation of oxidative stress accompanied by reduced mucin expression in the cigarette smoke in *Nrf-2* KO mouse model. Their results suggest that *Nrf-2* has an important role in the protection of the ocular surface against external factors.

Uchino et al.⁴⁰ showed in the mev-1 mouse model that oxidative damage in the mitochondria induced lacrimal gland damage associated with dry eye disease. Kawashima et al.⁴¹ reported that caloric restriction was associated with better

lacrimal functions in comparison to non-caloric-restricted controls. Kawashima et al.⁴² also showed that systemic administration of lactoferrin in mice resulted in improvements of oxidative damage in the lacrimal gland and tear functions. Recent efforts by Higuchi et al.⁴³ showed that selenium-binding lactoferrin was an effective treatment modality for oxidative stress related dry eye disease.

Such accumulating evidence in the literature suggests that oxidative stress may have a direct and/or indirect effect on ocular surface health and plays an important role in the pathogenesis of several forms of dry eye. Therapeutic modalities employing topical/systemic use of antioxidants may have a promising future in the treatment of dry eye disease.

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