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Analysis of the Pathogenic Factors and Management of Dry Eye in Ocular Surface Disorders

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ABSTRACT

The alteration of the delicate balance that regulates the secretion and distribution of the tear film determines the dry eye (DE) syndrome, because the tear film represents the interface between the eye and the environment. Despite having a multifactorial origin, the main risk factors for the emergence of the ocular disease are female gender and advanced age.

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Likewise, morphological changes in several glands and in chemical composition of their secretions such as proteins, mucins, lipidics, aqueous tears, and salinity, are highly relevant factors to maintain a condition of good health of the ocular anterior segment. Another key factor of recurrence and onset of the disease is the presence of local and/or systemic infiammation that reflex on the ocular surface. However, it is one of the most commonly encountered disease in clinical practice and many other causes related to daily life and to lengthen the average life will contribute to the beginning. This review will consider how and what disorders of the ocular surface are responsible for a widespread pathology so. In the end, the most appropriate and new therapies will be briefly exposed according to the specific pathology.

KEYWORDS: dry eye; lacrimal gland; lipids and lipidomics; Meibomian gland; ocular surface disorders; proteins and proteomics; tear film

INTRODUCTION

Dry eye (DE) syndrome or keratoconjunctivis sicca is an autoimmune disorders of the ocular surface that affects more than 35% of the population [1] particularly those most frequently human woman. It consists of qualitative and quantitative alteration of the tear film, whose main function is lubrification, nutrition, optical transparency, cleanliness and the main defense to bacterial infections corneal and conjunctival along the eyelid.

Dry Eye Workshop (DEWS) in 2007 defined DE as a multifactorial disease of tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instanbility with potential damage to the ocular surface which is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface [2].

The most common symptoms of the disease are burning, foreign body sensation, photophobia, itching, stinging, irritation, redness, blepharospasm, difficulty opening the eyelids on awakening and in severe cases, pain and blurred vision. Symptoms are subjective and increase in special environmental conditions, such as in exposure to wind or dry heat, in a little damp and in the presence of contact lens, smoke, air conditioning or heating (Table 1). Each specialised layer of the ocular surface contributes to a component of the tear film (Figure 1). In fact, the lacrimal and accessory glands produce the central nutritive aqueous component; the Meibomian glands (MGs) produce the oily outer lipid that provides tear film stability; and the conjunctival mucipare goblet cells (GCs) produce the inner 'surfactant' mucin that primarily improves the wettability of the ocular surface. The eyelids and lashes provide fundamental protection through the blink mechanism, as well as wiping away debris from the ocular surface and replenishing surface with a fresh layer of tears. Among these various factors the dysfunction of MGs is one of the most frequently causes found in DE.

In addition, DEWS recognizes two subgroups of DE based on the etiopathogenesis: evaporative and aqueous deficit. On the other hand, the **excessive evaporation,** caused by more dysfunction of MGs, and **mixed form** find in more than 80% of cases; whereas, approximately 10% of patients with DE have only an **aqueous deficit** [3].

In this review we have deepened the pathogenetic, biochemical and biophysical mechanisms responsible for the DE due to changes in the ocular surface. We have also approached and reported new therapeutic models in relation to the causes of tear discomfort.

METHODS

A literature study of relevant publications about DE syndrome was performed. Through a computerized search for review of several relevant articles in the PubMed database, published between 2000 and 2017 were identified. Key words used for the search included: 'Dry eye'

OR ‘Ocular surface disorders’ OR ‘Keratoconjunctivitis sicca’ OR ‘Sjögren’s syndrome’ AND ‘Tear film’ AND ‘Lacrimal gland’ OR ’Meibomian gland’ OR ‘Lipids and lipidomics’ AND ‘Protein and proteomics’ AND ‘Treatment’ OR ‘Therapy phase II study’. Potentially eligible articles were clinical trials or review article pertinent on treatment for DE and written in English. Relevant articles were manually searched and reviewed, and data concerning with DE were included into the manuscript.

1. DYSFUNCTION OF MEIBOMIAN GLANDS

1.1 Primary dysfunction of Meibomian glands

The dysfunction of MGs is the most frequent cause of evaporative DE disease. Localized at the level of the upper and lower eyelids, secrete lipids on the ocular surface, forming the outer most layer of the tear film, with lubrification functions during blinking and protection against the evaporation of tears [4-5]. In 2011 the International Workshop for MGs dysfunction with alteration of the tear film and symptoms of ocular surface inflammation proposed the following definition of evaporative DE disease *as a chronic, diffuse abnormality of MGs, characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion* [6]. The probable cause of this pathology is due to a meibum stasis secondary to obstruction, secretion, and infiammation of MGs that favors bacterial growth. This could lead to increase the release of esterase and lipase by commensal bacteria of the eyelids. As a consequence of this increased enzyme activity, the bacteria change meibum’s viscosity, leading to further stagnation of meibum within the MGs and generating free fatty acids, which in turn cause inflammation and hyperkeratinization [4,7]. This change of the lipid composition lead to the appearance of foam in the tear film, often visible on the eyelid margin of patients with MG dysfunction [8,9]. The absence of the normal meibum also would reduce the lipid content of the tear film, entering in the vicious circle of DE disease, in which

the lipid deficiency of the tear film promotes increased tear evaporation, hyperosmolarity, and inflammation. Furthermore, an infection with commensal bacteria such as Staphylococcus aureus is one of the causes of chronic blepharitis. Recent studies suggested a new term to describe it as an only chronic disease called *dry eye blepharitis syndrome* (DEBS) [10]. It's an inflammatory condition of the eyelid margin previously considered synonymous with MGs dysfunction but in recent years recognized as a clinical conditions that may develop in the later stages of MGs dysfunction or independently, where the DE is the ultimate manifestation of the disease.

1.2 Secondary dysfunction of Meibomian glands

1.2.1 Chronic inflammation and autoimmune systemic dysregulation

Skin diseases such as *rosacea* or *seborrheic dermatitis, rheumatoid arthritis, etc.* also play a role in the pathophysiology of MG dysfunction. Rosacea is a chronic skin disorder that affects the sebaceous glands, especially on the level of the face. It is characterized by erythema, telangiectasia, facial rash, inflammatory papules, occasionally hypertrophy of connective tissue [11]. It is more common among 50-60 years [11,12] and ocular manifestations may accompany those skin or appear independently. Although the etiology is uncertain, the latest findings show that microbial pathogenesis and mechanisms associated with chronic inflammation and autoimmune dysregulation may be involved. The primary defect in ocular rosacea is the MG infiammation with dilatation and obstruction of gland orifices leading to chronic dysfunction. Chronic inflammation can lead to hyperkeratinization of the ductal epithelium, loss of secretion, and then to blepharitis, meibomitis, chalasia, telangiectasias, and eyelid irregularities [13].

This stimulates the inflammatory cells to release of cytokines and activators of matrix metalloproteinases (MMP) leading to ocular surface damage [14,15]. Subsequently, punctate

keratopathy and superficial vascularization of peripheral cornea are also common findings [12,13].

2. DYSFUNCTION OF MEIBOMIAN GLANDS AND AQUEOUS DEFICIT: MIXED FORMS

2.1 Aging and lacrimal discomfort

Another major risk factor for DE is age. Modifications on MG structure have been identified with age as, an altered localization of the peroxisome proliferator-activated receptor-ɣ (PPAR-ɣ), a lipid-activated hormone receptor that regulates lipid synthesis and cell differentiation [16, 17] or MG atrophy [18]. In research on mouse model, some authors have found changes in the mucocutaneous junction and glandular atrophy through a loss of meibocyte progenitors [18]. The aging, in fact, leads to a decrease of the acinar diameter and an increase of acinar wall inhomogeneity without significant modifications of the glandular orifice diameter, altering qualitatively the meibum secretion. This is in agreement with the main clinical changes observed during aging, represented by tear break-up time (BUT) and Schirmer test scores reduction. Moreover, with the aging we observe the atrophic involution of the glandular unit, then a progressive dysfunction of the secretive activity. This appears to be in line with the involution of most parts of secretive structures, such as exocrine glands and lymphatics, observed in several tissues of the human body during aging [19].

2.2 Aging and associated factors

Advanced age is also often associated with several factors.

- The chronic use of *systemic medications* such as antidepressants, diuretics, dopamine such as those used for Parkinson's disease, and anti-metabolites commonly used to treat rheumatoid arthritis. Depending on their mechanism of action these drugs affect

lacrimal secretion. In Parkinson's patients, for example, dopaminergic dysfunction is thought to play a role in decreasing the blink reflex that leads to DE, in addition to physiological decrease of corneal sensitivity with age which increases the risk of exhibition keratopathy. In addition, the decrease in liver and kidney function increases the time of clearance of the systemic drugs.

- The chronic use of *topical medications* as for glaucoma. Beta blockers, alpha-adrenergic and prostaglandins can reduce tear production. They can cause chronic irritation of the ocular surface that compromises the integrity of the lacrimal glands. It has been highlighted a reduction of glandular density and area, and increased viscosity of the meibum. In addition the presence of the preservatives such as benzalkonium chloride (BAK) can cause tear film instability, lost of GCs, conjunctival squamous metaplasia and apoptosis, distruction of the corneal epithelium barrier, and damage to deeper ocular tissues even at low concentrations [20].
- *Abnormalities in the eyelid positioning,* as laxity, floppy eyelid syndrome, retraction, entropion, ectropion, and lagophthalmos. Horizontal lid laxity is the most common cause of involutional eyelid malposition. Eyelid malposition leads to corneal exposure, poor tear-film distribution, and abnormal tear outflow. As many as 50-70% of patients with this disease develops tear discomfort syndrome [21].
- *Conjunctivochalasis* is another notable contributor to poor tear outflow and is characterized by a redundant bulbar conjunctiva interposed between the globe and the eyelid [22]. Pathogenesis of conjunctivochalasis is under investigation; however, elastotic degeneration from cumulative sun exposure and infiammatory degeneration from delayed tear film clearance have been proposed [23]. Once formed, the redundant folds interfere with the inferior tear meniscus and, in some cases, cause occlusion of the inferior punctum. Elderly patients often have higher collective sun

exposure that can predispose to development of conjunctivochalasis and/or have aqueous tear deficiency that can be exacerbated by disruption of the tear meniscus.

- A gradual *reduction in corneal sensitivity* has been shown to occur with increasing age that predisposes older adults to DE. Roszkowska et al. reported that mechanical sensitivity of peripheral cornea decreases gradually through-out life, whereas central corneal sensitivity remains stable until age 60 and then decreases sharply subsequently [24].
- *Oxidative stress*, a counterpart of infiammation, occurs when antioxidants are unable to counteract reactive oxygen species (ROS) that are generated in normal metabolic processes. Production of aggressive oxygen species such as free radicals and peroxides leads to DNA damage over time, inducing cell necrosis and damage of the regenerative capacity of cells of the corneal epithelial layer. In younger, healthy human bodies, low levels of ROS are counteracted by antioxidant enzymes [25].

3. AQUEOUS DEFICITS IN AUTOIMMUNE DISEASES: SJOGREN'S SYNDROME AND NON-SJOGREN'S SYNDROME DRY EYE

EDWS divided aqueous deficits in two subclasses: Sjogren's syndrome (SS) and non-SSDE. SS is a chronic inflammatory disorder characterized by exocrine gland dysfunction. Lymphocytic infiltration of the lacrimal and salivary glands results in the classic sicca complex characterized by DE (keratoconjunctivitis sicca or xeroftalmia) and dry mouth (xerostomia). Then it can also affect the skin, trachea, vagina, nose and throat. Patients with DE associated with SS have been found to have elevated levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in their tears [26]. The IL-6 level is associated with disease severity and was found to correlate with tear film and ocular surface parameters, eg, tear film, lacrimal meniscus, BUT, Schirmer test, tear clearance, GC density, keratoepithelioplasty

score [26]. Recently, Zhu et al. found that co-stimulatory molecules Ox40 and Ox40L on peripheral blood mononuclear cells is higher in Sjögren patients than in normal controls. The levels correlated with clinical outcomes and therapeutic responses [27]. Even in patients with marked SS, biopsy specimens have revealed that 50% of glandular cells are still present. These results emphasize the importance of immune factors, such as cytokines, MMP, and autoantibodies, in decreasing neurosecretory circuits and inducing glandular dysfunction. The keratoconjunctivitis in SS is classically described as an aqueous tear deficiency. However, this assumption has been challenged in more recent years. Some studies suggest that pathologic changes induce global tear dysfunction, including alterations in MG function [28]. An analysis of patients affected from SS with aqueous tear deficiency compared to non-SSDE with aqueous tear deficiency demonstrated increased evaporation and also decreased MG expressibility with a deficient lipid layer in the first group [28]. A new laser scanning confocal microscopy approach recently found some differences in the MGs features in patients with primary SS, non-SSDE, and MG disease [27-29]. In fact, the pattern of inhomogeneity of the MGs walls and interstices was markedly higher in all groups of patients than in controls, with features more pronounced in primary SS compared to non-SSDE. This inhomogeneous appearance varies with the level of infiammation that is significantly higher in eyes with SS, because of the autoimmune pathogenesis of this condition. The presence of confocal signs of infiammation with the absence of dilative morphologic changes supports the occurrence of an infammatory/atrophic nonobstructive MG disease in the primary SS [30, 31]. Diagnosis of SS is generally based on the American-European Consensus Group (2002), and requires at least four out of six criteria, or three out of the four objective criteria. The six criteria include subjective and objective ocular dryness, subjective and objective oral dryness; presence of Sjogren-specific antibodies A (SSA)/RO and antibodies B (SSB)/LA, and positive minor salivary gland biopsy [32]. However in 2012, new classification criteria for SS

were approved by the American College of Rheumatology that requires at least two of the following three criteria: positive serum anti-SSA and/or anti-SSB or rheumatoid factor, or antinuclear antibody; total ocular surface staining score > 3, and presence of focal lymphocytic sialadenitis with a focus score >1/4 mm^2 in labial salivary gland biopsy samples [33]. According to the classification criteria from the European-American collaboration, secondary SS consists of the features of primary SS togheter with other features of an overt autoimmune connective tissue disease, the most common of which is rheumatoid arthritis. There is a known association of several systemic diseases with DE syndrome as SS, rheumatoid arthritis, scleroderma, polymyositis, lymphoma, amyloidosis, hemochromatosis, sarcoidosis, and systemic lupus erythematosus [34]. In particular rheumatoid arthritis presents the greatest risk of ocular complications such as scleritis, DE, and peripheral ulcerative keratitis [35].

4. OTHER MAJOR CAUSES OF MIXED FORMS

4. 1 Hormonal changes

DE disease affects women more frequently than men. Women are more likely to experience DE disease during certain periods of significant hormonal alteration, for example, during pregnancy, lactation, oral contraceptive use and after menopause, suggesting that DE may be related to sex hormones [36-39]. Receptor for androgens, progesterone, estrogen and prolactin has been found in several ocular tissues including the lacrimal gland and MG [36-39]. Androgens are steroid hormones that are produced by the adrenals (dehydroepiandrosterone sulfate [DHEAS], dehydro- epiandrosterone [DHEA], and androstenedione [A]), gonads (T), and peripheral tissues (dihydrotestosterone [DHT]). Experimental and human studies have demonstrated that androgen levels are essential for normal lacrimal gland function and structural organization and that prolactin and estrogens also play important roles in providing

an adeguate hormone provision for optimal lacrimal production [39-41]. In particular, the androgen receptor influence the MGs, this has led to the hypothesis that these glands are under hormonal control. Androgens in fact, act on epitelia acinar cells that contain receptors for messenger RNA and/or protein receptors for androgens. These cells respond androgen binding it to specific lipid-producing area on the cell surface which then transcribes specified genes to increase the lipid layer distribution over the ocular surface [36-39]. In particular, Suzuki et al. [38] demonstrated that testosterone stimulates genes related to lipid metabolic pathways and suppresses genes regulating epithelial keratinisation.

Moreover, the oestrogen produced after the menopause is primarily from the peripheral conversion of adrenal androgens to oestrone and occurs in the liver, kidney, brain, adrenal and peripheral adipose tissue. The absolute levels of oestrogen are influenced by weight, sex and age [39]. In postmenopausal androgens continue to be produced by the cells of the ovarian stromal and hilus cells in response to the increased levels of circulating lutein hormone [39]. There is a 25% increase in testosterone secretion by postmenopausal ovary. However, circulating testosterone levels decreased to slightly less than those of premenopausal women. This reduction has been attributed to a significant fall in the conversion of androstenedione to testosterone after the menopause [39,40]. The data suggest that sex hormone deficiency can not only cause a reduction in the production of aqueous tears leading to a deficiency but also to a dysfunction in the MG function resulting in an evaporative DE. In women with complete androgen insensitivity syndrome due to androgen receptor dysfunction, an alteration in neutral and polar lipid patterns of human MG secretion along with a keratinisation of the MG ductal epithelium (ie, orifice metaplasia) and the lid has been observed [38-41]. Particularly in these women there is a significant increase in signs and symptoms of DE. Not surprisingly, a high incidence of evaporative DE disease was found in hyperandrogenic women affected by syndrome of polycystic ovarian syndrome [40,41]. This allows us to speculate that for the tear

film to be stable it is necessary a perfect balance between androgens and estrogens for a normal function [40,41].

4. 2 Use of contact lenses, computer, and interactive environments

The LAC abuse helps to determine a syndrome of dysfunction of the tear film. It has been found a decreased basal epithelium cell density, reduced acinar unit diameters, higher glandular orifice diameters, greater secretion reactivity, and greater inhomogeneity of the periglandular interstices were the main observed findings. Morphologic changes in the MGs shown by contact lens wearers were interpreted by the authors as signs of MGs dropout, duct obstruction, and glandular infiammation caused by chronic mechanical contact lens irritation [12,19,20,30]. In such cases, therefore it is recommend the use of low or medium lenses hydration that would release liquid retaining its geometry, high permeability of oxygen, and the use of monodose artificial tears viscous.

The presence of environments at high temperatures and high humidity resulting in a greater rapidity of evaporation of tears. Not only the hot weather but also the wind, smoke, smog, air conditioning can help. The prolonged use of display screens, the gaze of fixity, the top position of the screen which doubles the exposed ocular surface, are all factors that may increase the evaporation of the tear film while the decreased frequency and regularity blink will reduce stability and efficiency.

4. 3 Dry eye and allergic diseases

Allergic diseases are among the most common ocular surface disorders, and allergy is one of the most frequent eye problems that occur in clinical practice. The prevalence of allergic diseases in children between 6 and 14 years varies from 0.3% to 20.5% and is gradually increasing [42]. This is due to genetic factors, environmental pollution and exposure in

children. The most common form of allergy is seasonal allergic conjunctivitis (AC), while the vernal keratoconjunctivitis (VKC) is considered a rare disease affecting in Europa most frequently within the second decade of life. VKC is a serious pediatric multifactorial illness, which compromises the quality of life of the children and can lead to severe ocular symptoms, with giant papillae on the upper tarsal conjunctiva (cobblestoning appearance) with gelatinous infiltrations to the limbus surrounding the cornea, called Horner-Trantas dots. If untreated, ocular surface remodeling leads to corneal ulcers and scars [43]. VKC can be associated with other serious disorders of allergic or autoimmune nature, such as asthma or collagenopathies [43].

There are many examples in the literature that connect the dysfunction of the lacrimal film with chronic forms of AC. Pflugfelder *et al*. [44] suggested that activated T-cells and an increase in inflammatory cytokines such as epidermal growth factor, IL-1, and IL-8, damage GCs and conjunctival epithelial, which account for a reduction of mucin production and subsequent decrease of tear film BUT.

These inflammatory changes lead to macroscopic modifications of the MG represented by glandular atrophy and ductal dilatation. The same morphofunctional alterations were described in seasonal AC where we observe a significant decrease in size and density of MG acinar units. MG appear small and irregular with periglandular fibrosis and a restricted and not well-defined lumen, occluded by hyperreflective and dense meibum [45,46,47]. In addition to the instability of the tear film, another risk factor is the use of antihistamines in the long term that have anticholinergic properties due to their high affinity for the muscarinic receptor [45,46,47].

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4.4 Dry eye and refractive surgery

Laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) are the two most commonly performed corneal surgeries used to correct refractive errors. Negative effects on the tear film are known after each refractive surgery, despite the great success in the result. Consequently, tear substitutes are needed in the first postoperative months [48]. The same applies in cataract surgery in which was found a decrease of BUT and squamous metaplasia in conjunctival impression cytology after phacoemulsification [49].

Yu and colleagues found that the 95% of patients who underwent LASIK had DE symptoms in the first postoperative day, and 85% continued to experience at first postoperative week. Over 60% of the patients still had complaints of DE at first postoperative month [50]. These symptoms improved over the course of several months, with reported rates of DE disease at 6 months postprocedure ranging from 12 to 48% [51]. The incidence of the DE symptoms after PRK is similar to that after LASIK except in the postoperative period. Indeed, immediately after PRK patients have a high discomfort due as the epithelium damage. Later symptoms are comparable to post-LASIK situation. Stephenson and colleagues showed that only 20% of patients post PRK complained of DE at the 3 to 6 months and one long-term study [52], only 3% of patients had symptons of DE 12 years later [53].

The genesis of DE postrefractive is depending on the type of technique causing injury to the nerve plexus and which can lead to neurotrophic deficits. In LASIK the nerve plexus is compromised with the execution of the corneal flap and the nervous integrity is preserved only in the point of the hinge. A further damage in depth is proportional to ablation and then to the defect to be corrected. In PRK the nerve endings are compromised over the entire surface ablation, and the greater the higher is the defect and therefore the ablation depth.

In cataract surgery the damage is related to corneal and limbal incisions. The damage to the nervous plexus involves a lesser corneal sensitivity with reduced blink [54], an increased

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evaporation of the lacrimal film itself, and an altered stimulus to its production. The destruction of corneal innervation also lacks of epitheliotropic factors, such as substance P, which have an important role for the regeneration and epithelial integrity. The never growth factor (NGF), important for corneal regeneration, may be present in small quantities after LASIK or PRK in cases of tear film deficiency, inducing a possible neurotrophic epitelopatia [55,56]. The increased evaporation or reduced tear production due to rise of the tear osmolarity and inflammatory origin of ocular surface. The many mediators released by damage to the nerve endings and cells from the corneal photoablation after cause the degranulation of mast cells and the recall of leukocytes, monocytes and macrophages with the effect of determining dilation of the blood vessels and hypersensitivity of the tissue [57,58]. In addition, the modification of the corneal profile alters the interaction between the upper eyelid and corneal curvature resulting in not perfect distribution of tear during blinking [59,60]. Despite the phenomena related to the nervous trophism tend to be exhausted in 6-8 months after surgery for the reconstruction of the nerve fibers sometime, the symptoms may last for a not perfect recovery of the nerve plexus or to the same phenomena already existing at the time of surgery. So adequate preoperative screening is required to identify and eliminate patients with compromised conditions.

5. TEAR FILM AND SPECIFIC PATHOLOGIES OF THE OCULAR SURFACE

5.1 Stevens-Johnson syndrome and toxic epidermal necrolysis

Stevens Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) characterize severe, immunologic dermato-bullous conditions with high morbidity and mortality that affect the skin and at least 2 mucous membranes. The diagnosis is made through clinical signs and skin biopsy demonstrating full-thickness necrosis of the epidermis and keratinocyte apoptosis, with minimal involvement of the underlying dermis. SJS is defined when the denuded

cutaneous surface covers less than 10% of the body surface area and TEN is when over 30% of the body surface area is denuded. The etiology of the diseases, to genetic susceptibility, is immune-mediated and can be triggered by drugs (most commonly sulfonamides, allopurinol, carbamazepine, etc.) or, less commonly, by systemic viral or mycoplasma pneumonia infection. The ocular surface represents one of the major targets in the disease, and patients may become irreversibly blind. Ocular surface inflammation can be intense, with pseudomembrane or membrane formation, symblepharon, corneal ulceration, and perforation. Acute ocular manifestations led to chronic ocular sequelae with visual significance in at least one-third of patients. Ectropion, entropion, trichiasis, distichiasis, corneal epithelial defects, infection, neovascularization, loss of conjunctival goblet cells, meibomian gland atrophy and inspissation, keratinization of the eyelid margin, tarsal and bulbar conjunctival surfaces, can be associated. Scarring in the fornices and in the lacrimal gland ducts cause severe aqueous, mucous, lipid tear deficiency and xerosis. Resultant corneal blindness, due to the absence of tears, eyelid malpositions, and tarsal conjunctival keratinization, is the most dreaded long-term complication among SJS/TEN survivors. These changes not only cause debilitating pain in affected patients, but also threaten vision and are correlated with development of late corneal blindness, due at least in part to chronic limbal stem cell dysfunction (LSCD). In general, SJS and TEN are considered T-cell mediated, type IV hypersensitivity disorders. There are elevated levels of TNF-α in blister fluid, skin, mononuclear cells, and blood of affected patients. TNF-α activates TNF-R1, which leads to activation of Fas-associated death domain protein and downstream caspase pathways. Furthermore, human ocular surface epithelial cells strongly express toll-like receptor 3 (TLR3), and its ligand, polyI:C. These were found to induce various molecules, such as proinflammatory cytokines and antiviral- and allergy-related molecules in SJS/TEN population. To conclude, keratinocyte and epitelial cells death in SJS/TEN appear to occur by apoptosis, thus, several mechanisms are likely

involved to varying degrees. Same find the autoimmune response in patients with to chronic graft-versus-host desease (GVHD), a major cause of morbidity and mortality in patients undergoing allogenetic hematopoietic stem cell transplantation for hematologic malignancies, where we find glandular dysfunction from glandular atrophy and fibrosis [61-63].

5.2 Keratoconus

Keratoconus (KC), by definition, is a progressive disorder characterized by non-inflammatory corneal ectasia, stromal thinning that leads to corneal surface distortion [64,65]. The etiology of this disease remains unknown, although there is evidence base is genetic and can bond with systemic diseases and obvious factors like excessive rubbing of the eyes, and wearing contact lenses [64,65]. The 80% of patients suffering from KC refers tired eye, irritation, and foreign body sensation, indicative elements of ocular dryness [64]. In fact, while the amount of the aqueous component remains comparable to that of healthy eyes, in functional studies evaluating the tear ducts on patients with KC the BUT values tests correlate in proportion to the degree of severity of the cone. Because of this is the conical morphology of the cornea with abnormal distribution of tears, alteration of the quality/quantity of mucin secretion and reduction of GCs which would lead to tear film instability [64]. Another rating is the level of adenosine tetraphosphate (Ap4A), proposed as potential molecular biomarkers for DE, the dosage which takes place at the processing of a strip used for the Schirmer 1 test analyzed by chromatographic technique. The concentration of Ap4A in tears increases proportionally to the degree of DE and patients with KC have concentrations 20 times higher than in healthy eyes [64]. The impression cytology reveals the high degree of squamous metaplasia conjunctival epithelium, and the marked reduction of GCs, detected in combination with confocal microscopy.

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Another element is the abnormal ratio between the cornea and ocular surface-eyelid complex. This, also by the more pronounced tendency of patients with KC to rub eyes. The morphological and functional alteration results in an abnormal distribution of a tear already altered in quality. In addition, the negative effect of shear forces operated from the upper eyelid leading to constant wear and tear of the surface epithelia, corneal and conjunctival, with consequent metaplasia reactive, scarring processes and loss of GCs from drying effect. The proof is given by fluorescein and rose bengal test: the increased corneal accumulation of these dyes is proportional to the damage caused to the whole of the ocular surface and are meaningful in patients with KC [65]. Several studies report a reduction in corneal sensitivity in patients with KC, especially if wearing contact lenses. This is related to morphological and structural changes in the cornea and to the alterations of the lacrimal function [66,67]. Hypothesis confirmed by histopathological studies and confocal microscopy, which have described and demonstrated the reduced density and the abnormal morphology of sub-basal and stromal nerves of the cornea in patients with KC, especially near the apex and with a decreasing gradient of the damage to the periphery [67]. The alteration structure relates in particular to the cold thermoceptors, reduced in number and in a chronic state excitatory. This alteration secondary excitability to ocular dryness and hyperosmolar tear film, to be the cause of the reduced sensitivity of DE sensation.

Furthermore, on the tears of patients with KC they are found reduced levels of lactoferrin, immunoglobulin A, lipofilina A and C. This deficit suggests the presence of inflammatory processes on the ocular surface in KC, together with the finding of elevated presence of serum albumin in the tear film that indicates alteration of the blood-ocular barrier, of the conjunctival vessels [68], and the presence of concomitant conjunctival inflammation. Increased levels of MMP-1, MMP-3, MMP-7, and MMP-9 are expressed in subjects with KC compared to healthy individuals [67,68]. MMP are responsible for the degradation enzymes

of matricolare protein extracellular and secreted in response to cytokines (IL-4, IL-5, IL-6, IL-8) and growth factors (TNF-α and TNF-β).

5.3 Pterygium

Pterygium is a common disease of the ocular surface characterized by the invasion of the fibrovascular tissue from the bulbar conjunctiva on the cornea. It can cause chronic ocular irritation, induced astigmatism, tear film disorders and descreased vision secondary to growth over the visual axis [69,70]. Although the exact etiology is unknown, the exposure to ultraviolet (UV) radiation is believed to be the main risk factor [69,70]. Age, chronic inflammation, microtrauma, and hereditary factor can contribute [70,71]. Studies believe that the genetic trauma mediated by UV rays could alter the expression of cytokines such as IL-6 and IL-8 in patients with pterygium [69]. Il-6 and IL-8 can induce the production of MMP, which are localized on the edges that are advancing pterygium [70]. The release of IL-6, IL-8, and MMP in the tear film could lead to damage to the surface and instability of the tear film, resulting in apoptosis of epithelial cells, loss of GCs, reduction of mucous secretion, and lacrimal hyperosmolarity [70,71]. Finally, it develops a vicious circle in which the tear hyperosmolarity same stimulates the expression of MMP and leads to inflammation of the ocular surface [71]. A study shows that excision of pterygium improves the osmolarity and function of the tear film, bringing an improvement of the tear BUT and ferning tests, due to increase in density of conjunctival GCs and lacrimal mucous component [72]. However, the tear osmolarity remains stable for the time in which no pterygium recurrence.

6. UP TO DATE ON BIOCHEMICAL MECHANISMS IN DRY EYE DISEASE

It is now established that DE is a disease caused by the presence of an inflammatory basis that destroys the normal homeostasis of the ocular surface. Thus, a common pathogenic

mechanism of DE is related to the tear hyperosmolarity that can activate a chain of events resulting in a vicious circle of inflammation which leads to further ocular surface injury (Figure 2). As a result demonstrated in DE patients by high levels of chemokines macrophage inflammatory proteins (MIP-1α), cytokines (IL-1β), MMP, TNF-α, lymphocytes infiltration and by increased of conjunctival human leukocyte antigen expression (HLA-DQ and HLA-DR), CD40, CD40 ligand ICAM-1 (cell surface receptors), and apoptotic marker APO2.7 [73]. In fact, studies confirmed the anti-inflammatory effects of CF-101, an A3 adenosine receptor (A3-AR) agonist, that binding to A3-AR mediates the downregulation of nuclear growth factor-κB-TNF-α (NF-kB-TNF-α) signaling pathway, the inhibition of cytokines secretion, and the apoptosis of inflammatory cells [73]. It has been suggested that the improvement of several pathological signs and symptoms in subjects with DE, such as Schirmer test and BUT scores, might be due to reduced ocular surface's inflammation following direct interaction between CF101 and its receptors on inflammatory cells [73]. Besides, other recent studies performed the essential role of inflammatory mediators. Since Omega 3 (ω3) and omega 6 (ω6) are polyunsaturated fatty acids (PUFAs) defined "essential", they cannot be synthesized within the human body; ω3 and ω6 derive from α-linolenic acid and linoleic acid, respectively, and are precursors for eicosanoids with potential anti-inflammatory (ω3 group) and proinflammatory effects (ω6 group). An equilibrate balance between ω3 and ω6 is necessary to avoid the prevalence of ω6 proinflammatory effect. In particular, proresolving lipid mediators derived from ω-3 precursors, such as docosahexaenoic acid (DHA) and eicopentaenoic acid (EPA), control epithelial wound healing, inflammatory cell migration, and nerve regeneration [74,75]. The prostaglandins, belonging to a class of inflammatory lipid mediators called eicosanoids, derive from the oxygenation of arachidonic acid (AA), an ω-6, that is enzymatically released from cell membranes of activated cells in response to environmental stress. The release of AA and the

subsequent generation of eicosanoid lipid mediators is responsible to trigger the acute inflammatory response to corneal injury [73]. But, its resolution is mediated by proresolving lipid mediators derived from ω-3 PUFA precursors, DHA and EPA. Consequently, it is hypothesized that the DE is a metabolic disorder characterized by an imbalance of ω-3 and ω-6 PUFA leading to underproduction of proresolving lipid mediators.

Corneal epithelial cells and resident regulatory polymorphonuclear (PNMs) leukocyte in the corneal limbus and lacrimal gland highly express 15-lipoxygenase (15-LOX), a key enzyme for generating and releasing specialized proresolving mediators which are critical to control the immunity of the ocular surface. Endogenous lipoxin A4 promotes corneal ephitelial wound healing [76], inhibits pathological angiogenesis and proinflammatory cytokine expression [77,78], and controls effector T-cell activation [79]. Neuroprotectin D1 is implicated in epithelial cell survival [80], recovery from oxidative stress and wound healing [81]; it has also been shown to promote corneal nerve regeneration and return of corneal sensitivity [81,82]. Resolvin E1 exerts proresolving effects directly through G-protein-coupled receptors [83] and indirectly through negative feedback on cyclooxygenase-2 (COX-2) expression, which play an important role in the synthesis of prostaglandins from arachidonic acid [84]. It is seen that these mediators are essential for the homeostasis of the tear film, as the survival of GCs and the secretion in response to stress. This underlines the biological significance of ω-3 proresolving lipid mediators in the tear film and lead support to the hypothesis that DE has a metabolic basis. Nutritional or metabolic deficiency of DHA and EPA may lead to underproduction of lipid mediators in tears. A higher ratio of ω-6 lipid mediators of ω-3 induces inflammation of the ocular surface. Recent studies and meta-analysis of randomized controlled trials support the supplement of ω-3 fatty acids for the DE syndrome; oral administration of ω-3 improves the Schirmer Test and BUT [81-84].

7. THERAPEUTICAL PRAGMATIC APPROACH

The purpose of recent researches was to promote ocular surface health reducing the inflammation and stabilizing tear film. Although several therapeutic approaches for DE are available, therapeutic strategies, like the use of artificial eye lubricants, focus on the treatment of symptoms guaranteeing just a temporary relief to the patient without having an effect on the implicit causes of DE [12]. DEWS report suggests to choose specifich treatments based on the severity of disease according to patients' clinical history and experience [2]. Nowadays, treatments of DE include the use of lubricating eye drops, unguents, dietary supplements associated with eyelid cleaning and, in severe cases, with punctual occlusion, topical and oral nonsteroidal anti-inflammatory (NSAID) and corticosteroids drugs.

7. 1 Topical nonsteroidal anti-inflammatory (NSAID)

Especially for SS are evaluated NSAID and steroids, also if a prolonged use of steroids may be associated with severe side effects such as onset of cataract, infections, and increases in intraocular pressure [12]. Currently, there are a few NSAID that do not seem to cause toxic effects on the cornea and, at the same time, they could lead to DE reducing eye sensitivity [85,86].

Pranoprofen and bromfenac sodium ophthalmic solution are NSAID with anti-inflammatory effect due to the inhibition of epoxidase and synthesis of arachidonic acid (AA). These drugs improved signs and symptoms and reduced conjunctival human leukocyte antigen II (HLADR) expression in the patients, also in cases of unresponsive to lubricants monotherapy [85,86].

Thymosin β4 is a naturally occurring 43 amino acid peptide found in high concentrations in most tissues, cells, blood plasma, and ocular surface fluid. Thymosin β4 possesses both wound-healing and anti-inflammatory properties [12,87]. This drug has been shown to have a

positive effect on epithelial migration and healing as well as an anti-inflammatory effect through a number of different pathways in vitro and in patients with diabetic neurotrophic corneal defects and in severe DE as graft-versus-host disease (GVHD), SJS, and TEN [87,61,63].

7. 2 New topical natural substance

An amphiphilic glycoprotein, proteoglycan 4 (PRG4) also called lubricin, plays a critical role as a boundary lubricant in several sites throughout the body [88,89].

Some researchers discovered the presence of lubricin mRNA in a number of exocrine and reproductive tissues. This superficial zone protein containing a 1404-aminoacid core, is produced by ocular surface epithelia, and acts to protect the cornea and conjunctiva against shear forces generated during an eyelid blink [88,89].

Deficit of this glycoprotein, natural boundary lubricant, promotes shear stress on the ocular surface and increases damage. On the contrary, exogenous application of lubricin, significantly reduces the friction between the cornea and conjunctiva. In a recent clinical trial, the researchers stated the efficacy and safety of recombinant human lubricin that was compared to a 0.18% hyaluronic acid (HA) eye drop in 39 subjects with moderate DE [89,90].

7. 3 Secretagogues

Different topical pharmacologic agents may potentially increase both aqueous and mucous secretion. Some preclinical studies demonstrated the nerve growth factor (NGF) efficacy in DE. In fact, NGF supports the lacrimal function stimulating the glycoconjugates' secretion of the conjunctival cell and promotes corneal homeostasis. NGF trophic function is related to tyrosine kinase receptor (TrkA) and p75 neurotrophin receptor (p75 NTR) stimulation. The presence of both TrkA and NGF on the ocular surface highlights their role in promoting

ocular homeostasis. Binding TrkA, NGF stimulates the release of mucin by conjunctiva stabilizing the tear film [91].

MIM-D3 is a small protein-like chain designed to mimic NGF, it activates TrkA receptor, but is not able to bind p75NTR receptor. In a phase II clinical trial, Meerovitch et al. demonstrated a considerable signs and symptoms improvement in DE, with both the 1% and 5% MIM-D3 ophthalmic solutions. One percent solution was related to the best result in fluorescein and lissamine green corneal and conjunctival staining, respectively [91].

More recently, 3% diquafosol tetrasodium, a P2Y2 purinergic receptor agonist, in ophthalmic solution has been launched in Asian countries. It acts through a novel mode of action by activating P2Y2 receptors of the ocular surface and stimulating the quantity and quality of tear fluid secretion, as determined by OCT and meniscometry in DE [92].

Diquafosol also stimulates the secretion of sialic acid, which is a mucinlike substance, in tears in healthy subjects after a single dosing. Therefore, clinical trials demonstrated a good safety profile with clinical improvement of the ocular surface in severe DE patients [92,93]

7. 4 Topical Immunomodulators

Cyclosporine A (0.05–2%), lipophilic cyclic polypeptide, is the first topical immunomodulator in ophthalmic emulsion, approved by the U.S. FDA in 2002 and by European Union in 2015. The cyclosporine A acts as immunosuppressive agent when administered systemically. It has been used topically for: blepharitis, dry eye, keratoconjunctivitis, post LASIK, GVHD, SJS, and TEN [94–97].

It has been shown to inhibit the production and/or release of proinflammatory cytokines and to upregulate the release of anti-inflammatory cytokines [94,96]. Moreover, inhibites the apoptosis and the number of Fas-ligand expression in infiltrating lymphocytes of human conjunctival epithelial cells [96,97]. The U.S. FDA approved on 11 July 2016 the first

medication of a new class of drugs for the treatment of DE. Lifitegrast, topical administration, is a novel integrin antagonist which inhibits the proinflammatory T-cell activity by T-cell adhesion to intercellular adhesion molecule-1 (ICAM-1) [94,98].

7. 5 Sistemic therapy

Some studies investigated the effects of dietary integration with PUFA as a possible substitutive therapy in the management of DE. The authors concluded that metabolic deficiency of ω3 tear film lipids can cause an inflammatory impact on the ocular surface and this could lead to a chronic inflammation in DE subjects. Further large-scale, multicenter studies are necessary to establish the guidelines for administration and dosing of PUFAs [12]. Recently, new biologic agents targeting B cells, such as rituximab, belimumab, and epratuzumab, have shown promising results in the treatment of autoimmune and progressive condition as SS. Achievements with rituximab, monoclonal antibody directed against CD20, and other B-cell-depleting therapies, such as interferon-α (INF-α) and anti-B-cell-activating factor (BAFF), have shown potential benefit even in severe DE [99,100- 102], thus, further studies are needed to validate the findings in these patients.

8. CONCLUSIONS

The DE syndrome consists of a wide spectrum of disorders with different causes. Nowadays, artificial tears that guarantee a transient improvement of DE patients' symptoms, represent the first and most used therapy for a distressing condition that significantly interferes with quality of life. However, inflammation if left untreated can lead to severe and permanent complications. The aim of new medications is to affect the various pathogenetic factors involved in the onset of DE. Nevertheless, transplantation of stem cells in recent results on clinical trials, has demonstrated the effectiveness of stem-cell-based treatments from human

embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) [103,104]. Stem cells hold promise for treating severe eye diseases, because it is an ideal organ for its relative immunological privilege, surgical accessibility, and its being a self-contained system [87,103,104].

Really, we think that a multidisciplinary approach based on both topical and systemic therapies should be considered for the clinical management of DE, in order to obtain a sustained relief to patients. In summary, further researches are needed to study the long-term effects of the new therapies planned for a chronic multivariate disease.

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Abbreviations

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Table 1. Dry eye symptoms and diagnostic tests.

COMMON DRY EYE SYMPTOMS	
▪ Irritation	▪ Burning or stinging sensation
▪ Dryness or grittiness	▪ Foreign body sensation
▪ Itching and Redness	▪ Tearing
▪ Fluctuation of vision	▪ Contact lens intolerance
▪ Increased blinking frequency	▪ Blurry vision
▪ Photophobia	▪ Mucous discharge
DRY EYE DIAGNOSTIC TESTS	
▪ Tear Break-Up Time (BUT)	▪ Tear film meniscus height
▪ Ocular surface staining with fluorescein, lissamine green, and rose bengal dye	▪ Schirmer test I and II
▪ Other tests: tear film osmolarity, lactoferrin, lysozyme immunoglobulin and albumin, and impression cytology.	

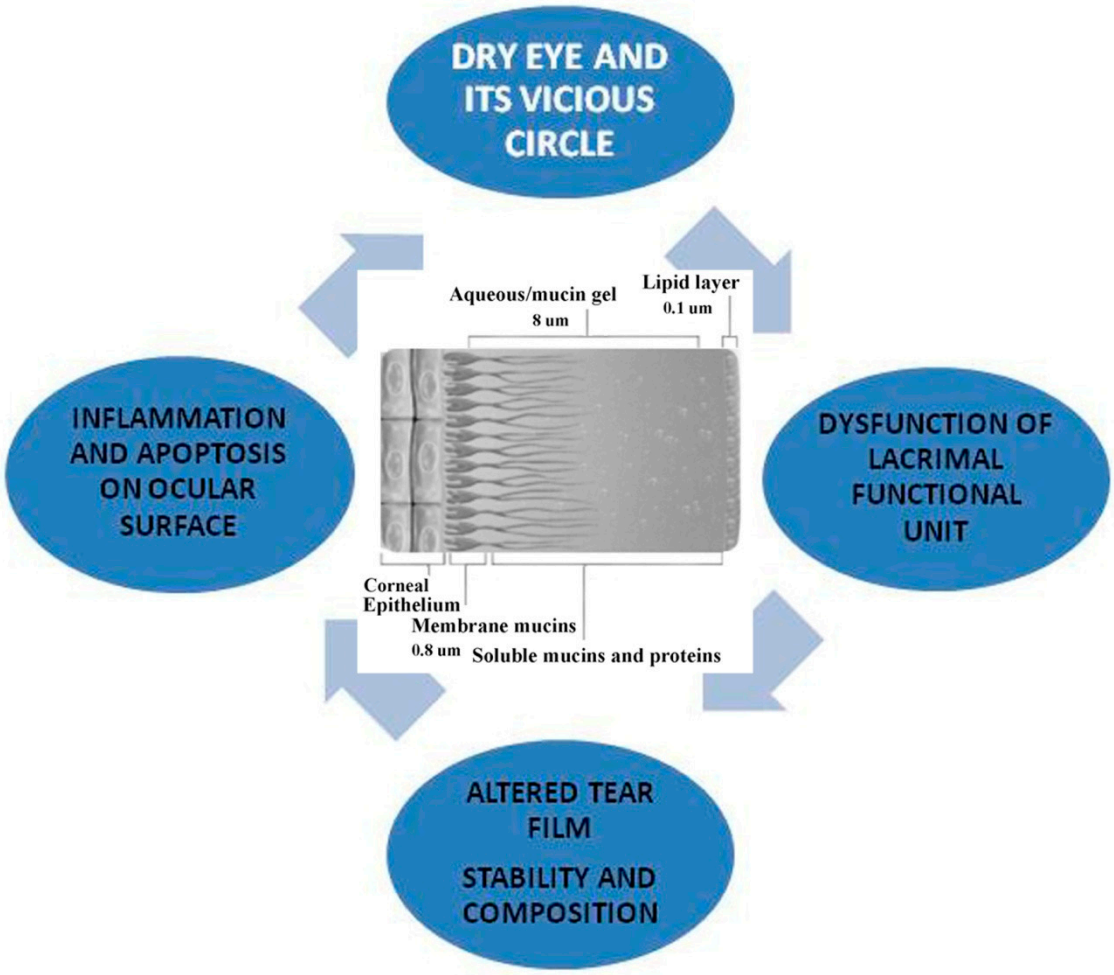

Figure 1. Dry eye and its vicious circle.

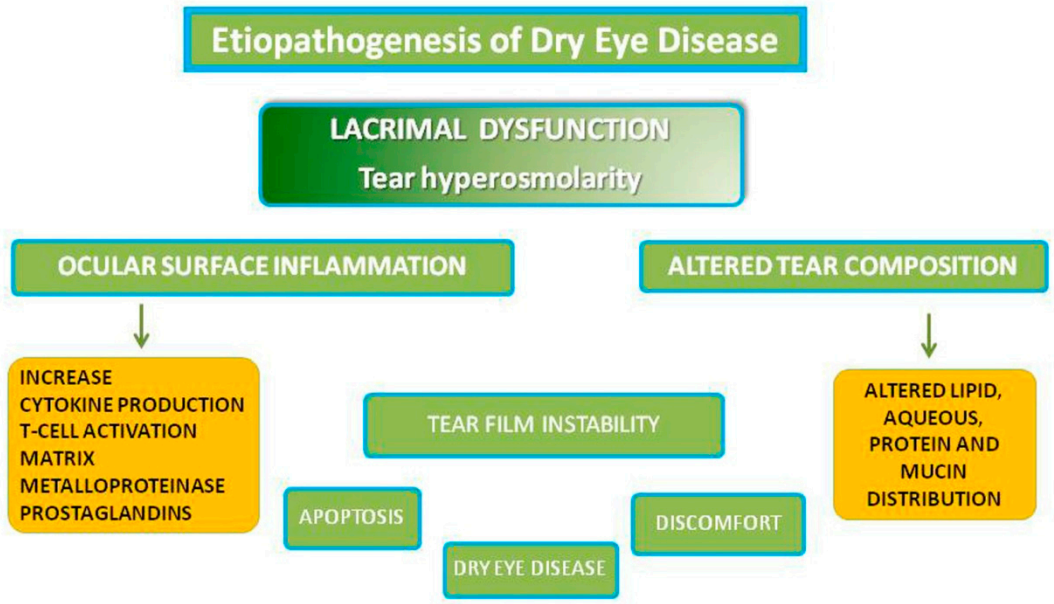

Figure 2. Etiopathogenesis of Dry Eye Disease.