Tear Biomarkers in Dry Eye Disease

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The diagnosis of dry eye disease (the early stages in particular) is important, but difficult, due to the lack of gold standards and poor correlation between tear biochemical changes and clinical signs. The current diagnostic tests (Schirmer's tests, tear film break-up time, and vital staining of the ocular surface) are more sensitive for severe cases. As a proximal fluid of the ocular surface, tear film analysis could be a promising area in the diagnosis and monitoring of dry eye because of the non-invasive nature of tear sampling procedures and the significant correlation between tear biochemical changes and progression of the disease. This article provides an overview of the most important tear biomarkers for dry eye disease (markers for lacrimal gland dysfunction, contact lens intolerance, inflammation, and oxidative stress) and their correlation with disease subtype and severity. The role of SDS-agarose gel electrophoresis of tear proteins (Hyrys-Hydrasys System, Sebia, Evry, France) as a potential routine test in diagnosis and management of dry eye disease and high-risk groups (computer users, contact lens wearers, cataract surgery, and glaucoma) is also detailed.

Keywords

Contact lens, dry eye, electrophoresis, glaucoma, inflammation, lactoferrin, lysozyme, oxidative stress, tear biomarkers, tear proteome

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Dry eye disease (DED) is a common ocular condition with a high impact on visual function and quality of life.[1] However, DED is one of the most misdiagnosed diseases because of a delay in symptoms and clinical signs, and the lack of unitary diagnostic criteria.[2,3] Moreover, current diagnostic tests are useful only in severe cases.[2] Thus, the identification of new tests for the diagnosis and management of DED is of great interest, and tear-biomarker assessment is a promising area, particularly in mild and moderate forms of DED.

According to the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) II Definition and Classification Subcommittee, "Dry eye is a multifactorial disease of the ocular surface characterised by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles".[4]

Initiated by the tear film hyperosmolarity and instability, the chronic immune-induced inflammation plays a pivotal role in DED pathogenesis.[5–8] There are certain inflammatory events that lead to cell apoptosis and loss of goblet cells, further altering the tear film, amplifying the inflammation, and creating a vicious cycle. These events are the alteration of epithelial immune receptors and antigen-presenting cells, the recruitment of inflammatory cells and dendritic cell maturation, the activation of T-lymphocytes, and the production of inflammation mediators and matrix metalloproteinases (MMP).[2,9] Recent studies have demonstrated the pathogenic role of transition from innate to adaptive immunity (induced by interleukin [IL]-6 and IL-6 soluble receptor) and autoimmunity.[10] Generated by inflammatory reactions, free radicals contribute to pathogenesis and/or self-propagation of disease by activation of nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasome and increasing of pro-inflammatory cytokine secretion (IL-1 β), or by leading conformational changes of tear proteins and subsequent protein aggregation.[11–14]

Two aetiological forms of DED have been described, depending on the origin of tear hyperosmolarity: aqueous deficient dry eye (ADDE) that results from a reduced tear production, and evaporative dry eye (EDE) in which the evaporation from the exposed tear film is excessive (*Figure 1*).[15] The TFOS DEWS II Pathophysiology Subcommittee emphasised that all forms of DED are evaporative since tear osmolarity depends on tear evaporation. Moreover, although EDE and ADDE are individual entities in the early stages of DED, both forms of DED have an evaporative component.[4]

The prevalence of DED increases with age and ranges between 5–50% depending on study and population studied.[15] DED can affect any race, but is more common in women than in men.[2,15] EDE (35% of DED cases) is more prevalent than ADDE (10% of DED cases), with the mixed forms affecting 25% of patients with DED.[2] The risk factors, which have been categorised as consistent, probable and inconclusive, are: age, female gender, ethnicity (e.g. Chinese, Hispanic and Asian populations), hormonal changes (menopause, pregnancy, androgen deficiency), environmental

Figure 1: Classification of dry eye disease

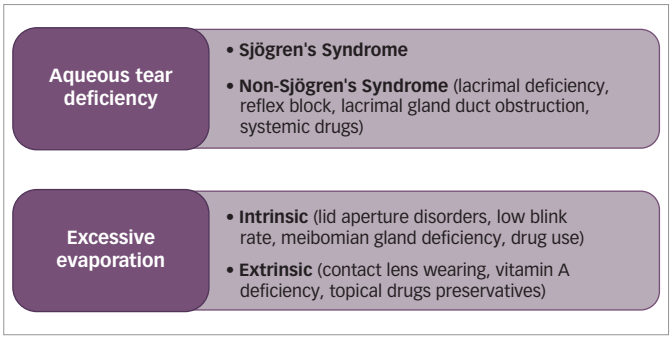
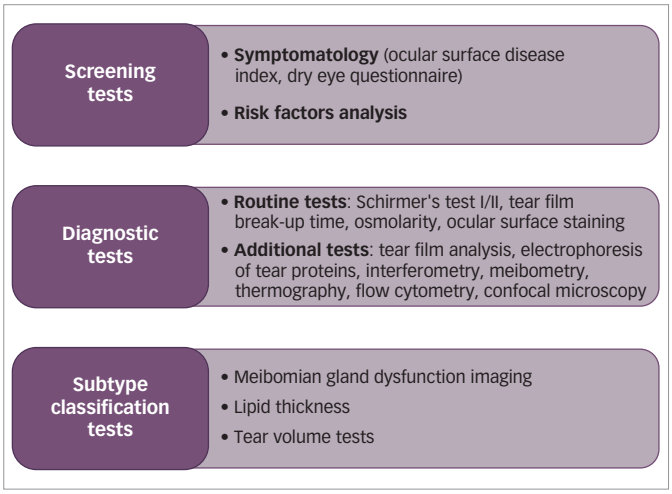


Figure 2: Diagnosis of dry eye disease



condition (pollution, low humidity), computer use, contact lens wear, some forms of ocular surgery, ocular (blepharitis, meibomian gland dysfunction [MGD]) or systemic disorders (thyroid disease, diabetes, dyslipidaemia, gout, osteoporosis), social and dietary habits (smoking or alcohol consumption), and medication use (antidepressants, anxiolytics, antihistamines or specific preservatives in topical medications).[15,16]

Diagnosis of dry eye disease

At present, DED diagnosis consists of a combination of the patient's symptoms, medical history and objective tests for tear function and ocular surface integrity (*Figure 2*).[8,9,17] The most commonly used tests include Schirmer's test, tear film osmolarity, meibomian grading, fluorescein tear film break-up time (TBUT) and vital staining of ocular surface epithelia (fluorescein, rose Bengal, lissamine green). Additional tests (interferometry, meibometry, thermography, flow cytometry and confocal microscopy) are used in order to improve the diagnosis accuracy. For the first time, the TFOS DEWS II Diagnostic Methodology Subcommittee have differentiated the diagnostic tests (e.g. symptoms, non-invasive break-up time, osmolarity and corneal/conjunctival/lid margin staining) from tests for subtype classification aetiologies (such as MGD imaging/observation and expression, lipid thickness and tear volume tests).[17]

Despite the complexity of diagnostic methodology, the diagnosis of DED is still a challenging task. This is due to the frequent discordance between patient symptoms and tear biochemical changes, the multifactorial aetiology of DED, the lack of consensus on the diagnostic criteria and standardisation of most routine tests, and the interference of mechanical or chemical stimulus in the results of measurements.[2,3] Moreover, the poor sensitivity of the conventional tests, their low positive-predictive value, and the limited availability of some innovative non-invasive procedures are strong evidence that the main interest in DED diagnosis should be the identification of disease-associated tear biochemical markers.

Tear film analysis in dry eye disease

Tear film analysis is a promising area in the diagnosis and prognosis of DED for two main reasons: the non-invasive nature of sample collection and the multiple origin of tear film biomolecules. It has been demonstrated that abnormal levels of many tear film biomolecules are related to dysfunction of the ocular surface.[18–20] Structured as two-phase body fluid (a lipid layer overlying a muco-aqueous phase), tears are a mixture of proteins, lipids, electrolytes and small molecule metabolites.[15,19,20] The lipid layer originates from meibomian and eyelid glands, and forms the barrier between the environment and the eye. The aqueous phase of tear film contains proteins, electrolytes, antioxidants, and growth factors arising both from the main and accessory lacrimal glands and ocular surface. It plays a central role in nutrition and protection of epithelia, ocular surface defence and maintenance of tear film stability. Proteins, which may be categorised as constitutive (sIg A), regulated (lysozyme, lactoferrin, lipocalin) and serum-derived (albumin), form the bulk of the aqueous layer.[21] The proportion of plasma-derived and conjunctival-derived proteins is correlated with tear-flow rate and the level of ocular surface stimulation.[22] Many gel-forming and transmembrane mucins were identified (such as soluble MUC5AC and transmembrane MUC1 and MUC16) in the muco-aqueous layer, playing a central role in protection, lubrication, barrier formation and hydration.[19] Decreased levels of tear mucins and alteration in the glycosylation pathway are common features in DED.[23]

Many technologies are now available for tear film analysis including agarose gel electrophoresis, enzyme-linked immunosorbent assay, mass-spectrometry based proteomic analysis, and the innovative multiplex bead analysis.[18,19,24]

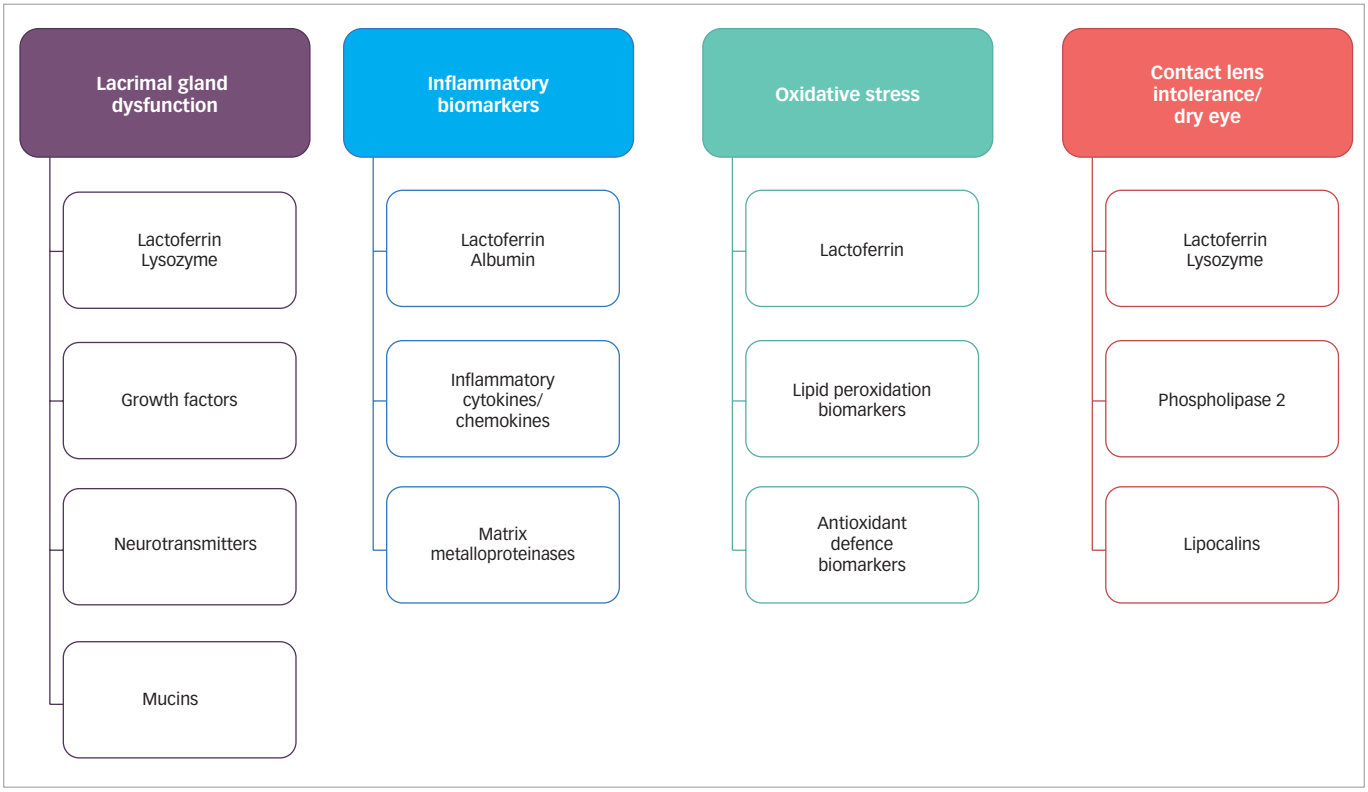
Tear collection methodology

Current methods for tear sampling include: collection from Schirmer strips; various types of collectors, such as sponges or rods positioned in the conjunctival meniscus to be impregnated by tears; and glass capillaries or micropipettes with a disposable, sterile mini-tip at the outer conjunctival cantus.[19,20] Although Schirmer strips are comfortable for the patients, the retention of some proteins with clinical significance, such as serum-derived proteins (e.g. albumin) and those with molecular weight <40 kDa (e.g. lysozyme), is the main limitation in tear protein analysis.[19–21,25] Because of the lower impact on tear protein profiles, tear collection using glass capillaries is considered the most appropriate for tear protein analysis, Schirmer strips being recommended only for analysis of multiple cytokines.[19,26] Tear protein analysis using Sebia agarose gel electrophoresis (Hyrys-Hydrasys System, Sebia, Evry, France) demonstrated that the electrophoretic pattern is not affected by the use of reflex tears, and both unstimulated (for mild and moderate forms) and reflex tears (for severe DED) can be used.[24] These results are in a good agreement with the TFOS International Workshop on Contact Lens Discomfort that emphasised no appreciable changes in lactoferrin, lipocalin 1 and lysozyme in closed-eye, basal and reflex tears.[21]

Traditional biomarkers for dry eye disease

There are two commonly accepted biomarkers for DED, one of which is lactoferrin, a multifunctional single-chain polypeptide with antimicrobial, antiviral, anti-inflammatory and antioxidant activities;

Figure 3: Tear biomarkers in dry eye disease



the other is lysozyme, a protein with antimicrobial properties.[26,27] As both lactoferrin and lysozyme are the main products of lacrimal glands, a decrease in their levels can be related to lacrimal gland dysfunction, ocular surface damage, inflammatory reactions, low antioxidant capacity, as well as low antimicrobial capacity (in particular lysozyme) (*Figure 3*).[26,28] Lysozyme and lactoferrin have been found to be decreased in patients with Sjögren's syndrome and/or glaucoma (with chronic medication-induced DED), with a higher specificity (95%) and sensitivity (72%) of lactoferrin compared to lysozyme.[26] Although some studies showed that tear lysozyme level did not differ between Sjögren's syndrome, non-Sjögren's syndrome DED and controls, this biomarker could be useful for monitoring the adverse effects of beta-adrenergic receptor-blocking drugs.[26] A cut-off value of 1.1 mg/mL for tear lactoferrin has been suggested by researchers for DED diagnosis, with high sensitivity (79.4%) and specificity (78.3%).[26] A new point-of-care test (Tear Scan™ Lactoferrin Diagnostic Test Kit, Advanced Tear Diagnostics, Raleigh, NC, USA) has been developed for the purpose of measuring lactoferrin levels.[19]

Tear proteome

Tear proteomic analysis has considerably improved the diagnosis and management of DED. Using mass spectrometry-based proteomic analysis, almost 1,800 proteins have been identified, more than 500 being recognised as candidate biomarkers (*Table 1*).[19] By removing the albumins and immunoglobulins from the analysis, these methods are the most appropriate for study of low weight molecular proteins.

Increased levels of annexin 2, enolase 1α, albumin, nerve growth factor, clusterin, β2 microglobulin, calgranulin A and B, cystatin SN, cathepsin S, defensins α and β, glycoprotein 340, secretoglobin 2A2, as well as decreased levels of lactoferrin, lysozyme, lipocalin, annexin 5, alpha 2-glycoprotein 1, lacritin, caspase 14, proline rich protein 3 and 4, cystatin S, cathepsin B, secretoglobin 1D1, prolactin inducible protein, and MUC5AC have been reported in DED.[19,24–26,28] Several proteins may distinguish between ADDE and EDE, and Sjögren's syndrome DED or non-Sjögren's syndrome DED (*Table 1*). For example, epidermal growth factor (EGF) is reduced in Sjögren's syndrome DED and ADDE, and increased in EDE due to MGD.[19,26,28] A significant decrease of lactoferrin, lipocalin 1 and lipophilin A and C levels, and a significant increase of albumin have been reported in EDE.[26,28] Lysozyme prolin-rich 4 is decreased both in ADDE and EDE, well correlated with disease severity.[28] Moreover, defensin 1, clusterin, and lactotransferrin were found to be unique in Sjögren's syndrome DED patients.[28,29]

Several tear protein panels have been suggested in order to improve the sensitivity and specificity, as well as the diagnostic accuracy.[29] A four-protein biomarker panel including α enolase, prolactin inducible protein, lipocalin 1, and calgranulin B demonstrated a diagnostic accuracy of 96% (91% sensitivity and 90% specificity).[28,29] An association of total protein content, albumin, and lipocalin 1 was suggested by Versura et al. with a high correlation with DED severity score.[29] A pentamarker panel, suggested by Soria et al., including S100 calcium-binding protein A6 (S100A6), annexin A1 and A11, cystatin S and phospholypase A2 activating protein, is able to discriminate between DED, MGD and control subjects.[30]

Despite the wide range of tear biomarkers identified using proteomic analysis, the small quantities of tears that can be collected, the lack of standardisation, and the limited availability of analytical procedures restrict the use of this analysis in clinical practice.[27]

Electrophoresis of tear proteins

Sodium dodecyl sulphate (SDS)-agarose gel electrophoresis using the Hyrys-Hydrasys system is able to remove most of the aforementioned limitations. For example, it has the following advantages: (1) the relative quantification of many proteins in a single

Table 1: Potential biomarkers in dry eye disease and their capability to distinguish between Sjögren's syndrome/non-Sjögren's syndrome dry eye disease and disease subtypes

Tear biomarker	Changes in DED	Differences between SS/non-SS DED	Differences between DED subtype	Contact lens intolerance
Lacrimal gland dysfunction				
Lactoferrin	Decreased	↓ SS-DED	↓ ADDE	
Lysozyme	Decreased	↓ SS-DED	↓ ADDE	
Calgranulin A/S100A8	Increased	↑ SS-DED	No	
Calgranulin B/S100A9	Increased	↑ SS-DED	No	
Annexin A2	Increased	↑ SS-DED	No	
Cystatin S	Decreased	No	No	
Cathepsin S	Increased	↑ SS-DED	No	
PRP4 kinase	Decreased	↑ SS-DED	No	Decreased
Tear lipocalin	Increased/decreased	↓ SS-DED	↓↑ ADDE	Increased
Secretoglobin family 1D member 1	Increased/decreased	No	↓↑ ADDE ↓ MGD	Decreased
Lacritin	Decreased	↓ SS-DED	↓ ADDE	Decreased
Secretoglobin family 2A member 2	Increased/decreased	No	↓ ADDE	Increased
Enolase 1α	Increased	No	No	
Mucin MUC5AC	Decreased	↓ SS-DED	No	
Neuromediators Nerve growth factor	Increased	No		
Calcitonin gene-related peptides	Decreased	No	No	
Neuropeptide Y	Decreased		No	
Serotonin	Increased	No	↓ ADDE	
Growth factors Epidermal growth factor	↓↑ ADDE and SS-DED; ↑ MGD	Yes	Yes	
Inflammatory biomarkers				
Interleukins	Increased	IL-2, 4, 6, 10, 17, 22, IFN-γ	IL-2, 5, 6, 9,10, 12, 15, 16	
Chemokines	Increased	CCL3, CCL4, CCL5, CXCL9, CXCL10	CCL3, CCL4, CCL5, CCL15, CXCL1, CXCL5, CXCL811	
Albumin	Increased	No	↑ ADDE	Increased

ADDE = aqueous deficient dry eye; DED = dry eye disease; IFN-γ = interferon gamma; IL = interleukin; MGD = meibomian gland dysfunction; SS = Sjögren's syndrome

analysis; (2) the small quantity of tears (5 µL) necessary for the test (unstimulated for mild and moderateforms or reflex tears for severe DED, with no significant differences between the electrophoretic patterns); (3) the short time in which the results are obtained (3 hours); and (4) assuming the instrument, which is commonly available in laboratories worldwide for routine electrophoresis of serum and urinary proteins, is available locally.[24,27] Lactoferrin (24.4–27.3% of total proteins), lysozyme (44.3–47.8%), albumin (1.4–2.6%), and proteins 20–60 kDa (7.4–10.0%) are the most important peaks that can be detected on Sebia electropherograms.[27] The levels of these biomarkers are correlated with DED severity or subtype.[24,27]

A four-tear protein panel consisting of lactoferrin, lysozyme, albumin and proteins 20–60 kDa has been shown, by several studies, to improve the diagnostic accuracy for DED.[24,25,29] In good agreement with the proteomic studies of Versura et al., the decrease of lactoferrin and lysozyme, along with an increase in albumin may reflect an early inflammatory reaction, and anticipates other clinical signs of DED.[25] Moreover, additional bands in the 20–60 kDa protein zone could be used as diagnostic criteria for lacrimal gland tumour or ocular complications for diabetes.[24,27] Well correlated with the level of glycated haemoglobin and microalbuminuria, a slight increase in the level of 20–60 kDa proteins is a common feature in diabetes.[24] By contrast, the fulminate variation of these proteins has been reported in lacrimal gland tumour.[24,27]

The individual assessment of lactoferrin, lysozyme and albumin could be useful in the management and monitoring of DED evolution, response to therapy (in particular, artificial tears, topical corticosteroids and cyclosporine A), and contact lens intolerance in some high-risk groups (computer users, contact lens wearers, glaucoma patients receiving chronic medication or patients undergoing cataract surgery).[24,27] Levels of lactoferrin <18%, lysozyme <35%, and albumin >15% are considered critical, being unique for severe DED. The best indicator for the efficacy of a given therapy is detecting an increase in the lactoferrin levels. In patients who use computers <3 hours/day, the lack of correlation between lactoferrin concentration in tears, Schirmer's test results and clinical signs suggest that the tear protein electrophoresis could be an important tool in early diagnosis of DED and prevention of complications. Decreased levels of lactoferrin and lysozyme in those who use computers for >3 hours/day have been correlated with ocular discomfort, supporting

the theory regarding the coexistence of ADDE and EDE in DED. Surgical procedures, such as cataract surgery, along with their associated use of topical anaesthesia and use of antibiotics may result in reflex hyposecretion with a subsequent inflammation and/or aggravation of a pre-existing DED. The decrease of lactoferrin and lysozyme, as well as the increase of albumin reflect the presence of an inflammatory reaction with a severity that is statistically correlated with changes to other tear biomarker levels. The amplitude of these changes improves over time in a favourable post-surgery evolution. Moreover, topical antiglaucoma therapy (in particular benzalkonium chloride, used as preservative in topical medication and ocular hypotensive active molecule) can induce or exacerbate a pre-existing DED. Thus, in glaucoma patients receiving chronic therapy, tear protein electrophoresis could be an important tool not only for monitoring the pre-existing DED, but also for early diagnosis of a therapy-induced DED in patients without an apparent DED-related problem.[24,27]

Tear inflammatory biomarkers

Identifying biomarkers to monitor ocular surface inflammatory reactions (*Figure 3*) not only improves DED diagnosis, the classification of disease severity and therapy outcome; but also provides important directions to develop effective anti-inflammatory treatments for patients with DED.[31]

In last 1–2 decades, multiplex bead assays have facilitated the identification of many cytokines, chemokines and chemokine receptors as tear biomarkers in DED. Increased levels of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-21, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, CXCL9, CXCL10, CXCL11, IL-1Ra, CCL5/RANTES, and fractalkine/CX3CL1 are common findings in DED.[26,28] These increased levels are mainly attributed to the upregulation of inflammatory genes in the conjunctival epithelium.[28] The cytokine/chemokine levels appear to be correlated with the type of DED and its severity. Thus, IL-17, IL-22, IL-6, IL-10, IL-4, IFN-γ, and TNF-α were increased in Sjögren's syndrome DED compared with non-Sjögren's syndrome DED.[26] Higher levels of TNF-α, IL-6, and IL-1β have been found in ADDE and mixed DED than in the EDE subtype.[32] Moreover, the levels of IL-6, IL-8/CXCL8, TNF-α, IL-1Ra, and CXCL11 have been correlated with DED severity.

Expression of MMP-9, a protease involved in induction of ocular surface damage and inflammatory signalling was also found to be significantly elevated in the tears of patients with DED.[2,26] However, the increase of MMP-9 activity was not specific to DED, being reported also in acanthamoeba/herpetic keratitis and ocular rosacea.[2] Thus, the MMP-9 expression in tears of patients with DED seems to be representative of specific ocular tissue damage or remodelling, and cannot be used as diagnostic tool for DED.[2] MMP-9 levels have been shown to be correlated with disease severity, and therefore can be used as a means of monitoring.[2] This is evidenced by development of the rapid point-of-care diagnostic test for tear MMP-9 (InflammaDry®, Quidel, San Diego, CA, USA), which has been commercially available, being able to detect levels of MMP-9 >40 ng/mL with an indicated sensitivity and specificity of 85% and 94%, respectively.[19]

Tear biomarkers for contact lens intolerance

Contact lens-related dry eye and contact lens intolerance are the most common complications among contact lens wearers.[21,22] As a result of a combined action of the lens and environmental factors (high air flow or low humidity), a contact lens is likely to alter the structure and stability of tear film, leading to DED by many potential mechanisms (increased tear evaporation and hyperosmolarity, lens dehydration, inflammation or dewetting related to lack of biocompatibility of the lens surface).[27] In turn, the resulting or pre-existing DED could lead to contact lens intolerance.

Although tear film stability, tear volume and other symptoms are recognised as the best variables for contact lens intolerance,[33] some tear biomarkers have been shown to differentiate between those who are tolerant and those who are intolerant to contact lenses (high levels of tear lipocalin and activation of phospholipase A2).[26,34] Tear biomarkers can also be used as potential tools for contact lens-related dry eye diagnosis, such as decreased levels of secretoglobin 1D1 (slightly reduced in soft contact lenses and significantly reduced in rigid gas permeable lenses), β2 microglobulin, proline rich protein 4 and lacritin, as well as increased levels of protein S100A8 in soft contact lenses, secretoglobin 1 A2, albumin, nerve growth factor, and prolactin inducible protein.[24,26–28,33,34] No correlation between cytokines and contact lens related discomfort has been demonstrated.[35] EGF, fractalkine, IL-10, IL-2, IL-4, IL-6, IL-8, IL-1 β, IL-1Ra, TNF-α, and MMP-9 are not affected by hydrogel contact lens wearing.[36]

Oxidative stress biomarkers

Environmental factors (pollutants, ultraviolet radiation and ozone), chronic therapy with preserved eyedrops in glaucoma, inflammatory reactions and decrease of antioxidant proteins (lactoferrin and lysozyme) are main contributors to oxidative stress on the ocular surface.[11] High levels of late lipid peroxidation markers 4-hydroxy-2-nonenal and malondialdehyde have been reported in tear film of patients with DED as indicators for oxidative damage, well correlated with ocular surface parameters (TBUT, Schirmer's test, corneal sensitivity).[37] Additionally, lactoferrin, S100A proteins, superoxide dismutase, peroxidase, catalase and mitochondrial oxidative enzymes are considered the most important antioxidant defence markers in DED. Although these biomarkers can be used for DED diagnosis, the sensitivity for discrimination between ADDE and EDE is low.[38]

Other tear biomarkers

Lacritin, a specific growth factor that promotes basal tearing when applied topically, is lacking in patients with DED.[39] Increased levels of aquaporin 5 (an integral protein located in the lacrimal glands and corneal epithelium) have been reported in patients with Sjögren's syndrome DED.[40] This is a result of aquaporin 5 release into the tears when acinar cells of the lacrimal gland are damaged by lymphocytic infiltration. Alteration of tear neuromediators has been also reported in DED.[41] Elevated levels of nerve growth factor, transforming growth factor and vascular endothelial growth factor, as well as decreased levels of calcitonin gene-related peptides, neuropeptide Y, and EGF, have all been suggested as potential biomarkers in DED.[26,41]

A cross-sectional study published in 2015 by Chhadva et al. reported high levels of tear serotonin in patients with DED symptoms and ADDE, compared with those with DED symptoms, but normal tear production and those without DED symptoms.[42]

Conclusions

Early diagnosis of DED is desirable, but due to the biochemical changes that can often occur before any signs of DED, and the fact that symptoms are not specific for DED, diagnosis can often be difficult. The routine tests, such as Schirmer's test, TBUT or vital staining, are invasive and/or only specific for severe DED. In the last 20 years, clinical interest in tear film analysis has increased due to the development of advanced methods of tear biochemical analysis, along with the non-invasive

nature of sampling methods. As a proximal fluid at the ocular surface and final output of the lacrimal functional unit, tears are a source of biomarkers which have multiple origins (lacrimal gland, ocular surface, epithelial cells, stromal immune cells, and meibomian gland acinar cells, as well as from blood) and whose changes reflect the condition of the lacrimal functional unit.[25,43] As DED has a multifaceted aetiology, many tear biomarkers have been identified. For example, lacrimal gland dysfunction has been associated with changes in the levels of lactoferrin, lysozyme, EGF and aquaporin 5; while inflammatory responses have been associated with changes in the expression of cytokines, chemokines and MMP-9. Other associations relate to oxidative stress (late lipid peroxidation products and antioxidant defence markers), and contact lens intolerance (lactoferrin, lysozyme, lipocalin, phospholipase 2).[19,26–28] Decreased levels of lactoferrin, lysozyme, lipocalin, growth factors and mucins, as well as increased levels of albumin, tear albumin, cytokines/chemokines and MMP activation are the main features for DED. The levels of these biomarkers may be used to distinguish between Sjögren's syndrome DED and non-Sjögren's syndrome DED, ADDE from EDE, or offer a measure of disease severity.[26,28] However, the lack of standardisation, the complexity of analytical procedures, and the small quantity of tears that can be collected, limit their use in clinical practice. SDS-agarose gel electrophoresis (Hyrys-Hydrasys system) can be used for routine analysis of tear fluid, and can be helpful in early diagnosis and the management of DED. This system has many advantages, including the quantification of many proteins using 5 µL of tears collected by a non-invasive procedure, the significant correlation with DED severity or subtype, the low cost of analysis, and the availability of the analytical instrument.[24,27] A four-tear protein panel consisting of lactoferrin, lysozyme, albumin and 20–60 kDa proteins, detected on Sebia electropherograms, has been successfully used for diagnosis of DED, as it is able to distinguish between disease sub-type and severity. The individual assessment of these biomarkers using SDS-agarose gel electrophoresis (Hyrys-Hydrasys system) could be useful in the management of DED and evaluation of response to therapy, particularly in high-risk groups (computer users, contact lens wearers, glaucoma patients receiving chronic therapy and patients who have undergone cataract surgery). Despite the multitude of tear biomarkers for DED, none of these has an absolute clinical value. For an accurate diagnosis, the levels of these biomarkers needs to be considered along with clinical history and other ocular surface investigations.

As the TFOS DEWS Tear Film Subcommittee recommends, further research directions should include the standardisation of tear collection and storage, as well as tear film metabolome studies with a particular focus on tear film amino acids and their derivates as possible markers of DED.[19] ❑

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