• Review Article •

New approaches for diagnosis of dry eye disease

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Abstract

• We reviewed the literature for different diagnostic approaches for dry eye disease (DED) including the most recent advances, contradictions and promising diagnostic tools and technique. We performed a broad literature search for articles discussing different methods for diagnosis of DED including assessment of tear osmolarity, tear film stability, ocular biomarkers and others. Articles indexed in PubMed and google scholar were included. With the growing cosmetic industry, environmental pollution, and booming of digital screens, DED is becoming more prevalent. Its multifactorial etiology renders the diagnosis challenging and invites the emergence of new diagnostic tools and tests. Diagnostic tools can be classified, based on the parameter they measure, into tear film osmolarity, functional visual acuity, tear volume, tear turnover, tear film stability, tear film composition, ocular biomarkers and others. Although numerous methods exist, the most accurate diagnosis can be reached through combining the results of more than one test. Many reported tests have shown potential as diagnostic/screening tools, however, require more research to prove their diagnostic power, alone or in combination. Future research should focus on identifying and measuring parameters that are the most specific to DED diagnosis.

• **KEYWORDS:** dry eye disease; tear film stability; tear osmolarity; ocular biomarkers
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INTRODUCTION

Dry eye disease (DED), also known as keratoconjunctivitis sicca, is one of the most common ophthalmic conditions, affecting hundreds of millions of people worldwide[1]. Recent technological advances and research targeting DED have led to the emergence of new definitions and new approaches for DED diagnosis and management. The two main subtypes of DED, which form an overlapping spectrum, include evaporative and aqueous-deficient DED[2]. Based on the Tear Film and Ocular Surface Society Dry Eye WorkShop II (TFOS DEWS II) definition for DED, multiple factors are involved that would ultimately lead to loss of tear film homeostasis together with abnormalities involving the ocular surface[3]. The main pathophysiology of the DED is based on evaporation-induced tear hyperosmolarity which leads to direct and indirect inflammatory damage and associated ocular symptoms Evaporative dry eye may be due to intrinsic factors, such as low blink rate, or extrinsic factors, such as contact lens wear[4]. Ocular symptoms may include discomfort symptoms and/or symptoms of visual disturbance[3]. However, chronic ocular pain is the most commonly reported symptom by DED patients. The long-term damage to the corneal nerves has been found to induce sensitization accounting for ocular pain[5]. DED symptoms can significantly impact patients' work productivity and quality of life with various effects on different life activities including, a delayed reaction time while driving, a decline in sustained reading performance, and a slowing of out-loud reading but to a lesser extent than silent reading[6]. DED has even been associated with depression and anxiety, especially in DED associated with Sjögren syndrome[1].

FACTORS RELATED TO DRY EYE DISEASE DEVELOPMENT

Role of Blinking Blinking plays a major role in maintaining the precorneal tear film and promotes the release of meibomian lipids that lubricate the ocular surface and delay tear film evaporation[7]. Many *in vitro* studies that have challenged the conventionally attributed function of the tear film lipid layer (TFLL) and demonstrated that the lipid layer may not inhibit

the rate of evaporation[8], but serve other functions such as to allow the spread of the tear film and to prevent collapse[9]. Forceful blinking has been shown to make the lipid layer of the precorneal tear film thicker[10] and has been reported to reduce dry eye symptoms[11]. However, a recent study, like multiple previous studies, demonstrated no correlation between TFLL thickness and non-invasive tear break up time (TBUT)[11]. Infants have a significantly lower spontaneous blink rate (1-6/min)[12-13] than adults (15-30/min)[13-14], attributed to a thicker lipid layer and higher tear film stability, and associated with a higher TBUT[15-16]. Decreased spontaneous blinking rate during visual tasks has been shown to be associated with increased tear film instability and subsequent symptoms of DED[17]. However, a recent study has shown no significant correlation between the frequency of blinking and any of the ocular surface parameters, including the Ocular Surface Disease Index (OSDI) questionnaire score[7]. Incomplete blinking, on the other hand, has been associated with greater meibomian gland dropout, poorer meibum quality and decreased tear film thickness, all of which accounting for about two-fold increase in the risk of developing DED[7]. As a result, more partial blinks were found in DED patients with shorter inter-blink periods compared to healthy subjects, and the number of partial blinks was positively associated with OSDI scores[18].

Environmental Factors Environmental pollution also exacerbates the manifestations of DED. Exposure to nitrogen dioxide was found to increase the frequency of eye irritation, and the OSDI score was found to be positively associated with the duration of exposure to environmental pollutants[19-20]. Other studies demonstrated that exposure to ozone gas and low humidity also carries a risk of developing DED symptoms[21]. Isotretinoin, used in dermatology creams, was found to cause the ductal epithelium of meibomian gland in animal models to thicken and the mature acini number to decrease; the ocular discomfort complaint is increasing because of the growing use of cosmetic products and associated accidental eye exposure[22]. Staring at digital screens for long time is another factor that contributes to the exacerbation of DED, as prolonged use was associated with higher OSDI scores and shorter TBUT; possibly due to the reduced spontaneous blinking rate during reading tasks, thus promoting tear fluid evaporation[23]. Higher OSDI Scores were noted in the smartphone users given the smaller screens which are usually held at a closer distance than other screens, and the large amount of blue light emitted increase oxidative stress relative to using computer displays[23]. Cessation of digital screens use in children decreased the punctate erosion, OSDI scores and increased TBUT[24].

Ambient temperature can also affect the rate of tear film evaporation. It was demonstrated that as the temperature of the air increases to 25°C the tear evaporation rate increases 3 folds[25]. In another study, ocular surface cooling (OSC) was observed to co-localize and precede the appearance of fluorescein tear thinning and breakup (FTBU) by 1 to 2s, and a direct positive correlation was found between the rates of OSC and FTBU formation, which was attributed to tear film evaporation[26].

Ocular Surgeries Ocular procedures can result in or exacerbate a preexisting DED[27].

Cataract surgery Phacoemulsification can result in reduction of the tear film secretion with subsequent DED development through its effect on the neurogenic response of the eye[27]. Although cataract-surgery-induced DED was reported to be improve after only one month postoperatively, others demonstrated that it may persist for up to 6mo[27-28]. Hence, the importance of ocular surface evaluation in prospective cataract surgery patients[28].

Refractive surgery Although commonly transient, lasting 6-9mo; post-LASIK dry eye may last more than a year in some patients due to the reduction of corneal innervation with subsequent reduction of tear secretion and alteration in tear film quality; and to the compromise of the corneal and conjunctival epithelium integrity including goblet cells; all resulting in reduced tear film stability[29]. Patients with prolonged dry eye after refractive surgery showed signs of lipid layer deficiency which improved with lid warming, suggesting the presence of meibomian gland dysfunction (MGD) as an underlying cause, that is possibly due to reduced corneal sensation causing reduced blink rate as well as incomplete blinking[29]. Induced flattening was found to generate incongruity between the corneal surface and the posterior surface of the eye lids[29].

Strabismus surgery DED symptoms were experienced by some patients after strabismus surgery due to changes in corneal sensitivity, tear film instability and goblet cell loss. However, some patients reported symptoms prior to surgery which was attributed to the larger area of bulbar conjunctiva opposite to the side of deviation and distortion of the normal relation between the lids and the globe, leading to microtrauma with increased friction between the lids and the globe[30].

Diseases and Treatments

Sjögren's syndrome Sjögren's syndrome is an autoimmune disorder characterized by exocrine gland dysfunction and predominantly associated with aqueous-deficient DED. Although the exact pathogenesis is unclear there is evidence of a concurrent evaporative mechanism, due to MGD, along with its principle etiology of autoimmune mediated lacrimal gland dysfunction[31].

Parkinson's disease Parkinson's disease (PD) has also been associated with a myriad of ocular manifestations and conditions including an increased risk of DED. The principal feature of DED in PD is decreased aqueous tear production, attributed to the autonomic dysfunction[32].

Hemopoietic stem cell transplant Graft versus host disease (GVHD) is a grave complication associated with allogeneic hematopoietic stem cell transplant. DED is the most common manifestation of ocular GVHD (oGVHD)[33]. The underlying mechanism is a combination of lacrimal gland destruction, severe meibomian gland damage and other factors, resulting in severe ocular surface abnormalities and symptoms[34].

Diabetes Diabetes has been recognized as one of the leading systemic risk factors for DED[35], however DED prevalence in this population may be underestimated due decreased symptom reporting associated with diminished corneal sensitivity in diabetic patients[1]. DED frequency was found to be positively associated with longer duration of the disease and significantly higher in diabetic retinopathy patients[36]. Furthermore, DED severity has been positively correlated with the severity of diabetic retinopathy[37]. The pathogenesis is multifactorial and stems from the vast complications of systemic hyperglycemia, ultimately leading to lacrimal dysfunction and associated deficiency in tear production, changes in tear film parameters and blinking abnormalities[35].

Other Factors

Contact lenses The mechanical and hypoxic stress caused by wearing contact lens, and the associated increased risk of microbial contamination, contribute to the development of subtle epithelial injury and severe infiltration and microbial keratitis[38]. The inflammation subsequently creates a vicious cycle of ocular damage making contact lens wear an additional factor related to the precipitation or exacerbation of DED[38].

Age Tear film stability decreases with advancing age[39] and major decline has been shown to occur between birth to age 25y; with infants having the most stable tears[40]. Signs of DED, such as decreased breakup time and increased blink rate, are the same changes observed with aging[39]. Age-related derangements affecting the lacrimal glands, meibomian glands, eyelids, conjunctiva, ocular surface and other ocular elements[41] including tear film compositional and structural changes[39] have all been associated with increased DED manifestations. Furthermore, the increased prevalence of certain ocular diseases with age, such as glaucoma[42] and systemic co-morbidities, such as hypertension and diabetes, and their treatments can all exacerbate dry eye with advancing age[41].

Occupation and others Occupation is another risk factor for DED especially among patients with indoor and sedentary jobs. Office workers with heavy digital screen usage have been shown to exhibit the highest risk[43]. In addition to the drying effects of the office environment, the underlying mechanism has been attributed to various effects of digital screen use including, incomplete blinking, diminished blink frequency and inappropriate gaze angle[44]. On the other hand, outdoor and active occupations, such as agriculture and fishing, were found to be protective against DED[43]. Other risk factors for DED include female sex, Asian race, medication use, allergies, estrogen replacement therapy, vitamin A deficiency, essential fatty acid (EFA) deficiency and hormonal imbalance such as androgen deficiency[1].

DRY EYE DISEASE AND HIGH ORDER ABERRATIONS

Tear film instability causes irregularity of the corneal surface resulting in development of higher order aberrations (HOA)[45]. Hartmann-Shack or double-pass aberrometers, can be used to evaluate the dynamic changes in image quality in DED patients[45]. Functional visual acuity assesses vision periodically. It can demonstrate reduction of visual acuity in DED patients with eye opening due to tear film irregularity and visual impairment experienced in daily tasks[46]. Studies using aberrometers have demonstrated higher HOA only in eyes having superficial punctate keratitis[47]. However, regardless of presence of superficial punctate keratitis, eyes with DED had higher straylight which is responsible for glare[47]. When compared to controls, patients with DED had lower contrast sensitivity[47].

APPROACHES FOR DRY EYE DISEASE DIAGNOSIS

According to the TFOS DEWS II report, DED is a self-perpetuating cyclic disease whose pathogenesis begins along the continuum of evaporative and aqueous-deficient DED, ultimately leading to the disruption of tear film homeostasis and a vicious cycle of events[3]. DED creates a diagnostic challenge which undermines attempts at its definition due to the various diagnostic criteria available[48]. Relying on symptoms alone for DED diagnosis was found to be inadequate, because symptoms, although reproducible, are similar to those found in a range of other ocular conditions, with objective signs of DED present only in 57% of symptomatic patients[49-50]. The discordance between signs and symptoms may be because the symptoms precede the signs or due to a different underlying etiology within the pathophysiology of dry eye[50]. Neurosensory abnormalities have also been newly identified to play a key role in the multifactorial etiology of DED. Dysfunctional sensation, for example, may lead to ocular surface signs without any symptoms, hence, diagnosis based on presenting symptoms or signs alone may lead to misdiagnosis[3]. A variety of diagnostic tests for DED diagnosis are available, however, their sensitivity and specificity vary significantly according to patient specific characteristics, disease severity and other factors[51].

Questionnaires are valuable tools for symptom screening that can capture the patient's experience and be easily implemented in everyday practice[44]. Furthermore, they can detect subclinical and unrecognizable cases of dry eye, which is especially important in patients who are planned to

undergo high visual expectation ophthalmic surgeries[52]. Many different questionnaires are available to assess and quantify DED symptom severity and effect on quality of life, however, a standardized questionnaire for diagnosis has not yet been developed. A comparative listing of DED questionnaires is included in the Epidemiology section of the International DEWS 2017 Report[1].

According to the TFOS DEWS II Report, diagnosis should begin with excluding other possible conditions that can mimic DED through triaging questions. Then, questionnaires, such as the OSDI, can be used for screening and grading of DED symptoms. A positive symptom score (≥13 points OSDI score) would then warrant minimally invasive tests for homeostatic markers including ocular surface staining, tear osmolarity and non-invasive tear breakup time. It is sufficient to confirm the diagnosis if any one of the homeostatic markers was found to be positive in either eye. Once diagnosis is confirmed additional more invasive tests, such as meibography and tear volume (TV) assessment, are instigated to assess DED severity and allow sub-classification of DED based on its etiological origin, hence leading to appropriate treatment[51]. A comprehensive listing of DED differential diagnosis is included in the DEWS 2017 diagnostic methodology report[51].

SLIT-LAMP EXAMINATION

After careful history taking and symptom screening, examination should begin with a thorough slit-lamp examination, with and without staining, to identify signs of DED. Ocular surface examination using vital stains, including fluorescein and lissamine green, have been used to assess dry eye. These tests are cheap and easy to perform; however, they assess surface damage so they can't be utilized as diagnostic tools for early DED[33]. Fluorescein staining can identify superficial punctate epithelial erosions and can be helpful in diagnosis, especially when present in specific patterns that reflect certain DED etiologies, such as inferior corneal erosions in lagophthalmos[52]. Low tear film meniscus height is another important marker for DED that can be measured by slit-lamp biomicroscopy without staining, however, measurements were found to be more stable with Tearscope-plus device[53]. Unlike other causes of conjunctival injection, DED associated conjunctival hyperaemia is usually subtle and involves the fine, horizontal vessels in the bulbar conjunctiva, where the ocular surface is exposed[54]. In addition to investigator grading scales, computer-automated redness grading scales have been developed to objectively assess dry eye-associated redness for potential diagnostic and treatment follow up purposes[54]. Slit-lamp examination of the eyelid margin, eyelashes and meibomian gland orifices is important to reveal possible meibomian gland impaction, gland dropout, telangiectasias, collarettes, and chalazia[52]. However, diagnosis and classification of MGD is limited by examiner variability and poor repeatability of the different MGD grading scales[52]. Although previously reported to have poor diagnostic value[55], Lid-parallel conjunctival folds (LIPCOFs), have been shown to be a promising diagnostic sign[56] and a potential screening tool[57] for DED. LIPCOFs can be easily detected and graded by slit-lamp examination, however, more objective methods such as optical coherence tomography (OCT) have been developed for its classification[58-59].

TEAR FILM OSMOLARITY

Tear film osmolarity reflects the balance between tear production, evaporation, drainage and absorption[60]. Quantitative measurements of these variables can identify any imbalance and thus the cause of DED due to associated derangement in the normal physiological parameters[60]. A single osmolarity reading maybe normal in DED patients and thus an average tear film osmolarity, rather than a static value, should be utilized for improved diagnosis[61]. Moreover, variability of tear film osmolarity has been shown to be directly correlated with DED severity[62].

Three main methods are used to measure the tear osmolarity: freezing point depression (the gold standard), vapor pressure method and electrical impedance of tear film[63-65].

Devices Using Electrical Impedance to Measure Tear Osmolarity Electrical conductivity of tear film can be easily utilized in clinical practice as it requires a small tear film volume and a short test time (30s). TearLab osmometer is a new device that measures tear film osmolarity based on the number of the charged particles in the tear film sample[66-67]. Without using anesthetic or manipulation of the eyelids, the device can collect a small, but sufficient, tear sample using a small chip touching the tear film[67]. It measures the electrical impedance in a 50-nL tear film sample[67]. The I-Pen® is a new device that measures the electrical impedance using flexible sensor touching the conjunctiva. However, it has been suggested to be deficient in differentiating between normal eyes and DED[68].

Vapor Pressure Concept Vapor pressure osmometry is a highly accurate technique utilized to measure tear osmolarity. The main disadvantages were previously related to the impracticality of the test due to tear evaporation and reflex tearing and the need of large TV (5 mL)[66]. Wescor 5520 model is a new device that depends on the concept of vapor pressure to measure the tear film osmolarity, however, it can process smaller sample volumes (up to 0.2 microns) using a specialized sample holder, allowing better clinical feasibility and decreased error[64].

Freezing Pressure Osmometers Freezing pressure osmometers have been the gold standard to measure tear film osmolarity[63]. They can work accurately on a very small sample size

up to 0.2 μL, allowing basal tears to be obtained rapidly minimizing the risk of contamination which may lead to falsely low osmolarity readings[63]. One limitation for its use includes being operator-dependent with resultant operator-bias. It also requires an extensive apparatus and is time consuming, requiring up to fifteen minutes per measurement[64].

TEAR FILM STABILITY

Tear film break up time (TBUT) is a commonly used technique to measure tear film stability. TBUT using fluorescein is more liable, repeatable and minimally invasive. Performing the test without using fluorescein can better asses the tear stability but still lacks the information on tear evaporation[69]. Fluorescein can destabilize the tear film affecting the results[69]. Non-invasive methods for evaluating the TBUT by capturing images of rings reflected by the tear film include the TFLL interferometry, the xeroscope and the Keeler tearscope and are superior to traditional methods in that the tear film is undisturbed by fluorescein; however, their results are not standardized[52,70-71]. Topographic analysis, which uses the corneal contour to provide data about regularity of the corneal surface and measures tear stability in patients with tear dysfunction, can give only one result at a time[72]. To overcome this limitation, a software was made to allow corneal topography instruments to take multiple measures and give dynamic results[70]. The system takes 10 consecutives corneal topograms over 10s period; and uses the intensity of the light reflected from each point to make a wave pattern that can be used to measure tear stability[70]. Instruments such as the Oculus Keratograph 5M and LipiView II can give objective measurements in addition to evaluating the meibomian gland among others; therefore, they are considered superior in the diagnosis of tear film stability[73]. In a study conducted to test the Oculus Keratograph results showed that noninvasive-TBUT had good correlation with traditional TBUT, Schirmer 1 and meniscus height[74]. The LipiView, on the other hand, measures the lipid layer thickness which was found to be negatively associated with symptoms of dry eye[73].

FUNCTIONAL VISUAL ACUITY

Another attempt to monitoring and reaching DED diagnosis was made by creating a device that relies on visual acuity[75]. Patients usually notice transient blurring of vision that is influenced by environmental factors, time of day, job requirements, and rate of blinking and visual demands[75]. FVA was defined as functional vision for daily activities[75].

The proposed method for measuring visual acuity with the eyes kept open without blinking (for 10 to 20s) after the instillation of 30 mL of 0.4% oxybuprocaine chloride into the eye[76]. However, the accuracy of measurements, inter-test variations and the timing of measurements all represented significant drawbacks to this method[77].

The Functional Visual Acuity Measurement System (SSC-350) may be used in patients who should refrain from blinking for 30s after a topical anesthetic instillation for the process[76]. DED patients were found to have a decreased FVA that can improve with treatment[78]. Accordingly, the device is thought to be useful in the diagnosis of DED and following up treatment.

MEIBOGRAPHY

Meibography is a valuable imagining tool that allows for direct clinical evaluation of MGD, which is at the root of evaporative DED. MGD can be broadly classified according to secretion rate into Low delivery (obstructive or hyposecretion) and high delivery (hypersecretory) states both having either primary or secondary underlying etiological origins[79]. MGD is more commonly associated with evaporative DED than aqueous-deficient dry eye[80]. The two principle meibography techniques are contact transillumination and the most recent non-contact meibography (NCT) which allows for non-invasive evaluation morphological abnormalities and quantification of MG loss (MGL)[81]. Infrared meibography is the most common meibographic technology utilized in both contact and non-contact techniques, however more recent technologies including laser confocal meibography and optical coherence tomographic meibography are available[82]. Recent advances have led to more compact and mobile devices that can be easily be used in common clinical practice[80]. Meibography alone cannot be used to diagnosis of DED however it is a useful clinical tool that can reinforce the diagnosis of evaporative DED[79].

TEAR VOLUME

The Schirmer test is one of the most commonly used tests for measuring tear production and diagnosis of dry eye given that it is simple, cheap and readily available, however, it has many limitations; and its results are non-reproducible and inaccurate due to the reflex tear component[83]. Furthermore, the test lacks standardization and can only quantify tear production in terms of volume without considering other etiologies that may contribute to the pathophysiology of DED, such as evaporative aspects and the actual tear constituents[83-84]. Although phenol red test, which measures TV production, is more difficult to perform compared to Schirmer test; it is less irritating and needs less testing time[69].

Tear film meniscus height reflects the overall TV, which in turns indicates the secretion and drainage rates of the lacrimal system[85]. The tear film meniscus can be measured quantitatively in a minimally invasive manner[86]. Classically, TV was measured using slit lamp, photography, video recording, or a tearscope. Recently, anterior segment optical coherence tomography (AS-OCT), is used as an accurate non-invasive method to measure the height of tear meniscus, which

in turn reflects TV[61]. It has been proven that tear meniscus measurements correlate with fluorescein staining and TBUT in DED patients[61]. AS-OCT captures and analyzes the tear meniscus image according to tear meniscus area, depth, and height. It can be used to differentiate between normal and DED patients based on the tear meniscus area and height[85]. AS-OCT can also measure epithelial thickness which is affected by tear film dysfunction[87]. AS-OCT ensures good repeatability and can be used to follow up changes of tear meniscus morphology after fluid instillation[88]. Thus, AS-OCT can be used as a rapid, qualitative and quantitative method of determining tear clearance rate. It has been suggested that changes in tear meniscus morphology and tear clearance measured by AS-OCT is a function of age[88]. Advanced AS-OCT technology can acquire 3D images of Meibomian glands which appear to be parallel to each other like clusters of grapes with clearly visible saccular acini in healthy individuals[89]. Analysis of tear film dynamics during blinking in DED patients using AS-OCT revealed reduced tear meniscus volume and height in DED patients compared to healthy individuals except for volume of the upper tear film meniscus[90]. Epithelial integrity factor has been proposed as an objective quantitative measure for corneal surface irregularities using high resolution AS-OCT with scores ranging from 0 to 4[91].

TEAR TURNOVER

Reduced tear turnover (TT) results in the ocular surface inflammation and positively correlated with DED symptoms[92]. Adequate physiological tear clearance depends on the integrity of the lacrimal system, and the rate of clearance is the summation of tear secretion by the lacrimal glands and ocular surface epithelia, fluid transudation through the conjunctiva, tear evaporation, tear drainage through the nasolacrimal system, and corneal and conjunctival permeability[49]. Tear clearance is used to reflect ocular surface irritation, severity of the ocular surface disease[49], MGD[93], and decreased ocular surface sensitivity[94].

Tear function index (TFI) and fluorescein clearance test (FCT) measure tear clearance by fluorescein instillation and a testing strip in the lower conjunctival fornix. Length of the wetted part is measured through serial measurements, and intensity of dye staining is compared to standard strip colors[78].

Fluorophotometery is the gold standard for measuring TT and TV; however, it is expensive and requires a lab and special expertise which makes it not practical clinically and limits its use to research purposes[73]. The International DEWS report presents assessment of TT rate with fluorophotometery as one of the additional measures of tear film that can be used to diagnose and monitor DED and addressed the need to develop cheaper, shorter and more simple methodologies[1].

TEAR FILM COMPOSITION

Meibum Lipid Composition, Structure and Function

The TFLL constitute 0.3% of the thickness of the tear film and is mostly derived from meibomian glands, with minor contribution from sebaceous glands[95]. In conjunction with other tear film components, the TFLL has a multitude of equally important functions, including suppressing evaporation, stabilizing the air/tear surface and serving as a first line of defense against bacterial invasion[96]. Multiple analytical tools, including infrared spectroscopy and H1-NMR spectroscopy can be used to uncover the compositional and structural details of human meibum. Human meibum's contribution to the tear film stability has been shown to decline with advancing age[39-40], MGD[97] and hematopoietic stem cell (HSCT) transplant patients[98]. This decline has been correlated to changes in the meibum composition and structure. Meibum lipid order (viscous structure), positively associated with lipid saturation and phase transition temperature, has been shown to increase in MGD patients[99] and HSCT transplant patients[98], however, contrasting studies have reported an increasing[40] *vs* a decreasing[39,99] lipid order with advancing age and in both cases attributed to the decreased tear film stability. Increased unsaturation, associated with decrease in lipid order, could contribute to decreased tear film stability with age[39]. Lipid order in infants is higher than adults and associated with enhanced tear film (TF) stability[39], whereas, an even higher lipid in MGD patients is associated with diminished stability[99]. The changes in meibomian lipid order can be used as a discriminatory marker for MGD with an accuracy of 93%, thus a promising diagnostic marker for DED[99]. Heating eye lids, associated with increased lipid disorder, has been shown to ameliorate symptoms in DED patients[100]. Furthermore, treatment with azithromycin, in addition to ameliorating symptoms, was associated with reduction of the meibum lipid order, which further stresses the relationship between lipid order and tear film stability[101]. Meibum should be balanced, such that it is fluid enough to exit the meibomian glands while at the same time rigid enough to form a continuous layer that can withstand shear stress and resist TF breakup[8]. Meibum compositional changes, such as lower cholesteryl esters in MGD patients, can also serve as diagnostic markers that can discriminate between normal and DED patients and can potentially be used to follow up efficacy of treatment[102].

Tear Film Biomarkers A biomarker is defined as a certain parameter that can be objectively measured and quantified to reflect a biological process whether physiological or pathological[103]. Biomarkers can be classified according to what they reflect for example predictive, diagnostic or for monitoring[103]. For a biomarker to be approved for clinical practice certain criteria should be met, such as sensitivity,

specificity, reproducibility and cost effectiveness[104]. However, the same diagnostic challenge of DED still stands regarding finding it suitable biomarkers and the multifactorial etiology of the disease creates an obstacle to look for a single parameter[104].

There is very strong and valid evidence that inflammation constitutes an important pillar in the pathophysiology of DED. Increased inflammatory cells, increased expression of immune activation and adhesion molecules, T-helper type 1 (Th-1) and Th-17 attracting immune pathways, cytokines, and chemokines are all evidence supporting the inflammatory pathology of dry eye[105].

Thus, in the ongoing attempts to diagnose this multifactorial disease, several studies attempted to tackle these specific proteins and inflammatory cells and compare their values between DED patients and healthy controls to serve as biomarkers for diagnosing and monitoring the disease[106].

Several studies have analyzed the protein profile in tears of dry eye patients and difference in certain proteins were found between DED patients and those of controls such as (S100A4, S100A8/calgranulin A, S100A11/calgizzarin, prolinerich protein 3, proline-rich protein 4, a-enolase, prolactin-inducible protein, mammaglobin B, lysozime C, or lactoferrin)[106].

In one study, tear samples from 24 patients with DED were compared to that of 18 control subjects[107]. The panel of proteins in each group was comparatively studied. The ratio of proteins from each of the 24 patients was calculated relative to that collected from the 18 healthy controls, using isobaric tagging for relative and absolute quantification (iTRAQ) technology[107]. The study showed increasing tear levels of S100A8 and S100A9 proteins correlated with the severity of MGD in DED[107]. The level of S100A8 protein was significantly correlated to grittiness, while levels of both S100A8 and S100A9 correlated to redness and transient blurring. Furthermore, they documented that the levels of Lipocalin-1 was associated with symptoms such as heaviness of the eyelids and tearing[107].

S100A8 and S100A9 proteins are linked to epidermal hyper proliferation, sequentially they maybe also be associated with meibomian ductal epithelium hyper-keratinization which is usually found in MGD[108]. The elevated proteins may be due to the damage sustained by the ocular surface epithelial cells from the effects of evaporative dry eye[109]. Another hypothesis proclaims that these proteins may originate from certain immune cells such as macrophages which are found to be increased in MGD[109].

A large study compared patients with dry eye to controls and found increased levels of IL-1b, IL-6, IL-8, TNF-α, and IFN-γ and increased expression of lipocalin, cystatin SN, and a-1 antitrypsin in tears of dry eye patients as compared to the control subjects[110]. Protein amounts were 2 to 2.5 times greater in dry eye patients as compared to controls. Cystatin SN and IL-6 showed the greatest differences when comparing values of DED patients to controls[110]. Subjects in the study were further subdivided into controls (CTRL), patients with aqueous-deficient dry eye (DRYaq), patients with changes of the lipid layer (DRYlip), and patients with a combination of both (DRYaqlip).Variation in protein profiles was most noticeable when comparing DED patients and controls in case of DRYaq or a DRYaqlip. On the other hand, minimal differences were observed in protein profiles when comparing of lipid layer deficiency patients with controls[110].

In aqueous deficient DED patients, cystatin SN was elevated up to 3-fold compared with controls, other proteins were increased between 2-folds to 3-folds. The cytokine with the most pronounced difference between DRYaq group and controls was TNF-α with a 2.5fold difference[110]. The significance of these findings is that proinflammatory cytokines are involved in the promotion of the differentiation of naive CD4 cells to Th17 T cells, which are important cells in autoimmune processes, thus fortifying the hypothesis of the inflammatory nature of DED[111].

Matrix metalloproteinases (MMPs) are enzymes found in high levels in cases of ocular inflammation, which contributes to the pathophysiology of DED[112]. It has been found that MMP-9 level is positively associated with decreased low-contrast visual acuity, scores of corneal and conjunctival fluorescein staining among other parameters. Also, it is negatively associated with fluorescein TBUT[113]. The InflammaDry test detects MMP-9 in the tear film. The examiner collects a tear sample, then activates it with a buffer solution. In 10min, the test will either show a single blue line (negative result) or a blue line with a red line (positive result). The test uses 40 μg/mL of MMP-9 as a cutoff point; anything above that will yield a red line[113].

Another study was preformed to study C-C chemokine receptor type 5 (CCR5) and its ligands on the ocular surface and how they correlate with the severity of symptoms in DED patients. They studied the expression of the MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/ CCL5, and CCR5 in the tear film and ocular surface of DED patients including both Sjögren's and non-Sjögren's syndrome (SS), and results were compared to healthy controls. The study showed elevated tear concentration of CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES and CCR5 expression in ocular surface in Sjogren syndrome patients compared to both non-Sjogren syndrome and controls. Higher levels were also demonstrated in in non-Sjogren syndrome as compared to controls[114]. CCL3/MIP-1α and CCL4/MIP-1β in patients with DED showed positive correlation with both the tear clearance rate and goblet cell density[114].

CCL5/RANTES levels is significantly and positively correlated with each of the following parameters TBUT, basal tear secretion, tear clearance rate, keratoepitheliopathy score and goblet cell density[114].

CCR5 is a receptor for the chemokine ligands CCL3, -4, and -5, it was found to be expressed on a number of different cells including activated (memory) Th-1 lymphocytes[115]. Thus, documented overexpression of CCR5 in tears and conjunctival epithelial cells of DED patients as compared to healthy controls validated and supports the hypothesis of the Th-1 mediated inflammation playing a role in the pathology of dry eye[114]. The study also assessed the CCR5 ligands CCL3, -4, and -5. CCL5 plays an important role in attraction of T cells to the sites of inflammation and plays an important role in their differentiation and maturation, thus it is considered to be the only cytokine involved in the overall process of T cell activation[116].

In the study CCL5 level showed significant correlation with different tear film and ocular surface parameters when compared to both CCL3 and CCL4[114]. Accordingly, CCL5 can be considered the most important cytokine for T cells involved in the pathophysiology of DED[114].

CONCLUSION

The prevalence of DED is increasing with the increasing number and extent of DED risk factors, however its diagnosis remains challenging given its multifactorial etiology and frequent discordance between signs and symptoms. Various diagnostic tools and techniques are available, however, combining results from more than one test, in addition to signs and symptoms, can lead to a more dependable diagnosis, especially that no single test can measure all the factors involved in the pathophysiology of DED. With that, DED diagnosis continues to be a challenge that invites for the development of new tools and technologies and conduction of further research in the field.

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