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Chapter

Diagnosis of Dry Eye

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Abstract

Dry eye is a multifactorial disease and hence single test cannot diagnose dry eye. Diagnosis of dry eye needs careful assessment of the symptoms along with battery of investigations. Many questionnaires have been developed to assess the symptoms of dry eye disease (DED). Some of the important questionnaires are Ocular Surface Disease Index (OSDI), Dry Eye Questionnaire (DEQ-5), Impact of Dry Eye on Everyday Living (IDEEL), National Eye Institute's Visual Function Questionnaire (NEI VFQ-25) and Dry Eye-Related Quality-of-Life Score (DEQS). Investigations for dry eye mainly target on the tear secretion, tear clearance, tear volume, tear film stability, tear evaporation, ocular surface damage, lipid layer of the tear film, chemical properties of the tear film and inflammation of the ocular surface. There are many investigations that target on the above parameters and helps in accurate diagnosis of Dry eye disease (DED).

Keywords: Dry eye disease, Ocular surface index (OSDI), Schirmers test, Phenol red test, Fluorescein

1. Introduction

1.1 Definition

Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEW) II amended the definition of dry eye into "Dry eye is a multifactorial disease of the ocular surface characterized 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" [1]. So, basically patients are not required to present with a particular set of symptoms to be diagnosed as dry eye disease (DED) but rather homeostasis of the tear film is more emphasized upon.

1.2 Classification

DED is classified into two main categories by TFOS DEW II. The two main types are the following-

1. Aqueous deficiency – It occurs due to deficient tear production.

2. Evaporative – Meibomian gland disease (MGD) lead to deficiency of lipid layer which ultimately leads to excessive evaporation of tears [1].

The diagnosis of DED is not only based upon investigations but rather it depends on both the investigations and signs and symptoms of DED.

2. Questionnaires

Numerous questionnaires have been developed till date to study dry eye symptoms for many purposes such as diagnosis and quantification of DED, to study epidemiology of the disease, to assess effects of the treatment and its impact on the quality of life. The questionnaires are as follows-.

2.1 Ocular Surface Disease Index (OSDI)

It is a questionnaire consisting 12 questions, developed by the Outcome Research Group at Allergan which was designed for quick assessment of the symptoms of DED and their impact on vision related problems such as visual disturbance (poor vision or blurred vision) and visual function problems (difficulty in watching TV. working on a computer, driving at night and reading) [2].

The response to all the questions is graded on a scale of 0 to 4 -

a. none of the time

b. some of the time

c. half of the time

d. most of the time

e. all of the time

The following formula is then used to calculate the OSDI [3].

$$\text{OSDI} = \frac{\left(\textit{sum} \text{ of scores for } \textit{all} \text{ questions answered}\right) \times 100}{\left(\text{total number of questions answered}\right) \times 4} \quad (1)$$

A randomized study was performed on 68 patients admitted in ophthalmology Polyclinic of the Dumlupinar University from December 2005 to April 2006. Patients of 18 years and above were included in the study. The history taking and OSDI calculation was done by the same physician. Then after the routine ophthalmic assessment, the Schirmer test and TBUT were performed by another physician. The correlation analysis was done between Schirmer test, TBUT and OSDI scores. The patients were divided into 3 groups according to the OSDI scores and they are as follows-

- Group 1 had patients with low OSDI score of 0–20 points
- Group 2 had patients with moderate OSDI score of 21–45 points
- Group 3 had patients with high OSDI score of 46–100 points [3].

The result showed there was a statistically significant difference between TBUT test scores of patients with low and high OSDI scores (p = .043), there was no significant difference between Schirmer test scores of the three groups. They concluded that although there is no internationally accepted criterion for the diagnosis of DED as of now, the OSDI is a standardized questionnaire to evaluate symptoms, and can easily be performed and used to support the diagnosis of DED [3].

2.2 Dry Eye Questionnaire (DEQ-5)

It is a questionnaire in which 4 dimensions are used to measure a series of symptoms. The 4 dimensions are -.

a. Frequency of watery eyes.

b. Degree of bother.

c. Late day intensity of discomfort and dryness (PM intensity).

d. Morning intensity of discomfort and dryness (AM intensity).

DEQ-5 scores of ≥ 6 establish suspicion of DED and indicates further clinical testing and of ≥ 12 establishes a suspicion of Sjogren syndrome (SS) [4].

A study reported that 10% of patients with non-Sjogren syndrome DED and 30% of patients with Sjogren syndrome complained of impaired vision while few of the other studies reported that 42% and 80% of patients with Sjogren syndrome experienced impaired vision [5–7].

It is believed that open-eye conditions might affect symptom progression as visual problems generally increase in intensity over the day [8].

2.3 Impact of Dry Eye on Everyday Living (IDEEL)

The questionnaire has 2 items related to visual problems.

a. Blurry vision

b. Sensitivity to light, glare, and/or wind.

Statistically significant differences were observed between various patients od DED with varying level of severity and in responses to the IDEEL questionnaire scores [9].

2.4 National Eye Institute's Visual Function Questionnaire (NEI VFQ-25)

NEI VFQ-25 is a questionnaire that checks visual function by focusing on seven visual domains including general vision, near vision, distance vision, peripheral vision, color vision, driving difficulties and ocular pain. DED patients have poor NEI VFQ-25 scores for the subscales of general health, general vision, ocular pain, short distance vision activities, long distance vision activities, vision-related social function, vision-related mental health, vision-related role difficulties, vision-related dependency, and driving [10, 11].

2.5 Dry Eye-Related Quality-of-Life Score (DEQS)

This questionnaire developed in Japan. It has shown strong correlations with 4 subscales of the NEI VFQ-25 namely Ocular Pain, Near Vision, Distance Vision, and Mental Health [12].

2.6 Computer-vision symptom scale (CVSS17)

It is a Rasch linear scale containing 17 items. It explores 15 different symptoms of computer-related visual and ocular symptoms and is considered very valuable in computer related ocular morbidities. The CVSS17 includes a broad range of symptoms such as photophobia and excessive blinking [13].

2.7 McMonnies' Questionnaire (MQ)

It is a screening instruments for DED that reported sensitivity to be varying between 87–98% and specificity between 87% and 97% [14, 15]. It consists of 12 questions. Every question has polytomous response options that vary in number and type [16].

2.8 Ocular Comfort Index (OCI and OCI-C)

It was developed by Johnson and Murphy in 2007. It allows the quick assessment of the ocular comfort and grading the severity of DED. It uses Rasch analysis to produce estimates on a linear scale. It contains 15 items [17].

2.9 Symptoms Assessment in Dry Eye (SANDE)

It is based on 100 mm horizontal linear visual analog scale that quantifies both severity and frequency of dry eye symptoms. It consists of 2 questions [18].

2.10 Standard Patient Evaluation of Eye Dryness (SPEED)

It is based on both frequency and severity of the symptoms of the DED. It was designed to track diurnal and long-term symptom changes over a period of 3 months. The total score was calculated by adding the scores from both the frequency and severity parts of the questionnaire.

The symptoms inquired by the SPEED questionnaire include dryness or grittiness or scratchiness, soreness or irritation, burning sensation or watering and ocular fatigue reported and scored as sometimes – 1, often – 2 and constant – 3 and whether these symptoms pose no problems –0, were tolerable - 1, uncomfortable - 2, bothersome - 3, or intolerable –4 [19].

There are 3 more questionnaires which were developed to diagnose DED in the contact lens wearers –.

2.11 Contact Lens Dry Eye Questionnaire (CLDEQ)

It was developed by Begley [20]. It was used to investigate the frequency and severity of the symptoms of DED in contact lens wearers. It is quite similar to DEQ but the only difference is that patients here are using contact lens. It consists of 36 items [21]. It divides symptoms into nine subscales -

- Dryness

- Discomfort
- Visual impairment
- Irritation and soreness
- Grittiness or scratchiness
- Burning sensation
- Foreign body sensation
- Itching
- Photophobia

2.12 8-Item Contact Lens Dry Eye Questionnaire (CLDEQ-8)

The CLDEQ-8 is a short form of the CLDEQ questionnaire that was designed to describe symptoms among contact lens wearers [21].

2.13 Contact lens impact on Quality of life (CLIQ)

It is a questionnaire containing 28 items. It is based on Rasch analysis and shows good validity and reliability. Boer suggested that the psychometric properties of CLIQ were of high quality [22].

It is important to emphasize that these Questionnaires are not diagnostic tool, however can give a good clinical assessment of the problem. It acts as preliminary tool of assessment and is meant for screening purposes. Proper diagnosis requires a battery of additional tests. These above questionnaires act as adjunct to the clinical tests and cannot replace them in any form.

3. Diagnosis and ancillary testing

3.1 Aim

Investigations of DED are set with following goals -.

a. To confirm the clinical diagnosis of DED.

b. To quantify the DED.

Various tests have been devised to diagnose DED but no test can singly give you a diagnosis of DED. The correct way to diagnose DED it to correlate between the signs and symptoms of patients and the investigations planned.

3.2 Parameters to be measured

The following parameters are measured by tests to diagnose DED –.

a. Tear secretion.

b. Tear clearance.

c. Tear volume.

d. Tear film stability.

e. Tear evaporation.

f. Ocular surface damage.

g. Tear film chemical properties.

h. Lipid layer.

3.3 Investigations

3.3.1 To test the tear secretion and tear volume

3.3.1.1 Schirmer test

It is a test to quantify the tear production.

It is done with a blotting paper strip of 5 X 35 mm which is popularly known as Whatman filter paper number 41.

Method of application- It is folded 5 mm from the inner end which is rounded and placed in the lower fornix at the junction of middle and outer one-third and kept for 5 minutes. Touching the cornea or lashes should be avoided. Eyes should be gently closed during the procedures [23].

The normal tear production varies between 0.5 to 0.67 ml of tears/day and that wets more than 15 mm of the strip.

The Schirmer test are basically of 3 types but the most important amongst them are the first two.

- Schirmer's test I –It is done without the use of topical anesthesia and measures maximum basic plus reflex tear secretion.

- Schirmer's test II - It is done with the help of anesthesia. A drop of anesthesia is put in the eye, excess is wiped out with the help of filter paper. Then Whatman strip is placed same as in Schirmer test I. It measures only the basal tear secretion.

- Schirmer's test III - The patient is advised to look directly in the sun and it is done to know about the reflex tear secretion. It is dangerous and of no diagnostic value, so not used.

The cut-off values for diagnosis have been proposed as ≤5 mm/5 min to ≤10 mm/5 min with 77–85% sensitivity and 70–83% specificity [23].

This test lacks repeatability and shows variable results, so a single test should not be used to diagnosis rather a series of abnormal results in Schirmer's test raise a suspicion of DED. However, low cost of this test makes it one of the most commonly used tests clinically.

- Variations

3.3.1.1.1 Strip meniscometry

A variation of the above test is available now-a-days, which is done by dipping a strip into tear meniscus for 5 seconds. 25 mm polyethylene terephthalate is used to make the strip and it is covered with a urethane-based material [24]. The value ≤ 4 mm raises suspicion of DED. When used alone it has a sensitivity and specificity of 84% and 58% respectively and when combined with Tear film break-up time test the sensitivity is reduced to 81% but the specificity increases to 99% [25].

3.3.1.1.2 1-minute Schirmer test

It was proposed by Nelson to decrease the ocular discomfort and save time by decreasing the time of performing the test from 5 minutes to 1 minute [26]. The cut-off value was set to be 6 mm. Bawazeer and Hodge et al. in 2003 concluded that the 1-minute Schirmer test with anesthesia highly correlates with the 5-minute Schirmer test with anesthesia [27]. In cases of severe dry eye, a value of ≤ 5.5 mm in a 5-minute Schirmer test highly correlates with 2 mm in a 1-minute Schirmer test while in cases of mild to moderate dry eye a value of 5–10 mm in a 5- minute Schirmer test corresponds with 3–6 mm in a 1-minute Schirmer test.

3.3.1.2 Phenol red thread test (PRT)

It uses a thin cotton thread impregnated with pH-sensitive dye "phenol red". When the dye is dry, the thread is of yellow color, but when the dry is moistened by tears, the thread turns red (as the tears has slightly alkaline pH between 7 and 8) [28].

Method of application- the folded end of the thread is hooked over the lower eyelid margin in the temporal one-third if the eyelid for 15 seconds.

It has few advantages, as it is small in dimension so less chance of reflex tearing and the minimal amount of dye on thread decreases the chances of reflex tearing [29, 30]. It suggests that the reading of PRT is indirect and realistic measure of the tear volume in resting phase [31, 32]. But despite these potential advantages it is rarely used in clinical practice as it is manufactured only in few countries which makes their supply costly.

A cut-off value of 20 mm is used for differentiation of DED with and without aqueous deficiency [33]. Sensitivity and specificity of a cut-off value of 10 mm are 25% and 93% respectively [34].

Doughty et al. concluded that there is no statistically significant difference in the PRT performed with open or closed eyes [35].

3.3.2 To test the tear clearance

3.3.2.1 Fluorescein clearance test

Method- 5 μl of 2% fluorescein dye is instilled in the eyes. One set of Schirmer papers are inserted for each 10-minute interval for 30 minutes. The amount of strip becoming wet and the disappearance of the dye were recorded. Nasal stimulation is done using a cotton tip along with the last strip to induce reflex tear secretion.

It is used to measure basal tears, reflex tears and tear clearance all at the same time.

A cut-off value of ≥ 3 mm at the 10-minute interval suggests normal tear secretion. If the dye cannot be detected at the 20-minute interval, it is known as "Clearance" [36].

It has many advantages such as it is inexpensive, easy to perform, availability of the materials used is adequate. The disadvantages are same as in standard Schirmer test. Jordan and Baum et al. in 1980 reported that the above disadvantages cannot be suppressed by use of topical anesthesia [37].

3.3.2.2 Tear function index (TFI)

Method- The procedure is similar to the Schirmer test with anesthesia, but it uses 10-µl of 0.5% fluorescein.

Fluorescein is instilled into the lower conjunctival fornix and after 5 minutes of instillation the length of the wetted portion of the strip is measured and the intensity of the staining of dye is compared to the standard strips. The rate at which the color of the fluorescein dye fades is used to determine the tear clearance rate (TCR). It is graded as 1, 1/2, 1/4, 1/8, 1/16 1/32, 1/64, 1/128 and 1/256.

$$TFI = \frac{\text{Values of Schirmer test with anesthesia}}{TCR} \tag{2}$$

Kaye et al. proposed a variation of TFI by suggesting use of prepared strip containing 1.3 µl of 0.5% fluorescein. They reported that 10 µl of fluorescein use increases the volume of tear and it may also act as a stimulant. This in turn limits the applicability of the TFI test [38].

3.3.2.3 Fluorophotometry

Fluorophotometry is useful clinical tool, because an increased corneal uptake of fluorescein demonstrates subtle damage to the corneal epithelium. In humans, measurements of the penetration of fluorescein across the corneal epithelium can be used in diagnosing or monitoring dry eye disease. It is an excellent test but the need of the machine itself makes it a costly test so it not much used in clinical practice.

3.3.3 To test the tear volume

3.3.3.1 Tear meniscus assessment by Meniscometry

Tear meniscus height (TMH), curvature (TMR), and cross-sectional area (TMA) are used in clinical practice widely and have good accuracy rate and correlate well with other tests of DED [39, 40]. However, they are very much operated dependent. They have other drawbacks such as dependency on time from blink, fluorescein instillation. It can be influenced by the temperature, humidity, air velocity, illumination and location of measurement along the lid margin.

Now-a-days portable digital meniscometry with application software being installed in the iPod touch are being used. They have good reproducibility, good correlation with both the conventional video and Optical coherence tomography (OCT) meniscometry and it detects tear meniscus changed after the instillation of artificial tears.

At present, OCT meniscometry studies parameters such as upper and lower TMH, TMA, TMR and tear meniscus depth most commonly. Intra-observer and inter-observer repeatability are good with spectral-domain OCT meniscometry. All

these measurements in the OCT meniscometry are machine dependent and can be influenced by the following conditions - conjunctivochalasis, LIPCOF, disorders of lid margin congruity, and apposition between the lid and ocular surface [41–44]. But it has many advantages such as it is non-invasive and image is taken rapidly and its simple but analysis of the image may take time [45]. It is an excellent but costly tool to test tear volume.

3.3.4 To test the tear film stability

3.3.4.1 Tear film break-up time (TBUT)

This is the time interval between the last complete blink and the appearance of the first randomly distributed dry spot [46, 47]. It is the most commonly done test for assessing the tear film stability.

It can either be done with or without fluorescein 2% dye. When done with dye it is known as Fluorescein break-up time (FBUT). The dye enhances the visibility of tear film but it also reduces the stability of the tear film and therefore the measurement may not be accurate [48, 49].

Method- Fluorescein 2% is instilled in the eye. It can be instilled in varying volume and concentrations either by impregnated strips or micropipette. A standardized method is to be followed every time and instructions are given to naturally blink thrice than stop blinking until instructed again [50].

A cut-off of < 10 seconds is used to diagnose DED. In patients of Sjogren syndrome, the sensitivity and specificity of the test have been reported to be 72.2% and 61.6%, respectively [7].

3.3.4.2 Non- invasive tear break-up time (NIBUT)

Tear film stability is believed to be affected by various factors such as temperature, fluorescein dye, humidity, air circulation so NIBUT is more reliable than the other tests.

Method –

- Placido disk -It can be measured with the help of a Placido disk images reflected over the anterior corneal surface with corneal topography systems [51].

- Keratography - Automated assessment is done with instruments having specific software such as keratography which detects and finds the location of tear film break-up over time [52, 53].

- High speed video keratography – The variance of the number of radial rings is estimated form center of the center image [54–56].

- Interferometry – It measures the time between the last blink and the appearance of first discontinuity in the lipid layer of the tear film. Recently instruments measuring the thickness of the lipid layer have also been developed [57–61].
  A cut-off of < 10 seconds is used to diagnose DED. The sensitivity and specificity of the NIBUT is reported to vary according to the technique used, with values of 82–84% and 76–94% respectively [53, 62, 63].

3.3.4.3 Thermography

When the tear film is evaporated it leaves the ocular surface cool [64]. Infrared thermography is used to measure the absolute temperature and the spatial and temporal changes in temperature during the inter-blink period. It can be used as an index of tear film stability.

Purslow and Wolffsohn reported that the ocular surface temperature measured by infrared thermography is related to the tear film [65]. The literature has given many evidences that indicates the cooling rate of the ocular surface is faster in individuals with DED than in normal eyes, which is assumed to be as a result of a greater rate of tear film evaporation [64, 66–68].

3.3.4.4 Osmolarity variability

Osmolarity in the patients of DED varies which in turn affects tear film stability. The inter-eye variability of osmolarity in patients of DED is greater than normal people [69–70]. As the severity of DED increases, this inter-eye difference of osmolarity also increases [71].

3.3.4.5 Tear evaporation rate

It is used as an indicator of tear film stability [72]. Lipid layer is necessary to prevent tear film evaporation. An absent and non-confluent lipid layer of tear film is thought to have association with a 4-fold increase in evaporation rate in normal patients and in patients of keratoconjunctivitis sicca, tear evaporation rate is thought to be increased by 2-fold [73, 74].

Method: Different techniques are used to measure tear film evaporation such as vapor pressure gradient and the velocity of increase in relative humidity (resistance hygrometry) [74–77].

3.3.5 To test the tear evaporation

Goto, Shimazaki et al. in 2002 reported the importance of evaluation of tear evaporation in dry eye assessment [78].

It is a non-invasive procedure and aim at assessing tear dynamics, differentiates the subcategories of DED and evaluating the treatment [79–84]. There are three methods for the measurement:

3.3.5.1 The evaporimeter system

The two humidity sensors are placed at different heights from the ocular surface and they are used to evaluate tear evaporation rates [79].

3.3.5.2 The closed-chamber system

At a given ambient humidity in a closed chamber, the velocity of the humidity increases and it is used to estimate the tear evaporation rate [80–82].

3.3.5.3 The ventilated chamber system

The evaporimeter consists of an eyecup in the form of a ventilated chamber which tightly covers the eye [85].

3.3.6 To test the ocular surface damage

3.3.6.1 Ocular surface staining

Vital and supra-vital stains are used to demonstrate the damaged epithelium. Staining occurs over cornea and conjunctiva in different fashion.

Cornea is stained in manner such that lower part (lower one-third) is stained more than upper part and nasal part stained more than temporal part.

Bulbar conjunctiva is stained nasally and temporally in a wedge-shaped zone [86]. It is a commonly used and cost -effective test.

The dyes used in the procedure are as follows:

- Fluorescein – 1% or 2% commercial preparation is used clinically for the ocular surface staining. It stains the surface when there is a disruption of cell junctions. It stains corneal epithelial damage better than conjunctiva. At physiologic pH, fluorescein is highly water soluble and hence poorly penetrates the lipid layer and doesn't stain normal cornea. It is orange in color and fluoresces green when excited by blue light.
  Yokoi and Kinoshita in 1998 reported that conjunctival damage precedes that of the cornea and is more severe.
  Method – Either it is instilled in form of a drop or impregnated strip. Excess dye is washed off if drop is instilled. Best results are obtained when viewed through a yellow barrier filter (such as Kodak Wratten 12 absorption filter) plus the standard blue exciter filter of the slit lamp [86].
  In the absence of yellow filter, the conjunctival stain is seen poorly.

- Rose Bengal (RB) – It is available as 1% commercial preparation.
  Method – Firstly, topical anesthesia is instilled in the eye to limit stinging with the dye. The dye is then instilled in the lower conjunctival sac. Excess dye is washed off with normal saline.
  The staining is dose-dependent, the more the dose of dye the more is the staining. Rose Bengal stains ocular surface epithelial cells that are unprotected by mucin or glycocalyx, as well as dead or degenerated cells [87].
  However, RB staining has many disadvantages as well which limits its association with dry eye. Schein et al. in 1997 reported that it stains in asymptomatic patients as well and does not correlate with the subjective symptoms [88]. RB causes staining of the Marx's line i.e., the mucocutaneous junctions of the lid margin. And thus, does not seem to have sufficient sensitivity and specificity [89].

- Lissamine Green – It is a synthetic acidic dye that stains similarly to RB. But it is not stringent to the eyes. Staining is dose- dependent. Staining should be checked at a proper time. It should neither be hasty nor be delayed as evaluating the staining too quickly does not allow the staining pattern to develop and if the evaluation is delayed the stain pattern starts fading. Ideally it should be checked between 1 and 4 minutes after staining [90].

- SCORING SYSTEM –

  Most commonly 3 methods are used to grade the ocular surface staining –

- Van Bijsterveld system – This system was developed in 1969. The whole ocular surface is divided into 3 zones – cornea, nasal bulbar conjunctiva and temporal

bulbar conjunctiva. Each zone is the scaled from 0 to 3 where 0 indicates no staining and 3 indicates confluent staining. The maximum possible score in 9 [91].

- NEI/Industry Workshop guidelines – This system was developed in 1995. The cornea is divided into 5 sectors namely central, superior, inferior, nasal and temporal, each of them is scored from 0 to 3. The maximum score is 15. Both the nasal and temporal conjunctiva is divided into 3 areas namely superior paralimbal area, inferior paralimbal area and peripheral area, each of which is scored from 0 to 3 with a maximum score of 9 for both nasal and temporal conjunctiva [92].

- Oxford scheme – This scheme was developed by Bron in 2003. There is a chart with series of panels labeled from A-E in order of severity- absent, minimal, mild, moderate and severe [93].

Recently, Miyata and coauthors described a new method for grading fluorescein staining in superficial punctate keratitis (SPK). Both the area and density of SPK were graded. The area was graded from A0 to A3 and the density was graded from D0 to D3 and then these two were combined in a single index [94].

3.3.6.2 Impression cytology

It is used to diagnose diseases like DED, limbal stem-cell deficiency, ocular surface neoplasia, and specific viral infections [95]. In patients of DED, it is used to study squamous metaplasia and goblet cell density of the conjunctiva for the diagnosis and monitoring of the disease [96].

Method - Cells from the first to third most superficial layers of the epithelium are removed by application of cellulose acetate filters or bio-pore membranes, and the cells can be subsequently analyzed by various methods including microscopy, immunocytochemistry, immunoblotting analysis, polymerase chain reaction, and flow cytometry, depending on the aim of the investigation [97].

Several squamous metaplasia grading systems by Nelson, Tseng and Blades are used to analyze the conjunctival impression cytology [98–100].

3.3.6.3 Lid Parallel Conjunctival Folds (LIPCOF)

These are the folds in bulbar conjunctiva in the lateral and lower quadrant which are parallel to the lower lid margin. They represent mild stage of conjuntivochalasis but clinically they are slightly different [101].

The tear meniscus height measurements may be underestimated due to LIPCOF [44].

3.3.6.4 In-vivo confocal microscopy (IVCM)

It is a non-invasive technique to evaluate the signs of ocular surface damage in DED patients at cellular level. The signs such as decreased corneal (apex and lower periphery), and conjunctival epithelial cell density, conjunctival squamous metaplasia (increased mean individual epithelial cell area, decreased nucleocytoplasmic ratio and goblet cell density), and corneal nerve changes (decreased sub-basal nerve density, increased tortuosity and increased number of bead-like formations) are evaluated [102–106].

3.3.6.5 Ocular surface sensitivity

The palpebral conjunctival sensitivity appears to be more critical than corneal sensitivity when assessing DED [107]. The instruments like Cochet-Bonnet or non-contact air-jet esthesiometers have been employed to evaluate ocular surface sensitivity.

3.3.7 To test the lipid layer of the tear film

The precorneal lipid layer assessment is done with the help of tear film interferometry.

The lid margin lipid layer assessment is done with the help of meibometry [108].

The meibomian gland assessment is done with the help of meibography [109].

3.3.7.1 Tear film interferometry

It is a non-invasive method that is used to visualize the translucent surface of the lipid layer of the tear film. McDonald in 1968 was first to analyze the tear interference images [110].

3.3.8 To test the chemical properties of the tear film

3.3.8.1 Tear film osmolarity

Interferon gamma is significantly increased in amount if the tear film on the ocular surface becomes hyperosmolar but other cytokines such as Th1, Th2 and Th17 have no significant increase in amount [111].

It has the highest correlation to disease severity of clinical DED tests [112].

Various literatures have proposed many cut-off values for DED from 305 mOsm/L to 316 mOsm/L [113], with reported sensitivities and specificities ranging from 64–91% and 78–96% respectively [113–117].

3.3.8.2 Tear film ferning

When tear film is dried on a glass plate, it causes ferning. There are few pre requisites for the process such as slow crystal growth rate, low solution viscosity and low impurity levels to permit free-solute diffusion. Seven to ten minutes under normal room temperature of 20 to 26°C and room humidity of (RH up to 50%) has been recommended [118].

The crystallization begins with the formation of a nucleus, due to the supersaturation of ions with solvent evaporation at the peripheral edge of the drop. Normal crystals are formed when the sample solute is able to diffuse into areas with a lower solute concentration [118].

Electrolytes may play a role in ferning as hyperosmolarity has been found to result in deteriorated ferns [113, 119].

Tear ferning changes with contact lens wear have been found to have a moderately high sensitivity (78.4%) and specificity (78.4%) for predicting contact lens tolerance in a clinical setting [120].

Healthy tear samples produce compact, dense ferning patterns, while in dry eye samples, the pattern is fragmented or absent.

3.3.8.3 Biochemical analysis of the tear composition

It includes lacrimal gland and serum protein analysis, mucin analysis and lipid analysis [36].

3.3.9 To test the inflammation of the ocular surface

Inflammation, although not specific, but is a recognized as one of the component of the pathophysiological mechanism of DED.

3.3.9.1 Ocular or conjunctival redness

This is the most common and consistent sign of ocular surface inflammation [121–123]. It can easily be detected with a pen torch or on slit lamp examination. It is not specific to DED and can occur in any disease with inflammation, for example, in response to chemical injury, infective conjunctivitis or allergic conjunctivitis.

3.3.9.2 Matrix metalloproteinases

They are secreted into the tears of a DED patients [72, 124–126]. It destroys the tight junctions of the ocular surface epithelium which in turn leads to loss of ocular surface barrier function. This assay produces a dichotomous outcome, with levels above 40 ng/ml producing a positive result, and is non-specific to the source of ocular surface inflammation.

3.3.9.3 Cytokines and chemokines

They reflect the level of epithelial disease. Elevation of Th1 and Th17 subclasses of cytokines suggest involvement of particular T lymphocyte differentiation pathways in the disease. Elevation of tear Th2 cytokines, on the other hand, may suggest a more allergic-based disease, although recent evidence suggests various aspects of T cell Th1, Th2 and Th17 exist across aqueous deficient, evaporative and mixed forms of DED, with a propensity towards Th1 type T cell responses as a more global indicator of DED [127].

3.3.9.4 Ocular sursface immune markers

The most commonly used ocular surface immune marker is HLA-DR expression, a Class-II MHC antigen, which indicates a loss of the normally immune-suppressed environment of the ocular surface.

Although the authors found increased expression of HLA-DR associated with increased clinical severity of DED [128], but in comparison with other studies the normal levels of HLA-DR expression showed high variability ranging from 5–54% and the study also suggested the weak correlation of HLA-DR and traditional clinical signs of DED [129]. Other relevant markers of apoptosis include CAM-1, CD14+, CD8+ and CD4+ cells [130, 131].

3.3.9.5 In vivo confocal imaging

Corneal sub-epithelial and stromal IVCM signs of inflammation have been hypothesized and studied in DED [132, 133].

4. Clinical protocol for dry eye

The recommended order and clinical practice procedural recommendations are as follows:

- Symptoms - DEQ-5 or OSDI are self-administered. The result is considered positive if the DEQ-5 score is 6 or if OSDI score is 13.

- Tear breakup time

  a. NIBUT - The cut-off for a positive finding can be as low as 2.7 seconds for automated algorithms, and up to 10 seconds for subjective observation techniques.

  b. FBUT - A positive finding has been reported to be a value < 10 seconds.

- Osmolarity - A positive result is considered to be 308 mOsm/L with the currently available device in either eye [69, 71] or an interocular difference >8 mOsm/L [112].

- Ocular surface staining – by lissamine green and fluorescein dye.

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