ECRONICON OPEN ACCESS

EC OPHTHALMOLOGY
Review Article

Tear Ferning Test as a Valuable Tool to Determine the Quality of Tear Film in Animals and Human

Mana A Alanazi[1]*, Gamal A El-Hiti[2], Rahaf Almaymuni[1], Mashaaer A Baashen[1], Raied Fagehi[1] and Ali M Masmali[2]

[1]Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[2]Cornea Research Chair, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

***Corresponding Author:** Mana A Alanazi, Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

Received: July 28, 2021; **Published:** August 30, 2021

Abstract

Background: Tear film stability is essential for healthy vision. More than one test should be used to examine tear film status. The tear ferning test is used as a valuable and repeatable tool to examine the quality of tears in animals and human. The current study aimed to report and discuss the most recent use of the tear ferning test to diagnose dry eye in animals and human.

Main text: The tear ferning test involves the collection of a tear sample using a capillary tube. The tears were allowed to dry in a controlled environment, and the patterns produced were observed and inspected using a light microscope. The four-point tear ferning grading scale that included type I or II (normal eye) and types III or IV (dry eye) is used to grade tear ferning patterns. Another grading scale is developed, which consists of five grades and is used in 0.1 increments. A grade > 2 is defined as dry eye. The tear ferning test has been applied to assess the quality of tears among birds, reptiles, and other animals, such as rabbits, cats, dogs, monkeys, horses, and camels. The tear ferning test has been used to investigate the effect of various disorders, such as the thyroid gland, diabetes, high body mass index, high blood cholesterol level, and vitamin deficiency on tear film. The effect of beverages such as green tea and peppermint that are rich in polyphenols on the tear film have been evaluated. Moreover, the effect of eyedrops on the tear film and addition of electrolytes on tear ferning patterns of eyedrops was studied.

Conclusion: The tear ferning test is an easy-to-perform and inexpensive tool to evaluate the quality of tears in animals and human. It can be used in combination with other tests to detect dry eye symptoms.

Keywords: *Tear Ferning Test; Ferning Patterns; Tear Ferning Grading Scales; Tear Film Stability; Dry Eye*

Introduction

The stability of the tear film is essential in healthy vision [1-5]. Various factors affect tear film structure, stability, and composition, such as rate of tear flow, ocular disease, age, and conjunctival stimulation [6,7]. The lipid content of the tear film, which is produced by the meibomian glands, is responsible for the prevention of tear evaporation [8]. The aqueous content of the tear film is rich in electrolytes in addition to large molecules, such as vitamins, proteins, and hormones [9]. The aqueous medium protects the tear film against microorganism infections and provides a smooth ocular surface [10]. The lacrimal glands and accessory glands of Krause and Wolfring are responsible for the production of aqueous content of the tear film [11]. Moreover, the tear film contains mucins that lubricate the tear film and helps the margin of the eye to be smooth during blinking [12]. The disturbance in tear film leads to dry eye due to either high rate of tear evaporation or shortage of aqueous phase production [13-17].

Dry eye is a common disorder of the ocular tear film [18-22]. Therefore, regular assessment of the tear film is important [23]. Many tests have been used to examine the tear film. For example, the tear volume and its production can be assessed using Schirmer and phenol red thread tests [24]. However, tear film stability can be assessed using noninvasive tear breakup (NITBUT) test [25]. Other tests that involve measurements of osmolarity and tear evaporation rate have been proven to be extremely valuable [26-29]. The correlation between the scores from different tests are generally poor since each test measures a different parameter [30]. Therefore, more than one test should be used rather than a single test to determine the tear film status [31]. The tear ferning (TF) test has can be used to detect dry eye and has been proven to be repeatable and useful [32-34].

The TF test evaluates the composition and quality of human and animal tears. The TF test is easy to run, inexpensive, quick, and useful [34]. Body fluids, such as saliva and tears, produce ferns when dried under normal temperature and humidity conditions [35]. The TF patterns reflect the quality of tears and provide information about the number of goblet cells, which are responsible for mucus production. Moreover, the ferns reflect the biochemical properties of large molecules (e.g., proteins) and electrolytes present in the tear film. The fern structure is highly dependent on the presence of organic and inorganic molecules and their concentrations [36]. The tear crystallization process was established in 2004 [37]. Fern formation has good sensitivity, specificity, and repeatability [38-40]. Crystal formation begins when the salt concentration becomes high due to water evaporation and continue to increase until complete dryness of tears. Such process required low solution viscosity, slow crystal growth rate, and low level of impurities. These conditions lead to the formation of dendritic crystals [41]. The fern-like dendritic growth can be enhanced by increasing the rate of water evaporation, temperature, and reduction of atmospheric humidity [43].

Electrolytes and sodium and chloride along with macromolecules, such as proteins and mucins, are responsible for fern formation. The concentration and ratio between sodium and potassium (monovalent cations) to calcium and manganese (divalent cations) play an important role in TF formation [42]. The concentration and divalent to monovalent cation ratio in tears are important and should be between 0.002 and 0.05 for fern production [42]. The scanning electron microscope reveals that the dendritic structure of ferns is composed of sodium and chloride, while the cubic crystals are composed of potassium and chloride [43]. No proteins or mucins participate in fern formation. The poor fern patterns for dry eye tears are produced due to the high concentration of inorganic salts that cause tear hyperosmolarity [44]. Humidity and temperature affect fern formation [45].

The TF test involves the collection of a small tear sample using several methods; however, the use of capillary tube is the most common method [34]. The tear sample can dry for 10 min over a clean glass microscopic slide at temperature of 20-25°C and humidity of 35-40%. The ferns produced are observed using a light microscope and graded using a number of TF grading scales [32-34,38,46]. Rolando identified the differences in TF patterns and introduced a scale that contains four grades [32]. The four-point TF grading scale is extremely common and has been proven to be reliable, subjective, and simple. It can be used to classify tears as normal and dry [32]. Type I and type II represent normal eye and are characterized by the presence of many ferns with little or no spaces between the branches. However, types III and IV represent dry eye in which few ferns were present with large spaces between the branches [47]. The five-point grading scale (grades 0, 1, 2, 3, and 4) was introduced in 2014 and is used in 0.1 increments [33,34]. Examples of TF patterns that represent normal and dry eyes based on the five-point TF grading scale are shown in figure 1. Both the four- and five-point TF grading scales are used to assess the quality of tears in animals and human.

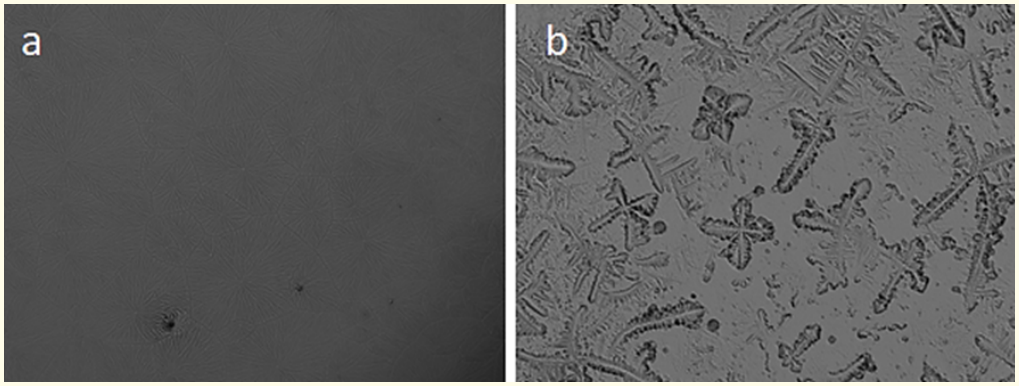

Figure 1: *TF images (1 mm) of normal eye (a) (TF = 0) and dry eye (b) (TF = 2.5) based on the five-point TF grading scale [33,34].*

TF test in animals

The patterns of TF due to tear crystallization are dependent on the composition of tears. The TF test was used along with other tools to evaluate the ocular tear film in birds, reptiles, rabbits, cats, dogs, monkeys, horses, and camels using both Rolando [32] and Masmali [33] TF grading scales. Moreover, it can be used in mouse with different ocular diseases for comparison analysis [48].

Birds and reptiles

The TF patterns of tears collected from birds and animals were observed using a polarized light microscope for each species and graded based on a TF grading scale (four- and five-point TF grading scales). The TF test was used to compare tear crystallization patterns and electrolyte content in birds and reptiles [49]. Tear samples were collected, and the concentration of anions (e.g. chloride), cations (e.g. phosphorus, calcium, iron, sodium, and potassium), urea, and protein were measured. The electrolyte concentration was similar in both birds and reptiles. The tears contain a high concentration of chloride, sodium, and iron [49]. However, a high protein level was seen in owl and a high concentration of urea was detected in the tears of sea turtles.

The tear ferns in birds showed normal TF grades (normal eyes), while the TF patterns of reptiles showed eye dryness [49]. Both tears of birds and reptiles have a higher chloride and sodium concentrations compared to human tears but similar ionic balance of the lacrimal fluids. The electrolyte concentrations were different for each species. Therefore, the differences in TF grades of birds and reptile tears seem to be due to the presence of different electrolytes in various concentrations, particularly chloride and sodium ions [49]. Moreover, the tears collected from wild species seem to have higher TF grades as a result of the presence of specific microelements. The balance between ions (cations and anions) in tears of birds and reptiles seems to be similar to that for humans. However, they contain high sodium and chloride concentrations compared with human.

Rabbits

The TF patterns collected from 10 rabbits were recorded and classified as normal based on Rolando [32] and Masmali [33] TF grading scales. Grades 1 was found in 50% of rabbits based on the five-point scale compared with 60% based on the four-point grading scale [50]. Rabbits have normal eye based on the TF test, which was consistent with the scores obtained from osmolarity measurements. The median ± semi-interquartile range (IQR) for the osmolarity scores was 283.5 ± 7.5 mOsm/L in rabbits. The chloride, sodium, and potassium concentrations in the tears of rabbits were found to be 196.51 mEq/L, 202.04 mmol/L, and 12.74 mmol/L, respectively.

Cats

The ocular tear film in healthy cats (*N* = 60) was assessed using the TF test based on four- and five-point TF grading scale. Based on the four-point TF grading scale, 96.6% of the cats (*N* = 58) showed normal eyes (types I and II). Only two cats showed type III TF patterns [51]. The use of five-point TF grading scale showed that all cats have normal eyes (grades ≤ 2). No significance difference was found between the TF grades of tears collected from both eyes (left and right) for the four-point (*P* = 0.225) and five-point (*P* = 0.368) TF grading scales. Clearly, both TF grading scales can be used efficiently to grade TF patterns of cats.

Dogs

The tears collected from dogs with normal eyes (*N* = 50) showed that the majority (78%) of them have normal TF grades (types I and II) based on Rolando TF grading scale [33]. However, all dogs with keratoconjunctivitis sicca (N = 30) have abnormal TF grades (types III and IV) [52]. The TF grades were correlated with scores recorded using Schirmer test. For example, the score of the Schirmer test was < 10 mm/min for abnormal TF grades and > 10 mm/min for type I or II [52]. Another study conducted on 90 healthy dogs showed that TF type I (based on the four-point grading scale) and grade 1 (based on the five-point grading scale) were noted in the majority of dogs (64.2% and 61.7%, respectively) [53]. Types I and II were noted in 95% of dogs compared with 77.5% for TF grades 0 and 5. No significant difference was found between the Schirmer test measurements and classification based on the TF test. Clearly, both grading scales provided similar results, and the TF scores agreed with the tear volume assessed using Schirmer test [53].

Monkeys

The TF patterns of tears collected from 11 capuchin monkeys (22 eyes) have been analyzed [54]. There was a significant (*P* < 0.05) difference between grades for tears collected from both eyes. The median ± semi-IQR was 3.0 ± 0.5 and 2.0 ± 0.0 for the right eye and 2.0 ± 0.5 and 2.0 ± 0.5 for the left eye based on Rolando and Masmali TF grading scales. Based on Rolando TF grading scale, 13 monkeys have type II, and 9 have type III. The type III found in tears of some monkeys can be attributed to species peculiarities and are similar to dry eyes in human subjects [54]. In contrast, 6 monkeys have TF grade of 1, and 16 monkeys have grade 2, based on Masmali TF grading scale [54]. The grades for the tears collected from monkeys were significantly (*P* < 0.05) higher than those of human tears. Moreover, tear osmolarity of the right and left eyes in monkeys were significantly higher (*P* < 0.05) (300.0 ± 9.8 mOsm/L and 304.5 ± 9.5 mOsm/L, respectively) compared with those for human (287.5 ± 8.4 mOsm/L and 293.0 ± 6.1 mOsm/L, respectively).

Horses

The patterns of tears collected 30 healthy horses (60 eyes) were graded based on the Rolando grading scale [32]. The TF grades showed types I and II in 30% and 51.7% of the eyes, respectively [55]. Only 11 eyes (18.3%) were dry (type III grade). No significant difference (*P* > 0.05) was noted between TF grades for both right and left eyes. Moreover, no significant correlation was found between TF test grades and Schirmer test scores.

Camels

The TF test was used to analyze the tears collected from camels and compare their compositions with those for artificial eyedrops and humans living in the desert of Saudi Arabia [56]. The grades of camels' tears ranged from grade 0 to 1 based on the five-point grading scale that indicated normal and healthy eyes. In contrast, the TF grades for human tears (*N* = 5) and Refresh Plus ranged from 1 to 2. Camels' tears showed the presence of oily droplets and other particles with small branches in the form of homogenous and thick layers

[56]. Furthermore, they showed a higher chloride level compared to human and artificial tears. The potassium level was higher in Refresh Plus eyedrops followed by camels and human tears [56]. Camels' tears contain higher levels of lubricants and mineral compared with those for artificial and human tears. It seems that the tear film in camels is more equipped than that in human tear film to adopt the harsh environment in the desert to reduce tear evaporation.

TF test in human

The TF test is used as a simple tool to assess the quality of tears in human [57-61]. The TF patterns and NITBUT scores are dependent on the age and independent of the race and sex [62]. Elderly individuals have higher TF grades and lower NITBUT scores compared with younger subjects. Recently, the TF test has been highly involved in assessment of tear films in different subjects. It has been used to detect the changes in patterns of artificial tears due to the addition of electrolyte solutions. Moreover, it is useful in assessing the changes in the quality of tears due to consumption of vitamins and beverages.

Effect of smoking on TF patterns

The TF test was used as an additional tool to assess the tear film in smokers. The TF grades (1.0 ± 0.5) recorded for tears collected from smokers (N = 30; 29.8 ± 5.1 years) was significantly (Kolmogorov–Smirnov test; $P < 0.05$) higher compared with those obtained for normal eye subjects (N = 30; 0.4 ± 0.4) based on the five-point grading scale [63]. The TF grades agreed with the scores obtained from other tools used to detect dry eye symptoms (e.g. McMonnies questionnaire, TBUT, and PRT tests) for smokers. Both TF and TBUT tests showed that 10% of smokers have dry eyes. Moreover, the scores from McMonnies questionnaire indicated that 26.7% of smokers had dry eye symptoms. However, the PRT measurements showed normal eyes in smokers. The scores from McMonnies questionnaire had strong correlations with both TF (r = 0.52) and PRT (r = 0.60) tests [63]. The TF images of tears obtained from two smokers and two non-smokers are shown in figure 2.

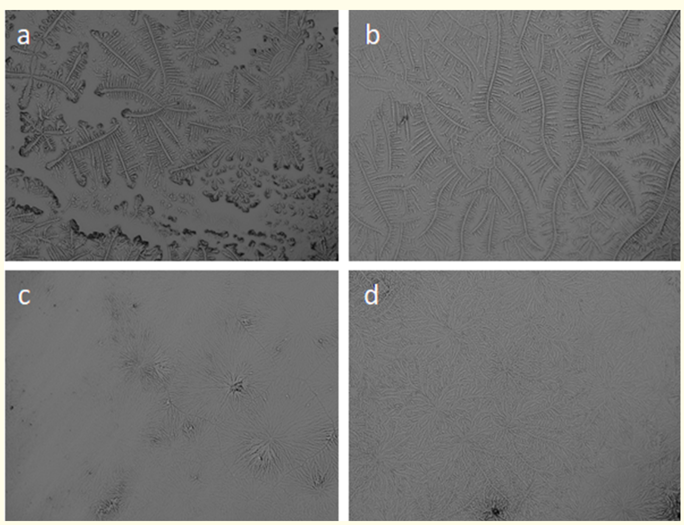

Figure 2: *TF images of tears from two smokers (a and b) and two normal eye subjects (c and d).*

Effect of vitamin D supplementation on TF patterns

TF test along with other tools were used to assess tear film in subjects supplied with oral vitamin D3 in the short term. Samples of tears were collected from subjects with dry eye (*N* = 30; 26.2 ± 5.0 years) and normal eye (*N* = 30; 25.6 ± 4.3 years) before and after receiving a dose of 400 IU of vitamin D3 for three consecutive days [64]. A significant (Wilcoxon test, $P < 0.001$) difference has been noted in the TF grades before (2.3 [0.6]) and after (1.8 [0.8]) supplementation. The TF grades were improved in the majority of dry eye subjects (*N* = 26; 86.7%) after the consumption of vitamin D3. Other tests, such as PRT and TBUT, showed some differences in the measurements before and after vitamin D3 consumption in subjects with dry eye; however, the differences were not significant. The TF test was sensitive in detecting the changes in the quality of tears due to the consumption of vitamin D3. For subjects with normal eyes, no significant differences were found between the TF grades before and after the oral consumption of vitamin D3 [64]. The TF images for a subject with dry eyes and normal eyes before and after the consumption of vitamin D3 are shown in figure 3.

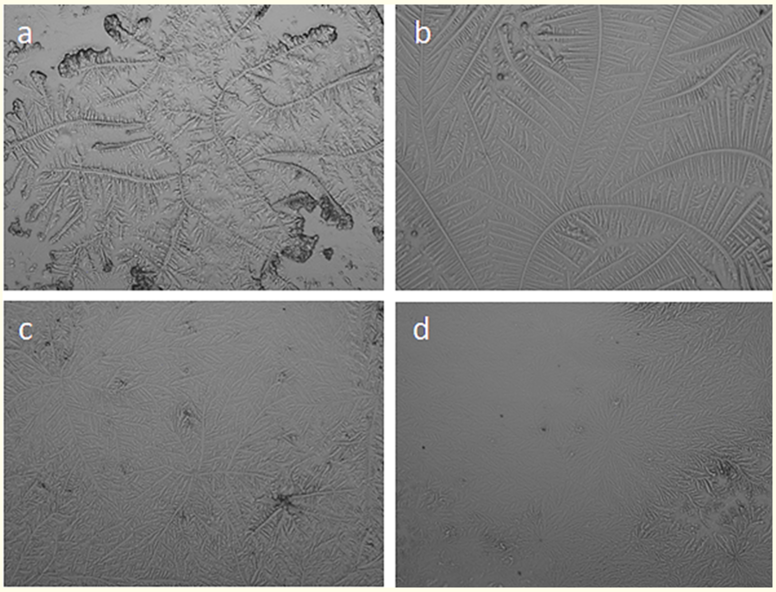

Figure 3: *TF images of tears from a subject with dry eyes (a and b) and a subject with normal eyes before and after consumption of vitamin D3.*

Effect of vitamin A supplementation on TF patterns

The effect of oral vitamin A supplementation on subjects with both dry (*N* = 30 men; 25.2 ± 2.8 years) and normal eye (*N* = 30 men; 24.5±2.3 years) using TF test has been investigated [65]. The subjects in both groups consumed a dose of 1,500 mg daily for three consecutive days. The TF test was performed before vitamin consumption and 24 h after the third dose of supplementation. In subjects with dry eye, the TF grades after the consumption of vitamin A has significantly improved (Wilcoxon test, $P = 0.01$). The mean TF grade has improved from 2.4 (0.5) before the consumption of vitamin A to 1.4 (1.1) after supplementation. No significant improvement in TF grades were recorded after vitamin A consumption in subjects with normal eyes. There was an increase in the PRT and TBUT scores in subjects with dry eyes after supplementation; however, the improvement was not significant [65]. Again, the TF test has been proven to be more sensitive than other tools in detecting changes in the ocular tear film after vitamin A consumption. Figure 4 shows the TF patterns for a subject with dry eye before and 24 h after the consumption of the third dose of supplementation.

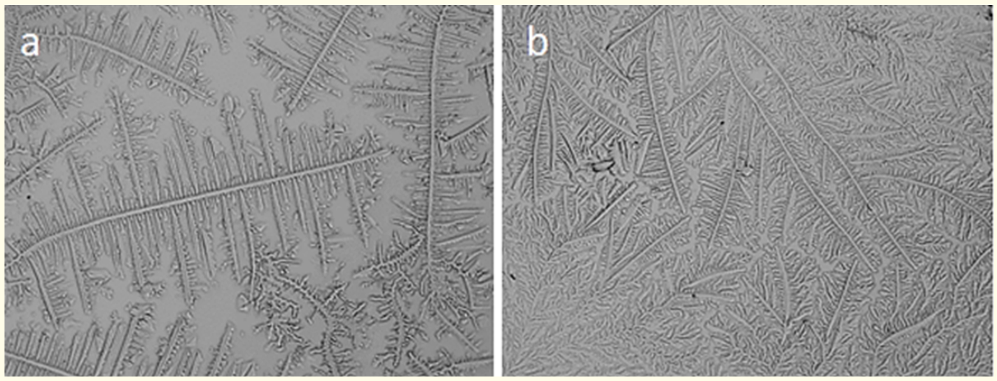

Figure 4: *TF images of tears from a subject with dry eye (a) before and (b) 24 h after vitamin A consumption.*

Effect of thyroid gland disorder on TF patterns

The TF patterns for tears collected from six men and 14 women with thyroid gland disorder (34.3 ± 3.2 years) and those obtained from normal eye tears (eight men and 12 women; 31.3 ± 2.9 years) were graded based on the five-point grading scale. The median TF grade score was significantly (Kolmogorov–Smirnov test; P < 0.05) higher in patients with thyroid gland disorder (2.0 [2.2]) compared with subjects with normal eye (1.2 [0.9]) [66]. Similar observations were noted for the average PRT and TBUT test and OSDI scores. The mean PRT score was significantly ($P < 0.05$) lower in the study group (OD = 11.7±8.1 mm and OS = 10.5 ±7.4 mm) compared with subjects with normal eye (OD = 22.2±6.5 mm and OS = 20.7±5.2 mm). Moreover, the mean TBUT score indicated eye dryness in subjects with thyroid disorder (OD = 4.9±1.6 s and OS = 4.2±1.9 s) compared with the control group (OD = 13.2±2.6 s and OS = 12.3±2.2 s). Clearly, the tools used, including the TF test, indicated that the level of eye dryness was significantly higher in patients with thyroid disorder. The TF images for tears collected from two patients with thyroid gland disorder and two subjects with normal eye are shown in figure 5.

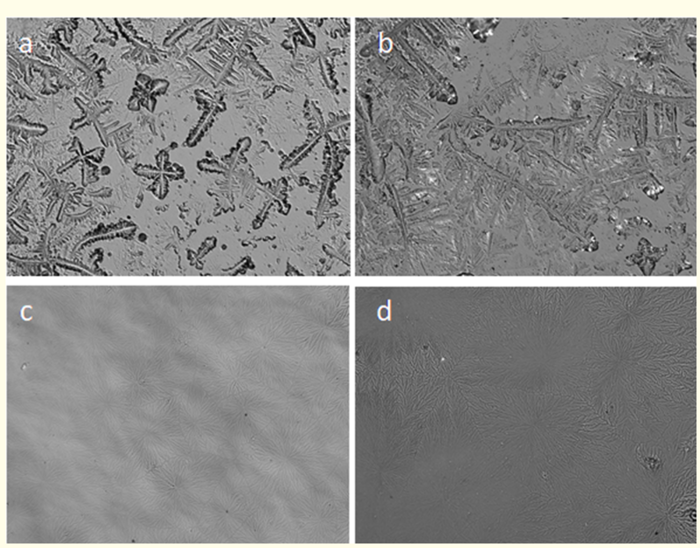

Figure 5: *TF images of tears from two subjects with thyroid gland disorder (a and b) and two subjects with normal eye (c and d).*

Effect of high body mass index (BMI) on TF patterns

The quality of tears collected from subjects (N = 20 men; 31.3 ± 5.7 years) with a high BMI (31.8 [5.2] kg/m^2) was assessed and compared with those with normal BMI (20.5 [2.8] kg/m^2) and healthy eye subjects (N = 20 men; 30.2 ± 4.9 years) [67]. The mean TF grade for subjects with a high BMI was significantly (Kolmogorov-Smirnov test; $P < 0.05$) higher (2.0 ± 1.1) compared with those for subjects with normal eye (0.7 ± 0.6). The TF test showed that the majority (75%) of subjects in the study group have dry eye. Such results were in agreement with the scores recorded from the NITBUT test (8.5 ± 2.8 s and 14.7 ± 2.8 s for the study and control groups, respectively). The high BMI affects the quality of tear film and tends to increase TF grades and reduce the tear quality. The images for tears collected from two subjects with a high BMI are shown in figure 6.

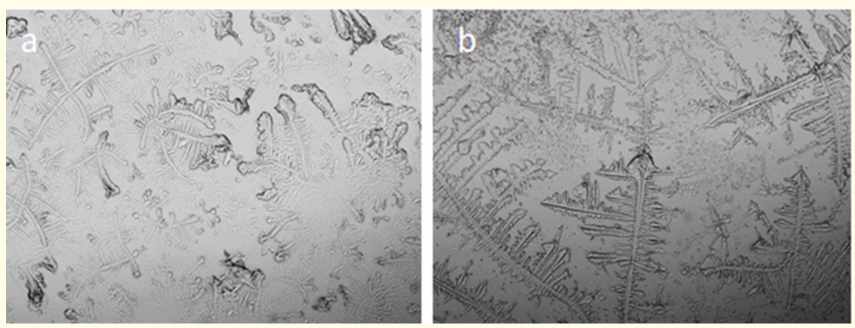

Figure 6: *Images of tears collected from two subjects with high BMI.*

Effect of high blood cholesterol level on TF patterns

The quality of tears collected from 20 male subjects (32.5 ± 7.3 years) with a high cholesterol level (6.2 ± 0.7 mmol/L) was compared with those of healthy subjects (N = 20 men; 31.1 ± 4.6 years) with normal cholesterol level (3.9 ± 0.6 mmol/L) using TF test [68]. The mean TF test score for subjects with a high cholesterol level was found to be significantly ($P < 0.05$) higher (2.2 [1.8]) compared with that for the control group (0.5 [1.5]). The high cholesterol level has a significant negative effect on tear film stability. Such results were in agreement with those obtained from both TMH and NITBUT tests. The mean scores for the TMH and NITBUT tests were significantly ($P < 0.05$) lower in the study group (0.15 [0.12] mm and 7.5 [3.3] s, respectively) compared with those for the control group (0.27 [0.06] mm and 15.3 [4.1] s, respectively). The images for tears collected from two subjects with high blood cholesterol level are shown in figure 7.

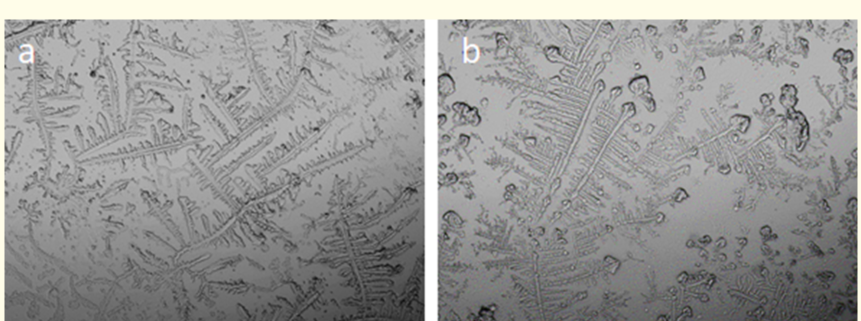

Figure 7: *TF images from two subjects with a high blood cholesterol level.*

TF patterns of stimulated and non-stimulated tears

The patterns of tears collected from 35 male subjects (23.4 ± 2.5 years) with normal eyes indicated that there was no significant (Wilcoxon test, $P > 0.05$) difference between stimulated and non-stimulated tears. The median TF grades recorded for stimulated tears from the right and left eyes were 0.65 (0.99) and 0.70 (1.15), respectively. For non-stimulated tears, the median TF grades were 0.55 (0.89) and 0.50 (1.11) for the right and left eyes, respectively [69]. Clearly, such result indicated that the chemical components of both stimulated and non-stimulated tears are similar. No alteration in chemical composition of tears occurs when tears were stimulated. Clearly, the lipid and electrolyte concentrations were steady in both tears. Figure 8 shows the TF patterns for non-stimulated and stimulated tears (right eye) of a subject.

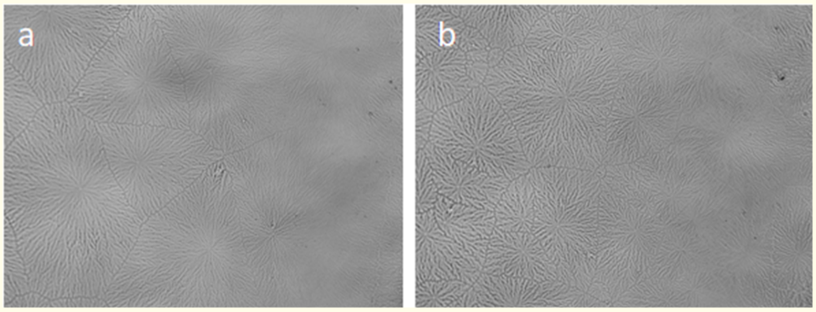

Figure 8: *TF images of(a) a non-stimulated and (b) stimulated tears (right eye) for a subject.*

Effect of beverages on TF patterns

The effect of hot green consumption on the tear film was tested in 40 subjects (22 men and 18 women; 26.0 ± 6.1 years) with normal eyes. The TF images were recorded 30 min before drinking hot green tea and 60 min after drinking hot green tea [70]. The median score for the TF grade was significantly (Wilcoxon test, $P < 0.05$) higher after consumption of green tea (2.65 [1.23]) than the one recorded before consumption of green tea (1.50 [0.88] mm). Similarly, the median PRT score was significantly higher after the drink, and the measurements showed normal tear volume before and after consumption of green tea. The TF grades increased in most subjects (97.5%) after the consumption of green tea, which agreed with the PRT measurements (80%). It has been suggested that high polyphenol content in green tea can oxidize lipids in rats [71]. Moreover, green tea can affect the cholesterol level in rats, which can distribute tear film [72]. Figure 9 shows the TF images recorded for a subject 30 min before the drink and 60 min after consumption of green tea.

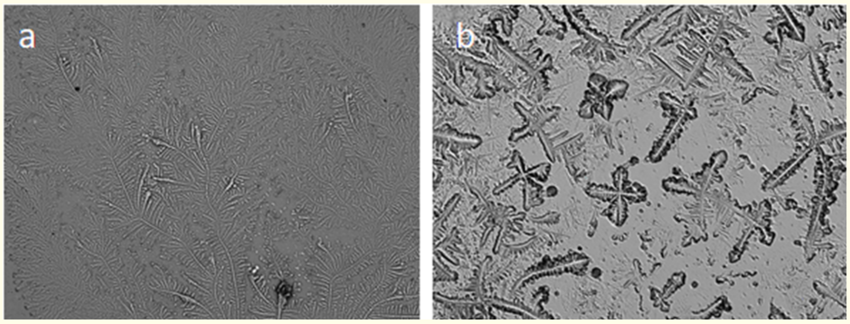

Figure 9: *Images of tears from a subject (a) 30 min before and (b) 60 min after the consumption of green tea.*

Peppermint contains a high level of polyphenols and therefore its consumption could have a negative effect on tear film. Indeed, the mean TF grade for tears collected from 30 subjects with normal eye (23.2 ± 2.2 years) after consumption of peppermint drink was significantly (Kolmogorov–Smirnov test, $P < 0.05$) higher (2.07 ± 1.20) compared with that recorded before consumption of peppermint drink (0.84 ± 0.71) [73]. A similar observation has been noted in TMH measurements (90%). Moreover, the NITBUT scores have decreased in 96.7% of subjects. The quality and quantity of tears significantly decreased after the consumption of hot peppermint. Polyphenols have a negative effect on lipids and electrolytes, such as iron and aluminum that alter the crystallization patterns of tears [74]. Figure 10 shows the TF images recorded for a subject 30 min before consumption of hot peppermint and 60 min after consumption of hot peppermint. The TF grades increased after consumption of hot peppermint in most subjects (93.3%).

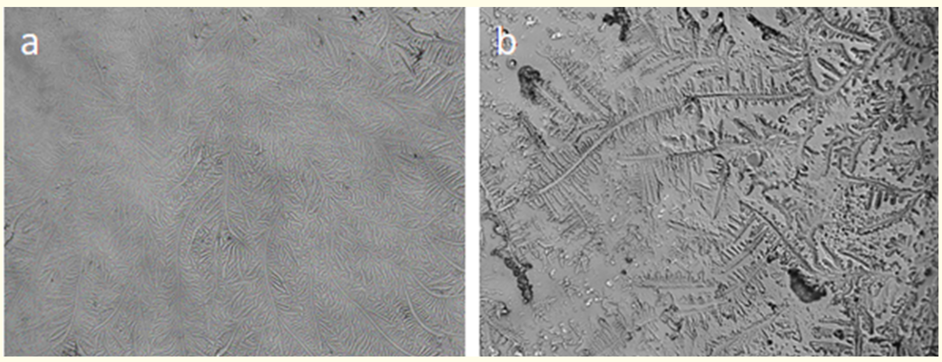

Figure 10: *TF images of tears from a subject (a) 30 minutes before and (b) 60 minutes after the consumption of hot peppermint.*

Effect of eyedrops on TF patterns

The effect of Refresh Plus eyedrops on the ocular tear film in subjects with normal (N = 30; 23.9 ± 3.2 years) and dry eyes (N = 30; 22.1 ± 2.3 years) has been tested using TF test [75]. The tears (right eye) were collected from the subjects, and TF images were recorded before and after (30, 60, 120, and 180 min) the application of lubricant eyedrops. A significant difference (Wilcoxon test; P = 0.02) was found between the TF grades before and after the use of eyedrops in subjects with dry eye. Figure 11 shows the TF images of tears (right eye) collected from a subject before and after Refresh Plus eyedrops.

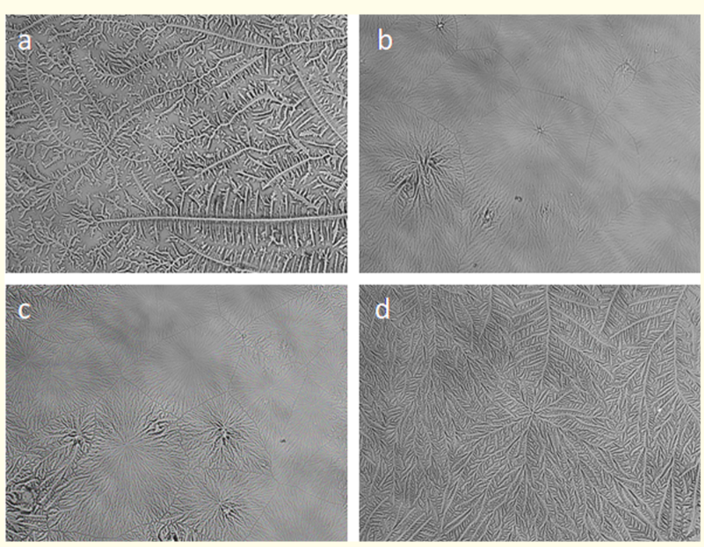

Figure 11: *TF images of tears (right eye) from a subject (a) before Refresh Plus eyedrops, (b) 30 min after Refresh Plus eyedrops, (c) 60 min after Refresh Plus eyedrops, and (d) 120 min after Refresh Plus eyedrops.*

In subjects with dry eye, a medium correlation (r = 0.484, P = 0.049) was found between TF grade recorded before and after 1h of application of eyedrops. Moreover, strong correlations were found between the TF grades before and those after artificial tears after 2 h (r = 0.560, P = 0.019) and 3 h (r = 0.726, P = 0.001) [50]. For subjects with normal eye, a medium correlation (r = 0.407, P = 0.029) was found between the TF grades obtained before and 1h after the application of eyedrops. Moreover, a strong correlation (r = 0.532, P = 0.003) was found between TF grades after 2 h and 3 h of application of eyedrops. Clearly, the TF test indicated that there is improvement in the quality of tears after the use of eyedrops. Refresh Plus contains sodium carboxymethylcellulose and other electrolytes that might be responsible for the improvement in TF grade [76]. Several eyedrops can be used to relieve symptoms of eye dryness [77-79].

Effect of electrolytes on TF patterns of eyedrops

The effect of the addition of electrolyte solutions on the TF grades of Blink Contact Soothing Eye Drops (TF grade = 1.2) and Refresh Plus Tears (TF grade = 1.5) has been investigated [80]. The TF grades of both artificial tears have been improved after the addition of electrolyte solutions. The addition of calcium chloride ($CaCl_2$) solution led to the greatest improvement in TF grades of both eyedrops. Similarly, the addition of different volumes of basic tear solution and sodium carboxymethylcellulose solutions leads to an improvement in the grades of artificial tears [80]. Figure 12 shows the TF patterns of Blink eyedrops and its mixtures with different proportions of various electrolyte solutions.

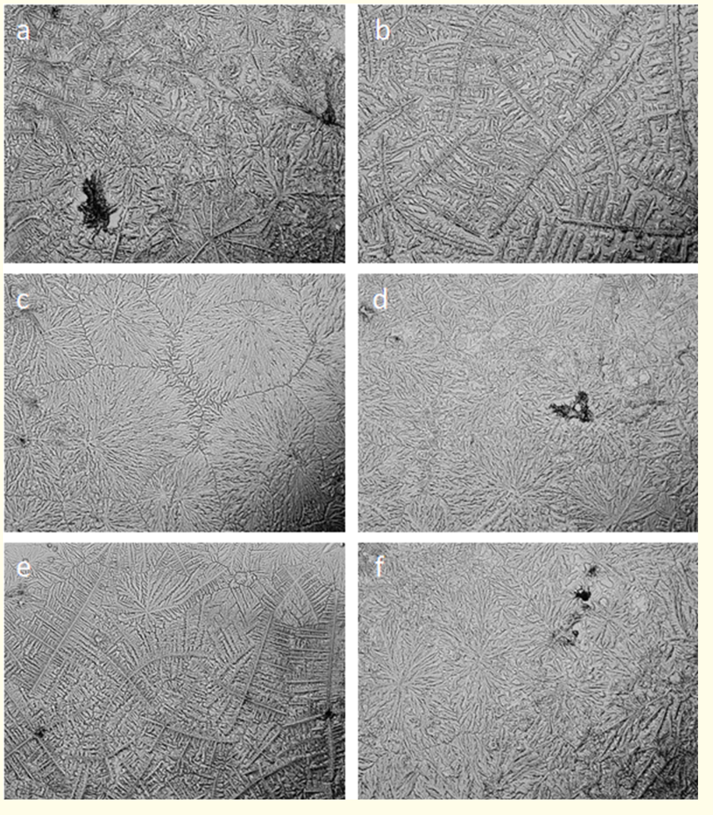

Figure 12: *TF images of (a) Blink eyedrops, (b) a mixture of Blink and KCl (10:6 by volume), (c) a mixture of Blink and CaCl2 (10:6), (d) a mixture of Blink and MgCl2 (10:9), (e) a mixture of Blink and NaHCO3 (10:3), and (f) a mixture of Blink and NaH2PO4 (10:3).*

Conclusion

The TF test is an inexpensive and easy-to-perform tool to evaluate the quality of tears. It can be used in combination with other tests to detect eye dryness. The TF test is used to assess the tear film in birds, reptiles, and animals (e.g. rabbits, cats, dogs, monkeys, horses, and camels) and human. It has been used to test the effect of smoking, thyroid gland disorder, diabetes, high BMI, high blood cholesterol level, vitamin supplements (vitamins A and D_3), and beverages (e.g. green tea and peppermint) on the quality of tear film. Moreover, the test turns to be useful in the detection of changes in patterns of artificial tears due to the addition of electrolyte solutions.

Acknowledgements

The authors are grateful to the Deanship of Scientific Research, King Saud University, for funding through Vice Deanship of Scientific Research Chairs.

Disclosure

The authors report no conflicts of interest in this work.

Bibliography

1. Sweeney DF., *et al.* "Tear film stability: a review". *Experimental Eye Research* 117 (2013): 28-38.
2. Mcmonnies CW. "Tear instability importance, mechanisms, validity and reliability of assessment". *Journal of Optometry* 11.4 (2018): 203-310.
3. Li N., *et al.* "Tear film instability and meibomian gland dysfunction correlate with the pterygium size and thickness pre- and postexcision in patients with pterygium". *Journal of Ophthalmology* (2019): 5935239.
4. Yokoi N and Georgiev GA. "Tear film–oriented diagnosis and tear film–oriented therapy for dry eye based on tear film dynamics". *Investigative Ophthalmology and Visual Science* 59.14 (2018): DES13-DES22.
5. Sun C-C., *et al.* "Effect of warming eyelids on tear film stability and quality of life in visual display terminal users: a randomized controlled trial". *Scientific Reports* 10 (2020): 16919.
6. McGill JI., *et al.* "Normal tear protein profiles and age-related changes". *British Journal of Ophthalmology* 68.5 (1984): 316-320.
7. Johnson ME and Murphy PJ. "Changes in the tear film and ocular surface from dry eye syndrome". *Progress in Retinal and Eye Research* 23.4 (2004): 449-474.
8. McCulley JP and Shine WE. "The lipid layer of tears: dependent on meibomian gland function". *Experimental Eye Research* 78.3 (2004): 361-365.
9. Holly FJ. "Tear film physiology". *American Journal of Optometry and Physiological Optics* 57.4 (1980): 252-257.
10. Korb DR., *et al.* "The Tear Film". Structure, Function and Clinical Examination". 1st edition, Elsevier (2002).
11. Milder B. "The Lacrimal Apparatus". In: Moses RA and Hart WM. Adler's Physiology of the Eye". St Louis, Missouri: The CV Mosby Co (1987).
12. Dilly PN. "Structure and function of the tear film". *Advances in Experimental Medicine and Biology* 350 (1994): 239-247.
13. Brewitt H and Sistani F. "Dry eye disease: the scale of the problem". *Survey of Ophthalmology* 45.S2 (2001): S199-S202.
14. Baudouin C. "The pathology of dry eye". *Survey of Ophthalmology* 45.S2 (2001): S211-S220.
15. McGinnigle S., *et al.* "Evaluation of dry eye". *Survey of Ophthalmology* 57.4 (2012): 293-316.
16. Kaštelan S., *et al.* "Diagnostic procedures and management of dry eye". *BioMed Research International* (2013): 309723.
17. Tomlinson A., *et al.* "The International Workshop on Meibomian Gland Dysfunction: Report of the Diagnosis Subcommittee". *Investigative Ophthalmology and Visual Science* 52.4 (2011): 2006-2049.
18. Stapleton F., *et al.* "TFOS DEWS II epidemiology report". *The Ocular Surface* 15.3 (2017): 334-365.
19. Bhavsar AS., *et al.* "A review on recent advances in dry eye: pathogenesis and management". *Oman Journal of Ophthalmology* 4.2 (2011): 50-56.
20. Phadatare SP., *et al.* "A comprehensive review on dry eye disease: diagnosis, medical management, recent developments, and future challenges". *Advances in Pharmaceutics* (2015): 704946.
21. Lin H and Yiu SC. "Dry eye disease: a review of diagnostic approaches and treatments". *Saudi Journal of Ophthalmology* 28.3 (2014): 173-181.

22. Thulasi P and Djalilian AR. "Update in current diagnostics and therapeutics of dry eye disease". *Ophthalmology* 124.11S (2017): S27-S33.

23. Willcox MDP., *et al.* "TFOS DEWS II Tear film report". *The Ocular Surface* 15.3 (2017): 366-403.

24. Masmali A., *et al.* "Comparative study of repeatability of phenol red thread test versus Schirmer's test in normal adults in Saudi Arabia". *Eye and Contact Lens* 40.3 (2014): 127-131.

25. Pauk SV., *et al.* "Noninvasive tear film break-up time assessment using handhold lipid layer examination instrument". *Acta Clinica Croatica* 58.1 (2019): 63-71.

26. Masmali A., *et al.* "Investigation of tear film using the TearLabTM osmolarity system in normal adults in Saudi Arabia". *Eye and Contact Lens* 40.2 (2014): 74-78.

27. Fagehi R., *et al.* "Correlation between tear osmolarity measurements using TearLab and I-Pen osmolarity systems in normal young Saudi subjects". *EC Ophthalmology* 12.2 (2021): 40-46.

28. Abusharaha A., *et al.* "Assessment of tear-evaporation rate in thyroid-gland patients". *Clinical Ophthalmology* 13 (2019): 131-135.

29. Alanazi SA., *et al.* "Assessment of the tear evaporation rate in chronic smokers using Delfin Vapo Meter". *International Journal of Ophthalmology and Visual Science* 4.2 (2019): 37-41.

30. Kyei S., *et al.* "Association between dry eye symptoms and signs". *Journal of Current Ophthalmology* 30.4 (2018): 321-325.

31. Wood SD and Mian SI. "Diagnostic tools for dry eye disease". *European Ophthalmic Review* 10.2 (2016): 101-107.

32. Rolando M. "Tear mucus ferning test in normal and keratoconjunctivitis sicca eyes". *Chibret International Journal of Ophthalmology* 2.4 (1984): 32-41.

33. Masmali A., *et al.* "Development of a new grading scale for tear ferning". *Contact Lens and Anterior Eye* 37.3 (2014): 178-184.

34. Masmali A., *et al.* "The tear ferning test: a simple clinical technique to evaluate the ocular tear film". *Clinical Experimental Optometry* 97.5 (2014): 399-406.

35. Tabbara KF and Okumoto M. "Ocular ferning test: a qualitative test for mucus deficiency". *Ophthalmology* 89.6 (1982): 712-714.

36. Golding TR and Brennan NA. "The basis of tear ferning". *Clinical Experimental Optometry* 72.4 (1989): 102-112.

37. Murube J. "Tear crystallization test: two centuries of history". *The Ocular Surface* 2.1 (2004): 7-9.

38. Vaikoussis E., *et al.* "Tear mucus ferning in patients with Sjögren's syndrome". *Documenta Ophthalmologica* 87.2 (1994): 145-151.

39. Norn M. "Quantitative tear ferning. Clinical investigations". *Acta Ophthalmologica* 72.3 (1994): 369-372.

40. Ravazzoni L., *et al.* "Forecasting of hydrophilic contact lens tolerance by means of tear ferning test". *Graefe's Archive for Clinical and Experimental Ophthalmology* 236 (1998): 354-358.

41. Buckley HE. "Crystal Growth". New York: Wiley; London: Chapman and Hall Ltd (1951).

42. Kogbe O., *et al.* "Factors responsible for tear ferning". *Cornea* 10.5 (1991) :433-444.

43. Pearce EI and Tomlinson A. "Spatial location studies on the chemical composition of human tear ferns". *Ophthalmic and Physiological Optics* 20.4 (2000): 306-313.

44. Rolando M., *et al.* "The effect of hyperosmolarity on tear mucus ferning". *Fortschritte der Ophthalmologie* 83.6 (1986): 644-646.

45. Horwath J., *et al.* "Ocular ferning test – Effect of temperature and humidity on tear ferning patterns". *Ophthalmologica* 215.2 (2001): 102-107.

46. Norn M. "Ferning in conjunctival-cytologic preparations. Crystallisation in stained semiquantitative pipette samples of conjunctival fluid". *Acta Ophthalmologica* 65.1 (1987): 118-122.

47. Pensyl CD and Dillehay SM. "The repeatability of tear mucus ferning grading". *Optometry and Vision Science* 75.8 (1998): 600-604.

48. Tang Y-J., *et al.* "Establishment of a tear ferning test protocol in the mouse model". *Translational Vision Science and Technology* 19.13 (2020): 1.

49. Oriá AP., *et al.* "Comparison of electrolyte composition and crystallization patterns in bird and reptile tears". *Frontiers in Veterinary Science* 7 (2020): 574.

50. Lantyer-Araujo NL., *et al.* "Rabbit as an animal model for ocular surface disease, tear osmolarity, electrolyte, and tear ferning profiles". *Optometry and Vision Science* 97.10 (2020): 847-851.

51. Veloso JF., *et al.* "The use of tear ferning test in cats for evaluation of ocular surface". *Acta Veterinaria Scandinavica* 62 (2020): 23.

52. Williams D and Hewitt H. "Tear ferning in normal dogs and dogs with keratoconjunctivitis sicca". *Open Veterinary Journal* 7.3 (2017): 268-272.

53. Oriá AP., *et al.* "Tear ferning test in healthy dogs". *Veterinary Ophthalmology* 21.4 (2018): 391-398.

54. Raposo ACS., *et al.* "Evaluation of lacrimal production, osmolarity, crystallization, proteomic profile, and biochemistry of capuchin monkeys tear film". *Journal of Medical Primatology* 47.6 (2018): 371-378.

55. Silva LR., *et al.* "Tear ferning test in horses and its correlation with ocular surface evaluation". *Veterinary Ophthalmology* 19.2 (2016): 117-123.

56. Masmali AM., *et al.* "Structure and microanalysis of tear film ferning of camel tears, human tears and refresh plus". *Molecular Vision* 24 (2018): 305-314.

57. Tabbara KF and Okumoto M. "Ocular ferning test". *Ophthalmology* 89.6 (1982): 712-714.

58. Norn M. "Quantitative tear ferning. Methodologic and experimental investigations". *Acta Ophthalmologica* 66.2 (1988): 201-205.

59. Puderbach S and Stolze HH. "Tear ferning and other lacrimal tests in normal persons of different ages". *International Ophthalmology* 15.6 (1991): 391-395.

60. Horwath J., *et al.* "Ocular ferning test - effect of temperature and humidity on tear Ferning patterns". *Ophthalmologica* 215.2 (2001): 102-107.

61. Nebbioso M., *et al.* "Tear ferning test and pathological effects on ocular surface before and after topical cyclosporine in vernal kerato-conjunctivitis patients". *Journal of Ophthalmology* (2018): 1061276.

62. Sharanjeet-Kaur., *et al.* "The relationship between tear ferning patterns and non-invasive tear break-up time in normal Asian population". *Journal of Optometry* 9.3 (2016): 175-181.

63. Masmali A., *et al.* "Assessment of tear film quality among smokers using tear ferning patterns". *Journal of Ophthalmology* (2016): 8154315.

64. Alanazi SA., *et al.* "Effect of short-term oral vitamin D3 supplementation on tear film in dry eye subjects". *International Journal of Ophthalmology and Visual Science* 4 (2019): 51-57.

65. Alanazi SA., *et al.* "Effects of short-term oral vitamin A supplementation on the ocular tear film in patients with dry eye". *Clinical Ophthalmology* 13 (2019): 599-604.

66. Alanazi SA., *et al.* "An assessment of the ocular tear film in patients with thyroid disorders". *Clinical Ophthalmology* 13 (2019): 1019-1026.

67. Alanazi SA. "Assessment of tear film in subjects with a high body mass index". *Clinical Optometry* 11 (2019): 77-84.

68. Alanazi SA. "Assessment of ocular tear film stability in subjects with high total cholesterol levels". *The Medical Journal of Cairo University* 87.9 (2019): 3109-3116.

69. Alanazi SA., *et al.* "A comparative study of the quality of non-stimulated and stimulated tears in normal eye male subjects using the tear ferning test". *Clinical Optometry* 11 (2019): 65-71.

70. Masmali AM., *et al.* "The acute effect of a single dose of green tea on the quality and quantity of tears in normal eye subjects". *Clinical Ophthalmology* 13 (2019): 605-610.

71. Cheng H., *et al.* "Epicatechin regulates blood lipids and attenuates hepatic steatosis in rats fed high-fat diet". *Molecular Nutrition and Food Research* 61 (2017): 1700303.

72. Muramatsu K., *et al.* "Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats". *Journal of Nutritional Science and Vitaminology* 32 (1986): 613-622.

73. Masmali AM., *et al.* "Assessment of the tear film in normal eye subjects after consumption of a single dose of hot peppermint drink". *Clinical Optometry* 11 (2019): 39-45.

74. Chacko SM., *et al.* "Beneficial effects of green tea: a literature review". *Chinese Medicine* 5 (2010): 8546513.

75. Alanazi SA., *et al.* "Effect of Refresh Plus® preservative-free lubricant eyedrops on tear ferning patterns in dry eye and normal eye subjects". *Clinical Ophthalmology* 13 (2019): 1011-1017.

76. Norn MS. "Tear fluid pH in normals, contact lens wearers, and pathological cases". *Acta Ophthalmologica* 66.5 (1988): 485-489.

77. Simmons PA and Vehige JG. "2Investigating the potential benefits of a new artificial tear formulation combining two polymers". *Clinical Ophthalmology* 11 (2017): 1637-1642.

78. Simmons PA., *et al.* "Efficacy and safety of two new formulations of artificial tears in subjects with dry eye disease: a 3-month, multicenter, active-controlled, randomized trial". *Clinical Ophthalmology* 9 (2015): 665-675.

79. Labetoulle M., *et al.* "Carboxymethylcellulose and hyaluronic acid multiingredient eye drop: a randomized controlled trial in moderate to severe dry eye". *Eye* 31.10 (2017): 1409-1416.

80. Masmali AM. "Improvement of ferning patterns of lubricant eye drops mixed with various electrolytes and carboxymethylcellulose". *Contact Lens and Anterior Eye* 42.6 (2019): 633-639.

Volume 12 Issue 9 September 2021