A comparative study of the quality of non-stimulated and stimulated tears in normal eye male subjects using the tear ferning test

Saud A Alanazi  
Mohammed A Aldawood  
Yousef S Badawood  
Gamal A El-Hiti  
Ali M Masmali

Cornea Research Chair, Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Saudi Arabia

Purpose: To evaluate and compare the tear ferning patterns of non-stimulated and stimulated tears in normal eye male subjects using the tear ferning test.

Methods: Thirty-five male subjects aged 20–38 years with normal eyes were enrolled in the study. Subjects with dry eye or with a risk factor for eye dryness (eg, recent ocular surgery, contact-lens wearers, smokers, anemic, diabetes, and thyroid gland problems) were excluded from the study. Dry eye subjects were excluded based on the ocular surface diseases index scores, non-invasive tear break-up time, and tear meniscus height measurements from the right and left eyes. A tear sample was collected from both eyes in each subject to perform the tear ferning test for non-stimulated tears. After 10 mins, the tears were stimulated using a Schirmer strip, a tear sample was collected from both eyes in each participant, the tear ferning test was performed, and the ferns were graded based on the five-point tear ferning grading scale.

Results: The average tear ferning grade [median (interquartile range)] for non-stimulated tears from the right and left eyes was 0.60 (1.25) and 0.50 (1.31), respectively. For stimulated tears, the average tear ferning grade from the right and left eyes was 0.50 (1.05) and 0.75 (1.25), respectively. There was no significant difference in the tear ferning grades obtained from non-stimulated and stimulated tears (Wilcoxon test, P>0.05).

Conclusion: This study found no significant difference in the tear ferning patterns obtained from non-stimulated and stimulated tears, or between the tear ferning patterns in the tears collected from the right and left eyes before and after stimulating tears. It seems that there were no changes in the chemical composition of tears before and after stimulation, possibly due to the steady concentration of electrolytes and lipids within the tear film for both non-stimulated and stimulated tears.

Keywords: tear ferning patterns, tear quality, tear film, eye dryness, non-stimulated and stimulated tears

Introduction

Tear film consists of three layers known as the mucin (inner), aqueous (middle), and lipid (outer) layers and has a complex structure.1–3 The mucin layer is produced by goblet cells and the lacrimal glands, while the meibomian glands are responsible for the production of lipids.4,5 Tear film covers the anterior surface of the eye, protects the cornea, provides the cornea with oxygen, lubricates and moistens the eye surface, and washes foreign bodies from the eye.5 The lipid layer influences tear film stability, and dysfunction of meibomian gland secretions leads to changes in
both the quality and quantity of tears.6 Instability in the
tear film leads to a dry eye, a disturbance in the visual
process, causing eye pain, dryness, and inflammation, and
negatively affecting the quality of life in 10–30% of the
global population.7 The cost of the treatment of dry eye
syndrome across the globe is estimated to be over $100
billion.8,9 Thus, many studies have been conducted on the
tear film, and on the lipid layer in particular, to investigate
which factors could negatively affect the ocular surface.10
Such studies found strong associations between the normal
structure of tear film and thickness of the lipids layer,
smoothness of the ocular surface, low evaporation rate of
tears, high viscosity of tears, and the efficiency of ocular
surface as antimicrobial barrier.5

Deficiency in tear secretion is common in approximately
50% of patients who experience dry eye symptoms,
which is mainly due to dysfunction of the lacrimal glands.6
The aqueous layer of the tear film is water-rich and con-
tains various types of proteins and electrolytes. The reg-
ulation of these tear proteins and electrolytes is vital to the
control of tear secretion.11,12 However, a high tear eva-
poration rate is common due to dysfunction of the meibo-
mian glands, leading to a reduction in lipid production.13
The quality and quantity of tears can be measured using
various tests. The most common diagnostic tests used to
assess the quantity of tears are the phenol red thread,14
Schirmer,14 tear meniscus height (TMH),15 and tear
evaporation rate16 tests. Other tests can be used to detect
the quality of tears, such as tear ferning (TF),17,18
osmolarity,19 and non-invasive tear break-up time
(NITBUT).20 In addition, dry eye symptoms can be
assessed with the use of dry eye questionnaires such as
the ocular surface disease index (OSDI).21 Eye drops
can be used to improve the tear film stability in patients
with moderate eye dryness.22 Further, mechanical massage
and warming eye lid devices could help patients with reduced
lipid secretion.23,24

The TF pattern reflects the chemical composition of
tears when dried. The five-points grading scale includes
five TF grades (grades 0 to 4) in which grade 0 shows a TF
pattern with no gaps or spaces between the branches and
ferns.18 Grade 1 TF pattern shows the presence of small
gaps between the branches ferns. The gaps and the thick-
ness of the branches increased in Grade 2 and increased
further in Grade 3.18 Grade 4 shows no TF pattern at all.18
Recently, we demonstrated that the TF test can be used to
detect eye dryness along with other diagnostic tests.25–29
The TF test was proved to be a valid and a reliable to
detect eye dryness.30 To our knowledge, the TF test has
not been used to compare the quality of non-stimulated
and stimulated tears among normal eye subjects. In addi-
tion, the TF patterns provide information about the chemi-
cal composition in non-stimulated and stimulated tears.
Therefore, the purpose of the current research was to
evaluate and compare the TF patterns of non-stimulated
and stimulated tears obtained from both the left and right
eyes of normal eye subjects.

Materials and methods
Subjects
Thirty-five male subjects aged 20–38 years (mean ± standard
deviation =23.36±2.48 years) with normal eyes were
enrolled in this study. The subjects were students at the
College of Applied Medical Sciences, King Saud
University, Riyadh. Subjects with dry eyes or a risk factor
for eye dryness (eg, those who had undergone recent ocular
surgery or who were contact-lens wearers, smokers, anemic,
had diabetes, had high body mass index, high cholesterol,
or had thyroid gland problems) were excluded from the study.
Dry eye subjects were excluded based on the OSDI score,
NITBUT, and TMH measurements from the right and left
eyes. Ethical approval was obtained from the College of
Applied Medical Sciences Ethics Committee, King Saud
University. Written informed consent was obtained from
each participant prior to the commencement of the research
and the subjects were treated according to the tenets of
Helsinki Declaration.31 All tests were performed by the
same examiner in a controlled environment at a temperature
of 23°C and a humidity of <40%. The OSDI was completed
by each participant followed by the TF, TMH, and NITBUT
tests. A 10 mins rest period was allowed between tests.

OSDI
The OSDI was first completed by each participant and a
score of <13 was considered indicative of a normal eye.21

TMH and NITBUT tests
The TMH and NITBUT tests were performed on
OCULUS Keratograph® 4 (OCULUS Inc., Wetzlar,
Germany). Fluorescein was added to the subject’s eye.
For NITBUT, the subject was asked to refrain from blink-
ing while the tear film was observed. A yellow barrier filter
was used to enhance the visibility of the tear film break-
up. The tear break-up time was recorded as the number of
seconds that elapsed between the last blink and the
The tears were collected by the investigators and the averages were calculated.

The current study suggested that there were no significant differences between the TF grades obtained from non-stimulated and stimulated tears collected from both the right and left eye of the study subjects ($P>0.05$). A previous study conducted on non-stimulated ($n=17$) and

### Table 1 Ocular surface disease index, tear meniscus height, non-invasive tear break-up time, and tear ferning scores

<table>
<thead>
<tr>
<th>Test</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSDI</td>
<td>40.00 (6.00)</td>
</tr>
<tr>
<td>TMH (OD; mm)*</td>
<td>0.25 (0.06)</td>
</tr>
<tr>
<td>TMH (OS; mm)*</td>
<td>0.25 (0.06)</td>
</tr>
<tr>
<td>NITBUT (OD; s)*</td>
<td>9.80 (5.33)</td>
</tr>
<tr>
<td>NITBUT (OS; s)*</td>
<td>10.00 (5.90)</td>
</tr>
</tbody>
</table>

**Note:** *P*<0.05.

**Abbreviations:** IQR, interquartile range; OSDI, ocular surface disease index; TMH, tear meniscus height; NITBUT, non-invasive tear break-up time; OD, right eye; OS, left eye.

### Table 2 Tear ferning grades

<table>
<thead>
<tr>
<th>TF grade*</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stimulated tears</td>
<td>Stimulated tears</td>
</tr>
<tr>
<td>TF (OD)</td>
<td>0.55 (0.89)</td>
</tr>
<tr>
<td>TF (OS)</td>
<td>0.50 (1.11)</td>
</tr>
</tbody>
</table>

**Notes:** *P*<0.05. TF patterns were graded using 0.1 increments.

**Abbreviations:** IQR, interquartile range; TF, tear ferning; OD, right eye; OS, left eye.

The results are provided in Figures 1. Representative TF images obtained from the right and left eyes for non-stimulated and stimulated tears obtained from the same subject are provided in Figures 2 and 3, respectively.

The correlations among the scores collected from the OSDI and TMH and NITBUT measurements in the right and left eyes are recorded in Table 3. There were strong correlations between the TMH ($r=0.577$, $P=0.001$) and NITBUT ($r=0.610$, $P=0.001$) measurements in the right and left eyes. No correlations were found among the OSDI scores and the TMH and NITBUT measurements.

The correlations between the TF grades for non-stimulated and stimulated tears obtained from the right and left eyes are recorded in Table 4. There was a strong ($r=0.554$, $P=0.007$) correlation between the TF grades for non-stimulated tears obtained from the right and left eyes. In addition, there was a medium ($r=0.491$, $P=0.002$) correlation between the TF grades for stimulated tears obtained from the right and left eyes.

### Results

The median scores for the OSDI and the TMH and NITBUT measurements obtained from both the right and left eyes are recorded in Table 1. There were no significant differences between the scores collected from the OSDI and the measurements obtained from TMH and NITBUT for both the right and left eyes (Wilcoxon test, $P>0.05$). The median (IQR) TF grades for the non-stimulated and stimulated tears obtained from the right and left eyes in each subject are reported in Table 2. There were no significant differences between the TF grades obtained from non-stimulated and stimulated tears and those obtained from both the right and left eyes (Wilcoxon test, $P>0.05$). Box plots for the TF grades for the non-stimulated and stimulated tears in the right and left eyes for the

### Discussion

The current study suggested that there were no significant differences between the TF grades obtained from non-stimulated and stimulated tears collected from both the right and left eye of the study subjects ($P>0.05$). A previous study conducted on non-stimulated ($n=17$) and
stimulated \((n=19)\) tears found no significant difference in the viscosity of tears.\(^{33}\) In addition, the surface tension for non-stimulated (43.0±2.1) and stimulated (46.0±1.5) tears were similar.\(^{33}\) Such results suggested that the viscosity and surface tension are not dependent on the mucin level.\(^{33}\) Therefore, the mucin level does not play a primary role in the differentiation of non-stimulated and stimulated tears.\(^{33}\) Another study suggested that both non-stimulated and stimulated tears have the same concentration of mucins, and therefore have similar viscosity.\(^{34}\)

**Figure 1** Box plots for the tear ferning grades for non-stimulated and stimulated tears.

**Note:** TF patterns were graded using 0.1 increments.

**Abbreviations:** TF, tear ferning; NTF (OD), tear ferning for non-stimulated tears collected from the right eye; NTF (OS), tear ferning for non-stimulated tears collected from the left eye; STF (OD), tear ferning for stimulated tears collected from the right eye; STF (OS), tear ferning for stimulated tears collected from the left eye.

**Figure 2** Representative tear ferning images of non-stimulated tears collected from the right and left eyes of the same subject.

**Abbreviations:** NTF (OD), tear ferning for non-stimulated tears collected from the right eye; NTF (OS), tear ferning for non-stimulated tears collected from the left eye.

**Figure 3** Representative tear ferning images of stimulated tears collected from the right and left eyes of the same subject.

**Abbreviations:** STF (OD), tear ferning for stimulated tears collected from the right eye; STF (OS), tear ferning for stimulated tears collected from the left eye.
Non-stimulated and stimulated tears collected from 30 normal eye subjects were previously analyzed by high-performance liquid chromatography, in which 12 types of protein were identified. The concentrations of lacrimal gland proteins, tear-specific prealbumin, lysozyme, and lactoferrin were similar in non-stimulated and stimulated tears. However, the remaining types of protein, such as immunoglobulin A and immunoglobulin M, were higher in non-stimulated tears than in stimulated tears. Such results suggested that the rate of tear flow affects the produced protein profile.

On the other hand, an enzyme-linked immunosorbent assay indicated that the concentrations of lysozyme, lactoferrin, serum albumin, and immunoglobulin A were similar in non-stimulated tears (n=12). Clearly, the control of the rate of tear flow in the collection process is important.

High-flow mechanical and intense cooling pulse stimulation of the cornea leads to a significant increase in tear secretion (P<0.05), while stimulation of the conjunctiva is not effective. However, moderate mechanical and cold stimulation of either the conjunctiva or the cornea does not significantly alter tear secretion. Electrical stimulation of the cornea, anterior ethmoid nerve, lacrimal gland, and lacrimal nerve leads to increased tear volume, and normal tear proteins and lipids, and a reduction in the osmolarity of tears. For example, electrical (30–90 Hz) stimulation of the anterior ethmoid nerve leads to a 92–133% increase in tear volume (P<0.01). The level of tear secretion returned to the baseline volume after 7 mins of stimulation. The osmolarity of the tear film was reduced.
by 7 mOsmol/L and the concentration of both tear lipids (24–36%) and proteins (48%, \(P<0.05\)) was increased.\(^2\) In addition, regular (≥4 times daily) electrical stimulation of the anterior nerve can reduce dry eye symptoms.\(^4\) Thus, tear stimulation can improve tear secretion, but does not affect the quality or chemical composition of the tears. In addition, the stimulation does not alter the component of tears, as the current study suggested. Indeed, the average scores for the Schirmer test were found to be significantly higher in tears stimulated with a nasal electrical device than in non-stimulated tears in dry eye subjects (\(n=40\)).\(^5\)

**Limitations**

The current study has some limitations such as the relatively small number of subjects, and the participants were all normal eye males who reside in Riyadh City. Therefore, future studies should involve a larger sample size that contains both males and females and normal and dry eye subjects, and represent different geographic areas within Saudi Arabia.

**Conclusions**

The current study found no significant difference in the tear ferning patterns obtained from non-stimulated and stimulated tears of male subjects in Riyadh City. In addition, there were no significant differences between the tear ferning patterns of the tears collected from the right and left eyes before and after stimulating tears. It seems that there were no changes in the chemical composition of tears before and after stimulation, possibly due to the steady concentration of electrolytes and lipids within the tear film before both non-stimulated and stimulated tears. The tear ferning test has the potential to be used in clinics to assess the quality of tears.

**Abbreviation list**

TMH, tear meniscus height; TF, tear ferning; NITBUT, non-invasive tear break-up time; OSDI, ocular surface disease index; IQR, interquartile range; \(r\), Spearman correlation coefficient; OD, right eye; OS, left eye.

**Ethics approval and informed consent**

Written informed consent was obtained from each participant prior to the commencement of the research, and the subjects were treated according to the tenets of Helsinki Declaration.

**Acknowledgments**

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**Disclosure**

The authors report no conflicts of interest in this work.

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