Differentiation of Lipid Tear Deficiency Dry Eye by Kinetic Analysis of Tear Interference Images

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Objective: To use kinetic changes to characterize tear interference images in patients with lipid tear deficiency (LTD) dry eye.

Methods: We used a DR-1 camera to digitize and analyze sequential images of tear interference on the central 8 mm of the cornea at the start of complete blinking in 11 healthy volunteers and 8 patients with LTD and noninflamed meibomian gland dysfunction.

Main Outcome Measures: We studied tear lipid spread time and pattern, stability of the lipid after spread, and distribution of thickness in a prospective, case-control study.

Results: On complete lid closure, the lipid spread was horizontal in healthy eyes but vertical in LTD (P < .001). Mean ± SD lipid spread time was 0.36 ± 0.22 seconds in healthy eyes but 3.54 ± 1.86 seconds in LTD (P < .001). Mean ± SD lipid film thickness in healthy eyes was 74.5 ± 6.9 nm, thicker than the 43.8 ± 10.6 nm in LTD (P < .001), and this result was confirmed by qualification with intensity histogram (P < .001).

Conclusions: Kinetic analysis of the tear interference revealed distinctive differences in the time and pattern of lipid spread and the distribution and stability of resultant lipid thickness between healthy subjects and patients with LTD. This method can be coupled with others for formulating effective therapies for patients with dry eye.

Arch Ophthalmol. 2003;121:173-180

O n lid blinking, the tear fluid, including the superficial lipid layer, spreads into a thin film. The capability of maintaining a rather stable tear film dictates the ocular surface comfort, clear vision, and effective defense. Dry eye with the hallmark of unstable tear film can be subdivided into aqueous tear deficiency (ATD) and lipid tear deficiency (LTD). With respect to ATD, the diagnostic criteria are straightforward and rest on the measurement of aqueous tear production. With respect to LTD, the diagnosis is indirect, based on examination of the morphological change of meibomian gland by means of meibography, meibum expressibility by means of digital compression, or semi-quantification of lid margin meibum by means of meibometry, or is inferred by showing rapid tear evaporation or a combination of dye staining and impression cytology.

One noninvasive method of investigating the lipid tear film on the precorneal tear film is tear interference. A single static image of tear interference is obtained to semiquantify the lipid film thickness. Such a static image is assumed to be representative and the data of the lipid film thickness to be informative to study contact lens wear, to show the beneficial effect of digital expression in patients with meibomian gland dysfunction (MGD) with LTD, to differentiate ATD from healthy eyes, and to correlate with evaporative MGD.

Because a static image has been obtained without being consistently timed from the onset of the blink, it is impossible to ascertain such information as the speed of lipid spread and difficult to compare among different studies or individuals. Although previous reports have described the continuous video recording of tear interference during blinking, these video images are too fast to be analyzed and the results have not been described in a kinetic manner. We have developed a new method to overcome this deficiency by means of direct digitization.
of sequential images for kinetic analysis. With the help of sophisticated image analysis, we report herein distinctive differences between healthy subjects and patients with LTD. The significance of this method and finding is further discussed.

METHODS

Unless otherwise indicated, data are expressed as mean ± SD.

PATIENTS

We consecutively included 11 healthy asymptomatic volunteers (7 men and 4 women; mean age, 38.7 ± 14.3 years; 6 Asian, 3 white, 1 Indian, and 1 Arabic participant) and 8 patients with noninflamed MGD from an ophthalmology subspecialty clinic (3 men and 5 women; mean age, 61.9 ± 15.4 years; 3 Asian and 5 white participants). The diagnosis of MGD was based on the criteria previously used.6 Only 1 eye of each person was randomly chosen for analysis. Eyes with MGD included the presence of meibomian gland dropout by means of meibography via transillumination through the tarsus, no or poor meibum expression by means of digital compression, no or negligible inflammation in the lid margin, and the presence of meibomian gland orifice squamous metaplasia. All patients with LTD and healthy subjects had normal aqueous tear secretion verified by results of a fluorescein clearance test.23 Mean tear breakup time was 11.7 ± 1.5 for healthy subjects and 3.0 ± 0.8 for patients with LTD (P = .03, Mann-Whitney U test). Results of dye staining were negative in healthy subjects, whereas results of fluorescein and rose bengal staining were positive at the non-exposure zone in 1 (13%) of 8 patients with LTD.6 No participant had any evidence of ocular infection or inflammation, received punctal occlusion, wore contact lenses, or had blepharospasm or abnormal blinking. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the Ocular Surface Research and Education Foundation.

INSTRUMENT SETUP

In an examination room set at the same light intensity (350 lux), humidity (45.2%-54.0%), and temperature (21.0°C-22.7°C), we used a DR-1 tear interference camera (provided by Kowa, Nagoya, Japan) that provided a sharp focus on the tear lipid without the iris background, a high-quality 3-chip charged-coupled device camera, and a full-speed National Television Systems Committee video output. We set the magnification at ×12, which allowed observation of an 8-mm diameter of the cornea, and used the same light source intensity without causing patient discomfort throughout the study. The DR-1 video output was grabbed with a video frame grabber (FlashBus MV Lite; Integral Technologies, Indianapolis, Ind) and digitized into sequential video images in uncompressed AVI (audio video interleaved) format using image analysis software (Image-Pro 4.1; MediaCybernetics, Silver Spring, Md). The frame rate was set at 5.18 frames per second (0.193 seconds per frame). The recording was obtained for 29 seconds in a single session, which generated 150 frames (131-megabyte video file). These sequential video images were then extracted in uncompressed tag image file format, which could be made into a thumbnail composite and subjected to subsequent image analysis without the loss of image quality and change of the color information.

KINETIC ANALYSIS OF INTERFERENCE IMAGES

Among several sets of blinks, we chose the representative blink interval that started with a complete eye closure, of which the first full eye-opening frame was set as time 0 and encompassed the entire time interval defined as the interblinking time (IBT) before the next blink.24 All data were obtained by a non-masked observer (E.G.). We performed a preliminary comparison of the data between 1 representative blink interval and the average of the first 3 blink intervals from 150 frames, excluding those with irregular and incomplete blink, and confirmed that the representative blink interval we chose indeed represented the kinetics of tear lipid layer (Wilcoxon signed rank test, P = .33 in healthy subjects and P = .18 in patients with LTD).

Pattern of Lipid Spread

After blinking, the sequential images allowed us to discern the pattern of lipid spread before it reached a stable image. This pattern was recorded as horizontally propagating, vertically streaking, or mixed horizontal and vertical by 3 masked observers, and a complete agreement was reached among them.

Lipid Spread Time to Reach a Stable Image

The interval from time 0 to the time of the frame, which first showed a stable interference image, was defined as lipid spread time. We determined the first stable image by playing it frame by frame on a liquid crystal display screen to see if there was any noticeable movement between frames. When there was no noticeable movement, we defined it as the first stable image. This measurement was conducted by 2 nonmasked observers, and the longer time measured was chosen if there was a disagreement between them. Throughout our analyses, both observers agreed completely 65% of the time. Among the 35% of frames in which there was a discrepancy, the difference was limited to 2 frames in 80% of the cases and to 3 frames in 20% of the cases. If the image did not achieve a stable pattern throughout the entire IBT, the entire IBT was used to calculate the spread time.

DR-1 Grading Used for Dry Eye

For comparison, we also used the grading system reported by Yokoi et al20 for the static tear interference image in patients with dry eye based on the first stable frames from the DR-1 camera. Under this system, grade 1 indicates somewhat gray color and uniform distribution; grade 2, somewhat gray color and nonuniform distribution; grade 3, a few colors and nonuniform distribution; grade 4, many colors and nonuniform distribution; and grade 5, corneal surface partially exposed.

Lipid Layer Thickness Estimated From the Look-Up Table

We used the same conventional color comparison method using the look-up color table reported by Korb and Greiner17 and King-Smith et al25 to estimate lipid layer thickness. We chose the time of 0.4 seconds to calculate thickness in healthy subjects and patients with LTD, because this was the average time in which the healthy subject first reached a stable image. We also compared these values with those measured when the first stable image was achieved.

Thickness Comparison Using Intensity Histogram in a Defined Area

Because the look-up color table comparison method is semiquantitative, we decided to adopt a more quantitative method.
To quantify the interference color brightness, which is known to correlate with the film layer thickness based on the physics principle of white-light source thin-film interference phenomenon, we compared the intensity histogram of the images. To do so, we defined a polygonal area (defined within the green line shown in Figure 1A and C) that excluded the upper eyelashes in the selected images of all patients. We then used the imaging software to display the intensity histogram of all the pixels in this area, which gave an average of the brightness value of the interference image (range, 0-255; Figure 1B and D).

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STATISTICAL ANALYSIS

We collected the data from prospectively completed data forms. For data of pattern of spread, we used a χ² test for independence; for data of ordinal and interval scale (spread time, DR-1 grading, estimated thickness from the look-up color table, and intensity at the defined area), Mann-Whitney U test. We performed these statistical tests using StatView 5.0 computer software (SAS Institute Inc, Cary, NC). P<.05 was considered statistically significant.

RESULTS

REPRESENTATIVE KINETIC IMAGES

Figure 2 shows a representative example with consecutive images obtained from a healthy subject (Figure 2A) and a patient with LTD (Figure 2B). The first frame is the last frame when the lid closed, and the second frame is the first frame of IBT and set as time 0. Each frame interval is 0.193 seconds (5.18 frames per second). Each figure includes 16 frames encompassing a period of 2.895 seconds. Figure 3 shows the representative images of 11 healthy subjects (Figure 3A) and 8 patients with LTD (Figure 3B). These representative images were chosen from...
Figure 2. Representative sequential images of a healthy subject (A; healthy subject 3 in the Table) and a patient with lipid tear deficiency (LTD) (B; patient 6 in the Table). In the healthy eye, lipid spread is seen in a horizontal pattern and reaches a stable image at the frame marked X for a total of 0.39 seconds. In LTD, a spread is seen in a vertical manner and took 2.32 seconds to reach a stable image in the frame marked X. Asterisk indicates the first frame within blinking time; arrowhead, the images start from upper left to lower right with a 0.193-second interval; and 0, the first interblinking interval frame. R indicates right.
the frame first showing a stable image. If the image never reached a stable one, the last image of IBT was chosen.

**KINETIC IMAGE ANALYSES**

The results of kinetic analyses are summarized in the Table.

**Healthy Subjects**

After blinking, the lipid film spread rapidly in horizontal propagating waves from the lower to the upper cornea (Figure 2A) in healthy subjects (Figure 3A). Three subjects (27%) showed a mixed lipid spread pattern. Within a mean period of 0.36±0.22 seconds, the tear interference reached a stable image and remained so thereafter (Table). During this time, the interference of the lipid film yielded a color of gray to gray-white, and was uniformly distributed throughout the entire central 8 mm of the corneal surface. The mean DR-1 grade was 1.7±0.5, falling in the range defined as normal by Yokoi et al.\textsuperscript{5,20} The mean estimated thickness was 74.5±6.9 nm for these 11 healthy subjects. The mean color intensity from the intensity histogram was distributed at 151.3±11.7 in the defined polygonal area shown in Figure 1.

**Patients With LTD**

After blinking, faint vertical streaking waves spread immediately from the lower to the upper cornea (Figure 3B). Patients 2, 4, 7, and 8 show faint vertical streaking. R indicates right; L, left.

![Figure 3. Representative static tear interference images of all healthy subjects at a stable image (A) and all patients with LTD (B). Healthy subjects 1 through 6, 8, and 10 show a horizontal spreading pattern with rather even distribution. Interference images showed the coloration of gray to gray-white, suggesting the thickness of 70 to 90 nm. All patients with LTD showed a vertical pattern with the coloration of dark gray to gray-white, suggesting the thickness of 40 to 70 nm. Patients 2, 4, 7, and 8 show faint vertical streaking. R indicates right; L, left.](image-url)
2B) in all 8 patients with LTD (Figure 3B). This pattern was entirely different from that of the healthy subjects (P < .001). Among the 8 patients, images from 2 (25%) never reached a stable stage. For them, we used the last IBT frame available for the analysis of the lipid spread time. For the remaining 6 patients, the interference image reached a stable stage at a much later time (Figure 2B). As a group, the lipid spread was retarded to a mean of 3.54 ± 1.86 seconds, which was significantly delayed compared with that of the healthy subjects (P < .001). Thus, the tear lipid film of the patients with LTD was significantly unstable. The dominant color of the tear interference images was dark gray to gray-white, which indicated a rather thin lipid layer. The mean DR-1 grade was 2.0 ± 0.0, which could not be distinguished from that of the healthy subjects (P = .32). The estimated mean thickness was 43.8 ± 10.6 nm, which was significantly thinner than that of the healthy subjects (P < .001). This value, calculated using the frame taken at 0.4 seconds, did not show any difference compared with the frame taken at the time when the image first reached a stable stage. The mean color intensity histogram was 121.9 ± 10.8, which was significantly darker than that of the healthy subjects (P < .001), indicating a thinner film presence in LTD.

We have demonstrated for the first time that kinetic analysis of tear interference images yields the information that has not been obtained previously to our knowledge by means of conventional methods. The conventional grading system published by Yokoi et al.6,21 based on the first stable frames from the DR-1 camera. Grades are described in the “Kinetic Analysis of Interference Images” subsection of the “Methods” section.

The estimated mean thickness was 43.8 ± 10.6 nm, which was significantly thinner than that of the healthy subjects (P < .001). This value, calculated using the frame taken at 0.4 seconds, did not show any difference compared with the frame taken at the time when the image first reached a stable stage. The mean color intensity histogram was 121.9 ± 10.8, which was significantly darker than that of the healthy subjects (P < .001), indicating a thinner film presence in LTD.

### COMMENT

We have demonstrated for the first time that kinetic analysis of tear interference images yields the information that has not been obtained previously to our knowledge by means of conventional methods. The conventional grading system published by Yokoi et al.6,21 for dry eye cannot distinguish healthy eyes from LTD. Moreover, our new method provides more quantitative measurements to differentiate LTD from healthy eyes. Besides the thickness, we were able to generate additional information such as the speed and pattern of lipid spread after eye opening and the stability of the lipid film after spread. Our data showed that the lipid spread on blinking was rapid in healthy subjects, but slow in patients with LTD. The pattern of lipid spread was horizontally wavy in the healthy eyes but vertically streaking in LTD. The lipid film was rather uniform and became stable after rapid spread in the healthy eyes. For LTD, the resultant film was rather nonuniform, and images in only 6 of 8 patients reached a stable pattern. Collectively, these 2 distinctively different patterns help to differentiate LTD from healthy eyes, and will permit us to explore how a stable lipid film is maintained in healthy eyes but lost in LTD.

Kinetic analysis timed with the onset of the eyelid blinking is important. Blinking facilitates meibum excretion from meibomian glands26 and generates upward excursion of the upper lid, an important driving force that helps the spread of aqueous and lipid tears over the corneal surface.27 Using an in vitro device to simulate eyelid blinking, Brown and Dervichian28 were the first to demonstrate that such upward excursion pulls up the first phase of aqueous fluid by means of capillary force. This is followed by the spread of the test oil, which then helps pull the second phase of aqueous fluid, resulting in a thicker aqueous layer. When extrapolated from this concept, our

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### Summary of Kinetic Analysis of Tear Interference Images

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pattern of Spread</th>
<th>Lipid Spread Time, s</th>
<th>DR-1 Grading*</th>
<th>Estimated Lipid Thickness From LUT, nm</th>
<th>Intensity at Defined Area†</th>
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</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
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<tr>
<td>1 H</td>
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<tr>
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<td>70</td>
<td>151.3</td>
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<td>142.0</td>
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<tr>
<td>Mean ± SD</td>
<td>0.36 ± 0.22</td>
<td>1.7 ± 0.5</td>
<td>74.5 ± 6.9</td>
<td>151.3 ± 11.7</td>
<td></td>
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<td>LTD</td>
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<td>1 V</td>
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<td>70</td>
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<td>2 V</td>
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<tr>
<td>Mean ± SD</td>
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<td>2.0 ± 0.0</td>
<td>43.8 ± 10.6</td>
<td>121.9 ± 10.8</td>
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<td>P Value</td>
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<td>&lt;.001</td>
<td>.32</td>
<td>&lt;.001</td>
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</table>

Abbreviations: H, horizontal; LTD, lipid tear deficiency; LUT, look-up color table; M, mixed horizontal and vertical; V, vertical.

*Derived by Yokoi et al.6,21 based on the first stable frames from the DR-1 camera. Grades are described in the “Kinetic Analysis of Interference Images” subsection of the “Methods” section.

†Range, 0-255.
data can be interpreted as follows: When the meibum is sufficiently present and evenly distributed on the lid margin, the lipid should spread in a horizontally propagating wave as observed in the healthy subjects (Figures 2A and 3A). This horizontal wavy pattern was also observed by Brown and Dervichian28 in their in vitro experiments.

When the meibum is insufficient on the lid margin, the upward excursion pulls up the first phase of aqueous fluid without accompanied lipid spread. This might be the reason why the vertical streaking pattern developed (Figures 2B and 3B). We speculate that this vertical streaking pattern may well represent the first phase of aqueous fluid spread previously described.28 During this time, the lipid film is extremely thin, giving rise to a dark gray color (Figures 2B and 3B). The reason that this vertical streaking was observed for the first time in vivo is in part because sequential images were taken from the onset of the blink and the subsequent lipid spread was rather slow, and in part because the lid margin of patients with LTD might be irregular and/or coated with uneven and deficient meibum. Future studies are needed to determine whether such a streaking pattern may also reflect the mechanical friction of the upper lid, which is normally exerted onto the corneal surface. In the healthy eye, such a friction is neutralized by the lubricating lipid film, whereas in LTD the friction can be significant enough to cause ocular surface irritation.

The lipid spread was very rapid in the normal tear film and completed in 0.36±0.22 seconds. This duration might not be precise, as the frame grab rate of 0.193 seconds per frame might not have caught the exact point when the lipid spread was completed. To resolve this potential error, a faster frame grab rate that does not lose image quality will have to be used. Regardless of this concern, the lipid spread of LTD was very slow, at a mean of 3.5±1.86 seconds (P<.001), and 2 of 8 cases never reached a stable interference image during the entire IBT. Several in vitro studies have confirmed that the initial phase of the lipid spread on an aqueous phase is conducted by polar lipids.20-24 Therefore, we speculate that the slower lipid spread in LTD might result from the lacking of polar lipids, a notion supported by published biochemical data.25-27 We thus believe that the measurement of the speed of the lipid spread may be used to determine the amount and quality of polar lipids in patients with MGD.

On a dish containing aqueous fluid, Brown and Dervichian28 noted that a meibum film is very thin when only polar lipids complete the rapid spread. The lipid film increases its thickness when nonpolar lipids subsequently move over the polar lipid film. In the healthy eye, the thickness was estimated at a mean of 74.5±6.9 nm, which was comparable with the values reported by others.9,10,12,14,16,17 In patients with LTD, the resultant film was estimated at a mean of 43.8±10.6 nm, significantly thinner than in the healthy eye (P<.001). When we used a more objective method of quantifying the intensity histogram, the thickness difference between the healthy eye and LTD was also significant (P<.001). Korb and Greiner21 first reported that patients with MGD had a lipid layer thickness of 60 nm or less, which was thinner than that of the healthy subjects. Nevertheless, their measurements were semiquantitative and might have suffered from the fact that their optical system only visualized a limited area of the peripheral cornea and could not eliminate the background iris coloration. Using the same DR-1 camera, Yokoi et al21-22 found that patients with LTD had a mean score of 2.5±0.2, which was between the scores for healthy subjects and patients with ATD. This unusual finding was caused by the design of the grading system for quantification of severity in ATD. Our study dealt with not only color (thickness) but also uniformity (evenness) of the lipid film. That was why we could successfully differentiate LTD from healthy eyes. A static interference image may be regarded artificially, even when there is a very little amount of lipid to yield an abnormal uneven image. This issue can be avoided by kinetic analysis, which shows a vertical streaking pattern. Furthermore, our image analysis using intensity histograms helped to objectively resolve confusing coloration ranging across dark gray, intermediate dark gray, intermediate bright gray, bright gray, and white (ie, covering the range of interference order from 0 to about 0.5, and the range of lipid thickness from 0 to about 92.5 nm).25

Because the final thickness is influenced a great deal by the interaction between the polar and nonpolar lipids, we wonder whether such interactions may be investigated by the measurements of the thickness and uniformity of the lipid film after spread. After the lipid film reached a stable interference image, we noted that subsequent images of the healthy subjects had a wide range of variability throughout the rest of the IBT. In contrast, patients with LTD reached a stable image during a longer period or did not reach such a stable interference image at all during entire IBT. Therefore, it is conceivable that measurements of spread time with blink analysis may be used to quantify the severity of LTD.

Collectively, this new way of analyzing tear interference can help correlate with xeroscopy38,39 to know how the tear film stability is influenced by the lipid film. Furthermore, it can also be used to correlate with tear evaporation45 to determine if indeed a stable lipid film dictates the rate of tear evaporation. Together, these tests may enhance our capability of investigating the mechanism by which an unstable tear film is formed in dry eye. By extrapolating lipid spread time and pattern and the distribution and uniformity of the thickness, we also believe that kinetic analysis of tear interference will help us devise and monitor future therapies directed to restoring meibomian gland functions.

Submitted for publication October 30, 2001; final revision received May 15, 2002; accepted August 8, 2002.


We thank P.-S. Tsai, PhD, of Intel, Santa Clara, Calif, for his advice in this project.

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REFERENCES


From the Archives of the Archives

My experience has taught me that the foreign body is best removed through the enlarged initial opening and that the giant magnet can be relied upon to do its work from start to finish.

First, whilst in London both Mr. Lawford and Mr. Treacher Collins of Moorfield gave me account of cases that had come under their care, where with very powerful giant magnets, they did not succeed in dislodging the foreign body within the eye. They had Mr. Mackenzie Davidson use X-rays for them, and through this the foreign body was located, the eye was cut down upon at the point designated by the X-ray, and the foreign body promptly removed, in one of the cases with the hand magnet.