A Novel Method for Pachymetry Mapping of Human Precorneal Tear Film Using Pentacam with Fluorescein

Hong Zhuang, Xingtao Zhou, and Jianjiang Xu

PURPOSE. To report a novel method for pachymetry mapping of human precorneal tear film using Pentacam.

METHODS. Precorneal tear film is routinely undetected by Pentacam but could be well visualized with the aid of fluorescein. Twenty patients with dry eye and 20 age-matched control subjects with normal eyes were enrolled in this prospective study. The right eye of each subject was scanned once with Pentacam and rescanned after instillation of 1 μL 0.1% fluorescein, and the differential map of corneal thickness between the two measurements was identified as the pachymetry map of tear film. Then the central tear film thickness was evaluated, and the pattern of each pachymetry map was determined.

RESULTS. Mean central tear film thickness in dry eyes (22.2 ± 4.5 μm) was less than in normal eyes (24.7 ± 3.9 μm) (Student’s t-test, \( P = 0.0614 \)). Additionally, the tear film pachymetry map could be classified into three patterns: pattern 1, thickening upward; pattern 2, uniform distribution; pattern 3, thickening downward. Tear film pachymetry maps of normal eyes consisted of pattern 1 (40%), pattern 2 (40%), and pattern 3 (20%), whereas those of dry eyes consisted of pattern 1 (70%), pattern 2 (20%), and pattern 3 (10%). Dry eyes tended to have a higher proportion of pattern 1 pachymetry maps than normal eyes, though still no significant difference was found between the two groups (Cochran-Mantel-Haenszel \( \chi^2 \) test, \( P = 0.0852 \)).

CONCLUSIONS. This novel method feasibly could be used to map tear film thickness, and it provides a valuable means to investigate the spatial distribution of tear film. (Invest Ophthalmol Vis Sci. 2010;51:156–159) DOI:10.1167/iovs.08-3265

Cornea

Laser interferometry has become a promising method to study tear film because of its noninvasiveness.4–7 In 1992, Prydal6 reported tear film thickness to be 40 μm using interferometry, and this value was further confirmed by confocal microscopy. Afterward, Danjo7 and King-Smith6 adopted interferometry to measure tear film thickness, but they gained discrepant results (Danjo, 11 μm; King-Smith, 3 μm). In the recent past, indirect measurements with optical coherence tomography (OCT) gave a value of approximately 3 μm.8–10

Actually, most studies referred only to the measurement of central tear film thickness. For one thing, tear film in this region serves a crucial role in the ocular optical system; for another, there has been, to date, no effective method to evaluate tear film thickness at different locations simultaneously. Few studies have attempted to discuss the spatial distribution of tear film over the corneal surface. A fluorophotometric study by Benedetto11 implied that inferior tear film was possibly thinner than superior tear film. King-Smith et al. (IOVS 2003; 44:ARVO E-Abstract 2476) measured tear film thickness with interferometry at different points along the corneal vertical meridian (one by one, not simultaneously) and preliminarily observed the phenomenon as Benedetto implied. However, the spatial variations in tear film thickness are still uncertain.

The purpose of this study was to report a novel method for pachymetry mapping of human precorneal tear film using Pentacam with fluorescein. Moreover, the spatial distribution of tear film in both normal eyes and dry eyes was investigated.

SUBJECTS AND METHODS

Subjects

Twenty patients (16 women, 4 men; average age, 47.7 ± 12.8 years) with dry eye consecutively referred to our clinic were enrolled in this study. Diagnoses of dry eye were made according to dry eye-related symptoms, Schirmer I test value <10 mm, and tear film breakup-time shorter than 5 seconds. Exclusion criteria included corneal erosion under slit-lamp examination, abnormality of lacrimal drainage apparatus, punctal plug insertion, and history of contact lens wear.

For comparison, 20 asymptomatic subjects (14 women, 6 men; average age, 44 ± 14.9 years) with normal tear function were recruited as a control group. Subjects with normal eyes had no ocular or systemic diseases with known association of ocular surface disorder, no abnormality of lacrimal drainage apparatus, and no history of contact lens wear.

Informed consent was obtained from each subject after explanation of the nature and possible consequences of the study. The research followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Shanghai Eye and ENT Hospital of Fudan University.

Apparatus

Pentacam (Oculus, Duedenhofen, Germany) is usually used to measure corneal thickness with Scheimpflug imaging technology during refractive surgery as a three-dimensional comprehensive scanner for anterior corneal thickness.
eye segment. A Pentacam-rotating Scheimpflug camera with a blue light source (light-emitting diode [LED], 475 nm) takes 25 or 50 cross-sectional images without contact within 2 seconds (25 images per second was chosen; Pentacam Instruction Manual; Oculus). Every sectional image consists of 500 true elevation points that allow high-resolution measurement. The high accuracy and repeatability of the Pentacam mean only one time scan is needed to evaluate corneal thickness.12,13 It can precisely locate and monitor the apex of the cornea, contributing to the avoidance of errors in comparison with two corneal pachymetry map of each eye (Pentacam Instruction Manual; Oculus).

**Principle**

Precorneal tear film is routinely undetected with the Pentacam Scheimpflug camera. In addition, corneal thickness is measured with the Pentacam from the corneal epithelium to the endothelium but does not include the tear film.14 After fluorescein staining, tear film can be activated by the Pentacam blue light source (LED at 475 nm) and emit green fluorescence. Consequently, fluorescent tear film can be visualized by Scheimpflug imaging. High-bright visualized tear film enhances the reflective optical intensity of the corneal surface after fluorescein instillation (Fig. 1). In this case, corneal pachymetric measurement includes the full-thickness of visualized tear film. Therefore, the differential map of corneal thickness between two measurements (with and without fluorescein) using Pentacam is identified as the pachymetry map of precorneal tear film.

**Procedure**

The right eye of each subject was scanned once with Pentacam to obtain a baseline corneal pachymetry map. Then 1 μL 0.1% fluorescein in unpreserved balance saline was softly instilled into the inferior cul-de-sac with a micropipette. Pentacam scanning was performed again after several natural blinks. During each test, the seated subject was instructed to keep both eyes open and to fixate on the center of the blue light source for the duration of the scanning. The real-time image of the subject’s eye should be quickly focused and aligned to reach the point of automatic release. To avoid interoperator confounding factors, all tests were performed by one trained operator. In addition, to avoid the influence of other tests on the measurements of tear film pachymetry map by Pentacam, tear function and slit-lamp examinations were conducted at least 1 day before Pentacam measurements or at least 1 hour later.

Internal software (version 1.16r04) in the Pentacam system could rapidly generate a color-coded pachymetry map of the precorneal tear film through comparison of two corneal pachymetry maps (with and without fluorescein) of each eye (Figs. 2–4). Tear film thickness is automatically displayed in concentric rings of 2-mm diameter increments and at any point can be evaluated by manually placing the cursor at the target point. When the precorneal tear film is covered by eyelids or eyelashes, this zone will be marked by black dots. All tear film pachymetry maps have shown zones of greater than 4-mm diameter with good quality.

**Data Analysis**

Tear film thickness at the corneal apex was evaluated, and the pattern of each pachymetry map was determined.

Data analysis was performed with statistical software (Stata 7.0; Stata Corp., College Station, TX). Two-tailed Student’s *t* test was used to analyze the differences in tear film thickness between patients with dry eye and subjects without normal eyes. Cochran-Mantel-Haenszel \( \chi^2 \) test was used to analyze the differences in patterns of tear film pachymetry maps between the two groups. \( P < 0.05 \) was considered significant.
RESULTS

Mean central tear film thickness in normal eyes was $24.7 \pm 3.9\ mu m$ (range, $17-32\ mu m$), and mean thickness in dry eyes was $22.2 \pm 4.5\ mu m$ (range, $15-30\ mu m$), which was smaller than in normal eyes (Student’s t-test, $P = 0.0614$).

In light of the spatial distribution of the overall precorneal tear film, pachymetry maps of tear film could be generally classified into three patterns: pattern 1, thickening upward (inferior tear film is thinner than superior; Fig. 2C); pattern 2, uniform distribution (thickness at inferior region is similar to superior in the uniformly distributed tear film; Fig. 3C); pattern 3, thickening downward (inferior tear film is thicker than superior; Fig. 4C).

Twenty pachymetry maps of tear film of normal eyes consisted of pattern 1 (8 maps, 40%), pattern 2 (8 maps, 40%), and pattern 3 (4 maps, 20%), whereas those of dry eyes consisted of pattern 1 (14 maps, 70%), pattern 2 (4 maps, 20%), and pattern 3 (2 maps, 10%). Dry eyes tended to have a higher proportion of pattern 1 pachymetry maps than normal eyes, though still no significant difference was found between two groups (Cochran-Mantel-Haenszel $\chi^2$ test, $P = 0.0852$).
DISCUSSION

Our study demonstrated that Pentacam could feasibly be used to map the precorneal tear film thickness in humans with the aid of fluorescein. We chose to instill 1 μL 0.1% fluorescein according to this new method because at lower concentrations it was difficult to visualize tear film with the Scheimpflug camera and at higher concentrations obvious magnification effects resulted in overestimations of tear film thickness.

The average value (24.7 μm) of central tear film thickness with the new method was among the wide range (3–40 μm) reported in published studies. However, our measured value was inconsistent with that of previously reported results, possibly because of the limitations of those methods. The classic value of 7 μm, based on early studies with invasive methods, was challenging by a reported value of 40 μm using laser interferometry. However, the difficulty in discerning the definite corresponding boundary of tear film in interferogram would lead to discrepant results gained by interferometry. In the recent past, Wang used OCT to indirectly measure tear film thickness at approximately 3 μm with the aid of rigid contact lenses, and the influence of contact lenses should be noted.

The drawback of this novel method is the requirement of fluorescein, which may cause reflex tear secretion, but none of our subjects experienced irritation after instillation of low-dose fluorescein. In addition, the possibility of a magnification effect cannot be ruled out even when applying 1 μL 0.1% fluorescein.

The difference in tear film thickness between normal eyes and dry eyes is always an interesting question. Scant literature and almost no systematic research relevant to this question exist. We tried to compare the tear film thickness of normal eyes with that of dry eyes in our study. Mean central tear film thickness in dry eyes (22.2 ± 4.5 μm) was less than in normal eyes (24.7 ± 3.9 μm), and the P value was near 0.05. Only a small difference was found between the two groups, and that could be partially explained by noncorrelation between the tear film thickness and the tear meniscus.

Tear film thickness depends on tear volume, tear surface tension, tear viscosity, and upper lid speed, and all these factors vary from each other. Previous studies using interferometry or OCT have shown considerable interindividual variability in tear film thickness. Wide interindividual variability was also found by this new method in both normal and dry eyes.

Few studies have attempted to discuss the spatial distribution of tear film on the corneal surface. According to the fundamental theory that fluorescence intensity is proportional to the sample thickness at a certain fluorescein concentration, provided fluorophotometric evidence that the tear film thickened upward. King-Smith et al. (IOVS 2003;44:ARVO E-Abstract 2476) preliminarily observed by interferometry that the inferior tear film was thinner than the superior tear film. However, the spatial variations in tear film thickness are still uncertain. The key advantage of the new method is the ability to map tear film thickness and then analyze its spatial distribution. Pachymetry maps of tear film obtained in this study primarily showed thickness variations in the vertical direction. Therefore, in light of tear film spatial variations, pachymetry maps could be classified into three patterns. We found that the tear film maps of normal eyes were composed primarily of pattern 1 (thickening upward) and pattern 2 (uniform distribution). Our finding, combined with those of Benedetto et al. and King-Smith et al. (IOVS 2003;44:ARVO E-Abstract 2476), confirmed that tear surface tension plays a more important role than gravity in the process of tear spreading after a blink. Moreover, we found pattern 1 increased to be the predominant part of tear film maps of patients with dry eyes. The higher proportion of pattern 1 could explain why the inferior cornea has a higher rate of dry spot formation and, subsequently, more frequent staining in dry eyes.

In conclusion, this new method for pachymetry mapping of tear film may provide information about tear film spatial distribution. Further studies with this new method will be helpful in our understanding of the physiologic and pathologic aspects of tear film.

References