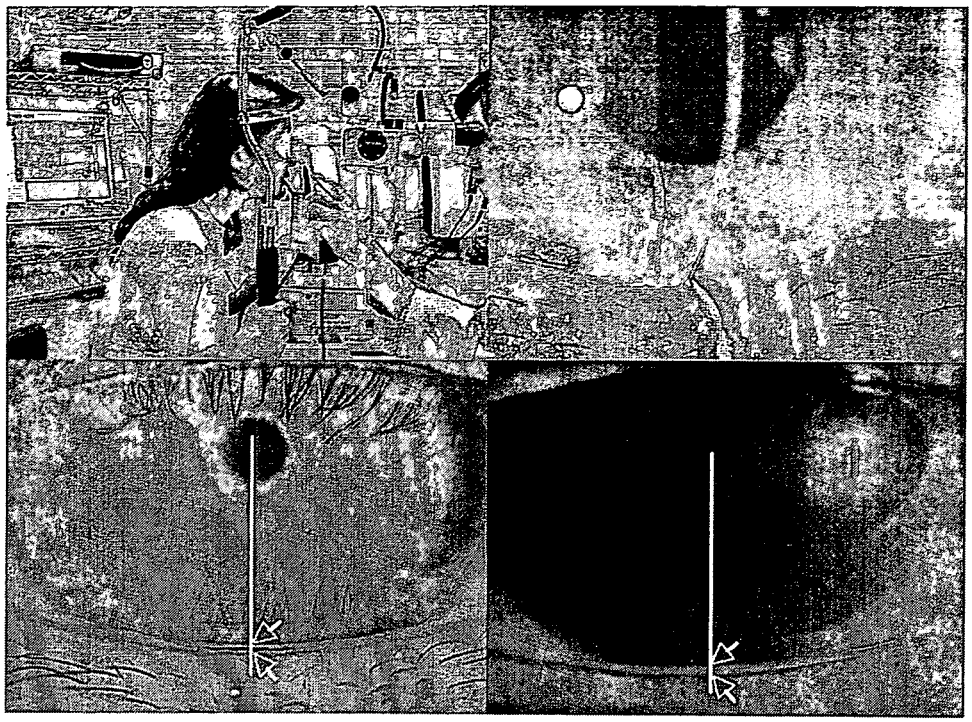

FIGURE 1. Measurement of noninvasive tear meniscus height (NI-TMH) using Tearscope Plus. (Top left) Tearscope interference device is set between the subject's eye and the slit-lamp. Precorneal tear interference image and tear meniscus interference image could be observed through the slit-lamp, and was recorded to the computer through the mounted digital video camera. Tear interference image with meniscus could also be seen on the computer screen. (Top right) Slit-lamp image of tear meniscus with diffuser light is shown. Tear meniscus of the same subject in Top left image is noninvasively visualized (Bottom left) and is also visualized with fluorescein staining (Bottom right). (Bottom left) Using image analysis software, the height of noninvasively visualized tear meniscus (between upper and lower white arrow) in central area (vertical white line) was measured. NI-TMH was quantified as 0.21 mm. Note that surface lipid layer of both tear meniscus and precorneal tear film is visualized by the tear interference device. (Bottom right) Using image analysis software, the height of fluorescein stained tear meniscus (between upper and lower white arrow) in central area (vertical white line) was measured in the same image capturing system. Fluorescein-stained tear meniscus height was quantified as 0.24 mm.

METHODS

• MEASUREMENT OF NONINVASIVE TEAR MENISCUS HEIGHT USING TEAR INTERFERENCE DEVICE: Tearscope Plus tear interference device was attached to the slit-lamp (SL130, Zeiss, Jena, Germany, magnification fixed to 12 ×, Figure 1). The tear interference image of the lower tear meniscus could be observed noninvasively when focusing at the lower lid margin. The image was captured using a high quality digital video camera (SP-321, JFC Sales Plan Co, Tokyo, Japan) attached through the beam-splitter of the slit-lamp and recorded using an image capturing system (P4m/MaxFile, P4 Medic Co, Kobe, Japan) in 720 × 480 pixels sized JPEG format. NI-TMH was measured using the ImageJ 1.32 image analysis software (National Institutes of Health, Bethesda, Maryland, USA). None of the subjects received any eye drop instillations at least six hours before the measurement.

As NI-TMH measurement with Tearscope Plus device has not been reported, it was compared concomitantly with conventional f-TMH in 31 eyes of 16 subjects. Nine eyes of five dry eye patients with SS (five females, mean age, 64 ± 8 years) and 22 eyes of 11 normal subjects (five males and six females, mean age, 34 ± 12 years) were measured. Initially, NI-TMH was measured with the Tearscope Plus tear interference device. Then, f-TMH was measured one minute after instillation of 2 μl of fluorescein solution with a micropipette. The images of NI-TMH and f-TMH were recorded and measured using exactly the same set-up as described above. The mean NI-TMH was 0.20 ± 0.09 mm, and f-TMH was 0.26 ± 0.11 mm. Images of the representative cases of NI-TMH and f-TMH are shown in Figure 1. The correlation between NI-TMH and f-TMH was also calculated with linear regression analysis. A significant correlation was found between NI-TMH and f-TMH ($r = .79$, $P < .0001$).

• SUBJECTS AND ASSESSMENT OF TEARS AND OCULAR SURFACE: We examined a consecutive series of 27 dry eye patients with SS (46 eyes, all female, mean age, 62 ± 10 years), as well as 17 normal subjects (28 eyes, all female, mean age, 52 ± 16 years). SS patients were diagnosed

FIGURE 2. Noninvasive tear meniscus height (NI-TMH) between normal subjects and dry eye with Sjögren syndrome (SS). (Left) NI-TMH of a representative normal subject (0.28 mm). (Right) NI-TMH of a representative dry eye subject with SS (0.094 mm).

FIGURE 3. Noninvasive tear meniscus height (NI-TMH) before and after punctal occlusion. (Left) NI-TMH of a representative dry eye patient with Sjögren syndrome (SS) before punctal occlusion (0.17 mm). (Right) NI-TMH after punctual occlusion of the same patient (0.56 mm).

according to the criteria of Fox and associates.[27] Among the SS patients, eyes with a Schirmer I test value less than or equal to 5 mm were included in the study as they were considered to have ATD dry eye according to the Japanese dry eye criteria.[28] Eyes with a history of punctal occlusion, conjunctivochalasis, corneal transplantation, or corneal perforation were excluded from the study. In addition, eyes with anterior blepharitis and infectious conjunctivitis were also excluded. No patients used contact lenses in this study.

NI-TMH was assessed as described above before any invasive procedure. After that, the cornea was examined by fluorescein staining. A 2-μl volume of preservative-free solution consisting of 1% fluorescein dye was applied to the conjunctival sac. The intensity of the actual fluorescein staining of the cornea such as superficial punctate keratopathy was rated from a minimum of zero to a maximum of three, in each upper, middle, and lower cornea. Thus, the maximum total staining score was 9.[29] Tear film break-up time (BUT) was measured three times, and the measurements were averaged.[29] 2 μl of preservative-free solution consisting of 1% Rose Bengal dye was then applied to the conjunctival sac. The intensity of rose bengal staining in the cornea and conjunctiva was recorded, with the maximum score rated as nine points.[30] The Schirmer I test was then performed to measure the tear secretion volume.[31] NI-TMH was compared between dry eye subjects and normal controls.

• CHANGE OF TEAR MENISCUS HEIGHT AFTER PUNCTAL OCCLUSION: All dry eye patients received treatment with non-preserved artificial tears, and 0.1% non-preserved hyaluronic acid eye drops as necessary for at least two months. These subjects who were refractory to this treatment protocol underwent punctal occlusion. NI-TMH was compared before and three weeks after punctal occlusion or punctal plug insertion for both superior and inferior puncta in 11 eyes of eight subjects in an additional interventional case series (eight females, mean age, 69 ± 8 years). Flex plugs (Eagle Vision, Memphis, Tennessee, USA) were used for punctal occlusion in three eyes of three subjects, and punctal cauterization using Optemp 2 (Alcon, Fort Worth, Texas, USA) was performed in eight eyes of five subjects. Tseng's method was performed in punctal occlusion surgery[32] and the operation was successful in all cases without re-canalization.

• STATISTICAL ANALYSIS: All data are shown as means ± standard deviation. The Mann-Whitney *U* test was applied to the comparison of NI-TMH, fluorescein staining, rose bengal staining, tear film BUT, and Schirmer I test between SS and normal subjects. Wilcoxon matched pairs

test was applied to the comparison before and after punctal occlusion at each examination. A level of $P < .05$ was accepted as statistically significant. Graphpad Instat 3.0 (Graphpad Software Inc, San Diego, California, USA) was used for statistical analysis.

RESULTS

THE MEAN NI-TMH IN NORMAL SUBJECTS WAS 0.22 ± 0.065 mm. On the contrary, it was significantly lower (0.13 ± 0.042 mm, $P < .0001$) in dry eye patients with SS. The representative cases are shown in Figure 2. Corneal fluorescein staining mean score was significantly lower (0.46 ± 0.64) in normal subjects compared to dry eye patients with SS (4.0 ± 2.1, $P < .0001$). Rose Bengal staining mean score was significantly lower in normal subjects (0.18 ± 0.48) compared to dry eye patients with SS (4.6 ± 1.8, $P < .0001$). Similarly, tear film BUT was 5.9 ± 3.0 seconds in normal subjects, and it was significantly longer than in dry eye patients with SS (2.3 ± 1.4 seconds, $P < .0001$). Schirmer I test result was 13.9 ± 9.4 mm in normal subjects, and it was significantly longer than in dry eye patients with SS (1.7 ± 1.5 mm, $P < .0001$).

Images of NI-TMH before and after punctal occlusion in the representative case are shown in Figure 3. The mean NI-TMH significantly increased from 0.12 ± 0.026 mm to 0.42 ± 0.21 mm after the punctal occlusion procedure ($P = .001$). NI-TMH was increased after both punctal cauterization or punctal plug insertion procedures. In addition, corneal fluorescein staining mean score significantly decreased from 4.5 ± 2.3 to 0.27 ± 0.65 ($P = .002$), tear film BUT was prolonged from 0.91 ± 0.30 seconds to 5.2 ± 2.8 seconds ($P = .001$) and the Schirmer I test result increased from 2.8 ± 2.0 mm to 6.8 ± 4.2 mm ($P = .005$). On the contrary, Rose Bengal staining mean score decreased, but not significantly, from 5.0 ± 1.7 to 2.5 ± 2.0 ($P = .06$).

DISCUSSION

IN THE PRESENT STUDY, USING THE TEAR INTERFERENCE device, tear meniscus was successfully visualized in a noninvasive manner in all cases. We showed that NI-TMH measurement could be as relevant as the conventional f-TMH measuring method in the diagnosis of dry eye syndromes, could differentiate between normal subjects and ATD dry eye patients with SS, and could help in the evaluation of the change of meniscus height after punctal occlusion.

NI-TMH was significantly lower in dry eye patients with SS (0.13 ± 0.042 mm) compared with normal controls, (0.22 ± 0.065 mm) along with higher fluorescein and rose bengal staining, shortened tear film BUT, and lower Schirmer I test result. After punctal occlusion, NI-TMH significantly increased from 0.12 ± 0.026 mm to 0.42 ± 0.21 mm along with the improvement of corneal fluorescein staining, tear film BUT, and Schirmer I test result. NI-TMH was increased after both punctal cauterization or punctal plug insertion procedures. We believe that NI-TMH accurately reflects the deficiency of tear volume on the ocular surface in ATD dry eye patients with SS.

The values of NI-TMH in this study are low compared with the previous studies on TMH.[9,11–15,33] The previous data related to TMH mainly measured with fluorescein dye. In this study, a significant correlation was found between NI-TMH and f-TMH, and NI-TMH was slightly lower than f-TMH. This was possibly because of the addition of a minimal amount of water added to the fluorescein dye. The other merit of the present method is visualization ability even when the TMH is very low. In a previous study, using direct observation of the TMH with the slit-lamp, Oguz and associates reported that tear meniscus could not be observed when it was too low in dry eye subjects.[10] Our method using interference phenomena could visualize clearly such low tear meniscus even in ATD dry eyes with SS (Figures 2 and 3). Furthermore, in the principle of tear interferometry, reflectance is ranged approximately from 2% to 6%.[17,18,34] Thus, tear interference image of tear meniscus could be visualized even in dry eye cases with lipid tear deficiency. Using optical coherence tomography in ATD dry eyes, Savini and associates recently reported that mean NI-TMH was significantly lower in patients with ATD dry eyes (0.13 ± 0.07 mm) than in the control group (0.25 ± 0.08 mm).[35] We considered that their results strongly support the relevance of our method.

Compared with fluorescein-stained tear meniscus observation, noninvasive tear meniscus observation using the interference device has one demerit in terms of the limitation in the observation area. As shown in the figures, this method could visualize frontal tear meniscus at a limited observation angle. To observe all lower and upper tear meniscus areas from the inner to outer canthi, we considered that the fluorescein staining method still has some advantages.

Recently, another tear meniscus measuring device to measure meniscus radius curvature has been reported by Yokoi and associates.[36,37] This noninvasive method, however, is not widely available yet, and we chose the Tearscope interference device for the evaluation of tear meniscus in this study. Furthermore, height and radius of tear meniscus have been reported to have a positive correlation by Yokoi's group.[10] Thus, we also believe that the measurement of the NI-TMH is important, as well as tear meniscus radius measurement.[38]

In this study, we compared NI-TMH of normal and dry eye patients with SS who are representing ATD dry eyes. In the future, NI-TMH measurement of the other dry eye subtypes such as non-SS dry eye, meibomian gland dys-

function,[39] or dry eye with only decreased tear film BUT[40] would be highly anticipated. Furthermore, observation of the upper NI-TMH using Tearscope in superior limbic keratoconjunctivitis,[41] or lid-wiper syndrome[42] would be also interesting.

As many clinicians are aware, the diagnosis of ATD dry eyes is sometimes difficult owing to the variability of the Schirmer I test results by its invasive nature. In the future, we expect that NI-TMH measurement by the tear interference device would become an established tear volume evaluation test such as the Schirmer I test.[31]

In conclusion, NI-TMH measurement using the tear interference device could be considered to have similar clinical relevance compared with conventional f-TMH measurement. Not only did this method evaluate tear aqueous volume noninvasively, but it could also indicate significantly lower NI-TMH in ATD dry eye patients with SS and, was useful for indicating the increase of NI-TMH after the punctal occlusion procedure. The difference of NI-TMH in normal and dry eye groups was considered to reflect the difference of tear volume, which is responsible for moistening and maintaining the ocular surface.

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Biosketch

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