



Grading reliability of the tear film viscosity examination

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Abstract

Purpose To assess the reliability of a tear film (TF) viscosity video grading system.

Methods Thirty-four dynamic TF viscosity videos were obtained by a clinically available TF analyzer and objectively sorted according to the movement speed of three arbitrary reflective light particles. A 4-grade system was constructed on a specially designed window for simultaneous comparison with the three standard videos. Two masked graders were invited to grade these videos under a randomized procedure. Observer reliabilities were determined by Spearman's correlation analysis and Bland-Altman agreement analysis.

Results For this four-grade system, the intra-observer correlation was very strong in the two graders ($\rho = 0.96$ and 0.82 ; both $P < 0.0001$). However, the inter-observer correlation showed moderate strength in normal playback speed ($\rho = 0.53$, $P = 0.002$ and $\rho = 0.52$, $P = 0.003$ for 1st and 2nd gradings, respectively). In slower playback videos, the inter-observer correlation of the two graders was higher ($\rho = 0.70$ and $P < 0.0001$) when reduced to 0.8-times playback speed. Moreover, the 0.8-times mode was also significantly better than normal playback mode ($P = 0.0204$) in terms of inter-observer agreement.

Conclusions The dynamic 4-grade system has an excellent intra-observer reliability and a good inter-observer reliability under 0.8-times playback speed. The grading system established in this study provides a promising solution for rapidly determining the level of TF viscosity.

Key messengers:

- The tear film viscosity is a dynamic parameter to evaluate homeostasis of tear film.
- We proposed a novel tear film viscosity examination to determine the dynamic homeostasis of the tear film. It can be constructed on a specially designed window with a 4-grade system.
- The tear film viscosity grading system can be adopted in routine clinical practice to assess tear film homeostasis.

Keywords Tear film viscosity · Tear film homeostasis · Dry eyes · Orthokeratology · Reliability

Meeting presentation A part of our result has been presented in the 60th Annual Meeting of the Ophthalmological Society of Taiwan in December 2019.

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Introduction

Tear film (TF) acts as a buffering interface between the outer environment and ocular surface and is essential for lubricating and protecting the ocular surface [1]. Loss of TF homeostasis is the core pathophysiological mechanism of dry eye disease (DED), which is highly prevalent and multifactorial [2]. The DED should meet a symptomatic survey (e.g., Ocular Surface Disease Index) and one of the three homeostasis markers of TF: noninvasive tear breakup time (NITBUT), TF osmolarity, and ocular surface staining [2]. However, only NIKBUT could really assess the dynamic expression of TF homeostasis,

but it assesses in an unnatural way in which the subject must forcefully keep his or her eyes open for at least 10 s. Children, in particular, often fails to complete this test. Thus, a better method is needed for evaluating TF homeostasis in children and other populations.

We previously proposed a novel model using TF viscosity examination to determine the dynamic TF homeostasis based on a commercialized TF analyzer (Keratograph® 5M, Oculus) [3]. This examination determined the TF viscosity by tracking the reflective light particles spreading on the cornea, of which the speed of reflective light particle was inversely proportional to the TF viscosity. This method was initially proposed by Owens and Phillips [4] to evaluate TF viscosity of DED and applied alternatively by Varikooty [5] to evaluate the hydrodynamics of pre-contact lens TF, both of which were assessed with a self-made slit lamp camera platform. It possessed noninvasive and dynamic evaluation properties and therefore could naturally reflect the TF homeostasis with the least interference. However, obtaining the results using this examination was a tedious process that involves decomposing videos to image frames, tracking the position of light particle for each image frame, and estimating the movement speed of light particle from these annotated coordinates. Consequently, it was preferred for research purposes over routine clinical practices.

A grading system based on a set of standard images was adopted in several clinical tests, including conjunctival bulbar redness, corneal epithelial dye staining, meibography, and tear ferning test [6–9]. These have provided objective assessment in our clinical practice for the diagnosis and follow-ups of ocular surface diseases. Hence, in order to make the TF viscosity test applicable in clinical practices, a grading approach may be a solution. However, there was no dynamic grading systems proposed previously.

Thus, we aimed to validate our newly proposed dynamic 4-grade system on TF homeostasis with the focus on myopic children and to evaluate the clinical feasibility of this test as a routine examination.

Methods

Participants

Dynamic TF videos were obtained from 34 children who participated in a prospective observational study for investigating the tear film stability in children asking for myopic assessment and treatment between December 2017 and May 2018 in Kaohsiung Chang Gung Memorial Hospital (CGMH). All procedures involving human participants adhered to the tenets of the Declaration of Helsinki and the ARVO statement on human subjects. Institutional review board/ethics committee approval (No. 201701393B0) was obtained from the

committee of medical ethics and human experiments of CGMH. These participants were healthy individuals aged from 6 to 15 years old without ocular or systemic diseases. All participants and their legal representatives were clearly informed about the aim and procedure of this study and signed the informed consent. Each subject received complete ocular examination and dynamic TF examinations by masked examiners.

Examination of the dynamic TF viscosity

The right eye of each subject was selected for the TF viscosity examination. Before the examination, each subject was instructed to blink naturally during examination. With a TF analyzer (Keratograph® 5M, Oculus), the moving reflective light particles were observed under two illumination sources of white diode. At the beginning of this examination, the examiner focused on the anterior corneal surface under $\times 1.4$ magnification to make mobile reflective light particles clearly visible on the TF. The recorder was then started to obtain a dynamic TF viscosity video. Generally, three to four blink cycles were recorded in a 10-s video record.

Determination of the objective moving speed of reflective light particles

The movement speeds of reflective light particles were determined according to our previous study [3]. In brief, the first qualified blink cycle was adopted for assessment, and the trajectories of particles were analyzed by decomposed image frames with a time interval of 0.1 s. The qualified sequence was defined as clearly visible TF particles under stable and undistorted superior and inferior reflective ellipses. By tracking three reflective light particles on the cornea starting from opening cycle of the qualified blink for 1 s, the movement speeds of three reflective light particles were averaged to obtain the momentary movement speed (MMS) of each subject. A power-law fitting operation $MMS(t) = \alpha \times t^{-\beta}$ was used to obtain the objective movement speed of reflective light particles, MMS (0.5), for each subject. The 34 videos from 34 subjects were then sorted from fastest to slowest objective movement speed for the purpose of establishing the subjective grading system in the section below.

Subjective grading system for the TF viscosity examination

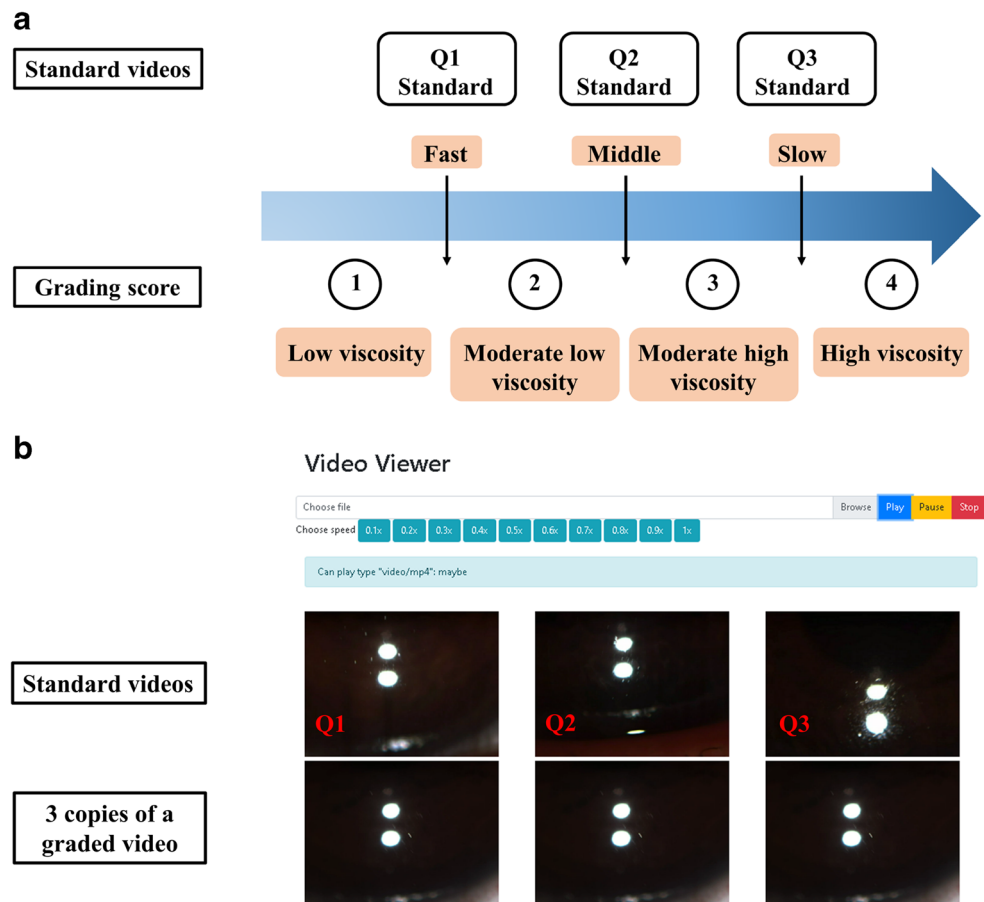
The first, second, and third quartiles (Q1, Q2, and Q3) of the sorted videos were adopted as standard videos for comparison during subjective grading. This 4-grade system was constructed on a specially designed window environment (application video viewer in the Supplementary file 1 and instructions for use in the Supplementary file 2) for simultaneous comparison

(demonstration in the Supplementary file 3) with the three standard videos (Supplementary file 4–6). Subjects were labeled as grades 1, 2, and 3 (lowest TF viscosity) if their particles moved faster than that of the Q1, Q2, and Q3 videos, respectively. Subjects were labeled as grade 4 if their particles moved slower than the Q3 (Fig. 1). The remaining videos ($n = 31$) were rearranged under a randomized procedure. After instructing the above grading rule and establishing the grading environment, two masked graders who were novel to this study were recruited to determine the grading of TF viscosity for each subject. After 2 weeks, the videos were regraded by the same masked graders after rearrangement of the video sequence under the same randomized procedure. In addition, the two graders were asked to regrade the same videos under slower playback speeds of 0.8-, 0.5-, and 0.4-times after the same rearrangement procedure on 4th–6th, 8th–10th, and 12th–14th weeks, respectively.

Statistical analysis

The objective movement speeds of reflective light particles, MMS (0.5), were estimated by the Microsoft Excel 2016, where the add-in program, Solver, was used to perform the power-law fitting and extract the model parameters α and β .

Fig. 1 A schematic diagram for illustrating a tear film viscosity grading system. **a** The level of tear film viscosity was determined by the movement speed of tear film particles. **b** The window environment for grading. The Q1, Q2, and Q3 videos were videos for grading standards, which represented tear film with fast-, middle-, and slow-moving particles. Subjects were labeled as grades 1, 2, and 3 (lowest TF viscosity) if their particles move faster than that of the Q1, Q2, and Q3 videos, respectively. Subjects were labeled as grade 4 if their particles moved slower than that of the Q3



The free online statistical software (Social Science Statistics; <https://www.socscistatistics.com/>) was used to calculate Spearman’s correlation coefficient ρ , which revealed the intra- and inter-observer reliabilities by correlating the grading scores of the same video for each subject. In addition, the Bland-Altman plot was used to determine the mean deviation and 95% limits of agreement (LoA) between two observers. We chose the Bland-Altman plot to directly visualize both the magnitude of bias and 95% limits of agreement between the two methods. The free online software GraphPad QuickCalcs was used to perform Fisher exact test for determining the statistical improvement of intra- and inter-observer grading concordance in lower playback speeds. $P < 0.05$ was recognized statistically significant.

Result

The ocular surface performance of the participants

The 34 dynamic TF viscosity videos were obtained from 34 children. The basic characteristics and representative ocular surface parameters of the participant are shown in Table 1. There were no significant differences between boys and girls

Table 1 Demographic data of pediatric subjects

Characteristics of subjects	
Number of subjects (<i>n</i>)	34
Age (year)	12.1 ± 2.9
Male (%)	44.1
Tear meniscus height (mm)	
Nasal meniscus	0.33 ± 0.07
Central meniscus	0.24 ± 0.05
Temporal meniscus	0.33 ± 0.07
Bulbar redness (a.u.)	
BN score	0.68 ± 0.43
BT score	0.72 ± 0.29
LN score	0.32 ± 0.25
LT score	0.25 ± 0.24
Mean redness score	0.72 ± 0.27
Assessable area	
Assessable area	8.58 ± 3.13
Tear breakup time (s)	
NIK BUT first	10.39 ± 6.8
NIK BUT avg	13.69 ± 6.00
Assessable time	21.07 ± 5.39

a.u., arbitrary unit; *BN*, bulbar nasal; *BT*, bulbar temporal; *LN*, limbal nasal; *LT*, limbal temporal; *NIK BUT first*, first noninvasive keratograph breakup time; *NIK BUT avg*, average noninvasive keratograph breakup time

in the tear meniscus height, bulbar redness, and tear breakup time. In addition, there was no correlation among age and the ocular surface parameters of tear meniscus height, bulbar redness, and tear breakup time.

Intra-observer reliability in normal playback speed of the TF viscosity video

After eliciting three videos as the grading standards, the Spearman correlation coefficients between first grading scores and second grading scores of 31 remaining TF viscosity videos of both graders ($\rho = 0.96$, P value < 0.001 for grader no. 1, and $\rho = 0.82$, p value < 0.001 for grader no. 2, respectively) were very strong (Fig. 2a, b). All videos were graded within the upper and lower limits of the Bland-Altman plot, and therefore, a high intra-observer agreement was concluded (Fig. 2c, d). Although both graders had good intra-observer reliability, they had discrepant grading performance. Grader no. 1 was apparently more consistent than grader no. 2.

Inter-observer reliability in normal playback speed of the TF viscosity video

For the 31 TF viscosity videos, the grading scores obtained from the two graders showed significantly positive correlation with Spearman's correlation coefficient and good inter-observer agreement with Bland-Altman plot (Fig. 3).

Fig. 2 The intra-observer reliability of the tear film viscosity grading system at normal playback speed. **a, b** The Spearman correlation between the 1st grading and 2nd grading of graders no. 1 and 2, respectively. **c, d** The agreement analysis based on the Bland-Altman difference plot between the 1st grading and 2nd grading of graders no. 1 and 2, respectively. The central horizontal dash line represented the mean deviation (MD). The upper and lower dash lines represented the 95% limits of agreement (LoA). The numbers in the graph were overlapping of gradings

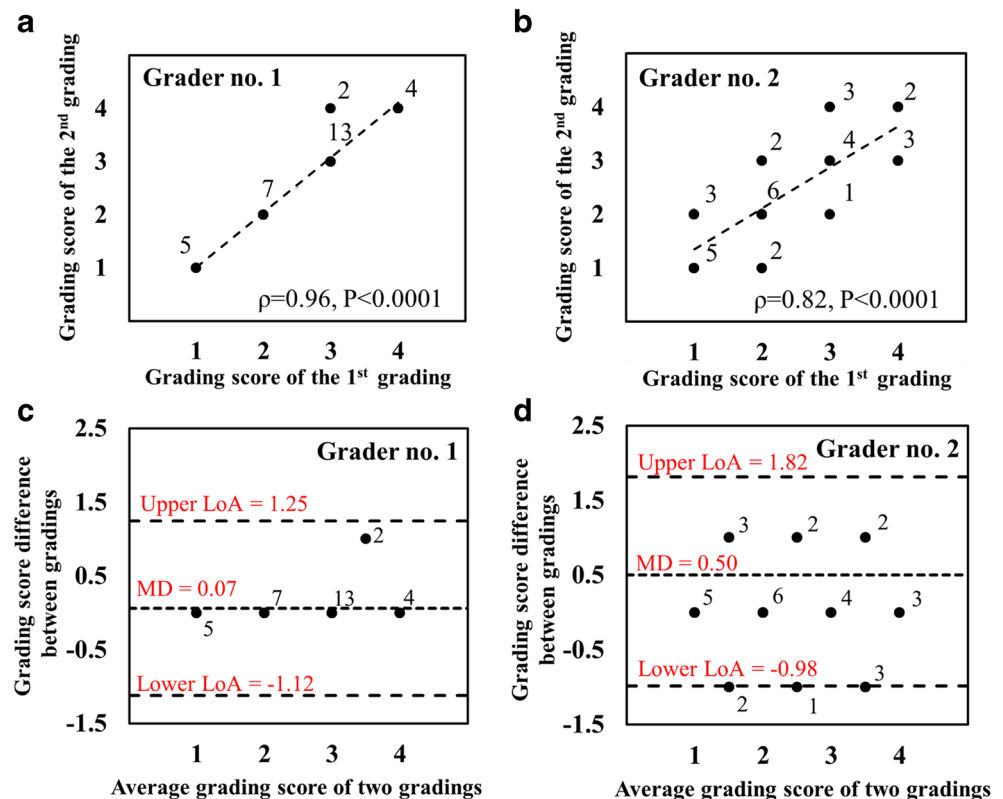
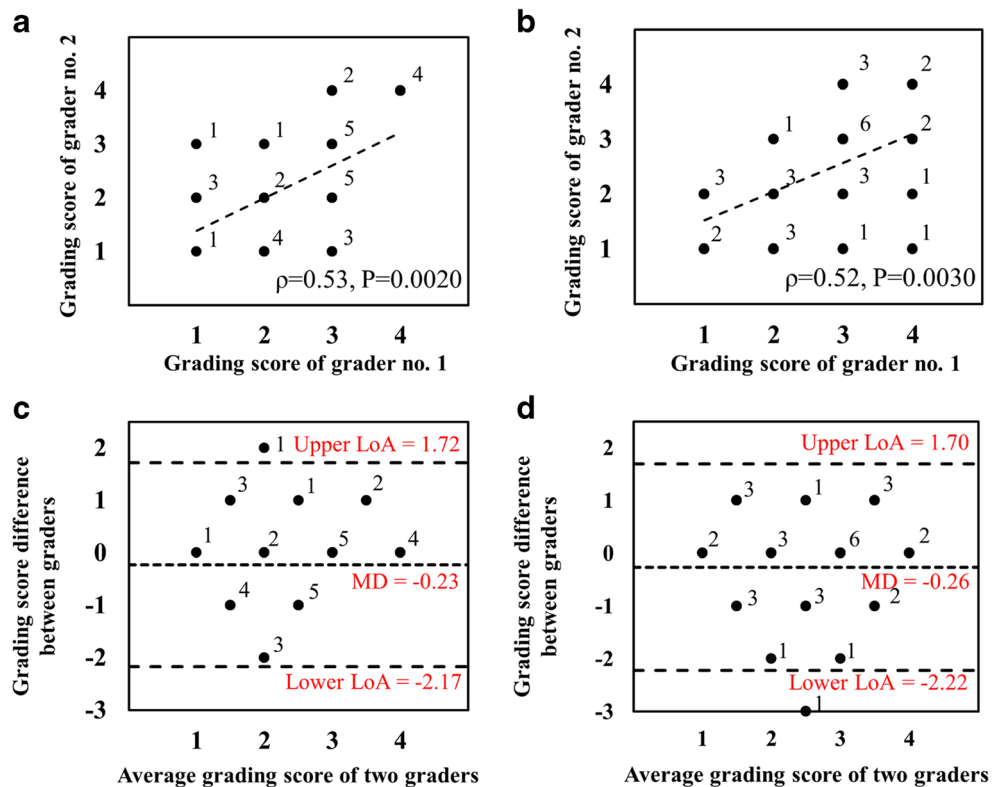


Fig. 3 The inter-observer reliability of the tear film viscosity grading system at normal playback speed. **a, b** The Spearman correlation between graders no. 1 and 2 of the 1st grading and 2nd grading, respectively. **c, d** The Bland-Altman analysis between graders no. 1 and 2 of the 1st grading and 2nd grading, respectively. The numbers in the graph were overlapping of gradings



However, the inter-grader correlation was moderate and consistent in the first and second gradings ($\rho = 0.53$ and 0.52 , respectively) in the normal playback speed (Fig. 3a, b). Only one video was outside the agreement limits of the Bland-Altman plot in the first and second gradings (Fig. 3c, d).

Intra- and inter-observer reliabilities in slower playback speed of the TF viscosity video

Intra- and inter-observer reliabilities were reassessed by using slower playback modes. For the intra-observer correlation, the grading by grader no. 1 did not change at slower playback speeds (Fig. 4a, d, g), while that of grader no. 2 had stronger correlation at 0.8-times but was decreased at 0.5-times and 0.4-times playback speeds (Fig. 4b, e, h). The inter-observer correlation of the two graders was increased at 0.8- and 0.5-times playback speeds but decreased at 0.4-times playback speed (Fig. 4c, f, i).

Although there was no significant intra-observer difference in different playback modes, grader no. 1 showed a relation between decreased playback speeding and increased consistency, of which the absolute values of MD approached zero and the amplitudes of LoA were less than that of the normal playback mode (Fig. 5). As for grader no. 2, the intra-observer agreement was significantly increased ($P = 0.0485$) at the 0.8-times mode, whereas no significant improvement was reached at both the 0.5-times and 0.4-times modes. Moreover, the inter-observer agreement was also significantly improved (P

$= 0.0204$) in the 0.8-times mode instead of the two slower modes. Therefore, the 0.8-times playback mode had the best intra- and inter-observer reliability and is recommended as a routine grading setting for this video grading system.

Discussion

Currently available TF tests cannot guarantee a comprehensive determination for TF homeostasis. In addition, present modalities for monitoring TF homeostasis may not be suitable for children due to their poor cooperation during tear breakup time measurements and tear collection for osmolality analysis. Consequently, we proposed a 4-grade system to rapidly determine the level of TF viscosity of a subject by an examiner through the comparison of TF particles with three standard videos under a specially designed window environment. We found this novel grading system to be highly reliable in both intra- and inter-observer tests especially under 0.8-times playback mode, of which we recommend for routine clinical practice.

We found discrepant performance between graders using intra-observer reliability test (Fig. 2). Tatsuto et al. found that visual motion perception is different in each individual [10]. There are some factors that may influence motion perception. For example, the attention and age would influence motion perception. Massimo et al. revealed that the perceived speed of moving object is directly proportional to a person’s visual attention [11]. Moreover, previous studies showed that the

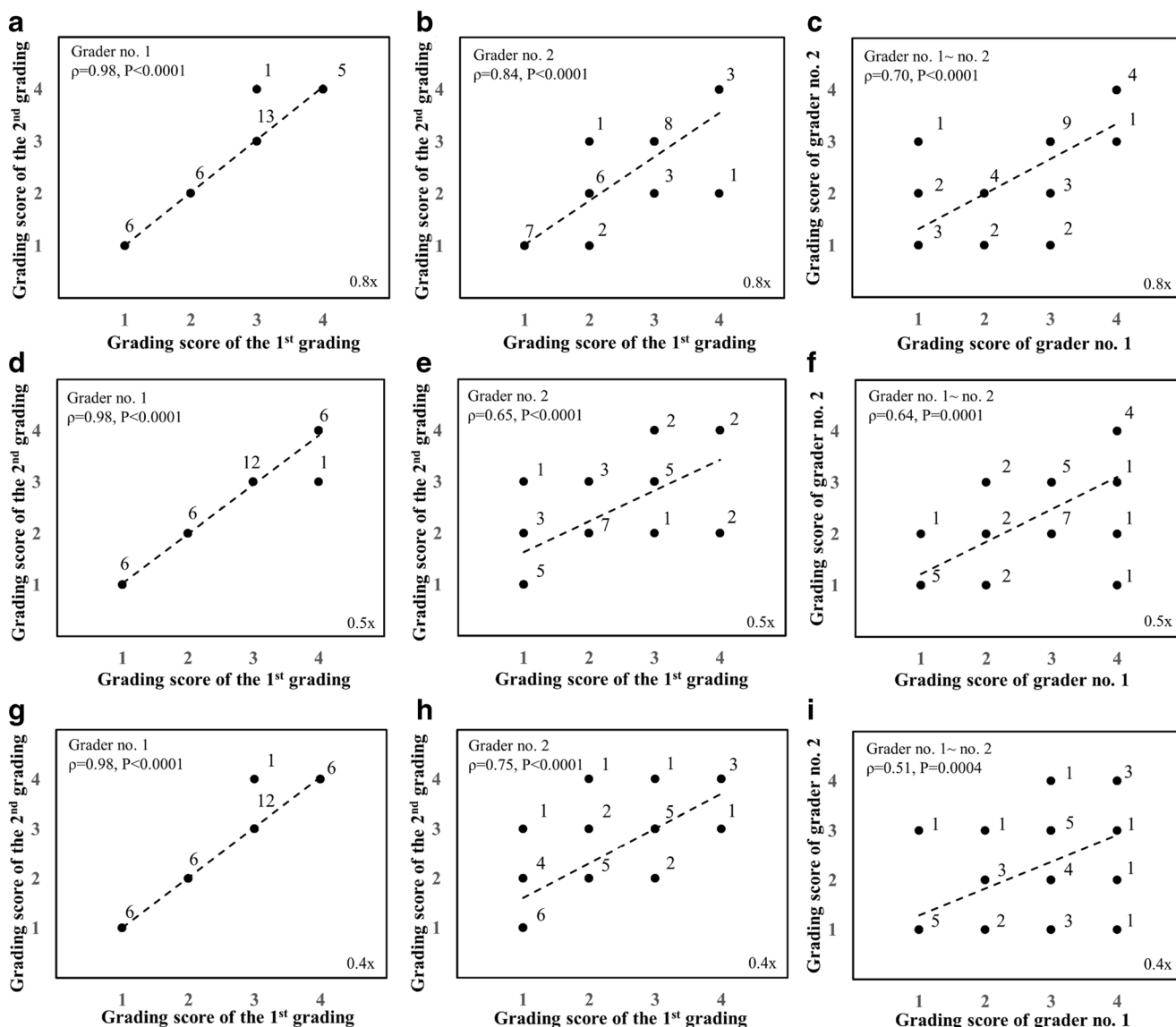


Fig. 4 The intra- and inter-observer correlations of the tear film viscosity grading system at slower playback speeds. **a, d, g** The intra-observer correlations of grader no. 1 were respectively evaluated at the 0.8-, 0.5-, and 0.4-times playback speeds. **b, e, h** The intra-observer correlations of

grader no. 2 were determined respectively at the same three playback modes. **c, f, i** The inter-observer correlations for the two graders were estimated under the same three modes

ability to detect fast visual motion develops earlier in life than that of the slower visual motion [12]. The state of birth also affects the processing of visual information. The prospective control study showed that pre-term infants had poor visual perception [13]. The above evidences may be the reasons that two graders have discrepant intra-observer reliabilities in this video grading system, which may also lead to the moderate inter-observer reliability (Fig. 3). Though the inter-observer correlation showed moderate strength in normal playback speed, we could improve the strength from moderate to strong using slower playback speed of 0.8-times. We could utilize the slower playback function in this specially designed window environment to advance the inter-observer reliability to make this method more clinically dependable and available.

To minimize the difference of visual motion perception in graders, we compared the grading reliabilities of the two graders in decelerated video. Grader no. 1 had very strong intra-observer reliability and the correlation was slightly increased when the playback speed was reduced (Fig. 4a, d, g). However, grader no. 2 had inconsistent intra-grader reliabilities, which were improved in 0.8-times playback modes (Fig. 4b) but worse in 0.5- and 0.4-times playback modes (Fig. 4e–h). The possible reason is that the decrease in video speed is associated with worsening of video resolution, resulting in blurred light particles that are more difficult to track. Above all, clinically, we could do the repeatability analysis first to find better graders to perform this examination and use slower playback speeds of 0.8-times to advance inter-observer reliability.

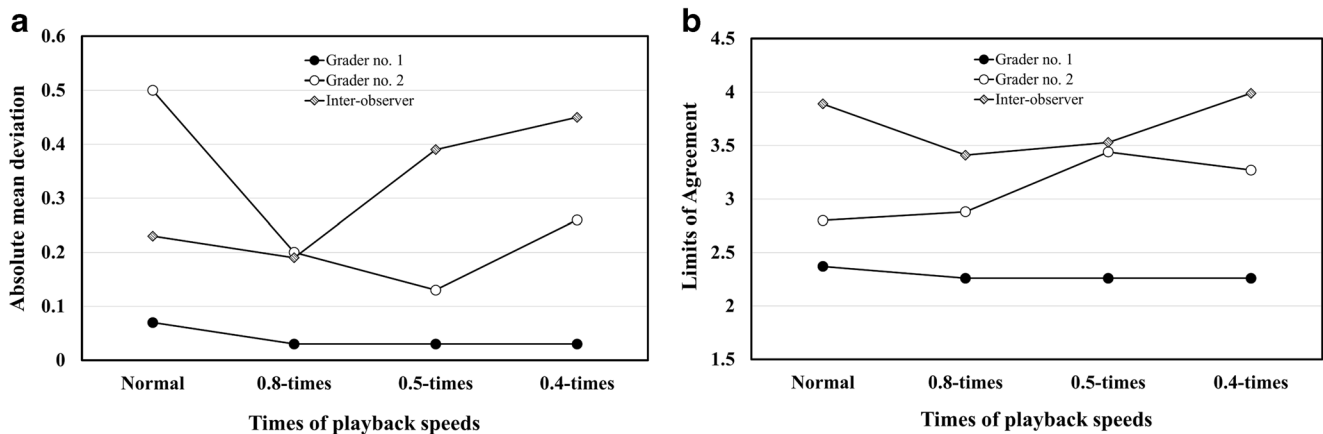


Fig. 5 The Bland-Altman analysis for intra- and inter-observer agreements of the tear film viscosity grading system at different playback speeds. **a** The absolute value of mean deviation of intra- and inter-observer agreement at the 1.0-, 0.8-, 0.5-, and 0.4-times playback speeds. **b** The amplitudes of 95% limits of agreements of intra- and inter-observer

The reliability of this dynamic grading system was highly dependent on each individual's visual motion perception, which possibly led to discrepancies in intra- and inter-observer agreements. However, both intra- and inter-observer agreements improved under 0.8-times playback speed. For graders with weaker visual motion perception, the specially designed window environment could provide a customized adjustment to improve their grading reliability. There was currently no agreement on the average movement speed of light particles to represent TF viscosity. The MMS (0.5) was used as the standard light particle speed in this study for sorting all TF viscosity videos and selecting the three standard videos to determine the grading of TF viscosity in orthokeratology wearers. For subjects with other ocular surface disease, such as DED, the optimal speed of TF viscosity videos may need to be adjusted accordingly. However, this grading system provides a highly flexible environment to adopt new standard videos for grading subjects with different ocular surface disorders.

Diagnosing dry eye disease or evaluating TF homeostasis in children is particularly challenging due to children's poor cooperation during examinations [14]. Thus, a novel and clinically feasible technique to easily evaluate TF homeostasis is needed for children with myopia for pre-treatment assessment and long-term monitoring. Our newly proposed grading system can rapidly determine the level of TF viscosity. We believe this system can assist eye care practitioners in the assessment of TF homeostasis for myopic children in routine clinical settings.

One of the potential limitations is that we chose the right eye of each subject for the TF viscosity examination in this study. Many studies investigating dry eye disease chose unilateral eye for analysis and drew conclusions based on monocular results [15, 16]. It may be argued that the evaluation of only one eye per subject may result in a potential waste of information or cause a bias due to non-random selection. However, inclusion of both eyes may also cause overlapping results due to high

similarity of both eyes in many cases [17]. We previously found that the assessment of tear film stability of the first eye could influence that of the fellow eye [18]. Thus, we ultimately decided to choose the right eye, which is the first eye that we routinely examine. Second, the participants were all children aged from 6 to 15 years, which may have different TF presentation compared to the adults. Third, the participants were all children for myopic assessment and treatment, which results in selection bias. In conclusion, the grading system established in this study provides a promising solution for rapidly determining the level of TF viscosity in myopic children necessitating interventions. This system has an excellent intra-observer reliability and a good inter-observer reliability under 0.8-times playback speed. We believe it can be used as one of the routine examinations for assessing the TF homeostasis in myopic children.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00417-021-05176-2>.

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Author contribution Conceptualization, MTK; methodology, HYL and MTK; validation, PCF and AC; formal analysis, HYL; investigation, HYL and PCF; resources, PCF and MTK; data curation, HYL; writing—original draft preparation, HYL; writing—review and editing, MTK and AC; visualization, HYL and AC; supervision, MTK; project administration, MTK; funding acquisition, MTK

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Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Code availability Not applicable.

Declarations

Ethics approval Institutional review board/ethics committee approval (no. 201701393B0) was obtained from the committee of medical ethics and human experiments of CGMH.

Consent to participate All participants and their legal representatives were clearly informed about the aim and procedure of this study and signed the informed consent.

Consent for publication All authors have read and agreed to the published version of the manuscript.

Conflict of interest The authors declare no competing interests.

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