Contact Lens and Anterior Eye xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability

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ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 22 December 2016
Accepted 11 January 2017

Keywords:
Tear film
Tear film stability
Fluorescein
Break-up time

ABSTRACT

Purpose: To compare clinical tear film break-up time measurements obtained non-invasively, with those measured following minimal and conventional volumes of fluorescein instillation.
Methods: Forty-one subjects (20 male, 21 female, mean ± SD age 34 ± 11 years), with or without dry eye, participated in a prospective cross-over study. Tear film break-up time was measured by the Tearscope Plus™ with fine grid insert. Measurements were made in triplicate, with no fluorescein instillation (NIBUT), then following application of a minimal volume of 1 μl fluorescein from the Dry Eye Test™ (mTBUT), and finally with 15–30 μl of fluid instilled via a conventional fluorescein strip (TBUT). A fifteen-minute interval between each set of measurements minimised the risk of residual contamination effects.
Results: All three techniques displayed statistically significant pairwise correlation (all $p < 0.001$). TBUT values were significantly shorter than both NIBUT (geometric mean 8.6 s versus 10.9s, $p = 0.03$) and mTBUT (geometric mean 8.6 s versus 10.6s, $p = 0.03$), and demonstrated narrower spread (both $p < 0.05$). No significant differences were detected between NIBUT and mTBUT (all $p > 0.05$).
Conclusions: Tear film break-up time values measured with conventional fluorescein instillation were shortened, while minimal fluorescein instillation and non-invasive methods produced comparable readings. This suggests that minimising instilled volumes can reduce the impact of fluorescein on clinical measurements of tear film stability.

1. Introduction

Tear film stability assessment is an essential component of the diagnosis and evaluation of dry eye [1,2]. It can be measured by the tear film break-up time, defined as the interval between a blink and the first occurrence of dry spots on the cornea [3]. Tear film stability is generally reduced irrespective of the aetiology of dry eye [4].

Tear film stability is commonly measured in the clinical setting with the fluorescein break-up time test [5–7]. Instilled aqueous fluorescein sodium enhances tear film visibility under blue light, thus simplifying the measurement [8]. However, conventional saline-wetted fluorescein-impregnated strips deliver a variable volume of fluid, typically around 15–30 μl, and in excess of the natural tear volume, which can destabilise the tear film [9,10]. Shaking off excess fluid from wetted fluorescein strips prior to application can reduce the volume instilled and improve the resulting visualisation [8].

Non-invasive measurement techniques using reflected mires to facilitate observation of tear break-up are considered to be superior, by avoiding the presumed destabilising action of fluorescein. Non-invasive measurements have previously been reported to be significantly greater than conventional fluorescein break-up times measured from the same tear film [9,10]. However, the conventional fluorescein test remains widely used clinically [5–7], largely on account of its relative convenience of measurement, ease of interpretation and low cost, relative to that perceived to exist with non-invasive instrumentation.

The Dry Eye Test™ (Amcon Laboratories, St Louis, MO, USA) provides a modified form of tear stability assessment, using a proprietary strip design that delivers consistently smaller volumes (1 μl) of instilled fluorescein than conventional strips. The Dry Eye Test has previously been reported to have greater measurement reliability and precision than conventional fluorescein strips [11], however its comparability with non-invasive techniques is not currently known.

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http://dx.doi.org/10.1016/j.clae.2017.01.004
1367-0484/

This crossover study assessed the invasiveness of Dry Eye Test relative to a non-invasive technique and the conventional fluorescein test, in the clinical setting, and investigates whether minimising the instillation volume of aqueous fluorescein can diminish the level of tear film disruption.

2. Methods

2.1. Subjects

The prospective crossover study followed the tenets of the Declaration of Helsinki and was approved by the institutional Human Participants Ethics Committee. Participants were required to be between 18 and 40 years of age, non-contact lens users, with no history of major systemic or ocular disease (other than dry eye), no previous ocular surgery, and no topical or systemic medications affecting the eye. Eligible participants were enrolled after providing written informed consent.

A total of 41 eligible participants (20 male, 21 female), with a mean ± SD age of 34 ± 11 years, were recruited. This exceeded the sample size requirement for the desired study power. Power calculations showed that a minimum of 33 participants was required, to detect a clinically significant difference of 5 s, with 80% power ($\beta = 0.2$), at a two-sided statistical significance level of 5% ($\alpha = 0.05$). The SD of normal values was estimated to be at 7 s [12]. Sample size estimates were determined using a uniform non-parametric adjustment, with PASS 2002 (NCSS Statistical Software LLC, Utah, USA).

2.2. Measurements

All subjects were assessed in the same location, with a mean ± SD room temperature of 22.0 ± 0.5 °C and a mean ± SD relative humidity of 49.0 ± 6.0%. Measurements were conducted by a trained research technician and therapeutically qualified optometrist.

Tear film stability measurements were performed on the right eye of each participant, using the Tearscope Plus™ (Keeler Ltd, UK), with fine grid insert in place, in all cases [13]. The tear film break-up time was measured in the same order for each subject under three different clincial scenarios: non-invasively with no fluorescein instillation (NIBUT); following application of a minimal volume of approximately 1 μl fluorescein from the Dry Eye Test (mTBUT); and following instillation of fluorescein in 1 drop (15–30 μl) of saline from a conventional (Haag-Streit, UK) fluorescein strip (TBUT), each applied according to the respective manufacturer's instructions, with excess fluid shaken off prior to application. The fluorescein was applied to the superior-temporal bulbar conjunctiva while participants were instructed to look infero-nasally.

Participants were instructed to blink naturally for 1 min to facilitate even distribution of fluorescein over the ocular surface. Subjects were then instructed to refrain from blinking while the examiner observed the reflected grid pattern. The tear film break-up time was recorded as the time between the blink and the first sign of distortion in the grid pattern. A mean of three measurements was recorded for each technique. Fifteen minutes were allowed to elapse between each set of measurements to minimise the risk of residual contamination effects.

2.3. Statistical analysis

Statistical analyses were performed using Graph Pad Prism version 6.02 (http://www.graphpad.com) and IBM SPSS Statistics for Windows version 19.0. The distributions of tear film stability measurements were assessed using the D'Agostino-Pearson omnibus normality test. Consistent with previous reports, tear film stability measurements were non-normally distributed [14–16], and thus both the geometric mean and the median are presented. The non-normally distributed measurements were then logarithmically transformed before further analysis. Comparisons of means were performed using repeated measures analysis of variance (ANOVA). Post-hoc analyses for pairwise comparisons were conducted using multiplicity adjusted Tukey tests. Comparisons for variances were undertaken using the *F*-test. For each pairwise comparison, the intra-class correlation co-efficient (ICC) was calculated, and Bland-Altman analysis performed [17]. All tests were two-tailed and $p < 0.05$ was considered significant.

3. Results

Tear film stability data from all three techniques were positively skewed, and failed normality testing (all $p < 0.001$). The distributions of tear film break-up time measurements obtained from the three techniques are illustrated in Fig. 1 and Table 1, and pairwise analyses are shown in Table 2.

Following logarithmic transformation, the distribution of values from each technique did not differ significantly from normal distributions (all $p > 0.05$). Significant differences were detected between the measurements obtained from the three techniques ($p = 0.01$). Post-hoc analysis showed that both NIBUT and mTBUT measurements were significantly longer than TBUT (all $p < 0.05$), while there were no significant differences between NIBUT and mTBUT ($p = 0.84$). Both NIBUT and mTBUT measurements demonstrated a larger spread than those of TBUT (all $p < 0.05$), although NIBUT and mTBUT did not differ significantly ($p = 0.87$).

In all three pairwise analyses, the stability techniques were found to be significantly correlated (all $p < 0.001$). The intraclass correlation coefficient was greater for NIBUT versus mTBUT, than for mTBUT versus TBUT, and NIBUT versus mTBUT. Bland-Altman analysis also demonstrated a smaller bias and narrower limits of agreements for NIBUT versus mTBUT (Fig. 2), than the other two pairwise comparisons (Figs. 3 and 4). Adjusting the Bland-Altman mean biases to the pre-transformed equivalents, NIBUT values were on average 1.04 times that of mTBUT, while mTBUT was 1.22 times that of TBUT, and NIBUT was 1.26 times that of TBUT.

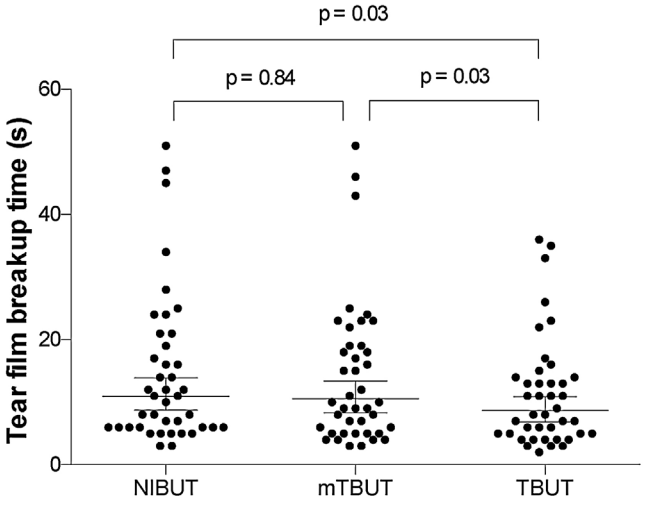

Fig. 1. The distribution of tear film break-up time measurements obtained from the non-invasive (NIBUT), minimal (mTBUT) and conventional (TBUT) fluorescein instillation techniques. Each point represents the tear film break-up time measurement of an individual eye. Bars represent the geometric mean and 95% confidence interval.

Please cite this article in press as: J.K. Mooi, et al., Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability, Contact Lens & Anterior Eye (2017), http://dx.doi.org/10.1016/j.clae.2017.01.004