SOUTH-EAST EUROPEAN JOURNAL of OPHTHALMOLOGY 2018; 1 (1) 41–8

Non-invasive tear film tests in post-keratoplasty eyes

Ágnes Füst, László Imre, Zoltán Zsolt Nagy

Department of Ophthalmology, Semmelweis University

Keywords
break-up time, keratoplasty, lipid interferometry, tear film

Abstract

Purpose: To examine the tear film in post-keratoplasty eyes with non-invasive methods and compare it with normal eyes.
Methods: Three consecutive readings were carried out with the following three tests each: non-invasive break-up time measurement, lipid layer interferometry and tear meniscus test using Polaris tearscope (bonOptic Vertriebsges. mbH). A control group (14 eyes) and 3 post-keratoplasty groups - patients after Descemet stripping and automated endothelial keratoplasty (4 eyes), patients after perforating keratoplasty sutures out (8 eyes) and patients after perforating keratoplasty sutures in (19 eyes) – were examined.
Results: The intraclass correlation of the three non-invasive break-up time measurements exceeded 0.8 in each group. There was no significant difference in the non-invasive break-up time values between the Descemet stripping and automated endothelial keratoplasty (15.6±9.7 sec) and the perforating keratoplasty sutures out (22.8 8.6 sec) groups as compared to the control group (22.0±7.6 sec), however, the respective value was significantly lower in the perforating keratoplasty sutures in group (11.4±8.9 sec) ($p=0.002$). The proportion of eyes with thin lipid layer pattern was significantly higher in the perforating keratoplasty sutures in group than in the control group.
Conclusion: In the present study the non-invasive tear film break-up time measurements had good intrasession repeatability in post-keratoplasty eyes. The tear film stability was decreased and the lipid layer was thinner in a higher proportion of post-keratoplasty eyes with sutures in place.

Corresponding author: Ágnes Füst, email: fustagi@yahoo.com,
Address: Semmelweis University, Department of Ophthalmology, Üllői út 26. 1085, Budapest, Hungary.
Phone: +36 20 8258457.

Introduction

The stable tear film is one of the most important requirements for clear vision. Tear film stability can be characterized with the break-up time. Traditionally break-up time (BUT) is measured by staining the tear film with sodium fluorescein dye and measuring the time until the first black spot appears. However, BUT has some limitations, e.g. it uses a foreign material, the fluorescein dye, which changes the original composition of the tear, and its reproducibility is low (1–4). With the use of non-invasive tear break-up time (NIBUT) tests these disadvantages can be overcome. During NIBUT measurement "a regular image of the reflected target indicates a stable tear film and the time in seconds from the last blink to the appearance of the first discontinuity or break in the reflected image is recorded" (5). NIBUT is longer than fluorescein break-up time and it is considered to have higher repeatability and precision (6, 7).

During perforating keratoplasty (PK) and deep anterior keratoplasty (DALK) the original corneal surface is replaced by the graft's surface. The regularity of the new corneal surface and the tear film over it has great influence on the postoperative best corrected visual acuity (BCVA) (8). The stability of the tear film has importance in maintaining a healthy corneal epithelium as well.

In the present study the purpose was to examine the tear film in post-keratoplasty eyes with non-invasive methods such as the NIBUT test, the lipid layer interferometry and tear meniscus test. The objective was to evaluate the repeatability of the NIBUT measurements and to investigate whether the results obtained with these measurements are comparable with normal findings.

Methods

The observational case-control cross sectional study was performed in Department of Ophthalmology, Semmelweis University in October and November 2016.

Patients

Three groups included patients who had undergone keratoplasty earlier: one with patients after Descemet stripping and automated endothelial keratoplasty (DSAEK group), one with patients after perforating keratoplasty, in whom the sutures had already been removed (PKP suture-out (PKPso) group) and one with patients after perforating keratoplasty, in whom the sutures were not removed (PKP suture-in (PKPsi) group). The donor graft size varied between 7.5 and 10.0 millimetres. Indications for keratoplasty were Fuchs dystrophy, pseudophakic bullous keratopathy, infectious non-healing corneal ulcer, stromal dystrophy, corneal scar and iridocorneal endothelial syndrome. Uncorrected and best corrected visual acuity test, refraction and slit lamp examination was performed in each patient. The inclusion criteria were the following: age over 18 years, at least 3 months after surgery, eyes without any postoperative complication, clear graft and apparently smooth surface on slit lamp examination. Patients with known dry eye or dry eye symptoms and patients with blepharitis were excluded. The control group consisted of people without any eye disease involving the ocular surface.

Demographic data are shown in *Table 1*.

Table 1. Demographic data of participants in the different groups

	Control	PKPsi	PKPso	DSAEK
No of eyes (patients)	14(14)	19(17)	8(8)	4(4)
Age: years, mean±SD	52.1±14.8	61.8±19.3	53.5±16.9	71.5±5.0
Time after surgery: months, (mean, min-max)	–	12.2 (3-30)	48.1 (16-125)	15.3 (3-36)

Tear film assessment

The tear film test was performed using the Polaris tearscope (bonOptic Vertriebsges. mbH) attached to a Haag Streit slit lamp. All examinations were performed by the same examiner.

Non-invasive tear break-up time: Three consecutive NIBUT measurements were conducted using the grid insert of the Polaris tearscope. The grid was projected onto the corneal surface and tear break-up was represented as discontinuity to any line in the grid's image. The time between the last blink and the first appearance of a break in any gridline was measured. If break-up didn't occur in 30 seconds then it was recorded accordingly (i.e. 30 seconds). We didn't extend the measurement any further as according to literature data the normal range of the NIBUT measured with Tearscope is between 5.0 s and 30.8 s (9), and the Polaris equipment works on the same principles as Tearscope.

Test repeatability was determined statistically in all patients and in each of the four groups separately.

The NIBUT results in each postoperative group were compared to that of the control group.

Correlation of NIBUT values to BCVA was also calculated in the four groups separately. In this calculation only those cases were included where no other disease resulting in impaired visual acuity was present.

Lipid layer interferometry pattern: In line with the classification recommended by Guillon the patterns seen were classified into one of the 5 different categories (10): 1: open meshwork, 2: closed meshwork, 3: wave, 4: amorphous, and 5: colour fringes pattern. The thicker the lipid layer the higher the scores were. The

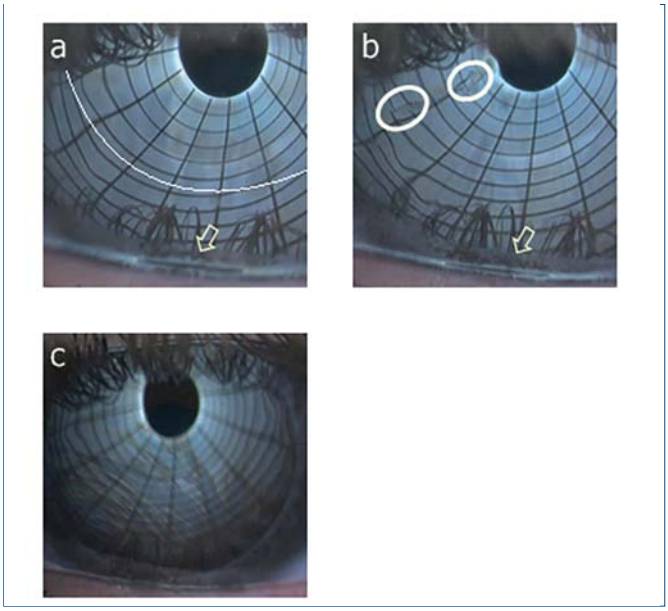

Fig.1 Examples of non-invasive tear film tests. a. Example of corneal grid reflex image in the beginning of NIBUT measurement. (White line: external border of the evaluable area). b. First breaks of the corneal grid reflex lines (circled). Regular tear meniscus morphology (a, b: arrow). c. Lipid interferometry image. Example of wave pattern (score 3)

number of eyes with a score less than or equal to 3 (open meshwork, closed meshwork, wave) was compared to the number of eyes with a score exceeding 3 (amorphous, colour fringes) in each group.

Tear meniscus morphology: By examining the morphology of the tear meniscus the amount of tear fluid can be assessed (10). The lower tear meniscus, when observed with a tearscope, is represented as a white strip with a thinner black zone in the middle. As described by Guillon (10) in an ideal case the central black zone is regular and continuous (pattern 1) (see *Figure 1).* Group of blocked Meibomian gland ducts results in a deformed central black zone (pattern 2). In case of severe dry eye, the height of the tear meniscus becomes significantly reduced or even fractured (pattern 3). The number of eyes with 'normal' (patter 1) and 'abnormal' (pattern 2, 3) tear meniscus was compared to each other in all groups.

Statistical analysis

IBM SPSS 20.0 software was used for the statistical analysis. Distribution of NIBUT values was analysed with Kolmogorov–Smirnov test. Mann–Whitney U test was applied to compare the NIBUT values in the patient groups to the respective value in the control group. Fisher's exact test was used for comparison of discrete values (lipid layer thickness, tear meniscus). Intraclass correlation was calculated to evaluate the repeatability of NIBUT measurements. The correlation between NIBUT and BCVA was analysed using the Spearman's correlation. The significance level was set at 5%.

Results

In post-perforating keratoplasty (PKPsi, PKPso) eyes a clear corneal grid reflex image for the NIBUT measurements could only be detected in the transplant area within the ring of sutures or at the former site of the ring of sutures which had been removed. The grid lines were distorted and curved over the ring-shaped area of sutures already at the start of the examination. Evaluable lipid interferometry pattern could be detected inside the ring of sutures, too. Illustrating examples are shown in *Figure 1.*

The intraclass correlation calculated for characterization of intrasession repeatability of NIBUT measurements exceeded 0.8 in each group *(Table 2).*

The results of NIBUT, lipid interferometry and tear meniscus measurements in the four groups, respectively are presented in *Table 3.* The NIBUT values were significantly lower in the PKPsi group, compared to the control group (p=0.02). No significant difference was found in the other two postoperative groups (PKPso, DSAEK) compared to the control group (see *Figure 2*). Very weak and not significant correlation was found between NIBUT and BCVA readings in each group: the correlation coefficient was –0.162, 0.115, 0.163, and the significance level was 0.616, 0.708, 0.758 in the

Table 2. Intrasession reproducibility of the NIBUT measurements in the four groups

	Control	PKPsi	PKPso	DSAEK
Intraclass correlation	0.848	0.822	0.924	0.881

Table 3. The results of non-invasive tear film assessments in the four groups

	Control	PKPsi	PKPso	DSAEK
NIBUT: sec, mean±SD (n)	22.0±7.6 (14)	11.4±8.9 (19) *	22.8±8.6 (8)	15.6±9.7 (4)
Lipid layer: No. of eyes with score ≤3/>3	10/4	6/13 **	5/3	2/2
Tear meniscus morphology (normal [pattern 1] / abnormal [pattern 2])	13/2	19/3	7/1	3/0 †
BCVA, mean±SD (n)	0.98±0.06 (12)	0.52±0.23 (13)	0.64±0.35 (6)	0.8±0.0 (3)

*: significant difference compared to the control group (p=0,02), **: significant difference compared to the control group (p=0,037), †: In one case the tear meniscus morphology couldn't be assessed due to excessive conjunctivochalasis

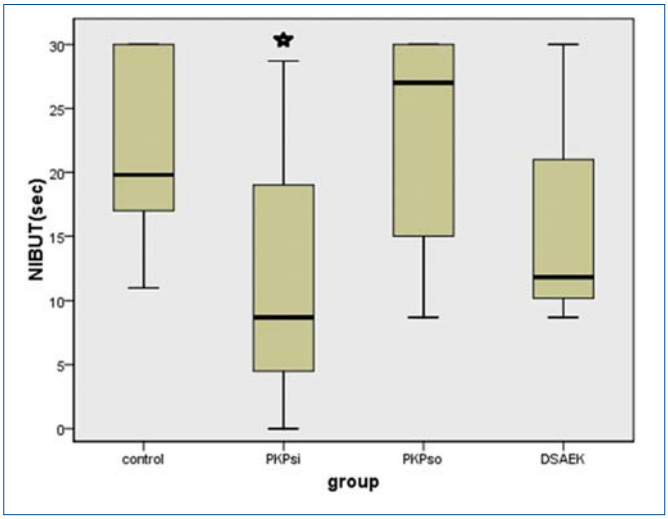

*Fig.2 Boxplot chart of NIBUT results in the four groups. * indicates significant difference from the control group. The central lines in the boxes represent the median*

control group, PKPsi group and PKPso group, respectively. In the DSAEK group the data was too few and equal to perform the correlation test.

The proportion of eyes with thicker lipid layer (score >3) was significantly higher in the PKPsi group compared to the control group (p=0.037). The other two postoperative groups (PKPso, DSAEK) didn't differ significantly from the control group.

There was no difference between the postoperative groups and the control group in terms of normal to abnormal tear meniscus morphology ratio.

Discussion

In our study the precorneal tear film was evaluated in post–keratoplasty eyes with non-invasive methods.

We found that the NIBUT measurement could be performed on the corneal graft surface of post-perforating keratoplasty eyes and on the corneal surface of post-DSAEK eyes. In post-PK eyes the lines of the projected grid at the site of the ring of sutures was distorted already when the tear film was yet intact, as this part of the cornea was slightly elevated from the level of the corneal surface. In some instances this observation remained true even after removal of the sutures. Therefore, we were confined to exclude the peripheral concentric corneal zone from the NIBUT examination and restrict the measurements to the central area inside the ring. For the same reason special attention was required to ensure stable fixation during the examination so that the same image would fall to the same, relatively limited area of the graft in the consecutive measurements.

The intrasession reproducibility of the NIBUT measurements can be considered good (ICC over 0.8) not only in control eyes but in post-keratoplasty corneas, as well. The repeatability of tear film stability measurement with Tearscope was examined by Elliott et al (11) in healthy eyes and the ICC was found to be 0.82 in asymptomatic subjects, which is comparable to our results. We haven't found any study in the literature on the repeatability of tear break-up time assessment in post-keratoplasty patients.

The NIBUT values in normal and post-keratoplasty eyes were compared in our study. It was found that although the NIBUT values were within the normal range – between 5 s and 30,8s (9) – they were significantly lower in post-PK eyes with the sutures in. The same observation was made by other authors (12, 13), who examined BUT using an "invasive" method, 1 to 12 months after surgery, when the sutures were of course still in place.

The non-invasive break-up time may be affected by partial blinking, uneven tear mixing, tear osmolarity and the smoothness of the ocular surface (14, 15). The most likely cause of the difference between suture-in and suture-out cases in terms of tear film over corneal grafts is the "ridge of suture area", which probably changes the distribution of the tear film over the cornea (13,16). Although the "ridge" did not disappear even after suture removal, as it disturbed the evaluation of the test, it did not cause significant changes in the tear film over the graft. It is known that some factors influencing the tear film could improve over time after surgery, such as corneal sensitivity, the improvement of which leads to the normalisation of blink rate (13, 16). Therefore, we also need to take into account that the time after surgery in the group of suture-out patients was of course usually longer.

In our study the proportion of eyes with thicker lipid layer was significantly higher in the suture-in PKP group as compared to the control group. To the best of our knowledge, the lipid layer thickness after keratoplasty has not yet been evaluated. Nevertheless, the change of lipid layer thickness after phototherapeutic keratectomy (PTK) was studied (17). Before treatment the corneal surface was uneven – similarly to our post-PK cases –, and lipid layer interferometry showed a pattern characteristic for dry eye. Over the months following PTK the interferometry pattern gradually improved and became similar to that found in the control eyes in our study. It seems that the corneal smoothness influences the tear film lipid layer.

We found very weak and not significant correlation between NIBUT and BCVA. This is in agreement with the study of Mohidin (18), in which the BUT did not correlate with BCVA at 6 and 12 months, only at 1 and 3 months after corneal transplantation, and in our study the measurements were performed more than 3 months after the keratoplasty. It is known that break-up of the tear film makes the corneal surface uneven, which entails the increase of the surface regularity index (SRI) of corneal topography and decrease of BCVA (19). However, as we measured the BCVA during nor-

mal blinking it is likely that the patients blinked before the tear broke up.

The tear meniscus morphology did not show difference in any of the 3 post-keratoplasty groups compared to the control group. Uneven tear meniscus (pattern 2) was found in some patients in every group, indicating that small groups of the Meibomian gland ducts were blocked. However, these cases did not meet the criteria of Meibomian gland dysfunction, as this was an exclusion criterion in the study.

There are some limitations of our study. The non-prospective nature of the study is to be mentioned first, as this was a cross sectional study. Due to the relatively low patient numbers caution needs to be exercised in drawing conclusions. Only few post-DSAEK patients were included as we did not expect any change in the tear film after surgery.

Conclusions

In our observational study the non-invasive tear film measurements, though with some limitations, were suitable to assess the tear film in post-keratoplasty eyes. The tear film stability was decreased and the lipid layer was thinner in a higher proportion of post-keratoplasty eyes with sutures still in place.

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