



Evaluation of the Ocular Surface and Tear Film Quality in Post-keratoplasty Patients

Miklós Ágoston Lukács¹ · László Módis¹

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Abstract

Purpose To evaluate the ocular surface and tear film quality in post-penetrating keratoplasty patients in comparison with healthy individuals.

Methods Slit lamp examination of lid parallel conjunctival folds (LIPCOF), tear osmolarity measurement, Schirmer I, fluorescein break-up time (FBUT) and corneal fluorescein staining tests were performed on 38 eyes of 34 patients underwent penetrating keratoplasty for different indications. In addition, 34 eyes of 18 healthy age-matched individuals served as normal controls.

Results Average age was 55.86 ± 16.75 years in the post-keratoplasty group and 51.05 ± 18.85 years in the control group ($p = 0.289$). There were significant differences in LIPCOF (1.45 ± 0.76 vs. 1.03 ± 0.83 ; $p = 0.035$) and FBUT values (5.82 ± 4.48 s vs. 11.03 ± 6.72 s; $p < 0.001$) between the operated and control eyes. Schirmer I and tear osmolarity tests demonstrated no significant differences. The frequencies of corneal staining ($p < 0.001$), abnormal LIPCOF ($p = 0.030$) and artificial tear use ($p < 0.001$) were significantly higher in the post-keratoplasty group. However, proportions of abnormal FBUT and Schirmer I test were similar in the two groups. Tear osmolarity was slightly higher in the post-keratoplasty group (311.53 ± 23.75 mOsm/l vs. 308.12 ± 15.83 mOsm/l), but the difference was not significant ($p = 0.524$). There were no significant differences in the proportion of hyperosmolar ocular surfaces between the two groups using cut-off values either ≥ 308 mOsm/l ($p = 0.635$) or ≥ 316 mOsm/l ($p = 0.805$).

Conclusions Abnormal dry eye tests are more prevalent in patients after penetrating keratoplasty. Reduced tear film stability, conspicuous corneal staining and lid parallel conjunctival folds represented further deterioration of the ocular surface homeostasis despite normal tear production and osmolarity. These findings highlight the importance of comprehensive ocular surface assessment and management in the postoperative care of keratoplasty patients.

Keywords Ocular surface · Tear film · Osmolarity · Keratoplasty · Dry eye disease

1 Introduction

Dry eye is a common phenomenon, previous papers focusing on symptoms suggest that 5 to 50% of the population is affected by this disease [1]. In other studies where diagnosis was based primarily on signs reported higher and more variable rates of dry eye, up to 75% in certain populations [2]. As shown by the new TFOS DEWS III report, prevalence of dry eye disease (DED) is greatly affected by the diagnostic

criteria used in studies; some of these showed a female predominance and correlation with age [3]. Furthermore, meibomian gland dysfunction (MGD) was age-related as well and was more likely in males [3]. DED varies from patient to patient and ranges from slight discomfort to serious corneal complications. Several factors contribute to the pathogenesis of DED, instable tear film, loss of goblet cells and glycocalyx, epithelial damage and release of inflammatory cytokines especially IL-1, IL-17, TNF-alpha, INF-gamma, and matrix metalloproteinases (MMPs), however the main trigger is tear hyperosmolarity [4].

All these factors contribute to the altered tear film homeostasis, inflammation and damage of the ocular surface causing subjective symptoms of dryness, blurry vision, burning and foreign body sensation. An additional factor can be

✉ Miklós Ágoston Lukács
lukacs.miklos@med.unideb.hu

¹ Department of Ophthalmology, Faculty of Medicine,
University of Debrecen, Nagyerdei Krt. 98, 4032 Debrecen,
Hungary

previous ocular surgery [5]. Disruption of corneal innervation may lead to the impairment of ocular surface lacrimal functional unit and to morphological alterations of the ocular surface, especially surface epithelium [6, 7]. Penetrating keratoplasty can completely abort the integrity of the sensory innervation of the cornea and may lead to tear film instability. This tear film instability is one of the core mechanisms to trigger inflammation and hyperosmolarity as key steps in a vicious circle that can result in definitive clinical signs of DED [8, 9]. According to the TFOS DEWS II report, subjective triaging questions of dry eye symptoms need to be confirmed by at least one objective tear homeostasis marker for the proper diagnosis of DED [10]. Among the many tests available, these can be fluorescein (or non-invasive) tear film break-up time, ocular surface staining or tear osmolarity. The recently published TFOS DEWS III report introduced a three-step diagnostic algorithm in which OSDI-6 questionnaire is followed by BUT or tear osmolarity measurement and by ocular surface staining [11].

The aim of this study was to evaluate ocular surface and tear film quality in patients after penetrating keratoplasty and compare the results with healthy individuals. In addition to osmolarity measurement, lid parallel conjunctival folds (LIPCOF) score, Schirmer I test value, tear film break-up time and the presence of ocular surface staining were determined.

2 Patients and methods

Conventional dry eye tests, evaluation of LIPCOF score, and tear osmolarity measurement were conducted on 38 eyes of 34 patients underwent penetrating keratoplasty and 34 eyes of 18 individuals as controls. Individuals in the control group did not have any corneal or other ocular disease, did not have any systemic condition affecting the ocular surface and did not undergo any eye surgery (including refractive surgery). Average time passed between the surgery and the examination was 47.8 ± 54.6 months (all sutures out). The clinical indications for corneal transplantation are listed in Table 1. Of the 38 eyes, eight underwent a repeated keratoplasty and the remaining 30 were primary keratoplasty. In the eight repeat keratoplasty cases, indications of the first grafting were keratoconus ($n=4$), bullous keratopathy ($n=2$), Fuchs endothelial dystrophy ($n=1$) and stromal dystrophy ($n=1$). Repeat keratoplasty was necessary due to various reasons, such as allograft rejection ($n=2$), late endothelial failure ($n=2$), graft infection ($n=1$), graft degeneration and scarring ($n=1$), and ectatic graft with high astigmatism ($n=2$). Ocular surface assessment and OCT imaging were performed 63.8 ± 49.0 month after surgery in these eight cases. During each keratoplasty, single 16 bites continuous running suture technique was used by

Table 1 Clinical indications for keratoplasty in the study group

Indication for keratoplasty	Number of patients
Repeated keratoplasty	8
Pseudophakic bullous keratopathy	8
Corneal scarring/degeneration	8
Keratoconus	7
Corneal dystrophy	3
Non-infectious keratitis	2
Infectious keratitis	2

the same surgeon (L. M.). After surgery, patients received a standard treatment protocol. They were treated with topical antibiotics (levofloxacin) for ten days and topical corticosteroids (dexamethasone) for six months. Dry eye tests were performed in the following order beginning from the less invasive to the more invasive ones: slit lamp examination of LIPCOF, measurement of tear osmolarity, Schirmer I test, fluorescein break-up time (FBUT) and evaluation of corneal staining [10, 11]. Conjunctival folds were evaluated in the lower temporal quadrant and rated on a 0–3 scale as described previously [12]. A LIPCOF score of ≥ 1 was rated as abnormal. Tear osmolarity was measured with the TearLab osmolarity system (TearLab Corporation, San Diego, CA, USA). The lab-on-a-chip test card collected 50 nanoliter tear sample without anaesthesia from the lower tear meniscus near the lateral canthus by capillary action. Osmolarity analysis was completed by electrical impedance measurement automatically within a few seconds. Cut-off values for tear osmolarity were ≥ 308 mOsm/l and ≥ 316 mOsm/l as suggested by previous studies [9–11, 13]. Schirmer I test was performed without topical anaesthesia using a standardized filter strip by inserting into the lower conjunctival sac (I-DEW Tearstrips, Entot Research Cell UK Ltd.). After 5 min, the amount of wetting was measured in millimeter (mm) unit, and a result of < 10 mm in 5 min was considered as abnormal. For tear film break-up time evaluation, the ocular surface was stained by using a fluorescein strip, and the time interval between the last blink and the appearance of the first dry spot was recorded. A FBUT of < 10 s was used as a threshold value for dry eye. Fluorescein staining of the corneal epithelium was examined under cobalt blue filtered light. Corneas stained at least grade 1 on the Oxford scheme were grouped as abnormal [14]. Use of artificial tears as well as relevant ophthalmic medical history were also recorded. To assess the precise anatomical and functional condition, we recorded the best-corrected visual acuity (BCVA), and measured the curvature and thickness of the cornea with the aid of anterior segment optical coherence tomography (AS OCT, Anterior, Heidelberg Engineering, Heidelberg

Germany). In addition, endothelial cell density (ECD) was determined on each corneal graft before transplantation using contact specular microscopy (Tomey, Nurnberg, Germany). Descriptive statistical analysis and Mann–Whitney tests were conducted for continuous and ordinal variables. Fisher exact tests were performed for nominal variables and Spearman's rank correlation for continuous variables. All statistics were calculated with the aid of IBM SPSS Statistics software (Version 29.0.1.0 (171) for Windows).

3 Results

Descriptive statistics of dry eye tests for both groups are summarized in Table 2. Average age was 55.86 ± 16.75 years in the post-keratoplasty group and 51.05 ± 18.85 years in the control group ($p = 0.289$). There were significant differences in LIPCOF (1.45 ± 0.76 vs. 1.03 ± 0.83 ; $p = 0.035$) and FBUT values (5.82 ± 4.48 s vs. 11.03 ± 6.72 s; $p < 0.001$) between the operated and healthy eyes (Mann–Whitney). The frequencies of corneal staining ($p < 0.001$), abnormal LIPCOF ($p = 0.030$) and artificial tear use ($p < 0.001$) were significantly higher in the post-keratoplasty group (Fisher exact).

However—using the previously mentioned cut-off values—the proportion of abnormal FBUT ($p = 0.091$) and Schirmer I ($p = 1.000$) were similar in the two groups (Fig. 1). Of the following three tests—FBUT, LIPCOF, Schirmer I—at least one positive result was present in 59% of control eyes while 92% in the post-keratoplasty group met the same criteria revealing a significant difference between the two groups ($p = 0.002$). Tear osmolarity was slightly higher in the post-keratoplasty group (311.53 ± 23.75 mOsm/l vs. 308.12 ± 15.83 mOsm/l), however the difference was not significant ($p = 0.524$). There were no significant differences in the proportion of hyperosmolar ocular surfaces between the two groups using cut-off values either ≥ 308 mOsm/l ($p = 0.635$) or ≥ 316 mOsm/l ($p = 0.805$). Schirmer I test correlated significantly with age in post-keratoplasty patients (-0.321 ; $p = 0.04$ Spearman) as well as in controls (-0.488 ; $p = 0.003$). LIPCOF correlated significantly with age (0.426 ; $p = 0.008$) and FBUT (-0.376 ; $p = 0.020$) in eyes with corneal graft. In addition, FBUT correlated with osmolarity, Schirmer I, LIPCOF and age in the control group (Table 3). In the post-keratoplasty group, BCVA was 0.56 ± 0.33 (decimal unit) with a mean spherical correction of -1.84 ± 5.05 D and a mean refractive cylinder of -2.25 ± 2.63 D. BCVA

Table 2 Results of dry eye diagnostic tests in both study groups

	Post-keratoplasty group (n = 38)	Control group (n = 34)	P*
Age (years)	55.86 ± 16.75 (50.17–61.18)	51.05 ± 18.85 (44.47–57.62)	0.289
LIPCOF	1.45 ± 0.76 (1.20–1.70)	1.03 ± 0.83 (0.74–1.32)	0.035
Osmolarity (mOsm/l)	311.53 ± 23.75 (303.72–319.33)	308.12 ± 15.83 (302.60–313.64)	0.524
Schirmer (mm)	20.78 ± 10.17 (17.43–24.12)	21.42 ± 10.42 (17.79–25.06)	0.875
FBUT (sec)	5.82 ± 4.48 (4.34–7.29)	11.03 ± 6.72 (8.68–13.37)	<0.001

Mean $\pm$ SD (95% confidence interval)

*Mann–Whitney test in the two study groups

Fig. 1 Positive dry eye signs in age-matched post-keratoplasty and control groups

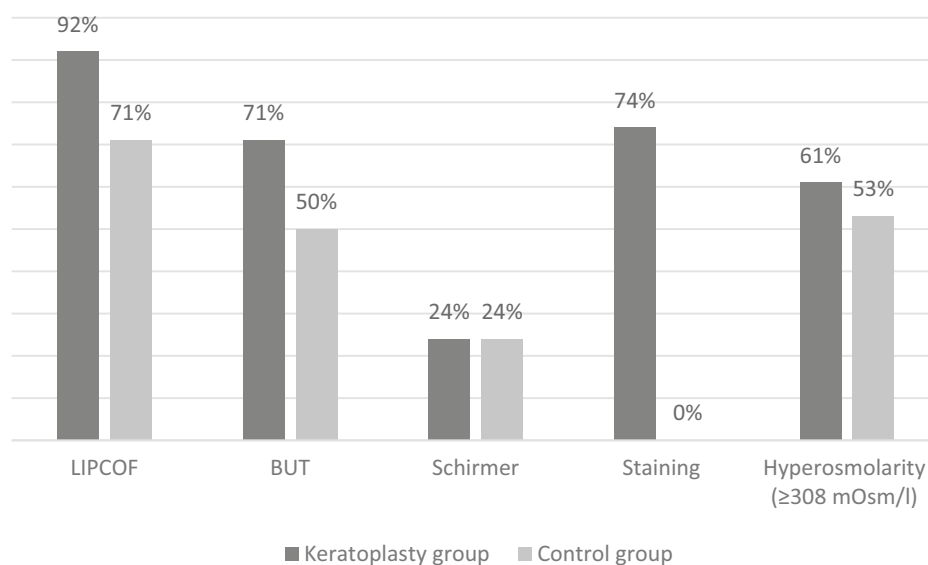


Table 3 Correlations between age and dry eye tests in the control group (n = 34)

	Age	Osmolarity	LIPCOF	Schirmer I	FBUT
Age	–	0.313 (0.072)	0.587 (<0.001)	–0.488 (0.003)	–0.436 (0.010)
Osmolarity	0.313 (0.072)	–	0.475 (0.005)	–0.316 (0.069)	–0.402 (0.019)
LIPCOF	0.587 (<0.001)	0.475 (0.005)	–	–0.201 (0.254)	–0.442 (0.009)
Schirmer I	–0.488 (0.003)	–0.316 (0.069)	–0.201 (0.254)	–	0.382 (0.026)
FBUT	–0.436 (0.010)	–0.402 (0.019)	–0.442 (0.009)	0.382 (0.026)	–

Spearman's correlation (P value)

was higher in the control group (0.72 ± 0.27 , $p < 0.001$) and the mean refraction was 1.68 ± 2.67 spherical D ($p = 0.010$) and -0.20 ± 1.91 cylindrical D ($p = 0.038$). Mean anterior surface axial curvature of the grafts was 48.3 ± 3.5 D at the steep meridian and 43.5 ± 2.9 D at the flat meridian (Fig. 2). In the control group, keratometry was lower at the steep meridian 44.4 ± 3.0 D, $p < 0.001$) and was comparable at the flat meridian (42.9 ± 2.2 D, $p = 0.302$). Furthermore, mean central corneal thickness was higher in the post-keratoplasty group than in the control group (567.0 ± 49.9 μm vs. 537.2 ± 48.8 μm , $p = 0.016$). The mean ECD of corneal grafts was 2235.6 ± 158 /mm² before transplantation and all grafts remained clear during the observation period.

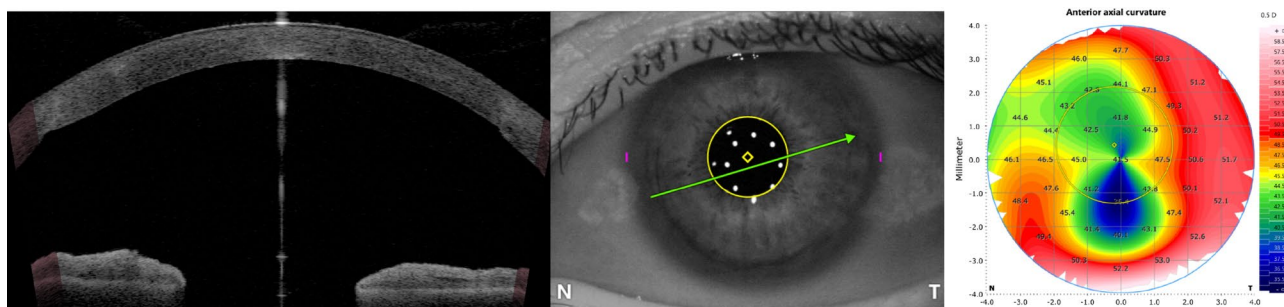
4 Discussion

This paper demonstrated with conventional tests, that dry eye signs are more common after penetrating keratoplasty. In addition, tear osmolarity measurement disclosed no significant difference between healthy and post-keratoplasty eyes.

Dry eye is a multifactorial disease affecting the tear film and ocular surface. Elevated tear osmolarity and tear film instability are thought to play a significant role in the ocular surface inflammation and epithelial damage [15–17]. Tear film hyperosmolarity induces osmolar stress in the surface epithelia which promotes an inflammatory cascade and the release of cytokines generating apoptosis in epithelial cells [18–20]. Human cornea contains sensory nerve fibers originated from the trigeminal nerve and sympathetic axons from

the superior cervical ganglion. Stromal nerve bundles enter the cornea at its periphery and compose the subepithelial plexus [21]. Corneal nerves have a pivotal role in maintaining the functional and morphological integrity of the ocular surface. Penetrating keratoplasty is a significant risk factor for dry eye as a result of the disrupted corneal innervations [22, 23]. The potential role of neuropathic mechanisms may also contribute to reduced corneal sensitivity and development of dry eye signs and symptoms [24]. Multiple studies have evaluated clinically the structure and function of corneal nerves in DED with the aid of confocal microscopy and corneal aesthesiometry [25]. Lower nerve density and nerve sensitivity were found in most studies in patients with aqueous tear deficient DED.

Several dry eye tests were applied in our study, such as tear osmolarity measurement, Schirmer I test, fluorescein break-up time, corneal staining and LIPCOF evaluation. Sensitivity and specificity of these tests were assessed previously by multiple studies. Szalai et al. demonstrated a sensitivity of 34.62% and specificity of 95.0% for Schirmer as well as a sensitivity of 95.95% and specificity of 54.55% for FBUT for the diagnosis of dry eye syndrome. The same study also evaluated the diagnostic power of LIPCOF which could distinguish healthy and dry eyes with a sensitivity of 89.74% and a specificity of 43.18% [26]. In a multicenter Sjögren syndrome study, a sensitivity of 76.9% and specificity of 72.4% for Schirmer, and a sensitivity of 77.8% and specificity of 38.9% for FBUT were found by Vitali et al. [27]. Both studies used 5 mm/5 min threshold for Schirmer and a ≤ 10 s for FBUT. In accordance with our

**Fig. 2** AS OCT cross-sectional image and anterior corneal axial curvature map of a post-keratoplasty patient

study, Meyer et al. demonstrated poorer non-invasive tear film breakup time (5.1 ± 3.6 vs. 7.6 ± 4.5 ; $p = 0.02$) as well as lower corneal sensitivity, lower sub-basal nerve density and more severe fluorescein staining scores in post-keratoplasty eyes than in unoperated fellow eyes [28]. In contrast, slight improvement in BUT, corneal staining and ocular surface disease index (OSDI) score was found after keratoplasty in a retrospective case-series study by Kim et al. [29]. The post-keratoplasty BUT and Schirmer values (12 months after keratoplasty: 4.3 ± 0.7 s, 16.5 ± 2.2 mm/5 min, respectively) were comparable with the same parameters of our study group. The same study disclosed worsening in parameters describing Meibomian gland function (Meibum quality, Meibomian gland expressibility) as well [29].

Tear osmolarity was also measured in our study as one of the main diagnostic tests for dry eye and ocular surface evaluation. Point-of-care testing of tear hyperosmolarity became available in the past two decades as a promising single marker for DED [30]. Although osmolarity value seems to have the same drawbacks as the conventional dry eye tests (i.e. osmolarity values in dry eye patients may overlap with values in normal population), assessment of tear hyperosmolarity provide further information on the homeostasis of the ocular surface and aids the diagnosis of DED as a fast and objective test [10, 31]. Tear osmolarity measurement has a well-established place in the current DEWS III diagnostic algorithm [11]. TearLab employs microfluidic lab-on-a-chip technology and takes up a small amount of tear sample by passive capillary action. Higher osmolarity was found in the post-keratoplasty group but the difference was not significant. Current cut-off values for tear osmolarity are designed to distinguish between normal and dry eyes. For assessing tear hyperosmolarity, a cut-off value of 308 mOsm/l was used by Sullivan et al. [30, 32]. This cut-off was applied in the DEWS II and is still used in the new DEWS III diagnostic criteria [10, 11]. Osmolarity of 316 mOsm/l was also used in our study as a more specific threshold recommended by Tomlinson et al. [13]. Tear film osmolarity generally increases with disease severity, however, more severely affected patients exhibit an increased average and increased variability between eyes and between follow-ups [30, 33, 34]. Various cut-off values for DED have been proposed ranging from 305 to 316 mOsm/L, with reported sensitivities ranging from 64 to 91% [35–38].

Limitations of this study could be its cross-sectional fashion. Long-term fluctuation as well as reproducibility of tear osmolarity in this special population were also not covered by this study and there is no relevant data currently on these in scientific literature.

To summarize, ocular surface abnormalities detected by conventional dry eye tests were more frequent in patients underwent keratoplasty. Tear film instability was the most prominent difference in tear film quality. In addition, pathologic corneal staining and lid parallel conjunctival folds

were also represented further deterioration of the ocular surface homeostasis despite normal tear production and osmolarity. Clinical implementation of these tests may also help to administer the appropriate tear substitution, since long-term tear supplementation is recommended to protect the denervated corneal graft.

Author Contributions All authors contributed to the study design, material preparation, data collection and analysis as well as to the manuscript writing. All authors read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval The study was performed in accordance with the tenets of the Helsinki Declaration. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Research identification number at the Regional Research Ethical Committee of the University of Debrecen: DE RKEB/KEB 6290–2023.

Informed consent Informed consent was obtained from all individual participants included in the study.

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