SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Our results with modern diagnostic and therapeutic methods in ectatic corneal diseases and in refractive surgery

by

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The Examination takes place at 10 a.m., November 29, 2013.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Medical and Health Science Center, University of Debrecen, at 12 a.m., November 29, 2013.

INTRODUCTION AND OVERVIEW

I. Corneal ectasia

Corneal ectasia (keratectasia) is the non-inflammatory, heritable and progressing thinning of the corneal tissue. Several forms of keratectasia are known. The most frequent corneal ectasia is keratoconus. Less frequent types are keratoglobus, pellucid marginal degeneration and Terrien's marginal degeneration. Iatrogenic keratectasia is also known developing due to refractive surgical interventions, mainly to laser in situ keratomileusis (LASIK).

Keratoconus

Keratoconus is a corneal disorder characterized by stromal thinning and bulging, which mostly develops in young adulthood. It is a progressive, non-inflammatory and congenital corneal disease which is usually bilateral and affecting paracentral cornea. Advanced keratoconus is due to the sliding of corneal layers towards the periphery and the change in the direction of the collagen fibres which result in the thinning of the central cornea and its bulging. What allows for corneal layer movement is the enhanced enzymatic degradation of interlamellar bounds and the increased oxidative stress.[1] Increased stress due to corneal extension leads to central, vertical corneal lines in half of the cases, called Vogt's striae.

Its exact pathogenesis still remains unclear, its possible causes may range from the enhanced proteinase activity, a defective mechanism of an antioxidant and also the etiological role of eye-rubbing.[2] Oestrogen may also play a role in facilitating processes provoking keratoconus, especially in biomechanically weakened cornea after refractive interventions such as LASIK and photorefractive keratectomy (PRK).[1]

Its prevalence is most common above the age of 14 usually at around 16. Its population based incidence is an average 1 out of 2000. In 85% of the cases it occurs bilaterally. It is classified as nipple, oval and globus: the most common is the global form. Progressive irregular astigmia is considered to be the hallmark of keratoconus. Its symptoms include: blurred vision, eye strain, frequent headache, widening of light sources, myopia or even photophobia. Iron deposites at the apical cornea (Fleischer's-ring) is a usual sign; in at advanced cases we can see bulging of the lower eyelid during downward gaze (Munson's signl). Acut inflammatoric signs and pain are observed in acut keratoconus leads to corneal oedema and scar formation.

Pellucid marginal degeneration

One of the rare types of keratectasia is known as pellucid marginal degeneration (PMD). Similarly to keratoconus, PMD involves the inferior cornea. It is a progressive and noninflammatory disorder. Clear corneal thinning involves the areas between 4- o'clock and 8 o'clock positions typically 1-3 mm from the limbus. Histopathologically, it is considered to

be a variation of keratoconus, however, the observed corneal thinning is significantly more peripheral than detected in cases of keratoconus.[3]

Diagnosis of corneal ectasias

Due to the rapid development of technology, more and more diagnostic devices have been introduced in ophthalmology. With the old and traditional equipments and in the lack of reliable imaging devices, keratectasia could be diagnosed only in an advanced stage. With the advance of technology, new treatments have been gradually introduced using mostly the non-contact method. Ultrasound biomicroscopy, corneal topography, refractokeratometry, optical coherence tomography, devices measuring corneal biomechanics and wavefront aberrometry allow the diagnosis of keratectasia already in an early stage thus early management can be achieved.

Scheiner was the first to try to assess the radius of curvature of the cornea in 1619. He compared the size of pictures reflected from the cornea to the size of the pictures reflected from a glass ball of a given radius of curvature. The first real keratometer was made by Ramsden in 1769, which was further developed by von Helmholtz and Javal in the middle of the XIXth century. Henry Good, who invented the first keratoscope, reported on his observations in eyes with astigmatism in 1847. Antonio Placido introduced photokeratoscope in 1880, applying black and white concentric circles which could be projected onto the cornea. Blix was the first to report on central corneal thickness measurements based on optical methods in 1880, which technique was further modified by Mishima, Hedbys and Ehlers thus developing the presently known pachymeters which can be fixed on slit-lamps.[4,5] The ultrasound pachymetry was first reported by Kremer in 1985.[6] Orbscan was the first non-contact device capable of capturing 3D images, which was developed in the late 1990s. It could assess a map-like pattern of both the refractive power and the thickness of the cornea.[7-10] Using a video camera, Orbscan projects a slit light onto the surface of the cornea. The slit lamp scans the cornea including the anterior chamber 40 times. The camera transmits 240 points from a sheet of light in 3 dimensions to the computer, thus is the topogram of the anterior segment obtained.

This was soon followed by another device, the Pentacam (Oculus, Wetzlar, Germany), allowing the examination of the anterior segment in 3 dimensions which was further developed into a higher resolution device, the Pentacam HR providing the possibility of using the Scheimpflug imaging based on optical principles in a non-contact way.[11,12] The device allows for conducting tangential and sagittal topography, measuring corneal thickness, assessing anterior chamber characteristics and the density of the lens. Ectatic and thinned cornea in keratoconus can be clearly observed by Pentacam.

Management of corneal ectasia

Several methods are known for managing astigmatism and corneal ectasia, thus keratoconus and pellucid marginal degeneration. Early keratoconus is most commonly managed using spectacles. Later, due to the progression of astigmatism, refractive error can be corrected only by contact lenses. The contact lens covers the irregularity of the cornea and acts as a refraction surface on the eye. In cases, where the eye with keratoconus can no longer tolerate contact lens wearing and the cornea is clear with an appropriate thickness,

intracorneal ring implantation is suggested. With the application of this invasive intervention, progression is stabilized, astigmatism can be reduced and vision can be improved.[13] In cases where the cornea is not clear, it lacks the sufficient thickness and vision is poor due to scars and ruptures, perforating or lamellar keratoplasty should be conducted.[14] The management of progressive pellucid marginal degeneration is similar, but still remains unclear.

Collagen cross-linking treatment of the cornea

Collagen cross-linking (CXL) is presently one of the top non-invasive procedures which combines riboflavin and ultraviolet-A light (UV-A).[15-17] The primary aim of the treatment is to delay or stop keratoconus progression and to improve vision quality. The procedure can prolong the period of contact lens wearing thus keratoplastic surgery can be avoided.

It has long been known that natural UV light has an effect that causes photochemical changes in collagen and its fiber producing characteristics with advancing age. CXL technique for keratoconus was described by Wollensak in 2003.[17] He used UV-A light, which is less malignant than UV-B and UV-C lights and avoids penetrating into deep layers, in combination with riboflavin also known as B2 vitamin. Riboflavin is a photosensitizer, which allows UV-A absorption to increase from 32% to 95%. The molecule is activated by UV light, which results in the formation of reactive oxygen radicals. These incline collagen fibres to form covalent cross-bonds thus increasing the stiffness of the cornea and its resistance to collagenase. Though corneal elasticity can be observed, Young modulus, which is inversely proportional to it increases up to 300 % after CXL treatment.[18,19] In such way, keratoconus progression can be delayed at an early stage.

Other structural stromal changes are generated by the treatment as well: due to cross-bond formation fibrillogenesis is altered. Riboflavin binds to the collagen molecule close to the area responsible for the initialization of fibrillogenesis, thus alters kinetic process. As a result, fibres shorter but bigger in diameter size are developed.[20] The evolving hydroxyl radicals can cause degradation in collagen chains. In addition, irradiation has a cytotoxic effect, too; and in the treated area, a temporary, dose-dependant apoptosis develops, which is followed by cell repopularisation.[18] Regarding biophysical characteristics, cornea presents further changes after CXL treatment. A decrease in hydration ability can be observed, cells become less swollen, which results in smaller corneal thickening, thus cornea retains its transparency. An increase in heat stability can be described, namely the shrinkage of cornea tissue occurs at higher temperature.[21] Changes mentioned above affect the anterior stroma only.

During and subsequent to CXL treatment, changes in corneal thickness occur[22-25] so under a preoperative corneal thickness of 400 µm the protecting effect of riboflavin is failed. At a sufficient corneal thickness the posterior stroma remains unaffected. The treated and untreated tissues are clearly separated by a demarcation line, which can be observed using the slit lamp measurement 2 weeks after treatment.[26] The investigation of Spoerl and Wollensak on the assessment of corneal shrinkage in treated and untreated layers under thermal effect, clearly demonstrates that cross-linking is safe for the endothelium, and has a positive effect on the anterior curvature of the cornea.[27] According to previously obtained data, 65% of UV-A light is absorbed in the anterior 200 µm substance of the cornea, and only 25-30% reaches further 200 µm. The maximum cross-linking effect can be applied till 300 µm.[28]

Consequently, riboflavin activation due to the effect of UV-A light promotes the release of oxygen radicals leading to the formation of covalent cross-bonds between weaker collagen fibres in keratoconus. Thus corneal substance and its structure are strengthened, cornea becomes more resistant, corneal curvature and visus can be stabilized.

The technique of collagen cross-linking

At first, topical anaesthesia is performed using drops (Humacain 1%, oxybuprocainium chloratum, TEVA Pharmaceutical Factory, Hungary) and sedatives can also be administered at request. Subsequently, the corneal epithelium within a 7-8 mm zone is removed using a spatula. 5 minutes prior to treatment Riboflavin solution (0.1% riboflavin-5-phosphat, 20% Dextran, Single use isotonic eye drops, MedioCross; Peschke Meditrade GmbH, Huenenberg, Switzerland) is instilled on the corneal surface. Then UV-A light irradiation with a radiant energy of 3 mW/cm^2 begins focusing on the centre of the cornea or on the paracentral zone. During the 30 minutes of irradiation the Riboflavin solution is instilled every 2 minutes. The entire procedure takes an average of 50 minutes. Riboflavin penetration into the stromal layers can be clearly seen in our images obtained during treatment, which appears as a hyper-reflective section in the Pentacam HR images.

Methods avoiding the removal of the corneal epithelium have already been released, such as the trans-epithelial treatment, the hypotonic „thickening" solution, iatrogenic ectasia in keratoconus and a special solution treating pellucid marginal degeneration. An accelerated therapy called „flash-linking" is also available, in which the treatment using special photoactive crosslinking agents is followed by an UV-A irradiation of 30 seconds. Conducting wave-elastomeric measurements, results similar to the efficiency of the traditional CXL treatment were reported.[29]

Indications for collagen cross-linking therapy can be primary and secondary. Primary indications include: age between 14-45 years, progression clinically observed within 1 year, corneal thickness above 400 µm and clear cornea. Further primary indications are when the patient is no longer capable of wearing contact lenses and a decrease in visual acuity is recorded. Secondary indications include: age between 25-35 years, an average keratometry data below 53-55 D, Vogt striae and anisometropy below 4.0 D. Therapy is contraindicated when central corneal thickness is below 400 µm, central corneal opacities are recorded or no progression is found within 1 year.

Prior to treatment the clinical anamnesis of the family is recorded, uncorrected and corrected visual acuities are assessed. This is followed by refracto-keratometry, slit-lamp measurement, pachymetry, corneal topography, funduscopic examination, intraocular pressure evaluation and dry-eye assessment. Corneal endothelial cell density is obtained with specular microscope.

After treatment the treated eye is dressed with either a protective bandage or a soft lens. Home treatment includes antibiotic eye drop administration 5 times a day for 5 days then patients are medicated 5 times a day with nonsteroidal anti-inflammatory eye drop for further 3 months. Artificial tear drops are suggested in case of dry eyes.

II. Tear-osmolarity

The increased osmolarity of the tear and tear film instability are regarded to play an important role in eye-dry syndrome development. Tear film hyperosmolarity leads to inflammatory processes in the corneal epithelium and causes a decrease in the number of the goblet cells in patients with keratoconjunctivitis sicca.[30-34] In patients waiting for refractive surgery dry eye syndrome is of a high significance. Due to the flap created during laser in situ keratomileusis the intact innervation of the cornea is damaged which causes further injuries in the functional unit on the eye surface responsible for normal tear production.

Formerly, tear osmolarity could be assessed only by laboratory techniques; today however, the measurement can be easily conducted using a special device, the TearLab osmometer. It is an easy-to-use device allowing for rapid and non-invasive tear osmolarity measurements.

OUR PURPOSES

1. ***Investigation of the effect of collagen cross-linking:***

    a. in keratoconus, analyzing the changes in corneal topographic indices
    b. in keratoconus, analyzing intra- and postoperative corneal thickness changes
    c. in a rare corneal ectasia called pellucid marginal degeneration

2. ***Measuring tear osmolarity using a new diagnostic device prior- and subsequent to LASIK surgery.***

PATIENTS AND METHODS

Prospective studies were carried out to assess the effect of collagen cross-linking treatment and to conduct tear osmolarity measurements at Orbi-Dent Health and Laser Centre. All our studies were conducted in accordance with the tenets of the Declaration of Helsinki and the Local Clinical Ethics Committee. Prior to treatments and measurements a complete ophthalmological examination was performed and patients were informed about the procedures.

Ophthalmic diagnostic devices used in our studies:

1. Corneal topography

Apart from conducting visual acuity assessment, slit-lamp examination, corneal thickness and intraocular pressure measurements, with corneal topography (TMS-4; Tomey, Erlangen, Germany) we also investigated several numerical data obtained by using mathematical methods and the Klyce-analysis conducted by the device. During the follow up period we monitored the following data: standard diopter value of simulated keratometry in the flat axis (SK1), standard diopter value of simulated keratometry in the steep axis (SK2) and cylinder value (CYL). We also investigated the changes in surface asymmetry index (SAI), irregular astigmatism index (IAI), surface regulatory index (SRI), average corneal power (ACP) and corneal eccentricity index (CEI) after the treatment.

2. Pentacam

The device applies the Scheimpflug imaging principle to obtain images of the anterior segment. In traditional photography, the planes of the object, the camera lens and the film are parallel. In the Scheimpflug technique these planes intersect each other in one point, thus the depth of field of the image is significantly improved.

In Scheimpflug images the software fits elevation points onto the edges of the optically different substances and out of these 25000 true elevation points it creates 3 dimensional, rotatable images and topograms of the anterior segment. The software helps to present the entire corneal thickness, the sagittal, tangential and elevation maps of both anterior and posterior surfaces. The software allows for further measurements in the images, we can rate of crystalline lens density on a numerical scale from 0 to 100.

3. The TearLab device

TearLab (TearLab Corporation, San Diego, USA) uses only 50 nanoliters of tear to analyse tear osmolarity applying a single-use test card. The device measures electrical impendence and belongs to the so called lab-on-a-chip applications. The sample is taken from the tear meniscus near the lateral canthus using the capillary action. The chip fixed on the test card performs the analysis itself. The system reader converts the electrical signals transmitted by the card into numerical data. The data of osmolarity is displayed on the screen of the system reader. The limit value of hyperosmolarity has been set by the manufacturer at 316 milliosmol/liter.

Statistical analysis

Statistical analysis was carried out with SPSS (version 13.0) and MedCalc (version 10.2.0) statistical softwares. Descriptive statistical results were described as mean and standard deviation (mean±SD). At tear osmolarity measurements a mean confidence interval of 95% was described (95% CI). Wilcoxon paired sample test and Mann-Whitney U test were carried out for the comparison between groups or variables. Correlation between data was analysed using the Spearman rank correlation. A P value of <0.05 was considered statistically significant.

I. a.

CHANGES IN CORNEAL TOPOGRAPHIC INDICES AFTER COLLAGEN CROSS-LINKING IN KERATOCONUS

Patients and methods

38 eyes of 25 patients (14 women and 11 men; mean age: 29.36 years, between 16 and 42) were treated with CXL technique. Follow-up period was 36 months. Subsequent to local anaesthesia, the central and paracentral epithelial layers within 7-8-mm-diameter zone were removed and 0.1 % riboflavin solution was instilled on the de-ephithelizated surface, in a way so as to cover its entire surface, 15 minutes before irradiation began and every five minutes thereafter. In every 2 minutes physiological saline was instilled to retain corneal moisture. During the procedure UVA light 370 nm in wavelength was irradiated with a radiant energy of 3 mW/cm² (=5.4 J/cm^2) for 30 minutes. The UV lamp was approximately 4-5 centimetres from the corneal apex. After treatment each patient was medicated with antibiotic eye drops, were given therapeutic contact lenses or bandage till the epithelial defect had closed; and non-steroid anti-inflammatory drugs were prescribed at least for one month. Therapeutic contact lenses were removed 5 days after treatment. CXL treatments were conducted with In-Pro CCL-Lix (Norderstadt, Germany).

Inclusion criteria were: cornea thicker than 400 µm clinically tested by pachymetry (Pentacam HR and Oculus Park 1), age between 14-45, significant decrease in visual acuity within the past 6-12 months or cases where keratometry value recorded by topography became worse by 1.0 D within 6 months or by more than 2.0 D within 12 months. Further inclusion criteria were clear cornea without opacities and corneas without Vogt-striae or anterior stoma scars.

Visual acuity tests, slit-lamp examination, corneal thickness and intraocular pressure measurements were performed; and to identify numerical data corneal topography (TMS-4; Tomey, Erlangen, Germany) was used. Data were obtained and calculated by the built-in software application of the device, by Klyce corneal statistics. Changes in the values of simulated keratometry, cylinder, surface asymmetry index, irregular astigmatism index, surface regularity index, average corneal power and corneal eccentricity index were monitored. Corneal topographic measurements were carried out subsequent to a thorough ophthalmologic examination, prior to and in 1, 3, 6, 12, 18, 24 and 36 months after surgery.

Results

Subsequent to CXL treatment, values of both uncorrected and corrected vision remained stable in the follow-up period of 36 months. Changes in keratometry values, in SAI, SRI, ACP, CEI and IAI indices were not statistically significantly different compared to values obtained prior to treatment. No changes in intraocular pressure were observed (p=0.79). All corneas remained clear, no significant change in pachymetry values was recorded (p=0.78) during the follow-up period.

Discussion

In the late 1970's, with the emergence of refractive surgery, corneal topography became more advanced, as focus was driven to the causes of the deviations that may contribute to the development of irregular astigmatism. The first topographical index measuring these deviations was SRI. Its values were correlated to the visual acuity in healthy, keratoconus and post-keratoplasty patients.

The prognosis of keratoconus highly depends on the onset of disease development, that is, in case it develops in childhood, progression is faster, prognosis is worse than in adult cases. In general, we can say, that progression gradually delays and stops after 10 years, but severe irregularities still reside. Collagen-cross linking is aimed to affect this progression in time. Results being in agreement with former studies are really promising, though more long-term researches with more patient populations are needed to be involved.[35] Collagen cross-linking may serve as the first choice in keratoconus therapy, by which progression can be reversed or at least delayed.

During the investigation of the intracorneal ring's therapeutic effect it was also observed that its efficiency is significantly increased, when it is combined with CXL.[36] CXL can be used in the treatments for corneal ulcers (particularly in cases of peripheral corneal dissolutions due to rheumatoid arthritis, Mooren ulcer, ulcers induced by herpes or alkali metals) and for bullous keratopathy.[37,38] In addition, its future potential can be well applied in the prevention and management of keratectasia subsequent to excimer laser treatment.

UV-A irradiation at a given stromal depth is calculated by the Lambert-Beer law, according to which transmission depends on the surface and the absorption coefficient of the cornea. The value of intensity (irradiation dose) is obtained when this value is multiplied by irradiation time (mW/cm^2 x 30x60 sec). At standard 3 mW/cm^2 dose the endothelial cytotoxic level is 0.36 mW/ cm^2, though this level can be reached only in corneas of 400 µm thickness, or in those thicker than that. At least 400-800 endothelial $cell/mm^2$ is needed to the sustenance of corneal transparency. Doses lower than that may result in damage when corneal thickness is reduced. In case of severe keratoconus, when corneal power is below 62 D (Amsler stage 4), special regard is needed as cornea may become significantly thin. Keeping the restraints and performing operation on cornea thicker than 400 µm, no change in endothelial cell number is detected.[39] In cases of keratoconus and corneal ulcers, corneal thickness can be below 400 µm, thus alternative treatment methods or CXL with reduced dose can be performed: on corneas of 350 µm 2 mW/ cm^2=3.6 J/cm^2 is applied. This is the minimal dose which is still efficient.

Subsequent to treatment, corneal oedema or corneal haze may develop which may absorb without treatment. It is more common that they develop on corneas where reticular hyporeflective microstria is presented combined with Vogt striae, or without. Subsequent to treatment, minimal overestimation of intraocular pressure may also occur.[40]

6 months after treatment the repopulation of stromal keratocytes and an increase in stromal fibre density could be observed by confocal microscopy, whereas no damage could be detected in corneal endothelial cells. These changes were specific after the different types of CXL treatments.[41,42]

According to Wollensak's early results, a 100% stop and a 70% delay of progression in keratometric values (a mean of 2.01 D decrease), and light fraction error (a mean of 1.14 D decrease) were achieved in the first 6 months, while in endothelial cell number and in intraocular pressure no change was observed.[39] Long-term researches involving 3-5 years in Dresden, published a decrease of 2.87 D and an increase by 1.4 Snellen lines in the best

corrected visual acuity.[43] At Siena University, Caporossi and his co-workers experienced an increase of 2.1 D in keratometric values, symmetry growth of nearly 70%, and a decrease in coma aberration; and they observed uncorrected visual acuity ameliorating by 3.6 lines and best corrected visual acuity by 1.66 lines.[35]

During the follow-up period of 36 months no significant change was found in the numerical data of the corneatopograph, which allows us to conclude that the continuous decrease of the otherwise progressive disease could be stopped and collagen-cross linking may be efficient. Our data, which did not show significant changes after collagen cross-linking therapy during 36 months, did not match particularly with the literature. Some authors, listed previously, described improvements in certain parameters.[35,39,43] The probable reasons for this difference are the data assessment in various stages and severity of the disease.

I.b
INTRA- AND POSTOPERATIVE CHANGES DURING AND AFTER COLLAGEN CROSS-LINKING THERAPY

Patients and methods

41 eyes of 41 keratoconus patients (mean age 27.97±6.97 years, ranging from 18.0 to 44.06 years) were examined in our study. Prior to CXL treatment detailed above, Pentacam HR measurements were conducted and apical, central and the thinnest corneal thickness were recorded in all cases.

When preoperative corneal thickness was below 400 µm we conducted a corneal soaking in a bulking hypotonic riboflavin solution for an hour (single use hypotonic eye drops; Medio Cross Medizin Produkte GmbH, Germany). At 15 and 30 minutes of the treatment with isotonic riboflavin solution the cornea was rinsed and further corneal thickness measurements were conducted using the Pentacam HR device. Postoperative examinations were carried out 3 days, 1 week then 1, 3, 6 and 12 months after CXL treatment capturing further Pentacam HR images.

Results

Compared to preoperative data, corneal thickness decreased by 108.95±48.6 µm at 15 minutes and by 112.35±47.3 µm at 30 minutes intraoperatively ($p<0.001$). The rate of the decrease showed no correlation with the values of the preoperative corneal thickness ($r=0.16$; $p=0.7$). 3 days after procedure, no further statistically significant deviations in corneal thickness were found compared to preoperative data ($p=0.17$). During the follow-up period corneal thickness remained stable and correlated well with the preoperative values.

Discussion

For the protection of the endothelium a minimum of 400 µm corneal thickness is suggested prior to CXL treatment.[44] In cases where corneal thickness is lower, a pretreatment of the cornea with hypotonic solution or transepithelial CXL is suggested.[45] Our

study comprised 2 cases where patients were administered a pretreatment with hypotonic riboflavin solution for an hour, after which corneal thickness reached the safety zone.

Using Pentacam, Greenstein et al.[46] conducted corneal thickness measurements administering isotonic riboflavin and measured corneal thickness thinner by 23-24.6 µm compared to preoperative values 1 month after treatment and their gradually increasing values reached preoperative values only 1 year after operation. In literature a decrease in corneal thickness is uniformly observed during CXL treatment.[22-25,47]

There are publications suggesting that changes in corneal thickness observed with Pentacam can be an artefact[48,49] of the postoperative corneal haze,[23,24,50] however, they published results correlating well with data obtained by ultrasound pachymetry.

In our study, no Pentacam HR images were captured after de-epithelisation. As the normal epithelial thickness is 53.4±4.6 µm,[51] and 43 µm,[52] the rate of the measured decrease allows for the conclusion that an actual decrease in stromal corneal thickness takes place due to the effect of the isotonic riboflavin.

Vinciguerra et al.[53] found a significantly thinner cornea in the pupil centre even 1 year after central CXL treatment, however it showed no significant change on the thinnest pachymetry location. In our study, initial corneal thickness was measured already 3 days after treatment, which remained stable in each of the 3 measured points even at the end of the 1-year follow-up.

Only a few reports were carried out on the changes of corneal thickness during CXL treatment.[52,54,55] Using isotonic solution, Kymionis et al. detected a mean of 75 µm CCT decrease subsequent to epithelial removal with ultrasound pachymetry measurement.[56] Other publications observed a mean decrease in corneal thickness by 50-65 µm at 10 and 30 minutes after isotonic riboflavin application.[54] Holopainen et al. using ultrasound pachymeter found a thinning of a mean 87 µm during the 1-hour operation.[55] However they reported a significantly lower value in corneal thickness 1 month after treatment compared to preoperative values, which contradicts our data, as we detected an initial value already on postoperative day 3.

In summary, significant decrease in corneal thickness can be observed during CXL treatment due to the effect of the isotonic riboflavin solution. An initial corneal thickness was reached already within 3 days after treatment which remained stable till the end of the 1 year follow-up period.

I. c.

MANAGEMENT OF PELLUCID MARGINAL DEGENERATION USING COLLAGEN CROSS-LINKING

Case report and discussion

In our study, a case diagnosed as bilateral, clinically and topographically pellucid marginal degeneration was reported, where CXL treatment was performed on one eye.

A 55-year-old man presented at our department for an ophthalmological examination with a progressive bilateral visual impairment mostly in the right eye. The patient with a history of primary open angle glaucoma and pseudophakia in the right eye was diagnosed with diabetes mellitus 9 years before. Prior to the examination period, he had worn rigid contact lens which he was no longer capable of wearing.

A bilateral PMD was demonstrated on the basis of slit-lamp, Pentacam HR examinations and corneal topography. A pachymetry map was created of the cornea using Pentacam HR: a significant corneal thinning was observed in the inferior segment of the right eye. In logMAR scale, best corrected vision of +0.8 was recorded in the right eye and that of +0.1 in the left eye. The topographic map showed corneal ectasia in the right eye.

The collagen cross-linking treatment was performed in the right eye. During the follow-up period of 8 months the best corrected vision was +0.25 and an improvement in SK1 and SK2 values were also detected. No progressive ectasia was detected on the posterior cornea according to the Pentacam HR findings. 8 months after CXL treatment central corneal thickness showed a minimal increase (from 520 μm to 528 μm).

Like those of the other corneal ectasia, the pathogenesis of PMD still remains unclear. Corneal topography verifies the thinning of the inferior cornea spreading towards the central part. In our case both corneas were involved. PMD management may include intrastromal ring implantation,[57] lamellar, crescent lamellar or perforating keratoplasty using contact lens, though conservative ways of management are also know.[58] Stojanovic has already reported on the combination of topography-guided ablation and CXL in PMD patients, after which an improvement in vision and a decrease in the asymmetry of keratometric values could be achieved.[59] Spadea reported on the eccentric CXL treatment also conducted in our study.[60] Kymionis[61] carried out simultaneous photorefractive keratectomy and CXL in PMD patients achieving outstanding results. In our study eccentric CXL treatment itself could stop progression and could significantly improve visual acuity.

To summarize our investigations about keratectasia, we experienced the efficiency of CXL treatment in managing both keratoconus and pellucid marginal degeneration. With corneal topography the decrease in the progression of ectatic diseases can be well demonstrated or could be as well stopped. Also, a decrease in refraction power and a subjective/objective improvement in vision could be detected. The period of contact lens wearing could be prolonged and surgical intervention could be avoided. No complications could be observed, the deeper regions were not damaged. Corneal thickness shows a significant change during treatment, but its value reaches the preoperative one already on the third postoperative day and remains unchanged in the first postoperative year. The method strengthening the anterior 200-250 μm of the cornea could be applied for treating not only keratoconus but corneal ulcers and bullous keratopathy, and for preventing keratectasia developing after excimer laser procedures. It is a technically simple, but time-consuming treatment.

II.

TEAR OSMOLARITY PRIOR- AND SUBSEQUENT TO LASIK

Patients

In present study 15 young patients prior to LASIK treatment were investigated. Patients had no history of ophthalmologic events, damage, operation, or ophthalmologic symptoms due to other systemic diseases other than refractive error. Thirty eyes of the 15 patients (10 women and 5 men) were examined with TearLab osmometer; their mean age was 30.55±11.79 years (range from 18 to 56).

Treatments were conducted using In-Pro Gauss excimer laser device (Norderstedt, Germany) and Zyoptix XP microkeratome (Bausch & Lomb Inc., Germany). After treatment, artificial tear were also administered in both eyes every hour on the operation day, then minimum 5 times daily for 90 days. In addition, patients received a combined treatment including tobramycine and dexamethasone eye drops 5 times for 1 week.

Methods

After taking the history, subjective dry eye complaints were evaluated using the Ocular Surface Disease Index Questionnaire (OSDI). The questionnaire measures the frequency of individual dry eye symptoms.[62] Subsequent to visual acuity measurement, tear osmolarity was assessed with the TearLab Osmolarity Test device. Using no topical anaesthesia, the system pen was placed onto the inferior lateral tear meniscus. Using the capillary action, the device takes a sample of 50 nanoliters of the tear, and displays the measured value in milliosmol/liter (mOsm/l) unit. An osmolarity result of ≥316 mOsms/L has been suggested for differentiating dry eye patients.[63]

At slit-lamp examination, the state of lid parallel conjunctival folds (LIPCOF) was observed and their absence and presence in the lower temporal quadrant were scored on a 0-3 scale according to Höh's qualification.[64] LIPCOF score is based on tear meniscus height and the separately calculated number of conjunctival folds where 0 represents the absence of the folds and 3 represents multiple folds with bigger height than tear meniscus. In our study, a LIPCOF score of ≥1 was rated as abnormal. Slit-lamp examination was used to analyse the state of the Meibomian glands and their pipes which was rated on a numerical scale (healthy eyelid=0, Meibomian glands dysfunction=1). The classical Copenhagen tests were conducted in a given order. At first Schirmer I test was performed without topical anaesthesia using a standardized filter strip (Bausch&Lomb Inc., Berlin, Germany) by inserting into the lower conjunctival sac. After 5 minutes, a result of ≤10 mm was used as a threshold value for dry eye. This was followed by the evaluation of tear film break-up time (BUT), fluorescein staining of the eye surface and the observation of corneal staining. A BUT of ≤10 seconds and the presence of more than 4 stained points on the cornea were recognized as abnormal.

Results

Preoperative OSDI results were 10.59±8.97 which significantly improved by follow-up day 60 (p=0.001). Tear osmolarity value was 303.62±12.29 mOsm/l prior to LASIK (95% CI:

299.66-309.54 mOsm/l) and 303.58±20.14 mOsm/l on postoperative day 60 (295.08-312.09 mOsm/l) (p=0.69). Tear osmolarity values were within the normal range 60 days postoperatively. LIPCOF score was 0.68±0.68 preoperatively and 0.58±0.65 (p=0.25) on postoperative day 60. No meibomian gland dysfunction was observed on day 1, day 30 and day 60 (p=0.40; p=0.25; p=0.52; respectively). Schirmer-test value was 25.55±8.06 mm preoperatively, on postoperative day 60 it was 22.71±11.09 mm (p=0.36). Preoperative tear film break-up time was 12.52±6.35 second, on postoperative day 60 it was 12.25±9.02 second (p=0.51). No significant corneal staining was detected prior to operation. On the first postoperative day corneal staining could be still observed, but this was due to the absence of the corneal epithelium. No further staining could be observed during the rest of the follow-up period.

Discussion

Tear film hyperosmolarity play an important role in the progression of clinical signs and subjective symptoms in dry eye syndrome. The hyperosmolar stress of the corneal epithelium leads to surface inflammation and morphological alteration of the epithelial cells.[65-67]

Dry eye is a syndrome affecting 5-30 % of the population and is common in patients waiting for refractive surgeries.[68-70] Patients who underwent LASIK surgery and with basically dry eyes often present severe post-LASIK dry eye syndrome. With these patients LASIK induced neurotrophic epitheliopathy is more common compared to LASIK patients with normal eyes.[70] With confocal microscopy greater impairment of corneal innervation can be observed in patients subsequent to LASIK intervention.[71] Traditional eye dry tests (Schirmer test, BUT) showed no correlation with the regeneration of corneal nerves after refractive surgeries, however, significant correlation is assumed between tear osmolarity and nerve regeneration.[71]

In our study, tear osmolarity measurement was based on the limit value set in the metaanalysis containing the reviewed values of literature of 27 years, as suggested by Tomlinson and associates.[63] Since the introduction of TearLab osmometer a number of results have been reported and several authors have suggested a value above 308 mOsm/l for differentiating dry eye syndrome.[72-74] TearLab is a portable, easy-to-use and non-invasive diagnostic device. It allows for quick measurements, however is rather expensive. Studies conducted with the device also calls the attention to the assessment of the difference in tear osmolarity between the two eyes.[72]

The application of TearLab in clinical practice is emphasized by most authors, and due to the outstanding efficacy of the test, its introduction to every-day usage is strongly suggested. According to our findings, LIPCOF, Schirmer- and BUT tests are of appropriate efficiency for diagnosis. In the research of Mesmer and his co-workers, no correlation was found between the outcomes of tear osmolarity and classical tests.[75]

It is needed to be emphasized that TearLab provides information about tear meniscus osmolarity conditions, and its results allow us only to deduce the real osmolarity of the tear film. For that reason, the reading of TearLab device in itself can not be evaluated but only when it is combined with conventional dry eye tests. As hyperosmolarity plays a crucial central role in the progression of symptoms and clinical signs, the test can call attention to a possible dry eye development already at an early stage. Versura and associates employed TearLab combined with conventional tests for osmolarity measurements for the diagnosis of dry eye, and strongly suggested its application for clinical purposes.[76]

Post-LASIK dry eye is a highly decisive factor for patient satisfaction and the outcome of the intervention. Therefore, it is not only the recognition of a dry eye syndrome that is of a great importance prior to surgery but also its appropriate treatment after operation.[77]

Lee and co-workers conducted tear osmolarity measurements with traditional tests prior to and after LASIK and PRK procedures for 3-6 months, observed a higher decrease in tear production after LASIK than PRK, and suggested the administration of artificial tears for a minimum of 6 months after procedure.[78]

In our investigation, no pathological abnormality in values of dry eye syndrome was detected, neither prior to- nor subsequent to LASIK procedure involving regular administration of tear drops. Tear film hyperosmolarity is regarded to play a key role in the progression of clinical signs and subjective symptoms in dry eye syndrome. We highly recommend conducting tear osmolarity measurements using the TearLab device in combination with traditional dry eye tests. Our results showed no significant correlation between tear osmolarity and the results obtained with classical tests in our proband group. LASIK is considered be a safe procedure for dry eye syndrome, when adequate artificial tear film is administered for a minimum period of 3 months.

SUMMARY OF NEW RESULTS

1. Corneal collagen cross-linking stopped keratoconus progression for at least 36 months which was proved by the monitoring of topographic indices. Moreover, postoperative visual acuity showed no further decrease.
2. A significant decrease in intraoperative central corneal thickness was observed when using isotonic riboflavin solution which was not detectable 3 days after CXL treatment and remained stable for at least one year.
3. We reported a rare case of collagen cross-linking treatment in pellucid marginal degeneration, which proved its efficiency in inhibiting ectatic progression.
4. We proved that tear osmolarity does not increase significantly after LASIK surgery in non-dry eye patients, measured by TearLab device.

References

1. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998;42:297-319.
2. Karseras AG, Ruben M. Aetiology of keratoconus. Br J Ophthalmol 1976;60:522-555.
3. Krachmer JH. Pellucid marginal degeneration. Arch Ophthalmol 1978;96:1217-1221.
4. Eysteinsson T, Jonasson F, Sasaki H, Arnarsson A, Sverrisson T, Sasaki K, Stefansson E, Reykjavik Eye Study Group: Central corneal thickness, radius of the corneal curvature and intraocular pressure in normal subjects using non-contact techniques. Acta Ophthalmol Scand 2002;80:11-15.
5. Marsich MW, Bullimore MA. The repeatability of corneal thickness measures. Cornea 2000; 19:792-795.
6. Kremer FB, Walton P, Gensheimer G. Determination of corneal thickness using ultrasonic pachometry. Ann Ophthalmol 1985;17:506-507.
7. Yaylali V, Kaufman SC, Thompson HW. Corneal thickness measurements with the Orbscan Topography System and ultrasonic pachymetry. J Cataract Refract Surg 1997;23:1345-1350.
8. Liu Z, Huang AJ, Pflugfelder SC. Evaluation of corneal thickness and topography in normal eyes using the Orbscan corneal topography system. Br J Ophthalmol 1999;83:774-778.
9. Módis L, Berta A, B Seitz. Az Orbscan cornea-topográf. Szemészet 2002;139:23-28.
10. Gonzalez-Meijome JM, Cervino A, Yebra-Pimentel E, Parafita MA. Central and peripheral corneal thickness measurement with Orbscan II and topographical ultrasound pachymetry. J Cataract Refract Surg 2003;29:125-132.
11. Rüfer F, Schröder A, Arvani MK, Erb C. Central and peripheral corneal pachymetry-standard evaluation with the Pentacam system. Klin Monbl Augenheilkd 2005;222:117-122.
12. O'Donnell C, Maldonado-Codina C. Agreement and repeatability of central thickness measurement in normal corneas using ultrasound pachymetry and the OCULUS Pentacam. Cornea 2005;24:920-924.
13. Colin J, Malet FJ. Intacs for the correction of keratoconus: Two-year follow-up. J Cataract Refractive Surg 2007;33:69-74.
14. Brierly SC, Izquierdo L Jr, Mannis MJ. Penetrating keratoplasty for keratoconus. Cornea 2000;9:329-332.
15. Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen Fiber Diameter in the Rabbit Cornea After Collagen Crosslinking by Riboflavin/UVA. Cornea 2004;23:503-507.
16. Mencucci R, Mazzotta C, Rossi F, Ponchietti C, Pini R, Baiocchi S, Caporossi A, Menchini U. Riboflavin and ultraviolet A collagen crosslinking: In vivo thermographic analisis of the corneal surface. J Cataract Refractive Surg 2007;33:1005-1008.
17. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A -induced crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620-627.
18. Spörl E, Raiskup-Wolf F, Pillunat LE. Biophysical principles of collagen cross-linking. Klin Monatsbl Augenheilkd 2008;225:131-137.
19. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A-induced collagen cross linking. J Cataract Refract Surg 2003;29;1780-1785.
20. Menter JM, Patta AM, Sayre RM, Dowdy J, Willis I. Effect of UV irradiation on type I collagen fibril formation in neutral collagen solutions. Photodermatol Photoimmunol Photomed 2001;17:114-120.

21. Wollensak G, Aurich H, Pham DT, Wirbelauer C. Hydration behavior of porcine cornea crosslinked with riboflavin and ultraviolet A. J Cataract Refract Surg 2007;33:516-521.
22. Caporossi A, Baiocchi S, Mazzotta C, Traversi C, Caporossi T. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A rays induced cross-linking of corneal collagen; preliminary refractive results in an Italian study. J Cataract Refract Surg 2006;32:837-845.
23. Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet A corneal collagen cross-linking for keratoconus in Italy: the Siena Eye Cross Study. Am J Ophthalmol 2010;149:585-593.
24. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: longterm results. J Cataract Refract Surg 2008;34:796-801.
25. Grewal DS, Brar GS, Jain R, Sood V, Singla M, Grewal SP. Corneal collagen crosslinking using riboflavin and ultraviolet-A light for keratoconus; one-year analysis using Scheimpflug imaging. J Cataract Refract Surg 2009;35:425-432.
26. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. Cornea 2006;25:1057-1059.
27. Spoerl E, Wollensak G, Dittert DD, Seiler T. Thermomechanical Behavior of collagen-cross-linked porcine cornea. Ophtalmologica 2004;218:136-140.
28. Schilde T, Kohlhaas M, Spoerl E, Pillunat LE. Enzymatic evidence of the depth dependence of stiffening on riboflavin/UVA treated corneas. Ophtalmologe 2008;105:165-169.
29. Rocha KM, Ramos-Esteban JC, Quian Y, Herkar S, Krueger RR. Comparative study of riboflavin-UVA cross-linking and „flash-linking" using surface wave elastometry. J Refract Surg 2008;24:S748-751.
30. Gilbard JP, Dartt DA. Changes in rabbit lacrimal gland fluid osmolarity with flow rate. Invest Ophthalmol Vis Sci 1982;23:804-806.
31. Gilbard JP, Rossi SR, Gray KL. A new rabbit model for keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci 1987;28:225-228.
32. Módis L , Fodor M , Berta A. A conjunctivalis impressziós citológia szerepe a száraz szem diagnózisában. Szemészet 2007;144:171-175.
33. Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease. Arch Ophthalmol 1984;102:1049-1051.
34. Ralph RA. Conjunctival goblet cell density in normal subjects and in dry eye syndromes. Invest Ophthalmol 1975;14:299-302.
35. Caporossi A, Baiocchi S, Mazzotta C, Traversi C, Caporossi T. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A rays induced cross linking of corneal collagen: preliminary refractive results in an Italian study. J Cataract Refract Surg 2007;32:837-845.
36. Chan CCK, Charma M, Wachler BS. Effect of inferior-segment Intacs with and without C3R on keratoconus. J Refract Surg 2007;33:75-80.
37. Spoerl E, Wollensak G, Seiler T. Increased resistance of crosslinked cornea against enzymatic digestion. Current Eye Research 2004;29:35-40.
38. Wollensak G, Aurich H, Wirbelauder C, Pham DT. Potential use of riboflavin/UVA cross-linking in bullous kerathopathy. Ophtalmic Res 2008;41:114-117.
39. Wollensak G, Spoerl E, Wilsch M, Seiler T. Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit. J Cataract Refract Surg 2003;29:786-1790.
40. Romppainen T, Bachmann LM, Kauffmann C, Kniestedt C, Mrochen M, Thiel MA. Effect of riboflavin-UV-A-induced collagen cross-linking on intraocular pressure measurement. Invest Ophtalmol Vis Sci 2007;48:5494-5498.

41. Mazzotta C, Balestrazzi A, Traversi C, Baiocchi S, Caporossi T, Tommasi C, Caporossi A.Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructural analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. Cornea 2007;26:390-397.
42. Touboul D, Efron N, Smadja D, Praud D, Malet F, Colin J. Corneal Confocal Microscopy Following Conventional, Transepithelial, and Accelerated Corneal Collagen Cross-linking Procedures for Keratoconus. J Refract Surg 2012;28:769-776.
43. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol 2006;17:356-360.
44. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA-riboflavin crosslinking of the cornea. Cornea 2007;26:385-389.
45. Kymionis GD, Diakonis VF, Coskunseven E, Jankov M, Yoo SH, Pallikaris IG. Customized pachymetric guided epithelial debridement for corneal collagen cross linking. BMC Ophthalmol 2009 Aug 28;9:10. doi: 10.1186/1471-2415-9-10.
46. Greenstein SA, Shah VP, Fry KL, Hersh PS. Corneal thickness changes after collagen crosslinking for keratoconus and corneal ectsia: one-year result. J Cataract Refract Surg 2011;37:691-700.
47. Koller T, Iseli HP, Hafezi F, Vinciguerra P, Seiler T. Scheimpflug imaging of corneas after collagen cross-linking. Cornea 2009;28:510-515.
48. de Sanctis U, Missolungi A, Mutani B, Richiardi L, Grignolo FM. Reproducibility and repeatability of central corneal thickness measurement in keratoconus using the rotating Scheimpflug camera and ultrasound pachymetry. Am J Ophthalmol 2007;144:712-718.
49. Grewal DS, Brar GS, Grewal SPS. Assessment of central corneal thickness in normal, keratoconus, and post-laser in situ keratomileusis eyes using Scheimpflug imaging, spectral domain optical coherence tomography, and ultrasound pachymetry. J Cataract Refract Surg 2010;36:954-964.
50. Mazzotta C, Traversi C, Baiocchi S, Caporossi O, Bovone C, Sparano MC, Balestrazzi A, Caporossi A. Corneal healing after riboflavin ultraviolet-A collagen cross-linking determined by confocal laser scanning microscopy in vivo: early and late modifications. Am J Ophthalmol 2008;146:527-533.
51. Reinstein DZ, Archer TJ, Gobbe M, Silverman RH, Coleman DJ. Epithelial thickness in the normal cornea: three-dimensional display with Artemis very high-frequency digital ultrasound. J Refract Surg 2008;24: 571-581.
52. Kymionis GD, Kounis GA, Portaliou DM, Grentzelos MA, Karavitaki AE, Coskunseven E, Jankov MR, Pallikaris IG. Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation. Ophthalmology 2009; 116:2336-2339.
53. Vinciguerra P, Albè E, Trazza S, Rosetta P, Vinciguerra R, Seiler T, Epstein D. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. Ophthalmology 2009;116: 369-378.
54. Kaya V, Utine CA, Ylmaz OF. Intraoperative corneal thickness measurements during corneal cross-linking with hypoosmolar riboflavin solution in thin corneas. Cornea 2012;31:486-490.
55. Holopainen JM, Krootila K. Transient corneal thinning in eyes undergoing corneal cross-linking. Am J Ophthalmol 2011;152:533-536.
56. Kymionis GD, Kounis GA, Portaliou DM, Grentzelos MA, Karavitaki AE, Coskunseven E, Jankov MR, Pallikaris IG. Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation. Ophthalmology 2009;116:2336-2339.

57. Ertan A, Bahadit M. Intrastromal ring segment insertion using a femtosecond laser to correct pellucid marginal corneal degeneration. J Cataract Refract Surg 2006;32:1710-1716.
58. Forooghian F, Assaad D, Dixon WS. Successful conservative treatment of hydrops with perforation in pellucid marginal degeneration. Can J Ophthalmol 2006;41:74-77.
59. Stojanovic A, Zhang J, Chen X, Nitter TA, Chen S, Wang Q. Topography-guided transepithelial surface ablation followed by corneal collagen cross-linking performed in a single combined procedure for the treatment of keratoconus and pellucid marginal degeneration. J Refract Surg 2010;26:145-152.
60. Spadea L. Corneal collagen cross-linking with riboflavin and UVA irradiation in pellucid marginal degeneration. J Refract Surg 2010;26:375-377.
61. Kymionis GD, Karavitaki AE, Kounis GA, Portaliou DM, Yoo SH, Pallikaris IG. Management of pellucid marginal corneal degeneration with simultaneous customized photorefractive keratectomy and collagen crosslinking. J Cataract Refract Surg 2009;35:1298-1301.
62. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 2000;118:615-621.
63. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. Invest Ophthalmol Vis Sci 2006;47:4309-4315.
64. Höh H, Schirra F, Kienecker C, Ruprecht KW. Lid-parallel conjunctival folds are a sure diagnostic sign of dry eye. Ophthalmologe 1995;92:802-808.
65. Gilbard JP, Rossi SR, Gray KL. A new rabbit model for keratoconjunctivitis sicca. Invest Ophthalmol Vis Sci 1987;28:225-228.
66. Gilbard JP, Carter JB, Sang DN, Refojo MF, Hanninen LA, Kenyon KR. Morphologic effect of hyperosmolarity on rabbit corneal epithelium. Ophthalmology 1984;91:1205-1212.
67. Lemp MA, Baudouin C, Baum J et al. The definition and classification of dry eye disease: Report of the definition and classification subcommittee of the international Dry Eye WorkShop (2007). Ocul Surf 2007;5:75-92.
68. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. Ophthalmology 1998;105:1114-1119.
69. Shimmura S, Shimazaki J, Tsubota K. Results of a population-based questionnaire on the symptoms and lifestyles associated with dry eye. Cornea 1999;18:408-411.
70. Toda I, Asano-Kato N, Hori-Komai Y, Tsubota K. Laser-assisted in situ keratomileusis for patients with dry eye. Arch Ophthalmol 2002;120:1024-1028.
71. Lee SJ, Kim JK, Seo KY, Kim EK, Lee HK. Comparison of corneal nerve regeneration and sensitivity between LASIK and laser epithelial keratomileusis (LASEK). Am J Ophthalmol 2006;141:1009-1015.
72. Eldridge DC, Sullivan BD, Berg MD, Lemp MA, Durrie DS. Longitudinal Variability of Tear Film Osmolarity in Normal and Dry Eye Patients. ARVO Abstract 3379/D965, 2010.
73. Foulks GN, Lemp MA, Berg M, Bhola R, Sullivan BD. TearLab™ Osmolarity as a biomarker for disease severity in mild to moderate dry eye disease. AAO Abstract PO382, 2009.
74. Sullivan BD, Eldridge DC, Berg M, Kosheleff V, Porreco A, Truitt J, Lemp MA. Diagnostic performance of osmolarity combined with subset markers of dry eye disease in an unstratified patient population. ARVO Abstract 3380/ D966, 2010.
75. Messmer EM, Bulgen M, Kampik A. Hyperosmolarity of the tear film in dry eye syndrome. Dev Ophthalmol 2010;45:129-138.

76. Versura P, Profasio V, Campos EC. Performance of tear osmolarity compared to previous diagnostic tests for dryeye diseases Curr eye Res 2010:35:553-564.
77. Ambrósio R Jr, Tervo T, Wilson SE. LASIK-associated dry eye and neurotrophic epitheliopathy: pathophysiology and strategies for prevention and treatment. J Refract Surg 2008;24:396-407.
78. Lee JB, Ryu Ch Kim J, Kim EK, Kim HB. Comparison of tear secretion and tear film instability after photorefractive keratectomy and laser in situ keratomileusis. J Cataract Refract Surg 2000;26:1326-1331.

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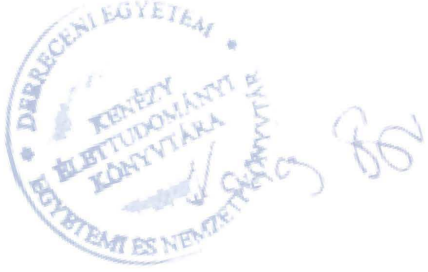
List of publications related to the dissertation

1. **Hassan, Z.**, Módis, L., Szalai, E., Berta, A., Németh, G.: Intra- and postoperative corneal thickness changing after collagen cross-linking therapy.
   Eur. J. Ophthalmol. "accepted by publisher", 2013.
   IF:0.912 (2012)

2. **Hassan, Z.**, Németh, G., Módis, L., Szalai, E., Berta, A.: Collagen cross-linking in the treatment of pellucid marginal degeneration.
   Indian J. Ophthalmol. Epub ahead of print (2013)
   IF:0.797 (2012)

3. **Hassan, Z.**, Szalai, E., Módis, L., Berta, A., Németh, G.: Assessment of corneal topography indices after collagen crosslinking for keratoconus.
   Eur. J. Ophthalmol. 23 (5), 635-640, 2013
   DOI: http://dx.doi.org/10.5301/ejo.5000249
   IF:0.912 (2012)

4. **Hassan, Z.**, Szalai, E., Berta, A., Módis, L., Németh, G.: Assessment of tear osmolarity and other dry eye parameters in post-LASIK eyes.
   Cornea. 32 (7), e142-145, 2013
   IF:1.746 (2012)



List of other publications

5. Németh, G., **Hassan, Z.**, Szalai, E., Berta, A., Módis, L.: Scheimpflug imaging in anterior megalophthalmos.
Indian J. Ophthalmol. 61 (1), 32-35, 2013.
DOI: http://dx.doi.org/10.4103/0301-4738.105052
IF:0.797 (2012)

6. Németh, G., **Hassan, Z.**, Csutak, A., Szalai, E., Berta, A., Módis, L.: Repeatability of ocular biomechanical data measurements with a Scheimpflug-based noncontact device on normal corneas.
J. Refract. Surg. 29 (8), 558-563, 2013
IF:2.474 (2012)

7. Németh, G., **Hassan, Z.**, Szalai, E., Berta, A., Módis, L.: Anterior segment parameteres measured with two optical devices compared to ultrasonic data.
Eur. J. Ophthalmol. 43 (3), 177-182, 2013
DOI: http://dx.doi.org/10.5301/ejo.5000214
IF:0.912 (2012)

8. Németh, G., **Hassan, Z.**, Szalai, E., Berta, A., Módis, L.: Analysis of Age-Dependence of the Anterior and Posterior Cornea With Scheimpflug Imaging.
J. Refractive Surg. 29 (5), 326-331, 2013
DOI: http://dx.doi.org/10.3928/1081597X-20130301-01
IF:2.474

9. Szalai, E., Berta, A., **Hassan, Z.**, Módis, L.: Reliability and repeatability of swept source Fourier domain optical coherence tomography and Scheimpflug imaging in keratoconus.
J. Cataract. Refract. Surg. 38 (3), 485-494, 2012.
DOI: http://dx.doi.org/10.1016/j.jcrs.2011.10.027
IF:2.527

10. Németh, G., **Hassan, Z.**, Módis, L., Szalai, E., Katona, K., Berta, A.: Comparison of anterior chamber depth measurements conducted with Pentacam HR(®) and IOLMaster(®).
Ophthalmic Surg. Lasers Imaging. 42 (3), 144-147, 2011.
DOI: http://dx.doi.org/10.3928/15428877-20110210-03
IF:1.042

11. Szalai, E., Berta, A., Németh, G., **Hassan, Z.**, Módis, L.: Anterior chamber depth measurements obtained with Pentacam HR(®) imaging system and conventional A-scan ultrasound.
Ophthalmic Surg. Lasers Imaging. 42 (3), 248-253, 2011.
DOI: http://dx.doi.org/10.3928/15428877-20110210-04
IF:1.042

12. Németh, G., **Hassan, Z.**, Szalai, E., Berta, A., Módis, L.: Comparative analyses of white-to-white and angle-to-angle distance measurements with IOLMaster and Visante OCT.
J. Cataract. Refract. Surg. 36 (11), 1862-1866, 2010.
DOI: http://dx.doi.org/10.1016/j.jcrs.2010.05.017
IF:2.942

13. Csutak, A., Silver, D.M., Tőzsér, J., Steiber, Z., Bagossi, P., **Hassan, Z.**, Berta, A.: Plasminogen activator inhibitor in human tears after laser refractive surgery.
J. Cataract. Refract. Surg. 34 (6), 897-901, 2008.
DOI: http://dx.doi.org/10.1016/j.jcrs.2008.02.024
IF:2.508

14. Módis, L., Sohajda, Z., Komár, T., **Hassan, Z.**, Berta, A.: Die klinisch-pathologischen Merkmale des Keratoglobus.
Klinische Monatsblat. Augenheilkunde. 222 (6), 505-508, 2005.
DOI: http://dx.doi.org/10.1055/s-2005-858171
IF:0.412

15. Csutak, A., Silver, D.M., Tőzsér, J., **Hassan, Z.**, Berta, A.: Urokinase-Type Plasminogen Activator to Prevent Haze after Photorefractive Keratectomy, and Pregnancy as a Risk Factor for Haze in Rabbits.
Invest. Ophthalmol. Vis. Sci. 45 (5), 1329-1333, 2004.
DOI: http://dx.doi.org/10.1167/iovs.03-0881
IF:3.577

16. Csutak, A., Tőzsér, J., Békési, L., **Hassan, Z.**, Berta, A., Silver, D.M.: Plasminogen activator activity in tears after excimer laser photorefractive keratectomy.
Invest. Ophthalmol. Vis. Sci. 41 (12), 3743-3747, 2000.
IF:4.373

17. **Hassan, Z.**, Lampé, Z., Békési, L., Berta, A.: Excimer laser photorefractive keratectomy with different ablation zones.
Acta Chir. Hung. 36, 122-124, 1997.

Total IF: 29.447

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