Evaluation of changes in corneal morphology and sensory functions in patients with keratoconus

PhD thesis

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Budapest
2017

Introduction

Keratoconus has been recognized for more than 150 years by ophthalmologists as part of the group called corneal „thinning disorders”. The name keratoconus comes from the Greek word (kerato: Cornea; konos: Cone). Exact definition of the disease is not easy, but key findings for diagnosis are bilateral clinical non-inflammatory posterior ectasia with abnormal corneal thickness distribution which involves the central two-thirds of the cornea. Modern and more precise diagnostic tools such as corneal tomography, has increased the ability of ophthalmologist to recognize keratoconus and corneal ectasia at a much earlier stage than previously possible. Regarding the increasing diagnostic potential the previously established prevalence of keratoconus in the general population has changed from 50/100 000 to a much higher prevalence rate of 50-230/100 000.

Global prevalence of refractive errors are also increasing. Solely myopia will affect an estimated 4758 million people globally (and moreover 938 million with high myopia) by 2050. Hyperopia (8.4 % of the USA population of age 40 and older) and corneal astigmatism (1 in 3 people in the USA) also affect a significant population worldwide.

Nowadays, in everyday life and during work one have to face a high amount of visual information. There is a need that people could process and respond to stimuli very fast during our accelerated life pace. Most of the stimuli comes through the visual system. These high standards and the spread of refractive laser procedures generate the need for perfect vision. An estimated 8.4 million people in the USA from 1995 to 2013 had undergone refractive surgery (including all types of refractive procedures). The most feared post-operative complication for laser refractive surgery is corneal ectasia after treatment. The pre-operative risk factors for post-treatment corneal ectasia are high myopia, low preoperative corneal thickness, residual stromal bed (RSB) thickness less than 250 μm, younger age and keratoconus (especially forme fruste keratoconus). Present time there is a lack of consensus in the ultimate diagnostic criteria for keratoconus (subclinical forms), although early recognition of keratoconus is curtail in the screening of refractive surgery candidates.

Corneal nerves plays an important role in maintaining the integrity of the human cornea. The vast majority of corneal nerves are sensory types, and their main function is to protect the ocular surface against harmful impacts. Changes in the anterior segment of the keratoconic cornea also influence the corneal nerves and their functions. Nerve dysfunction in advanced keratoconus is well known for decades, but the exact origin and the correlation with the disease severity is unclear. Weather sensory dysfunction is a cause or a consequence is still unknown. Corneal esthesiometry could give exact and comparable information about the

different type of sensory nerve functions, and could give additional information during decision making/screening.

Keratoconus screening and early diagnosis is mandatory when laser refractive surgery candidates are selected.

Objectives

Our research group aimed to evaluate and to compare the tomographic and topographic corneal values of normal and early stage keratoconus patient's eyes. Our aim was to find a reliable method to recognize keratoconus as early as possible with high accuracy. Secondly, our purpose was to evaluate corneal sensitivity changes in keratoconus patients, and to assess the relationship between keratoconus severity and corneal sensitivity. The purpose of these investigations was to study keratoconus from both functional and morphological aspects. Our focus was on the relation between keratoconus severity, corneal sensory changes and dry eye symptoms connected with tear film dynamics. Whether functional changes like corneal sensory disturbances are a cause or a consequence? The purpose of our research was:

- To assess the relationship between keratoconus severity and intereye asymmetry of corneal tomography values.
- Evaluate their combined accuracy in discriminating normal corneas from those with early signs of keratoconus.
- To investigate changes in corneal sensitivity to selective mechanical, chemical, and thermal stimulation in keratoconus.
- To asses if there is any correlation present between different stages of keratoconus and changes in corneal sensitivity.
- Evaluate the relation between dry eye symptoms and changes in corneal sensitivity in patients with keratoconus.

Methods

The clinical studies were performed at the Semmelweis University, Department of Ophthalmology between 2012 and 2015. The studies were conducted in compliance with the Declaration of Helsinki, applicable national and local requirements regarding the ethics committee and institutional review boards. Ethical approval was obtained from the Institutional Review Board (Semmelweis University Regional and Institutional Committee of Sciences and Research Ethics). A written informed consent was obtained before the examination from each patient or from the parent on behalf of the minors/children.

A) *Evaluation of intereye corneal asymmetry in patients with keratoconus vs. healthy patients, with the guidance of Scheimpflug imaging*

The keratoconus group comprised 64 eyes of 32 patients (15 men, 17 women) with a mean age of 36.98±12.34 years. The control group comprised 130 eyes of 65 patients (29 men, 36 women) with a mean age of 39.95±15.44 years.

B) *Evaluation of corneal sensitivity and dry eye symptoms in patients with keratoconus vs. healthy patients with Belmonte's gas esthesiometer*

The keratoconus group (KC group) included one randomized eye of 19 patients (28.9±6.3 years) with bilateral mild or moderate keratoconus and in the control group 20 eyes of 20 healthy refractive surgery candidates were enrolled (30.2±5.3 years) of both sexes.

Patients

Eyes with severe keratoconus were excluded because of difficulties in topographic map acquisition and potential stromal haze or scar formation, which can alter the optical transparency of the cornea and thus Scheimpflug imaging. Both eyes of each patient had a complete ophthalmologic evaluation including slit lamp biomicroscopy, keratometry, retinoscopy, slit lamp indirect ophthalmoscopy, and Placido disk–based videokeratography (TOMEY TMS-4 corneal topographer; TOMEY Corp., Nagoya, Japan). Diagnosis was based on classic corneal biomicroscopic and topographic findings in accordance with the criteria of Rabinowitz et al. Inclusion criteria for the control group included a refractive error less than +/– 5.00 diopters (D) sphere and astigmatism less than +/– 3.00 D. None of the control patients had a history of previous ocular disease, surgery or trauma. Rigid contact lenses were not worn for 4 weeks and soft contact lenses for at least 1 week before assessment in either

group. Patients were asked whether they rubbed their eyes or experienced previous ocular trauma.

Participants in the control group (esthesiometry study) did not have any clinical signs and/or symptoms of dry eye (ocular surface disease index—OSDI score <10) or significant ocular surface disease and were not using eye drops. Subjects with ophthalmic conditions other than keratoconus including blepharitis, meibomitis, lid abnormalities as well as contact lens wearers were also excluded. Both eyes of each patient had a complete ophthalmologic evaluation including slitlamp biomicroscopy, ophthalmoscopy, Scheimpflug imaging and assessment of tear flow and non-invasive tear film breakup time were performed. Subjects who showed significant corneal staining (>Grade 2, Oxford Scale) were excluded because corneal epitheliopathy could potentially be a confounding factor affecting the ocular surface sensory responses.

Scheimpflug imaging in evaluation of intereye corneal asymmetry

All eyes were examined with the Pentacam HR Scheimpflug camera, used by three trained examiners without application of dilating or anaesthetic eye drops or previous tonometry. The readings were taken as recommended in the instruction manual. The measurement results were checked under the quality specification (QS) window, only the correct measurements ('QS' reads OK) were accepted; if the comments were marked yellow or red, the examination was repeated. In all cases one reading taken from an eye was saved and processed for further statistical analyses. For local posterior elevation measurements, the reference surface was set to best fit sphere (BFS) with fixed 8- mm-diameter settings. Keratometry at the steep (K_s) and flat (K_f) meridians, central corneal thickness (CCT), pachymetry at the thinnest point (ThCT) and posterior elevation at the thinnest point of the cornea (PE) were measured in both eyes. Intereye asymmetry of pachymetry and elevation data was determined by subtracting the lower value from the higher value for each variable. The better and worse eyes were designated for each keratoconus patient based on each variable (i.e. the worse eye is with higher K_s, K_f, PE and lower CCT and ThCT).

Statistical analysis in evaluation of intereye corneal asymmetry

Statistical analysis was performed with SPSS software (version 15.0, SPSS, Inc.). The Shapiro-Wilk W test was used to confirm normal distribution of the variables. Paired samples t-test was used to compare means between eyes of the same subject (within-subject variance). Linear regression was used to test significant correlation between parameters of the two eyes

of the same subject (within-subject correlation). The repeated measures analysis of variance test (ANOVA) was used to analyze the differences between group means and their associated procedures (within-group and between-group variances). This test allows to compare within-subject parameters (better eye vs. worse eye) in the two study groups by taking into account between-eye correlations by treating data from eyes of patients in statistical analysis as repeated measures. Correlation between keratoconus severity and intereye asymmetry was tested using linear and non-linear regression analysis in each group. In this study keratoconus severity was assessed by corneal thickness values as it was suggested previously. Receiver operator characteristic curves (ROCs) with covariate adjustment were used to compare discriminating ability of posterior elevation and pachymetry data after adjustment for the correlation between keratoconus severity and between-eye asymmetry. In ROC analysis, covariate adjustment is recommended when the accuracy of the test result is dependent on patient characteristic, similarly as adjusting for confounders in multivariable regression. In all analyses, a P value less than 0.05 was considered as statistically significant.

Statistical analysis in the esthesiometry study was performed with SPSS software (version 21.0, IBM Inc., Chicago, IL, USA). The Shapiro-Wilk W test was used to assess normal distribution of the variables. Due to non-normality of data the Mann–Whitney U test was used for group comparisons. Spearman correlation analysis was used to determine the correlation between corneal sensitivity and age or pachymetric severity of keratoconus. In all analyses a p value less than 0.05 was considered as statistically significant.

Corneal esthesiometry

Mechanical, chemical, and thermal (hot and cold) thresholds were determined at the center of the cornea using a Belmonte's gas esthesiometer. This is a safe and reproducible, well documented technique. Belmonte’s esthesiometer cause no alterations of the ocular surface (like Cochet-Bonnet esthesiometer) with respect to conjunctival hyperemia and corneal fluorescein staining regarding to studies. Finally as a noncontact instrument, it avoids the risk of producing mechanical damage in hypoesthesic and/or fragile corneas as can occur with contact esthesiometers, hence this device is an excellent candidate for investigating corneas with keratoconus. The Belmonte non-contact esthesiometer allows exploration of different types of sensory fibers. It is known that during mechanical stimulation, when air at increasing flow rates (0-264 ml/min) is applied to the central corneal surface (5mm from corneal surface) at a temperature of 34°C, the corneal mechanoreceptors are activated predominantly. During

the stimulations three-second air pulses of filtered medicinal air were used. After assessing the mechanical threshold, thermal thresholds were determined by heating or cooling the air (-10°C - +70°C) to produce changes in basal corneal temperature (-5°C - +3°C change), with air pulses of 10 ml/min flow below mechanical threshold. With gas mixtures of increasing CO_2 concentration, a proportional decrease in pH occurs at the corneal surface acting as a specific stimulus for polymodal nociceptors of the cornea with an intensity proportional to the local pH reduction. A specific instrument with a rotary potentiometer was built to record intensity rating immediately after stimulation. Subjects were instructed to adjust the potentiometer to the corresponding intensity of the sensations arising during stimulation. A specific computer software written in MatLab program (The MathWorks, Natick, MA) was used to sample the data acquired from the potentiometer and to convert it to numeric values on a 10 unit scale. We measured with the potentiometer the intensity of the irritation sensation evoked by selective mechanical, chemical, and thermal stimuli applied on the central cornea of participants using the gas esthesiometer.

Assessment of dry eye symptoms with OSDI score

All patients completed a questionnaire to assess dry-eye disease symptoms (ocular surface disease index—OSDI, Allergan Inc., Irvine, CA) using a validated Hungarian translation of the original OSDI questionnaire. This questionnaire is comprised of 12 questions and evaluates the frequency of symptoms over the preceding week. The scores range from 0 to 100. Based on the score, the patients' symptoms can be categorized as normal (0–12), mild dry eye (13–22), moderate dry eye (23–32), or severe dry eye (33–100). None of the subjects received any drops at least 6 hours before the measurements.

Measuring non-invasive tear film breakup time (NI-BUT)

The non-invasive tear film breakup time (NI-BUT) was measured using the Keeler Tearscope Plus immediately after a complete blink. The Keeler Tearscope Plus was attached to a slit lamp (Topcon SL-D2, Topcon Medical Systems, Oakland, NJ, USA) in a fixed position to obtain a full coverage of the cornea. The measurement of non-invasive tear film breakup time with Tearscope Plus is based on the projection of a cylindrical source of cool white fluorescent light onto the cornea so that tear film breakup could be observed at any point over the corneal surface. The tear film was recorded by a digital camera attached to the slit lamp, captured videos were exported at a spatial resolution of 1024 × 768 pixels and were analyzed by a masked observer. The non-invasive tear film breakup time was defined as the time from

the last blink when visible deterioration of the projected rings was detectable during the continuous recording. In each subject, NI-BUT was averaged from three consecutive measurements.

Schirmer test

Schirmer I test was performed without anesthesia. Briefly a small strip of filter paper was placed inside the lateral 1/3 of the lower eyelid (inferior fornix). Then the patient was asked to close the eyes for 5 minutes, then the paper was removed, the amount of moisture was measured:

Evaluation of dry eye according to Schirmer I test result

1. Normal:≥15 mm wetting of the paper after 5 minutes.
2. Mild: 14-9 mm wetting of the paper after 5 minutes.
3. Moderate: 8-4 mm wetting of the paper after 5 minutes.
4. Severe: <4 mm wetting of the paper after 5 minutes.

The test was executed 15 min after the esthesiometry measurement.

Results

Asymmetry evaluation with Scheimpflug imaging

There were no statistically significant differences between the keratoconus and the control groups in age or sex distribution ($p>0.05$). Values of topographic, posterior elevation and pachymetry parameters in the two groups showed significant difference. We have found no significant correlation between self-reported eye rubbing or ocular trauma and the presence of keratoconus in a given eye ($p>0.05$).

There was a statistically significant difference in keratometric, CCT, ThCT and PE values between worse eye and better eye in the keratoconus group (Kf (D): 44.90±3.09 vs. 47.42±4.58; Ks (D): 46.84±4.23 vs. 51.33±5.56; PE (μm): 32.60±29.51 vs. 68.00±51.24; CCT (μm): 493.73±26.04 vs. 463.60±33.53; ThCT (μm): 493.53±47.07 vs. 453.83±47.59; for all parameters $p <0.001$). In contrast, there was no significant difference in these parameters between the right eye and the left eye of controls ($p>0.05$).

Mean intereye difference was significantly higher for all of the variables when comparing keratoconus eyes with normal eyes ($p<0.001$ for all parameters; Kf (D): 2.70±3.57 vs. 0.37±0.39; Ks (D): 4.37±5.14 vs. 0.43±0.44; PE (μm): 35.4±37.31 vs. 3.13±3.71; ThCT(μm): 39.70±36.42 vs. 6.57±5.30; CCT(μm): 30.13±35.80 vs. 5.59±4.90).

Correlation analysis showed significant correlation between data from the worse eye and data from the better eye in the keratoconus group ($p<0.001$). Data from the right eye and data from the left eye in the control group also showed strong correlation ($p<0.001$). The difference between correlation coefficients was significant for each variable. Intereye asymmetry of pachymetry significantly correlated with decreasing thinnest pachymetry ($r=-0.40$; $p=0.03$) or central pachymetry ($r=-0.72$; $p=0.002$) in the keratoconus group but not in the control group ($p>0.05$). Similarly, correlation was found between intereye asymmetry of PE and increasing posterior elevation ($r=0.82$; $p<0.001$) in the keratoconus group but not in the control group ($p>0.05$). The relationship between intereye asymmetry and keratoconus severity could best be described by an exponential regression model across the two groups with an r value of 0.74 for steep keratometry ($r^2=0.55$, $p<0.001$), with an r value of 0.62 for CCT ($r^2=0.39$, $p<0.001$), an r value of 0.69 for ThCT ($r^2=0.48$, $p<0.001$) and an r value of 0.80 for PE ($r^2=0.64$, $p<0.001$).

To identify the best parameter to characterize intereye corneal asymmetry in keratoconus, receiver operator characteristic curves with adjustment for keratoconus severity was used. This ROC analysis showed, that asymmetry in thinnest pachymetry had the highest accuracy (AUROC: 0.99) and significantly better discriminating ability for keratoconus than posterior elevation (AUROC: 0.96), ThCT (AUROC: 0.94) or CCT had (AUROC: 0.92; pairwise comparison $p<0.05$).

Corneal sensitivity evaluation with Belmonte's gas estehsiometer

There was no significant difference in age and gender between the keratoconus and the control group ($p>0.05$). Patients with keratoconus had significantly higher steep and flat keratometry values and significantly lower thinnest corneal thickness compared to normal ($p<0.05$). Patients with keratoconus had significantly decreased tear secretion and significantly higher OSDI scores compared to controls ($p<0.001$ for all parameters; Schirmer I test (mm): 5.27 ± 2.19 vs. 13.22 ± 1.99; OSDI score: 26.81 ± 15.82 vs. 8.11 ± 2.31). There was no significant difference in tear film breakup time between the two groups ($p>0.05$, NI-BUT (sec): 9.82 ± 4.83 vs. 10.67 ± 3.81).

The threshold sensitivity to mechanical stimulation with air pulses of neutral temperature applied to the center of the cornea in the patients with KC was significantly higher than those observed in the control subjects ($p<0.001$). No correlation was found between mechanical threshold and age in the patients with KC ($r = 0.13$, $p = 0.58$), whereas in the control subjects, mechanical threshold increased proportionally with age ($r = 0.52$, $p = 0.02$).

The mean sensation threshold for selective chemical stimulation was significantly higher in patients with KC than in the control group ($p<0.001$). Chemical thresholds did not tend to increase with age in the subjects with KC ($r = -0.17$, $p = 0.46$), contrary to the responses of the control subjects ($r = 0.47$, $p = 0.04$).

A significantly higher threshold value was obtained with heat stimulation in patients with KC than in the control group ($p<0.001$), with no correlation between threshold and age ($r = 0.01$, $p = 0.98$) contrary to the responses of the control subjects, in whom threshold and age correlated positively ($r = 0.26$, $p = 0.04$).

Similarly, an elevated threshold value to cold stimulation was observed in patients with KC compared to the control individuals ($p = 0.001$). Cold threshold responses did not correlate with age in patients with KC ($r = -0.09$, $p = 0.69$), whereas in control subjects the correlation was significant ($r = 0.40$, $p = 0.03$).

In the keratoconus group, corneal thickness did not correlate significantly with threshold values of mechanical, chemical, heat or cold stimulation ($p>0.05$ for all variables). Similarly, threshold values of mechanical, chemical, heat or cold stimulation did not correlate to tear flow ($p>0.05$ for all variables), NI-BUT ($p>0.05$ for all variables) or OSDI score ($p>0.05$ for all variables). In the keratoconus group, there was no correlation between thinnest corneal thickness and tear flow, NI-BUT or OSDI values ($p>0.05$ for all variables).

Conclusions

As a conclusion, in this study we have shown that in comparison to healthy subjects, there is a greater intereye asymmetry regarding to tomographic parameters in patients with keratoconus. In case of corneal topography, pachymetry and elevation outcomes, the degree of intereye asymmetry is associated with disease severity. Regarding to our results when intereye pachymetric asymmetry is taken into account, the diagnostic accuracy of keratoconus can be significantly improved. Increasing pachymetric asymmetry could be thus considered as a warning sign for keratoconus (mainly for subclinical forms), and could serve as an indicator for disease progression and as therapy indication.

Our study group also found that decreased corneal sensitivity in all aspects of the sensory functions (cold-, mechano-, nociceptors) is present in keratoconic corneas. Regarding to the significantly decreased corneal sensitivity one might conclude that axon damage and/or altered ion channel expression (in the cell membrane) is involved in this process.

The corneal hypoesthesia was independent from age and disease severity, and did not show correlation with tear dysfunction, so from these results one could suppose that sensory nerve dysfunction might be present in early stages of keratoconus.

As a summary our group studied the keratoconus disease from different aspects, the clinical relevance of our results is that the diagnostics of subclinical keratoconus could be significantly improved if these morphological and functional features are taken into account in the screening of early forms of this ectatic disease.

New results

- We have shown that intereye asymmetry in keratoconus is significantly increasing with disease severity.
- Our group shown for the first time, that when intereye asymmetry is taken into account the diagnostic accuracy of keratoconus can be improved significantly.
- We described for the first time, that an intereye asymmetry beyond 12 μm in ThCT could predict keratoconus with high accuracy independently from disease severity.
- We described for the first time, that the optimal cut-off point for posterior elevation asymmetry was 7 μm and showed 97% sensitivity and 93% specificity in predicting keratoconus.
- Our group have shown for the first time, that sensory nerve impairment in keratoconus affects significantly all types of corneal sensory nerve endings.
- We demonstrated for the first time, that the decrease of corneal sensory functions are present in early stages of keratoconus and are independent of age, disease severity or tear film quality.

Bibliography of the candidate's publications

List of publications related to the thesis:

Dienes L, Kránitz K, Juhász E, Gyenes A, Takács A, Miháltz K, Nagy ZZ, Kovács I. (2014) Evaluation of intereye corneal asymmetry in patients with keratoconus. A scheimpflug imaging study. *PLoS One*, 8;9(10):e108882.

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