Significance of laser confocal tomography in diagnosis and monitoring of keratoconjunctivitis sicca

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Laser confocal tomography of the cornea enables studying ultrathin sections of corneal layers, which provides additional reliable information on tissue changes in keratoconjunctivitis sicca (KCS). Aim – to assess the significance of laser confocal tomography of the cornea in the diagnosis and monitoring of KCS. Material and methods. We investigated 38 eyes of 30 patients with severe KCS. The patients were divided into two groups. Group 1 (15 patients, 19 eyes) was prescribed cyclosporine А 0.05% instillations 2 times daily, artificial tears, and soft contact lenses. Group 2 (15 patients, 19 eyes) received only instillations of cyclosporine А 0.05% 2 times daily and artificial tears. Besides standard ophthalmic examination, additional tests were performed, namely Schirmer’s test, tear break-up time test, fluorescein eye stain test, tear osmolarity test (TearLab System, USA), and Heidelberg retinal tomography of the cornea (HRT, Heidelberg Engineering GmbH, Germany). Results. HRT findings revealed a 3 times shorter epithelization period and faster recovery of corneal transparency in group 1 as compared to group 2 (1.5 and 4.5 months, respectively). There was also an evident reduction in the number of immune cells in the cornea, most pronounced in group 1 at 3 months, which is indicative of inflammation termination. Conclusion. The use of HRT of the cornea in KCS patients allows real-time cellular level observation of corneal changes, which together with clinical findings and diagnostic tests not only confirms the diagnosis but also determines treatment effectiveness. It has been also found that soft contact lenses accelerate epithelization of the cornea and relieve inflammation of the ocular surface in KCS patients under cyclosporine A 0.05% instillation therapy. Transparency of financial activity: the authors have no financial interest in the submitted materials and methods. Keywords: keratoconjunctivitis sicca, laser confocal tomography, cornea, cyclosporin A, contact lenses.

Material and methods

We investigated 38 eyes of 30 patients with severe KCS aged 27–76 years. The patients were divided into two groups with equal number of participants and statistically comparable structure in terms of patient age and sex. Group 1 (15 patients, 19 eyes) was prescribed cyclosporine А 0.05% instillations 2 times daily for no less than 6 months, artificial tears, and soft contact lenses (SCLs). Group 2 (15 patients, 19 eyes) received the same therapy, but did not wear contact lenses. Cyclosporine А 0.05% was started only if no pathological growth occurred after plating conjunctival swabs. Of all available tear substitutes, we used only hyaluronic acid-containing preservative-free products biocompatible with SCLs. The latter were individually produced from the Definitive 65% clear silicon hydrogel (Si-H) material by our local optical group (Department of refractive errors of the Research Institute of Eye Diseases). For a better fit, lens edges were additionally chamfered. Lens wear was continuous with duration depending on the state of the cornea.

All patients underwent standard and extended ophthalmic examinations, including Schirmer’s test, Norn’s test, tear osmolarity test (TearLab System, USA), vital staining tests (evaluated using the Oxford scale), and Heidelberg retinal tomography with Rostock Cornea...
Module (HRT III/RCM). The latter involves confocal scanning laser ophthalmoscopy that allows optical sectioning of the cornea, automated determination of the number and density of corneal epithelial cells, accurate pachymetry, and high-resolution in vivo histology at different depths. HRT III/RCM utilizes a 670-nm diode laser as the light source. The resultant two-dimensional image consists of 384 x 384 pixels corresponding to a 0.4 x 0.4 mm area of the cornea. A three-dimensional image is formed from 40 consecutive image planes with 2-μm increments. Acquisition time is 0.024 sec for a 2-D image and 6 sec — for a 3-D. The procedure is controlled through a CCD camera (480 x 460 pixels, RGB, 15 frames/sec) on one side of the cornea module [3].

The scanning was performed according to the standard protocol. Before the examination, viscous ophthalmic gel (Corneregel, Bausch & Lomb) was placed on the tip of objective lens and covered with a disposable sterile cap. The patient was then given a drop of topical anesthetic agent and Corneregel in both eyes in order to prevent blinking. Another bit of the gel was added on top of the tip touching the cornea. Further scanning provided a stack of in vivo corneal images from epithelium to endothelium with the possibility to assess individual layers.

The reasons to choose Corneregel as the optical medium include its high viscosity, transparency, hypoallergenicity, and good tolerance. Corneregel contains dexamethasone, a potent stimulator of corneal regeneration, and a polyacrylic polymer that ensures ocular surface moistening and prolongs the effect of dexamethasone. The use of Corneregel made the procedure more safe and comfortable and enabled reduction in rehabilitation time. Treatment results were evaluated at months 1, 3 and 6.

**Results and discussion**

On first presentation, all patients had their severe KCS verified basing on the findings of both standard and extended examinations, including HRT of the cornea. In both groups, visual acuity ranged from 0.1 to 1.0 with the median of 0.5, Schirmer’s test values averaged 3.3±1.8 mm, Norn’s test values — 2.9±1.0 sec, tear osmolarity lied within the range of 308-389 mOsms/L, and the grade of corneal and conjunctival staining was about 2.8±0.7.

In both groups, HRT images demonstrated an increased desquamation of epitheliocytes (89.5% of cases, 34 eyes), cellular polymorphism (92% of cases, 35 eyes), vague or hyperreflective cell nuclei (60.5% of cases, 23 eyes), pycnosis of epithelial cell nuclei, increased desquamation of epitheliocytes; a — epithelial cell polymorphism; c — swelling in the wing cell layer; d — increased number of Langerhans cells in the basal layer of the epithelium; e — increased tortuosity of sub-basal nerves, leukocytic infiltration of the basal epithelium; f — anterior stromal edema; g — stromal microdeposits; h — posterior stroma; i — degenerative changes in the endothelium.

**Fig. 1. Corneal HRT findings before the treatment.**

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eyes), swelling of the wing epithelial cells (65% of cases, 25 eyes), leucocytic infiltration of the basal epithelium (34% of cases, 13 eyes), increased number of Langerhans cells in the basal and sub-basal layers of the epithelium (60.5% of cases, 23 eyes), increased tortuosity of sub-basal nerves (55% of cases, 21 eyes), stromal edema (55% of cases, 21 eyes), microdeposits in the anterior stroma (15% of cases, 6 eyes), and degenerative changes in the endothelium (31% of cases, 12 eyes) (Fig. 1).

According to the literature, morphological changes in epithelial cells result from ocular surface xerosis. Demarcation of the nuclei is due to karyopycnosis and accumulation of chromatin. Infiltration of the epithelium by leukocytes and Langerhans cells (antigen-presenting immune cells) depends on the inflammation activity [4–6]. Having analyzed the HRT findings, we conclude that the total number of infiltrating cells directly correlates with KCS severity and inflammation intensity. As to qualitative changes in sub-basal corneal nerves that manifest with an increased tortuosity of the latter in HRT images, some authors consider them to reflect the damage caused by proinflammatory agents as well as the consequences of regeneration [5, 6]. Quantitative parameters of sub-basal nerves, however, showed no correlation with process severity, which may be explained by wide scattering of the obtained data. We have also noticed an uneven distribution of epitheliocytes, which is an attribute of stromal edema. According to J. Jester [7], corneal swelling occurs with the loss of transketolase and aldehyde dehydrogenase class 1.

By the end of the first month after the beginning of the treatment, clinically significant changes have only occurred in group 1, namely, reduction in the number of pyknotic nuclei (21% of cases, 4 eyes) and polymorphous epithelial cells (52% of cases, 10 eyes) as well as resolution of swelling within the wing cell layer (89.5% of cases, 17 eyes) and stroma (100% of cases, 19 eyes). Thus, the cornea became more structured, however, with no reduction in the number of inflammation cells (Fig. 2).

The said changes in group 1 were supported by an increase in visual acuity by the average of 0.1±0.05. There was also a trend toward lower tear osmolarity, which

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**Fig. 2.** Corneal HRT findings at 1 month after the beginning of the treatment in group 2.

a — less desquamation of the superficial epithelial cells; a-c — less pronounced cellular polymorphism; c — reduction of swelling in the wing cell layer; d — Langerhans cells in the basal layer of the epithelium; e-g — reduction of stromal edema; h — degenerative changes in the endothelium.
ranged from 275 to 360 mOsms/L with the median of 289.5 mOsms/L. Other functional parameters showed no dynamics in either group.

Three months after the beginning of the treatment, in group 1, there was neither excessive desquamation of epitheliocytes, nor karyopycnosis, nor corneal edema, nor anterior stromal deposits (100% of cases, 19 eyes). Epithelial cells were polymorphous in only 31% of cases (6 eyes). In group 2, corneal edema got generally reduced: only 2 eyes (10.5% of cases) still had swollen wing cells and 5 eyes (26% of cases) — swollen corneal stroma. Leucocytes and Langerhans cells were less in number in both groups, particularly, in group 1, leucocytes were only seen in 17% of cases (3 eyes), Langerhans cells — 21% of cases (4 eyes), while in group 2 the figures were 26% (5 eyes) and 42% (8 eyes), respectively. The absence of degenerative changes in corneal endothelium was also reported (Fig. 3).

The described HRT findings were accompanied by certain clinical changes. Thus, tear osmolarity decreased in both groups ranging from 275 to 300 mOsms/L (median of 275 mOsms/L) and from 275 to 380 mOsms/L (median of 289 mOsms/L), respectively. Group 1 also demonstrated an increase in visual acuity by the average of 0.25±0.07 and a decrease in the grade of corneal and conjunctival staining by the average of 0.8±0.6 points. Other functional parameters underwent no statistically significant changes.

At 6 month follow-up, changes were reported in all studied categories: tomographic, clinical, and functional parameters. HRT images showed no leucocytic infiltration, or stromal microdeposits, or edema, or signs of endothelial degeneration (100% of cases, 38 eyes). Judging from other parameters, group 1 demonstrated a generally greater improvement than group 2. Thus, in group 1, epithelial cell polymorphism was only reported in 16% cases (3 eyes), Langerhans cells — 21% of cases (4 eyes), while in group 2 the figures were 26% (5 eyes) and 42% (8 eyes), respectively. The absence of degenerative changes in corneal endothelium was also reported (Fig. 3).

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there was also an increase in Schirmer’s and Norn’s tests values (up to 4.8±1.2 mm and 6.0±2.2 sec, respectively). In group 2, patients’ visual acuity increased by the average of 0.1±0.05 and so did the tear tests values (up to 4.6±2.7 mm and 5.8±2.2 sec, respectively), while the grade of corneal and conjunctival staining decreased down to 1.7 ± 0.6 point (according to the Oxford grading scale). Other differences in functional parameters were not statistically significant.

We may, therefore, conclude that introduction of corneal HRT has enabled objective evaluation of treatment results in KCS patients, previously impossible with standard methods. By analyzing HRT findings in the two groups we have found that, in group 1, the time required for corneal re-epithelization and restoration of its transparency was about 3 times as short as in group 2 -1.5 and 4.5 months, respectively.

The combination of cyclosporine A 0.05% and preservative-free tear substitutes together with original therapeutic SCL wearing has proved highly effective in shortening re-epithelization periods and relieving inflammation of the ocular surface. The lens was able to create optimal conditions for epithelial repair, prolonged the effect of cyclosporine A, and protected the cornea from exogenous irritative factors. The proof is that the number of immune cells within the cornea decreased significantly after 3 months of treatment in group 1 indicating termination of inflammation process. As to clinical and functional parameters, the improvement was also more pronounced in group 1 as compared to group 2.

Hence, corneal HRT, unlike routine examinations, provides an opportunity to assess the extent of corneal involvement at the cellular level, particularly, epithelial changes and the tortuosity of sub-basal nerves as well as the number of immune cells within the cornea that correlates with the severity of KCS signs and symptoms. The data on disturbed morphology of corneal cells allows proper diagnosis and pathogenetic therapy. The advantages of corneal HRT over cytology include the absence of direct contact with the cornea and the possibility of real-time evaluations.

Fig. 4. Corneal HRT findings at 6 months after the beginning of the treatment in both groups.
a-c — less pronounced cellular polymorphism within the epithelium; d — a few Langerhans cells in the basal layer of the epithelium; e-g — corneal stroma is not swollen; h — corneal endothelium.
Conclusion

1. Corneal HRT allows real-time assessment of corneal involvement at the cellular level and together with other clinical diagnostic tests ensures accurate diagnosis and evaluation of treatment effect.

2. The use of therapeutic soft contact lenses in KCS patients under cyclosporine A 0.05% therapy accelerates corneal re-epithelization and relieves inflammation of the ocular surface.

Author contributions:
Study conception and design — T.S., O.G.
Acquisition and handling of data — T.S., O.G., V.B.
Statistical analysis of data — O.G.
Drafting of manuscript — O.G., T.S.
Critical revision — T.S.

The authors declare no conflict of interests.

REFERENCES


