Regenerative Aspects of Excimer Laser Ablation

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Refraction abnormalities prevail in the world profile of ophthalmic diseases and require timely correction, including surgery. Both ophthalmologists and patients have shown increasing interest to different keratorefractive interventions. However, some authors consider that in patients with ametropia, routine methods, i.e. spectacle and contact lenses, can provide high functional results. It means that surgery aims at a cosmetic effect. And only in astigmatism, keratorefractive operations (KRO) are considered to be pathogenetically substantiated. Thus, the requirements of the results of correction should be rather high.

Despite high level of modern KRO, adequate equipment and minimal possible trauma, any operation causes complex biochemical, immunological, and morpho-functional alterations in eye tissues, that can provoke in certain conditions development of postoperative complications.

In all cases, compensatory mechanisms start which are directed towards restoration of homeostasis but in some cases they appear to be insufficient.

In this case, postoperative complications of KRO caused by failure of regenerative process develops which includes the following:

- neurotrophic epitheliopathy
- edema and non-infectious inflammation of corneal flap
- non-specific diffuse intralamellar keratitis
- early subepithelial fibroplasias – haze
- certain forms of secondary syndrome of dry eye
- allergic kerato-conjunctivitis
- retardation of re-epithelialization of the zone of surgery
- hyperplasic processes (for example, epithelial hyperplasia) and some other effects.

Epidemiological data about KRO complications vary within a wide range because of, in particular, different approaches to interpretation of the term “complication”. Routine criterion for development of KRO complication implies deterioration of corrected visual acuity as compared to preoperative values. Thus, published data on postoperative complications are large.

Lately, more rigid criteria have been formulated for the term: “KRO complication” which means any aberration in the normal course of surgery or postoperative period, which requires additional manipulations or drug therapy even without deterioration of final result of the surgery.

According to this approach, the rate of complications is rather higher:

1) The rate of subepithelial fibroplasias (haze) one month after PRK achieves 60% (in patients with high degree of ametropia). Under the influence of intense drug therapy (corticosteroids, enzyme therapy (Lidaza), and application of anti-proliferative agents such as mytomycin) and as a
result of spontaneous regression, rate of residual fibroplasias one year after PRK does not exceed 9% (in initial mixed or stromal forms of haze) and requires repeated surgery in not more than 3% of cases.5,36,53

2) Neurotrophic epitheliopathy (NE), according to different authors, is found in 11.2-48% of cases. Some authors do not distinguish NE as separate complication but include it into complex of symptoms of secondary dry eye induced by KRO on the basis of the fact that the rate of NE is significantly higher in the group of patients with impairment of lacrimation. However, in some cases, signs of secondary dry eye do not accompany NE. The cause of more frequent detection of NE in this group of patients is common etiology of complications: development of both NE and secondary impairments of lacrimation are caused by mechanical damage to intrastromal corneal nerves in the course of KRO. The difference is that NE is mainly caused by failure of neurotrophic function of intrastromal nerves, while development of secondary dry eye is mainly caused by separation of neuronal connections of receptor areas and glands, which produce lacrimal fluid.9,54,59

3) Transitory secondary dry eye forms in 8.2-45% of cases after LASIK and somewhat rarely after PRK (up to 17% of cases) 18,39,44.

4) Non-specific diffuse lamellar keratitis (DLK) – syndrome “Sahara sands” develops in 1.3-1.9% of cases. Till now, there is no generally accepted concept of DLK etiology, it is supposed to be caused by powder from surgical gloves, metal microparticles from cutting edge of microkeratome, lipid and mucin secretions of conjunctival glands, autoimmune reactions, and recently there are some articles about failure of local metabolic processes induced by KRO as important factor of DLK development 33,55,60.

As a rule, complications, listed above, are rather successfully cured but they require long-term application of drugs, which are cumbersome for the patient on the whole.5,27. This prolongs significantly the period of visual and social rehabilitation of patients, deteriorates life quality of active working people, and prolongs sick-list time.42.

It was noted that these KRO complications are recorded more frequently in patients with certain ophthalmic and system diseases.

This was the base for determination of the following risk factors for KRO complications.

- long-term application of contact lenses;
- preceding surgeries on the cornea;
- aggravated ophthalmic anamnesis (particularly, infectious keratoconjunctivitis);
- age before 18 years and after 40 years;
- long-term hormone substitutive therapy;
- inclination to keloid formation;
- allergic and autoimmune diseases (bronchial asthma, neurodermatitis, psoriasis, atopic dermatitis, rhinitis, etc.)

Due to different mechanisms, these risk factors interfere in general and local (in eye tissue) metabolic, hormonal, and immune processes. This decreases significantly compensatory abilities of the organism to restore homeostasis after the influence of exogenous destabilizing factors such as surgery or trauma. Initiation and persistence of metabolic and immune misbalance cause development of certain postoperative complications of KRO 1,4,14,31,40.

Many authors have shown that excimer laser ablation of the cornea is accompanied by development of surgically induced oxidative stress (SIOS) at the level of tissue. It aggravates the course of posttraumatic inflammatory reaction and is one of the main pathophysiological mechanisms of disregenerative postoperative complications 16,21,28.

SIOS is the impairment of balance between pro- and anti-oxidative systems in tissues of the anterior eye segment. Among causes of SIOS, the main one is generation of free radicals and active forms of oxygen under the influence of excimer laser.

Besides, influence of excimer laser leads to inhibition of glutathione-dependent antioxidative system of the cornea. In the case of insufficient activation of other chains of anti-oxidative protection, it leads to aggravation of SIOS 16.
SIOS is intensified by chronic psycho-emotional tension and unbalanced nutrition with deficiency of bioantioxidants typical of urbanization.

SIOS produces multifactor pathological influence on eye tissues.

1) Intensification of lipid peroxidation (LPO) leads to increased cell membrane permeability, ion misbalance, separation of tissue respiration and oxidative phosphorylation in mitochondria, and, as a result, decreases ATP production. Energetic starvation interferes into all energy-dependent processes. Impairment in function of transport protein aquaporin-5, which provides energy-dependent trans-membrane transportation of water molecules, results in long-term aseptic edema of the corneal flap. Regeneration of quickly renewing tissues is affected that is accompanied by retarded re-epithelialization of corneal erosions, long-term neurotrophic epitheliopathies, etc.

2) Oxidative modification of DNA causes abnormal regeneration of corneal cells with altered cytophysical and antigenic properties. This initiates cascade of autoimmune reactions, which play the role in formation of DLK. Besides, altered keratocytes synthesize abnormal collagen, which is deposited chaotically and is visualized as the component of early haze.

3) Lipoperoxidation of proteins of cytoplasmic membranes and direct cytotoxic influence of LPO induces cytolysis of epithelium and keratocytes that is manifested by retardation of re-epithelialization and formation of so called acellular zone along both sides of interface lacking in keratocytes. This phenomenon was first diagnosed with the help of confocal microscopy. There are hypothesis that long-term existence of acellular zone alters biomechanical properties of the cornea and may be the cause of iatrogenic keratectasia.

4) Irreversible conformation of glycosaminoglycans molecules, for example, increase of number of cross-links in hyaluronic acid, causes alteration in mucin layer of lacrimal film that leads to alteration of its stability and induces development of a special form of secondary dry eye.

The factors mentioned above indicate that SIOS plays the main role in formation of certain postoperative complications of photorefractive surgery.

Impairment of protein metabolism with prevalence of catabolic reactions over anabolic ones is another factor induced by KRO and aggravated by secondary alteration by SIOS. This leads to impairment of the balance between cytolysis and cellular regeneration, synthesis and inactivation of enzymes and other protein-containing substances playing an important role in cellular metabolism.

Thus, KRO has multifactor influence, which causes the development of the complex of alternative-regenerative processes. They are reflected in deep biochemical reconstructions at the regional level, first of all in the cornea. They are specific and precede the development of clinical picture of postoperative complications.

Lacrimal fluid is an available diagnostic medium for evaluation of metabolic processes in the eye as it is constant, dynamically renewing micro-medium of the anterior segment of the eye. It is tightly connected with local metabolic processes. On the other hand, non-invasion method of lacrimal fluid collection is an important advantage.

Besides, objective evaluation of dynamics of regenerative processes after KRO and search for subclinical signs of postoperative complications are impossible without precise methods of visualization of corneal ultrastructure. Confocal microscopy, which is recently widely introduced into different fields of ophthalmology, provides valuable assistance in examination of corneal morphology in vivo.

Confocal microscopy allows examination of biological tissues at the cellular level at the state of physiological activity and demonstration of results in three dimensions – height, width, depth, and time.

For the first time, principle of confocal microscopy was described by Minsky in 1957. He proposed the system, where the lenses of illuminator and objective focused in one point (had common focal points) that gave the name of “confocal” microscopy (Fig. 1). Confocal microscopy allowed significant increase of axial (5-10 um) and lateral (up to 1-2 um) resolution of microscopy due to exclusion from focal points of...
information from adjacent areas. This makes possible 600 times magnification of image without the lost of contrast and clearness.

White light passing through the first perforation in the disk is focused on the focal plane in the cornea with the help of collecting (convex) lens. Reflected ray is refracted on the lens of the objective and, passing through the outlet in the disk, achieves camera-detector. All rays, which are focused above and under the focal plane, are cut off with the help of perforations in the disk and do not achieve the camera.

Increasing interest to KRO and successes in the study of histomorphology of the cornea in vivo using confocal microscopy open wide prospects for the study of the cornea after different types of surgery: evaluation of cellular reactions related to healing process, migration of different types of cells and cornea remodeling, process of re-innervation of the cornea, formation of Haze, and cicatrisation of the cornea, reasons of formation of iatrogenic keratectasia in the case of preservation of sufficient thickness of residual stroma and several other questions that can be answered by confocal microscopy.

Modern confocal microscopes allow one to visualize cellular composition of different corneal layers, to measure thickness of the corneal valve and residual stroma, to determine localization and length of subepithelial fibroplasia, to measure thickening of the cornea, which causes regress of refractive effect after PRK, and to analyze the type of inclusions in the interface.

Although several studies on this topic have been published, there are no integrative studies connecting histomorphological alterations in the cornea of patients in vivo with metabolic processes in eye tissues in the course of reparation after KRO and during the development of complications.

All facts mentioned above, and twenty years experience of active scientific and surgical activity in the field of excimer laser surgery gave us an ideas to study morphological and metabolic features of typical and pathological regenerative process in the cornea after different keratorefractive interventions, to develop objective methods of evaluation of individual reaction of eye tissues on surgical intervention, and to propose algorithm of diagnosis, prophylaxis, and correction of disregegenerative complications.

Materials and Methods

Clinical characteristics of examined patients

We studied 213 patients (394 eyes) with myopia to solve different tasks of this study (table 1).

There were the following principles of formation of groups:

1. **Control group** included patients with myopia who used spectacles for optic correction.

2. **The first main group** was formed to study specific features of typical postoperative course of different KRO. It comprised patients with myopia, initially unaltered cornea, and uncomplicated postoperative period. Based on the type of surgical correction, the group was divided into two subgroups:
   - 1a – patients with myopia, who have undergone LASIK;
   - 1b – patients with myopia, who have undergone PRK.

3. We formed **the second main group** to study specific features of atypical postoperative period of different types of KRO. This group comprised patients with myopia, initially unaltered cornea, and disregegenerative postoperative complications recorded three days to four months after surgery. Based on the type of surgical correction, the group was divided into two subgroups:
   - 2a – patients with myopia, who have undergone LASIK;
   - 2b – patients with myopia, who have undergone PRK.

4. We formed **the third main group** to prove effectiveness of the algorithm of prediction,
diagnosis, and correction of dismetabolic complications of KRO, developed in the course of the study. It comprised patients with myopia and initially altered cornea because of long-term history of contact lenses with development of neovascular keratopathy or KRO, who were intended for LASIK.

5. Additionally, we examined healthy volunteers with emmetropia (to develop the method of examination and to determine normal biochemical parameters of lacrimal fluid).

**Table 1.** Characteristic of groups of observed patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Criteria of inclusion</th>
<th>Number of patients (number of eyes)</th>
<th>Mean age, M±s</th>
<th>Type of ametropia correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The main group 1</td>
<td>Uncomplicated myopia</td>
<td>20 (40)</td>
<td>24.0±1.8</td>
<td>Spectacles</td>
</tr>
<tr>
<td></td>
<td>1a – myopia, initially unaltered cornea, unaltered cornea, uncomplicated postop period</td>
<td>50 (100)</td>
<td>23.5±1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1b – myopia, initially unaltered cornea, uncomplicated postop period</td>
<td>30 (60)</td>
<td>26.2±2.4</td>
<td>LASIK</td>
</tr>
<tr>
<td>The main group 2</td>
<td>2a – myopia, initially unaltered cornea, dismetabolic complications</td>
<td>37 (49)</td>
<td>24.7±2.2</td>
<td>LASIK</td>
</tr>
<tr>
<td></td>
<td>2b – myopia, initially unaltered cornea, dismetabolic complications</td>
<td></td>
<td></td>
<td>PRK</td>
</tr>
<tr>
<td>The main group 3</td>
<td>Myopia, initially altered cornea (because of preceding surgeries or long-term use of contact lenses)</td>
<td>26 (45)</td>
<td>27.1±3.4</td>
<td>PRK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 (100)</td>
<td>31.2±4.2</td>
<td></td>
</tr>
</tbody>
</table>

**Screening system for studying functional tear complex**

Lately in refractive surgery, much attention is paid to examination of condition of functional tear complex (FTC), which is implied to consist of eye surface, tear-producing organs, and their neuroreflexive interactions. We used the following diagnostic tests to evaluate condition of FTC:

1. **Schirmer test-1** – evaluation of total (basal and reflexive) tear production. The test is based on moistening of standard sterile strips of filter paper during a certain time. We used ready-to-use test strips “Bausch&Lomb” (USA). Results were evaluated in millimeters of moistened part of the strip during five minutes.

   We used the following criteria to interpret the data obtained:
   - more than 25 mm during 5 minutes – hypersecretion;
   - 15-25 mm during 5 minutes – normosecretion;
   - 10-15 mm during 5 minutes – intermediate condition;
   - less than 10 mm during 5 minutes – hyposecretion of lachrymal fluid.

2. **Schirmer test-2 (modification by Jones)** – examination of value of basal tear production.

   Method of testing: after preliminary instillation of anesthesia, lacrimal fluid and residual anesthetics were accurately absorbed by cotton tampon from inferior fornix of conjunctiva. Then filter paper strip was placed under the lower lid of the patient for 5 minutes (as in Schirmer test-1). Moistening of more than ten millimeters of standard test strip during five minutes was considered to be normal.

3. **Test for evaluation of tear film break-up time (Norn’s test)** – examination of tear film stability indicating condition of its mucin and lipid layers.

   Method of testing: 0.2 % sodium fluorescein solution was instilled into conjunctival cavity with subsequent examination of patient’s eye using slit-lamp with cobalt filter. Time interval between the last blinking and appearance of first dry spots was evaluated. Parameters for evaluation of results: norm – from 15 to 45 sec., 10-15 sec. – intermediate
4. **Evaluation of the corneal condition** is based on the ability of fluorescein solution instilled into conjunctival cavity to indicate epithelial defects.

Method of testing: condition of the corneal epithelium is evaluated after instillation of 0.2% sodium fluorescein solution into conjunctival cavity using biomicroscopy with cobalt filter. For quantitative evaluation of epithelial damage, the cornea was divided into five zones. Staining in each zone is evaluated using a four-points scale:

1. dotted defects (to ten spots);
2. moderate;
3. average;
4. severe alteration. Then marks for each zone are summarized. Maximal mark is twenty.

**Method of investigation of biochemical composition of lacrimal fluid (LF).**

LF was collected from inferior fornix of conjunctiva using laboratory micropipette with disposable sterile tips or glass microcapiller without preliminary stimulation of lacrimation (Fig. 3).

To exclude influence of drugs on composition of lacrimal fluid, samples were collected at the same time in all patients (from 8.30 to 9.00 a.m.).

Biochemical examination of LF was performed using automatic analyzers “Express Plus” (Bayer, USA), “Hitachi-912” (F. Hoffmann-La Roche LTD, France), and spectrophotometer. The following parameters were studied: parameters of free-radical oxidation (malonic dialdehyde), anti-oxidative protection (superoxide dismutase), protein synthetic activity of cells (total protein), and activity of protein degradation (urea). To evaluate severity of damage to the cornea after KRO, in all patients pre- and postoperatively, we calculated values of earlier developed biochemical coefficients of SIOS and degree of impairment of synthesis/degradation of protein (SDP) using the following formulas:

1) \[ K_1 = \frac{MDA \times 1000}{SOD} - 54.0 \], where
   - MDA – content of malonic dialdehyde, parameter of activity of free-radical oxidation;
   - SOD – activity of superoxide dismutase, the most active enzyme of anti-oxidative protection of the cornea;
   - 54 is the mean ratio of MDA x 1000/SOD in healthy people.

   If \( K_1 \) lower than 8, corneal damage is absent, \( K_1 \) is from 8 to 38 – light damage of the cornea, \( K_1 \) is from 38 to 55 – average damage, \( K_1 \) is from 55 to 75 – severe damage, \( K_1 \) is from 75 and higher – extremely severe damage.

2) \[ K_2 = 4.9 - \frac{P}{U} \], where
   - \( P \) – content of the protein, an indicator of protein-synthetic activity of cells;
U – content of urine – the product of biodegradation of proteins,
– average value of ratio P/U in tears of healthy people;
If K lower than 0.7, corneal damage is absent,
K is from 0.7 to 1.4 – light damage of the cornea,
K is from 1.4 to 2.8 – average damage,
K is from 2.8 to 4.1 – severe damage,
K is from 4.1 and higher – extremely severe damage.

**Method of confocal microscopy of the cornea**

We used confocal microscope Confoscan 4 (Nidek, Japan) with the following parameters: lens for examination through immersion gel – 40x, NA 0.75, WD 1.98, Zeiss; examined zone of the cornea was 460x345 um, image obtained was 768x576 pixel, lateral resolution – 0.6 um/pixel, and speed of scanning was 25 images per second. We used automatic mode for examination of the whole thickness of the cornea, manual mode for visualization of certain corneal structures, automatic calculation of density of endothelial cells with evaluation of polymorphism and size of cells, and optic pachymetry (using Z-ring).

Examination was performed after one instillation of local anesthetic through immersion gel.

*All the special examinations was performed in all patients before surgery and one hour to 12 months postoperatively.*

**Technology of keratorefractive surgeries**

Leading ophthalmosurgeons of excimer laser refractive department of the Center of Laser Surgery of Eye Microsurgery Complex operated all patients in the various groups.

Standard preoperative preparation in all types of KRO was identical and consisted in antibiotic installations three times a day two days prior to operation.

**Technology of LASIK procedure**

LASIK procedure was performed using standard technology accepted in Eye Microsurgery Complex with the use of modern home excimer ArF laser “MicroScan” created in collaboration with the Center for Physics and Instrument-making Industry of the Institute of General Physics of Russian Academy of Sciences. The device functions at frequency of 100 Hz, it is equipped by formation system according to the technology of “flying spot” with diameter of 1.0 mm and highly sensitive system of control over the movements of patient’s eye – “eye tracking system”.

The corneal flap was formed by microkeratome “Zyoptix” (Bausch & Lomb, USA) with head “120”, which allows one to form the flap 100±20μm thick, according to the data of the producing company. Our previous studies on flap thickness with different microkeratomes performed with the use of optical coherence tomograph “OCT Visante” (Carl Zeiss Meditec Inc., Germany) showed that thickness of the corneal flap, which is formed by keratome “Zyoptix” with the head “120” is 105.3 μm, on average (95 to 110 μm) (Fig. 5).

Standard postoperative therapy consisted of regular instillations of:

- antibiotics three times a day up to seven days postoperatively (3-5 days, on average);

![Fig. 4. Excimer laser device “MicroScan”](image)

![Fig. 5. Measurement of thickness of the corneal flap and residual stroma using optic coherent tomograph OCT Visante in the patient after LASIK.](image)
- corticosteroid medicines during 2-3 weeks postoperatively according to a decreasing scheme beginning with three times a day.

**Technology of PRK operation**

PRK was also performed using excimer laser “MicroScan”. In all patients, we used an original transepithelial technology of ablation – without preliminary scarification of epithelium. We have developed special algorithm of the first stage of PRK, which allows us to achieve even removal of epithelium on the whole area of correction (area of de-epithelialization zone depends on the diameter of transition zone of operation) The system of interactive control over the process of epithelial ablation provides total differentiated removal of epithelium without refractive effect. This allows us to use standard nomograms of the laser for refractive keratectomy itself at the second stage of correction.

Transepithelial technology of PRK decreases the risk of development of subepithelial fibroplasia due to decrease of stimulating effects of products from destroyed epitheliocytes on synthesis of non-organized collagen by stromal fibroblasts. The operation was completed by application of bandage contact lens, which decreases postoperative pain syndrome and stimulates re-epithelialization.

Standard postoperative therapy consisted of two stages: 1) the first stage (before re-epithelialization of the corneal erosion) during 3-5 days:

- antibiotic – three times a day;
- non-steroidal anti-inflammatory drug – three times a day;

3) the second stage – up to two months postoperatively:
- corticosteroid medicines in tapering doses scheme.

**Results and discussion**

At first examination, parameters of FTC and biochemical tests of LF of control group and the first main group did not differ significantly (p<0.5) (Tables 2, 3). Confocal microscopy showed that corneas were intact in all patients. This indicates homogeneity of groups and gives grounds for further correct comparison and interpretation of results.

All patients of the first and second main groups underwent KRO without intra-operative complications. Results of complex dynamic examination of patients of the first main group after KRO:

**Investigation of FTC**: In all patients in early postoperative period (from one hour to three days), we found intensification of reflexive tear production that distorted results of examination of basal secretion of LF and Break-up Time Test, and different degree of damage to corneal epithelium (from 4.9 points after LASIK to 12.3 points after PRK according to twenty points scale). Later on, we noted general tendency to decrease of total (Schirmer test-1) and basal (Schirmer test-2) tear production (maximally pronounced after

| Table 2. Results of FTC analysis in patients of control group and the first main group at first examination (M±s) |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Shirmer-1 Test, in mm for 5 min (I±o) | 20,5±1,5 | 21,0±1,2 | 20,7±0,9 |
| Shirmer-2 Test, in mm for 5 min (I±o) | 12,3±0,5 | 12,2±0,3 | 12,3±0,3 |
| Break-up time test, sec (I±o) | 19,1±0,7 | 18,9±0,7 | 19,0±0,6 |
| Corneal Epithelium Assessment, points (I±o) | 1,9±0,5 | 2,1±0,4 | 2,0±0,3 |

| Table 3. Results of biochemical analysis of LF in patients of control group and the first main group at first examination (M±s) |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Total Protein, g/l | 19,1±1,8 | 18,8±2,0 | 19,3±1,8 |
| Urea, mmole/l | 3,87±0.5 | 3,90±0.3 | 3,88±0.4 |
| Malonic dialdehyde, μmole/l | 1,39±0,22 | 1,41±0,22 | 1,40±0,15 |
| Superoxide Dismutase, Un/l | 25,1±2,0 | 25,6±2,2 | 25,7±2,5 |
LASIK) accompanied with decrease of stability of tear film (Break-up time test) (also maximally pronounced after LASIK) with gradual normalization of parameters by 6 (after PRK) and 8 months (LASIK) postoperatively. Degree of damage to epithelium after all types of KRO gradually decreased and reached initial values by month 1-3 of postoperative period (Figs. 6-9).

The study showed that analysis of FTC allows quick (to 15 minutes) evaluation of severe alterations of eye surface but has low specificity and does not meet the requirements of subclinical diagnosis of postoperative complications.

Investigation of biochemical coefficients of degree of corneal damage in dynamics of postoperative period of KRO was most interesting for us. It was noted that acquisition of reliable data on metabolic status of the anterior eye segment is possible from the second day after LASIK and third day after PRK (i.e. after cessation of pronounced reflexive tear production, which coincide with re-epithelialization of the area of surgery).

Results of dynamical coefficients in patients with uncomplicated postoperative period after KRO are presented on figures:

Dynamic study of coefficients in patients after KRO showed the following:

1) In uncomplicated course of PRK, values of coefficient evaluating SIOS (K1) in early postoperative period were within ranges of severe degree, impairments of SDP were of average degree. Values of coefficients reached norm by eight (K1) and six (K2) months postoperatively.

2) After LASIK, alterations of metabolic status (K1 and K2) are minimal, and achievement of initial level was observed by eight months postoperatively (Fig. 10, 11).
Confocal microscopy in dynamics of uncomplicated postoperative period allowed us to visualize the following features of corneal regeneration.

After LASIK, foreign inclusions of different origins were visualized in the interface of 97% of eyes (in 91.2% of cases they were metal, in 33.4% - lipid and mucin, and in 12.3% there were inflammatory macrophage-like cells and erythrocytes) (Fig. 12).

By days 10-14 of postoperative period, acellular zone began to form along both sides of the interface. It represented the area lacking differentiated cells, which gradually decreased in length and disappeared by 6-8 months postoperatively (Fig. 13).

In 78% of cases, microstripes of the corneal flap were visible (fig. 14). Re-innervation of the central zone of the cornea occurred by 8-12 months postoperatively. However, abnormal branching of newly formed nervous fibers and abundant anastomoses did not allow one to consider it to be full (fig. 15).
a – “scraps” of the nerve fibers of the superficial nerve plexus (arrow) damaged by microkeratome during corneal flap creation

b – re-innervation of the central corneal optical zone

Quantitative and qualitative analysis of endothelium revealed cell loss within 2.2-2.6 % without alteration of cellular morphology.

After PRK, epithelial defect was substituted by migration of wing-shaped epitheliocytes from intact zone of the cornea (Fig. 16).

Fig. 16. Substitution of epithelial defect with wing-shape epitheliocytes.

Thickness of newly formed epithelium was significantly higher (76.3±9.8 mm) as compared to intact cornea (52.1±6.5 mm).

Length of acellular zone was less (to 68 mm) than that after LASIK (to 160 mm), and re-innervation of the central optic zone occurred earlier (by 5-6 months). Loss of endothelial cells was 2.5-2.7 % by one year postoperatively.

Complex dynamic examination of patients with disregenerative KRO postoperative complications (main group 2) gave the following results:

1. Study of FTC parameters allowed us only to register complications but did not have essential prognostic value.

2. Calculation of values of biochemical coefficients of degree of corneal damage degree showed their significant difference from values typical of uncomplicated postoperative period: coefficient of SIOS (K1) was increased in 97.9 % of cases, coefficient of SDP (K2) – in 84.0 % of cases, both coefficients (K1 + K2) – in 76.6 % of cases, that confirms important role of these pathophysiological mechanisms in pathogenesis of disregenerative complications of KRO. Besides, we noted that in all cases, increase of these coefficients preceeded clinical manifestation of complications that allowed us to include them in predicting system of disregenerative complications of KRO.

3. Almost in all cases, confocal microscopy of the cornea in patients with disregenerative complications revealed specific pathomorphological signs of the forming complication at subclinical stage (Fig. 17-19).

Fig. 17. Confocal microscopy of the cornea of the patient with aseptic edema of the corneal valve (day 3 after LASIK).

Fig. 18. Confocal microscopy of the cornea of the patient with neurotrophic epitheliopathy (day 7 after LASIK).

Fig. 19. Confocal microscopy of the cornea of the patient with subepithelial fibroplasia (one month after PRK).

4. Based on the pathophysiological mechanisms revealed, we include the following medicaments into complex therapy:
<table>
<thead>
<tr>
<th>Complication</th>
<th>eye N</th>
<th>Time of finding</th>
<th>K1/K2 FTC in (average)</th>
<th>Specific features of FTC in (average)</th>
<th>Specific features of confocal microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotrophic epitheliopathy (NE)</td>
<td>22</td>
<td>Day 7-14</td>
<td>59,6 / 2,6</td>
<td>Epithelium condition (EC):</td>
<td>↓ number of basal epitheliocytes,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.7 points</td>
<td>local defects of epithelium</td>
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<td></td>
<td></td>
<td></td>
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<td>21.7 / 1.04</td>
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<td></td>
<td></td>
<td></td>
<td>2.9 points</td>
<td></td>
</tr>
<tr>
<td>Aseptic edema of corneal flap</td>
<td>2</td>
<td>Day 3</td>
<td>59.0 / 1.4</td>
<td>EC: 4.1 points</td>
<td>Diffuse edema of all layers of the</td>
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<td></td>
<td></td>
<td>cornea, thickening of the flap to 150</td>
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<td></td>
<td></td>
<td></td>
<td>um</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>28.8 / 1.05</td>
<td></td>
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<td></td>
<td></td>
<td>3.6 points</td>
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<tr>
<td>Dry-eye syndrome (DES)</td>
<td>19</td>
<td>Day 7 – one month</td>
<td>62.3 / 1.8</td>
<td>Schirmer test-1 (ST-1):</td>
<td>Increase of number of inflammatory</td>
</tr>
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<td></td>
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<td></td>
<td>8.2</td>
<td>cells</td>
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<td></td>
<td></td>
<td></td>
<td>14.1 / 0.8</td>
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<td>17.4</td>
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<tr>
<td>NE+DES</td>
<td>13</td>
<td>Day 7-14</td>
<td>64.3 / 2.5</td>
<td>EC / ST-1:</td>
<td>Local defects of epithelium + many</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>6.2/ 9.5</td>
<td>inflammatory cells in stroma</td>
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<tr>
<td>Subepithelial fibroplasia of the cornea</td>
<td>38</td>
<td>1-3 months</td>
<td>69.2 / 2.6</td>
<td>No specific features</td>
<td>There is an additional pike on the</td>
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<td>curve of optic density (behind</td>
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<td></td>
<td></td>
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<td>epithelium), ↑ reflective ability of</td>
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<td>extracellular matrix, ↓ of cell</td>
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<td></td>
<td>number in stroma</td>
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<td></td>
<td></td>
<td>13.3 / 0.7</td>
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<td>4.4 points</td>
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</tbody>
</table>

- antioxidants in patients with high values of coefficient of evaluation of SIOS;
- antioxidants + reparative drugs in patients with combined increase of both coefficients.
- reparative drugs in patients with high coefficient of evaluation of SDP evaluation;
- In all patients, we recorded quick regress of clinical signs of complications accompanied by decrease of biochemical coefficients.
Analysis of results obtained proposed the following diagnostic algorithm of early detection of complicated postoperative course (scheme 1).

Scheme 1

Final section of the study is a clinical proof of effectiveness of the proposed algorithm of predicting and correction of excessive lesion of the cornea resulted from KRO.

We selected group of patients (50 patients – 100 eyes) with myopia who were intended for LASIK (main group 3). To increase probability of signs of atypical postoperative course, patients with initially altered cornea because of long-term use of contact lenses (neovascular keratopathy) or previous KRO were included into the group. Patients were divided into two equal subgroups. LASIK was uncomplicated in all patients.

Design of the study: in all patients, pre- and postoperative examination was performed according to the proposed algorithm but in patients of the first subgroup, drug therapy was carried out in standard way and in patients of the second subgroup, we carried out differentiated correction of revealed lesions (scheme 2).

Results of the study represent at the scheme 3 and 4.

Thus, in the first subgroup with initially altered cornea, average degree of corneal lesion revealed by calculation of biochemical coefficients on day 2 postoperatively was accompanied by development of complications in 83.3% of cases, while severe degree of corneal lesion – in 100% of cases. In all cases, confocal microscopy confirmed the diagnosis.

In the second subgroup, drug correction (antioxidants and reparative drugs) was performed according to
Table 5. Algorithm of prophylaxis of complications after KRO

<table>
<thead>
<tr>
<th>Algorithm of prophylaxis</th>
<th>Criterion of effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Before KRO in patients with unaltered cornea: study of FTC and correction of deviations revealed</td>
<td>Normal value of FTC parameters</td>
</tr>
<tr>
<td>2. Before KRO in patients with initially affected cornea: FTC + biochemical coefficients + confocal microscopy of the cornea and correction of deviations revealed</td>
<td>Normal value of all examined parameters</td>
</tr>
<tr>
<td>3. After KRO in all patients: FTC and in the case of deviations of parameters and/or unclear clinical picture biochemical coefficients confocal microscopy in all patients with excessive corneal damage (even in absence of clinical signs – metabolic correction)</td>
<td>Decrease of biochemical coefficients to values typical of uncomplicated postop course</td>
</tr>
</tbody>
</table>

scheme described above in patients with average and severe degree of corneal lesion revealed on day 2 postoperatively by data of biochemical coefficients. This allowed us to achieve uncomplicated course during the whole period of observations in 94.4% of patients with initially altered cornea and excessive corneal damage by KRO.

The results obtained suggest the following algorithm of preventing dismetabolic complications of KRO based on early detection and correction of excessive damage to the cornea (table 5).

Thus, the study revealed morphological and metabolic features of uncomplicated course of PRK and LASIK and specific subclinical markers of excessive corneal damage causing disregenerative postoperative complications. Algorithm of prediction and correction of postoperative disregenerative complications of KRO, developed on the basis of these markers, will improve quality of rehabilitation of young socially active patients with ametropia who decide to get rid of spectacles or contact lenses with the help of excimer laser correction.

References


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