ORIGINAL ARTICLE

UDC: 617.7-089
DOI: 10.2298/VSP131203033R

Levels of interleukin-6 in tears before and after excimer laser treatment

Nivoi interleukina-6 u suzama pre i posle tretmana *excimer* laserom

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Abstract

Background/Aim. Immune response and consequent inflammatory process which originate on ocular surface after a trauma are mediated by cytokines. Photoablation of corneal stroma performed by excimer laser causes surgically induced trauma. Interleukin-6 (IL-6) is mostly known as a proinflammatory cytokine. However, it also has regenerative and anti-inflammatory effects. It is supposed that this cytokine is likely to play a significant role in the process of corneal wound healing response after photoablation of stroma carried out by laser *in situ* keratomileusis (LASIK) or photorefractive keratectomy (PRK) methods. The aim of this study was to determine and compare the levels of IL-6 in tears before and after treatment with LASIK and PRK methods. **Methods.** The study included 68 shortsighted eyes up to -3.0 diopter sphere, i.e. 198 samples of tears (*per* three samples taken from each of the eyes), divided into two groups according to the kind of excimer laser intervention performed: the group 1 – eyes treated by LASIK method (n = 31), and the group 2 – eyes treated by the PRK method (n = 37). The samples of tears were taken from each eye at the following time points: before excimer laser treatment (0 h, the control group), 1 h after the treatment (1 h) and 24 h after the treatment (24 h). The patients did not use anti-inflammatory therapy 24 h after the intervention. Tear samples were collected using microsurgical sponge. Level of IL-6 in tear fluid was determined by the flow cytometry method, applying a commercial test kit which allowed cytokine detection from a small sample volume. **Results.** The values of IL-6 were detectable in 16% of samples before LASIK treatment and in 30% of samples before PRK treatment. One h after the treatment IL-6 was detectable in 29% of samples for the LASIK group and 43% of samples for the PRK group, and 24 h after the treatment it was detectable in 19% of samples for the LASIK group and in 57% of samples for the PRK group. When we analyzed the dynamics of IL-6 production in particular groups, we noticed that both in the LASIK and PRK group the number of samples with increased values of IL-6 after 1 h, and after 24 h, was considerably larger than the number of samples with decreased values of IL-6 after the intervention. Analyzing the dynamics of IL-6 concentration changes in the 1 h samples *vs* 24 h samples there was a statistically significant increase in the number of samples with IL-6 concentration decline in the LASIK group, while at the same time no considerable changes occurred in the PRK group. Comparing average IL-6 values between the two treatment groups in all tear samples at 0 h, 1 h and 24 h after intervention a significantly higher level in the PRK group 24 h after procedure ($p = 0.0031$) was detected. **Conclusion.** IL-6 level in tears increases 1 h and 24 h after LASIK and PRK treatments. This increment is significantly larger 24 h after the treatment with the PRK method than with the LASIK method. Changes of IL-6 production levels in tears after excimer laser treatment indicate that this cytokine takes part in the corneal recovery process after stromal photoablation.

Key words:
keratomileusis, laser in situ; photorefractive keratectomy; interleukin-6; tears; laser therapy; treatment outcome.

Apstrakt

Uvod/Cilj. Imunski odgovor i posledični inflamacijski proces koji nastaju na okularnoj površini nakon dejstva traume, pod uticajem su citokina. Fotoablacija strome rožnjače dejstvom *excimer* lasera dovodi do hirurški nastale traume. Interleukin-6 (IL-6) poznat je kao proinflamacijski citokin, ali on ispoljava i regenerativna i antiinflamacijska dejstva. Pretpostavlja se da bi ovaj citokin mogao imati značajnu ulogu u procesu zarastanja rane rožnjače nakon izvršene fotoablacije strome LASIK (laser *in situ* keratomilensis) i PRK (fotorefraktivna keratektomija) metodom. Cilj ovog rada bio je

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određivanje i upoređivanje nivoa IL-6 u suzama pre i posle lečenja LASIK i PRK metodama. **Metode.** U studiju je bilo uključeno 68 kratkovidih očiju do -3,0 dioptrija sfere, tj. 198 uzoraka suza (iz svakog oka po 3 uzorka suza), podeljenih u 2 grupe, zavisno od vrste izvedene *excimer* laser intervencije: grupa 1 – oči lečene LASIK metodom (n = 31) i grupa 2 – oči lečene PRK metodom (n = 37). Uzorci suza su uzeti iz svakog oka i to: pre izvođenja lečenja *excimer* laserom (0 h, kontrolna grupa), 1 h posle i 24 h posle lečenja *excimer* laserom. Pacijenti nisu koristili antiinflamacijsku terapiju 24 h nakon intervencije. Uzorci suza su prikupljani mikrohirurškim sunđerom. Nivo IL-6 u suznoj tečnosti određivan je metodom protočne citometrije, primenom komercijalnog kompleta za testiranje koji omogućuje detekciju citokina iz malog volumena. **Rezultati.** Vrednosti IL-6 bilo je moguće otkriti u 16% uzoraka pre LASIK i u 30% uzoraka pre PRK lečenja. Jedan sat nakon lečenja IL-6 je bilo moguće otkriti u 29% uzoraka LASIK grupe i 43% uzoraka PRK grupe, dok je 24 h nakon lečenja bilo moguće otkriti u 19% uzoraka LASIK grupe i 57% uzoraka PRK grupe. Kada je analizirana dinamika promene vrednosti koncentracije IL-6 u pojedinim grupama, zapaženo je da je i u LASIK grupi i u PRK grupi broj uzoraka u kojima je došlo do porasta vrednosti IL-6 posle 1 h, odnosno posle 24 h, bio značajno veći nego broj uzoraka u kojima su vrednosti IL-6 bile snižene. Analizirajući dinamiku promene koncentracije IL-6 u uzorcima 1 h prema 24 h, u LASIK grupi došlo je do statistički značajnog porasta broja uzoraka u kojima je registrovan pad koncentracije IL-6, dok u PRK grupi nije bilo značajnih promena. Poređenjem srednjih vrednosti IL-6, u svim uzorcima suza u okviru termina 0 h, 1 h, i 24 h, između LASIK i PRK grupe nađen je značajno viši nivo ovog citokina samo 24 h posle tretmana u PRK grupi (p = 0,0031). **Zaključak.** Nivo IL-6 u suzama raste 1 h i 24 h nakon LASIK i PRK tretmana. Ovaj porast je značajno veći 24 h nakon PRK tretmana u poređenju sa LASIK tretmanom. Promene u nivoima produkcije IL-6 u suzama nakon *excimer* laser lečenja ukazuju na učešće ovog citokina u procesu oporavka rožnjače nakon fotoablacije strome.

Ključne reči:
keratomileusis, laser in situ; fotorefraktivna keratektomija; interleukin-6; suze; lečenje laserom; lečenje, ishod.

Introduction

Excimer laser keratectomy implies a remodelling of cornea by photoablation, thus removing its stroma [1]. The photoablative process removes tissue material of corneal stroma with great precision, leaving the surface behind perfectly smooth [2]. Laser *in situ* keratomileusis (LASIK) and photorefractive keratectomy (PRK) are the two most frequently performed refractive surgical procedures using excimer laser. Corneal wound healing response after photoablation of stroma by excimer laser is the determinant of efficiency and safety of these procedures. Clinical outcomes as well as numerous complications of the procedures (hypercorrection, hypocorrection, regression, stromal haze) are directly related to the processes of reparation and the complex nature of corneal cell response. Both methods apart from their positive effect in terms of correction of existing ametropia, lead to surgically induced trauma. Trauma response consists of a complex cascade of cellular interactions mediated by cytokines, growth factors and chemokines. Corneal trauma response is intertwined by interactions of epithelial, stromal, neural, lacrimal cells and immune system cells. Interactions between these cells determine corneal wound healing response and they help regenerate and maintain anatomy and normal physiology of cornea [3, 4].

When responding to wound healing, in the PRK method cornea may more frequently react in subepithelial haze than in the LASIK method. The main cause of the haze is the interaction between epithelium and stromal keratocytes mediated by cytokines, which activates keratocytes and causes degradation of stromal extracellular matrix. Compared to the PRK method, corneal wound healing response after the LASIK method is featured with a weaker interaction between the epithelium and stromal keratocytes since the epithelial surface remains generally intact in the LASIK method [5].

Immune response and the consequent inflammatory process occurring on the ocular surface after the effect of trauma are mediated by cytokines. Interleukin-6 (IL-6) is produced by the following cells from the ocular surface: macrophages, mast cells, epithelial cells of conjunctiva and cornea, keratocytes, fibroblasts and vascular endothelial cells [6]. This cytokine is mostly known as proinflammatory cytokine. However, it also has regenerative and anti-inflammatory effects [7]. For these reasons it is interesting to examine the role of IL-6 in corneal wound healing response after stromal photoablation. The aim of this study was to determine and compare the levels of IL-6 in tears before and after the treatment with LASIK and PRK methods.

Methods

This clinical randomized prospective cohort study was carried out, with the permission given by the Ethical Board of Military Medical Academy in Belgrade. With a notified consent from each of the participants the study included 68 shortsighted eyes up to -3.0 diopter sphere, i.e. 198 samples of tears (*per* 3 samples taken from each eye) divided into two groups based on the kind of excimer laser intervention performed: the group 1 – eyes treated with the LASIK method (n = 31), and the group 2 – eyes treated with the PRK method (n = 37).

Each group was then divided into three subgroups based on the time of observations, i.e. time of tears sampling. Tear samples were taken from each eye as follows: before excimer laser treatment (0 h, the control group), 1 h after the treatment (1 h) and 24 h after the treatment (24 h).

The inclusion criteria were that patients did not use anti-inflammatory therapy 24 h after the intervention and had no presence of general or eye disorders. The exclusion criterion was a dioptric error greater than -3.0 diopter sphere.

There were 35 participants included in the study, that is 68 eyes were treated (two patients had one eye operated). There was a total of 16 patients in the LASIK group (9 men and 7 women), and a total of 19 patients in the PRK group (14 men and 5 women).

The LASIK and PRK methods were performed by a Wavelight Allegretto (400 Hz) excimer laser. In the PRK method the energy of excimer laser is applied directly onto deepithelialized corneal stroma (Figure 1). In the LASIK method the energy of excimer laser is applied on deeper layers of stroma, i.e. at a larger distance from corneal epithelium (Figure 2). To create a flap we used a Moria microkeratome during the LASIK method procedure, and when we applied the PRK method we removed corneal epithelium with an Amoils rotational brush.

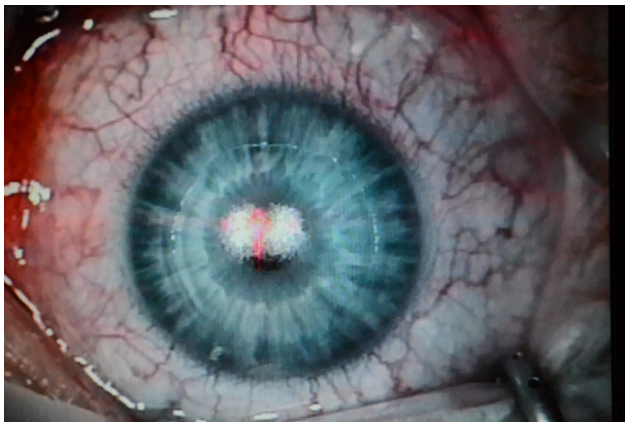

Fig. 1 – Corneal stroma after photoablation during photorefractive keratectomy method.

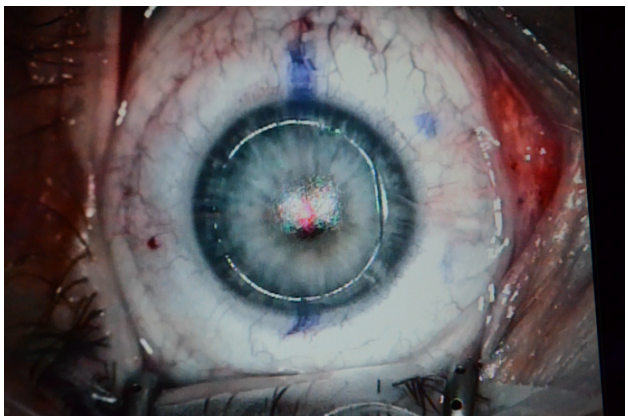

Fig. 2 – Corneal stroma after photoablation during laser *in situ* keratomileusis method.

A procedure described in the study by Acera et al. [8] was applied to collect the samples of tears. The tear fluid was sampled from lower lateral tear meniscus with minimal irritation of ocular surface and the edge of eyelids, with no use of anesthetics (Figure 3). Each tear sample was taken by using a cellulose microsurgical sponge (Alcon, USA). After sampling, the tear fluid was separated by centrifugation of the sponge in a 0.5 mL volume of phosphate buffered saline (PBS). The samples were centrifugated at 13,000 rpm for 15 min at 4°C (MPW-350 r, Med. Instruments, Poland). Collected samples were kept at -80°C until the final examination.

IL-6 level in tear fluid was determined by the flow cytometry method, and we used the commercial test kit (Human Th1/Th2 11 plex FlowCytomix Multiplex) intended for cytokine detection from a small sample volume.

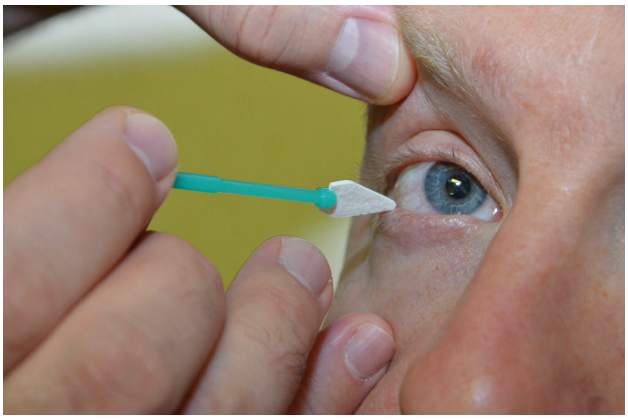

Fig. 3 – Tear fluid sampling from lower lateral tear meniscus using a cellulose microsurgical sponge with minimal irritation of ocular surface.

Methods of descriptive and inferential statistics were used to process statistical data: mean, standard deviation, maximum and minimum values, mode and median for descriptive statistics, and Wilcoxon signed-rank test, ANOVA-Bonferroni test, Mann-Whitney test and Chi square test for inferential statistics.

Results

The average age of the patients in the LASIK group was 34 (33.81 ± 6.52) years, and in the PRK group it was 33 (33.05 ± 6.11) years.

IL-6 concentration was estimated in samples obtained from 31 eyes treated with the LASIK method and 37 eyes treated with the PRK method, at the following time intervals: before the intervention (0 h), 1 h after and 24 h after the intervention (Table 1).

IL-6 was detectable in 16% of samples before LASIK treatment and 30% of samples before the PRK treatment. One h after the treatment IL-6 was detectable in 29% of the LASIK group samples and in 43% of the PRK group samples, and 24 h after the treatment IL-6 was detectable in 19% of the LASIK group samples and 57% of the PRK group samples (Figure 4).

The analysis of tear samples with a detectable IL-6 concentration showed a significant increase in the PRK treated group when we compared posttreatment levels (1 h and 24 h) to the control (pre-treatment level, 0 h). Namely, IL-6 concentration changes showed a significant increment in the PRK treated patients in both time intervals after the treatment compared to pretreatment (0 h/1 h, $p = 0.0031$; 0 h/24 h, $p = 0.0059$). IL-6 concentration average value ratios were 2.365 *vs* 13.01 pg/mL in 0 h/1 h samples and 2.365 *vs* 19.09

pg/mL in 0 h/24 h samples. There was no significant difference between IL-6 levels in 1h and 24 h samples. In the LASIK treated group there was no significant increase in concentration of IL-6 in serial tear samples (Table 2).

Table 1

The levels of IL-6 (pg/mL) in tear samples from the participants treated with the LASIK and PRK methods

ID	LASIK (n = 31)			ID	PRK (n = 37)		
	0 h	1 h	24 h		0 h	1 h	24 h
34	0.00	0.00	0.00	1	0.00	0.00	12.61
35	0.00	0.00	0.00	2	0.00	0.00	18.90
40	0.00	17.13	0.00	3	0.00	0.00	0.00
41	0.00	15.20	0.00	4	0.00	3.85	2.63
42	0.00	9.32	0.00	5	11.12	18.31	27.44
43	0.00	0.00	0.00	6	4.52	25.71	21.38
44	0.63	0.00	0.00	7	0.00	3.52	91.95
45	0.00	0.00	0.00	8	0.00	0.00	0.00
46	0.00	0.00	0.00	9	1.90	0.00	0.00
47	0.00	0.00	0.00	10	0.00	0.00	4.66
48	0.00	0.00	0.00	11	0.00	0.00	116.82
49	0.00	10.75	0.00	12	0.00	0.00	86.10
50	0.00	0.63	0.00	13	1.90	0.00	0.00
51	0.00	0.00	0.00	14	0.00	0.00	0.00
52	0.00	0.00	0.00	15	0.00	0.00	0.00
53	14.07	27.44	14.92	16	26.57	2.55	0.00
54	0.00	0.00	0.00	17	1.90	86.55	0.00
55	0.00	0.00	87.12	18	0.00	6.54	0.00
56	18.31	0.00	0.00	19	0.00	0.00	2.96
57	0.00	0.00	0.70	20	0.00	0.00	0.00
58	0.00	0.00	0.00	21	0.00	0.00	0.00
59	0.00	0.00	3.64	22	0.00	0.00	4.66
60	0.00	0.00	0.00	23	0.00	0.00	94.49
61	87.59	111.79	99.01	24	0.00	0.00	0.00
62	7.23	22.36	17.76	25	0.00	7.92	0.00
63	0.00	17.52	0.00	26	6.88	86.09	0.00
64	0.00	0.00	0.00	27	7.70	17.52	0.00
65	0.00	0.00	0.00	28	0.00	0.00	22.22
66	0.00	0.00	0.00	29	0.00	16.74	85.90
67	0.00	0.00	0.00	30	0.00	18.31	8.18
68	0.00	0.00	0.00	31	0.00	86.89	0.00
				32	0.00	7.23	1.63
				33	0.00	0.00	8.18
				36	3.20	5.87	3.98
				37	19.90	87.88	2.96
				38	0.00	0.00	2.63
				39	1.90	0.00	86.03

LASIK – laser *in situ* keratomileusis; PRK – photorefractive keratectomy; IL-6 – interleukin 6.

Table 2

Statistical significance of differences in production of IL-6 in tear samples with detectable IL-6 values collected 1 h and 24 h after treatment by LASIK and PRK methods, compared to the control (0 h)

Colection time of tears samples	LASIK *(p)*	PRK *(p)*
0 h/1 h	> 0.05	0.0031*
0 h/24 h	> 0.05	0.0059*
1 h/24 h	> 0.05	> 0.05

***Statistically significant difference (Wilcoxon test); LASIK – laser *in situ* keratomileusis; PRK – photorefractive keratectomy; IL-6 – interleukin 6.**

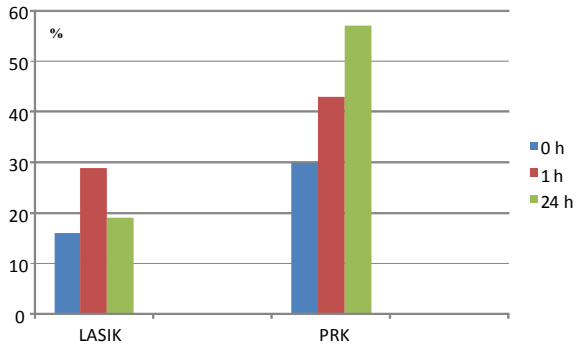

Fig. 4 – Percentages of samples of detectable concentration of IL-6. Tear samples were collected before (0 h) and after (1 h and 24 h) LASIK and PRK treatments.

LASIK – laser *in situ* keratomileusis; PRK – photorefractive keratectomy; IL-6 – interleukin 6.

When we analyzed all tear samples (including samples with undetectable concentration of IL-6) we found a significant difference in the production of IL-6 in tear samples collected 24 h after the treatment compared to the control (0 h) only in the PRK group (Table 3).

values for all the shortsighted eyes included in the study were up to -3.0 diopter sphere in order to expose corneas of every eye to a similar photoablative trauma. Efficiency and safety of the LASIK and PRK method in shortsightedness correction are shown in the studies of Vukosavljević et al. [9] and Resan et al. [10].

Table 3

Statistical significance of differences in production of IL-6 in all analyzed tear samples collected 1 h and 24 h after treatment by LASIK and PRK methods, compared to the control (0 h)

Colection time of tears samples	LASIK (*p*)	PRK (*p*)
0 h/1 h	> 0.05	> 0.05
0 h/24 h	> 0.05	< 0.05*
1 h/24 h	> 0.05	> 0.05

***Statistically significant (ANOVA, Bonferroni test)**
LASIK – laser *in situ* keratomileusis; PRK – photorefractive keratectomy; IL-6 – interleukin 6.

Comparison of average IL-6 values in all tested samples between the two treatment groups showed a significantly higher level of IL-6 in the PRK group 24 h after procedure ($p = 0.0031$) (Table 4).

Table 4

Statistical significance of differences in average IL-6 values in all tested samples between the treatment groups, LASIK and PRK, at the different points of time

Colection time of tears samples	LASIK *vs* PRK *(p)*
0 h	> 0.05
1 h	> 0.05
24 h	0.0031*

***Statistically significant difference (Mann-Whitney test).**
LASIK – laser *in situ* keratomileusis; PRK – photorefractive keratectomy; IL-6 – interleukin 6.

Frequency of IL-6 value change showed similar characteristics both in the LASIK and PRK group when we analyzed posttreatment (1 h and 24 h) to pretreatment (0 h) numbers of increments. Almost 80% and 70% of all samples collected 1h and 24h after treatments, respectively in both groups had an increase in IL-6 concentration compared to 0 h (before the treatment). When we analyzed 1 h *vs* 24 h numbers of increments, we found a significantly lower number of samples with increased IL-6 concentration in the LASIK group in comparison to the PRK group (Table 5).

In our study values of IL-6 were detectable in 16% of tear samples before the LASIK treatment and in 30% of tear samples before PRK treatment. One h after the treatment IL-6 was detectable in 29% of the LASIK group samples and 43% of the PRK group samples, while 24 h after the treatment IL-6 was detectable in 19% of the LASIK group samples and 57% of the PRK group samples. When we analyzed dynamics of IL-6 production in separate groups we noticed that the number of samples with increased values of IL-6, 1 h and 24 h after the treatments, was larger than the number of samples with decreased values of IL-6 in both groups (LASIK and PRK) compared to the control (0 h). Analyzing the dynamics of IL-6 production in samples with detectable level of cytokine, collected 1 h and 24 h after the treatments, we found a significantly higher number of samples of lower concentration of IL-6 in the LASIK group 24 h after the treatment compared to 1 h. No significant changes between the number of samples with decreased or increased concentration of IL-6 were observed in the PRK group at the same time points.

Leonardi et al.[11] in their study examine levels of different cytokines and chemokines in tears from shortsighted eyes before and after LASIK intervention as well as in corneal fibroblast cultures before and after excimer laser treatment. Tears were sampled by a glass capillary micropipette from the eyes of 15 shortsighted patients before, 1 h after and 24 h

Table 5

Dynamics of IL-6 production in tear samples from the patients treated with the LASIK and PRK methods (only samples with detectable IL-6)

Colection time of tears samples	LASIK (%)		PRK (%)	
	▲	▼	▲	▼
0 h/1 h	82	18	79	21
0 h/24 h	75	25	74	26
1 h/24 h	25	75	54	46

▲ – number (%) of samples with increased IL-6 level;
▼ – number (%) of samples with decreased IL-6 level.

Discussion

Our study included shortsighted eyes because shortsightedness is the most common ametropia and at the same time the most common indication for laser diopter removal. Diopter

after LASIK intervention. The levels of cytokine in tears were determined by the multiplex bead analysis system. In this study IL-6 was not detected in patients' tears before LASIK treatment. Postoperatively, 24 h after LASIK treatment, the level of IL-6 in tear samples rosen in 9 out of 15

patients (60%). The mean tear IL-6 value was in a significant correlation with the mean symptom score value 1 h after LASIK treatment. Corneal fibroblast culture had an elevated IL-6 level before excimer laser treatment. One hour after the culture was exposed to excimer laser the IL-6 level decreased. At 24 h after excimer laser treatment IL-6 level was significantly increased as compared with the baseline level and 1 h value. After the surgery, the symptom score was only in correlation with tear sample IL-6 values which showed direct involvement of this cytokine in postsurgical inflammation development and in the corneal wound healing processes [12]. In our study, compared with the Leonardi et al. [11], IL-6 was detectable 24 h after the treatment in 19% of tear samples of the LASIK treated patients.

Malecaze et al.[13] studied the role of IL-6 in corneal wound healing after PRK treatment. Similarly to our results, they obtained the increase in IL-6 level in tear samples 24 h after treatment and stated that IL-6 is probably produced by epithelial cells and keratocytes.

Prada et al. [14] analyzed the gene expression for TNF-α and IL-6 in corneas of rats after phototherapeutic keratectomy (PTK) treatment. Regarding IL-6, there was a significant rise of gene expression 1 h after PTK treatment compared to the control one. Twelve hours after the treatment there was an even larger elevation of IL-6 gene expression, only to decline 24 h after treatment. Still, gene expression remained significantly elevated compared to the control one. The IL-6 expression was detected not only in epithelial and endothelial cells but also in keratocytes of corneal stroma. In our study, there is a similar dynamism only in IL-6 detectability change in tear samples of the LASIK treated patients. The PTK treatment procedure is more similar to the PRK method than the LASIK method. However, it is to be mentioned that the Prada et al. [14] study was carried out on an animal model.

The results of our study indicate that the local production of IL-6 was of higher magnitude after the PRK comparing to the LASIK method. Higher local bioavailability of this cytokine after the PRK treatment could be the consequence of more intense injury of corneal epithelium influenced by the method itself.

Conclusion

IL-6 level in tears increases 1 h and 24 h after laser *in situ* keratomileusis and photorefractive keratectomy treatments. This increment is larger 24 h after the treatment in photorefractive keratectomy method than in laser *in situ* keratomileusis method. Changes of IL-6 production levels in tears after excimer laser treatment indicate that this cytokine takes part in the corneal recovery process after stromal photoablation.

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Received on December 3, 2013.
Revised on April 2, 2014.
Accepted on April 3, 2014.
On Line-First May, 2014.