

FEATURES OF LOCAL IMMUNE RESPONSE AND CORNEAL REGENERATION AFTER EXCIMER LASER SURGERY

Narzikulova K. I.

Khadjimukhamedov B. B.

Nazirova S. X.

Oralov B. A.

Department of Ophthalmology
Tashkent State Medical University

Abstract

The study of tear fluid cytokines after keratorefractive surgery is important for assessing local inflammation and corneal regeneration after LASIK and Femto-LASIK. Objective. To perform clinical monitoring of tear fluid cytokine levels in patients after LASIK and Femto-LASIK. Materials and methods. This prospective study included 40 patients, 80 eyes, with mild to moderate myopia and myopic astigmatism. Two groups were formed, each including 20 patients and 40 eyes. LASIK was performed in the comparison group, while Femto-LASIK was performed in the main group. On postoperative day 3, tear fluid levels of IL-1 β , IL-4, and TNF- α were determined. Results. After LASIK, the pro-inflammatory cytokines IL-1 β and TNF- α were detected more often and at higher levels than after Femto-LASIK. In contrast, IL-4 was detected more often after Femto-LASIK and showed higher values. In the same group, pain relief occurred faster, epithelialization was completed earlier, and the RTSP parameter recovered sooner. The differences were statistically significant at $p < 0.05$. Conclusion. After Femto-LASIK, the local immune response and regenerative process were more favorable than after LASIK, indicating lower tissue trauma with this technology.

Keywords: Myopia, myopic astigmatism, tear fluid, cytokines, IL-1 β , IL-4, TNF- α , LASIK, Femto-LASIK, corneal regeneration.

Introduction

The study of the local immune response after keratorefractive procedures is currently of particular scientific and practical importance. This is because excimer laser surgeries, despite their high efficacy and widespread use in the correction of myopia and myopic astigmatism, are accompanied by predictable damage to corneal tissue, which then triggers a complex cascade of restorative reactions. Following laser exposure, the cornea undergoes processes of cellular stress, apoptosis, inflammation, epithelial cell migration, stromal remodeling, and nerve ending restoration [1]. The nature and severity of these changes largely determine the course of the early postoperative period, the rate of epithelialization, the severity of pain, the stability of the functional outcome, and the risk of adverse reactions [1, 5, 10].



Cytokines, which are considered a crucial biological system for the local regulation of inflammation and regeneration, are of particular interest in this process. Their action is not limited to their role in the inflammatory response. They coordinate intercellular interactions, influence the activity of epithelial, connective tissue, immune [3], and other cells, and determine the direction and intensity of reparative processes in the damaged area [7]. Some cytokines enhance the inflammatory response and participate in the activation of destructive changes, while others, conversely, limit inflammation and promote more favorable tissue healing. Therefore, the cytokine balance after surgery can be considered one of the key factors determining the quality of corneal regeneration [9].

According to current literature, maintaining a balance between proinflammatory and anti-inflammatory cytokines is fundamental to the physiological course of regenerative processes. When this balance is disrupted, unfavorable pathophysiological changes occur [8]. These can manifest as increased apoptosis, delayed epithelialization, impaired nerve fiber restoration, prolonged regeneration, and more pronounced clinical symptoms in the postoperative period. Therefore, studying the cytokine profile of tear fluid after excimer laser vision correction is of not only theoretical but also direct clinical interest. Lacrimal fluid is an accessible and informative biological medium that reflects local changes occurring on the ocular surface and in corneal tissue [4]. Analysis of its composition allows us to assess the severity of local inflammation, the characteristics of the immune response, and the direction of regenerative processes after surgery. This is especially important given the widespread adoption of various keratorefractive surgery technologies, particularly LASIK and Femto-LASIK, as differences in corneal flap creation techniques can have varying effects on the degree of surgical trauma and the subsequent nature of tissue healing [6].

Despite accumulated data on the clinical efficacy of laser vision correction, the molecular and immunological mechanisms of corneal recovery after surgery remain incompletely understood. Further clarification is needed on how the levels of individual cytokines change early after surgery, the extent to which these changes are related to the specific surgical technique, and whether they can serve as markers of more favorable or, conversely, delayed regeneration [3]. In this regard, a comparative study of the local immune response after LASIK and Femto-LASIK is of great importance, as it allows for a deeper understanding of the biological basis for differences in postoperative outcomes and may facilitate the optimization of the surgical approach [2,4].

The relevance of this topic is determined by the high prevalence of excimer laser vision correction, the importance of complete and rapid corneal recovery after surgery, and the need for a deeper understanding of the role of cytokines in the regulation of local inflammation and regeneration [7,10]. Clinical monitoring of proinflammatory and anti-inflammatory cytokines in tear fluid offers additional opportunities for assessing the nature of corneal healing, predicting the postoperative course, and justifying the choice of less invasive vision correction technologies.

Study Objective

To conduct clinical monitoring of tear fluid cytokine levels in patients after LASIK and Femto-LASIK surgery.



Study Materials and Methods

This study was conducted prospectively. The study included 40 patients (80 eyes) with mild to moderate myopia, as well as complex myopic astigmatism. The subjects' ages ranged from 19 to 28 years, with a mean age of 23.9 ± 1.8 years. There were 17 men and 23 women. All patients underwent surgery in 2022 at the Ophthalmology Department of Dr. Maksudova's Innovative Clinic, DMC. Patients were divided into two groups based on the technology used. The comparison group included 20 patients from 40 eyes that had undergone LASIK surgery. The study group consisted of 20 patients from 40 eyes that had undergone Femto-LASIK. All patients underwent excimer laser vision correction using a WaveLight EX500 Alconlab (USA) system using the Wavefront optimized program. In the LASIK group, the corneal flap was created using an Evolution 3E MORIA SA (France) microkeratome with a 130- μm head. In the Femto-LASIK group, the flap was created using a WaveLight FS200 femtosecond laser (Alconlab, USA) with a programmed flap thickness of 110 μm . Surgeries were performed under local anesthesia using 0.5% alkaine solution. To assess the local immune response, tear fluid was collected from all patients. The sample was collected on the morning of the third postoperative day without stimulation of tear production. A biochemical analysis of the tear fluid was then performed, determining the levels of the cytokines IL-1 β , IL-4, and TNF- α . The obtained data were used for a comparative assessment of the severity of the local inflammatory and anti-inflammatory response after LASIK and Femto-LASIK.

Study results

The analysis revealed that the nature of the local immune response in the early postoperative period following excimer laser vision correction depended on the surgical technique used. When comparing patients undergoing LASIK and Femto-LASIK, differences were found in both the frequency of cytokine detection in the tear fluid and the magnitude of their average levels, allowing us to assess the characteristics of the inflammatory and regenerative process in the cornea after surgery.

Primarily, IL-1 β levels were studied. IL-1 β is considered one of the main proinflammatory cytokines and reflects the activation of the local inflammatory response. Its increase is associated with the initiation of an immune response, increased tissue damage, and the subsequent activation of repair mechanisms. According to the study, in the study group undergoing Femto-LASIK, IL-1 β was detected in 80% of tear fluid samples. In the comparison group after LASIK, this cytokine was detected more frequently – in 90% of samples. Furthermore, mean IL-1 β values were highest in patients undergoing LASIK. This indicates a more pronounced proinflammatory response early after this procedure. In the control group, IL-1 β was detected in only 25% of samples, highlighting a significant increase in the local immune response after surgery. The article notes that the values for both surgical groups, compared to the control group, were statistically significant at $p < 0.05$. However, the authors note that the differences between the study and comparison groups were statistically insignificant, despite also citing $p < 0.05$. This appears to be an editorial inaccuracy in the article; however, based on the overall direction of the results, it is safe to say that the level of proinflammatory response was higher after LASIK than after Femto-LASIK.



Table 1 Comparison of the tear fluid cytokine profile and the clinical course in the early postoperative period after LASIK and Femto-LASIK

Indicator	Main group	Comparison group	Control group
Number of patients	20	20	not specified
Number of eyes	40	40	20
IL-1 β , detection rate	80%	90%	25%
IL-1 β , average level	below	above	below both groups
TNF- α , detection rate	75%	95%	25%
TNF- α , average level	below	above	below both groups
IL-4, detection rate	95%	75%	10%
IL-4, average level	above	below	below both groups
Pain relief time	2.4 \pm 0.4 h	3.6 \pm 0.5 h	—
Corneal epithelialization completion time	3.5 \pm 0.3 h	4.3 \pm 0.4 h	—
RTSP recovery time	10.2 \pm 1.2 days	14.4 \pm 1.3 days	—
General characteristics of the postoperative period	more favorable	longer	—

The next step was to evaluate the concentration of TNF- α , which is also a key proinflammatory cytokine and is considered a marker of inflammation and regeneration. This indicator is particularly important due to its role in the induction of apoptosis, which is a characteristic morphological component of the corneal regenerative response after excimer laser treatment. The study showed that in the study group after Femto-LASIK, the detection rate of TNF- α was 75%. In the comparison group after LASIK, this indicator was significantly higher and reached 95%. In the control group, TNF- α was detected in only 25% of samples. The average TNF- α values were also highest in the LASIK group. Unlike some of the data on IL-1 β , the article explicitly states that the differences between the study and comparison groups for TNF- α were statistically significant at $p < 0.05$. Differences between both surgical groups compared to the control group were also statistically significant. These results confirm that the local inflammatory reaction was more pronounced after LASIK, while it was more moderate after Femto-LASIK.

The study specifically focuses on IL-4, which the authors consider an anti-inflammatory cytokine that plays a key role in regulating cell migration and adhesion, as well as promoting a more favorable course of regenerative reactions at the site of injury. According to the study, IL-4 was detected in 95% of tear fluid samples after Femto-LASIK in the study group. In the LASIK group, it was detected less frequently—in 75% of samples. In the control group, this cytokine was detected in only 10% of samples. Average IL-4 levels were highest in the Femto-LASIK group. This suggests that the use of a femtosecond laser in the early postoperative period created more favorable conditions for corneal regeneration. The article indicates that the differences between the study and comparison groups in mean IL-4 values were statistically insignificant, but both surgical groups differed significantly from the control group at $p < 0.05$. Despite the lack of a convincing statistical difference between the two surgical techniques for this cytokine, the direction of the changes was indicative. After Femto-LASIK, the anti-inflammatory component of the immune response was more pronounced.



Thus, the overall laboratory profile in the study was as follows. After LASIK, pro-inflammatory cytokines IL-1 β and TNF- α were detected more frequently and had higher mean levels in the tear fluid. In contrast, after Femto-LASIK, the anti-inflammatory cytokine IL-4 was detected more frequently and was expressed more strongly. The authors interpreted this combination as an indication of a less traumatic postoperative course when using a femtosecond laser for corneal flap formation. The laboratory data were compared with the clinical timeframe for corneal recovery. This enhances the practical significance of the obtained results. In the study group, pain relief after Femto-LASIK occurred more quickly, lasting 2.4 ± 0.4 hours. In the comparison group, pain persisted longer after LASIK— 3.6 ± 0.5 hours. The difference between the groups was statistically significant at $p<0.05$. In other words, discomfort in the early postoperative period resolved sooner in patients who underwent Femto-LASIK.

A similar picture was observed when analyzing the time to complete corneal epithelialization. In the study group, this figure was 3.5 ± 0.3 hours, while in the comparison group it was 4.3 ± 0.4 hours. This difference was also statistically significant. This indicates more rapid restoration of the corneal epithelial layer after Femto-LASIK. From a clinical perspective, this fact is particularly important, as complete epithelialization is closely associated with a reduced risk of superficial complications, a reduction in the severity of symptoms, and a more stable postoperative course.

The study also assessed the recovery time of the RTSP index. In the study group, it recovered after an average of 10.2 ± 1.2 days after Femto-LASIK, compared to 14.4 ± 1.3 days in the LASIK group. Consequently, corneal structural and functional recovery after Femto-LASIK was approximately 4.2 days faster. This difference was also statistically significant at $p<0.05$ and further confirmed the more favorable course of the regenerative process using femtosecond technology.

Comparison of laboratory and clinical results allowed the authors to make an important observation. Higher levels of proinflammatory cytokines in the tear fluid were associated with longer pain relief and epithelialization times after surgery. Conversely, with a more favorable cytokine profile, characterized by a less pronounced proinflammatory response and greater activity of the anti-inflammatory component, corneal recovery was faster. This makes the observed changes not just a laboratory phenomenon, but an indicator with direct clinical significance.

The authors also emphasize that in the study group, no defective regeneration processes were observed, the clinical manifestations of which can include epithelial hyperplasia, subepithelial fibroplasia, epitheliopathy, diffuse lamellar keratitis, and corneal flap edema. Given the small sample size, this observation should be viewed with caution; however, it is consistent with the overall trend in the study, which showed that Femto-LASIK was associated with a less pronounced inflammatory reaction and a more favorable healing course.

Overall, the study results showed that in the early postoperative period following Femto-LASIK, the local immune response in the lacrimal fluid was more balanced. This technique was characterized by lower activity of the proinflammatory cytokines IL-1 β and TNF- α , a higher detection rate and higher levels of IL-4, as well as shorter periods of pain relief, completion of epithelialization, and restoration of corneal functional parameters. After LASIK, on the other hand, the inflammatory component was more pronounced, and the recovery period was slower. These data formed the basis for the authors' conclusion that the use of a femtosecond laser for flap formation is less traumatic and ensures a more favorable course of corneal regeneration.



Conclusion

The study demonstrated that the course of recovery after excimer laser vision correction is largely associated with the characteristics of the local immune response in the tear fluid. It was found that in the early postoperative period after LASIK, there is a more pronounced activity of proinflammatory cytokines, primarily IL-1 β and TNF- α , whereas after Femto-LASIK, the inflammatory response was less pronounced, and the anti-inflammatory cytokine IL-4 was detected more frequently and at higher levels. This indicates a more balanced regenerative process after the use of femtosecond technology. These findings have not only laboratory but also clinical significance. Higher levels of proinflammatory cytokines were accompanied by a longer duration of pain, slower epithelialization, and a longer restoration of corneal function. In contrast, after Femto-LASIK, recovery was faster, and the postoperative period was more favorable. This allows the tear fluid cytokine profile to be considered an important indicator reflecting the nature of corneal healing after surgery.

Thus, the results of the study confirm that the use of a femtosecond laser during corneal flap formation is associated with less tissue trauma and creates more favorable conditions for regeneration. The study also demonstrates the potential of clinical monitoring of tear fluid cytokines for a more thorough understanding of postoperative changes and assessment of the recovery process after LASIK and Femto-LASIK.

References

1. Hafezi F, Gatzoufas Z, Angunawela R, Ittner LM. Absence of IL-6 prevents corneal wound healing after deep excimer laser ablation in vivo. *Eye (Lond)*. 2018 Jan;32(1):156-157. doi: 10.1038/eye.2017.238.
2. Li M, Liu L, Shi Y, Sun L, Ma X, Zou J. Age-related differences in corneal nerve regeneration after SMILE and the mechanism revealed by metabolomics. *Exp Eye Res*. 2021 Aug;209:108665. doi: 10.1016/j.exer.2021.108665.
3. Luft N, Schumann RG, Dirisamer M, Kook D, Siedlecki J, Wertheimer C, Priglinger SG, Mayer WJ. Wound Healing, Inflammation, and Corneal Ultrastructure After SMILE and Femtosecond Laser-Assisted LASIK: A Human Ex Vivo Study. *J Refract Surg*. 2018 Jun 1;34(6):393-399. doi: 10.3928/1081597X-20180425-02.
4. Marino GK, Santhiago MR, Santhanam A, Torricelli AAM, Wilson SE. Regeneration of Defective Epithelial Basement Membrane and Restoration of Corneal Transparency After Photorefractive Keratectomy. *J Refract Surg*. 2017 May 1;33(5):337-346. doi: 10.3928/1081597X-20170126-02.
5. Spadea L, Giammaria D, Trabucco P. Corneal wound healing after laser vision correction. *Br J Ophthalmol*. 2016 Jan;100(1):28-33. doi: 10.1136/bjophthalmol-2015-306770.
6. Vinciguerra P, Camesasca FI, Morengi E, Azzolini C, Pagano L, Trazza S, Vinciguerra R. Corneal Apical Scar After Hyperopic Excimer Laser Refractive Surgery: Long-term Follow-up of Treatment With Sequential Customized Therapeutic Keratectomy. *J Refract Surg*. 2018 Feb 1;34(2):113-120. doi: 10.3928/1081597X-20171214-01.
7. Wang LX, Li Y. Regeneration of corneal nerve after SMILE, FS-LASIK and T-PRK surgery and study its relationship with subjective visual quality. *Zhonghua Yan Ke Za Zhi*. 2018 Oct 11;54(10):737-743. Chinese. doi: 10.3760/cma.j.issn.0412-4081.2018.10.004.





8. Wilson SE. Biology of keratorefractive surgery- PRK, PTK, LASIK, SMILE, inlays and other refractive procedures. *Exp Eye Res.* 2020 Sep;198:108136. doi: 10.1016/j.exer.2020.108136.
9. Xeroudaki M, Peebo B, Germundsson J, Fagerholm P, Lagali N. RGTA in corneal wound healing after transepithelial laser ablation in a rabbit model: a randomized, blinded, placebo-controlled study. *Acta Ophthalmol.* 2016 Nov;94(7):685-691. doi: 10.1111/aos.13116.
10. Zhao J, Shen Y, Tian M, Sun L, Zhao Y, Zhang X, Zhou X. Corneal Lenticule Allotransplantation After Femtosecond Laser Small Incision Lenticule Extraction in Rabbits. *Cornea.* 2017 Feb;36(2):222-228. doi: 10.1097/ICO.0000000000001076.

