Effect of Suction Ring Application During LASIK on Goblet Cell Density

Jose Luis Rodríguez-Prats, MD, PhD; Islam M. Hamdi, FRCS, MD; Alejandra E. Rodriguez, BSc; Ahmed Galal, MD, PhD; Jorge L. Alio, MD, PhD

ABSTRACT

PURPOSE: To study the effect of LASIK surgery on conjunctival goblet cells as one of the proposed mechanisms for dry eye occurring after LASIK.

METHODS: This prospective study included 22 eyes (11 patients) that underwent LASIK for the correction of myopia. Three pairs of samples were taken from the bulbar conjunctiva of each eye. The first pair was taken preoperatively before application of the suction ring. The second and third pairs were taken from the same site at 1 week and 1 month consecutively. The first site was at 12 o’clock and the second at the inferotemporal quadrant between 7 and 8 o’clock. Time of suction was recorded.

RESULTS: Preoperatively, mean goblet cell density was 424±105 cells/mm² (range: 284 to 630 cells/mm²). All postoperative samples showed a statistically significant decrease in goblet cell count: 216±81 cells/mm² (range: 40 to 325 cells/mm²) at 1 week and 218±99 cells/mm² (range: 50 to 396 cells/mm²) at 1 month. Other parameters of conjunctival impression cytology were normal. The difference between the samples in the inferior conjunctiva preoperatively and 1 week postoperatively was greater than that of the superior conjunctiva. Recovery rate in both sites was similar and the damage did not correlate with the duration of suction.

CONCLUSIONS: The application of the microkeratome suction ring induced changes in the perilimbal conjunctiva. These changes contribute to the pathology of dry eye. Goblet cell count remains affected at 1 month postoperatively. [J Refract Surg, 2007;23:559-562.]

Asker in situ keratomileusis (LASIK) is the most standard procedure for the correction of mild to moderate myopia.1 Dry eye is a sign and symptom that occurs after LASIK.2 Many etiologies of pathogenesis have been suggested for this phenomenon. Among these are damage to the corneal nerve plexus,3 toxicity of topical medication, and damage to goblet cells at the site of the suction ring application.4 The suction ring provides a vacuum that reaches up to 765 mmHg on the external ocular wall and raises the intraocular pressure to 65 mmHg as measured by an external tonometer; however, in porcine eyes this was found to reach approximately 99 mmHg.5 This pressure is needed to maintain a tight grip on the globe so as to facilitate a smooth and perfect pass of the microkeratome blade and thus create an uneventful flap.

The introduction of impression cytology has facilitated study of the ocular surface. It is a histopathological technique used to study the epithelium found on any body surface, from the skin to the eye, including the gastrointestinal and genitourinary tracts.6 In 1977, Thatcher et al7 were the first to describe the technique that was eventually adopted by many authors. It involves the use of a cellulose acetate filter paper. Many pore-sized papers are used and most of them show the same efficacy. The paper is applied on the desired surface to be examined. Cells from the conjunctival epithelium adhere between the pores of the paper and remain on its surface when it is peeled off.8 The sample is transferred to 96% alcohol for fixation at 4°C. At the time of examination, alcohol is diluted for staining. The type of stain depends on the nature

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of the study. Specimens are prepared on a glass slide and examined under a light microscope with different magnifications. Although the technique removes the superficial cell layer, goblet cell density reduction and alteration of the epithelium structure have not been recorded in the literature.

This technique has revealed the mysteries of various diseases. Among these are dry eye syndrome, ocular surface neoplasia, ocular surface infections, and many others. Classifications have been made to evaluate the degree of harm to the ocular surface using specific parameters such as goblet cell density, where mucous-producing goblet cells are counted per millimeter squared, and other parameters describing individual epithelial cells in terms of cell size, nucleus/cytoplasm ratio, degree of keratinization, and power of cohesion.

This study evaluated the effect of LASIK on conjunctival goblet cell density.

PATIENTS AND METHODS

PATIENT POPULATION

This prospective noncomparative observational study included 22 eyes of 11 patients (7 [63.6%] women and 4 [36.4%] men) who underwent LASIK for myopia. All patients signed a written consent form in accordance with the Helsinki Declaration. No institutional review board approval was required for this study.

Mean patient age was 31.1±8.4 years (range: 22 to 48 years). Prior to surgery, all patients were fully examined. Eyes with pathologies (especially dry eye) or previous surgical intervention were excluded from the study. Individuals with contact lens wear in the past 6 months were excluded due to the marked alteration of the ocular surface. All surgeries were performed by one surgeon (J.R.P) using a 1.5 suction ring with an 8.5 stop (Moria, Antony, France) and the M2 microkeratome (Moria) with the 160-µm head. When the tip is blocked, pressure at the ring drops from ~730 mmHg (atmospheric) to ~110 mmHg. This creates a vacuum capable of holding the eye in place during rotation of the microkeratome head. The duration of the suction was recorded from the time of vacuum activation until vacuum release.

SAMPLE COLLECTION AND IMPRESSION CYTOLOGY

Two impression cytology samples were taken from each eye from the peribulbar conjunctiva (area of suction) at each visit. The first sample was collected from the superior quadrant sample at 12 o’clock and the second from the inferotemporal quadrant between 7 and 8 o’clock. Samples were taken immediately preoperatively as control samples. Postoperatively, patients received topical antibiotics and steroids for 1 week and artificial preservative-free tear drops of hydroxypropyl methylcellulose 0.3% 3 times a day for 1 month. Two additional examinations were performed at 1 week and 1 month to detect the degree of damage and recovery rate of the epithelium.

Impression cytology was performed using cellulose acetate filter paper with 0.45-µm pores (HAWP 02400; Millipore, Billerica, Mass). To differentiate between sample sites, the small pieces were cut in different shapes—superior samples were rectangular with one corner broken towards the limbus and inferotemporal samples were triangular with the tip broken and directed towards the limbus. After instillation of one drop of oxibuprocaaine and tetracaine (Colicurs Anestésico Doble; Alcon-Cusi Laboratories, Madrid, Spain) as a topical anesthetic, pieces of filter paper were placed in their respective positions for 2 to 5 seconds, which was a sufficient amount of time to adhere to the conjunctival surface. They were peeled off and fixed in a tube containing 96% alcohol and stored at 4°C. The samples were stained with Periodic Acid Schiff’s (PAS) Glemsa stain and examined in the laboratory with optic microscopy at 100× and 400×.

MICROSCOPIC EXAMINATION

All samples were examined for goblet cell density (cells/mm²), nucleus/cytoplasm ratio, and inflammatory cells. Goblet cell density values were calculated as separate samples (superior and inferior) and as an average count. Inflammatory cells were evaluated as normal, mild, moderate, and severe according to their quantity. These parameters together represent the degree of damage inflicted on the ocular surface.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS version 10.0 software (SPSS Inc, Chicago, Ill). Descriptive statistics were calculated in terms of range, mean±standard deviation, or standard error of the mean.

Analytical statistics were calculated in terms of Student t test to compare two independent means, correlation matrix and coefficient of correlation using Pearson’s method “r” to correlate two variables in the same group, and chi-square test for qualitative data.

The level of significance was indicated using P value, where >.05 was not significant, <.05 was significant, and <.01 was highly significant.

RESULTS

Preoperatively, the samples showed normal ocular surface and goblet cell density (Table 1). The nucleus/cytoplasm ratio of these samples was 0.54±0.18 (range: 0.33 to 1.00). Regarding inflammatory cells, 17.6% of
TABLE 1

Goblet Cell Density Before and After LASIK in 22 Eyes

<table>
<thead>
<tr>
<th>Goblet Cell Density (cells/mm²)</th>
<th>Preoperative</th>
<th>1 Week</th>
<th>1 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>359±98</td>
<td>204±77</td>
<td>215±100</td>
</tr>
<tr>
<td>(203 to 575)</td>
<td>(30 to 317)</td>
<td>(100 to 300)</td>
<td></td>
</tr>
<tr>
<td>Inferior</td>
<td>480±139</td>
<td>252±94</td>
<td>259±85</td>
</tr>
<tr>
<td>(283 to 850)</td>
<td>(50 to 380)</td>
<td>(108 to 405)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>424±105</td>
<td>216±81</td>
<td>218±100</td>
</tr>
<tr>
<td>count</td>
<td>(284 to 630)</td>
<td>(40 to 325)</td>
<td>(50 to 396)</td>
</tr>
</tbody>
</table>

TABLE 2

Difference in Goblet Cell Density in 22 Eyes That Underwent LASIK

<table>
<thead>
<tr>
<th>Goblet Cell Density (cells/mm²)</th>
<th>Preoperative vs 1 Week</th>
<th>1 Week vs 1 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>185±90</td>
<td>−37±133</td>
</tr>
<tr>
<td>(6 to 327)</td>
<td>(−270 to 301)</td>
<td></td>
</tr>
<tr>
<td>Inferior</td>
<td>308±150</td>
<td>−2±144</td>
</tr>
<tr>
<td>(3 to 649)</td>
<td>(−344 to 350)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>228±89</td>
<td>−12±120</td>
</tr>
<tr>
<td>count</td>
<td>(44 to 349)</td>
<td>(−216 to 326)</td>
</tr>
</tbody>
</table>

Figure 1. Impression cytology of the superior bulbar conjunctiva preoperatively. Goblet cell density = 513 cells/mm², nucleus/cytoplasm ratio 1:1, and no inflammatory cells (Periodic Acid Schiff’s Giemsa, original magnification ×400).

Figure 2. Impression cytology of the superior bulbar conjunctiva 1 week postoperatively of the same patient in Figure 1. Goblet cell density = 125 cells/mm², nucleus/cytoplasm ratio 1:2, and no inflammatory cells (Periodic Acid Schiff’s Giemsa, original magnification ×400).

samples showed normal to mild cells, 52.9% of samples showed moderate cells, and 29.4% showed severe cells. As the cases were selected depending on their clinical condition (no ocular surface problems), these results were considered normal and were compared to the samples after surgery. A microscopic image of one sample is shown in Figure 1.

Mean suction time of the microkeratome was 22.4±8.0 seconds (range: 13 to 50 seconds).

Goblet cell density at 1 week after surgery is shown in Table 1. The mean nucleus/cytoplasm ratio was 0.38±0.09 (range: 0.25 to 0.50). Regarding the inflammatory cells, all samples showed moderate cells. A microscopic image of the same patient in Figure 1 is shown in Figure 2.

Goblet cell density at 1 month after surgery is shown in Table 1. The mean nucleus/cytoplasm ratio was 0.32±0.08 (range: 0.25 to 0.50). Regarding the inflammatory cells, 20% of samples showed mild cells, 70% showed moderate cells, and 10% showed severe cells.

The mean change in goblet cell density between preoperative values and the values at 1 week postoperatively in the superior and inferotemporal samples and their average count were highly statistically significant (P<.01). However, no statistically significant differences were noted between 1 week and 1 month after surgery (P>.05).

The nucleus/cytoplasm changes in the three samples as well as the amount of inflammatory cells were not statistically significant (P>.05) (Table 2).

The mean change of goblet cell density was compared and a highly statistically significant difference was found between the values of the superior and inferotemporal samples preoperatively and at 1 week postoperatively (P<.01), with the damage in the inferotemporal samples being greater. This difference was not statistically significant between 1 week and 1 month (P>.05).
Effect of LASIK on Goblet Cell Density/Rodriguez-Prats et al

Figure 3. Average goblet cell density (GCD) (cell/mm²) preoperatively and 1 week and 1 month postoperatively in 22 eyes that underwent LASIK for myopia.

No statistically significant correlation was found between the time of suction and the decrease in goblet cell density in any sample (r=0.23).

DISCUSSION

Dry eye syndrome after LASIK usually is related to nerve plexus damage. Other mechanisms, such as toxicity from topical drugs and goblet cell damage from suction ring, have also been suggested.

In the present study, we tried to objectively confirm the mechanism causing dry eye after LASIK and determine the recovery rate of goblet cell density. Twenty-two eyes that underwent LASIK had a clinically normal ocular surface. Cytological samples taken a few minutes before the LASIK procedures showed normal conjunctivae. One month postoperatively, goblet cell density showed a significant decrease superiorly and inferotemporally without alteration of other parameters (Fig 3). Nelson’s classification could not be applied, as all parameters required alteration, not only goblet cell density. Goblet cell density was calculated without referring to Nelson’s classification, indicating that the damage was limited to goblet cells alone. We can therefore conclude that this change is due to the mechanical stress applied 1 month earlier and not due to the dry eye status induced by the denervation or by the effect of preservatives in the postoperative medications.

At 1 month, goblet cell density showed a slight improvement (see Fig 3); however, it was not significant. Other parameters remained normal, suggesting that goblet cell density requires more than 1 month to recover after trauma from the suction ring.

Changes at both sites were compared, and the major goblet cell density decrease occurred inferiorly. However, both sites continued to recover similarly (Table 1). In this study, the goblet cell decrease was not related to the duration of suction. In a study by Boira Cabre et al., this decrease was proven but not detailed. It also included other parameters such as squamous metaplasia. Another study by Albitz et al. showed that this goblet cell decrease continues up to 1 year with a slow rate of recovery.

Although not mentioned in previous literature or directly studied in this research, the damage limited to goblet cells might be related to the more fragile nature of this type of cell. Being a secretory cell, it has a less stable cell membrane, which degranulates with vacuum pressure.

Although the study confirms the damage of goblet cells in relation to the suction ring, it does not attribute this damage to the clinical condition. Samples were only taken from the area covered by the suction ring. They did not include further regions with different goblet cell density. Modification of the suction ring is suggested, such as use of polymeric materials, which would be more delicate on the ocular surface.

REFERENCES