Can tear film analysis predict post-lasik lactoferrin concentration?

Staci Renee Lupa
Pacific University
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Results: There was a significant decrease in tear lactoferrin concentration postoperatively for right and left eyes. The mean lactoferrin concentration for right eyes decreased from 1.12 mg/ml pre-operatively to 0.50 mg/ml post-operatively. The mean lactoferrin concentration for left eyes decreased from 0.97 mg/ml to 0.27 mg/ml post-operatively.

Conclusions: A decrease in tear lactoferrin concentration was observed after LASIK. In this study, the assumption was made that a decrease in tear lactoferrin levels reflects lacrimal gland malfunction, thereby increasing the risk of post-LASIK dry eye. Most subjects in this study had a decrease in tear lactoferrin after LASIK suggesting a correlative relationship between LASIK and tear lactoferrin decrease. This information is useful in establishing tear baseline levels for patients, as each patient can serve as his or her own control.

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CAN TEAR FILM ANALYSIS PREDICT POST-LASIK LACTOFERRIN CONCENTRATION?

By

STACI RENEE LUPA

A thesis submitted to the faculty of the
College of Optometry
Pacific University
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Advisors:
Salisa K. Williams, O.D.
Patrick J. Caroline, C.O.T., F.A.A.O
Doug Devries, O.D.
Paul Hiss, M.D.
Biography

Staci Lupa is a student at Pacific University College of Optometry in Forest Grove, Oregon. She has a bachelor’s degree in psychology and minor degree in biology from the University of Colorado, Boulder. Her undergraduate work focused on behavioral neuroscience. As an optometry student, her clinical studies focus on ocular disease and pathology. She plans to practice optometry in a co-management setting, where she can pursue her career in ocular disease. Staci Lupa is currently a member of the optometry honors association Beta Sigma Kappa.
Abstract

Purpose: To determine if tear lactoferrin levels decreases after laser-assisted in situ keratomileusis (LASIK).

Design: Prospective, non-comparative case series, pilot study

Participants: Thirty-one eyes of 8 females and 8 males (age range 33-63, mean 47.9 years) who underwent bilateral LASIK for myopia and astigmatism. One male had LASIK on only his right eye.

Methods: LASIK was performed on thirty-one eyes of 17 subjects. Tear film analysis was performed before and after LASIK, in order to determine the lactoferrin concentration in each subject’s tears. Each eye was evaluated one hour prior to LASIK, and then again one day after LASIK. A non-parametric t-test was used for statistical analysis. The results from 6 right eyes and 5 left eyes were eliminated due to poor tear collection, secondary to inadequate tear sample.

Results: There was a significant decrease in tear lactoferrin concentration post-operatively for right and left eyes. The mean lactoferrin concentration for right eyes decreased from 1.12 mg/ml pre-operatively to 0.50 mg/ml post-operatively. The mean lactoferrin concentration for left eyes decreased from 0.97 mg/ml to 0.27 mg/ml post-operatively.
Conclusions: A decrease in tear lactoferrin concentration was observed after LASIK. In this study, the assumption was made that a decrease in tear lactoferrin levels reflects lacrimal gland malfunction, thereby increasing the risk of post-LASIK dry eye. Most subjects in this study had a decrease in tear lactoferrin after LASIK, suggesting a correlational relationship between LASIK and tear lactoferrin decrease. This information is useful in establishing tear baseline levels for patients, as each patient can serve as his or her own control.

Key Words: LASIK, dry eye, tears, lactoferrin
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Introduction

Laser-assisted in situ keratomileusis (LASIK) is an effective treatment modality for the correction of refractive error. It is a surgical procedure capable of correcting hyperopia, myopia, and astigmatism with minimal pain, rapid recovery of vision, and good refractive stability. The procedure involves the use of a microkeratome to create a hinged flap of corneal epithelium. The creation of the flap, the keratectomy, allows for the underlying stromal bed to be exposed. The cornea's curvature is then altered during the ablation process, in which a 193-nm pre-programmed excimer laser treats the underlying stromal bed. After the appropriate treatment level is reached, the corneal flap is repositioned and the interface is irrigated. Within one day of treatment, the average patient achieves reasonably good visual recovery (20/20 to 20/50).

Most published literature proposes LASIK as effective and relatively safe. However, as with any surgical procedure, there are various post-operative complications that the LASIK patient may experience. It has been well established that an alarming number of patients who undergo LASIK experience intense dry eye symptoms afterwards. This post-LASIK dry eye condition is particularly difficult on patients. It often requires the use of extensive amount of topical lubricants and visits to eye care practitioners on a regular basis, both which can be very time consuming, distressful, and expensive.

Little is understood about the etiology of post-LASIK dry eye. Many researchers have suggested it differs from chronic dry eye in several ways. It has been proposed that poor tear spreading over the ablation zone of LASIK treated eyes may contribute to these dry
eye symptoms. Decreased corneal sensation is an additional factor that should be considered. During the surgical procedure, an unknown amount of corneal nerves are severed, thereby decreasing corneal sensation. Corneal sensation appears to be vital to normal tear function, consequently, some compromise to the corneal nerve integrity could contribute to post-LASIK dry eye. Abelson suggests that a weakened tear reflex is presumably accountable for post-LASIK dry eye, and a decrease in corneal sensitivity is both an effect and exacerbating cause of dry eye symptoms.

Dry eye is said to be a disorder of the tear film, usually due to tear deficiency or excess tear evaporation. It causes extreme discomfort in the eyes including symptoms of burning, itching, grittiness, and redness. It has been estimated that 20% of eye care practitioner visits are related to dry eye symptoms.

The tear film plays a primary role in dry eye syndrome. It removes foreign material, manifests antimicrobial properties, provides oxygen and nutrients to ocular epithelial cells, and acts to maintain a smooth ocular surface boundary. The tear film consists of three primary layers: the outermost lipid layer, the middle aqueous layer, and the innermost mucin layer. Each of these layers play a crucial role in the preservation of tear stability. Produced by the meibomian glands, the outer lipid layer consists of low-polarity lipids, such as wax and cholesterol esters. Its purpose is to act as a boundary between the air and the tear film, while providing a structural support system. The mucin layer is the innermost layer that attaches to microvilli. It protects the cornea by inhibiting
microbial adhesion. The middle aqueous layer of the tear film contains lactate dehydrogenase, IgA, epidermal growth factor, and a protective protein called lactoferrin.

Lactoferrin is an important biological protein that controls inflammatory responses. It is produced by the lacrimal gland and has significant antibacterial activity. Previous research has suggested that tear lactoferrin concentration is an important indicator of lacrimal gland function. The Lactoferrin MicroAssay System™ is an in vitro diagnostic device which directly measures lactoferrin concentration in human tears. According to their research, the Touch Scientific, Inc. suggests that the low value of normal tear lactoferrin levels is about 0.9mg/mL. They also maintain that the lower the lactoferrin value, the greater dysfunction of the lacrimal gland, thus leading to an exacerbation of dry eye symptoms.

The diagnosis of dry eye in the clinical setting has been somewhat controversial. Dry eye can be assessed with the Schirmer test, tear break-up time, tear mucin measurement, goblet cell count, tear osmolarity, rose bengal test, tear lysozyme measurement, and lactoferrin immunoassay analysis. Presently, there is no single test that completely evaluates the ocular tear film. Goren and Goren found lactoferrin immunologic assay to be the most reliable diagnostic test when compared with the Schirmer test, the rose bengal test, and the tear break-up time test in patients with keratoconjunctivitis sicca. Although the Schirmer test is the most frequently use examination for dry eye, it was found to have poor correlation with symptoms reported in previous studies.
The need for an appropriate and reliable dry eye test is crucial to LASIK patients. Although the Schirmer test has been shown to have poor correlation with symptoms, it had been demonstrated by the Shirmer test that values less than 10mm are especially at risk for experiencing post-LASIK dry eye symptoms. Recently, the Food and Drug Administration approved The Touch Tear Microassay System™ to rapidly measure the concentration of proteins in tears.

It is hypothesized that the concentration of tear lactoferrin will decrease after LASIK is performed on patients for correction of refractive error. If LASIK patients were to undergo tear film lactoferrin analysis prior to surgery, they could be treated differently. Tear film analysis could be an imperative procedure for eye care practitioners when profiling their patients prior to LASIK. This pilot study used the Touch Tear MicroAssay System™ to examine the changes in tear lactoferrin concentration in subjects before and after LASIK.
Methods

Laser-assisted in situ keratomileusis (LASIK) was performed in 33 eyes (17 patients) on March 27, 2002. Pre-operative LASIK evaluations were performed on March 27, 2002, and post-operative LASIK evaluations were performed on March 28, 2002. The mean age of the subjects was 47.9 years (range 33-63 years). Seven subjects were men and 3 subjects were women. All LASIK procedures were bilateral for myopia and astigmatism, with the exception of one male who had LASIK on his right eye only.

Pre-LASIK Procedure

A lactoferrin immunoassay test was performed on each subject’s eyes using the Touch Tear MicroAssay System™, as per manufacturer’s instructions. One hour prior to LASIK, tear samples were collected from each subject using the calibrated capillary tubes from the Touch Lactoferrin Kit™. The capillary tube was placed in each subject’s inferior tear meniscus in order to collect an adequate amount of tear sample for analysis. The capillary tubes were fully immersed in tear, filling the 0.5µL capillary tube completely with tear sample. After the tear samples were collected, they were transferred to vials and refrigerated, as per manufacturer’s instructions.

For the analysis of the tear samples, each sample was transferred onto a Lactoferrin Kit™ card as instructed by the package insert. A trained optometric technician also assisted in the tear analysis. A representative technician from the Touch™ company conducted the tear analysis in order to reduce possible sources of error. Specific calibrated wash, control, and conjugate solutions were added to the tear samples on the Lactoferrin Kit™
cards, and then transferred to and analyzed by the Touch Scientific™ M9 Reflectance Spectrophotometer. This device produced a print out for each subject, displaying the amount of tear lactoferrin present in each tear sample.

Post-LASIK procedure

One day after LASIK, tear samples were collected and analyzed by the Touch™ M9 spectrophotometer. This was performed following the identical pre-LASIK protocols. These post-operative lactoferrin levels were compared to pre-operative lactoferrin levels.
Results

The lactoferrin concentrations before and after LASIK are listed in Table 1 and Table 2. Statistical analysis included a non-parametric t-test on ranks. A non-parametric t-test analysis was chosen, as opposed to a standard t-test, because it is best suited for small sample sizes. The differences in lactoferrin concentration for each eye was ranked (including the sign), and a non-parametric t-test was conducted to determine if the mean rank was statistically significant from zero. Right and left eyes were tested separately because right and left eyes were likely to be correlated. Ten right eyes and 5 left eyes were analyzed for statistical purposes. The results from 6 right eyes and 5 left eyes were eliminated due to poor tear collection, secondary to inadequate tear sample.

There was a statistically significant within-subject decrease in tear lactoferrin concentrations after LASIK. The t-value was highly significant for right eyes ($t = 3.48$, $p < 0.01$) and left eyes ($t = 4.24$, $p < 0.01$). The mean lactoferrin concentration for right eyes pre-operatively was 1.108 mg/ml, which decreased to 0.503 mg/ml after surgery. The mean lactoferrin levels for left eyes before surgery was 0.956 mg/ml, and after LASIK those values decreased to 0.266 mg/ml. Since the differences were significant overall for right and left eyes, the data was grouped together (both eyes combined) and is shown in Figure 1.
Discussion

The definition of dry eye is still under investigation. This small pilot study attempted to predict post-LASIK dry eye syndrome based on the decrease in tear lactoferrin levels after surgery. After LASIK, subjects in the current study showed a significant decrease in tear lactoferrin concentration. It was assumed that a decrease in tear lactoferrin level reflects lacrimal gland malfunction, thereby increasing the risk of post-LASIK dry eye. Lacrimal gland degeneration has been shown to cause changes in the lactoferrin composition of the lacrimal fluid, as well as a diminished tear flow. Most subjects in the current study had a decrease in tear lactoferrin after LASIK, suggesting a correlational relationship between LASIK and tear lactoferrin decrease. This information is useful in establishing tear baseline levels for patients, as each patient can serve as his or her own control.

Touch Scientific Inc., reports mean values of lactoferrin levels as high, normal, or low (Table 3). According to Touch Scientific Inc., lactoferrin levels may be able to predict successful contact lens fits, healing responses for LASIK and photorefractive keratectomy (PRK), as well as confirm or rule out a diagnosis of dry eye. Further investigation is needed to explore these clinical applications.

There were several limitations to this study. First, the researchers were unable to follow subjects over time. Sufficed to say subjects’ corneas may not have had the proper healing time after LASIK. Future studies could investigate tear lactoferrin levels at one month,
three months, and six months post-LASIK. The correlation of dry eye symptoms and lactoferrin levels should be studied long-term. In addition, without prior training and experience, Touch Tear MicroAssay™ tear collection technique was quite tedious to perform. This study was also subject to potential measurement error due to the inexperience of the researchers in the tear collection process. With proper training, instruction, and experience, the researchers believe the Touch Tear MicroAssay™ could be a valid technique in the measurement of tear composition. Further studies are warranted.
Table 1. Lactoferrin concentrations in right eyes.

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Lactoferrin levels before LASIK (mg/ml)</th>
<th>Lactoferrin levels after LASIK (mg/ml)</th>
<th>Mean difference (before-after) (mg/ml)</th>
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<tbody>
<tr>
<td>2</td>
<td>1.39</td>
<td>1.13</td>
<td>0.26</td>
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<tr>
<td>4</td>
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<td>1.68</td>
<td>0.55</td>
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Table 2. Lactoferrin concentrations in left eyes.

<table>
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<tr>
<th>Subject number</th>
<th>Lactoferrin levels before LASIK (mg/ml)</th>
<th>Lactoferrin levels after LASIK (mg/ml)</th>
<th>Mean difference (before-after) (mg/ml)</th>
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</thead>
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<tr>
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<tr>
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<td>0.39</td>
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<tr>
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<td>0.86</td>
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<tr>
<td>7</td>
<td>0.64</td>
<td>0.28</td>
<td>0.36</td>
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<tr>
<td>8</td>
<td>0.78</td>
<td>0.01</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Figure 1. Mean tear lactoferrin concentration before and after LASIK.

Table 3. Mean lactoferrin levels, per Touch Scientific, Inc. manufacturer's brochure

<table>
<thead>
<tr>
<th>Level</th>
<th>Concentration (mg/ml)</th>
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<tbody>
<tr>
<td>High</td>
<td>2.0</td>
</tr>
<tr>
<td>Normal</td>
<td>1.4</td>
</tr>
<tr>
<td>Low</td>
<td>0.6</td>
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</table>
References

8. Touch Scientific™ package insert.