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ORIGINAL PAPER

Tear Martix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 in Post-Lasik Ectasia

Mohamed Nagy Elmohamady · Walid Abdelghaffar · Tamer Ibrahim Salem

Abstract

Purpose To estimate the concentrations of matrix metalloproteinase-9 (MMP-9) and tissue inhibitors of metalloproteinase-1 (TIMP-1) in the tear film of cases with post-Lasik ectasia (PLE) to spot any role of these mediators.

Settings Ophthalmology department, Benha University hospitals, Egypt.

Methods Twelve eyes of 12 patients with PLE, 30 eyes of 30 patients with KC, 25 eyes of 25 subjects with uncomplicated Lasik and finally 25 eyes of 25 healthy subjects as a control group were studied. Subjects with ocular surface diseases, previous ocular surgeries except for Lasik in PLE group and Lasik group, were excluded. All subjects had full ophthalmic examination and Pentacam imaging. The concentration of tear MMP-9 and TIMP-1 was measured by ELISA.

Results Our results showed a significant elevation in the level of MMP-9 and a significant reduction in the level of TIMP-1 in tear samples from PLE cases (MMP-9 was 59.17 ± 28.15 ng/ml, and TIMP-1 was 110.3 $\pm$ 50.6 ng/ml) and also in KC cases (MMP-9 was 53.12 ± 17.35 ng/ml, and TIMP-1 was 105.8 ± 56.3 ng/ml) when compared to post-Lasik group (MMP-9 was 35.65 ± 17.32 ng/ml, and TIMP-1 was 155.2 ± 39.4 ng/ml) and control group (MMP-9 was 31.92 ± 20.78 ng/ml, and TIMP-1 was 162.5 ± 48.2 ng/ml).

Conclusion The results pointed to potential role of MMP-9 in the pathogenesis of PLE and also referred to a biochemical similarity between PLE and KC. More studies are needed in the future to investigate larger number of tear mediators.

Keywords Post-Lasik ectasia · MMP-9 · TIMP-1 · Keratoconus · Lasik

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Introduction

In public health, corneal ectatic disorders have a substantial significance [1]. The progression is variable from one disease to the other and is linked with a rise in myopia and irregular astigmatism with subsequent decline of the visual acuity [2]. Corneal ectasia following corneal refractive surgeries is denoted as post-Lasik ectasia (PLE) when it occurs after Lasik. It can be distinguished by a progressive corneal steepening accompanied with a reduction in the visual acuity [3]. First described in 1998 [4], it is known as a serious complication of Lasik, one that may be

visually devastating and tough to manage [5]. Keratoconus (KC) is an asymmetric, progressive ecstatic disorder which might induce weighty visual disturbance. Though the precise pathogenesis of KC is not fully revealed, the disease is now supposed to be provoked by numerous genetics, along with the environmental impacts [6].

Many studies have been done to investigate the tear film biomarkers in KC and showed that there was disturbed expression of some inflammatory mediators like matrix metalloproteinases (MMPs; MMP-1, 3, 7, 9, 13) with their inhibitors (tissue inhibitor of metalloproteinases-1 (TIMP-1)) [7, 8]. MMP-9 catalyzes the cleavage of all forms of denatured collagens and the innate basement membrane components. This may give rise to continuing basement membrane remodeling, hence leading to extended keratocyte apoptosis, diminished production of extracellular components, corneal thinning and ectasia [9]. Consequently, MMP-9 can be considered as an indicator of ectasia [10]. Tear sampling possibly offers a suitable way of analyzing a accessible body fluid for the study of biomarkers [11].

A recent study evaluated tear cytokines in cases of PLE [12]. But, until the top of our knowledge, tear MMPs of subjects with PLE have not been studied yet. Therefore, we aimed to estimate the levels of MMP-9 and TIMP-1 in the tear film of cases with PLE to detect any rule of these mediators in the pathogenesis of this disease.

Patients and methods

This study tracked the assurances of the Declaration of Helsinki and was agreed by the Benha University Research Ethics Committee. It included 92 eyes of 92 subjects divided as follows:

• PLE group

Twelve eyes of 12 patients with post-Lasik ectasia.

• KC group

Thirty eyes of 30 patients with keratoconus matched with cases in PLE as for age and severity evaluated by Pentacam topographic indices.

• Lasik group

Twenty-five eyes of 25 patients who had uncomplicated Lasik more than 1 year ago. Cases in this group was matched with PLE group in age and in duration from Lasik surgery to time of examination.

• Control group

Twenty-five eyes of 25 normal subjects age matched to the other groups.

Subjects with ocular surface diseases, previous ocular surgeries, except for Lasik in PLE group and in Lasik group, any other ocular or systemic diseases, systemic or topical drug use were excluded.

Clinical evaluation

Clinical evaluation of all subjects was done in the form of unaided visual acuity (UAVA), best-corrected visual acuity (BCVA), slit lamp biomicroscopy.

Corneal imaging

Imaging of the cornea by Pentacam was performed on all subjects. We recorded K readings, anterior elevation maps, posterior elevation maps, pachymetric maps and corneal keratoconic indices.

Tear collection

After anterior ocular surface assessment of every patient, tear collection was done between 9.00 and 10.00 a.m. with sterilized microcapillary tubes, from the temporal part of the tear meniscus just above the lower lid margin for 2 min via a preweighted polyester wick (Transorb rods; American Filtrona, Richmond, VA) to get the tear samples. Eye drops were avoided 1 h before tear collection. No anesthetic eye drops were used. We tried not to touch the lids or the conjunctiva or the cornea during the tear sample collection. Wicks were then positioned into the end of a micropipette tip placed inside a 0.5-ml tube (Eppendorf, Fremont, CA). The collected amount of tears was measured by reweighting the tubes, and it was approximately 10 µl. The samples were frozen at − 80 °C within 30 min from collection waiting for the time they were utilized for enzyme-linked immunosorbent assay (ELISA).

Tear analysis

The samples were kept at room temperature for half an hour before using them for ELISA. The tears were then taken out from the soaked wicks by centrifugation at 12,000 rpm for 5 min inside the pipette tip, after adding a volume of ELISA buffer provided by the manufacturer. The total volume of tears and the added buffer is 20 µl. The tear fluid was aspirated, cautious removal of the rods and micropipette.

ELISAs for MMP-9 and TIMP-1 were done using commercial kits (Oncogene, Cambridge, MA). The samples were diluted again in the buffer to have a final volume of 100 µl. Assays were achieved as per the manufacturer's directions.

Statistical analysis

Only one eye per patient was analyzed to sidestep bias from inter-eye correlations. Data are shown as mean $\pm$ standard deviation on their innate scales. PLE patients' tear MPP-9 and TIMP-1 levels were plotted against mean K values, and Pearson's correlation coefficients were calculated, to assess the relation between them. Analysis was made by means of SPSS 18.0 software, and a P value of 0.05 or less was considered to be significant.

Results

This study included 92 eyes of 92 subjects (42 males and 50 females). Table 1 shows selected demographics and topographic criteria of the 4 groups. Age showed no statistically significant differences between the 4 groups because all groups are age matched. There were no statistically significant differences between PLE group and KC group as for K readings and Pentacam topographic indices, and this indicates that these two groups are properly matched in the severity of ectasia.

The levels of tear MMP-9 were significantly lower in Lasik and control groups, and the values of TIMP-1 were significantly higher in Lasik and control groups when compared to PLE and KC groups, as shown in Table 2 and Fig. 1.

The twelve PLE cases details are summarized in Table 3. Four cases were referred to us from other centers, so the preoperative and operative details are missing. PLE patients' tear MPP-9 levels were plotted against mean K values (Fig. 2), and Pearson's correlation coefficient was $r = 0.9029$ which indicates a strong positive correlation. PLE patients' tear TIMP-1 levels were plotted against mean K values (Fig. 3), and Pearson's correlation coefficient was $r = -0.8817$ which indicates a strong negative correlation.

**Table 1** Selected demographic and topographic characteristics

Parameters	PLE	KC	Lasik	Control
Male/female	5/7	14/16	11/14	12/13
Age	26.4 ± 5.3	28.8 ± 6.1	25.2 ± 5.8	26.3 ± 4.7
K1 (D)	44.9 ± 4.2	45.6 ± 3.7	39.4 ± 2.3	42.3 ± 1.7
K2 (D)	47.4 ± 5.7	50.4 ± 3.1	40.1 ± 2.1	43.2 ± 1.6
Astigmatism (D)	3.8 ± 2.1	6.1 ± 1.8	0.3 ± 0.06	0.8 ± 0.5
Pachy Min (µm)	428 ± 47	465 ± 45	478 ± 32	544 ± 30
ISV	68.3 ± 30.5	77.9 ± 28.7	12.4 ± 3.9	16.2 ± 5.2
IVA	0.81 ± 0.57	0.92 ± 0.46	0.11 ± 0.04	0.13 ± 0.08
IHA	19.3 ± 16.8	24.4 ± 11.9	3.2 ± 1.2	4.1 ± 1.9
IHD	0.10 ± 0.07	0.13 ± 0.04	0.01 ± 0.00	0.01 ± 0.00

Patients groups: post-Lasik ectasia (PLE), keratoconus (KC), more than 1 year after uncomplicated Lasik (Lasik) and control. Keratometric value at flat (K1) and steep (K2) meridian, corneal thickness at the thinnest point of the cornea (Pachy Min), index of surface variation (ISV), index of vertical asymmetry (IVA), index of height asymmetry (IHA), index of height decentration (IHD)

Table 2 Tear MMP-9 and TIMP-1 in the four groups

|	PLE	KC	Lasik	Control
MMP-9 (ng/ml)	59.17 ± 28.15	53.12 ± 17.35	35.65 ± 17.32* **	31.92 ± 20.78* **
TIMP-1 (ng/ml)	110.3 ± 50.6	105.8 ± 56.3	155.2 ± 39.4* **	162.5 ± 48.2* **
MMP-9/TIMP-1	0.54	0.50	0.23 * **	0.20 * **

Patients groups: post-Lasik ectasia (PLE), keratoconus (KC), more than 1 year after uncomplicated Lasik (Lasik) and control. Matrix metalloproteinase-9 (MMP-9), tissue inhibitor metalloproteinase-1 (TIMP-1), statistically significant difference from PLE group (*), statistically significant difference from KC group (**)
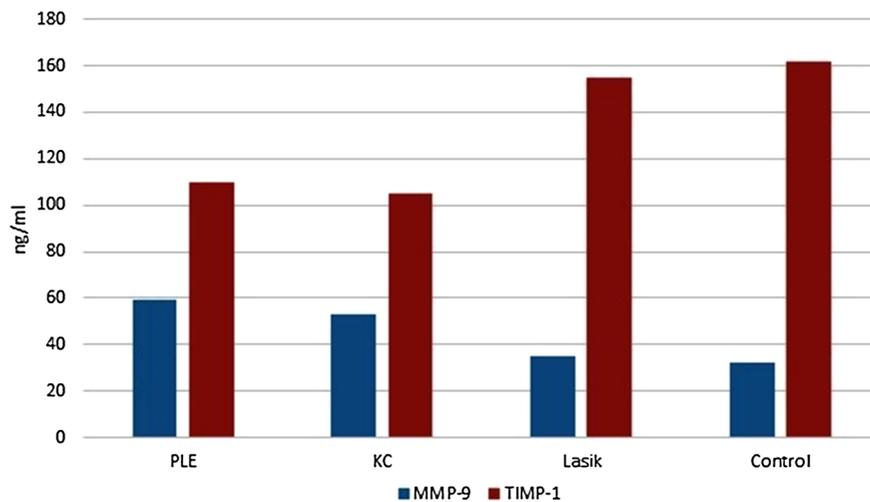
Fig. 1 Tear MMP-9 and TIMP-1 in the four groups

Table 3 Selected characteristics of PLE cases

Case	Type of operation	Flap creation	Flap thickness (μm)	Pre-op refraction (SE)	Duration from op to PLE (months)	Duration of PLE from diagnosis (months)
1	Lasik	Moria	110	− 5.25	12	1
2	Lasik	Moria	100	− 2.50	14	2
3	Lasik	Moria	100	− 7.00	15	1
4	Lasik	Unavailable	Unavailable	Unavailable	20	4
5	Femto-Lasik	Visumax	90	− 6.25	18	1
6	Lasik	Moria	110	− 5.00	11	1
7	Femto-Lasik	Unavailable	Unavailable	Unavailable	10	2
8	Lasik	Unavailable	Unavailable	Unavailable	17	3
9	Lasik	Moria	120	− 3.50	20	2
10	Femto-Lasik	Visumax	90	− 4.75	16	1
11	Lasik	Unavailable	Unavailable	Unavailable	16	3
12	Lasik	Moria	110	− 8.75	11	1
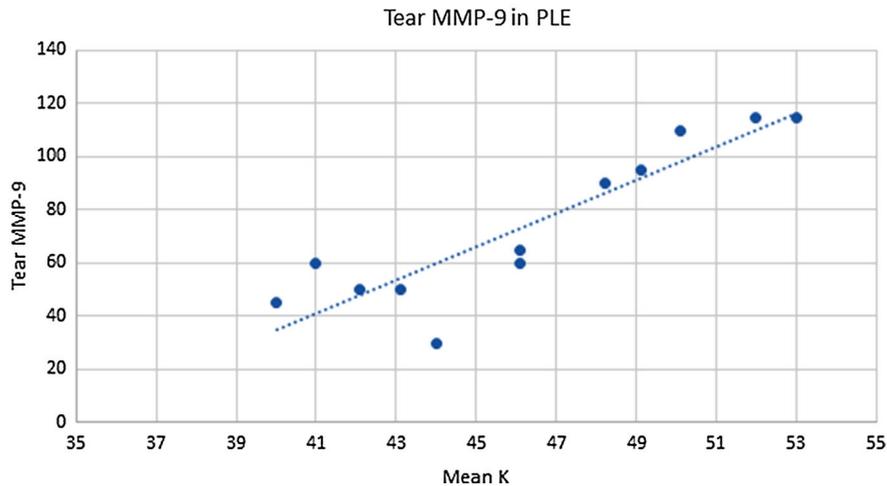
Fig. 2 A scatterplot showing a statistically significant positive correlation between tear MMP-9 and mean *K* in cases of PLE (Pearson correlation coefficient $r = 0.9029$)
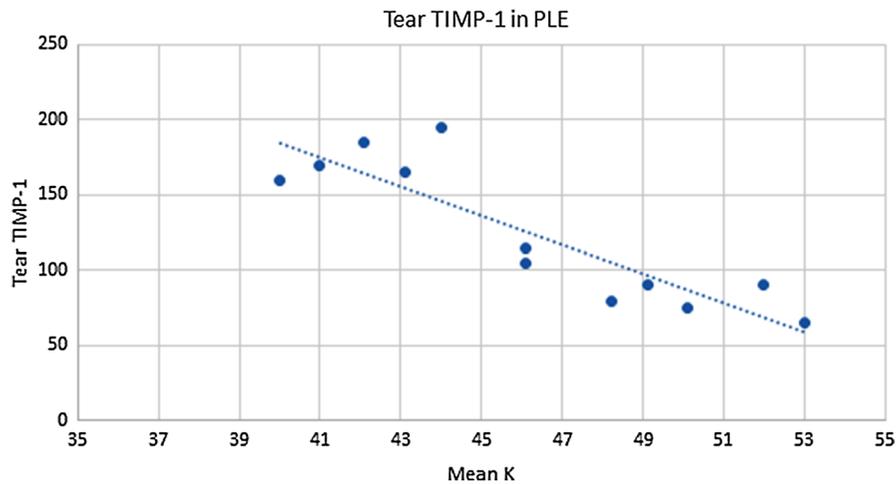
Fig. 3 A scatterplot showing a statistically significant negative correlation between tear TIMP-1 and mean *K* in cases of PLE (Pearson correlation coefficient $r = -0.8817$)

Discussion

Although post-Lasik ectasia (PLE) is a well-known complication of that procedure, its pathogenesis remains inadequately revealed. Better understanding of the mechanisms behind any disease is of great value in its management. Corneal ectasia has been considered to be a non-inflammatory condition [13]. Yet, recent evidence on the pathogenesis of KC proved it inflammatory in nature [14]. Additionally, lessening in the evolution rate of ectasia in keratoconus by the use

cyclosporine A, an immunomodulatory agent, was described [9].

Our study included 12 eyes of 12 patients with PLE, 30 eyes of 30 KC patients, 25 eyes of 25 subjects with uncomplicated LASIK and finally 25 eyes of 25 normal subjects as a control group. We found that both PLE group and KC group had significantly higher levels of tear MMP-9 and significantly lower levels of TIMP-1 when compared to the post-LASIK group and control group.

We planned to investigate tear MMP-9 and TIMP-1 in PLE, since many studies have linked MMPs to several systemic [11] and corneal diseases [11, 15] including some ectatic disorders [9, 16–19]. Smith et al. [15] estimated the MMP levels of samples obtained from the conjunctiva, corneal epithelium, plasma and tears of rheumatoid arthritis patients and others with diverse ocular surface diseases. They concluded that the accumulated MMPs in tears may be provoked by ocular disorders which bring an inflammatory response. Shetty R [9] and associates studied tear levels of MMP9 and other mediators using ELISA kits in 129 Indian patients with KC. Their data showed that corneal epithelium shares in raised MMP9 and cytokine appearance in tears of KC patients. Our results also indicated that there was a significantly higher level of tear MMP-9 in KC cases when compared to control but not when compared to PLE group. This similarity between both diseases may guide us to an improved comprehension of the mechanisms behind the PLE.

Also the MMP-9/TIMP-1 ratio was reported to be elevated in some corneal ectatic disorders. Pásztor D et al. [19] studied some tear mediators, including MMP-9 and TIMP-1 in 9 eyes of 7 pellucid marginal degeneration (PMD) patients, 55 eyes of 55 KC patients and 24 eyes of 24 normal subjects as a control. The ratio of MMP-9 to TIMP-1 was 2.45 in PMD, 0.40 in KC and 0.23 in controls, pointing to more tissue degradation in PMD and KC than in control. This was also the case in our study as the MMP-9/TIMP-1 ratio was 0.54 and 0.50 in PLE and KC, respectively, while it was 0.23 and 0.20 in Lasik and control groups, respectively. This point directly refers to the tissue damage that occurs during the pathogenesis of PLE as it is not only a protrusion due to corneal stretching.

Some studies have pointed at an elevation of tear MMPs in the complicated Lasik cases [20] and also in uncomplicated Lasik cases during the first year postoperatively. Ji H and associates [21] investigated the dynamic changes of MMP-9 level in tears in 22 myopic subjects within 12 months following eventless Lasik. They found that MMP-9 concentrations in the tears of post-Lasik subjects display a time-related discrepancy and remain more than the preoperative level, even at 12 months after the procedure, telling that corneal wound healing after LASIK lasts for more than 12 months. Thus, we preferred to have a group of post-LASIK subjects with uncomplicated LASIK at least 1 year earlier than the time of examination.

Esquenazi et al. [10] studied five corneas of subjects who had post-Lasik ectasia, and corneal buttons were collected after keratoplasty and studied by conventional histological analysis and immunofluorescence. The existence of MMP-9 at the edge of the wound years after LASIK, as proved by their study, demonstrated a dynamic remodeling of the wound that may elucidate the ongoing tissue destruction. Also, Maguen E and associates [22] performed an immunohistochemical evaluation of 3 corneal button specimens with INTACS one for post-Lasik ectasia and two for KC, obtained after corneal transplantation, and two postmortem corneas were studied as a control. They found that fibrotic extracellular matrix components and proteinases were abnormally accumulated close to INTACS suggesting a continuing lysis and remodeling of the stroma.

The lack of pre-LASIK values of tear MMP-9 and TIMP-1 is a limitation of this study. As it might give us a clue to know whether these changes started after the surgery or it is a progression of a preoperative condition and if it was the case, we may use the level of tear MMP-9 to predict the risk of developing PLE.

Our results showed a significant elevation in the level of MMP-9 and a significant reduction in the level of TIMP-1 in tear samples from PLE cases. These results indicate an inflammatory mechanism of PLE. So, controlling the inflammatory process may offer a valid option in the treatment of PLE. Moreover, the results pointed to a biochemical similarity between PLE and KC. So, all strategies of management of KC can be used in management of PLE. More studies are needed in the future to investigate larger number of tear mediators.

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