Galectin-3, IL-1A, IL-6, and EGF Levels in Corneal Epithelium of Patients With Recurrent Corneal Erosion Syndrome

Tuba Candar, MD, Leyla Asena, MD,† Husamadden Alkayid, MD,† and Dilek D. Altınörs, MD†*

Purpose: To determine the galectin-3 (Gal3), interleukin-1 (IL-1), interleukin-6 (IL-6), and epidermal growth factor (EGF) levels in corneal epithelium of patients with recurrent corneal erosion (RCE) syndrome and compare them with healthy controls.

Methods: In this prospective interventional case control study, 32 eyes of 32 patients with RCE syndrome who had corneal epithelial erosions and 28 eyes of 28 healthy participants scheduled for photorefractive keratectomy (control group) were included. Exclusion criteria included corneal dystrophies, ectasia, dry eye, previous ocular surgery or topical medications, and systemic diseases. Epithelial samples were obtained during epithelial debridement in the study group and mechanical epithelial keratectomy in the control group. Galectin-3 levels were studied by the chemiluminescent microparticle immunoassay method. IL-1, IL-6, and EGF levels were determined using corresponding ELISA kits.

Results: The median Gal3 levels were 132.25 ng/mL in the study group and 106.50 ng/mL in the control group. The median IL-1 and IL-6 levels were 6.24 pg/mL and 10.16 pg/mL, respectively, in the study group which were higher than that in the control group. The median EGF level in the study group was lower than that the control group with 1.30 pg/mL versus 2.67 pg/mL. In the control group, there was a significant positive correlation between EGF and IL-6 ($r = 0.554$; $P = 0.040$). A similar correlation was not observed in patients with RCE ($r = -0.071$; $P = 0.794$).

Conclusions: The lack of increased EGF expression and the imbalance between growth factors, adhesion molecules, and interleukins may be the reason for the impaired wound healing response in RCE syndrome.

Key Words: epidermal growth factor, galectin-3, interleukin-1, interleukin-6, recurrent corneal erosion

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Recurrent corneal erosion (RCE) syndrome is a chronic relapsing disease of the cornea, characterized by repeated breakdown of the corneal epithelium. It usually occurs secondary to a previous the corneal epithelial trauma or in eyes with epithelial basement membrane dystrophy. Other causes include corneal stromal dystrophies, corneal degenerations, previous ocular surgery, diabetes mellitus, and severe dry eye.[1,2]

The abnormality in the hemidesmosomes and fibril boundary complex between the basement membrane and epithelium plays the main role in the pathogenesis of RCEs.[1–4] Patients with RCE have upregulated matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), which promotes cleavage of collagen types IV, V, VII, and X, as well as the adhesion molecules, fibronectin and laminin.[5] Treatment options include intense ocular surface lubrication, topical antibiotic prophylaxis, bandage contact lenses, topical anti-inflammatory agents, and inhibitors of MMP-9 including systemic doxycycline and topical corticosteroids.[6]

When corneal injury or erosion occurs, epidermal growth factor (EGF), keratinocyte growth factor, hepatocyte growth factor, and cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6) are released to induce wound healing.[7] Because the epithelial cells migrate over a corneal wound, they must adhere to the underlying extracellular matrix (ECM) components of the basement membrane, such as laminin, fibronectin, and collagen IV. Galectin-3 (Gal3) is an ECM carbohydrate-binding protein expressed in the human cornea. In the wound healing process, it binds the glycans on the cell surface and contributes to the formation of new cross-links.[8] Galectin-3 promotes lamellipodia formation by interaction with complex N-glycans on integrin $\alpha_3\beta_1$ and promotes adhesion of corneal epithelial cells onto collagen IV, thereby enhancing wound healing in corneal explants.[9,10] It has been shown that exogenous Gal3 stimulates reepithelialization of corneal wounds in mice, and Gal3 knockout mice show significantly slower healing of corneal wounds.[9] Furthermore, Gal3 in the nucleus of cells may influence cell-matrix interactions indirectly by its effect on the expression of well-known cell adhesion molecules such as integrins and cytokines (eg, IL-1).[11] Therefore, exogenous Gal3 has been proposed as

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From the *Department of Biochemistry, Ufuk University Faculty of Medicine, Ankara, Turkey; and †Department of Ophthalmology, Baskent University Faculty of Medicine, Ankara, Turkey.
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Correspondence: Leyla Asena, MD, Baskent University Faculty of Medicine, Fevzi Çakmak Caddesi, 06490 Bahçelievler, Ankara, Turkey (e-mail: leylaerk@yahoo.com).

a candidate drug for enhancing epithelial cell wound healing in previous studies.[12,13]

Other molecules known to play a role in the corneal wound healing process are growth factors and interleukins.[14] EGF and proinflammatory cytokines such as IL-1 and IL-6 are mediators that have been shown to be increased during corneal wound healing.[15,16] EGF is a well-known stimulator of cell growth, proliferation, and differentiation through activation of the extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathway.[17] It has been tested for therapy of corneal wounds and has been found to increase proliferation in a dose-dependent manner at concentrations greater than 10 ng/mL in vitro.[18] In a study investigating the effects of EGF, IL-1, and their combination on in vitro corneal epithelial wound closure, it was found that they have an additive effect on corneal epithelial cell chemotactic migration.[19] In an in vitro experimental study,[20] IL-6-exposed cells showed faster wound healing than control cells, and in another in vivo experimental study,[21] exogenous application of IL-6 resulted in increased the corneal epithelial wound healing rate in rabbit corneas.

In RCE syndrome, corneal epithelial wound healing is dysregulated and impaired. The roles of Gal3, EGF, and cytokines in the pathophysiology of RCEs are currently not clearly identified, and the potential use of these mediators in the treatment still need to be investigated. In this pilot study, our aim was to determine the levels of Gal3, IL-1, IL-6, and EGF in corneal epithelium of patients with RCE syndrome and compare them with healthy controls.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board of Baskent University and conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from each participant.

Thirty-two eyes of 32 patients with RCE syndrome who had an acute corneal epithelial erosion and were admitted to the Baskent University Faculty of Medicine Department of Ophthalmology between October 2017 and May 2019 were included in this pilot study. Diagnostic criteria for RCE syndrome were as follows: patients with a history of painful recurrent attacks of corneal epithelial erosion that wakens the individuals from sleep at night and who have a basal membrane loosely bound to the epithelium as observed by biomicroscopic examination. Patients did not respond to conservative treatment, which was intense lubrication and bandage contact lenses, and they needed to undergo epithelial debridement. Twenty-eight eyes of 28 healthy participants scheduled for refractive surgery in the same clinic were included as the control group. Exclusion criteria for both groups included an underlying epithelial basement membrane dystrophy, other corneal dystrophies, corneal degenerations, ectasia, severe dry eye or inflammatory ocular surface disease, history of ocular surgery, topical antiglaucoma, or anti-inflammatory therapy. Patients with systemic diseases including diabetes mellitus, Sjögren's syndrome, or thyroid eye disease were excluded as well.

Corneal epithelial samples in the study group were obtained during corneal epithelial debridement for the treatment of recurrent erosion, where there was an epithelial defect with loose adhesion in the margins extending to the periphery of the cornea. In the control group, epithelial samples were obtained by mechanical epithelial keratectomy before photorefractive keratectomy. As a result, there was no deviation from standard treatment for the participants included in the current study. After topical anesthesia with proparacaine HCl 0.5%, the loose corneal epithelium was removed by stripping it with sterile forceps in patients with RCE syndrome. Because the epithelial attachments were intact in the control group, superficial keratectomy was performed by using a sterile blade, and the epithelium was removed with sterile forceps. The diameter of the debridement area was 9 mm in all participants.

Epithelial samples were stored in 1 cc of sterile saline at −80°C in Eppendorf tubes until analysis. On the day of analysis, the samples were homogenized by sonication in a Braun-Sonic 1510 (B. Braun Melsungen AG, Melsungen, Germany) at 400 watts for 1 minute. Galectin-3 levels were studied in the cell lysate by using the Abbott ARCHITECT $i1000_{SR}$ auto analyzer (Abbott Laboratories, Chicago, IL) by the chemiluminescent microparticle immunoassay method. Intra-assay and inter-assay coefficient variations (%CV) for Gal-3 were 4.7% and 5.3%, respectively. The results were recorded as ng/mL. Measurements of IL-1, IL-6, and EDGF levels were performed using Elabscience Human IL-1α, Elabscience Human IL-6, and Elabscience Human EGF ELISA kits, respectively, on an MRC UT6100 Microplate Reader system. The results were recorded as pg/mL for all 3 patients. Intra-assay and inter-assay %CV were <10% for all of them.

All data were tabulated and analyzed using the IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY) software. The test of normal distribution for continuous variables was performed by the Shapiro–Wilk test. The Levene test was used for the evaluation of homogeneity of variances assumption. Descriptive statistics for continuous variables were expressed as mean ± SD or median (interquartile range), where appropriate. The number of cases and percentages were used for categorical data. The mean differences between groups were compared by the Student t test. The Mann–Whitney U test was used for this comparison, when data were not normally distributed. Categorical data were evaluated by the continuity corrected χ^2 test. Degrees of association between continuous variables were evaluated by Spearman rank correlation analyses. A P value less than 0.05 was considered statistically significant.

RESULTS

The mean age of the participants was 35.1 ± 9.5 years. Male/female ratio was 28/32. Table 1 shows the demographic characteristics of the patients in the study and control groups.

The mean age of the study group was higher than the control group ($P < 0.001$). However, all study participants were healthy adults within the age range of 18 and 47 years. Gender distribution was similar among groups ($P = 0.149$). Table 2 shows comparison of Gal3, IL-1, IL-6, and EGF levels in the study and control groups.

The median Gal3 level was measured as 132.25 ng/mL in patients with RCE, whereas it was 106.50 ng/mL in the control

TABLE 1. Demographic Characteristics of the Patients in the Study and Control Groups

	Total (n = 60)	Control (n = 28)	RCE (n = 32)	*P*
Age (yr)	35.1 ± 9.5	29.2 ± 8.1	40.2 ± 7.6	<0.001*
Gender				0.149†
Male	28 (46.7%)	18 (64.3%)	10 (31.3%)	
Female	32 (53.3%)	10 (35.7%)	22 (68.7%)	

*Student *t* test.
†Continuity corrected χ^2 test.

group ($P = 0.667$). The median IL-1 and IL-6 levels were 6.24 pg/mL and 10.16 pg/mL, respectively, in RCE patients, which were also higher than the control group ($P = 0.077$ for IL-1 and $P = 0.257$ for IL-6). By contrast, the median EGF level in the study group was lower when compared with the control group with 1.30 pg/mL versus 2.67 pg/mL ($P = 0.154$). However, none of these differences reached a statistical significance.

Because we observed significant difference in mean age between control and study groups during data analysis, we performed multivariate linear regression analysis adjusting age between groups. We still did not find any statistically significant difference between groups for the primary outcomes that were Gal3, IL1, IL6, and EGF levels ($P > 0.05$).

In summary, although statistical significance could not be reached, patients with RCE displayed higher levels of Galectin-3, IL-1, and IL-6 and lower levels of EGF in corneal epithelial cells when compared with the control group (Fig. 1).

We also analyzed correlations between different laboratory measurements within the study and control groups (Table 3) to determine any possible effects of interleukins and growth factors on the levels of galectin expression during the corneal epithelial healing process in recurrent erosions. Galectin-3 levels did not show any correlation with other laboratory markers both in the study and control groups. In the healthy control group, there was a significant positive correlation between EGF and IL-6 ($r = 0.554$; $P = 0.040$). A similar correlation between EGF and IL-6 was not observed in patients with RCE ($r = -0.071$; $P = 0.794$).

DISCUSSION

In this pilot study, we studied the levels of different mediators that take place in the wound healing process of the corneal epithelium in RCEs. To our knowledge, this is the first study comparing corneal epithelial Gal3, growth factor, and interleukin levels of patients with RCE with healthy controls.

TABLE 2. Median (Interquartile Range) Values for the Levels of Galectin-3, Interleukin-1 (IL-1), Interleukin-6 (IL-6), and EGF in the Study and Control Groups

	Total (n = 60)	Control (n = 28)	RCE (n = 32)	*P**
Galectin-3 (ng/mL)	127.50 (207.00)	106.50 (20.75)	132.25 (242.93)	0.667
EGF (pg/mL)	1.40 (3.75)	2.67 (4.11)	1.30 (1.71)	0.154
IL-6 (pg/mL)	8.10 (7.39)	4.70 (7.55)	10.16 (8.93)	0.257
IL-1A (pg/mL)	6.21 (6.62)	5.16 (5.77)	6.24 (9.32)	0.077

*Mann Whitney *U* test.

Galectin 3 has been shown to be an important mediator taking part in corneal epithelial wound healing, enhancing epithelial cell migration and forming cross-links between epithelial cells and collagen type IV found in the ECM.[10–13] Saravanan et al[10] reported that Gal3 induces clustering of $\alpha_3\beta_1$ integrin on the cell surface. These clusters activate an intracellular signaling cascade, leading to lamellipodia formation, cell migration, and reepithelialization of wounds.[11–13] Increased Gal3 levels would be expected during the epithelial wound healing process in a healthy cornea. We found higher levels of Gal3 when compared with a healthy unabraded cornea, although this increase did not reach a significant level. According to our results, it could be postulated that the failure of increase in Gal3 levels to a threshold might be one of the reasons for loose adhesion of the epithelium to the Bowman membrane during the healing process in RCEs, besides other pathophysiological mechanisms. To test this hypothesis, in vivo experiments should be planned where epithelial samples would be obtained during the healing process of an abrasion in a healthy cornea and compared with patients with RCE syndrome.

EGF stimulates corneal epithelial cell proliferation, migration, fibronectin synthesis, and integrin activity and is particularly important for enabling ECM interactions.[22] It upregulates expression and activation of β_4 integrins and enhances degradation and reformation of hemidesmosomes during epithelial cell migration after injury.[23] However, treatment of corneal epithelial wounds with exogenous EGF has been found to cause corneal neovascularization.[22] We detected lower levels of EGF in patients with RCEs when compared with healthy corneas and postulated that this may be one of the reasons for the impaired wound healing response in RCE syndrome leading to a loosely attached epithelium to the basement membrane.

The major cytokines released after corneal epithelial injury are IL-1 and IL-6 and the increase in their level seems to be directly proportional to the severity of the injury.[24] IL-6 facilitates epithelial cell migration by upregulation of the integrin receptor for fibronectin and has been shown to have a synergistic effect with EGF during the wound healing process.[21,22] IL-1 has also been shown to promote wound healing synergistically with EGF in addition to upregulating the levels of other growth factors such as hepatocyte growth factor and keratinocyte growth factor.[19] In the current study, the levels of these interleukins were higher in patients with RCEs when compared with healthy unabraded corneas; however, the difference was not statistically significant. Another finding was that EGF levels did not increase in direct proportion to IL-6 levels, although there was a positive correlation between the levels of these mediators in healthy corneas.

Our results suggest that in patients with RCE syndrome, increased expression of Gal3, IL-1, and IL-6 levels occur in corneal epithelium during the epithelial healing process when compared with a healthy nontraumatized corneal epithelium. However, this increase cannot reach a statistically significant

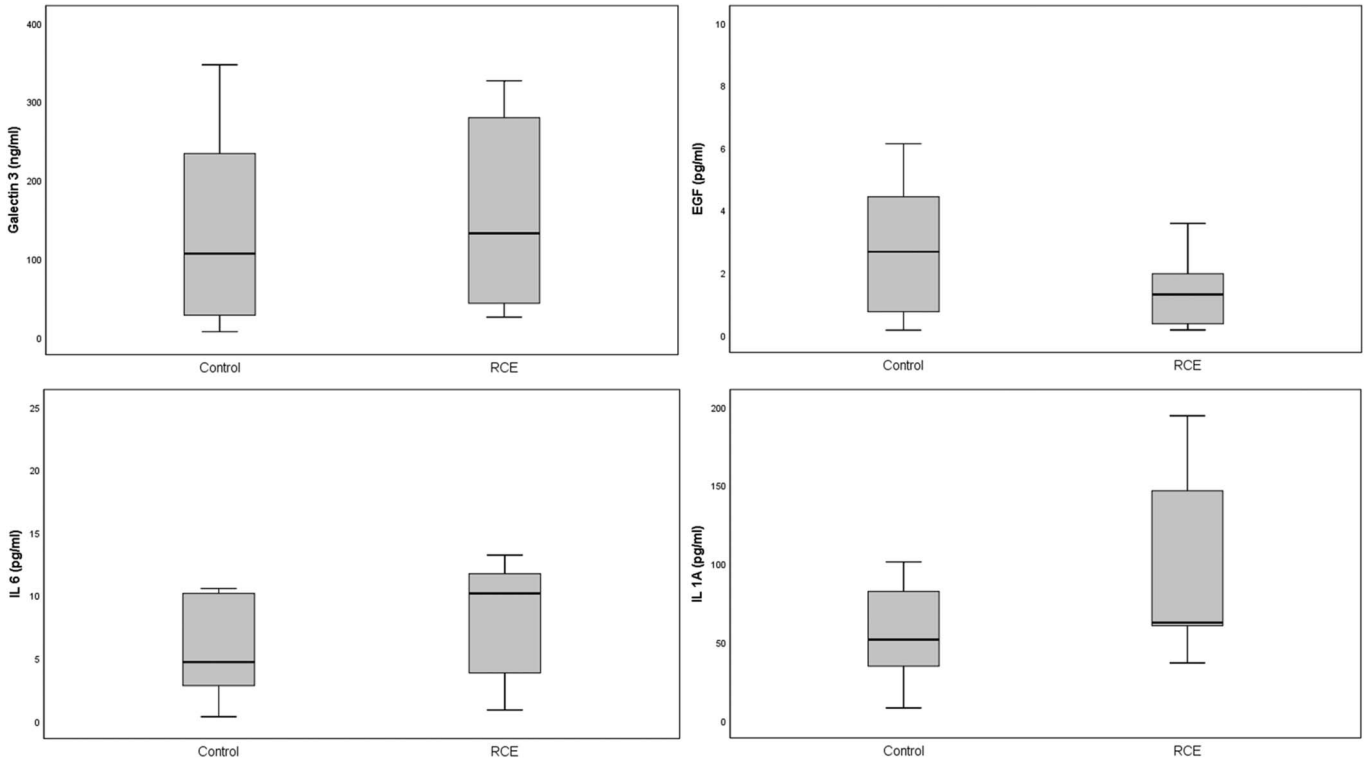

FIGURE 1. Comparison of Gal3, EGF, IL-6, and IL-1A levels between control and RCE groups. The horizontal lines in the middle of each box indicates the median, whereas the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the boxes mark the maximum and minimum Gal3, EGF, IL-6, and IL-1A levels.

level. In addition, EGF levels were found to be lower when compared with a healthy corneal epithelium. The lack of increased EGF expression and the imbalance between growth factors, cell-ECM adhesion molecules, and interleukins may be the reason for the lack of a good wound healing response in RCE syndrome. The statistical nonsignificance of the median values between control and RCE patients may be due to the relatively low number of participants, which is the major limitation of the current study. Further studies should be undertaken in a larger series of patients to differentiate the roles of Gal3, growth factors, and interleukins in pathogenesis of different corneal diseases including traumatic abrasions in healthy corneas, recurrent erosions, or persistent epithelial defects.

Experimental and clinical studies comparing the level of Gal3, growth factors, and cytokines between recurrent erosions and abraded, but otherwise healthy corneas with an ongoing healing process are needed to further clarify the pathophysiology and determine potential therapeutic agents in RCE syndrome. Because obtaining epithelial samples from an

TABLE 3. Correlations and Their Significance Between Different Laboratory Measurements Within the Study and Control Groups

	Total (n = 60)			Control (n = 28)			RCE (n = 32)		
	EGF	**IL-6**	**IL-1A**	**EGF**	**IL-6**	**IL-1A**	**EGF**	**IL-6**	**IL-1A**
Galectin 3									
Correlation coefficient	−0.002	0.080	−0.103	−0.132	0.169	−0.152	0.208	−0.047	0.003
*P**	0.992	0.673	0.588	0.653	0.563	0.605	0.440	0.862	0.991
EGF									
Correlation coefficient		0.131	−0.030		0.554	0.233		−0.071	−0.091
*P**		0.489	0.876		**0.040**	0.422		0.794	0.736
IL-6									
Correlation coefficient			0.043			−0.015			0.065
*P**			0.822			0.958			0.811

*Spearman rank-order correlation test.
Significant *p* values are indicated with boldface text.

abraded and otherwise healthy cornea with an ongoing healing process is associated with ethical concerns in human trials, animal or laboratory studies with a larger number of test subjects are needed for this purpose. Further investigations are needed as well to reveal the potential effects of exogenous Gal3, growth factor, and interleukin application or their combinations for the treatment of acute corneal erosions in RCE syndrome.

We hope that the results of this pilot study will contribute to the understanding of the pathophysiology of RCE syndrome and provide insight for future studies investigating the pathophysiology of RCE and the effect of exogenous Gal3, growth factor, and interleukin therapies for the treatment of this disease.

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