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## Tear lactoferrin concentration in patients with recurrent herpetic stromal keratitis and therapeutic effect of Lacto eyedrops in the multicomponent treatment for this disorder

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**Background:** Herpetic stromal keratitis (HSK) is an immunomodulatory disease that develops as a result of herpes simplex virus (HSV)1 or HSV2 reactivation. HSK is the most common form (20-50%) of recurrent herpetic keratitis (HK) that most commonly results in significantly reduced vision. It is well known that changes in biological compound levels in tear fluid can be indicative of the state of the ocular surface and determine whether ocular surface pathology is present, and, therefore, can be used as diagnostic markers of pathological changes in the ocular surface. Lactoferrin (LF) is one of these compounds and has been proven to have innate antibacterial, antifungal, antiviral, antiparasite, anti-inflammatory, immunomodulatory, antioxidative and other properties.

**Purpose:** To determine tear Lf concentration in patients with recurrent acute HSK, its effect on functional characteristics of the ocular surface, and the therapeutic effect of Lacto eyedrops in the comprehensive treatment for this disorder.

**Material and Methods:** The study was conducted at the site of Corneal Pathology Department and Immunology Laboratory of the Institute in January to December 2021. Seventeen patients (17 affected eyes and 17 fellow eyes) with recurrent acute HSK were included in the study. Mean patient age plus or minus standard deviation (SD) was  $48.7 \pm 16.0$  years. The ophthalmological examination included biomicroscopy of the bulbar conjunctiva and cornea, fluorescein test, determination of corneal sensation and basal tear production (Schirmer's II test), and microbiological examination of the conjunctiva. Tear Lf concentration was determined by a human Lf enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology, Inc., Wuhan, China). The results were photometrically measured at 450 nm with an ELISA reader (Stat Fax 2100, Awareness Technology Inc, Palm City, FL). Lacto eyedrops were administered two times a day for 30 days. Tear Lf concentrations were determined at baseline and at day 30.

**Results:** Mean tear LF concentration plus or minus SD in eyes of patients with recurrent HSK was  $1.21 \pm 0.52$  g/l, with no significant difference between the affected eye and fellow eye. However, a subnormal tear LF concentration was more common in the affected eye than in the fellow eye ( $\chi^2 = 4.24$ ,  $p = 0.04$ ). After a 30-day anti-inflammatory treatment with Lacto eyedrops, mean tear LF concentration plus or minus SD increased by 47% to  $1.78 \pm 0.7$  g/l in the affected eyes. A corneal sensation score of 1 to 9 was 12.4 times more common in HSK eyes than in fellow eyes (OR = 12.4; 95% CI, 2.0–76.8), which reflects neurosensory abnormalities in the affected eye. Corneal sensation loss, was, however, more common in HSK eyes with a low tear Lf concentration than in fellow eyes with a low tear Lf concentration. Median basal tear production (as assessed by Schirmer's II test) was as low as 7-8 mm in eyes with a low tear Lf concentration and as high as 13-16 mm in eyes with a high tear Lf concentration ( $p < 0.05$ ). There was a direct correlation between the basal tear production and the tear Lf concentration ( $r=0.45$ ;  $p < 0.05$ ). Among study patients with recurrent HSK, 24.9% showed baseline microbiological evidence of potentially pathogenic or fungal organisms in the conjunctiva. A course of multicomponent anti-inflammatory treatment with Lacto eyedrops contributed to complete removal of these organisms.

**Conclusion:** We determined mean tear Lf concentrations in affected and fellow eyes of patients with recurrent HSK, and established certain relationships between functional characteristics of the ocular surface and tear Lf concentrations in these eyes. A disinfecting effect of Lacto eyedrops against any microorganisms present in the conjunctival sac of patients with recurrent HSK was confirmed by the absence of culture growth on completion of the multicomponent anti-inflammatory treatment.

### Keywords:

herpetic keratitis, tear production, corneal sensation, lactoferrin, ELISA, conjunctival bacterial and fungal flora

## Introduction

Herpetic stromal keratitis (HSK) is an immunomodulatory disease that develops as a result of herpes simplex virus (HSV)1 or HSV2 reactivation. Cellular immunity to corneal antigens to HSV and side effects of proinflammatory cytokines secreted by infected corneal cells are believed to be implicated in the pathogenesis of HSK. The type of inflammatory response in the corneal stroma may have a substantial role in the pathogenesis of recurrent HSK [1]. HSK can be classified as either necrotizing or non-necrotizing (also known as interstitial or disciform). In non-necrotizing HSK, clinical symptoms include corneal stromal and epithelial edema in a round or oval pattern, associated with keratic precipitates underlying the zone of edema (these disc-shaped stromal edema and keratic precipitates appear out of proportion to the degree of anterior chamber reaction) and iridocyclitis. These changes are caused by HSV reactivation with proliferation of the virus deep in the cornea, and corneal immune response to the viral antigen. The potent immune response to viral proteins results in infiltration of leukocytes, damage to the corneal stroma and endothelium that combine to promote corneal opacity and edema. Stromal HSK causes 20% to 50% of recurrent disease, and it is the form of recurrent herpetic external disease associated with the greatest visual morbidity [2,3].

Several natural antimicrobial proteins and peptides have demonstrated the capacity to inhibit viral infection and block viral proliferation into host cells and exert effects on late phases of viral growth. One of these natural antimicrobial proteins is lactoferrin (Lf), which has bactericidal, fungicidal, antiviral, antiparasite, anti-inflammatory, immunomodulatory, antioxidant, anti-tumor and other properties. Lf is a monomeric, 80-kDa iron-binding glycoprotein, with a single polypeptide chain of about 690 amino acid residues. This glycoprotein belongs to the transferrin family, and its antimicrobial activity is associated with its chelating properties towards Fe<sup>3+</sup> ions. It is found in many mucosal secretions and performs multiple functions including immune stimulation and antiviral activity towards not only HSV1 and HSV2, but also rotavirus, respiratory syncytial virus, cytomegalovirus, parainfluenza virus, human papilloma virus, adenovirus, hepatitis C virus and human immunodeficiency virus. It has been reported on the antibacterial activities of Lactoferricin (Lf<sub>cin</sub>), a peptide generated by pepsin cleavage from the N-terminal part of lactoferrin. It has been shown that Lf<sub>cin</sub> inhibits HSV cell-to-cell spread through their interaction with the cell surface glycosaminoglycan heparan sulfate, and thus block the entry of the virus into the cell. It has been reported [4-7] that the antiviral effect of Lf lies in the early phase of infection, preventing the entry of a virus into the host cell, either by blocking cellular receptors, or by direct binding to the virus particles.

Through its unique combination of antimicrobial action and anti-inflammatory activities lactoferrin (Lf) in the tear film plays an important role in the maintenance

of ocular health. Virus particles' entry into epithelial cells is inhibited by Lf while an excess of Lf in tear film is thought to limit the opportunistic Lf-mediated bridging of adenovirus and host cell that occurs in other tissues [8]. Lf plays an important role in the defense against infections, including HSV keratitis [9]. In addition, it inhibits bacterial growth in the conjunctival sac and prevents HSV-1 plaque formation [10]. Lf aids in control of HSV infections, either by preventing HSV particles from binding target cells, or by interfering with intracellular trafficking of HSV virions [8,11].

In recent decades, acyclovir (ACV) has become the gold standard for the treatment of HSV infections. However, long-term antiviral treatment, as it is required mainly in immunocompromised patients, led to the emergence of resistances towards ACV and other antivirals. Therefore, there is a clear need for the development of new potent antivirals which combine good oral bioavailability and tolerability with low side effects [12].

In this context, Lf has drawn researchers' attention. Its efficacy as an antimicrobial agent has been widely reported, and novel interest has been rising towards its potential application in the setting of viral infections [13]. In *in vivo* studies on HSV1, it has been demonstrated that topical administration of 1% bLf, prior to the virus inoculation, suppressed HSV1 infection in the mouse cornea but not viral propagation [10]. It has been demonstrated that LF-loaded contact lenses could represent a new therapeutic approach to treat ocular surface pathologies characterized by high levels of oxidative stress [14]. Wakabayashi and colleagues [15] reported that orally administered Lf increases the splenocyte production of Th1 (IFN-gamma and IL-12) and also the serum level of IL-18 in response to HSV-1 infection of mice, which could improve host defense against HSV-1 infection. In a study by Krzyzowska and colleagues [16], the antiviral and cytotoxic activities of silver and gold nanoparticles modified with lactoferrin (LF-Ag/AuNPs) were tested in human skin HaCaT and vaginal VK-2-E6/E7 keratinocytes. It was concluded that LF-Ag/AuNPs could become effective novel antiviral microbicides with immune-stimulant properties to be applied upon the mucosal tissues. Researchers' interest has been drawn to the use of Lf preparations in viral conjunctivitis, which is a clinical symptom of COVID-19 [17-19].

Therefore, the endogenous biologically active compounds can have effects on the morphology and functions of the ocular surface system components, and be involved in the metabolic and cellular pathways maintaining homeostatic conditions. Consequently, Lf meets the demand for developing new methods to treat ocular surface infections. In this field, LF could be used as a stand alone treatment or in conjunction with other medications to improve efficacy [17]. Since changes in tear proteomes can reflect the state of the ocular surface and highlight disease state, they may be used as markers for diagnosing patients with ocular surface disease [20].

The primary purpose of this study was to assess tear Lf concentration in patients with recurrent HSK and to examine its relationship with the function of some ocular surface systems. In addition, we aimed to determine the therapeutic effect of LF-based eyedrops in the treatment of recurrent HSK.

The purpose of this study was to assess tear Lf concentration in patients with recurrent HSK in the acute phase, its effect on ocular surface function, and the therapeutic effect of LF-based eyedrops in multicomponent treatment for this disorder.

### Material and Methods

The study was conducted at the site of Corneal Pathology Department and Immunology Laboratory of the Institute in January to December 2021. This study was approved by the bioethics committee of the Filatov Institute and the principles of the Declaration of Helsinki and the European Convention on Human Rights, and relevant laws of Ukraine were followed throughout the investigation. Informed consent was obtained from all study participants after explanation of the nature and possible consequences of the study.

Seventeen patients with recurrent non-necrotizing HSK (17 affected eyes and 17 fellow eyes) were included in the study. Mean patient age plus or minus standard deviation (SD) was  $48.7 \pm 16.0$  years. They were diagnosed with HSK on the basis of history and clinical signs and symptoms. All patients had a history of at least two separate HSK recurrences. The ophthalmological examination included biomicroscopy of the bulbar conjunctiva and cornea, fluorescein examination, and determination of corneal sensation and basal tear production (Schirmer's II test).

Basal tear production was assessed using a 5-minute anesthetized Schirmer's II test. Wetting of more than 10 mm in 5 minutes was an indication of normal tear production.

A cotton wisp test was used to assess corneal sensation. A small fine-tipped cotton wisp is lightly touched first at the central cornea and then at four different quadrants. The patient feels intense irritation and tries to close the eyes if corneal sensation is normal. If this does not take place, thicker portions of the wisp are used to assess the reduction in corneal sensation. If no corneal reflex is elicited by touching the cornea, corneal sensation is believed to be absent. Corneal sensation was estimated in each of the five points using a 0 to 2 scale in which 0 was no sensation, 1 was decreased sensation, and 2 was normal sensation. The total score of corneal sensation was calculated ranging from 0 (no sensation) to 10 points (normal sensation).

Corneal and conjunctival xerosis was assessed with the scale of 0-9 using the method of van Bijsterveld (1969). The ocular surface was divided into three areas (nasal bulbar conjunctiva, cornea, and temporal nasal conjunctiva) that were assessed for fluorescein staining. Each area was given a staining score from 0 (no damage) to 3 (severe damage) points, and the total score of fluorescein staining was calculated ranging from 0 to 9 points. Conjunctival

and corneal epithelium staining was defined as (a) normal if a total score was 3.5 or lower and (b) pathological if a total score was higher than 3.5.

A routine microbiological examination was conducted before and after treatment for evidence of pathogenic or potentially pathogenic organisms in the conjunctiva.

Patients with history of surgery within prior 6 months, systemic autoimmune disease, ocular comorbidity requiring regular administration of eyedrops, or the presence of corneal epithelial defects, corneal erosion or ulceration were excluded from the study.

Patients with HSK were administered topical antiseptic (miramistin) four times daily recombinant human interferon alpha 2b four-five times daily, dexamethasone 0.1% three-four times daily, preservative-free hyaluronic acid artificial tears five times daily, and mydriatics one-two times daily, in the affected eye. In addition, they were administered systemic valacyclovir (500 mg twice daily) and intramuscular non-steroidal anti-inflammatory drugs. Moreover, they were administered Lacto eyedrops (manufactured by NOVAX® PHARMA and containing lactoferrin) twice daily over thirty days in both eyes.

Tear Lf concentration was assessed before administration and at day 30 of administration of Lacto eyedrops. An immunological study was conducted to assess tear Lf concentration in all patients with HSK. Tear samples for the determination of tear LF concentration were collected in the morning before diagnostic and treatment procedures were performed. For this purpose we used a sterile plastic tip attached to a pipette aid. The tear samples collected were stored at  $-20^{\circ}\text{C}$  until immunological testing. Concentration of LF in the tear samples was determined by a human Lf enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology, Inc., Wuhan, China). The results were photometrically measured at 450 nm with an ELISA reader (Stat Fax 2100, Awareness Technology Inc, Palm City, FL).

Statistical analyses were conducted using Statistica 9.0 (StatSoft, Tulsa, OK, USA). Normal distribution of quantitative data was assessed using the Shapiro-Wilk test. Variables without normal distribution are reported as median and interquartile range (IQR), and nominal data as absolute values and percentages. Mean and standard deviation (SD), and 95% confidence interval (CI) of differences were calculated for variables with a normal distribution. Mann-Whitney U test was used to compare variables without normal distribution, and t-test was used to compare variables with a normal distribution. Pearson's chi 2 test was used for frequency analysis. Odds Ratio (OR) was used as a quantitative measure of effect for the comparison of relative characteristics and defined as the ratio of the odds of an event occurring in the group affected by the risk to the odds of it occurring the control group. Odds ratios were calculated by means of a 2x2 contingency table.

## Results

Tear Lf concentrations were assessed in the eyes with HSK and fellow eyes. Figure 1 shows a histogram of the distribution of tear Lf concentration values across study eyes. Mean tear Lf concentration plus or minus SD was  $1.21 \pm 0.52$  g/l for the total sample of affected and fellow eyes. In addition, mean tear Lf concentration plus or minus SD was  $1.16 \pm 0.47$  mg/ml, and 95% CI, 0.91-1.14, for affected eyes only, and  $1.30 \pm 0.47$  mg/ml, and 95% CI, 0.89-1.7, for fellow eyes only. In 58 tear samples from healthy subjects an average Lf concentration of 1.42 g/l was found [8, 21–23].

After a 30-day treatment with Lacto eyedrops, mean tear Lf concentration plus or minus SD in affected eyes increased to  $1.78 \pm 0.7$  g/l (with a 95% CI ranging from 0.61 to 2.9 g/l), which was 53.4% and significantly higher than at baseline ( $p = 0.01$ ). In addition, mean tear Lf concentration plus or minus SD in fellow eyes  $1.34 \pm 0.2$  g/l ( $p = 0.4$ ), which was 32.8% and significantly lower than in affected eyes ( $p=0.01$ ) (Table 1).

A high corneal sensation score (score of 10) was much more common in fellow eyes than in affected eyes (72.7% vs 17.6%;  $\chi^2=8.5$ ,  $p = 0.004$ , Table 2). In addition, a high corneal sensation score (score of 10) was less common in affected eyes with a tear Lf concentration lower than 1.21 g/l than in fellow eyes with the same tear Lf concentration ( $\chi^2 = 5.4$ ;  $p = 0.02$ ; Table 2). We, however, found no significant difference between affected eyes and fellow eyes with a tear Lf concentration higher than 1.21 g/l in terms of corneal sensation score. Moreover, a high corneal sensation score was less common in affected eyes with a tear Lf concentration lower than 1.42 g/l than in fellow eyes with the same tear Lf concentration (Table 2).

Corneal sensation was lower in affected eyes with a low tear Lf concentration than in the fellow eyes with a low tear Lf concentration ( $\chi^2 = 8.65$ ,  $p = 0.003$ ; Table 2). Therefore, loss of corneal sensation was more severe in the affected eyes with a low tear Lf concentration than in

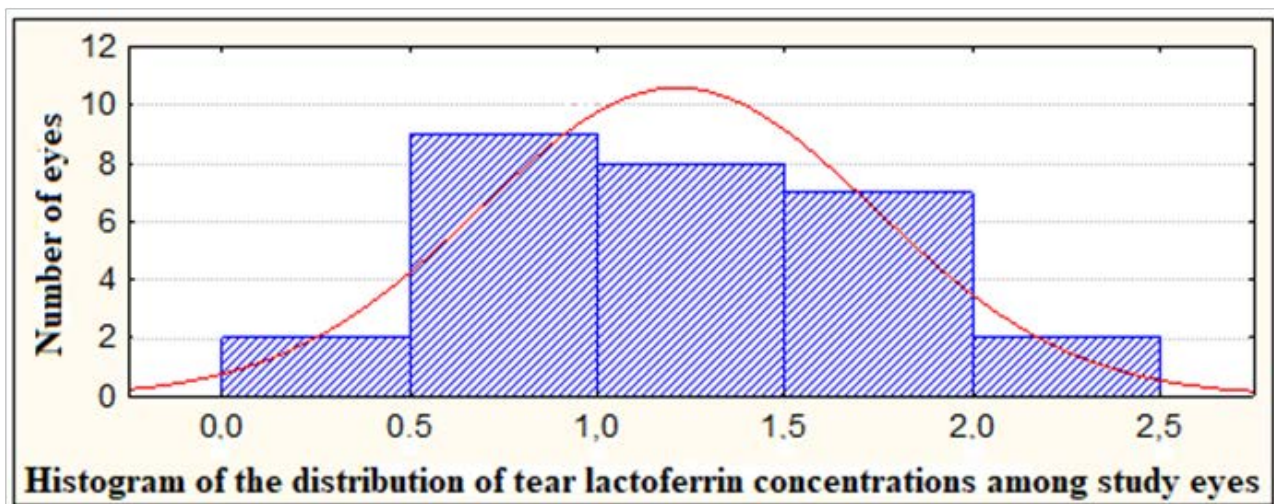
the fellow eyes with a low tear Lf concentration. A corneal sensation score of 1 to 9 was 12.4 times more common in eyes with HSK than in fellow eyes (OR=12.4; 95% CI, 2.0–76.8). This reflects neurosensory abnormalities in the eye with HSK.

There was no significant difference in corneal and conjunctival epithelial staining score between eyes with a tear Lf concentration below the median of 1.21 g/l and eyes with a tear Lf concentration above the median, and between eyes with a tear Lf concentration below the normal value of 1.42 g/l and eyes with a tear Lf concentration above the normal value (Table 3).

This is likely due to the fact that patients with corneal epithelial defects and corneal erosions were not included in the study, and no complications were seen during the course of treatment.

Median basal tear production (as assessed by Schirmer's II test) was as low as 7-8 mm in eyes with a low tear Lf concentration and as high as 13-16 mm in eyes with a high tear Lf concentration ( $p < 0.05$ ) (Table 4). There was a direct correlation between the basal tear production (as assessed by Schirmer's II test) and the tear Lf concentration ( $r = 0.45$ ;  $p < 0.05$ ). Therefore, in eyes with HSK with a low tear Lf concentration, basal tear production (as assessed by Schirmer's II test) was lower than in eyes with HSK with a high tear Lf concentration.

The examination found no microbial growth in the conjunctiva in 24 eyes. Staphylococcus epidermidis was found in 8 eyes, and Candida albicans, in two eyes (Table 6), and these ten eyes were those of 5/17 patients (24.9%). There was no significant difference in the incidence of microbial growth in the conjunctiva between the affected eyes and fellow eyes. At baseline, tear Lf concentration was 28.6% higher in the absence of potentially pathogenic organisms than in their presence ( $1.29 \pm 0.5$  g/l vs  $0.92 \pm 0.5$  g/l;  $p = 0.046$ ). No pathogens were found in the samples taken from the conjunctival sac after comprehensive treatment with Lacto eyedrops (Table 5).



**Figure 1.** Histogram of the distribution of tear Lf concentration values among a total sample of affected and fellow eyes of patients with herpetic stromal keratitis

**Table 1.** Tear lactoferrin concentrations in patients with herpetic stromal keratitis before and after treatment

	Numbers of affected and fellow eyes	Statistical characteristics	Tear lactoferrin concentration (g/l)	
			Before treatment	After treatment
Herpetic stromal keratitis	Affected eyes, n=17	M ± SD	1.16 ± 0.47	1.78 ± 0.7*
		(95% CI)	(0.91–1.4)	(0.61–2.9)
	Fellow eyes, n=17	M ± SD	1.30 ± 0.47	1.34 ± 0.2
		(95% CI)	(0.89–1.7)	(0.9–1.74)

Notes: M, mean value; SD, standard deviation; 95% CI, 95% confidence interval; \*, significant difference ( $p < 0.05$ )

**Table 2.** Frequency distribution of corneal sensation scores with respect to the mean or normal (1.42 g/l) tear lactoferrin concentrations among affected eyes and fellow eyes of patients with herpetic stromal keratitis

Parameter	Eye	Tear lactoferrin concentration below 1.21 g/l / 1.42 g/l			Tear lactoferrin concentration above 1.21 g/l / 1.42 g/l		
		Number of eyes			Number of eyes		
		A score of 0	A score of 1–9	A score of 10	A score of 0	A score of 1–9	A score of 10
Corneal sensation	Affected eye	0/0	7/10	2/2	0/0	7/4	1/1
	Fellow eye	0/0	1/1	5/6	0/0	2/2	3/2

**Table 3.** Frequency distribution of herpetic stromal keratitis (HSK) eyes with respect to the mean or normal tear lactoferrin concentrations based on the corneal and conjunctival epithelial staining score

Parameter	Tear lactoferrin concentration below 1.21 g/l / 1.42 g/l		Tear lactoferrin concentration above 1.21 g/l / 1.42 g/l	
	<3.5	>3.5	<3.5	>3.5
Corneal and conjunctival epithelial staining score				
Number of HSK eyes	9/10	1/1	6/5	1/1

**Table 4.** Median and interquartile range (IQR) for basal tear production (as assessed by Schirmer's II test) with respect to the mean or normal (1.42 g/l) tear lactoferrin concentrations in herpetic stromal keratitis (HSK) eyes

Parameter	Statistical characteristics	tear lactoferrin concentrations				p
		Below 1.21 g/l	Below 1.42 g/l	Above 1.2 g/l	Above 1.41 g/l	
		1	2	3	4	
Basal tear production (as assessed by Schirmer's II test), mm	Median	7	8	13	16	$p_{1-3}=0.03$ $p_{2-4}=0.04$
	Interquartile range (IQR)	(4.5–15)	(6–10)	(11–17)	(12–20)	

Note: p, significance of difference

Therefore, we identified the features of tear Lf concentration as a marker of certain underlying pathological conditions of the anterior eye and determined the therapeutic effect of Lacto eyedrops in multicomponent treatment for recurrent HSK.

## Discussion

Lf is a natural protective protein that has numeral protective effects, including biocidal effects and modulation of viral infections. The antiherpetic effect of Lf is based on its interaction with cell surface glycosaminoglycans, thus preventing virus attachment to the target cells by binding

**Table 5.** Results of the microbiological examination of conjunctival swabs in patients with herpetic stromal keratitis (HSK)

HSK or fellow eyes	No growth	<i>Staphylococcus epidermidis</i>	<i>Candida albicans</i>
	n	n	n
HSK eyes	11	4	2
Fellow eyes	13	4	-
Total	24	8	2

Note: n, number of eyes

to the virus receptor on the target cells or binding to the virus. Oral administration of Lf increases the production of Th1 cytokines (IFN-gamma and IL-12) and also the serum level of IL-18 in response to HSV-1 infection, which could improve host defense against HSV-1 infection [11,17,24]. Lf has been shown to be beneficial against corneal damage. Fujihara and colleagues [25] reported that Lf protects against UV-B irradiation-induced corneal epithelial damage in rats. There are numerous reports on tear Lf concentration in ocular disorders. Most of the reports are related mostly to dry eye and diabetic eye lesions, and there are isolated reports related to ocular inflammatory disorders. It has been concluded that a low Lf concentration may have a role in the inflammatory component of ocular surface disease [21,26,27]. There are, however, few reports on the role of Lf in the pathogenesis of HSK. Keijser and colleagues [9] noted that tear Lf concentrations were the same in patients with HSV keratitis and healthy controls and also did not differ among patients with various Lf genotypes. The current study included patients with recurrent non-necrotizing HSK, the most common form of recurrent HK leading to a substantially reduced vision. A HSK that persists for long duration can result in persistent bullous keratopathy, corneal endothelial decompensation and corneal opacity.

We determined tear Lf concentration in affected eyes and fellow eyes of patients with recurrent HSK. In the current study, we have taken into account an average normal tear Lf concentration of 1.42 g/l [21], and the median tear Lf concentration for our study sample. It should be noted that no standard deviation or confidence interval had been provided for an average normal tear Lf concentration of 1.42 g/l, posing difficulties in its use for statistical analysis.

A limitation of the current study is the absence of a control group. This is due to the fact that the study was conducted during the COVID-19 pandemic and tear sampling in healthy individuals could not be performed to compare them with patients with HSK in terms of tear Lf concentration.

In the current study, we found the baseline tear Lf concentration in affected eyes to be 18.3% lower than

normal. A tear Lf concentration lower than normal was more common in eyes with HSK than in fellow eyes.

After a 30-day treatment with Lf-based eyedrops, mean tear Lf concentration in eyes with HSK was 53.4% and significantly higher than baseline, whereas those in fellow eyes did not change much compared to baseline. A corneal sensation score of 1 to 9 was 12.4 times more common in eyes with HSK than in fellow eyes (OR=12.4; 95% CI, 2.0–76.8), which reflects neurosensory abnormalities in the affected eye. Corneal sensation loss, was, however, more severe in fellow eyes with a low tear Lf concentration. We have failed to find any report on this in the literature on HSK.

We found no changes in fluorescein staining score with changes in tear Lf concentration, likely due to the fact that patients with corneal epithelial defects and corneal erosions were not included in the study, and no complications were seen during the course of treatment. A lower basal tear production was, however, found in HSK eyes with a low tear Lf concentration. This is in agreement with findings of a dry eye study by Sonobe and colleagues [28] who reported that ELISA revealed Lf concentration correlated with the value of Schirmer test and tear film breakup time, whereas it was inversely correlated with fluorescein scores.

Although there have been numerous reports on the therapeutic effect of Lf in dry eye patients, no reports have assessed the therapeutic effect of Lf in HSK patients. It has been reported that Lf was found to be beneficial in improving ocular surface parameters like the value of Schirmer test and tear film breakup time [28,29]. Median basal tear production (as assessed by Schirmer's II test) in eyes with HSK tended to improve after comprehensive treatment with Lacto eyedrops; this warrants research on a more prolonged application of Lf-based eyedrops in patients with HSK.

Mixed (viral and bacterial or fungal) infections of the anterior eye are common in the clinical practice of ophthalmology and are more difficult to manage than viral only infections. In the current study, 35.2% of eyes with HSK showed microbiological evidence of potentially pathogenic and fungal organisms in the conjunctiva. At baseline, tear Lf concentration was 28.6% higher in the absence of potentially pathogenic and fungal organisms than in their presence in the conjunctiva. Application of Lacto eyedrops enabled complete removal of these organisms from the conjunctival sac. Lf is well known (1) to inhibit bacterial growth and bacterial biofilm formation independently from bacterial cell adhesion and bacterial entry into cells, and (2) increase the activity of various antimicrobial agents against suspended bacterial cultures, including *Staphylococcus epidermidis* [30]. In addition, Lf inhibits bacterial growth in the conjunctival sac and prevents HSV-1 plaque formation [10].

We have previously reported [31] on the beneficial effect of Lf-based eyedrops on eyes with chronic bacterial conjunctivitis. Therefore, we determined mean tear Lf

concentrations in affected and fellow eyes of patients with recurrent HSK, and established certain relationships between functional characteristics of the ocular surface and tear Lf concentrations in these eyes. Moreover, we noted a beneficial effect of the use of Lf-based eyedrops in the comprehensive anti-inflammatory and anti-viral effect treatment of HSK.

It should be noted that further observations are required to determine the effect of Lf on remission duration and severity of potential HSK recurrence.

### Conclusion

First, mean tear LF concentration plus or minus SD in eyes of patients with recurrent HSK was  $1.21 \pm 0.52$  g/l, with no significant difference between the affected eye and fellow eye. However, a subnormal tear LF level was more common in the affected eye than in the fellow eye ( $\chi^2 = 4.24$ ,  $p = 0.04$ ).

Second, a corneal sensation score of 1 to 9 was 12.4 times more common in eyes with HSK than in fellow eyes (OR=12.4; 95% CI, 2.0–76.8), which reflects neurosensory abnormalities in the affected eye and is associated with a subnormal tear Lf concentration in eyes with HSK.

Third, in patients with HSK, the basal tear production depended on the tear Lf concentration: eyes with a supranormal tear Lf concentration showed a high basal tear production (13–16 mm), and eyes with a subnormal tear Lf concentration, a low basal tear production (7–8 mm). There was a direct correlation between the basal tear production and the tear Lf concentration ( $r=0.45$ ;  $p < 0.05$ ).

Finally, after a 30-day anti-inflammatory treatment with Lacto eyedrops, mean tear LF level plus or minus SD increased by 47% to  $1.78 \pm 0.7$  g/l in the affected eyes. This was accompanied by a complete removal of any present microorganisms, which was confirmed by the absence of culture growth on completion of comprehensive anti-inflammatory treatment.

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## Disclosures

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**Abbreviations:** *CC, chronic conjunctivitis; CS, corneal sensation; HIV, human immunodeficiency virus; HK, herpetic keratitis; HSK, herpetic stromal keratitis; HSV, herpes simplex virus; Lf, lactoferrin; OR, odds ratio; SD, standard deviation.*