

# Tear lactoferrin levels in chronic meibomitis associated with acne rosacea

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**PURPOSE.** *To determine tear lactoferrin levels in patients with chronic diffuse meibomitis associated with acne rosacea and to investigate their role in the development and perpetuation of some inflammatory ocular surface complications.*

**METHODS.** *In this non-randomized, controlled trial performed in a tertiary care center, eight patients with biopsy-proven acne rosacea and blepharitis, and ten patients with seborrheic blepharitis not associated with acne rosacea formed the study groups and ten normal patients were used as controls. Schirmer I test results, tear break-up time and radial immunodiffusion (Lactoplate™) tests on tear samples of the three groups were compared.*

**RESULTS.** *Compared to controls, tear lactoferrin concentrations were lower in both the acne rosacea and seborrheic blepharitis groups. However, this decrease was significant in only the seborrheic blepharitis group ( $p = 0.026$ ). One patient in the acne rosacea group, with the lowest measurement, developed bacterial keratitis.*

**CONCLUSIONS.** *Though within the normal range, tear lactoferrin levels in patients with acne rosacea and seborrheic blepharitis were lower than controls. Low concentrations of lactoferrin may play a role in the ocular surface inflammatory components associated with these diseases. (Eur J Ophthalmol 2000; 10: 11-4)*

**KEY WORDS:** *Acne rosacea, Blepharitis, Lactoferrin, Meibomitis*

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## INTRODUCTION

Acne rosacea is a chronic cutaneous disease encountered in 10% of the population, characterized by facial erythematous telangiectasias, papules, pustules and hypertrophy of the sebaceous glands (1). Ocular manifestations are present in 58% of patients, with meibomian dysfunction being the hallmark of the disorder (1, 2). The lipid composition of the meibomian secretion is altered in such a way that some cholesterol esters are transformed into cholesterol by bacterial esterase and serve as a substrate, facilitating the development of other bacteria (1). The fatty acids liberated by the bacterial lipase have a negative impact on the protective action of the tear film over the ocular surface (1).

Lactoferrin is a tear glycoprotein secreted by the acinar cells of the main and accessory lacrimal glands and constitutes almost 25% of proteins in human tears (3-5). Lactoferrin has an antibacterial effect by virtue of its avid iron binding capacity, and helps control the surface bacterial flora (4-7). Lactoferrin also had a strong inhibitory effect on the classical complement cascade, thus potentially regulating inflammatory reactions (4-7).

The aim of this study was to measure tear lactoferrin levels in patients with chronic diffuse meibomitis associated with acne rosacea and seborrheic blepharitis unrelated to acne rosacea, and to check for any relationship between the amount of lactoferrin and inflammatory ocular surface complications.

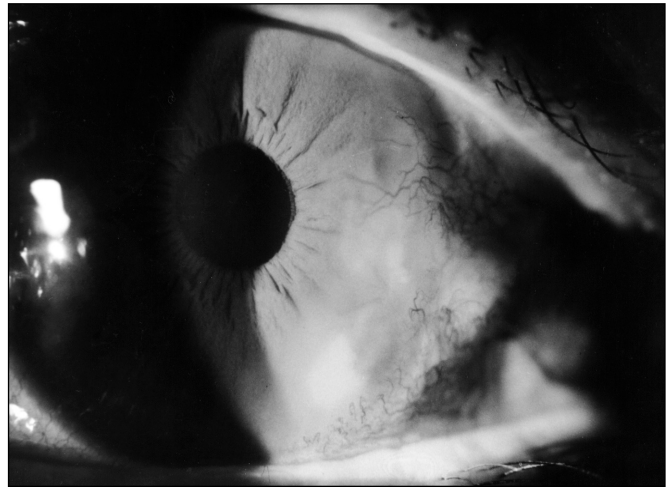
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**Fig. 1** - A typical patient with diffuse meibomitis in the acne rosacea group with crusty, thickened and erythematous upper eyelid margin.



**Fig. 2** - Patient 1 in the acne rosacea group, who had the lowest levels of tear lactoferrin, developed infectious keratitis in her right eye.

## MATERIALS AND METHODS

Eight patients with chronic diffuse primary meibomitis associated with cutaneous biopsy-proven acne rosacea (Fig. 1) and ten patients with anterior seborrheic blepharitis without any signs and symptoms of acne rosacea were consecutively recruited into the study. The McCulley chronic blepharitis classification system was used (2). Ten patients without any ocular diseases were selected as controls. The mean age in the acne rosacea group was 46 years (range 31-65) and 25.7 years (range 15-30) in the seborrheic blepharitis group. Females predominated in the acne rosacea group and males in the seborrheic blepharitis group.

Each patient underwent a thorough ophthalmic examination with special emphasis on the lid margins and ocular surface. The patients then had Schirmer I and tear break-up time (BUT) tests (8) and tear lactoferrin was determined by the radial immunodiffusion technique. The Schirmer I test was done using a commercially available Schirmer test strip with the eye closed. After 5 minutes, the results were recorded as the length (mm) of the wetted portion of the paper strip. Ten minutes later, 0.05% fluorescein was instilled on the conjunctiva and the tear BUT was observed.

Thirty minutes later, a filter paper disc 4 mm in diameter was placed on the temporal portion of the lower conjunctival fornix for five minutes. The disc was

then transferred to a Lactoplate™ (JDC, Culemborg, The Netherlands) which was kept at room temperature for 72 hours. The Lactoplate™ is composed of agarose gel with rabbit antiserum to human lactoferrin and 0.5% sodium azide added as preservative. The diameter of the precipitation ring on the plate was measured to calculate the concentration of tear lactoferrin (mg/ml) using the conversion table found in the package insert. Results within the range of  $1.5 \pm 0.4$  mg/ml were accepted as normal as suggested by the manufacturer. Readings below 1.1 mg/ml were considered definitely low (9).

Student's t-test was used for statistical analysis.

## RESULTS

The results of Schirmer I and tear BUT tests were normal in all patients (Tabs. I and II). The mean tear lactoferrin level was 1.63 mg/ml in the acne rosacea group compared to 1.88 mg/ml in the control group but this difference was not significant. One patient (No. 1, Tab. I) had bilaterally low levels of lactoferrin with bacterial keratitis in the right eye (Fig. 2).

In the seborrheic blepharitis group, mean tear lactoferrin was 1.26 mg/ml and the difference from the control group was significant ( $p = 0.026$ ). One patient had bilaterally low levels of lactoferrin but no ocular surface inflammation.

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**TABLE I - DATA ON PATIENTS WITH ACNE ROSACEA**

Patient	Sex	Age	Lactoferrin (mg/ml)		BUT (sec)		Schirmer (mm)	
			OD	OS	OD	OS	OD	OS
1	F	55	0.64	0.94	20	25	40	23
2	F	65	1.44	1.44	20	22	25	40
3	F	40	1.44	1.44	17	20	40	42
4	F	31	2.10	2.00	22	22	44	43
5	F	32	2.00	2.10	29	29	33	33
6	M	50	1.80	1.70	26	26	29	26
7	M	65	2.10	2.00	20	18	21	24
8	F	40	1.60	1.40	25	20	28	30

BUT= break-up time, OD = right eye, OS = left eye, F = female, M = male

**TABLE II - DATA ON PATIENTS WITH SEBORRHEIC BLEPHARITIS**

Patient	Sex	Age	Lactoferrin (mg/ml)		BUT (sec)		Schirmer (mm)	
			OD	OS	OD	OS	OD	OS
1	M	30	1.21	1.21	26	24	20	22
2	M	28	1.70	1.70	15	15	22	21
3	M	30	0.72	0.72	20	21	21	27
4	F	27	1.10	1.10	23	23	17	19
5	M	20	1.10	1.10	20	21	18	23
6	M	15	1.21	1.21	20	20	27	23
7	M	32	1.21	1.21	25	25	25	24
8	M	23	1.21	1.21	17	19	28	27
9	M	29	1.70	1.70	20	22	19	21
10	M	23	1.21	1.60	20	26	22	24

BUT= break-up time, OD = right eye, OS = left eye, F = female, M = male

## DISCUSSION

Lactoferrin secretion is encoded on chromosome 3 (3q21-q23). It has several important functions in the modulation of inflammatory response over the ocular surface (10). Lactoferrin inhibits C3 convertase formation and the deposition of C3 and C5 of the complement system and thus exerts antiinflammatory effects (11). Depending on the ocular surface environment, the amount of tear lactoferrin can be increased by cholinergic stimulation of the lacrimal gland (12). Exposure to ultraviolet irradiation may increase intracellular peroxide formation which is readily coun-

teracted by lactoferrin (13). Lactoferrin also has a protective effect against hydroxyl-induced insults resulting from oxidative stress (14).

Several methods quantitatively measure this key protein of the tear film. Currently, the radial immunodiffusion technique, ELISA-sodium dodecyl polyacrylamide gel electrophoresis, binding with lectins and high-pressure liquid chromatography are used (9, 15). The radial immunodiffusion test results were sometimes questioned since lactoferrin usually became bound to agar and agarose and this could lead to erroneous outcomes (6). Although this may be partly true, in a later study in which rocket electrophoresis and radial

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immunodiffusion techniques were compared, both tests produced consistent measurements (16). Our results are therefore comparable to those in the literature.

Tear lactoferrin levels are decreased in keratoconjunctivitis sicca, trachoma, bacterial, herpetic and other viral keratitis, and giant papillary conjunctivitis (17, 18). The underlying mechanisms of this decrease are gradually being clarified in a limited number of diseases. In Sjögren syndrome, the severity of lymphocytic infiltration into the lacrimal gland was related to the decrease of lactoferrin in tears (19). Also tear lactoferrin was significantly decreased starting from the first day after cataract surgery, which may in part account for the bacterial resistance or vulnerability of the eye during this critical period (20).

Our results suggest there is a mild depression of tear lactoferrin levels in patients with chronic meibomitis associated with acne rosacea and seborrheic blepharitis compared to normal controls. Although the significance of these findings has yet to be substantiated, tear lactoferrin might be one of the factors in the progression of inflammatory ocular surface changes associated with these chronic disorders.

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