

In Vivo Confocal Microscopy Evaluation of Meibomian Gland Dysfunction in Atopic-Keratoconjunctivitis Patients

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Purpose: To clarify meibomian gland (MG) alterations in atopic keratoconjunctivitis (AKC) patients and compare the findings with obstructive MG dysfunction (MGD) patients and control subjects using in vivo confocal microscopy (CM).

Design: Prospective, controlled, single-center study.

Participants: Twelve AKC patients (10 males, 2 females; mean age, 31.0 ± 16.5 years), 12 obstructive MGD patients (7 males, 5 females; mean age, 37.6 ± 5.6 years), and 26 control subjects (13 males, 13 females; mean age, 32.9 ± 5.7 years) were recruited. No significant age or gender differences were observed between the 3 groups.

Methods: All subjects underwent assessment of tear evaporation rate from the ocular surface (TEROS), slit-lamp examinations, tear break-up time (BUT) measurements, vital staining, Schirmer test I, meibography, MG expressibility, and CM examination of the MG (HRTII-RCM). Statistical analysis was performed using the Mann-Whitney test.

Main Outcome Measures: The MG acinar unit density, inflammatory cell density, MG acinar unit longest diameter, MG acinar unit shortest diameter, and MG acinar unit area as observed by in vivo CM, MG drop-out, MG expressibility grading, tear stability, tear evaporation, and vital staining scores.

Results: The TEROS values, mean BUT, vital staining scores, MG expressibility, and MG dropout grades were significantly worse in AKC patients compared with those in obstructive MGD patients and controls ($P < 0.05$). The mean values of the CM parameters in AKC patients were significantly worse than those observed in the obstructive MGD patients and controls ($P < 0.001$).

Conclusions: Changes in MG in AKC patients seem to be more severe than in patients with obstructive MGD and controls. In vivo CM is a noninvasive, efficient tool in the assessment of MG status and ocular surface disease in AKC.

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Atopic diseases represent an international health problem, with a 15.6% incidence in north Europe, an incidence of nearly 8% in Japan, and between 5% and 20% of the general population in the United States.^{1–6} Although atopy can present as allergic rhinitis, asthma, or allergic conjunctivitis, atopic keratoconjunctivitis (AKC) represents the most severe manifestation of the disease.⁷

A bilateral chronic hypersensitivity disease of the ocular surface, AKC is associated with systemic atopic dermatitis. The ocular complications in atopic dermatitis are increasing and account for 25% to 40% of patients with atopic dermatitis.^{8,9} Patients with AKC usually suffer from a wide variety of keratoconjunctival manifestations, including conjunctival papillary hypertrophy, cicatrizing conjunctivitis, superficial punctate keratitis, ulceration, eczema of the lids, and blepharitis.^{1,7} Several studies have been conducted to investigate the ocular surface disease in AKC patients; however, meibomian gland (MG) changes and the impact of such changes on ocular surface disease have not been reported so far.

Slit-lamp microscopy and meibography with or without infrared photography or videography have been reported to be useful in the assessment of MG status and function.^{10–14} Other studies showed that MG lipid secretions could be evaluated using meibometry directly from the eyelid.^{15,16} Confocal microscopy (CM) is an emerging imaging technique that can aid in the assessment of structural changes in several ocular surface diseases and anterior segment disorders at a cellular level.^{17–21}

We previously reported CM-based parameters to be invaluable tools for assessment and evaluation of ocular surface and MG status in patients with MG dysfunction (MGD).^{22,23} Parameters such as MG acinar unit density (MGAUD), inflammatory cell density (ICD), MG acinar longest diameter (MGALD), and MG acinar shortest diameter (MGASD) had an acceptable sensitivity and specificity in the diagnosis of MGD and correlated significantly with tear function tests, MG dropout, and meibum expression grades.²⁴ In this study, we investigated the applicability of CM in assessment of MG status in AKC patients. We also

investigated the MG changes in AKC patients by comparing the results with those of obstructive MGD patients and control subjects.

Materials and Methods

Subjects and Examination

Twelve right eyes of 12 AKC patients (10 males, 2 females; mean age, 31.0 ± 16.5 years), 12 right eyes of 12 obstructive MGD patients (7 males, 5 females; mean age, 37.6 ± 5.6 years), and 26 healthy control subjects (13 women and 13 men; mean age, 32.9 ± 5.7 years) were recruited for this prospective, single-center clinical study. There were no age or gender differences between the 3 groups. None of the subjects included in this study had a history of Stevens–Johnson syndrome; Sjögren syndrome; chemical, thermal, and radiation injury; or had undergone any ocular surgery or procedure that would create an ocular surface problem. No participant in this study had a history of systemic drug or contact lens use. During ocular examination, particular attention was paid to eyelid margins, tarsal and bulbar conjunctiva, and cornea. Assessment of MG status was observed by careful slit-lamp examination of the glandular orifices, mucocutaneous junction changes, and digital expression of the meibomian lipids. To express the meibomian lipids, the upper and lower eyelids were pressed gently. Meibum viscosity was graded as described by Bron et al.²⁵

The criteria for diagnosis of AKC patients included patients with symptoms of allergic conjunctivitis, such as ocular itching, redness, tearing, or ocular pain without seasonal aggravation, and presenting with conjunctival papillae and keratopathy. All AKC patients included in this study had active atopic dermatitis. Only patients with obstructive MGD who met all of the following criteria were included in this study: (1) occlusion of MG orifices at slit-lamp microscopy described as loss of circular concave formations at the lid margin^{14,25}; (2) cloudy or inspissated glandular secretion with lack of clear meibum secretion after the application of moderate digital pressure on the tarsus of the upper and lower eyelid¹⁴; (3) presence of keratinization or displacement of the mucocutaneous junction on slit-lamp examination^{25,26}; (4) absence of inflammatory lid disease such as blepharitis as well as inflammatory skin disorders such as atopic dermatitis, seborrhea sicca, and acne rosacea; cases with anterior blepharitis as well as posterior blepharitis with secondary meibomitis were excluded²⁷; (5) absence of a history of cicatricial eyelid and conjunctival diseases such as trachoma, erythema multiforme, ocular cicatricial pemphigoid, and chemical, thermal, or radiation injury; and (6) absence of excessive copious meibomian lipid secretion with mild digital pressure on slit-lamp microscopy (seborrheic MGD).²⁶ The study was conducted in compliance with the tenets of the Declaration of Helsinki and the examination procedures were board reviewed. Written informed consent was obtained from all subjects.

Both eyes of all subjects included in this study underwent tear evaporation rate measurements from the ocular surface (TEROS), slit-lamp examinations including tear film break-up time (BUT), fluorescein (FL) and Rose-Bengal (RB) staining of the ocular surface, Schirmer test² without anesthesia, in vivo laser CM, transillumination of the lids with a transilluminator (meibography), and MG expressibility test, in this respective order. We performed RB staining right after FL staining and before the Schirmer test. Taking into consideration the irritation effect of RB dye, which would affect the Schirmer testing, the dye was washed away in each patient after each staining session with nonpreserved artificial tears (Soft Santears, Santen Pharmaceuticals, Tokyo, Japan) and

then the patient was asked to wait for ≥ 30 minutes for the Schirmer test. Confocal microscopy, meibography, and MG expressibility tests were performed at the end so that expressed MG secretions would not affect the BUT.

Data from only the right eyes were analyzed by a researcher who was masked to whether the data belonged to patients or controls, according to the protocol of the current study.

Tear Film Evaporation Rate Measurement

We measured the TEROS with a quartz crystal humidity sensor (Kao Analytical Research Center, Tochigi, Japan).²⁸ The temperature and humidity of the examination room were maintained at a range from 20°C to 25°C and from 30% to 50%, respectively, during all examinations.

Tear Stability and Ocular Surface Staining Examinations

The standard tear film BUT measurement was performed after instillation of 2 μ L of 1% FL preservative-free solution in the conjunctival sac with a micropipette. Patients were instructed to blink several times for a few seconds to ensure adequate mixing of the dye. The interval between the last complete blink and appearance of the first corneal black spot in the stained tear film was measured 3 times, and the mean value of the measurements was calculated. A BUT value of < 5 seconds was considered abnormal.

For the assessment of FL staining, the cornea was divided into 3 equal upper, middle, and lower zones. Each zone had a staining score ranging between 0 and 3 points; the minimum and maximum total staining scores ranged between 0 and 9 points. Likewise, the presence of scarce staining in 1 zone was scored as 1 point, whereas punctate staining covering the entire zone was scored as 3 points. A score of > 3 points was regarded as abnormal. After assessment of BUT and FL staining, 1% RB dye was instilled into the conjunctival sac and scored according to the van Bijsterveld scoring system.

Briefly, the ocular surface was divided into 3 zones: nasal conjunctival, corneal, and temporal conjunctival areas. A staining score between 0 and 3 points was employed in each zone, with the minimum and maximum total staining scores ranging between 0 and 9 points. The presence of scarce punctate staining received 1 point. The presence of denser staining not covering the entire zone received 2 points, and the presence of RB staining over the entire zone received 3 points. A score of > 3 points was regarded as abnormal.

Tear Quantity Evaluation

A Schirmer test² without topical anesthesia was performed for further evaluation of tears. The sterilized strips of filter paper (Showa Yakuhin Kako Co. Ltd., Tokyo, Japan) were placed in the lateral canthus away from the cornea and left in place for 5 minutes. Readings were recorded in millimeters of wetting for 5 minutes. A value of < 5 mm was considered abnormal.

In Vivo Laser Confocal Microscopy Examination

All subjects were examined using in vivo laser CM, Heidelberg Retina Tomograph II-Rostock Cornea Module (Heidelberg Engineering GmbH, Dossenheim, Germany), as described previously.^{22,24} Briefly, after the eyelid was everted, the center of the Tomo-Cap was appanated onto the palpebral conjunctiva and the MG were scanned while moving the appanating lens from the lid margins toward the fornix with minute vertical movements. The

MG was also scanned while moving the applanating lens along the entire lid length with minute horizontal movements. None of the subjects complained of discomfort; no adverse effect was observed after an examination in this series. The CM examination performed by the same researcher on all study participants.

We measured the MGALD, MGASD, periglandular ICD, MGAUD, and MG acinar unit atrophy (MGAUA), to evaluate the morphologic changes in the MG, as described previously.²² The density of glandular acinar units and ICD were measured with the internal software. Clearly visible acinar units were all counted in a 400×400-μm frame, and the acinar density was described as the number of units per square. Within a 400×400-μm frame, MGALD and MGASD were assessed by measuring the longest and shortest axes of clearly visible acinar units, and MGAUA was assessed by drawing a line around the inner lumen of clearly visible acinar units. The MGALD and MGASD were measured in μm whereas MGAUA was measured in square micrometers using Image J software (Java software program developed by the National Institutes of Health, Bethesda, MD; available at: <http://rsb.info.nih.gov/ij/>; accessed December 20, 2011). Three randomized, nonoverlapping, high-quality digital images of the nasal, middle, and temporal lower eyelids (total of 9 images per eyelid) were used for calculation of the CM parameters.

Meibomian Gland Dropout Grades (Meibography)

Transillumination examination (meibography) was performed by using a transilluminator (Medicospot; MS-H, Neitz Instruments Co. Ltd., Tokyo, Japan). Transillumination of the MG through the lids was achieved using an illuminated probe placed on the skin of the eyelid, which was everted; the glandular morphology could be photographed along the entire length of the eyelid by the slit lamp, including the nasal, temporal, and central lid areas. This allowed quantification of the meibomian glandular rows and their loss. Loss of visible MG structure ("gland dropout") revealed by meibography was considered as evidence of MGD because this finding has been reported to be a good parameter for obstructive MGD-associated ocular surface disease.^{11,29} The degree of MG dropout was scored as described previously (Pflugfelder grading): grade 0, no gland dropout; grade 1, loss of ≤33% of the glandular structures; grade 2, loss of 34% to 66% of the glandular structures; grade 3, loss of ≥67% of the glandular structures.³⁰

Meibomian Gland Expressibility and Eyelid Assessment

After digital pressure application on the lid tarsus,^{10,29} meibum expression was evaluated semiquantitatively according to Bron's grading.²⁵ Additional evaluation included eyelid margin assessment (vascularity, hyperkeratinization, irregularity); assessment of glandular orifices (capping, degree of obliteration, narrowing, loss of cuffing definition, opaque/scarred, pouting); and quality of expressed secretions (clear, cloudy, peritubule, toothpaste).²⁵ Each finding was graded from 0 to 3 points.

Statistical Analysis

The Mann–Whitney test was applied to test the statistical differences between TEROS, BUT, vital staining, Schirmer test², the MG acinar unit longest and shortest diameters, density and area of MG acinar unit, and ICD values. The Mann–Whitney test was also used to study the differences between the FL staining score, RB staining score, and gender differences between the study groups. Graphpad Instat 3.0 (Graphpad Software, Inc., San Diego, CA) was used for the analyses.

Table 1. Comparison of Tear Evaporation Rates, Tear Function, and Ocular Surface Tests between Atopic-Keratoconjunctivitis, Obstructive Meibomian Gland Dysfunction Patients and Normal Controls

Examinations	AKC (n = 12)	Obstructive MGD (n = 12)	Controls (n = 21)
TEROS (10 ⁻⁷ g/cm ² /second)	9.8±5.0	7.4±2.7	3.3±1.5
BUT (seconds)	3.1±1.2	6.0±2.1	8.3±2.1
RB score (points)	4.5±2.0	1.4±0.7	0.1±0.2
FL score (points)	6.2±2.3	2.4±1.8	0.3±0.7
Schirmer test (mm)	12.6±6.9	15.1±8.5	14.0±7.7

BUT = tear break-up time measurements; FL = fluorescein staining; MGD = meibomian gland dysfunction; RB = Rose-Bengal staining; TEROS = tear evaporation rate from the ocular surface.

Note the elevated tear evaporation rates, tear stability and vital staining in Atopic-Keratoconjunctivitis (AKC) patients compared to the other groups. Schirmer test didn't show a statistical significant difference between the three groups.

*P value considered significant ($P<0.05$). Mann Whitney test; **P value considered very significant ($P<0.005$); ***P value considered extremely significant ($P<0.0001$).

Results

Tear Evaporation Rate Measurement

The mean tear evaporation rate in AKC patients was significantly higher than the control group ($P = 0.0002$; Table 1). There was no difference between the TEROS values in AKC patients compared with obstructive MGD patients ($P = 0.2317$; Table 1).

Tear Stability and Ocular Surface Staining Examinations

The mean tear stability (BUT) and ocular surface staining scores were significantly worse ($P<0.05$) in AKC patients compared with obstructive MGD patients and controls (Table 1).

Tear Quantity Evaluation

The mean Schirmer test value was not significantly different between the 3 groups included in this study ($P>0.05$; Table 1).

In Vivo Laser Confocal Microscopy Examination

The CM examination revealed extensive fibrotic changes in MG acinar units of AKC patients (Fig 1). The fibrosis in MG of AKC patients seemed to be more extensive than that observed in obstructive MGD patients. In vivo laser CM images disclosed morphologic alterations of MG in patients with AKC, including extensive periglandular inflammatory cell infiltration and decrease in size of MG acinar units (Fig 2A) compared with patients with obstructive MGD and control subjects (Fig 2B, C). The mean ICD was significantly higher (1192.0 ± 432.1 cells/mm²) in AKC patients than in obstructive MGD patients (712.9 ± 376.5 cells/mm²; $P = 0.0346$) and controls (60.6 ± 30.4 cells/mm²; $P<0.0001$; Fig 2). The mean MGAUD was 40.1 ± 10.8 U/mm² in AKC patients. This value was significantly lower than the mean values measured

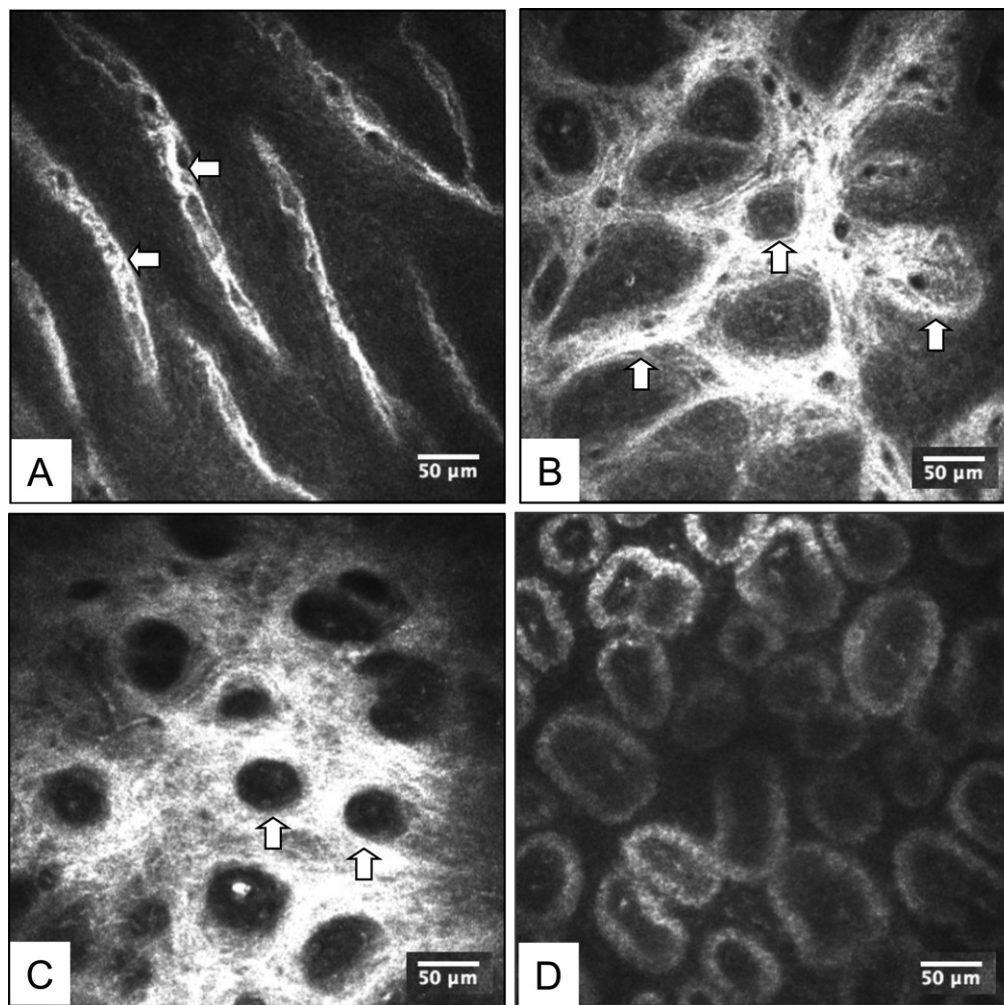


Figure 1. In vivo confocal microscopy images of meibomian gland (MG) changes in atopic keratoconjunctivitis patients compared to a normal control subject. **A**, Linear streaks of fibrosis of MG. **B**, Loss of MG architecture with extensive fibrotic tissue surrounding the atrophic remnants of MG. **C**, Dense fibrotic changes observed in MG and the nearby conjunctival tissues. White arrows point to areas of fibrosis in MG. **D**, Representative image of MG acinar units in a normal subject.

in obstructive MGD patients (70.3 ± 20.4 U/mm²; $P = 0.0017$) and normal controls (120.1 ± 38.6 U/mm²; $P < 0.0001$; Fig 3). The MGAUA mean value was significantly lower in AKC patients (646.0 ± 82.4 µm²) compared with patients with obstructive MGD (2119.5 ± 716.5 µm²) and controls (870.0 ± 191.8 µm²). Similarly, the MGALD mean value was significantly lower (43.8 ± 5.1 µm) in AKC patients than in MGD patients (98.9 ± 24.1 µm) and controls (59.9 ± 9.7 µm; $P < 0.0001$; Fig 4). The MGASD was also significantly shorter (17.4 ± 4.4 µm) in AKC patients than in obstructive MGD patients (34.9 ± 9.0 µm; $P = 0.0005$; Fig 4). There were no significant differences between the mean MGASD values of AKC patients and controls (17.9 ± 3.7 µm; $P = 0.9120$).

Meibomian Gland Dropout Grades (Meibography)

Patients with AKC had a significantly higher MG dropout grading than patients with obstructive MGD and controls ($P = 0.02$). Patients with MGD had, in turn, significantly higher grades of dropout compared with normal subjects ($P = 0.03$; Table 2).

Meibomian Gland Expressibility and Eyelid Evaluation

The MG expressibility grading was significantly worse in AKC patients compared with those in obstructive MGD patients and normal subjects ($P < 0.05$; Table 2). A similar significant increase in eyelid margin vascularity and hyperkeratinization grade was observed in AKC patients compared with obstructive MGD patients and controls (Table 2). The orifices of MG also showed a significant grade of obliteration, narrowing, and loss of cuffing in AKC patients (Table 2).

Discussion

The MGs secrete lipids onto the ocular surface. These lipids constitute the outer layer of the tear film, act as a barrier to cutaneous sebum to prevent contamination, prevent rapid evaporation of the tear film, facilitate lubrication to reduce friction from blinks on the ocular

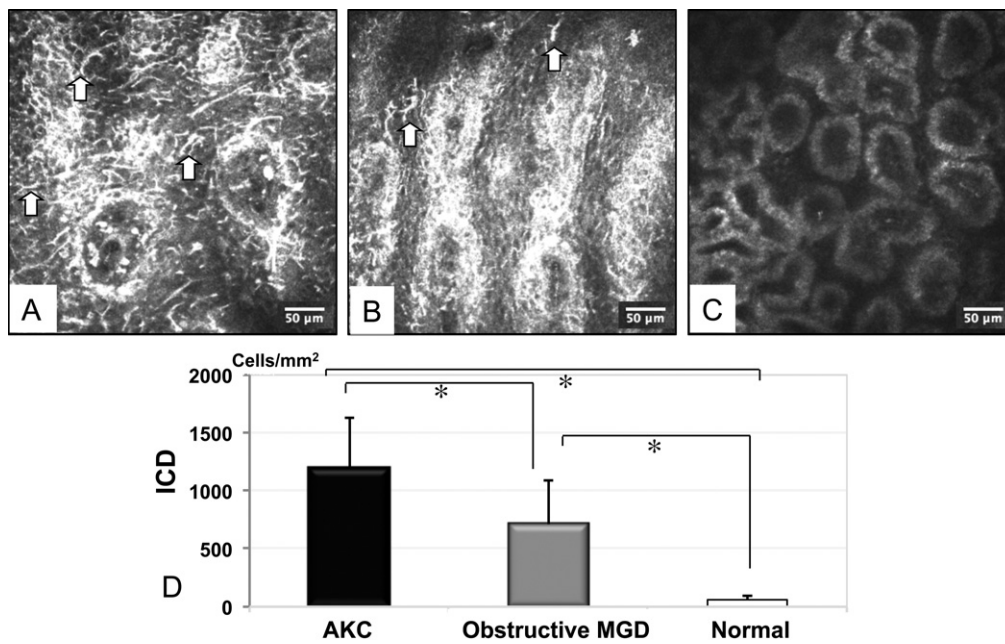


Figure 2. In vivo confocal microscopy (CM) representative images. **A**, Atopic keratoconjunctivitis (AKC) patient. **B**, Obstructive meibomian gland dysfunction (MGD) patient. **C**, Normal control subject. Note the more extensive periglandular inflammatory cell infiltration observed in AKC patients, as indicated by white arrows, compared with obstructive MGD patients. The conjunctival inflammatory cell density (ICD) assessed by in vivo CM was significantly higher in AKC and obstructive MGD compared with normal controls (**D**). * $P < 0.05$, Mann-Whitney test.

surface, and provide a smooth optical surface.³¹ Although several studies reported that AKC patients suffer from compromised tear film and ocular surface, there has been no report investigating the MG alterations and their impact on the ocular surface disease so far.

The recent technological progress in CM enabled us to investigate the pathophysiologic changes in the ocular surface in vivo and noninvasively. In vivo CM proved to be an invaluable clinical tool for assessment and evaluation of several pathologic conditions, including aqueous deficiency and evap-

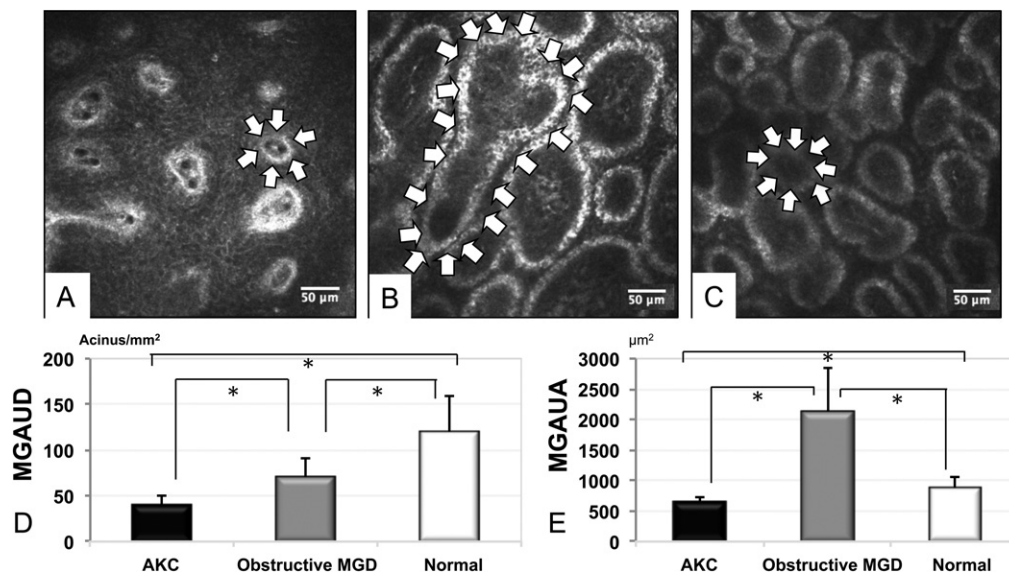


Figure 3. Comparison of confocal microscopy (CM) parameters; meibomian gland acinar unit density (MGAUD) and meibomian gland (MG) acinar unit area (MGAUA) between atopic keratoconjunctivitis (AKC) patients, obstructive MG dysfunction (MGD) patients, and normal controls. In vivo CM revealed shrinkage and decrease in size of the MGs, as shown in the representative image from an AKC patient (**A**). Enlargement of MG acinar units was observed in obstructive MGD patients (**B**) compared with normal controls (**C**). The mean MGAUD parameter value was significantly lower in AKC patients compared with its value in obstructive MGD patients and normal controls (**D**). The mean MGAUA parameter value was significantly lower in AKC patients compared with obstructive MGD patients and normal controls (**E**). The mean MGAUA parameter value was significantly larger in obstructive MGD patients compared with the controls. * $P < 0.05$, Mann-Whitney test.

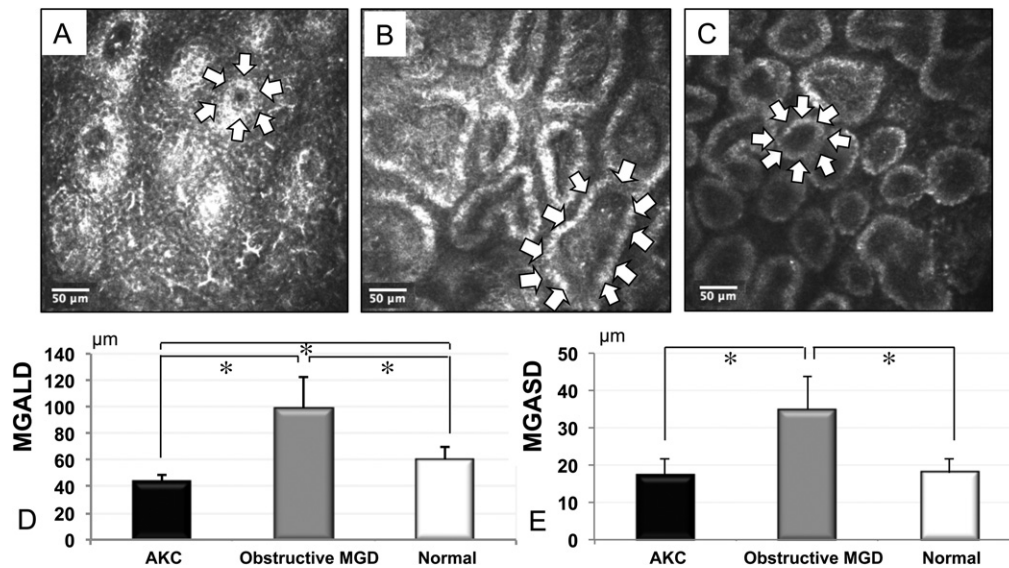


Figure 4. Comparison of in vivo confocal microscopy (CM) parameters; meibomian gland acinar unit longest diameter (MGALD) and meibomian gland acinar unit shortest diameter (MGASD) between atopic keratoconjunctivitis (AKC) patients, obstructive meibomian gland dysfunction (MGD) patients, and normal controls. **A**, Representative CM image for meibomian glands (MGs) in an AKC patient; note the decrease in MG acinar unit diameters. **B**, Representative CM image for MG in an obstructive MGD patient; note the increase in MG acinar unit diameters. **C**, A CM image of a normal subject showing numerous and compact MG acinar units. White arrows depict a typical acinar unit. (**D** and **E**) The mean MGALD and MGASD parameters were significantly shorter in AKC patients compared with obstructive MGD patients and normal controls. The same parameters were significantly larger in obstructive MGD patients compared with normal controls. * $P < 0.05$, Mann-Whitney test.

orative types of dry eye associated with primary Sjögren syndrome, acanthamoeba keratitis, and obstructive MGD.

We reported CM parameters such as acinar unit density and acinar unit diameter to be of value in understanding histopathologic changes like glandular atrophy and acinar/ductal dilatation in obstructive MGD.²² In addition, we also described periglandular ICD as another CM parameter that can differentiate inflammatory obstructive MGD from non-inflammatory subtypes. These parameters were character-

ized by high sensitivity and specificity in the diagnosis of MGD and correlated well with the ocular surface status.²⁴

In this study, we investigated the tear functions and MG alterations in AKC patients and compared the findings with those in obstructive MGD patients and control subjects. Although the tear quantity in AKC patients was not significantly lower than in obstructive MGD patients and controls, tear function tests, including BUT and vital staining, were significantly worse in AKC patients compared with obstructive MGD and control groups, in accordance with previous reports.³² Dogru et al^{2,33} reported the presence of disturbances in tear quality and quantity in AKC patients with a decrease in conjunctival goblet cell population. The tear film instability in those patients was attributed in part to disturbances in tear mucins resulting from decreased goblet cell density. Our observations in this study suggest that MG alterations in AKC patients may have aggravated the tear stability and ocular surface epithelial damage, possibly because of the changes in the lipid layer of the tear film, which may have resulted from a greater extent of MG dropout and lid margin changes in AKC.

In vivo CM examinations in this study revealed a severe degree of fibrosis and atrophy in the MG and a decrease in the size and density of the MG acinar units. Although the MGALD and MGASD in obstructive MGD patients were significantly larger than controls, as we previously reported, we observed significant decrease in the same parameters in the AKC patients. This can be attributed to the more severe degree of fibrosis and atrophy in the MGs of AKC patients.

A previous study by us and other studies showed that the tears of AKC patients harbor inflammatory cytokines,

Table 2. Comparison of Meibomian Gland Dropout, Expressibility and Eyelid Assessment between Atopic-Keratoconjunctivitis, Obstructive Meibomian Gland Dysfunction Patients and Normal Controls

Grade	AKC	Obstructive MGD	Controls
MG dropout	2.0±0.8	1.0±0.5	0.3±0.5
Eyelid margin	3.1±1.3	1.6±1.3	0.4±0.5
MG orifices	4.2±0.4	3.0±1.0	0.1±0.3
MG expressibility	2.3±0.4	1.4±0.5	0.1±0.3

Meibomian gland (MG) dropout and expressibility grades were significantly worse in Atopic-Keratoconjunctivitis (AKC) than in obstructive meibomian gland dysfunction (MGD) patients and normal controls. Each category of the MG and eyelid evaluation in AKC patients was significantly worse than the values measured in other groups. * P value considered significant ($P < 0.05$). Mann Whitney test.

including tumor necrosis factor- α , interleukin-4, and interleukin-5.^{34–37} These cytokines play an important role in the production and maintenance of conjunctival inflammation responses and subsequent conjunctival scarring in patients with AKC. Tumor necrosis factor- α and interleukin-4 are well known inducers of fibrosis in soft tissues.^{37–39}

We also reported MGAUA as a new CM parameter, which we believe to be a good parameter for the assessment of the process of acinar unit atrophy. The MGAUA values were significantly lesser in AKC patients compared with obstructive MGD and control subjects. We observed a significant increase of periglandular ICD in AKC patients compared with those in obstructive MGD and normal controls.

The CM parameters in AKC showed potential differences from those in patients with obstructive MGD. Contrary to the enlarged MG acinar units found in obstructive MGD patients, AKC patients had shrunken MGs, with extensive periglandular fibrosis. The mechanism of MGD in AKC patients has not been fully understood, although Arita et al⁴⁰ reported MG duct distortion and increased MG dropout in patients with perennial allergic conjunctivitis by infrared meibography.

We have previously reported the presence of extensive inflammatory cell infiltrates with stromal edema in the conjunctival papillae and conjunctiva of AKC patients by *in vivo* CM microscopy.^{41,42} Further studies at the cellular level showed an increase in inflammatory infiltrates and the presence of increased lipid oxidation in patients with AKC.³⁴

This study confirmed the presence of an ocular surface disease state, which was characterized by tear instability and significantly higher ocular surface vital staining scores in patients with AKC and obstructive MGD when compared with healthy subjects, indicating the important role of MG in maintaining the health and integrity of the ocular surface.

It was harder to express the MG secretions in the AKC patients of this study than obstructive MGD and controls, as evidenced by the significantly higher expressibility grades. Meibography showed significant glandular loss in the AKC patients compared with obstructive MGD patients and normal controls.

The mechanism of MGD in AKC patients could be explained through direct inflammatory damage to the cornea and conjunctiva due to elevated inflammatory status because of the presence of dermatitis, as well as through an indirect effect due to increases inflammatory cytokine and cells in the ocular surface of the AKC patients, leading to alterations in MG structure and function. We believe that MGD, in turn, leads to a deterioration in the quantity and quality of meibum, which may lead to increased tear film instability and aggravation of dry eye disease. Alteration in MGs may well be secondary to the AKC inflammation, as are the tear and surface changes—in parallel fashion rather than sequential causation.

Our study suggests that MG status in AKC patients requires careful attention from clinicians to avoid the disturbances in the tear film, which can increase the inflammatory status through a vicious circle. Future research looking into the differences in MG status between AKC and

vernal keratoconjunctivitis using *in vivo* CM and infrared meibography technologies will add immensely to the literature. In addition, investigating the tear osmolarity changes in the tear film of AKC patients can provide further information about mechanism of dry eye disease in AKC patients.

In summary, we have reported the first study to elucidate the alterations of MG morphology in AKC disease by *in vivo* CM. Our study revealed new evidence regarding the pathologic changes of MG in AKC and provided strong *in vivo* evidence for the role of inflammation in the ocular surface disease process. *In vivo* confocal scanning laser microscopy used in this study was an effective and a non-invasive method for elucidating the structural and functional changes of MG in AKC.

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