In Vivo Evaluation of Ocular Demodicosis Using Laser Scanning Confocal Microscopy

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PURPOSE. To investigate the applicability of in vivo laser scanning confocal microscopy in the diagnosis and follow-up of ocular demodicosis infestation in a prospective controlled study.

METHODS. Fifteen right eyes of 15 patients with blepharitis associated with cylindrical dandruff (10 males, 5 females; mean age: 62.9 ± 9 years) and eight right eyes of eight age- and sex-matched control subjects underwent HRT II/RCM, evaluation of ocular symptom scores, tear function tests including vital stainings, Schirmer test, and tear clearance test, and evaluation of mite numbers in the eyelids.

RESULTS. In vivo confocal microscopy effectively disclosed the mites in the terminal bulbs of the eyelashes, which were not observed after treatment. Eyelids with demodicosis infestation showed marked inflammatory infiltrates around the meibomian glands and conjunctiva, which cleared with tea tree oil treatment.

CONCLUSIONS. Laser scanning confocal microscopy seems to be an efficient noninvasive tool in the diagnosis and follow-up of ocular demodicosis infestation. (Invest Ophthalmol Vis Sci. 2011;52:565–569) DOI:10.1167/iovs.10-5477

The term “demodicosis” has been used to describe cutaneous diseases caused by Demodex mites, among which Demodex folliculorum and Demodex brevis are common commensals of the pilosebaceous units in human beings. The face, scalp, and upper chest are reportedly common sites for infestation. D. folliculorum is occasionally found in the follicular infundibulum, whereas D. brevis is most commonly encountered in sebaceous ducts and meibomian glands. Demodex mites may play a pathogenic role when they are in excessive numbers or penetrating into the dermis and in dermatology practices, they have been implicated in papulopustular rosacea, pityriasis folliculorum, rosacea-like demodicosis, demodicosis gravis (granulomatous rosacea-like demodicosis), and blepharitis.

Kheirkhah et al. provided strong evidence supporting the notion that blepharitis was frequently associated with mite-harboring cylindrical dandruff in eyelashes and that Demodex infestation in eyelashes also manifested trichiasis, meibomian gland dysfunction with lipid tear deficiency, and conjunctival inflammation, together with features of corneal disease at presentation that were serious enough to prompt the referring physician to suspect limbal stem cell deficiency.

The presence and density of Demodex mites can be studied by potassium hydroxide (KOH) preparations of follicular plugs, skin scrapings, and skin biopsy specimens. Kheirkhah et al. recently reported that addition of fluorescein solution after mounting further increased the proficiency of detecting and counting mites embedded in cylindrical dandruff of epilated eyelashes.

Confocal microscopy is a new emerging noninvasive technology for evaluating the tissue structure and cell phenotype in vivo, which is useful as a supplementary diagnostic tool for the assessment of the histopathological processes in many ocular surface diseases and anterior-segment disorders, including the in vivo examination of the cornea, bulbar and palpebral conjunctiva, and meibomian glands. The process has been reported to be useful in the investigation of the morphology of normal human corneas and pathologic alterations in dry eyes, diabetes, acanthamoeba keratitis, infectious corneal ulcers, herpetic keratitis, keratoconus, aging, contact lens wear, and refractive surgical procedures.

In this prospective controlled study, we investigated the applicability of in vivo laser scanning confocal microscopy in the diagnosis and follow-up of ocular demodicosis infestation.

PATIENTS AND METHODS

Fifteen right eyes of 15 patients with blepharitis associated with cylindrical dandruff (10 males, 5 females; mean age: 62.9 ± 9 years) and eight right eyes of eight age- and sex-matched control subjects were studied in this study. Subjects who were referred to us with chronic blepharitis associated with cylindrical dandruff not responding to a previous treatment consisting of infant shampoo lid scrubbing, topical steroids, and antibiotic eye drops for more than 8 weeks were included in this study. Those patients who had any history of Stevens-Johnson syndrome, chemical, thermal, or radiation injury, keratoconus, ocular or systemic disease including atopic keratoconjunctivitis, a history of ocular surgery, or contact lens or drug use that would alter the ocular surface were excluded. No patient was being treated with systemic steroids, prostaglandin inhibitors, or topical or systemic immunosuppressants at the time of inclusion in the study. A conventional slit-lamp microscopic examination was initially performed. The subjects then underwent tear function and ocular surface examinations, including tear film break-up time measurements, fluorescein and Rose Bengal staining, vital staining with trypan blue, and Schirmer test.

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Submitted for publication March 5, 2010; revised June 15, 2010; accepted August 1, 2010.

Disclosure: T. Kojima, None; R. Ishida, None; E.A. Sato, None; T. Kawakita, None; O.M.A. Ibrahim, None; Y. Matsumoto, None; M. Kaido, None; M. Dogru, None; K. Tsubota, None

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staining of the ocular surface, the Schirmer test I, and finally the confocal scanning laser microscopy of the entire eyelid. Confocal laser scanning examinations were done on the control subjects as well by the same investigator, who was masked to the diagnosis of the subjects.

The examination procedures were approved by the Institutional Review Board, and the study conformed to the ethical principles for research involving human subjects as outlined in the tenets of the Declaration of Helsinki. Informed consents were obtained from all subjects after receiving an explanation of the nature and possible consequences of taking part in the study.

**Assessment of Ocular Symptom Visual Analog Scale Scores**

Patients were asked about the symptoms of itching and foreign body sensation, and the intensity of symptoms was evaluated before and after the eye oil treatment using visual analog scales (VAS) scores. Absence of itching or foreign body sensation constituted a score of zero points on the visual analog pain scales, and the presence of intense, unbearable symptoms was considered a full pain score of 100 points. Briefly, the visual analog symptom scales were prepared as 10 cm lines, and the patients were asked to check a point on the line corresponding to their degree of symptoms.

**Ocular Surface Vital Staining**

The ocular surface was examined by fluorescein staining. Briefly, 2 μL of preservative-free 0.5% fluorescein dye was instilled in the conjunctival sac. The fluorescein staining was scored according to the protocol described by Shimmura et al. The cornea was divided into three equal areas of upper, middle, and inferior corneal compartments. Each compartment was graded on a scale of zero (no staining) to three points (intense staining). A fluorescein staining score above one point was considered abnormal (maximum: nine points).

**Tear Quantity Evaluation**

To evaluate the tear quantity, a Schirmer test without anesthesia was performed. Briefly, the sterilized Schirmer strip (Showa Yakuhin Kako Co., Tokyo, Japan) was placed in the lateral conjunctival fornix for 5 minutes. The length of the wet portion was measured. A value of <5 mm was considered abnormal.

**In Vivo Laser Scanning Confocal Microscopy**

In vivo laser confocal microscopy was performed on all subjects with a new-generation confocal microscope (Rostock Corneal Software Version 1.2 of the HRT-II-RCM; Heidelberg Retina Tomograph II Rostock Cornea Module, Heidelberg Engineering, Dossenheim, Germany). After topical anesthesia with 0.4% oxybuprocaine, the subject’s chin was placed on the chin rest. The objective of the microscope was an immersion lens covered by a polymethylmethacrylate cap (Tomo-Cap; Heidelberg Engineering). Comfort gel (Bausch & Lomb GmbH, Berlin, Germany) was used as a coupling agent between the applanating lens and the corneal surface. After an examiner asked the patient to look downward, the center of the polymethylmethacrylate cap was appplicated onto the eyelid margins by adjusting the controller, and the digital images of the underlying eyelash bulbs of three central eyelashes, palpebral conjunctiva, and meibomian glands were observed on the computer screen. These eyelashes were also marked before confocal examination on the eyelid so that the same eyelashes could be removed for light microscopy examination. When the first superficial epithelial cells were visualized, the digital micrometer gauge was set at zero, and then by pressing on the foot pedal, sequence images were recorded while gradually moving the focal plane into the subepithelial tissue. The eyelids were scanned all along while moving the applanating lens from the nasal toward the temporal lid margin with minute horizontal movements. Ten sequences each containing 100 frames were taken in each eye, and ten non-overlapping frames with the best resolution were selected from each sequence. The length of a single confocal microscopy examination session was approximately 10 minutes. None of the subjects complained of discomfort, nor was any adverse effect observed after an examination in this series.

**Light Microscopic Evaluation of Ocular Demodicosis**

Ocular demodicosis was confirmed by microscopic examination of epilated, previously marked lashes according to a modified method recently reported by Gao et al. Briefly, under a slit-lamp biomicroscope at a magnification of ×25, three central lashes with cylindrical dandruff from the superior eyelid were removed by fine forceps, placed separately on each end of a glass slide, and mounted with a separate coverslip. The number of mites was counted immediately, and the Demodex count was recorded as the mean number of mites/lash and compared after tea tree oil treatment using the Wilcoxon test. Weekly lid scrubs with 50% tea tree oil and daily lid scrubs with tea tree shampoo also were advised for the patients for a minimum of 6 weeks, according to a regimen recently reported by Gao et al. but the healthy control subjects were not given any treatment. The medical records, including the history of present illness, complete eye examination results, and external photographs, also were reviewed for the first visits and subsequent follow-up visit to compare the changes in subjective symptoms, objective ocular surface signs documented by external photography, and Demodex count.

**Statistical Analyses**

A Wilcoxon test was performed to analyze the differences in symptom scores and tear function examinations between subjects and controls before and after treatment with tea tree oil. Age and sex differences were studied by χ² analysis. A P value of <0.05 was considered statistically significant (Instat software for Macintosh; GraphPad Software, La Jolla, CA).

**Results**

All patients in whom both in vivo confocal microscopy and light microscopy examinations revealed *Demodex* infestation had cylindrical dandruff of the eyelashes. The dandruff observed before treatment cleared up with 6 weeks of tea tree oil treatment. All patients had notable conjunctival inflammation and eyelid margin injection at presentation as evidenced by redness involving bulbar areas and tarsal areas. All patients in this series had meibomian gland dysfunction, defined by cloudy secretions and poor expression of the meibum on digital expression. None of the control subjects were observed to have dandruff on their eyelashes.

**Ocular Symptom VAS Scores**

The mean VAS scores for itchiness and foreign body sensation were significantly higher in patients with demodicosis infestation before treatment compared with posttreatment scores and VAS scores of healthy control subjects (P < 0.05). The mean VAS scores for itchiness and foreign body sensation decreased significantly after 6 weeks of treatment (P < 0.05; Table 1).

**Ocular Surface Vital Staining**

The mean fluorescein staining score was significantly higher in patients with demodicosis infestation before treatment compared with posttreatment scores and scores of healthy control subjects (P < 0.05). The mean fluorescein staining score decreased significantly after 6 weeks of treatment in patients with demodicosis infestation (P < 0.05; Table 1).
Control subjects

Demodex patients before treatment
Demodex patients after treatment
Control subjects

Itchiness

Foreign Body

Fluorescein

VAS

Sensation

Staining

Score

Score

Score

92 ± 2.5

6.5 ± 6

3.0 ± 1.5

15 ± 5.5

1.0 ± 1.0

0.5 ± 0.5

1.0 ± 1.0

0.5 ± 1.0

0.25 ± 0.15

VAS, visual analog scale.

Tear Quantity Results

Schirmer test results were above 10 mm in all patients with demodicosis and healthy control subjects. There were no significant differences in Schirmer test values between patients and controls as well as the Schirmer test values before and after treatment in patients (data not shown).

In Vivo Confocal Microscopy Evaluation of Demodicosis Infestation

In vivo confocal microscopy examination effectively disclosed the mites in all patients with cylindrical eyelash dandruff. Representative in vivo confocal microscopy images of the eyelash bulbs in a 72-year-old female patient with demodicosis and a healthy female control subject are shown in Figure 1. In vivo confocal microscopy examination showed consistent dilatation of meibomian gland acinar units surrounding the infested eyelashes with periglandular inflammatory infiltrates mainly consisting of dendritic cells in all patients with demodicosis. Similarly, the palpebral conjunctiva adjacent to the eyelid margin showed marked inflammatory infiltrates in all patients. The acinar dilatation appeared to improve with tea tree oil treatment together with resolution of periglandular and conjunctival inflammatory cell infiltrates. Representative in vivo confocal microscopy images of the meibomian gland acinar units before and after treatment, as well as the palpebral conjunctiva in the same patient shown in Figure 1A, are shown in Figures 1C–F. The anterior segment photograph of the eyelid, in vivo confocal microscopy, and light microscopy images of a central lash in another 68-year-old female patient with demodicosis before treatment are shown in Figures 2A, 2C, and 2E, respectively. Posttreatment anterior segment photograph of the eyelid, in vivo confocal microscopy, and light microscopy images of a central lash are shown in Figures 2B, 2D, and 2F, respectively.

The mean mite count/lash was observed to decrease significantly with treatment in both in vivo confocal microscopy and light microscopy examinations ($P < 0.05$; Table 2). Although the mean mite count/lash tended to be higher in confocal microscopy examinations when compared with the mite counts in light microscopy, there were no statistically significant differences in mean mite counts between the two methodologies ($P > 0.05$; Table 2).

**DISCUSSION**

The *Demodex* is a microscopic, elongated mite that is a very common parasite, with only *D. folliculorum* and *D. brevis* found in the human skin.\(^5\) The mite has a head-neck part and a body-tail part, with four pairs of stumpy legs. The adult *D. folliculorum* has a length of 0.35–0.4 mm and is found in small hair follicles. *D. brevis* is 0.15–0.2 mm long and lives embedded in the sebaceous glands. Both species appear to coexist in the same skin area and especially tend to gather in the forehead, nose, cheeks, and external ear tract.\(^5\)

Previous ophthalmological studies showed *D. folliculorum* to be attached to the lash follicle, whereas *D. brevis* appears to be embedded deep into the lash’s sebaceous gland and the meibomian glands.\(^5\)

Several pathogenic mechanisms have been proposed for demodicosis, including (1) blockage of hair follicles and sebaceous ducts by the mites or the reactive hyperkeratosis, (2) stimulation of the host’s humoral and cell-mediated immune reactions by the mites and their waste products, (3) a foreign body granulomatous reaction to the mite’s chitinous skeleton, and (4) a vector role for bacteria.\(^1\)\(^4\)\(^5\)\(^3\)

Recently, Lacey et al.\(^3\)\(^4\) reported that antigenic proteins related to *Bacillus oleronius* isolated from *D. folliculorum* mites are capable of stimulating an inflammatory response in

**FIGURES 1.** (A, B) Representative in vivo confocal microscopy images of the eyelash bulbs in a 72-year-old female patient with demodicosis and a healthy female control subject. Note the heavy *Demodex* infestation of the eyelash in the patient and the absence of mites in the control subject. (C–F) Representative in vivo confocal microscopy images of the meibomian gland acinar units before and after treatment as well as the palpebral conjunctiva in the same patient shown in (A). Note the dilatation of meibomian gland acinar units surrounding the infested eyelashes with periglandular inflammatory infiltrates mainly consisting of dendritic cells (C). Similarly, the palpebral conjunctiva adjacent to the eyelid margin showed marked inflammatory infiltrates (E). The acinar dilatation appeared to improve with tea tree oil treatment together with resolution of periglandular and conjunctival inflammatory cell infiltrates (D, F).
patients with papulopustular rosacea. In relation to ocular inflammation, Kheirkhah et al.\textsuperscript{35} described clinical features of inflammation including blepharitis, meibomitis, bulbar and palpebral conjunctival injection, and a wide spectrum of corneal lesions, including superficial corneal vascularization, marginal corneal infiltration phlyctenule-like lesions, and nodular corneal scars. Akilov and Mumcuoglu\textsuperscript{36} showed that an increasing density of the mites was associated with an increasing trend of perifollicular inflammation and clinical manifestations.\textsuperscript{3} A density of $>$5 mites/follicle or 5 mites/cm\textsuperscript{2} of skin biopsy specimens has been considered to be pathogenic.\textsuperscript{2,3} Until now, the density of Demodex mites has been traditionally studied by KOH preparations of follicular plugs, skin scrapings, and skin biopsy specimens in dermatology literature and light microscopic examination of epilated eyelashes in ophthalmology.\textsuperscript{14,15} Kheirkhah et al.\textsuperscript{36} suggested among other methods that fluorescein dye staining improved microscopic evaluation and counting of Demodex in blepharitis with cylindrical dandruff. We tried to find out whether in vivo confocal microscopy would be effective in the diagnosis and follow-up of the eyelid disease in patients with blepharitis associated with cylindrical dandruff. Our results suggested that this technology not only effectively disclosed the mites embedded in the bulbs but also provided additional useful information on the meibomian gland/conjunctival disease, the features of which were acinar dilatation, periglandular inflammation, and conjunctival inflammatory infiltrates. In vivo confocal microscopy was also helpful in following the course of the eyelid-meibomian-conjunctival disease with tea tree oil treatment revealing resolution of inflammatory cells, resolution of acinar dilatation, and clearance of the mites with an obvious and significant decrease in mean mite counts in the eyelashes. Further improvements in relation to resolution of this new technology might allow us to visualize the eggs of the novice mites and tailor our treatment strategies accordingly. Likewise, future studies on larger populations establishing the cutoff value for mite density that causes clinically significant ocular surface disease and studies looking into the correlation between eyelid mite density assessed by confocal microscopy and corneal epithelial and meibomian gland disease severity will provide invaluable information. Although statistically insignificant, it was of interest that the mean mite counts in confocal microscopy were higher than the counts obtained by light microscopy, which may be owing to the in vivo examination of the mites, while the mites could be lost during epilation or coverslipping for light microscopy examination. Some patients may experience pain during epilation for light microscopy evaluation of the eyelashes. The painless nature of in vivo confocal microscopy examination compared with epilation may be another advantage of this technology. On the other hand, it is sometimes quite difficult to show the mites embedded in dandruff attached to the midportion of the eyelashes because of lack of stability of the examination background.\textsuperscript{56} We tried to find out whether in vivo confocal microscopy where mites embedded within the dandruff can be visualized with ease ex vivo when epilated and coverslipped with this technology. Finally, in accordance with data coming mostly from studies by Gao et al.,\textsuperscript{13,29} we found 50% tea tree oil treatment to be effective based on our in vivo observations on the improvement of meibomian gland disease, eyelid inflammatory status, and clearance of the mites from the base of the eyelashes. In conclusion, we found laser scanning confocal microscopy to be an efficient noninvasive approach.

![Figure 2](image_url)

**FIGURE 2.** (A, B) Representative anterior segment photographs of the eyelid in a 68-year-old female patient with demodiconis before and after treatment. Note the clearance of dandruff and resolution of eyelid injection. (C, D) In vivo confocal microscopy images of a central lash before and after treatment. Note the disappearance of the mites with tea tree oil treatment. (E, F) Light microscopy images of epilated central lashes before and after treatment. Note the disappearance of the mites with tea tree oil treatment.

| Table 2. Mean Mite Counts in Eye Lashes Assessed by Confocal and Light Microscopy before and after Tea Tree Oil Treatment |
|---------------------------------|---------------------------------|
| Mean Mite Count/Lash before Treatment | Mean Mite Count/Lash after Treatment |
| Confocal microscopy | 4.0 ± 0.5 | 0.5 ± 0.5 |
| Light microscopy | 3.0 ± 1.0 | 0.5 ± 0.5 |

Previous histopathology studies in the dermatology literature revealed dense perivascular and perifollicular infiltrates, often with abundant neutrophils and occasionally with multinucleated histiocytes, excessive Demodex mites in follicular infundibula, and infundibular pustules containing mites in perifollicular inflammatory infiltrates.\textsuperscript{56} In a study by Vollmer,\textsuperscript{39} 83% of the follicles with mites showed inflammation. It has been suggested that an increasing density of mites correlated with an increasing perifollicular inflammation and clinical manifestations.\textsuperscript{3} A density of $>$5 mites/follicle or 5 mites/cm\textsuperscript{2} of skin biopsy specimens has been considered to be pathogenic.\textsuperscript{2,3} Until now, the density of Demodex mites has been traditionally studied by KOH preparations of follicular plugs, skin scrapings, and skin biopsy specimens in dermatology literature and light microscopic examination of epilated eyelashes in ophthalmology.\textsuperscript{14,15} Kheirkhah et al.\textsuperscript{36} suggested among other methods that fluorescein dye staining improved microscopic evaluation and counting of Demodex in blepharitis with cylindrical dandruff. We tried to find out whether in vivo confocal microscopy would be effective in the diagnosis and follow-up of the eyelid disease in patients with blepharitis associated with cylindrical dandruff. Our results suggested that this technology not only effectively disclosed the mites embedded in the bulbs but also provided additional useful information on the meibomian gland/conjunctival disease, the features of which were acinar dilatation, periglandular inflammation, and conjunctival inflammatory infiltrates. In vivo confocal microscopy was also helpful in following the course of the eyelid-meibomian-conjunctival disease with tea tree oil treatment revealing resolution of inflammatory cells, resolution of acinar dilatation, and clearance of the mites with an obvious and significant decrease in mean mite counts in the eyelashes. Further improvements in relation to resolution of this new technology might allow us to visualize the eggs of the novice mites and tailor our treatment strategies accordingly. Likewise, future studies on larger populations establishing the cutoff value for mite density that causes clinically significant ocular surface disease and studies looking into the correlation between eyelid mite density assessed by confocal microscopy and corneal epithelial and meibomian gland disease severity will provide invaluable information. Although statistically insignificant, it was of interest that the mean mite counts in confocal microscopy were higher than the counts obtained by light microscopy, which may be owing to the in vivo examination of the mites, while the mites could be lost during epilation or coverslipping for light microscopy examination. Some patients may experience pain during epilation for light microscopy evaluation of the eyelashes. The painless nature of in vivo confocal microscopy examination compared with epilation may be another advantage of this technology. On the other hand, it is sometimes quite difficult to show the mites embedded in dandruff attached to the midportion of the eyelashes because of lack of stability of the examination background.\textsuperscript{56} We tried to find out whether in vivo confocal microscopy where mites embedded within the dandruff can be visualized with ease ex vivo when epilated and coverslipped with this technology. Finally, in accordance with data coming mostly from studies by Gao et al.,\textsuperscript{13,29} we found 50% tea tree oil treatment to be effective based on our in vivo observations on the improvement of meibomian gland disease, eyelid inflammatory status, and clearance of the mites from the base of the eyelashes. In conclusion, we found laser scanning confocal microscopy to be an efficient noninvasive approach.

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tool in the diagnosis and follow-up of ocular demodicosis infestation.

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