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 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 breakup-time measurements, fluorescein and Rose Bengal
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 tea tree 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 Shimura 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 (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 Cornea Software Version 1.2 of the HRTII-RCM; Heidelberg Retina Tomograph II- Rostock Cornea Module, Heidelberg Engineering, Dossenheim, Germany). After topical anesthesia with 0.4% oxybuprocaine, the subject's chin was positioned onto the eyelid margins by adjusting the controller, and the ocular surface. After an examiner asked the patient to look downward, the center of the polymethylmethacrylate cap was approximated to the eyelid margin 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).
Table 1. Ocular Symptom Visual Analog Scale Scores and Fluorescein Staining Scores in Control Subjects as well as Patients with Demodicosis Infestation before and after Treatment

<table>
<thead>
<tr>
<th></th>
<th>Itchiness VAS Score</th>
<th>Foreign Body Sensation VAS Score</th>
<th>Fluorescein Staining Score</th>
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<tr>
<td>Demodex patients</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>before treatment</td>
<td>92 ± 2.5</td>
<td>96.5 ± 0.6</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>after treatment</td>
<td>15 ± 5.5</td>
<td>1.0 ± 1.0</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1.0 ± 1.0</td>
<td>0.5 ± 1.0</td>
<td>0.25 ± 0.15</td>
</tr>
</tbody>
</table>

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 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. *D. brevis* has a length of 0.35–0.4 mm and is found in small folliculorum embedded deep into the lash follicle, whereas *D. brevis* appears to be attached to the lash follicle. Both species appear to coexist in the same skin area and especially tend to gather in the forehead, face, nose, cheeks, and external ear tract. Previous ophthalmological studies showed *D. folliculorum* to be a common parasite, with only *D. brevis* appearing to be embedded deep into the lash’s sebaceous gland and the meibomian glands.

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. Recently, Lacey et al. reported that antigenic proteins related to *Bacillus oleronius* isolated from *D. folliculorum* mites are capable of stimulating an inflammatory response in demodicosis.
Although our study could not provide information on heavily infested, or when the mites penetrate into the dermal tissue. It has been suggested that an increasing density of mites correlated with an increasing perifollicular inflammation and clinical manifestations. A density of >5 mites/follicle or 5 mites/cm² of skin biopsy specimens has been considered to be pathogenic. 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. Kheirkhah et al. 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 in 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., 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 technique.

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. In a study by Vollmer, 83% of the follicles with mites showed inflammation. Confocal microscopy 4.0 ± 0.5 * 0.5 ± 0.5

### TABLE 2. Mean Mite Counts in Eye Lashes Assessed by Confocal and Light Microscopy before and after Tea Tree Oil Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Mite Count/ Lash before</th>
<th>Mean Mite Count/ Lash after</th>
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<tbody>
<tr>
<td>Confocal microscopy</td>
<td>4.0 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Light microscopy</td>
<td>3.0 ± 1.0</td>
<td>0.5 ± 0.5</td>
</tr>
</tbody>
</table>
tool in the diagnosis and follow-up of ocular demodicosis infestation.

References