Demodex Mite Infestation and its Associations with Tear Film and Ocular Surface Parameters in Patients with Ocular Discomfort

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• PURPOSE: **The presence of Demodex species can be associated with blepharitis. Their pathogenic potential in meibomian gland dysfunction is discussed herein. The purpose of this study was to determine the prevalence of Demodex mites in eyelashes of Austrian patients with ocular discomfort and to evaluate associated changes of the lid margins and meibomian glands.**

• DESIGN: **This is a case-control study.**

• METHODS: **Two hundred twenty-nine consecutive patients with ocular discomfort from an Austrian dry eye clinic were investigated for the presence of Demodex mites on sampled eyelashes. Associations of a mite infestation with individual dry eye and lid parameters were assessed. Lid margins were evaluated for scales, vascularization, Marx line, expressibility and quality of meibum, and drop-out of meibomian glands.**

• RESULTS: **Demodex mites were identified in 40.2% of patients suffering from ocular discomfort (mean mite count 3.3 ± 2.9 per patient). Infestation with mites was associated with the presence of significantly more cylindrical scales (sleeves), a higher Marx line score, and a lower quality of meibum compared with mite-free patients. There were no significant associations with the expressibility and the drop-out of meibomian glands.**

• CONCLUSION: **The prevalence of Demodex mites in patients with ocular discomfort is high. The mean mite count per patient in this Austrian dry eye unit population is lower compared with previously published data from Asian regions. The infestation of the eyelids with Demodex species is associated with changes of the anterior and posterior lid margin, suggesting a pathogenic role in blepharitis and meibomian gland dysfunction. (Am J Ophthalmol 2019;204:7–12. © 2019 Elsevier Inc. All rights reserved.)**

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MITES SUCH AS *DEMODEX BREVIS* AND *DEMODEX folliculorum* are among the most frequent permanent ectoparasites of humans, who are often the only hosts. The 150- to 350-μm long parasites are primarily found on the eyelids and nose, usually in the openings of the hair follicles, but also within meibomian glands. They penetrate cell membranes and feed on the cytoplasm and the surrounding secretions.[1–6]

D folliculorum was discovered in humans as early as 1841; in 1875, the mite was found in the excretory duct of a meibomian gland (MG) for the first time.[7] Despite the early description, its pathogenicity remained controversial. *D folliculorum* has been identified as a vector for different kinds of bacteria, fungi, and viruses in the development of diseases.[1,8] *D brevis* has especially been associated with the development of chalazia because it usually resides deep within sebaceous glands.[9,10]

These mites are also attributed to the development of MG dysfunction (MGD) and anterior blepharitis (AB).[7,11] Inflammation of the eyelids caused by a Demodex infestation is usually characterized by redness and encrustation of the lid margins, viscous and yellowish discharge, the loss of eyelashes, and the formation of sleeves, as well as increased pigmentation around the roots of the remaining cilia. Higher numbers of mites in the follicles of the eyelashes and in the MGs are often associated with a reactive conjunctivitis and keratitis. The subjective symptoms are mostly similar to those of dry eye disease but are frequently accompanied by an increased feeling of burning and itching in the area of the lid margins.[4]

Dry eye disorders are a group of diseases resulting from certain pathophysiological pathways, e.g., hyperosmolarity, inflammation, and reduced tear volume and/or function, as well as disturbances of the eyelids. Ocular symptoms triggered by dry eye provide a broad clinical spectrum ranging from mild transient to persistent irritation, such as burning, itching, redness, pain, ocular fatigue, and visual disturbances.[10–15]

We assessed the prevalence and grade of Demodex infestations of the eyelids of patients suffering from ocular discomfort in an Austrian dry eye clinic population. Associations of a mite infestation with individual dry eye parameters, especially lid parameters, were evaluated.

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METHODS

LOCAL INSTITUTIONAL REVIEW BOARD APPROVAL WAS OBtained for this case-control study to analyze the records of patients with symptoms of ocular discomfort, such as dryness, foreign body sensation, burning, increased sensitivity to light, sensation of pressure, and frequent blinking, in patients who either showed a mite infestation (cases) or those who did not (control subjects). All patients were investigated at the dry eye unit of the Department of Ophthalmology at the Medical University of Graz, Graz, Austria, between 2011 and 2012. The research followed the tenets of the Declaration of Helsinki. Mentally impaired patients, supervised patients, and patients who had undergone ocular surgery in the previous 6 months were excluded.

Objective tear film and ocular surface parameters were analyzed, including an evaluation of the lid margins, noncontact infrared meibography, and an assessment of subjective symptoms.

A grading of lid margin parallel conjunctival folds was performed (score 0-4).[12]

Fluorescein tear film break-up time was determined after the application of dye into the tear film by a fluorescein strip (Haag-Streit Diagnostics, Köniz, Switzerland) moistened with physiological saline. The patient was instructed to blink a few times and then to keep their eyes open. Fluorescein tear film break-up time was always assessed in the right eye first. The precorneal tear film was observed at 10-fold magnification using a slit lamp with cobalt blue illumination. By a stopwatch, the time until the break-up of the tear film was measured 3 times and the mean time was documented.[13]

Subsequently, the extent of fluorescein staining of the cornea was reported using an area and density (score 0-3).[13]

The Schirmer test was carried out without prior application of a local anesthetic. Filter paper strips (Clement Clark International Ltd., Harlow, United Kingdom) were bent at the notch and hooked over the lateral lower lid margin for 5 minutes, with the patient instructed to keep the eyes gently closed. The wetting length of the filter paper strip was read from the calibrated scale in millimeters and was documented. Diagnosis of aqueous tear deficiency (ATD) was defined by Schirmer test values ≤5 mm in at least 1 eye. Sjögren syndrome was diagnosed according to the American-European Consensus Group.[14]

Further evaluation of the ocular surface was performed by lissamine green staining. The dye was always applied in the same way. The lissamine green strips (HUB Pharmaceuticals, Rancho Cucamonga, CA, USA) were moistened with 1 drop of physiological saline and dye was introduced into the lower conjunctival sac. Staining of the nasal, central, and temporal third of the ocular surface was scored according to van Bijsterveld: all 3 regions each having a scale of 0 to 3 points and the results added, resulting in a maximum total score of 9 points per eye.[15]

Lissamine green also stains the so-called Marx line (ML). It is usually located on the conjunctival side of the orifices of the MGs. In MGD and AB, the ML may be totally or partially located on the cutaneous side of the orifices of the MGs. A score was assigned for the outer third, the middle third, and the inner third of the upper and lower lids, respectively. According to Yamaguchi and associates,[16] the scores for the 3 portions were summarized and defined as the total score of each lid; the maximum possible ML score was 9 per lid.

Symptom intensity was assessed using a visual analogue scale (VAS) from 0-100 mm, where 0 represents no complaints and 100 represents the maximum amount of discomfort. In addition, symptom severity was assessed by the Ocular Surface Disease Index (OSDI) questionnaire. Patients were asked to grade the average intensity of their dry eye symptoms within the last week.[17]

Patients presenting with telangiectasia, erythema, and irregularity of the lid margins and a shifting of the openings of the MGs together with changes in the expressibility and quality of the meibum, e.g., waxy secretion or no secretion at all, or plugging of the orifices were diagnosed with MGD.[18] Expressibility and quality of the meibum were evaluated after applying pressure to the skin of the middle of the lower and upper lid (the 10 central MGs) with a cotton tip (score 0-3).[18–20]

Patients were diagnosed with blepharitis anterior (AB) in case of erythema of the lid margin, the presence of squamous debris and/or crusts at the cilia, and inflammation anterior to the grey line. The crusts were differentiated into cylindrical dandruffs (sleeves) and other scales (flakes and collarettes).

Noncontact meibography was performed with a Heidelberg Retina Angiography camera (Heidelberg Engineering, Heidelberg, Germany). The drop-out of meibomian glands was graded according to Arita and associates.[21] The scores of the lower and upper eyelid were added (score 0-6).

To check for a possible allergic disposition, smears from the inferior fornix were taken and investigated after Giemsa staining.

For the determination of a possible infestation of the eyelids with mites, in addition to slit lamp biomicroscopy, 4 eyelashes per eyelid were epilated and subsequently examined by light microscopy. One eyelash was epilated from every quadrant of the according eyelid. As proposed by Gao and associates,[22] lashes presenting with cylindrical dandruff (sleeves) were preferred. Fluorescein dye was added onto the slide to increase visibility. A possible Demodex infestation was documented in the patient records, together with the number of mites whether they were dead or alive and the number of larvae or eggs present.

The presence of systemic diseases (arterial hypertension, diabetes, and skin disease) was recorded.

The diagnostic tests were performed in the following chronological order: assessment of subjective symptoms

TABLE 1. Patient Characteristics and Diagnoses for Demodex Mite Infested, Noninfested, and Total Patients

	Total Patients (n = 229)	Mite-Infested Patients (n = 92)	Noninfested Patients (n = 137)	*P* Value
Age (y), mean (SD)	56.3 (15.8)	59.2 (14.9)	54.4 (16.1)	.026[a]
Female, n (%)	172 (75.1)	68 (73.9)	104 (75.9)	.757
VAS, mean (SD)	53.51 (22.2)	52.49 (22.5)	54.17 (22.1)	.510
OSDI, mean (SD)	47.36 (22.7)	43.52 (21.4)	49.83 (23.2)	.035[a]
SS, n (%)	47 (20.5)	15 (16.3)	32 (23.4)	.243
ATD, n (%)	120 (52.4)	49 (53.3)	71 (51.8)	.893
MGD, n (%)	210 (91.7)	85 (92.4)	125 (91.2)	.812
AB, n (%)	51 (22.3)	31 (33.7)	20 (14.6)	.001[a]
Allergic disposition, n (%)	40 (19.1)	18 (22.0)	22 (17.3)	.472

P value of Mann-Whitney *U* test for continuous parameters and of χ^2 test for categorical parameters. Because of some missing values, the statistics for VAS are based on 203 patients, OSDI on 207 patients, and allergic disposition on 209 patients.

AB = anterior blepharitis; ATD = aqueous tear deficiency; MGD = meibomian gland dysfunction; VAS = visual analog scale; OSDI = Ocular Surface Disease Index; SD = standard deviation; SS = Sjögren syndrome.

[a]Statistically significant differences between mite-infested and noninfested patients.

(VAS and OSDI), slit lamp examination, lid margin parallel conjunctival folds, fluorescein tear film break-up time, corneal fluorescein staining, Schirmer test, lissamine green staining, lid margin evaluation (including ML score, meibum expressibility and quality, and documentation of crusts at the cilia), noncontact meibography, conjunctival smear, and epilation of eyelashes for microscopic evaluation.

Continuous parameters are described as appropriate either as means and standard deviations in parentheses or as medians with range (minimum to maximum). Categorical parameters are reported as frequencies (with percentage). Differences in categorical parameters are assessed with exact χ^2 tests and for continuous parameters we used the Mann-Whitney *U* test. Odds ratios (ORs) with 95% confidence intervals (CIs) derived from multivariable logistic regression are used to describe the odds of a Demodex infestation among the diagnosis groups (ATD, SS, MGD, and AB) adjusted for age and sex.

$P < .05$ was considered statistically significant. All computations were performed with SPSS software (releases 19.0.0 [2010] and 25.0.0.1 [2017]; IBM Corp., Armonk, NY, USA).

RESULTS

THE RECORDS OF 229 CONSECUTIVE PATIENTS WITH DRY EYE symptoms who were investigated at the dry eye unit of the ophthalmological department, Medical University of Graz, Austria, between 2011 and 2012 were analyzed. The mean age was 56.3 ± 15.8 years and 172 (75.1%) were female. Demodex species were found in 92 patients (40.2%) suffering from ocular discomfort. Details of patient characteristics for Demodex infested, mite-free, and total patients are shown in Table 1.

Patients with Demodex infestation were significantly older (59.2 ± 14.9 years) than patients who were negative for a mite infestation (54.4 ± 16.1 years; $P = .026$). There was no difference in the occurrence of Demodex species between males and females.

Patients showed a high level of discomfort with a mean VAS value of (53.5 ± 22.2) with no difference among infested and noninfested patients. However, mite-free patients reported higher OSDI values (49.8 ± 23.2) than mite-infested patients (43.5 ± 21.4; $P = .035$). The proportion of Giemsa staining was comparable in infested and noninfested patients (18 [22.0%] and 22 [17.3%], respectively).

Diagnosis of AB was significantly more frequent in mite-infested patients (31 [33.7%]) than in noninfested patients (20 [14.6%]; $P = .001$). Sjögren syndrome was diagnosed more frequently in noninfested patients (32 [23.4%]) than in mite-infested patients (15 [16.3%]), but this difference was not statistically significant. ATD and MGD were diagnosed in 120 (52.4%) and in 210 (91.7%) patients of the total study group, respectively, and were equally distributed in Demodex infested and noninfested patients.

In terms of mean mite count, there was no statistically significant difference between the diagnoses, although it was highest within patients diagnosed with AB (ATD 3.2 ± 3.1 [0.2/lash], Sjögren syndrome 3.7 ± 2.6 [0.2/lash], MGD 3.3 ± 2.9 [0.2/lash], and AB 4.0 ± 3.8 [0.25/lash]).

Table 2 shows the results of the analysis of the specified tear film, ocular surface, and lid parameters in the mite-infested, noninfested, and total patient groups. Information on the prevalence of the different kinds of scales (crusts) is given. The frequencies of sleeves (cylindrical dandruff) and other scales (flakes and collarettes), as well as of all scales together (sleeves, flakes and collarettes), are displayed.

TABLE 2. Tear Film, Ocular Surface, and Lid Parameters of Demodex Mite Infested, Noninfested, and Total Patients

	Total Patients (n = 229)	Mite-Infested Patients (n = 92)	Noninfested Patients (n = 137)	*P* Value
Sleeves, n (%)	35 (20.8)	27 (41.5)	8 (7.8)	<.001[a]
Other scales, n (%)	78 (44.8)	39 (54.9)	39 (37.9)	.030[a]
All scales, n (%)	90 (51.7)	50 (70.4)	40 (38.8)	<.001[a]
Lid-parallel conjunctival folds (score 0-4), median (range)	2.0 (0-4.0)	2.0 (0-3.5)	2.25 (.5-4.0)	.981
Fluorescein staining (score 0-3), median (range)	.25 (0-3.0)	.25 (0-3.0)	.25 (0-3.0)	.979
Fluorescein break-up time (seconds), median (range)	3.5 (0-14.9)	3.35 (0.5-12.3)	3.7 (0-14.9)	.971
Schirmer (mm), median (range)	7.5 (0-40.0)	7.0 (0-35.0)	8.0 (0-40.0)	.646
Lissamine green staining (score 0-9), median (range)	1.5 (0-8.6)	1.5 (0-8.5)	1.5 (0-8.6)	.878
Lid margin vascularization (score 0-3), median (range)	1.5 (0-3.0)	1.5 (0-3.0)	1.5 (0-3.0)	.109
MG secrete quality (score 0-3), median (range)	1.0 (0-3.0)	1.5 (0-3.0)	1.0 (0-3.0)	.018[a]
MG expressibility (score 0-3), median (range)	1.5 (0-3.0)	1.5 (0-3.0)	1.5 (0-3.0)	.943
Marx line (score 0-3), median (range)	3.75 (0-9.0)	4.5 (0-9.0)	3.4 (0-9.0)	.016[a]
MG drop-out (score 0-6), median (range)	2.0 (0-6.0)	2.0 (0-6.0)	2.0 (0-6.0)	.687

P value of Mann-Whitney *U* test for continuous parameters and of χ^2 test for categorical parameters. Because of some missing values, the statistics for lid-parallel conjunctival folds is based on 187 patients, lid margin vascularization on 209, all scales on 174, other scales on 174, MG secrete quality on 169, sleeves on 168, and MG drop-out on 129 patients. All other parameters have fewer than 10 patients with missing data.

MG = meibomian gland.

[a]Statistically significant differences between mite-infested and noninfested patients.

Within infested patients, the mean mite count was 3.3 ± 2.9 per patient. *D folliculorum* was the main species found in epilated lashes. It was detected in 91 of 92 patients (98.9%) with positive ocular Demodex infestation. In contrast, *D brevis* was found in 1 of 92 patients (1.1%). Compared with the noninfested group, patients with Demodex mite infestation had significantly more scales formed as sleeves ($P < .001$), more other scales ($P = .030$) and more scales in total ($P < .001$), a higher ML score ($P = .016$), and a lower quality of MG secretion ($P = .018$). No significant association was observed with lid margin vascularization, expressibility, and drop-out of MG, as well as other tear film and ocular surface parameters (Table 2).

A multivariable logistic regression to predict mite infestation by diagnosis groups (ATD, SS, MGD, and AB) controlled for age and sex revealed that patients with diagnosis AB show a threefold risk for mite infestation (OR 3.03 [95% CI 1.54-5.93]). No other diagnosis nor sex shows a statistically significant influence on infestation. However, the risk for a mite infestation increases 2.6% with each year of age (OR 1.026 [95% CI 1.01-1.05]).

DISCUSSION

IN THE PRESENT STUDY, THE MEDICAL RECORDS OF 229 consecutive patients suffering from ocular discomfort were analyzed retrospectively at a dry eye unit in Austria. A high prevalence of Demodex mites in Caucasian populations has been reported.[23,24] To the best of our knowledge, we are the first to report the prevalence and grade of Demodex infestations of the eyelids and their associations with individual dry eye and lid parameters in Caucasians. Demodex species were found in 92 patients (40.2%) of this heterogenous study population. Wesolowska and associates[23] found a prevalence of 41%, whereas Sędzikowska and associates[24] report a prevalence of 47% within their Caucasian population. The comparison of the prevalence of an infestation with Demodex mites to other studies remains difficult because distinct populations with different ages were studied so far. Furthermore, the methods for lash sampling and mite counting vary within the current literature. This might affect the sensitivity in the detection of the mites of the published studies.[25–28] We decided to apply the methods for the detection of Demodex mites as proposed by Gao and associates[22] for the routine diagnostic at our dry eye unit.

The mean age of the study population was 56.3 ± 15.8 years. Our data confirm previous findings that the prevalence of a mite infestation increases with age.[23,24] Patients positive for the presence of Demodex mites were older than the mite-free patients.

Most of our study population was female (75.1%). This is consistent with the higher prevalence of dry eye disease in females, which is well-known.[29] Within our study population there was no difference in the occurrence of Demodex species between the sexes.

The presence of Demodex mites was associated with changes of the anterior lid margin such as an increased scale intensity and more cylindrical dandruff or sleeves, explaining the significant association to the diagnosis of AB. Sleeves are especially pathognomonic for the presence

of Demodex mites, whereas other crusts, like flakes and collarettes, are mainly associated with AB itself. Collarettes are thought to be small pieces of a bacterial biofilm adhering to the lashes, which seem to be pulled of the lid margin as the lashes grow.[30,31] Patients with the diagnosis of AB showed a threefold risk for a mite infestation (OR 3.03). In these terms our results corroborate previous studies.[25–27]

Demodex mites were also associated with the changes of the posterior lid margin, such as a higher ML score and a low MG secretion quality, which implies a role in MGD although the presence of Demodex was not statistically significant associated to the diagnosis MGD within this study population. No significant associations were found with meibomian gland expressibility and drop-out, which might be related to the low number of *D brevis* within the patients infested by Demodex mites.[25]

We almost exclusively identified *D folliculorum* on the lashes sampled. Only 1 of 92 patients (1.1%) was found to be infested by *D brevis*. Therefore, the question arises whether there is a difference in the prevalence of *D brevis* between Asians and Caucasians or if this was related to our cohort being older than other study cohorts. According to Liang and associates,[10] *D brevis* has often been found in younger patients suffering from recurrent hordeola and chalazia.[10] A significant correlation between MGD and keratitis with ocular demodicosis has been reported.[25] The lack of patients infested by *D brevis* might be the reason why we did not find any association between a Demodex infestation and the diagnosis MGD. In addition, one must keep in mind that the identification of *D brevis* may be difficult, and it is important not to confuse it with shorter *D folliculorum* species. Short mites that are present together with *D folliculorum* eggs are likely to be *D folliculorum*, not *D brevis*.[3]

There were no significant associations with the other tear film and ocular surface parameters and the diagnoses of ATD, Sjögren syndrome, and MGD themselves. Concerning systemic diseases, we could not observe any association with arterial hypertension, diabetes, or skin disease. This is especially interesting because skin diseases are known to be associated with the presence of AB.[32]

Within this study population the mean mite count was 3.3 ± 2.9 mites, resulting in only 0.2 mites per epilated lash. Liang and colleagues[25] found a higher mite count, up to 5.6 ± 3.5 (0.35 mites per epilated lash). The number of mites was found to increase with age, corroborating the work of Wesolowska and associates.[23] Although our analysis did not reveal statistically significant differences between dry eye diagnoses, the mean mite count was highest within patients with AB.

Concerning subjective symptoms, surprisingly, the OSDI scores were statistically significantly lower in patients infested by Demodex mites compared with noninfested patients suffering from ocular discomfort. This may be because OSDI questions specifically target the ocular surface more than lid disease. Within the OSDI questionnaire there is not a single question asking for lid problems, e.g. reddened, itchy, or encrusted lid margins. Further studies should use a questionnaire that might be more suitable to assess subjective symptoms of lid disease. There was no significant difference in VAS between infested and mite-free patients with dry eyes.

A possible limitation of our study is that patients with mite infestation were compared with other patients suffering from ocular discomfort. No comparison with healthy people without complaints or normal tear film and ocular surface parameters were made. This might be a reason why some of the parameters were not different between the groups. Furthermore, we find it difficult to point out which associations are causal and which are secondary.

Our data suggest that Demodex mites are associated with changes of the anterior and posterior lid margin and therefore may play a pathogenic role in AB and MGD. They seem to be a widespread problem among patients who have ocular discomfort. Therefore, we recommend examining the eyelashes for Demodex, especially in patients with cylindrical dandruff, and advise lid hygiene and a special treatment by Demodex lid scrubs with tea tree oil or the use of special cleaning wipes containing terpineol.[33,34]

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