

The association between *demodex* infestation and ocular surface manifestations in meibomian gland dysfunction

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Abstract

• **AIM:** To investigate the association between ocular *demodex folliculorum* infestation and ocular surface manifestations in meibomian gland dysfunction (MGD).

• **METHODS:** Eight-six patients with MGD were enrolled. All enrolled subjects were tested in the following sequence: ocular surface disease index (OSDI), slit-lamp biomicroscope examination, corneal surface regularity index (SRI) and surface asymmetry index (SAI), tear fluid collection, fluorescein tear film break-up time (F-BUT), corneal fluorescein staining (CFS), Schirmer I test (SIT) and *demodex folliculorum* counting. Tear matrix metalloproteinase (MMP)-9 activity was assessed using MMP-9 activity assay kit.

• **RESULTS:** Among 86 MGD patients, 40 were positive for *demodex*. The ocular *demodex*-positive group showed significantly increased scores of OSDI (25.96 ± 13.74 vs 18.07 ± 11.55 , $P=0.01$), lid margin abnormality (2.38 ± 0.87 vs 1.98 ± 0.91 , $P=0.04$) and CFS (1.28 ± 2.00 vs 0.94 ± 1.36 , $P=0.01$) compared to the ocular *demodex*-free group. The tear MMP-9 activity was higher in the ocular *demodex*-positive group (102.9 ± 32.4 ng/mL) than the ocular *demodex*-free group (46.2 ± 19.2 ng/mL, $P=0.03$). There was no significant difference in meibum quality and expressibility, SRI, SAI, F-BUT and SIT between the two groups ($P>0.05$ for each). No significant correlation was noted between the number of *demodex* and ocular surface parameters in *demodex*-positive MGD group ($P>0.05$ for each).

• **CONCLUSION:** Ocular *demodex folliculorum* infestation may be associated with ocular discomfort and ocular surface damage in MGD.

• **KEYWORDS:** *demodex*; meibomian gland dysfunction; ocular surface

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INTRODUCTION

Meibomian gland dysfunction (MGD) is a chronic disorder of the meibomian gland usually due to compromised quality/quantity of glandular secretion and obstructive terminal duct^[1]. MGD appears to affect the well-being and activities of routine life. The prevalence rate of MGD has been reported to be 3.5% to almost 70% worldwide^[2-3]. Persistent MGD can lead to various clinical conditions such as altered tear film stability, dry eye symptoms, inflammation and ocular surface damage^[1,4].

Demodex mites are microscopic ectoparasites harbour the human skin. Humans are infested by two distinct species namely: *demodex folliculorum* and *demodex brevis*. *Demodex folliculorum* infest the eyelash follicles, and *demodex brevis* are capable of burrowing deep into sebaceous glands and meibomian gland. *Demodex* is not only involved in the etiology of blepharitis, but also in other ocular surface manifestations such as superficial corneal neovascularization, marginal corneal infiltration, phlyctenule-like lesions, superficial corneal opacity, and nodular corneal scars, mainly in patients with ocular rosacea^[5-9]. Until now, the role of *demodex* in the etiology of MGD related ocular surface damage remains elusive.

The purpose of this study was to investigate the relationship between ocular *demodex folliculorum* infestation and ocular surface manifestations in MGD.

SUBJECTS AND METHODS

Subjects The present study was performed according to the Helsinki Declaration of Human Studies and was approved by the Ethics Committee of He's Eye Hospital. A total of 86 MGD patients were recruited from July 12, 2014 to January 15, 2015. MGD was diagnosed as previously reported, in brief^[10]: 1) presence of ocular symptoms; 2) at least one lid margin abnormality including irregular lid margin, vascular engorgement, plugged meibomian gland orifices and anterior or posterior displacement of the mucocutaneous junction; and 3) impaired meibum expression. Ocular symptoms were

determined by ocular surface disease index (OSDI), and subject with a score of >12 was considered abnormal. All participants were diagnosed as MGD at the Clinic of Shenyang He Eye Hospital. The exclusion criteria were: infectious keratitis or conjunctivitis, intraocular surgery within three months, ocular trauma within 6mo, and history of ocular burn, pterygium and serious systematic diseases.

Experimental Procedure All enrolled subjects were assessed in the following sequence: OSDI, slit-lamp biomicroscope examination, corneal surface regularity index (SRI) and surface asymmetry index (SAI), tear fluid collection, fluorescein tear film break-up time (F-BUT), corneal fluorescein staining (CFS), Schirmer I test (SIT) with anesthetic and finally *demodex folliculorum* counting. All examinations were assessed for one eye of each subject randomly.

Slit-lamp Biomicroscope Examination Lid margin abnormality observations include: lid margin irregularity, plugging of the meibomian orifices, lid margin vascular engorgement, and anterior or posterior replacement of mucocutaneous junction, giving a score of 0 to 4 as previously reported^[11]. Meibum quality: each of the 8 glands of the upper and lower eyelid was graded on a scale from 0 to 3 as follows^[11]: grade 0, clear; grade 1, cloudy; grade 2, cloudy with granular debris; grade 3, thick, like toothpaste. The scores of the 8 glands were summed to obtain a total score (maximum score for each eye, 24). Meibum expressibility: the degree of meibum expressibility was assessed using firm digital pressure applied over central 5 upper lid glands or lower lid glands as follows^[11]: grade 0, all glands expressible; grade 1, 3-4 glands expressible; grade 2, 1-2 glands expressible; grade 3, no glands expressible. The scores of the 5 glands were summed to obtain a total score (maximum score for each eye, 15).

Videokeratoscopy Videokeratoscopic examination (TMS-4; Japan) was performed to test corneal SRI and SAI. The patients placed the head against the support rest of the topograph, and look straight ahead. The subject was then asked to make a complete blink and subsequently to keep the eyes open. At the same time, acquisition of video images was initiated. The measurement was repeated for thrice, and the mean value was calculated.

Sampling and Counting Demodex Counting of parasite was performed as described previously^[5]. In brief, under a slit lamp microscope, two lashes, one from each half of each lid, were removed by fine forceps and placed separately on each end of glass slides. Total 20 μ L of 100% alcohol (If the eyelash covered with dandruff, 70% glycerin was used) was added and mounted with coverslip. After 10min, the samples were analysed under a light microscope. The *demodex folliculorum* mites were recognized according to its morphology and peculiar movement.

Tear Fluid Collection Tears was collected as previously reported^[12]. Briefly, tears was collected with a 0.5- μ L glass capillary tube (Drummond Scientific, Broomall, PA, USA) from the inferior tear meniscus of each eye. Tear samples from both eyes (1 μ L total) were eluted into a sterile tube containing 9 μ L of PBS and 0.1% bovine serum albumin. The tubes were immediately stored at -80°C .

Tear Matrix Metalloproteinase-9 Activity Matrix metalloproteinase (MMP)-9 enzyme activity was tested as previously reported^[12]. MMP-9 enzyme activity was tested with a MMP activity assay kit (Biotrak; Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's protocol.

Statistical Analysis The data was analyzed using SPSS 17.0 (Inc., Chicago, Illinois, USA). Data was expressed as mean \pm standard deviations. Except of OSDI and CFS scores in which the Mann-Whitney *U* test was used, unpaired *t*-tests were performed to determine the differences of variables between the two groups. $P < 0.05$ was considered significant.

RESULTS

Prevalence of Ocular Demodex Folliculorum Mites in Meibomian Gland Dysfunction Patients Ocular *demodex folliculorum* mite was found in 40 of 86 MGD patients (46.5%). The number of mite count was 2.4 \pm 0.33/8 lashes. No significant difference was found in age ($P=0.46$) or gender ($P=0.82$) between the ocular *demodex folliculorum*-positive group (26 females and 14 males; aged 36.5 \pm 9.6y) and ocular *demodex folliculorum*-negative group (28 females and 18 males; aged 38.2 \pm 11.2y).

Ocular Surface Manifestation of Demodex Folliculorum Infection in Meibomian Gland Dysfunction Statistical difference was noted in OSDI, lid margin abnormality and CFS between the two groups ($P < 0.05$ for each; Table 1). Based on the data of OSDI, the power is 0.83 under the type I error rate of 0.05. No significant difference was found in meibum quality and expressibility, SRI, SAI, F-BUT, and SIT between the two groups ($P > 0.05$ for each; Table 2).

Tear Matrix Metalloproteinase-9 Activity In *demodex folliculorum*-positive group, the level of tear MMP-9 activity was significantly increased compared with *demodex folliculorum*-negative group (102.9 \pm 32.4 ng/mL versus 46.2 \pm 19.2 ng/mL, $P=0.03$). The level of tear MMP-9 activity in *demodex folliculorum*-positive group was significantly higher than that in *demodex*-free group ($P=0.03$).

The Association Between the Number of Demodex and Ocular Surface Parameters in Demodex Folliculorum-positive Meibomian Gland Dysfunction No significant correlation was noted between the number of *demodex* and ocular surface parameters in *demodex*-positive MGD group ($P > 0.05$ for each). No significant correlation was noted between the number of *demodex* and the level of tear MMP-9 activity in *demodex*-positive MGD group ($P > 0.05$; Table 2).

Table 1 Ocular surface manifestation of *demodex* infection in MGD

Parameters	<i>Demodex</i> positive (n=40)	<i>Demodex</i> negative (n=46)	P
OSDI	25.96±13.74	18.07±11.55	0.01
Lid margin abnormality	2.38±0.87	1.98±0.91	0.04
Meibum quality			
Upper eyelid	12.55±4.14	12.43±5.00	0.91
Lower eyelid	11.88±4.83	11.78±4.92	0.93
Meibum expressibility			
Upper eyelid	1.03±0.89	0.74±1.08	0.19
Lower eyelid	1.18±1.06	0.80±1.11	0.12
SRI	0.27±0.20	0.32±0.20	0.29
SAI	0.32±0.25	0.33±0.19	0.28
F-BUT (s)	2.93±2.10	3.26±2.42	0.50
CFS	1.28±2.00	0.94±1.36	0.01
SIT (mm)	6.45±5.01	6.74±5.26	0.80

Table 2 The association between the number of *demodex* and ocular surface parameters in *demodex*-positive MGD

Parameters	R	P
OSDI	0.03	0.88
Lid margin abnormality	0.13	0.42
Meibum quality		
Upper eyelid	0.11	0.50
Lower eyelid	0.06	0.73
Meibum expressibility		
Upper eyelid	0.14	0.38
Lower eyelid	0.13	0.43
SRI	0.07	0.68
SAI	0.35	0.23
F-BUT (s)	-0.16	0.31
CFS	0.09	0.60
SIT (mm)	-0.01	0.97
Tear MMP-9 activity (ng/mL)	0.14	0.42

DISCUSSION

Though the role of ocular *demodex* infestation in the etiology of blepharitis has been debated since decades, it has been reported the incidence of *demodex* infestation is relatively higher in blepharitis patients compared to the patients without ophthalmic diseases^[13]. Studies have also proved that the corneal and conjunctival pathologies were significantly reduced after *demodex* eradication^[6]. In the present study, ocular *demodex folliculorum* infestation in MGD patients is related to ocular discomfort, lid margin abnormality and corneal epithelial barrier disruption in MGD. The evidence above mentioned, taken together, implied that *demodex folliculorum* may be related to ocular surface disorders.

In this study, the lid margin abnormality was more severe in *demodex*-infested MGD patients than in *demodex*-negative MGD patients. Though the definite mechanism of lid margin disorders exacerbated by *demodex folliculorum* infestation remain elusive, we speculate that the host inflammatory

responses elicited by *demodex* mites in lid margin could be the possible mechanism. Studies have proved that *demodex folliculorum* infestation can directly damage the epithelial cells of the eye lash follicle and are capable to induce delayed type hypersensitivity in the host meibomian gland, lid margin and ocular surface^[6-8]. Indeed, lid margin inflammation shown by crusting and reddening of lid margin has been noted in the *demodex*-infected patients^[9]. In addition, the association between lid margin inflammation and facial rosacea also suggests that *demodex* infestation may result in lid margin inflammation which eventually results in exacerbated MGD^[9]. Lee *et al*^[14] has shown that the number of *demodex folliculorum* was proportional to the severity levels of ocular discomfort and the OSDI score. Similarly, in our study, the OSDI was higher in the *demodex folliculorum*-positive MGD patients compared to *demodex folliculorum*-negative MGD patients. Taken together, these evidences suggests that eradication of *demodex folliculorum* mites may be beneficial in relieving ocular surface discomfort related to MGD.

In our study, CFS was more severe in *demodex folliculorum*-infested MGD patients compared to *demodex folliculorum*-negative MGD patients. Fluorescein staining is an important indicator of the integrity of the corneal epithelial barrier function. Increased fluorescein staining is usually observed during the disruption of the superficial epithelial cell-cell junctions and superficial corneal epithelial cells damage^[15]. Interleukin (IL)-17/MMP-9 signaling has been found to be the key factor in disrupting corneal epithelial barrier function in ocular surface diseases, such as dry eye^[16-20]. MMP-9 can rapidly degrade the corneal epithelial basement membrane components and tight-junction proteins such as Zonula occludens-1 and occludin that are involved in maintaining the corneal epithelial barrier function^[17]. Studies show that MMP-9-deficient mice were resistant to corneal epithelial barrier disruption in experimental dry eye^[16]. In addition, it has been proved that T helper-17 cytokine, IL-17, can disrupt the corneal barrier *via* upregulating the expression of MMP-9^[18-20]. Previously, it has been confirmed the association between tear IL-17 level and *demodex folliculorum* infection as eradication of *demodex folliculorum* significantly decreased tear IL-17 level^[8,21]. In our study, *demodex*-positive group showed significantly increased levels of tear MMP-9 activity compared to *demodex*-negative group. These results suggest that *demodex folliculorum* may exacerbate corneal epithelial barrier function *via* activation IL-17/MMP-9 signaling.

In our study, no significant difference was noted in meibum quality and expressibility between the two groups, suggesting *demodex folliculorum* infection has no obvious effect on meibum quality and expressibility. Further studies are required to investigate the effect of *demodex brevis* infection in meibomian gland on meibum quality and expressibility as

they are capable of borrowing deep into the meibomian gland. Significant difference in F-BUT and SIT values were not observed between the two groups in our study, indicating that *demodex folliculorum* infection has no obvious effect on tear film stability and tear production. The SRI represents surface regularity within the central area, and the SAI is a measure of central corneal asymmetry. No significant difference was observed in SRI and SAI values between the two groups, indicating that *demodex folliculorum* infection has no obvious effect on the irregularity and asymmetry of corneal surface in MGD patients.

Our study focused on the relationship between *demodex folliculorum* infestation and ocular surface damage in MGD, and found that ocular *demodex folliculorum* infestation is related to ocular discomfort, lid margin abnormality, corneal epithelial barrier disruption, and tear MMP-9 activity. Our findings suggest that treatment of *demodex folliculorum* is necessary and critical in patients with MGD.

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