Original Article

Comparison of Clinical Manifestations between Blepharitis Patients With and Without *Demodex* Infestation

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Background: *Demodex* mites are common ectoparasites of the skin that are implicated as a cause of blepharitis. However, it is difficult to differentiate *Demodex* infestation from other causes of blepharitis due to the lack of distinctive symptoms and requirement of a microscope examination to confirm the diagnosis.

Objective: To compare signs and symptoms between blepharitis patients with and without *Demodex* infestation.

Materials and Methods: Sixty-one consecutive patients with symptomatic blepharitis were enrolled. The patients completed the Ocular Surface Disease Index (OSDI) questionnaires and underwent standard eye examination, Schirmer I test, tear break-up time (TBUT), and ocular surface staining. Four eyelash samples were epilated from each eye and examined for *Demodex* mites using a microscope. Symptoms and signs of blepharitis between patients with and without *Demodex* infestation were compared.

Results: The prevalence of *Demodex* infestation among blepharitis patients was 63.9%, with an average *Demodex* count of 7.2 mites per patient. Common symptoms of *Demodex* blepharitis included ocular irritation (82.1%), itching (79.5%), and blurry vision (76.9%), but no significant difference was found when compared to non-*Demodex* blepharitis. Cylindrical dandruff (CD) was significantly more prevalent in blepharitis with *Demodex* infestation (74.4%) than those without (22.7%) ($p<0.001$). TBUT was significantly shorter in the blepharitis with *Demodex* infestation (3.6 second) than those without (4.3) ($p=0.04$). There was no significant difference in age, sex, duration of symptoms, OSDI score, Schirmer I test, ocular staining score, and severity of meibomian gland dysfunction between the two groups.

Conclusion: Symptoms of *Demodex* blepharitis are non-specific, similar to blepharitis without *Demodex* infestation. The presence of CD and short TBUT strongly suggest *Demodex* infestation and should warrant further diagnostic evaluation.

Keywords: Blepharitis, Cylindrical dandruff, *Demodex*, Demodicosis, Meibomian gland dysfunction

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Demodex mites are common ectoparasites of the skin that are implicated as a cause of blepharitis. Two distinct *Demodex* species have been identified, *Demodex folliculorum*, which typically clusters in the eyelash follicles, is responsible for anterior blepharitis, and *Demodex brevis*, which lives solitarily in the sebaceous or meibomian glands, is associated with posterior blepharitis or meibomian gland dysfunction (MGD). It has been proposed that the pathogenesis of *Demodex* blepharitis may be attributed to direct damage, hypersensitivity reaction, and vector for bacteria[1]. However, their pathogenic role remains controversial because *Demodex* mites can be found in healthy individuals. Therefore, *Demodex* mites are considered harmless but may induce ocular symptoms when the population becomes excessive.

Symptoms of *Demodex* blepharitis are itching, irritation, redness, burning, foreign body sensation, eyelid crusting, blurry vision, and mucous discharge, which are non-specific and overlap with other forms of anterior blepharitis. Signs include blepharoconjunctivitis, keratitis, nodular corneal scar, eyelashes abnormalities, MGD, with cylindrical dandruff (CD) being considered as the most characteristic feature of *Demodex* infestation[1-3]. Other associated pathologies such as chalazion and rosacea were also reported[3,4]. *Demodex* blepharitis is usually

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refractory to conventional blepharitis treatment, requiring targeted therapies against *Demodex* mites such as tea tree oil lid scrub, systemic ivermectin, or metronidazole[5,6].

Blepharitis is a very common problem in the ophthalmic practice. However, it is difficult to differentiate *Demodex* infestation from other causes of blepharitis due to the lack of distinctive symptoms and requirement of a microscope examination to confirm the diagnosis. Therefore, it is often overlooked and can result in treatment failure. At present, most studies have focused on the prevalence of ocular demodicosis but information regarding distinctive features are rarely pointed out. The aim of the present study was to evaluate whether there were any helpful clinical features that could differentiate blepharitis with *Demodex* infestation from those without.

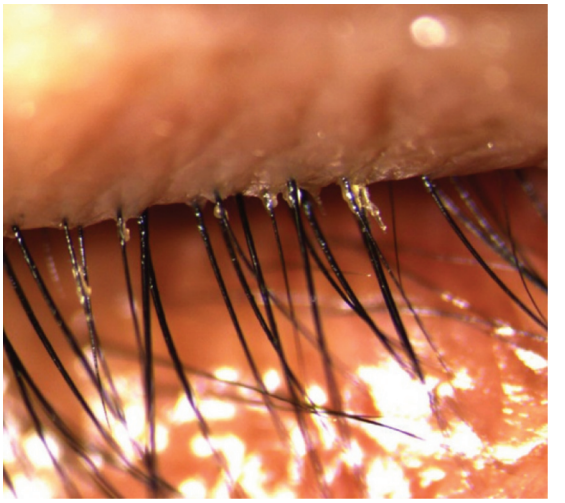

Figure 1. Cylindrical dandruff (CD).

Material and Methods

This cross-sectional study followed the tenets of the Declaration of Helsinki and was approved by the ethic committee of the HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University. Consecutive patients diagnosed with anterior blepharitis and/or MGD with at least one symptom of ocular discomfort were recruited between March and December 2017. Written informed consents were obtained from all patients. Exclusion criteria were active ocular inflammation other than blepharitis, used of topical eye drop excepted artificial tears, a history of ocular or eyelid surgery within the preceding six months, previous diagnosis of eyelid malposition, chemical burns, Steven-Johnson syndrome, lacrimal passage obstruction, and contact lens wearer.

The patients completed the Ocular Surface Disease Index (OSDI) questionnaire prior to further ophthalmic examination. The OSDI is a 12-item questionnaire evaluating five symptoms of ocular discomfort (light sensitiveness, grittiness, soreness, blurry vision, and poor vision) and seven items of their impact on daily activities and by environmental factors (reading, night driving, computer use, watching TV, windy condition, low humidity, and air conditioning area). The authors modified the questionnaire by adding symptoms of blepharitis including irritation, itching, burning, tearing, mucous discharge, and red eye[7]. Therefore, the questionnaire included 18 items. The total score was calculated by the following formula: OSDI = [(sum of scores for all questions answered) × 25] / total number of questions answered. The score was assessed on the scale of 0 to 100, with higher scores representing greater ocular discomfort.

All patients underwent complete ophthalmic evaluation under a slit lamp microscope, Schirmer I test, and tear break-up time (TBUT). Corneal and conjunctival staining were scored from 0 to 12[8]. MGD was evaluated by expressibility and quality of meibum as followed, 1) clear fluid expressed on mild digital pressure, 2) cloudy fluid expressed on moderate digital pressure, 3) cloudy fluid with particles expressed on moderated to hard pressure, and 4) toothpaste-like fluid expressed or could not be expressed even with hard pressure[9,10].

Demodex infestation was confirmed by microscopic examination of epilated eyelashes following the method describe by Gao et al[2]. In all patients, eight eyelashes (two eyelashes from each eyelid) was epilated in a rotating manner. Eyelashes with CD was intentionally epilated (Figure 1). If CD was absent, random epilation from medial and lateral half of the eyelid was performed. Each eyelash was placed on a glass slide, added with a drop of saline, mounted with a cover slip, and inspected under a microscope to identify *Demodex* mites. For eyelashes with retained compact CD, 100% alcohol was added to dissolve the CD and stimulate embedded *Demodex* mites to migrate out, and then re-evaluated within the following 20 minutes (Figure 2). *Demodex* blepharitis is defined as presence of at least one of any life cycle stage of the *Demodex* spp. All life cycle stages were counted and recorded as the total number of mites found per eight eyelashes for each patient.

Patients were divided into *Demodex*-positive blepharitis group and *Demodex*-negative blepharitis group. Demographic data including age, sex, duration

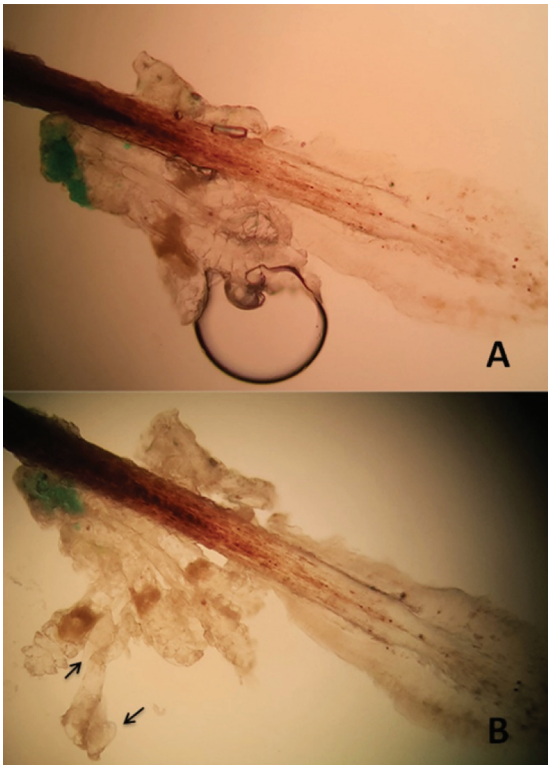

Figure 2. Modified counting method with added alcohol. Clustered *Demodex* mites within in the cylindrical dandruff (CD). (A) Alcohol dissolved the CD and stimulated *Demodex* mites to migrate out, allowing better visualization. (B) Two eggs were noted (arrows).

of symptoms, clinical symptoms and signs between the two groups were compared.

Sample size was calculated based on previous report to detect clinically significant difference in itching between the case and control with an 80% power and 5% significance[11], yield a minimum sample size of 37 participants. Data were expressed as number (%), mean ± standard deviation (SD), median, range, and interquartile range (IQR). Data between the two groups were assessed by independent t-test and Mann-Whitney U test for continuous variables, and chi-square test for categorical ones. A p-value less than 0.05 was considered statistically significant.

Results

Sixty-one consecutive patients with symptomatic blepharitis were included in the study. Forty-three (70.5%) patients were female and 18 (29.5%) were males with a mean age of 68.0±11.6 years (range 20 to 87). Of the total, 37 (60.7%) patients had mixed blepharitis, 20 (32.8%) had MGD, and 4 (6.6%) had anterior blepharitis. Median duration of symptom was six months (IQR 3 to 12).

The prevalence of *Demodex* infestation among blepharitis patients was 63.9% (39/61). *Demodex* mites were detected only in patients age over 50 years, with a 30% prevalence at age 60 and 28% after 70 years of age. *Demodex* mites were found in 66% of those who had symptoms over six months. Mean *Demodex* count was 7.2 mites (range 1 to 46) per person. Of all the *Demodex* spp. detected, one *D. brevis* was identified.

The most common ocular symptoms in *Demodex* blepharitis were irritation, itching, and blurry vision. However, when compared to those without *Demodex* infestation, all symptoms were not significantly different. There was no significant difference in age, sex, duration of symptoms, OSDI score between the two groups.

Among clinical signs, CD was significantly more prevalent in blepharitis patient with *Demodex* infestation than those without ($p<0.001$). In addition, TBUT in blepharitis patient with *Demodex* infestation was significantly shorter than those without ($p=0.04$). Schirmer I test, ocular staining score and severity of MGD were not significantly different between the two groups (Table 1). Trichiasis was found in two patients with *Demodex* infestation and one patient without infestation.

Discussion

The prevalence of *Demodex* infestation varies widely depending on the studied population and eyelash sampling method. In Thailand, the prevalence of ocular demodicosis in a hospital-based population was 42% microscopically and up to 79% when determined by semi-nested polymerase chain reaction (PCR)[12]. Several case-control studies have shown that the prevalence rates of *Demodex* infestation in blepharitis patients are almost always higher than those of the control subjects[11,13-15]. According to the meta-analysis, the pool prevalence of *Demodex* infestation was 45% in blepharitis patients versus 17% in the controls[16]. A modified eyelash sampling and counting method describe by Gao et al[2] has shown an extremely high detection rate of *Demodex* mites when selectively epilating eyelash with CD. Using this modified counting method, the present study found a relatively high prevalence of *Demodex* infestation (63.9%) among blepharitis patients. Bhandari and Reddy also reported a prevalence of 78.7% of *Demodex* infestation among blepharitis patients[14]. In addition, *Demodex* was detected in 70% to 99% of the patients presented with other various ocular

Table 1. Comparison of clinical features between two groups of blepharitis

	Demodex-positive (n=39) n (%)	*Demodex*-negative (n=22) n (%)	p-value
Age (years), Mean±SD	68.46 ± 8.37	67.05 ± 15.93	0.70
Sex			0.77
Male	11 (28.2)	7 (31.8)	
Female	28 (71.8)	15 (68.2)	
Duration of symptom (months), Median (IQR)	6 (3 to 12)	9 (2.75 to 12)	0.93
Symptoms			
Irritation	32 (82.1)	16 (72.7)	0.52
Itching	31 (79.5)	17 (77.3)	1.00
Blurry vision	30 (76.9)	19 (86.4)	0.51
Tearing	28 (71.8)	12 (54.5)	0.17
Photophobia	26 (66.7)	14 (63.6)	0.81
Gritty	24 (61.5)	12 (54.5)	0.59
Decrease vision	21 (53.8)	9 (40.9)	0.33
Mucous discharge	19 (48.7)	12 (54.5)	0.66
Soreness	14 (35.9)	12 (54.5)	0.16
Burning	13 (33.3)	8 (36.4)	0.81
Red eye	11 (28.2)	5 (22.7)	0.64
OSDI score, Mean±SD	22.93±12.25	29.87±16.53	0.67
Cylindrical dandruff	29 (74.4)	5 (22.7)	<0.001*
Schirmer I test (mm), Median (IQR)	12 (6 to 20)	9.5 (7 to 20)	0.75
TBUT (second), Mean (range)	3.6 (1 to 10)	4.3 (0 to 10)	0.04*
Staining score, Mean (range)	1.2 (0 to 6)	1.0 (0 to 7)	0.81
MGD			
Meibum expressibility and quality, Mean	2.06	1.95	0.41
MGD grade 3-4	17 (43.6)	7 (17.9)	0.58

SD=standard deviation; IQR=interquartile range; OSDI=ocular surface disease index; TBUT=tear break up time; MGD=meibomian gland dysfunction

* Statistically significant

surface diseases[7,17,18].

Demodex can affect any age group with increasing prevalence with age, occurring in 40% by age 60, and increasing to 70% after 80 years of age[12]. In the present study, only symptomatic blepharitis patients over 50 years of age had *Demodex* infestation, with a 30% prevalence at age 60, and 28% after 70 years of age.

Demodex has been described to be related to sex. Roihu and Kariniemi reported that males (59%) were more frequently infested than females (30%)[19]. Aylesworth and Vance also reported a higher frequency in males, especially by the *D. brevis*[20]. In contrast, females were more infested in 71% by Inceboz et al and 75% by Nicholls et al[13,18]. In the present study, 71.8% of infested patients were females. However, there was no gender differences in infestation rate when compared to those without *Demodex* infestation (p=0.77). This is in agreement with the results from previous studies that *Demodex* was not associated with gender[7,15,17].

Median duration of symptoms before the diagnosis of *Demodex* blepharitis was not significantly different from non-*Demodex* blepharitis. However,

Demodex mites were found in 66% of those who had symptoms over six months.

There is no conclusive evidence suggesting the minimum number of *Demodex* mites required to induce symptomatic demodicosis. In dermatology, Sattler et al proposed as a criterion of infestation with at least four mites detected in one follicle within a 5×5-mm mosaic[21]. For eyelash, Coston had suggested if six or more mites (per 16 eyelashes) presence, especially four to five mites cling to one eyelash would considered overpopulation[22]. Previous studies found ocular discomfort patients had a mean *Demodex* count of 3.4 to 4.4 per eight eyelashes[7,17]. The present study found a mean number of 7.2 mites per patient. However, counting method by epilation technique may not extract all mites, as some mites may be left inside the eyelid. Further investigation to examine mites that buried deep in the sebaceous glands such as confocal microscopy may be needed[23].

Comparing the symptoms and signs between blepharitis with and without *Demodex* infestation show both similarities and important differences. Itching has been described as the most prominent symptom of *Demodex* infestation[11,24]. The present study found that ocular irritation (82.1%), itching (79.5%), and blurry vision (76.9%) were the most common presenting symptoms in *Demodex* blepharitis. However, there was no difference in the prevalence rates of these symptoms when compared to those without *Demodex* infestation. This is in accordance to the study by Inceboz et al that itching and redness were not different between both groups[13]. Moreover, Bhandari and Reddy reported ocular irritation was the most common presenting symptom in *Demodex* blepharitis and also correlated with a high *Demodex* count[14].

Overall symptom of blepharitis was expressed as OSDI score, and the score was not significantly different between the groups. In contrast, Lee et al have reported that OSDI score was correlated with the number of *Demodex*[7], however, this previous study included patients with various ocular surface problems other than blepharitis.

CD is known as the characteristic feature of *Demodex* blepharitis. Some clinicians even consider CD as the pathognomonic sign of the disease[1,2]. The present study found CD was significantly more common in blepharitis patients with *Demodex* infestation (74.4%) than those without (22.7%), ($p<0.001$), in accordance with previous studies[11,12,14]. It was believed that CD consists of lipid and keratin that result from irritation of mite's claw scrapping the hair follicle[1,2]. Recent controversy has arisen as to whether CD was produced by the mites. Rynerson and Perry has stated that CD most likely represents biofilm that consisted of a mixture of bacteria and polysaccharide that accumulates around the eyelash[25]. This biofilm provides nutritious food for *Demodex* mites and cause mite overpopulation. Slow eyelash growth and abundant biofilm stacking up at the root of eyelash result in a sleeve appearance, rather than directly formed by the mites. Although controversy exists, the present study has demonstrated that CD is still a clinical important sign indicative of ocular *Demodex* infestation.

The present study found *Demodex* infestation was associated with reduced TBUT ($p=0.04$) but had no effect on the Schirmer test. Although not significantly different between the groups, *Demodex* mites were detected more in cases of severe MGD. The results are similar to previous studies showing that *Demodex* may damage the meibomian glands causing lipid tear film instability but does not affect the lacrimal gland[7,11,26]. However, their role in the pathogenesis of MGD has not yet been convincingly established.

Limitation of the present study was the eyelash sampling method by epilation as some *Demodex* mites may be left in the eyelid, especially those that buried deep in the follicle. Further diagnostic investigations such as confocal microscopy that provides superior detection of the *Demodex* mites in the follicle may be needed.

Treatment was not included in the study protocol. Nonetheless, the authors treated all *Demodex* blepharitis with 50% tea tree oil lid scrub. All patients had significantly decreased *Demodex* counts, reaching zero in 87.1% with clearing CD after one to four scrub treatments. Ocular symptoms significantly improved in all patients even those who had a small number of *Demodex* mites remaining. Therefore, recognizing *Demodex* blepharitis is essential to provide proper treatment.

Conclusion

Main symptoms of *Demodex* blepharitis include irritation, itching, and blurry vision, which are similar to blepharitis without *Demodex* infestation. The presence of CD and short TBUT strongly suggest *Demodex* infestation and should warrant further diagnostic evaluation.

What is already known on this topic?

Demodex blepharitis has no-specific symptom and is associated with cylindrical dandruff.

What this study adds?

There is a relatively high prevalence of *Demodex* infestation among blepharitis patients, and *Demodex* blepharitis is associated with shorter TBUT when compared to non-*Demodex* blepharitis.

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Conflicts of interest

The authors declare no conflict of interest.

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