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Research Article

Prevalence and Load of *Demodex folliculorum* and *Demodex brevis* (Acari: Demodicidae) in Patients With Chronic Blepharitis in the Province of Erzincan, Turkey

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Abstract

Chronic blepharitis is an ocular disease frequently encountered by ophthalmologists. *Demodex* mites can play a role in the pathogenesis of blepharitis along with bacterial agents, especially in treatment-resistant cases or recurrent cases after treatment. This study was performed to determine the prevalence and load of *Demodex folliculorum* (Simon) and *Demodex brevis* Akbulutova in chronic blepharitis patients and to assess the relationship between the prevalence and load of *Demodex* species and ocular symptoms. The study included 365 patients diagnosed with chronic blepharitis in clinical examination, and 175 controls without any chronic or ocular disease. In the study, two eyelashes were sampled from the lower and upper lids of the right and left eyes (a total of eight samples) of the participants. Eyelash samples were examined under a light microscope, and *Demodex* species were identified and counted. *Demodex* were detected in 79.2% (95% CI: 75–83%) of patients and 31.4% (95% CI: 24–38%) of controls in this study. *D. folliculorum* alone (mean: 4.96; min: 1; max: 17; $P < 0.001$) was detected in 72.3% of patients, in 0.7% *D. brevis* alone (mean: 1.00, $P > 0.05$), and in 27% both *D. folliculorum* and *D. brevis* (mean: 21.65; min: 2; max: 79; $P < 0.001$). In *Demodex* positive controls, only *D. folliculorum* (mean: 2.38; min: 1; max: 6) was detected while there was no *D. brevis*. Nevertheless, mean ocular symptom scores were significantly higher in *Demodex* positive patients than in *Demodex* negative patients ($P < 0.001$). Itching, foreign body sensation, and redness were the most common complaints in *Demodex* positive patients. As a result, *Demodex* mites were high in numbers in patients with chronic blepharitis in Erzincan. There was a positive correlation between *Demodex* mites and chronic blepharitis and ocular symptoms. It may be helpful to consider these findings in clinical assessment of blepharitis patients.

Key words: Acari, blepharitis, ocular symptom, eyelash, epidemiology

Demodex mites (Acari) are microscopic arthropods organisms belonging to the Demodecidae family of the Trombidiformes order. Among *Demodex* mites two species that can only survive in humans have been described: *Demodex folliculorum* Simon and *Demodex brevis* Akbulutova (Desch and Nutting 1972, Rufli and Mumcuoglu 1981). *D. folliculorum*, which has a long opisthosoma, lives alone or in groups in hair follicles, and *D. brevis*, which has a short opisthosoma, lives mainly alone in sebaceous and meibomian glands (Rufli and Mumcuoglu 1981, Aytekin et al. 2017). Their main food sources are follicular and sebaceous epithelial cells and sebum. They have a cigar-shaped body, a chitinous external skeleton, a perforating mouthparts, and four pairs of legs with a pair of claws at the terminal end (Desch and Nutting 1977, Rufli and Mumcuoglu 1981).

Chronic blepharitis is an ocular disease frequently encountered by ophthalmologists in clinical practices. Its treatment is difficult for both the patient and the physician, because often it recurs despite being treated. Eyelash dandruff, adhesion of eyelashes, mild papillary conjunctivitis, meibomian gland dysfunction, and telangiectasia may be seen in chronic blepharitis, as well as irregularity of the lid edge in frequently recurring cases (Liu et al. 2010, Küçümen et al. 2015, Altınkurt et al. 2017, Luo et al. 2017). *Demodex* mites can play a role in the pathogenesis of blepharitis along with bacterial agents, especially in treatment-resistant cases or recurrent cases after treatment. Indeed, some researchers have reported that *Demodex* mites are vectors for the transport of some bacteria such as bacillus, staphylococcus, and streptococcus to humans (Lacey et al. 2009, Liu et al. 2010, Cheng et al. 2015, Luo et al. 2017). Szkaradkiewicz et al. (2012) reported that *Demodex* mites play an independent etiopathogenetic role in chronic blepharitis, and are the vector of *Bacillus oleronius* bacteria that function as

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an assistant pathogen in the development of severe blepharitis symptoms.

Numerous epidemiological studies of *Demodex* mites have been performed in blepharitis patients, as well as in healthy individuals and in patients of various diseases (Sümer et al. 2000, Arici et al. 2005, Kheirkhah et al. 2007, Türk et al. 2007, Emre et al. 2008, İnceboz et al. 2009, de Venecia and Siong 2011, Yula et al. 2013, Bhandari and Reddy 2014, Altınkurt et al. 2017, Alver et al. 2017, Kabataş et al. 2017, Kasetsuwan et al. 2017, Lopez-Ponce et al. 2017). However, in most of the studies carried out, the *Demodex* species is not specified or mites are only identify up to genus. In this context, separately detection of the prevalence and load of *Demodex* species will may allow better understanding of the pathogenesis of the disease and better evaluation of the clinical picture. Furthermore, in most of studies about the prevalence and load of *Demodex*, different sampling methods have been used, possibly leading to different outcomes. Therefore, use of a standard method might allow for more accurate estimation of the *Demodex* prevalence and load in patients with blepharitis.

In our previous studies (Tilki et al. 2017, Zeytun 2017, Zeytun et al. 2017), we focused on the prevalence and load of *Demodex* in healthy individuals in Erzincan, Turkey. However, no studies have been performed on blepharitis patients in this province, Turkey. This study was carried out to separately determine the prevalence and load of *Demodex* species with a standard method, and also assess the relationship between *Demodex* mites and ocular symptoms.

Materials and Methods

This study was performed between March 2016 and February 2017 in the Erzincan province in the Eastern Anatolia region of Turkey, having with a population about 140,000 inhabitants. In total, 365 patients diagnosed with chronic blepharitis in their clinical examination in Erzincan University Mengücek Gazi Education and Research Hospital Ophthalmology Clinic, and 175 healthy controls without any chronic or ocular disease were enrolled in this study. Controls were randomly selected from among volunteer participants in Erzincan province. Patients who had any ocular or systemic disease other than chronic blepharitis, who had undergone ocular surgery, and who had received any systemic or topical treatment were excluded from the study. All patients and controls were having similar demographic characteristics (age, sex, ethnicity, etc.). The study was approved by Erzincan University Clinical Research Ethics Committee, and all participants read and signed the informed consent form in accordance with Helsinki Declaration.

In addition, ocular symptoms such as itching, redness, burning, watering, burring, and foreign body sensation (stinging) from all patients were recorded in detail. Patients were asked to rate their symptoms as none (asymptomatic), mild (occasionally disturbing, not interfering with daily activities), moderate (often disturbing, intermittently interfering with daily activities), or severe (constantly disturbing and often interfering with daily activities). Symptom scores were obtained as 0 for no symptoms, 1 for mild symptoms, 2 for moderate, and 3 for severe symptoms (Gao et al. 2012, Arrua et al. 2015).

In total, eight eyelashes (two eyelashes for each from the lower and upper lids) were obtained using the 'epilation method'. Firstly, pure sterile olive oil was applied to the eyelid skin of all participants with a sterile cotton applicator; later eyelashes were collected with sterile optical tweezers. Eyelash samples were made into preparates in Hoyer medium, separately for each eye, and they were examined within 1 h at 4×, 10×, 40× magnification on a light microscope (Leica DM750, Heerbrugg, Switzerland). All counting and identification of mite species were performed by the same investigator according to the literature for *Demodex* mites (Desch and Nutting 1972, 1977). Patients from whom larval, nymph, or adult stages of *Demodex* mites were detected in at least one of the eyelashes were considered to be *Demodex* positive. The mean number of *Demodex* mites was calculated by dividing the total number of *Demodex* mites to the number of *Demodex* positive patients and also the range of the load of *Demodex* was provided (Arıcı et al. 2005, İnceboz et al. 2009, Wesolowska et al. 2014).

Statistical evaluation of the data was performed using the SPSS 23.0 (Statistical Package for Social Sciences, Chicago, IL) program. The suitability of the variables against normal distribution was examined using the Kolmogorov–Smirnov test. Nonparametric Mann–Whitney *U* and Kruskal–Wallis tests were used for intergroup comparisons. Spearman correlation test was used for the relationship between ocular symptoms and *Demodex* load. Chi-square test was used to evaluate categorical data. *Demodex* prevalence rates were calculated using maximum likelihood estimation method with 95% confidence intervals (CIs). A *P*-value less than 0.05 was considered to be statistically significant.

Results

In total, 540 participants, consisting of 365 patients (220 women, 145 men, mean age 53.23 yr) and 175 healthy controls (92 women, 83 men, mean age 51.97 yr), were enrolled in this study (Table 1).

Demodex was detected in 79.2% (95% CI: 75–83%) of the patients and 31.4% (95% CI: 24–38%) of the controls in the study. In total, 2,727 (mean: 9.44; min: 1; max: 79) mites were isolated from patients who infested with *Demodex*. In total, 1,036 (mean: 4.96; min: 1; max: 17) mites (Fig. 1) were isolated from individuals who infested with *D. folliculorum* only, 2 (mean: 1.00) mites were isolated from individuals who infested with *D. brevis* only, and 1,689 (mean: 21.65; min: 2; max: 79) mites were isolated from individuals who infested with both species (Table 2). In the controls who infested with *Demodex*, only *D. folliculorum* was detected while there was no *D. brevis*. In total, 131 (mean: 2.38; min: 1; max: 6) mites were isolated from these controls. Except for patients with only *D. brevis*, differences in the prevalence and load of *Demodex* between patients and controls were statistically significant ($P < 0.001$).

The prevalence and load of *Demodex* in the eyes of patients and controls are detailed in Tables 3 and 4. *Demodex* prevalence and load in the patients who infested with *Demodex* were 18.3% (mean 4.13), 22.9% (mean 3.76), and 58.8% (mean 13.29), respectively, in the right eye, left eye, and both eyes. Whereas, in the controls who infested with *Demodex*, *Demodex* prevalence and load were 47.3% (mean 2.04), 30.9% (mean 1.82) and 21.8% (mean 3.92), respectively, in the right eye, left eye and both eyes.

Table 1. Age and sex of patients and controls

	Patients (*n*: 365)	Controls (*n*: 175)
Age (yr)		
Mean ± SD	53.23 ± 16.08	51.97 ± 16.79
Median (min.–max.)	56.00 (12–86)	49.00 (13–85)
Sex		
Female	220/365 (60.3%)	92/175 (52.6%)
Male	145/365 (39.7%)	83/175 (47.4%)

SD: standard deviation; min: minimum; max: maximum.

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The ocular symptoms of patients and their relationship to *Demodex* mites are detailed in Tables 5 and 6. Mean ocular symptom scores were significantly higher in *Demodex* positive patients than in *Demodex* negative patients ($P < 0.001$). Ocular complaints with the highest mean score in *Demodex* positive patients were itching, foreign body sensation, and redness. The relationship between the age and sex of participants, and the prevalence and load of *Demodex* is given in Table 7. The prevalence and load of *Demodex* were constantly increase with age, and also were higher in the male patients.

Discussion

To date, a number of epidemiological studies have been performed to determine the prevalence of *Demodex* mites both in Turkey and in other countries. The prevalence of *Demodex* mites in patients with blepharitis and healthy controls from studies performed in Turkey has been reported as; 62.9% and 26.7% in Sivas; 68.3% and 4.1% in İzmir; 56.9% and 10% in Malatya; 67.2% and 54.9% in Ankara; 66.7% in Istanbul; 73.9% in Bursa, respectively (Sumer et al. 2000, Arici et al. 2005, Türk et al. 2007, Emre et al. 2008, İnceboz et al. 2009, Altınkurt et al. 2017, Alver et al. 2017, Kabataş et al. 2017). In addition, *Demodex* prevalence was reported in 81.2% of 96 blepharitis patients and 27.9% of 197 controls in Hatay. The same study reported that *D. folliculorum* alone was detected in 50% of the patients, the coinfection of *D. folliculorum* and *D. brevis* was determined in 26.04% of the patients, and none were detected with only *D. brevis* (Yula et al. 2013). In other countries, *Demodex* prevalence in patients with blepharitis and controls has been reported as; 97% and 34% in Manila, Philippines; 78.7% and 18% in Tamil Nadu, India; 83.7% in Santiago, Chile, respectively (de Venecia and Siong 2011, Bhandari and Reddy 2014, Lopez-Ponce et al. 2017). In a study conducted by Kheirkhah et al. (2007) in Florida, United States, *D. folliculorum* was detected in all of six blepharitis patients and *D. brevis* was detected in 50%. In a study conducted in Bangkok, Thailand, *D. folliculorum* and *D. brevis* were reported in 42% and 1% of 100 blepharitis patients, respectively (Kasetsuwan et al. 2017). In addition, in a meta-analysis of 13 separate articles (a total of 2,098 patients with blepharitis and 2,643 controls) from four countries (China, France, Germany, Turkey), *Demodex* prevalence was reported as 44.52% in patients with blepharitis and 16.69% in controls, and a positive correlation between *Demodex* mites and blepharitis was reported in 11 of 13 articles (Zhao et al. 2012). Considering the epidemiological studies summarized above, the prevalence of *Demodex* in blepharitis patients and controls ranged from 28.8% to 97% and 4.1% to 54.9%, respectively. In our study, two eyelash samples were randomly taken from the lower and upper lids of the right and left eyes (a total of eight lashes) of the patients. Eyelash samples were examined in detail separately for each eye with regard to the presence of *D. folliculorum* alone, the presence of *D. brevis* alone, and the coinfection of *D. folliculorum* and *D. brevis*. In the present study, *Demodex* was detected in 79.2% (95% CI: 75–83%) of the patients and in 31.4% (95% CI: 24–38%) of the controls. About 72.3% of the patients in which *Demodex* was detected were infested with *D. folliculorum* only, 0.7% with *D. brevis* only, and 27% with both species.



Fig. 1. *Demodex folliculorum* (at 40× magnification).

Table 2. Prevalence and load of *Demodex* mites in patients and controls

	Patients	Controls	*P*-value
Demodex infestation	289/365 (79.2%) (95% CI: 75–83%)	55/175 (31.4%) (95% CI: 24–38%)	<0.001[a]
Prevalence of *Demodex*[b]			
DF only	209/289 (72.3%) (95% CI: 67–78%)	55/55 (100%) (95% CI: 88–100%)	<0.001[a]
DB only	2/289 (0.7%) (95% CI: 0–2%)	0/55 (0%)	>0.05[a]
Both DF and DB	78/289 (27.0%) (95% CI: 22–32%)	0/55 (0%)	<0.001[a]
Total	289/289 (100%)	55/55 (100%)	-
Mean load of *Demodex*[b] (min–max)			
DF only	4.96 (1–17)	2.38 (1–6)	<0.001[c]
DB only	1.00 (1–1)	0	>0.05[c]
Both DF and DB	21.65 (2–79)	0	<0.001[c]
Total	9.44 (1–79)	2.38 (1–6)	-
Total load of *Demodex*[b]			
DF only	1,036	131	<0.001[c]
DB only	2	0	>0.05[c]
Both DF and DB	1,689	0	<0.001[c]
Total	2,727	131	-

DF: *D. folliculorum*; DB: *D. brevis*; min: minimum; max: maximum; CI: confidence interval.

[a]Chi-square test.

[b]Only positive participants have been taken into accounts.

[c]Mann–Whitney *U*-test.

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Table 3. Prevalence of *Demodex* mites in eyes of patients and controls

	Patients	Controls	*P*-value[a]
Prevalence of *Demodex*[b]			
Right eye only	53/289 (18.3%) (95% CI: 14–23%)	26/55 (47.3%) (95% CI: 34–61%)	<0.001
Left eye only	66/289 (22.9%) (95% CI: 18–28%)	17/55 (30.9%) (95% CI: 18–44%)	>0.05
Both eye	170/289 (58.8%) (95% CI: 53–65%)	12/55 (21.8%) (95% CI: 11–33%)	<0.001
Total	289/289 (100%)	55/55 (100%)	-
Right eye only[b]			
DF only	49/53 (92.5%) (95% CI: 85–98%)	26/26 (100%) (95% CI: 83–100%)	>0.05
DB only	0/53 (0%)	0/26 (0%)	-
Both DF and DB	4/53 (7.5%) (95% CI: 2–15%)	0/26 (0%)	>0.05
Total	53/53 (100%)	26/26 (100%)	-
Left eye only[b]			
DF only	57/66 (86.4%) (95% CI: 78–95%)	17/17 (100%) (95% CI: 85–100%)	>0.05
DB only	2/66 (3.0%) (95% CI: 1–7%)	0/17 (0%)	>0.05
Both DF and DB	7/66 (10.6%) (95% CI: 3–18%)	0/17 (0%)	>0.05
Total	66/66 (100%)	17/17 (100%)	-
Both eye[b]			
DF only	103/170 (60.6%) (95% CI: 53–68%)	12/12 (100%) (95% CI: 84–100%)	0.006
DB only	0/170 (0%)	0/12 (0%)	-
Both DF and DB	67/170 (39.4%) (95% CI: 32–47%)	0/12 (0%)	0.006
Total	170/170 (100%)	12/12 (100%)	-

DF: *D. folliculorum*; DB: *D. brevis*; CI: confidence interval.

[a]Chi-square test.

[b]Only positive participants have been taken into accounts.

Table 4. Load of *Demodex* mites in eyes of patients and controls

	Patients		Controls		
	Mean (min–max)	Total	Mean (min–max)	Total	*P*-value[a]
Demodex load[b]					
Right eye only	4.13 (1–21)	219	2.04 (1–5)	53	0.041
Left eye only	3.76 (1–19)	248	1.82 (1–6)	31	0.031
Both eye	13.29 (2–79)	2,260	3.92 (2–5)	47	<0.001
Total	9.44 (1–79)	2,727	2.38 (1–6)	131	
Right eye only[b]					
DF only	3.08 (1–8)	151	2.04 (1–5)	53	>0.05
DB only	0	0	0	0	>0.05
Both DF and DB	17.0 (13–21)	68	0	0	>0.05
Total	4.13 (1–21)	219	2.04 (1–5)	53	
Left eye only[b]					
DF only	2.70 (1–10)	154	1.82 (1–6)	31	>0.05
DB only	1.00 (1–1)	2	0	0	>0.05
Both DF and DB	13.14 (10–19)	92	0	0	>0.05
Total	3.76 (1–19)	248	1.82 (1–6)	31	
Both eye[b]					
DF only	7.10 (1–17)	731	3.92 (2–5)	47	>0.05
DB only		0	0	0	>0.05
Both DF and DB	22.82 (2–79)	1,529	0	0	>0.05
Total	13.29 (1–79)	2,260	3.92 (2–5)	47	

DF: *D. folliculorum*; DB: *D. brevis*; min: minimum; max: maximum.

[a]Mann–Whitney *U*-test.

[b]Only positive participants have been taken into accounts.

Although the same method (epilation) was used to collect eyelash samples from patients, a standard method has not been used regarding which eyelids (right eye, left eye, lower eyelid, or upper eyelid), which eyelashes (cylindrical eyelashes with dandruff, healthy eyelashes, or random), and how many eyelashes (one each, two each, three each, or four each) will be sampled. Thus, these sampling differences between studies may have led to obtaining different outcomes for the prevalence and load of *Demodex* in blepharitis patients. For example, the greater the number of eyelashes collected from patients, the greater the likelihood of encountering *Demodex* mites. Similarly, the greater the number of examined eyelid, the greater the likelihood of encountering *Demodex* mites also. If only the eyelashes with cylindrical dandruff are examined, the possibility of encountering *Demodex* will be higher. Actually, sampling should

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Table 5. Comparison of ocular symptoms of patients with *Demodex* positive and negative

	Ocular symptom scores[a] [mean (min.–max.)]		
	Demodex positive (*n*: 289)	*Demodex* negative (*n*: 76)	*P*-value[b]
Ocular symptoms			
Itching	2.35 (0–3)	0.13 (0–1)	<0.001
Redness	2.10 (0–3)	0.09 (0–1)	<0.001
Burning	0.89 (0–3)	0.04 (0–1)	<0.001
Watering	1.25 (0–3)	0.05 (0–1)	<0.001
Burring	0.94 (0–3)	0.08 (0–1)	<0.001
Foreign body sensation	2.15 (0–3)	0.07 (0–1)	<0.001

min: minimum; max: maximum.
[a]The scoring method of ocular symptoms: 0 = none; 1 = mild; 2 = moderate; 3 = severe.
[b]Mann–Whitney *U*-test.

Table 6. Correlation between ocular symptoms and *Demodex* load in patients

	n/*N* (%)	Ocular symptom scores [mean (min.–max.)]	*Demodex* load [mean (min.–max.)]	*P*-value[a] (*r*)
Ocular symptoms				
Itching	280/289 (96.9%)	2.43 (1–3)	9.66 (1–79)	<0.001 (0.626)
Redness	275/289 (95.2%)	2.21 (1–3)	9.47 (1–79)	<0.001 (0.776)
Burning	239/289 (82.7%)	1.07 (1–3)	8.46 (1–50)	0.009 (0.169)
Watering	257/289 (88.9%)	1.40 (1–3)	8.21 (1–33)	<0.001 (0.415)
Burring	255/289 (88.2%)	1.07 (1–3)	8.39 (1–46)	>0.05 (0.095)
Foreign body sensation	283/289 (97.9%)	2.20 (1–3)	9.57 (1–79)	<0.001 (0.797)

n: *Demodex* positive patients who suffer from ocular symptoms; *N*: all *Demodex* positive patients; *r*: correlation coefficient.
[a]Spearman correlation test.

Table 7. The relationship between prevalence and load of *Demodex* and age and sex of participants

	Prevalence of *Demodex* [*n*/*N* (%)]			Load of *Demodex*[b] [mean (min.–max.)]		
	Patients	Controls	*P*-value[a]	Patients	Controls	*P*-value[c]
Age (yr)						
12–20	5/11 (45.5%) (95% CI: 14–75%)	0/4 (0%)	<0.001	3.80 (1–7)	0	<0.001
21–40	47/76 (61.8%) (95% CI: 51–73%)	9/41 (22.0%) (95% CI: 10–35%)		3.77 (1–13)	2.00 (1–5)	
41–60	112/137 (81.8%) (95% CI: 75–88%)	24/61 (39.3%) (95% CI: 28–53%)		8.34 (1–36)	2.33 (1–8)	
61 +	125/141 (88.7%) (95% CI: 83–94%)	22/69 (31.9%) (95% CI: 21–44%)		12.78 (1–79)	2.59 (1–5)	
Total	289/365 (79.2%) (95% CI: 75–83%)	55/175 (31.4%) (95% CI: 24–38%)		9.44 (1–79)	2.38 (1–8)	
Sex						
Female	168/220 (76.4%) (95% CI: 71–82%)	32/92 (34.8%) (95% CI: 26–45%)	<0.001	8.83 (1–79)	2.28 (1–8)	<0.001
Male	121/145 (83.5%) (95% CI: 77–89%)	23/83 (27.7%) (95% CI: 19–37%)		10.28 (1–50)	2.52 (1–6)	
Total	289/365 (79.2%) (95% CI: 75–83%)	55/175 (31.4%) (95% CI: 24–38%)		9.44 (1–79)	2.38 (1–8)	

n: *Demodex* positive participants; *N*: all participants; min: minimum; max: maximum; CI: confidence interval.
[a]Chi-square test.
[b]Only positive participants have been taken into accounts.
[c]Kruskal–Wallis test.

be done randomly to determinate of parasite prevalence in epidemiological studies. Therefore, obtaining eyelash samples separately and randomly from each eyelid (lower and upper lids of right and left eye) will allow for more accurate estimation of the *Demodex* prevalence and load in patients with blepharitis.

On the other hand, in the majority of the epidemiological studies, *Demodex* species have not been identified separately, the parasite is called *Demodex* sp. or only *D. folliculorum* is emphasized. But the presence of only *D. brevis* or the coinfection of *D. folliculorum* and *D. brevis* were not adequately investigated. Some researchers reported that *D. folliculorum* causes anterior blepharitis, and that *D. brevis* causes posterior blepharitis (Lacey et al. 2009, Liu et al. 2010, Cheng et al. 2015). Some researchers have reported that accumulation of *D. folliculorum* clusters in eyelash follicles creates plug and strain in the follicles, leading to deformations of the lashes. In addition, micro-abrasions caused by the feet of *D. folliculorum* result in epithelial hyperplasia and reactive hyperkeratinization in the eyelash, leading to the formation of cylindrical dandruff, and these were considered pathognomonical for *D. folliculorum* infestation (Gao et al. 2005, Lacey et al. 2009, Liu et al. 2010, Luo et al. 2017). It has been reported that *D. brevis* prevented lipid secretion by blocking the orifice of the meibomian glands, and that caused dysfunction of it (Lacey et al. 2009, Liu et al. 2010, Cheng et al. 2015, Liang et al. 2010). Thus, in the epidemiological studies on *Demodex* mites,

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the separate determination of the presence of *D. folliculorum* alone, *D. brevis* alone or the coinfection of *D. folliculorum* and *D. brevis*, and of their load may allow a better understanding of the blepharitis pathogenesis and a better evaluation of the clinical picture.

In our study, *D. folliculorum* was more common and intense than *D. brevis* in both patients and controls. This may be due to the fact that *D. brevis* lives in deeper structures of the skin surface (in the meibomian glands), and *D. folliculorum* lives in the outer surfaces of the hair follicles (closer to the skin surface). Therefore, *D. folliculorum* can be more easily isolated than *D. brevis*. Other studies supported our findings in this direction (Zhao et al. 2012, Wesolowska et al. 2014, Tilki et al. 2017, Zeytun 2017, Zeytun and Ölmez 2017, Zeytun et al. 2017).

Chronic blepharitis is characterized by symptoms such as itching, redness, foreign body sensation, burning, watering, burring, cylindrical dandruff, and photophobia. Some researchers have reported that the most common symptoms of blepharitis are itching, redness, and foreign body sensation (stinging) (Kheirkhah et al. 2007, de Venecia and Siong 2011, Wesolowska et al. 2014). Some researchers have reported that *Demodex* mites trigger itching and redness in blepharitis patients, and that the most common symptom in *Demodex* positive patients with blepharitis is itching followed by foreign body sensation, redness, burning, and watery eyes (Inceboz et al. 2009, Alver et al. 2017, Kabataş et al. 2017). In compliance with other studies, we determined that the most common complaint of the patients was itching, followed by foreign body sensation, redness, watering, burring, and burning. In *Demodex* positive patients with the complaints of itching, foreign body sensation, and redness, mean *Demodex* count was higher. Moreover, mean ocular symptom scores were significantly higher in *Demodex* positive patients than in *Demodex* negative patients.

All findings in our study confirm a positive correlation between the presence and load of *Demodex* mite, and chronic blepharitis. However, the fact that *Demodex* mites can also be found in healthy individuals and cause no clinical symptoms can lead to confusion. In this regard, many researchers have noted that *Demodex* mites destroy the follicular and sebaceous epithelial cells with perforating mouthparts and claws, break the skin barrier, and create a lymphocytic infiltrate around the follicle. Some researchers have reported that *Demodex* mites cause immune response after they penetrate into the dermis of the host. In addition to this, if the immune system is suppressed or inadequate (congenital or acquired), *Demodex* mites may increase in number and may be opportunistic pathogens (Forton 2012, Forton et al. 2015, Aytekin et al. 2017, Zeytun and Ölmez 2017). HLA (human leukocyte antigen) haplotypes, T and B lymphocytes, and NK (natural killer) cells play an important role in the formation of the immune response. In studies investigating the relationship between demodicosis (*Demodex* infestation) and HLA, it was emphasized that the HLA-A2 haplotype is protective against demodicosis. Individuals with this phenotype are three times more resistant to demodicosis, and individuals with HLA-CW2 and HLA-CW4 haplotypes are five times more likely to develop demodicosis and *Demodex* load increases due to the increase in lymphocyte and NK apoptosis (programmed cell death) in these individuals (Akilov and Mumcuoglu 2003, 2004; Mumcuoglu and Akilov 2005; Aytekin and Göktay 2015). Therefore, controls in our study could be asymptomatic despite being *Demodex* positive due to the genetic characteristics of these individuals and the HLA haplotypes they have. Despite the genetic component being a major conductor of *Demodex* pathogenesis, there are other possible causes: bacterial or viral infections could change the eyelid microenvironment favoring the proliferation of *Demodex*. They could also the immune response by reducing NK cells or lymphocytes, thus depleting the host from the main controllers of the mite. Immunosuppression by any cause could trigger disease in genetically resistant individuals. However, new studies are needed in this regard.

In the present study, the prevalence and load of *Demodex* in patients and controls increased with age in line with the literature (Arıcı et al. 2005, İnceboz et al. 2009, de Venecia and Siong 2011, Aycan-Kaya et al. 2012, Altınkurt et al. 2017, Kasetsuwan et al. 2017, Lopez-Ponce et al. 2017, Tilki et al. 2017, Zeytun 2017, Zeytun and Ölmez 2017, Zeytun et al. 2017). This finding can be explained by the fact that elderly have a weaker immune system than younger individuals and that the amount of sebum increases with age. In addition to this, the skin barrier function of elderly individuals may be disrupt due to weakening of skin repair or thinning of stratum corneum which the outermost layer of the epidermis (Blume-Peytavi et al. 2016, Demirdag et al. 2016, Zeytun 2017). On the other hand, *Demodex* prevalence and load was found to be higher in males than females in this study. While some studies also showed that *Demodex* prevalence is higher in male than female (Okyay et al. 2006, Durmaz et al. 2015, Tilki et al. 2017, Zeytun et al. 2017), in other studies the prevalence was higher in female (Ozdemir et al. 2005, Aycan et al. 2007, Zeytun 2017, Zeytun and Ölmez 2017) or equal among male and female (Godinez-Hana et al. 2004, Zhao et al. 2011). This may be related to personal care and hygienic practices. One of the important risk factors for *Demodex* mites infestation is hygienic behaviors of individuals. Forton et al. (2005) reported that the majority of patients with demodicosis did not use soap to wash their faces. Washing with soap and water probably helps fight mite proliferation by mechanical (face cloth) and chemical (soap) actions, those showing the importance of hygiene. Some researchers reported that *Demodex* mites were very prevalent in individuals who washed their face only once a day, did not use personal face towel (Zhao et al. 2011, Zeytun et al. 2017). However, some authors did not find correlation with regard to hygienic practices (Okyay et al. 2006). To summarize, *Demodex* mites were found to be very common and intense in patients with chronic blepharitis in Erzincan. Ocular complaints with the highest mean score in *Demodex* positive patients were itching, foreign body sensation, and redness. There was a positive correlation between *Demodex* mites and chronic blepharitis and ocular symptoms. It may be helpful to consider these findings in clinical assessment of blepharitis patients.

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