

Could ocular demodicosis be a risk factor for punctal stenosis, dry eye, and blepharitis?

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Purpose: *Demodex*, an ectoparasite, can threaten eye health by settling into different eyelash bases. It is particularly suggested to cause dysfunction in the Meibomian glands. This cross-sectional study aimed to investigate the relationships between ocular demodicosis, punctal stenosis, dry eye, and blepharitis. **Methods:** A total of 144 patients who presented to the Ophthalmology Clinic and volunteered to participate in the study were included. The demographic characteristics of the patients, presence of blepharitis, tear breakup time, eye-watering according to Munk's epiphora scoring, and punctal structures according to Kashkouli classification were recorded. Eyelash epilation was performed while preserving the root structure, and the eyelashes were examined under a light microscope. The relationship between *Demodex* presence and severity with punctal structure, dry eye status, and blepharitis was evaluated. **Results:** No significant relationship was found between punctal stenosis and *Demodex* spp. infestation in both eyes ($P > 0.05$). Overall, there was a statistical relationship between the presence of *Demodex* spp. settled in the eyelashes (right lower and upper, left upper eyelid lashes) and dry eye ($P < 0.05$), but there was no relationship with *Demodex* density (>3 /eyelid). In eyes with blepharitis, especially in patients with anterior blepharitis, the presence and density of *Demodex* spp. were higher than in patients without blepharitis ($P < 0.001$ for the right upper, left lower, and upper eyelids, $P = 0.001$ for the right lower eyelid). **Conclusion:** *Demodex* infestations may be associated with blepharitis and dryness of eyes, but there is no association with punctal stenosis.

Key words: Blepharitis, *Demodex*, ectoparasite, epiphora, inflammation, punctal stenosis

Demodex is the most common ectoparasite in humans. While numerous *Demodex* species are found in animals, only *Demodex folliculorum* and *Demodex brevis* have been identified in humans. *D. folliculorum* can be found singularly or in clusters in eyelash follicles or other hairy areas of the body, whereas *D. brevis* resides singly in areas abundant with sebaceous glands.^[1] These mites are assumed to have a symbiotic relationship with humans, feeding on sebum and living commensally. However, it is suggested that when mite numbers reach a critical level, they can potentially develop a pathogenic role in the host, although the host's immune system appears to tolerate their presence, indicating that they may be pathogenic if their numbers increase significantly.^[2] The sizes and preferred locations of *Demodex* species settled in humans vary.^[3] *D. folliculorum*, which is larger, typically covers the eyelash base and contributes to anterior blepharitis, while the smaller *D. brevis* inhabits the meibomian glands and contributes to posterior blepharitis.^[3-5]

Blepharitis is characterized by chronic inflammation manifested by eyelid redness, irritation, and discomfort.^[6] Clinically, blepharitis can be divided into anterior and posterior types, although both presentations often coexist. Posterior blepharitis is characterized by inflammation at the level of the

inner aspect of the eyelid involving the meibomian glands.^[7] Abnormal secretions from the meibomian glands have a direct toxic effect on the ocular surface.^[8] Additionally, meibomian gland abnormalities provide an environment supportive of bacterial growth. Prolonged inflammation leads to dysfunction of the glands and fibrosis, as well as damage to the eyelid and ocular surface. Anterior blepharitis is characterized by inflammation at the base of the eyelashes.^[9] Various factors such as allergens, bacterial exotoxins, parasitic infections, and various ocular surface disorders may contribute to the pathophysiology of anterior blepharitis.^[10] While blepharitis can result from various causes, one of the most common is *Demodex* infestation.^[6] The pathogenesis of *Demodex* blepharitis is explained by mechanisms such as direct damage by *Demodex*, serving as a vector for bacteria, and eliciting hypersensitivity reactions.^[11]

Apart from congenital causes, acquired anatomical punctal stenosis can arise due to various factors. Acquired punctal stenosis refers to the narrowing or obstruction of the external opening of the lacrimal canaliculus located at the nasal part of the upper and lower palpebral borders. Caesar and McNab

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defined it as a punctal diameter of less than 0.3 mm or the inability to intubate the punctum without dilation using a 26-gauge cannula.^[12] The causes of punctal edema or stenosis can be infectious or non-infectious. Inflammatory eyelid disorders, such as chronic blepharitis and ocular surface disease, especially systemic or topical medication use following cataract and glaucoma surgery, are among the most common causes of punctal stenosis.^[13] Aging has been described as a cause of punctal stenosis in various studies.^[14,15] Although a precise pathophysiological explanation has not been identified, chronic inflammation, leading to gradual fibrotic changes in the punctum ostium and subsequent progressive occlusion of the canal, is believed to be responsible for the possible pathogenesis.^[16-18] A study examining the histopathological features of 20 cases of punctal stenosis revealed widespread fibrosis in 30% of the samples, inflammation in 80%, and squamous metaplasia in 15%.^[18] Given the high prevalence of ocular demodicosis in both symptomatic and asymptomatic populations as a risk factor for blepharitis, its predisposing effect for punctal stenosis should be considered in the context of chronic inflammation.^[19]

Cylindrical dandruff (CD) consists of fine, waxy, and dry flakes concentrated at the base of the eyelashes and is considered pathognomonic for *Demodex*, indicating the presence of high-density *Demodex* mite infestation. Since CD indicates a higher level of mite detection, *Demodex* numbers can vary depending on the presence of CD at the epilated eyelash base.^[20] While there is a general consensus that CD is pathognomonic for *Demodex*, visual confirmation of the mite itself is required to confirm the diagnosis.^[1] Unlike the evaluation of facial demodicosis, there is no standardized mite counting technique for the eyelids. Despite the lack of standardization, eyelash epilation and *in vivo* confocal microscopy (IVCM) techniques are commonly used.^[21]

No study has been found in the literature that examines the relationship between ocular demodicosis and punctal structures. This study aims to investigate the relationship between ocular demodicosis and punctal stenosis, blepharitis, and dry eye and to contribute the resulting data to the literature.

Methods

Following ethical committee approval, a total of 144 patients presenting to the Ophthalmology Clinic of Akdeniz University who volunteered to participate were included in this study. Patients with a history of eye trauma, ocular malposition, ocular tumor, chronic ocular surface disorder, or topical and systemic medication use affecting the ocular surface or punctum (such as steroids, antiglaucomatous agents, antihistamines, etc.) were excluded. The patients' age, gender, known ocular and systemic diseases, smoking status, current complaints, tear breakup time (TBUT), and eye-watering according to Munk's epiphora scoring were recorded.^[22,23] Ophthalmic anterior segment examination was performed by an experienced ophthalmologist (Dr. I.B.). The measured TBUT value was recorded in seconds, and dry eye presence was considered in patients with a TBUT result of <10 seconds. Punctal structures were graded from 0 to 5 based on biomicroscopic appearance according to the Kashkouli classification and recorded (Grade 0 [punctal atresia], Grade 1 [papilla covered with a membrane or fibrosis], Grade 2 [smaller than normal

but recognizable], Grade 3 [normal size], Grade 4 [small fissure < 2 mm], and Grade 5 [large fissure ≥ 2 mm]).^[14]

Eyelash epilation was performed with the preservation of the root structure using forceps under biomicroscopy. Two eyelashes were collected from each eyelid. Eyelashes showing marked hyperemia and CD suspected for *Demodex* were preferred for sample collection. The collected samples were placed on a glass slide and immediately examined under a light microscope after fixation with cellophane tape [Figs. 1 and 2]. The identified *Demodex* numbers were recorded.

In the study, the total number of *Demodex* detected in each eyelid was used for parasitic quantification. Statistical analysis was performed by evaluating the *Demodex* quantification in two different ways. Similar to Randon *et al.*,^[24] patients with a *Demodex* count >3 were considered to have high infestation. Following a similar approach to Murphy *et al.*,^[25] the infestation was graded from 0 to 3 based on severity (Grade 0: No parasites detected, Grade 1: 1–3 parasites, Grade 2: 4–6 parasites, Grade 3: ≥7).

Ethical Approval: This study was conducted with the approval of the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee and in accordance with the guidelines of the Declaration of Helsinki.

Statistical Analysis: For statistical analysis, PASW Statistics for Windows, version 23.0 (SPSS Inc., Chicago, USA) was used. Descriptive statistics such as mean, standard deviation, frequency, and percentage were used to evaluate variables. Categorical data were compared using the Chi-square test, and a comparison of parametric values was performed using the Student's *t*-test. A *P* value < 0.05 was considered statistically significant.

Results

In the study, a total of 144 patients were included, of whom 90 (62.5%) were female, and 54 (37.5%) were male. Patients with detected punctal stenosis (*n*: 52) and those without punctal stenosis (*n*: 92) were statistically compared in terms of age, gender, smoking status, type of blepharitis, TBUT, eye-watering in the eyes, and other diseases. A statistically significant difference was found between the two groups in



Figure 1: *Demodex* spp. egg

terms of age, while no significant differences were observed in other variables [Table 1].

Punctal stenosis was evaluated for each eyelid. Stenosis was detected in 27.8% (n : 40) of the right upper, 26.4% (n : 38) of the right lower, 28.5% (n : 41) of the left upper, and 25.7% (n : 37) of the left lower eyelids in patients [Figs. 3 and 4]. When compared with individuals having more than three *Demodex* spp., statistically significant differences were not observed for the right upper (P : 0.554), right lower (P : 0.058), left upper (P : 0.732), and left lower (P : 0.831) eyelids. There was also no statistically significant difference observed when comparing the presence of punctal stenosis with the severity of *Demodex* spp. [Table 2].

In the examination of the relationship between TBUT value and the presence and severity of *Demodex*, statistically

significant relationships were found between having more than three *Demodex* counts on the lower left eyelid and dryness in the left eye (p : 0.044). Additionally, statistically significant relationships were found between *Demodex* severity and dryness in the right upper, right lower, and left lower eyelids (P : 0.008, P : 0.035, P : 0.012, respectively) [Table 3].

Demodex was detected in any eyelid of 100 (69.4%) out of a total of 144 patients. Blepharitis was detected in any eyelid of 92 (63.9%) out of a total of 144 patients. There was a statistically significant relationship between the presence and severity of *Demodex* and the presence of anterior or any type of blepharitis in both eyes (P < 0.001 and P : 0.001) [Table 4]. Additionally, a statistically significant relationship was found between the presence and severity of *Demodex* and posterior blepharitis in the left upper eyelid (P = 0.047).

Table 1: Comparison of demographic findings of patients with and without punctal stenosis

	Punctal stenosis (+) (n : 52)	Punctal stenosis (–) (n : 92)	P
Age (Mean \pm SD)	51.5 \pm 18.7	44.0 \pm 14.5	0.008
Sex (F), (n , %)	37 (71.1)	53 (57.6)	0.107
Cigarettes, (n , %)			
Didn't	42 (80.8)	68 (73.9)	0.435
Active	10 (19.2)	22 (23.9)	
Left	–	2 (2.2)	
Blepharitis type, (n , %)			
Right eye, Anterior	33 (63.5)	52 (56.5)	0.416
Posterior	17 (32.7)	28 (30.4)	0.779
Left eye, Anterior	33 (62.5)	50 (54.4)	0.288
Posterior	18 (34.6)	29 (31.5)	0.704
TBUT (n , %)			
Right (abnormal, low)	20 (38.5)	32 (34.8)	0.659
Left (abnormal, low)	21 (40.4)	33 (35.9)	0.591
Eye-watering (+), (n , %)	20 (38.5)	37 (40.2)	0.836
Comorbidity, (n , %)			
None	37 (71.1)	74 (80.4)	0.482
≥ 1 comorbidity	15 (28.9)	18 (19.6)	
Hypertension	8 (15.4)	10 (10.9)	
diabetes mellitus	6 (11.5)	10 (10.9)	
Asthma	1 (1.9)	–	
Epilepsy	–	1 (1.1)	
Hypothyroidism	1 (1.9)	–	
Dyslipidemia	–	1 (1.1)	
Prostate cancer	1 (1.9)	–	

Table 2: Relationship between punctal stenosis and *Demodex* spp

	Punctal stenosis							
	Right upper eyelid		Right lower eyelid		Left upper eyelid		Left lower eyelid	
	(+) (n : 40)	(–) (n : 104)	(+) (n : 38)	(–) (n : 106)	(+) (n : 41)	(–) (n : 103)	(+) (n : 37)	(–) (n : 107)
<i>Demodex</i> spp. number (>3), (n , %)	6 (15.0)	20 (19.2)	3 (7.9)	23 (21.7)	9 (21.9)	20 (19.4)	5 (13.5)	16 (14.9)
P	0.554		0.058		0.732		0.831	
<i>Demodex</i> spp. severity, (n , %)								
Absent (0)	22 (55.0)	55 (52.9)	27 (71.1)	57 (53.8)	21 (51.2)	52 (50.1)	20 (54.1)	55 (51.4)
Mild (1–3)	12 (30.0)	29 (27.9)	8 (21.1)	26 (24.5)	11 (26.8)	31 (30.1)	12 (32.4)	36 (33.6)
Moderate (4–6)	4 (10.0)	13 (12.5)	2 (5.1)	17 (16.0)	7 (17.1)	14 (13.6)	3 (8.1)	10 (9.3)
Severe (≥ 7)	2 (5.0)	7 (6.7)	1 (2.6)	6 (5.7)	2 (4.9)	6 (5.8)	2 (5.4)	6 (5.6)
P	0.948		0.207		0.940		0.992	

Table 3: Relationship between dryness in the eyes and *Demodex* spp

	Right eye dryness					Left eye dryness				
	TBUT <10 sn		TBUT ≥ 10 sn		P	TBUT <10 sn		TBUT ≥ 10 sn		P
	(n: 52)		(n: 92)			(n: 54)		(n: 90)		
	n	%	n	%		n	%	n	%	
<i>Demodex</i> spp. number (>3)										
Upper	13	25.0	13	14.1	0.103	14	25.9	15	16.7	0.180
Lower	13	25.0	13	14.1	0.103	12	22.2	9	10.0	0.044
<i>Demodex</i> spp. severity										
Upper										
Absent	18	34.6	59	64.1	0.008	22	40.7	51	56.7	0.204
Mild (1–3)	21	40.4	20	21.7		18	33.3	24	26.7	
Moderate (4–6)	8	15.4	9	9.8		9	16.7	12	13.3	
Severe (≥7)	5	9.6	4	4.3		5	9.3	3	3.3	
Lower										
Absent	23	44.2	61	66.3	0.035	22	40.7	53	58.9	0.012
Mild (1–3)	16	30.8	18	19.6		20	37.0	28	31.1	
Moderate (4–6)	8	15.4	11	11.9		5	9.3	8	8.9	
Severe (≥7)	5	9.6	2	2.2		7	13.0	1	1.1	

**Figure 2: *Demodex folliculorum* adults**

Discussion

Due to the absence of a known, validated scale for counting mites in eyelash follicles, there are variations among studies, making them often incomparable. While Aumond *et al.* and Murphy *et al.* collected a single lash per lid in their studies, some studies gathered 2–3 lashes per lid, while others only collected four lashes from the upper lid.^[21,26–28] In this study, two lashes were collected from each lid. Similar to the study by Randon *et al.*^[24] and Murphy *et al.*,^[25] data were evaluated using two different grading systems.

Acquired external punctal stenosis can arise from the toxic effects of topical or systemic medications, various infections, lid malposition, or different types of trauma and tumors. Aging changes can also lead to punctal stenosis.^[14] Chronic eyelid inflammation, especially chronic blepharitis, continues to be a widely recognized cause of acquired punctal stenosis. It has been proposed that chronic inflammation of the external punctum in the pathogenesis leads to gradual fibrotic changes in the ostium, followed by progressive canal occlusion.^[29]

**Figure 3: Right lower stenotic punctum**

Studies have shown that chronic inflammation plays a central role in the pathogenesis of punctal stenosis.^[17,18] Prior studies have extensively explored the role of *Demodex* in blepharitis and dry eye; however, its potential association with punctal stenosis has not been systematically evaluated. Upon review of the literature, no study comparing punctal stenosis with *Demodex* positivity could be found. Our study result is the first report to investigate the relationship between ocular demodicosis and punctal stenosis in the literature. Our study fills the gap by demonstrating no relationship between punctal stenosis and the presence and severity of *Demodex* spp. Considering that *Demodex* can cause pathology in the punctum both inflammatorily and mechanically, the presence and severity of *Demodex* with punctal stenosis were evaluated for each lid, and no relationship was found between the presence and severity of *Demodex* spp. and punctal stenosis. These findings suggest that while addressing *Demodex* may benefit patients with blepharitis and dry eye, additional etiological factors contribute to punctal stenosis that warrants further investigation.

Impairment of Meibomian gland function adversely affects both the quality and quantity of its secretion. This affects ocular surface health through changes in tear film composition.^[30] In a study evaluating changes in meibum

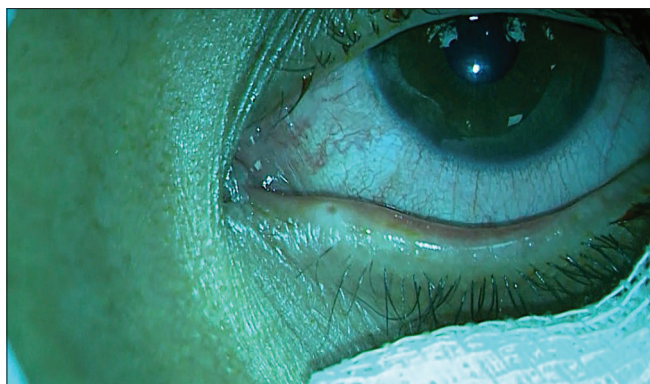


Figure 4: Left lower normal punctum

in patients with ocular *Demodex* infestation, a significant increase in 19 different types of OAHFA ([O-acyl]- ω -hydroxy fatty acids) associated with tear film stability was detected in the *Demodex*-positive group, suggesting that OAHFA may be associated with the progression of ocular *Demodex* infections.^[31] Mites' lipases and proteases found in cylindrical deposits cause irritation and inflammation on the ocular surface, leading to dryness, itching, foreign body sensation, and redness of the eyelid.^[32] Meibomian gland dysfunction (MGD) is considered a significant factor in dry eye disease. In a study by Hao Y *et al.*^[33] comparing 68 patients with MGD-associated dry eye disease and *Demodex* infestation to 51 patients without *Demodex* infestation, *Demodex folliculorum* infestation was reported to be one of the most significant contributions to the pathogenesis of MGD-associated dry eye. In our study, it was generally observed that more *Demodex* settled in dry eyes. However, a high *Demodex* count (>3) was not statistically significant. The rate of mild *Demodex* infestation (1-3) was higher in patients with dry eyes than others. Regardless of density, the presence of *Demodex* infestation on eyelashes may contribute to the development of dryness as a factor for MGD. Our findings complement those of Hao *et al.*

Demodex can have an effect on anterior blepharitis, posterior blepharitis, and MGD on the anterior ocular surface. Streptococci and staphylococci on the surface of mites are directly associated with both anterior and posterior microbial blepharitis. Additionally, it has been shown that bacteria harbored within mites (*Bacillus oleronius*) activate the host's immune response.^[20] The parasite can also block the openings of follicles and/or sebaceous ducts and cause epithelial hyperplasia and hyperkeratinization.^[1,32] Additionally, they can deposit their eggs at the base of lashes, leading to follicular swelling. Since mites lack an excretory organ and cannot expel their material, they have been reported to cause CD, a pathognomonic sign of demodicosis, by adhering to keratin and epithelial cell accumulations.^[32] Furthermore, these mites vomit fecal matter after death, which can cause an inflammatory response in the host.^[3] Various studies on patients with blepharitis have shown significantly higher *Demodex* counts compared to control groups. In a study by Akkucuk *et al.*,^[4] *Demodex* was found in 75.5% of the blepharitis group compared to 16.2% in the control group ($P < 0.001$), while in a study by Kabataş *et al.*,^[27] *Demodex* was found in 67.2% of the blepharitis group compared to 54.9% in the control group ($P = 0.18$). In a study of patients with chronic blepharitis, *Demodex* spp. was observed in 60% of patients, and the species

identified in all positive samples was reported as *D. folliculorum*.^[34] In a study by Bhandari *et al.*,^[35] *Demodex* infestation was found in 78.6% of symptomatic patients compared to 18% in the control group ($P < 0.001$), and the frequency of *Demodex* infestation was reported to be statistically significantly higher in cases of anterior blepharitis and mixed blepharitis. In our study, we also found significant associations between the presence of blepharitis in both eyes and *Demodex* infestation. We found that eyes with blepharitis, especially in the upper eyelid lashes, harbored more *Demodex* spp. Furthermore, *Demodex* presence and density may contribute more to anterior blepharitis compared to posterior blepharitis. *Demodex* infestations may be considered a factor or risk factor for blepharitis.

Conclusion

While our study found an association between blepharitis, dry eye, and *Demodex* infestations, the relationship between punctal stenosis and *Demodex* presence was not demonstrated. Additional studies are needed to elucidate the mechanisms by which *Demodex* spp. contribute to the development of blepharitis and dryness.

Ethical approval

This study was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (08/07/2020/Akdeniz University KAEK- 501). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from the patients involved in this study.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All the datasets obtained in the current study have been deposited in hospital archive.

Contributions

Methodology, I.B., H.Y and H.D.I.; investigation, I.B. and H.Y.; resources, O.K.O. and B.O.; data curation, I.B., H.Y. and H.D.I.; writing-original draft preparation, I.B. and H.Y.; writing-review and editing, H.D.I., O.K.O. and B.O.; supervision, H.D.I. and B.O.; project administration, I.B. and H.Y. All authors reviewed the manuscript.

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