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# Assessment of *Demodex* Sp. Prevalence in Blepharitis Patients: Comparison of Light and Fluorescence Microscopy Techniques

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## ABSTRACT

**Objective:***Demodex* mites, one of the most prevalent human ectoparasites, are frequently associated with blepharitis. Despite their widespread occurrence, *Demodex* blepharitis remains underdiagnosed and often overlooked in clinical practice. This study investigates the prevalence of *Demodex* sp. in blepharitis patients, compares the diagnostic effectiveness of light and fluorescence microscopy, and examines the relationship between *Demodex* infestation and associated clinical symptoms.

**Methods:**A total of 240 individuals, 120 patients with blepharitis and 120 controls, were included in the study. Eyelash samples from both groups were analyzed using light microscopy and fluorescence microscopy to detect *Demodex* sp. Additionally, clinical and demographic data were gathered through a structured questionnaire.

**Results:***Demodex* sp. were detected in 70.8% (85/120) of patients with blepharitis using light microscopy and in 80.8% (97/120) using fluorescence microscopy. In contrast, among patients without blepharitis, *Demodex* sp. were identified in 47.5% (57/120) of cases by both methods. The prevalence of *Demodex* sp. was significantly higher in patients with blepharitis compared to controls ($p<0.001$). Fluorescent microscopy exhibited a higher detection rate than light microscopy ($p=0.012$).

**Conclusions:***Demodex* mites play a significant role in ocular disorders, particularly blepharitis. Timely and accurate diagnosis, along with appropriate treatment, is essential to prevent the adverse mental and physical health impacts associated with *Demodex* blepharitis.

***Keywords***: *Demodex*, Blepharitis, Fluorescence Microscopy.

# Blefaritli Hastalarda Demodex Türlerinin Prevalansının Değerlendirilmesi: Işık ve Floresan Mikroskopi Tekniklerinin Karşılaştırılması

**Araştırma Makalesi**

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## ÖZ

**Amaç:***Demodex* sp., en yaygın görülen insan ektoparazitlerinden biri olup sıklıkla blefarit ile ilişkili bulunmaktadır. *Demodex* blefariti, yaygın görülmesine rağmen klinik uygulamalarda yeterince tanı konulamayan ve göz ardı edilen bir durumdur. Bu çalışmada, blefarit hastalarında *Demodex* sp. görülme sıklığının araştırılması, ışık mikroskopisi ile floresan mikroskopinin tanısal etkinliğinin karşılaştırılması ve *Demodex* enfestasyonu ile klinik semptomlar arasındaki ilişkinin incelenmesi amaçlanmıştır.

**Yöntem:**Çalışmaya 120 blefaritli hasta ve 120 kontrol grubu olmak üzere toplam 240 kişi dahil edilmiştir. *Demodex* sp. varlığını saptamak üzere, her iki gruptan alınan kirpik örnekleri, ışık mikroskobu ve floresan mikroskobu kullanılarak incelenmiştir. Tüm katılımcılara uygulanan anket aracılığıyla klinik ve demografik veriler elde edilmiştir.

**Bulgular:***Demodex* sp., blefaritli hastaların %70,8'inde (85/120) ışık mikroskobu ile, %80,8'inde (97/120) ise floresan mikroskobu kullanılarak tespit edilmiştir. Buna karşılık, blefariti olmayan kontrol grubunda her iki yöntemle olguların %47,5'inde (57/120) *Demodex* sp. saptanmıştır. *Demodex* sp. prevalansı, blefaritli hastalarda kontrol grubuna kıyasla anlamlı olarak daha yüksek bulunmuştur ($p<0.001$). Floresan mikroskopisi, ışık mikroskopisine göre daha yüksek bir tespit oranı göstermiştir ($p=0.012$).

**Tartışma:***Demodex* sp., özellikle blefarit olmak üzere oküler rahatsızlıklarda önemli bir rol oynar. *Demodex* blefaritin fiziksel ve zihinsel sağlık üzerine olumsuz etkilerini önlemek için zamanında ve doğru teşhis ile uygun tedavi büyük önem taşımaktadır.

***Anahtar Kelimeler***: *Demodex*, blefarit, floresan mikroskopi

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## Introduction

*Demodex* mites, first described in 1841, are among the most common human ectoparasites. Two species, *Demodex folliculorum (D. folliculorum)* and *Demodex brevis (D. brevis)* primarily clusters in hair follicles on the face, and resides in sebaceous and meibomian glands, respectively.[1,2] While *Demodex* infestation often remains asymptomatic, it can become pathogenic at high densities or in individuals with immune imbalances. Infestation with *D. folliculorum* is more prevalent than with *D. brevis*, although *D. brevis* has a broader distribution across the body. The prevalence of *Demodex* infestation increases with age, peaking in the 20-30 age group, coinciding with maximal sebum production.[3,4] High *Demodex* loads have been linked to dermatological conditions like rosacea, folliculitis, and pityriasis folliculorum, as well as ocular disorders including blepharitis, cylindrical dandruff, keratitis, and conjunctivitis.[5,6]

Blepharitis, characterized by eyelid and eyelash inflammation, manifests with symptoms such as itching, burning, photophobia, dryness, eyelash loss, and dandruff near eyelash bases. It sometimes mimics other ocular conditions, making effective treatment challenging. The involvement of *Demodex* sp., alongside bacterial agents, is believed to contribute to cases of treatment-resistant or recurring blepharitis, necessitates further investigation.[7-9]

There is general agreement that cylindrical dandruff is indicative of *Demodex* blepharitis; however, visual identification of the mite remains the diagnostic standard. Currently, diagnosis lacks standardized methodology, with two primary techniques reported: eyelash epilation with microscopic examination and in vivo confocal microscopy (IVCM). Compared to IVCM, lash epilation is more commonly used in research and is more accessible in clinical practice. The procedure involves selecting lashes under a slit lamp, extracting them with tweezers from the follicle, and placing them on a microscope slide for ex vivo examination. By using varying magnifications and adjusting the focal plane on a light microscope, the entire mite can be visualized.[10]

Under light microscopy, the mite appears as a semi-transparent organism characterized by two connected body segments and eight legs. *D. folliculorum* measures approximately 0.3–0.5 mm in length, whereas *D. brevis* ranges from 0.15–0.3 mm.[11] An important feature of *Demodex* sp. is their natural autofluorescence, which enhances their visibility in fluorescence microscopy and is critical for accurate detection.[12]

The objective of this study was to investigate the frequency of *Demodex* sp. among patients diagnosed with blepharitis, evaluate the diagnostic accuracy of direct and fluorescent microscopy techniques, and assess the relationship between parasite presence and symptoms.

## METHODS

### *Ethics Approval*

The study was approved by the Clinical Research Ethics Committee of Ankara Training and Research Hospital (date: 05.12.2019, number: E-19/127). Participants provided informed consent, and the study adhered to the Declaration of Helsinki principles.

### *Study Population*

A total of 120 individuals over the age of 18 years with a diagnosis of blepharitis who were admitted to the Ophthalmology Clinic of Ankara Training and Research Hospital, between January 2020 and March 2020 were included as the patient group. As a control group, 120 individuals over 18 years of age without a diagnosis of blepharitis were included in the study.

### *Questionnaire*

Participants completed a questionnaire detailing age, gender, health conditions, occupation, education, residence, household size, glasses use, facial cleansing habits, makeup practices, skin type, pet ownership, and symptoms like itching and redness.

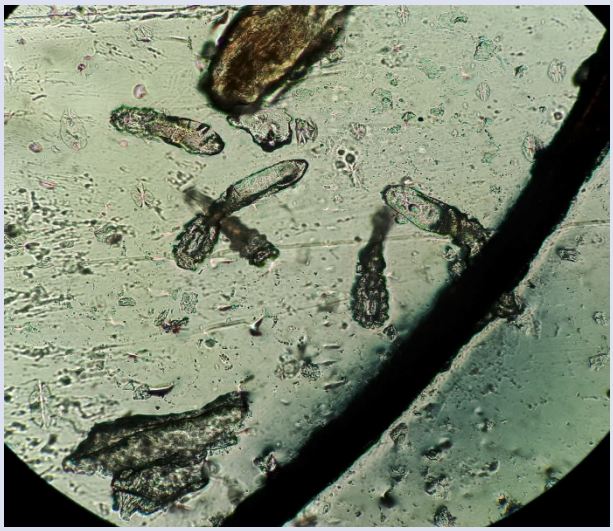

*Figure 1. Light microscopy image showing Demodex mites under direct observation (x400)*

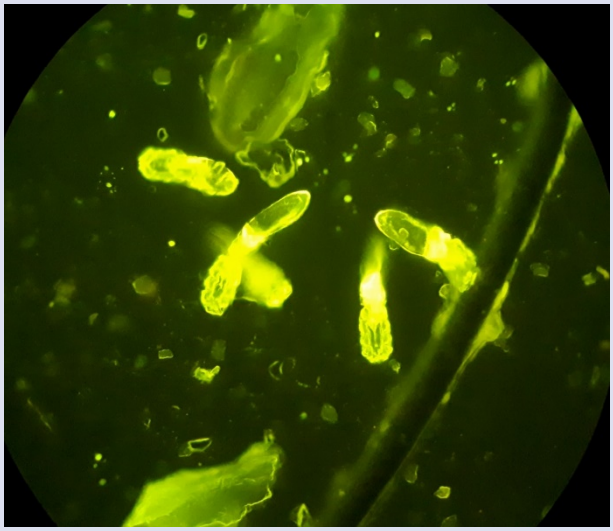

*Figure 2. Fluorescence microscopy image showing Demodex mites under direct observation (x400).*

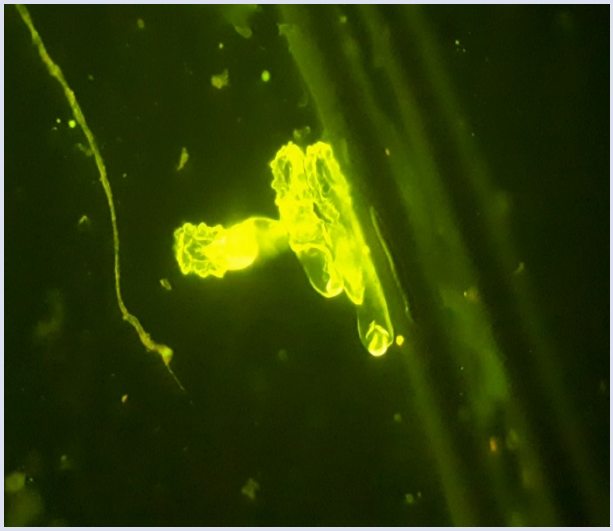

*Figure 3. Fluorescence microscopy image showing Demodex mites under direct observation (x400).*

*Figure 4. Fluorescence microscopy image showing Demodex mites under direct observation (x400).*

### *Eyelash Sampling and Microscopy*

To detect *Demodex* sp. in eyelash samples, all participants included in the study underwent eyelash epilation. For this purpose, eyelash specimens were obtained from patients examined by an ophthalmologist using sterile optical forceps. A total of eight eyelashes were collected from each individual: two from each eyelid, including both the upper and lower eyelids. The collected eyelashes were mounted on glass slides using cellophane tape for preparation. Upon arrival at the laboratory, the slides were examined without delay. Initially, all eyelash structures and their surrounding areas were thoroughly inspected under a light microscope at magnifications of 5×, 10×, 20×, and 40×. The presence and number of *Demodex* sp. at various developmental stages (larvae, nymphs, or adults) were recorded, along with the specific eyelid regions from which the samples were obtained. Subsequently, the same preparations were examined under a fluorescence microscope using blue light at a wavelength of 455 nm at magnifications of 5X, 10X, 20X, and 40X (Figure 1-4). The presence and number of autofluorescent mites, as well as the corresponding sampling sites, were similarly documented.

### *Statistical Analyses*

Data were analyzed using SPSS v22. The Mann-Whitney U test compared nonparametric numerical variables, and the Chi-Square test assessed categorical variables. The Wilcoxon test evaluated differences between microscopy methods, and the Spearman correlation test examined relationships. A p-value <0.05 was considered statistically significant.

*Table 1. Distribution of Demodex sp. positivity in patients with and without blepharitis by using light and fluorescent microscopic examination methods.*

| | ***Patients with blepharitis* (n=120)** | | ***Patients without blepharitis* (n=120)** | | p |
|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | |
| **Number of patients *Demodex* detected by light microscopy** | 85 | 70.8% | 57 | 47.5% | <0.001 |
| **Number of patients *Demodex* detected by fluorescent microscopy** | 97 | 80.8% | 57 | 47.5% | |

*Table 2. The age and gender distribution of Demodex-positive patients with and without blepharitis.*

| | ***Patients with blepharitis* (n=97)** | ***Patients without blepharitis* (n=57)** | **p** |
|---|---|---|---|
| **Age** | 58,30±14,63 | 54,04±16,17 | 0,069 |
| **Gender** | | | |
| Male | 29 (78.4%) | 17 (%) | 1,000 |
| Female | 68 (81.9%) | 40 (%) | |

## RESULTS

Among blepharitis patients, 30.8% (n=37) were male and 69.2% (n=83) female, with a mean age of 57.29±14.71 (12-86) years. In the control group without blepharitis, 36.7% (n=44) were male and 63.3% (n=76) female, with a mean age of 49.76±16.88 (12-85) years. No significant age or gender differences were observed between groups (p>0.05).

*Demodex* sp. prevalence in blepharitis patients was 70.8% (85/120) via light microscopy and 80.8% (97/120) via fluorescence microscopy. Among the patients with blepharitis, the detection of *Demodex* positivity was higher through fluorescence microscopy compared to light microscopy (p<0.001). In individuals without blepharitis, the prevalence of *Demodex* sp. was established at 47.5% (57/120) using both light microscopy and fluorescent microscopy. *Demodex* sp. was statistically more prevalent in the patient group compared to the control group (p<0.001) (Table 1).

The age and gender distribution of *Demodex*-positive patients with and without blepharitis is presented in Table 2. No significant relationship was found between gender and the presence of *Demodex* sp. (p>0.05). In the control group without blepharitis, *Demodex* positivity was notably higher in the older age groups, while no significant difference was observed between age groups in patients with blepharitis.

*Table 3. Comparison of daily habits of Demodex-positive patients with and without blepharitis.*

| | *Patients with blepharitis*(n=97) | | *Patients without blepharitis*(n=57) | | p |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Use of glasses** | | | | | |
| None | 46 | 47.4 | 20 | 35.1 | |
| Glasses | 50 | 51.6 | 37 | 64.9 | 0.171 |
| Contact lens | 1 | 1.0 | 0 | 0 | |
| **Residence** | | | | | |
| Village | 2 | 2.1 | 1 | 1.8 | |
| District | 5 | 5.1 | 11 | 19.3 | 0.016 |
| City center | 90 | 92.8 | 45 | 78.9 | |
| **Face washing frequency** | | | | | |
| Once | 4 | 4.1 | 1 | 1.7 | |
| Twice | 11 | 11.4 | 14 | 24.6 | 0.072 |
| More than twice | 82 | 84.5 | 42 | 73.7 | |
| **Face washing product** | | | | | |
| None | 84 | 86.6 | 43 | 75.4 | |
| Soap | 12 | 12.4 | 7 | 12.3 | 0.01 |
| Cosmetic product | 1 | 1.0 | 7 | 12.3 | |
| **Makeup usage** | | | | | |
| None | 88 | 90.7 | 46 | 80.7 | |
| Occasionally | 8 | 8.2 | 10 | 17.5 | 0.184 |
| Frequently | 1 | 1.1 | 1 | 1.8 | |
| **Skin type** | | | | | |
| Dry | 13 | 13.4 | 26 | 45.6 | |
| Oily | 5 | 5.2 | 3 | 5.3 | <0.001 |
| Combination | 79 | 81.4 | 28 | 49.1 | |
| **Pet ownership** | | | | | |
| Yes | 12 | 12.4 | 9 | 15.8 | 0.629 |
| No | 85 | 87.6 | 48 | 84.2 | |

*Table 4. Comparison of comorbidity, occupation, education level and symptoms between Demodex-positive patients with and without blepharitis*

| | *Patients with blepharitis* (n=97) | | *Patients without blepharitis* (n=57) | | p |
|---|---|---|---|---|---|
| **Comorbidity** | n | % | n | % | |
| Hypertension | 26 | 26.8 | 16 | 28.1 | |
| Diabetes Mellitus | 10 | 10.3 | 7 | 12.3 | |
| Hypothyroidism | 4 | 4.1 | 3 | 5.7 | |
| Cardiac Disease | 8 | 8.2 | 1 | 1.8 | |
| Cerebrovascular Event | 1 | 1.0 | 0 | 0 | 0.350 |
| Hypercholesterolemia | 0 | 0 | 1 | 1.8 | |
| Cancer | 4 | 4.1 | 1 | 1.8 | |
| Pulmonary Disease | 1 | 1.0 | 0 | 0 | |
| Gastrointestinal Disorder | 1 | 1.0 | 0 | 0 | |
| **Occupation** | | | | | |
| Housewife | 64 | 66.0 | 28 | 49.1 | |
| Officer | 13 | 13.4 | 10 | 17.5 | |
| Worker | 3 | 3.1 | 9 | 15.8 | 0.023 |
| Farmer | 6 | 6.2 | 1 | 1.8 | |
| Student | 1 | 1.0 | 0 | 0 | |
| Retired | 10 | 10.3 | 9 | 15.8 | |
| **Education Level** | | | | | |
| None | 28 | 28.9 | 12 | 21.1 | |
| Primary School | 39 | 40.2 | 18 | 31.6 | |
| Middle School | 8 | 8.2 | 7 | 12.3 | |
| High School | 14 | 14.4 | 9 | 15.8 | 0.118 |
| University | 6 | 6.2 | 11 | 19.3 | |
| Master's Degree | 2 | 2.0 | 0 | 0 | |
| Doctorate | 0 | 0 | 0 | 0 | |
| **Symptoms** | | | | | |
| Facial redness/acne | 30 | 31.0 | 10 | 17.5 | 0.087 |
| Facial itching | 18 | 18.6 | 6 | 10.5 | 0.251 |
| Ocular itching | 91 | 93.8 | 1 | 1.8 | <0,001 |
| Ocular burning | 92 | 94.8 | 0 | 0 | <0,001 |
| Ocular stinging | 94 | 97.0 | 0 | 0 | <0,001 |
| Ocular redness | 74 | 76.3 | 0 | 0 | <0,001 |

Comparison of daily habits of *Demodex*-positive patients with and without blepharitis is presented in Table 3. Patients with blepharitis were more likely to reside in city centers compared to those without blepharitis. The use of no facial cleansing product was significantly higher among patients with blepharitis, whereas cosmetic product use was more frequent among those without blepharitis. Skin type distribution differed markedly, with combination skin being predominant in the blepharitis group and dry skin more common in the control group.

Comparison of comorbidity, occupation, education level and symptoms between *Demodex*-positive patients with and without blepharitis is presented in Table 4. Occupational status varied significantly; housewives were more common in the blepharitis group, whereas workers were more frequent in the control group. Regarding symptoms, ocular manifestations such as itching, burning, stinging, and redness were significantly more prevalent among patients with blepharitis compared to controls.

## Discussion

*Demodex* is one of the most common ectoparasites in humans and its role in ocular diseases is still debated. While *Demodex* infestations are mostly asymptomatic, various studies have reported associations between the presence of *Demodex* and diseases such as blepharitis, meibomian gland dysfunction (MGD), keratitis, blepharokeratoconjunctivitis, chalazion, dry eye, and pterygium. *Demodex* mites have also been suggested as potential vectors for bacteria, transporting streptococci and staphylococci on their surfaces. This may lead to additional inflammatory responses in the surrounding tissues.[10] Additionally; *Demodex* blepharitis has been reported to have significant adverse effects on the mental and physical health of affected individuals, as well as their daily activities.[13]

A high prevalence of *Demodex* blepharitis has been reported globally, with rates ranging from 41% to 70%. Despite the widespread occurrence and high prevalence rates of *Demodex* blepharitis, patients may often go undiagnosed or misdiagnosed for years.[13] In the current study, the prevalence of *Demodex* sp. in patients with blepharitis was determined to be approximately 80%, which was found to be two times higher than in individuals without blepharitis. In Türkiye, *Demodex* detection rates in patients with blepharitis vary between studies. Turk et al. found the prevalence of *D. follicularum* to be 25% (12/48) in patients with blepharitis and 4.2% (2/48) in patients without blepharitis.[14] Tanrıverdi et al. reported the prevalence of *Demodex* sp. in patients with chronic blepharitis as 45.1% (69/153).[15] Similarly, in another study conducted by Demirkazık et al., *D. folliculorum* was detected in 42.6% (143/335) of the patients with blepharitis[16]. Akkucuk et al. found *Demodex* sp. in 75.5% (249/330) of the patients with blepharitis and 16.2% (21/130) of the control group.[17] In our study, a higher rate of *Demodex* positivity was observed compared to these studies. This could be attributed to the fact that the study was conducted in a hospital primarily serving patients with a low socioeconomic status, and a substantial number of eyelash examinations from each patient were performed.

In the current study, *Demodex* positivity was notably higher in advanced age in the control group, while no significant difference was observed in the patients with blepharitis. Although it is generally reported that the prevalence of *Demodex* sp. increases with age and *Demodex* infestation is rarely seen in paediatric age groups, a recent study has found no significant difference between young and elderly patients with ocular demodicosis in terms of *Demodex* counts, supporting our study.[18]

Rosacea, diabetes, local or systemic immunosuppression, stress, higher alcohol intake, increased sun exposure, and smoking have been reported as risk factors for the development of demodectic blepharitis.[19] There are several reports assessing the effect of chronic diseases on the etiopathogenesis of *Demodex* infestations. In a study, *Demodex* sp. was found in 27% of the patients with hypertension, 23.7% of the patients with hypothyroidism, 32.5% of the patients with chronic renal failure, and 18.5% of the patients with diabetes mellitus.[20] In another study, *D. folliculorum* was observed at a higher prevalence in individuals with metabolic syndrome compared to the healthy group, and it was suggested that high blood sugar levels in patients with metabolic syndrome make them more susceptible to *Demodex* infestation.[21] On the contrary, another study found metabolic syndrome had no effect on *Demodex* density but reported a significant relationship with diastolic hypertension and occurrence of the parasite.[22] In support of this report, in our study, the most frequently observed comorbid condition in the patients with *Demodex* blepharitis, was noted to be hypertension. Whereas hypertension in the patients with *Demodex* spp. was significantly higher compared to the healthy control group, no significant relationship was identified in patients with diabetes. More studies are needed to understand the roles of hypertension and diabetes in the pathogenesis of demodectic blepharitis.

Demodectic blepharitis is typically diagnosed clinically based on signs and symptoms such as cylindrical dandruff, and the diagnosis is confirmed through microscopic analysis of eyelash samples. Counting the number of *Demodex* mites in epilated eyelashes through microscopic observation is the most common laboratory diagnostic method used.[23,24]
The autofluorescence property of *Demodex* mites has been primarily observed in their eggs and larval stages. Niu et al. reported that eggs exhibit transient autofluorescence under blue light, which disappears within 24 hours, while larvae display weak autofluorescence that gradually diminishes within approximately five days.[12] Recent studies have also demonstrated that preparing sebum samples in saline solution and examining them under fluorescence microscopy represents an efficient and economical approach for the detection of *Demodex* mites, highlighting the diagnostic potential of this intrinsic optical property.[25]

In our study, the prevalence of *Demodex* sp. was significantly higher in patients with blepharitis compared to controls. Fluorescent microscopy exhibited a higher detection rate than light microscopy. The total number of *Demodex* sp. obtained from all eyelash regions was also higher in the examination performed by fluorescence microscopy than in the examination performed by light microscopy. The autofluorescence properties of *Demodex* mites make them much easier to see and identify in

fluorescent microscopy. Clanner-Engelshofen et al. also reported that utilizing the autofluorescence of mites with blue or green fluorescent light provides enhanced visualization of live mites.[26]

In conclusion, according to our study, the positivity for *Demodex* sp. in patients with blepharitis was significantly higher than in healthy volunteers. Additionally, the total amount of *Demodex* sp. detected in eyelash samples was significantly higher in fluorescent microscopy compared to light microscopy. It is believed that the use of fluorescent microscopy will be advantageous in investigating the presence of *Demodex* mites, especially in cases presenting with symptoms and signs of blepharitis.

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