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ORIGINAL ARTICLE

Prevalence of *Demodex* spp. in patients with chronic blepharitis

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Abstract Blepharitis is a very common disease in ophthalmology, dermatology and allergy practice. It generally follows a chronic course and is frequently associated with objective and/or subjective symptoms such as epiphora, red eye, dandruff, gritty sensation, itching, burning, photophobia, and blurred vision. The purpose of this study is to analyze the prevalence of *Demodex* spp. in patients with symptoms of chronic blepharitis. An analytical cross-sectional study was conducted in the period between 2016 and 2020. All patients with symptoms of chronic blepharitis who underwent a parasitological test of eyelashes (Rapitest) in the Dermatology Department of the *Hospital Italiano de Buenos Aires* were included. Those with previously established blepharitis due to another infectious cause were excluded. We analyzed 972 patients. Sixty percent (n = 585) underwent a positive Rapitest for the presence of *Demodex* spp. Seventy five percent (n = 728) were women. There were no significant differences in the prevalence associated with sex ($p = 0.38$). Among the patients positive for *Demodex* spp., 65% (n = 628) were older than 60 years old. The most frequently associated symptom was itching, present in 35% (n = 342). A statistically significant decrease in the number of consultations was observed during the cold months of the year (May–June–July–August). Our results show a high prevalence of *Demodex* spp. in patients with chronic blepharitis. As its presence reveals a direct association with age, we recommend looking for this parasite in this age group.

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PALABRAS CLAVE
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Ectoparásitos

Prevalencia de *Demodex* spp. en pacientes con blefaritis crónica

Resumen La blefaritis es una enfermedad muy frecuente en la consulta de oftalmología, dermatología y alergia. Generalmente, esta afección es de evolución crónica y, a menudo, se asocia con síntomas objetivos o subjetivos referidos por el paciente, como epífora, ojo rojo, caspa, sensación de arenilla, prurito, ardor, fotofobia y visión borrosa. El objetivo de este trabajo fue analizar la prevalencia de *Demodex* spp. en pacientes con síntomas de blefaritis crónica. Se realizó un estudio analítico de corte transversal en el período comprendido entre 2016 y 2020. Se incluyeron todos los pacientes con síntomas de blefaritis crónica que realizaron el estudio parasitológico de pestañas (Rapitest) en el Servicio de Dermatología del *Hospital Italiano de Buenos Aires*. Se excluyeron aquellos con blefaritis previamente establecida por otra causa infecciosa. El número total de pacientes incluidos fue de 972, el 60% de ellos (n = 585) presentaron Rapitest positivo para la presencia de *Demodex* spp. El 75% (n = 728) fueron mujeres. No hubo diferencias significativas en la prevalencia asociada al sexo (p = 0,38). Entre los pacientes en los que se detectó *Demodex* spp., el 65% (n = 628) eran mayores de 60 años, y el síntoma asociado con mayor frecuencia fue el prurito, presente en un 35% (n = 342). Se observó una disminución estadísticamente significativa en el número de consultas durante los meses más fríos del año (mayo-junio-julio-agosto). Los resultados del presente estudio evidencian una alta prevalencia de *Demodex* spp. en pacientes con blefaritis crónica. El hallazgo de este agente tuvo una relación directa con la edad mayor de 60 años, por lo que sugerimos su búsqueda en este grupo etario.

Introduction

Blepharitis is defined as an ocular surface disease commonly associated with eyelid inflammation and secondary eye irritation. It has a chronic clinical course with episodes of remission and exacerbation, and is usually difficult to manage[4].

It is a very frequent disease in ophthalmology, dermatology and allergy practice. The reported symptoms are: presence of scales, loss of eyelashes, photophobia, tearing, blurred vision, burning or eye itching. It is associated with other eye diseases such as dry eye, Meibomian gland dysfunction, hordeolum, among others, as well as skin diseases, with rosacea and seborrheic dermatitis being the most frequent[13]. Blepharitis has multiple etiologies. Among them viral, mycotic and parasitic infections stand out; the latter include infection by *Demodex* spp.

The genus *Demodex* spp. includes mites belonging to the *Animalia* domain, *Arthropoda* phylum, *Arachnida* class, *Demodicidae* family. It has a universal distribution and can colonize different mammals. They are the most common permanent ectoparasites in humans. The transmission can be by human–human contact or with fomites contaminated with mites that are in different stages of the evolutionary cycle of the parasite[7].

There are two species of *Demodex* (*D.*) spp. that can colonize humans: *D. folliculorum* and *D. brevis*. Their differential diagnosis is made under direct microscopic observation. The body of the adult *D. folliculorum* is between 250 and 400 μm. Its vermiform abdomen (opisthosoma) is striated, elongated and can cover more than 7/10 of the total length, while the body of *D. brevis* is somewhat shorter (200–300 μm) and its opisthosoma occupies up to 2/3 of the total body length[7,12,13]. *D. folliculorum* generally colonizes the pilosebaceous follicles of the seborrheic regions of the facial area, but can affect the anterior thorax, back, and extremities less frequently; while *D. brevis* lives in the Meibomian glands on the inner face of the eyelids[7,8,13].

The main purpose of this study is to estimate the prevalence of *Demodex* spp. in patients with chronic blepharitis. Furthermore, we want to assess the most frequent clinical manifestations and the different variables that could interfere in the prevalence of this disease.

Materials and methods

All patients with suspected blepharitis associated with *Demodex* spp. referred to the Dermatology Department of the *Hospital Italiano de Buenos Aires* to run a Rapitest of eyelashes in the period between January 1, 2016 and December 31, 2020 were included.

Description of the Rapitest procedure: five lashes from the upper and lower eyelids of both eyes were extracted by traction, using sterile stainless-steel tweezers. The extracted eyelashes were observed under an optical microscope (100× and 400×) after the addition of 10% potassium hydroxide (KOH) solution. Observation was conducted within 2 h of making the preparation. It was considered positive if at least one of the evolutionary stages of *Demodex* spp. (egg, larva, protonymph, nymph, adult) was detected (Figs. 1 and 2). The differentiation between *D. folliculorum* and *D. brevis* was conducted by the analysis of the morphological characteristics, such as the total length of the adults (in μm) and the size of the opisthosoma, which vary among

Figure 1 Microscope photography of an epilated eyelash showing adult *Demodex folliculorum* (100×).

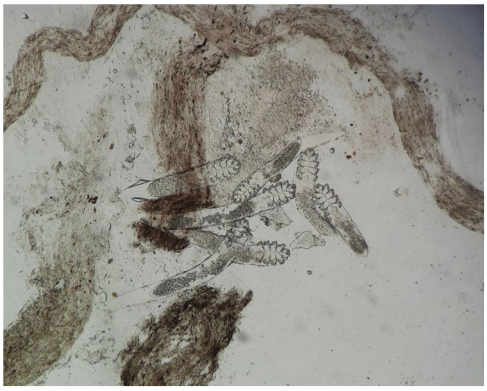

Figure 2 Microscope photography of epilated eyelashes showing adults and eggs of *Demodex folliculorum* (100×).

species. The measurement of the size of the mites was carried out using an ocular micrometer; those measuring more than 300 μm were considered *D. folliculorum*.

The following variables were collected: sex, age, month of visit, objective/subjective symptoms associated with the presence of the mite (meibomitis, epiphora, red eye, dandruff, sensation of grittiness, itching, burning, photophobia and blurred vision) and the association with dermatological conditions (rosacea, seborrheic dermatitis/oily skin).

For the purposes of the analysis, the months of the year in which the Rapitest sample was taken were divided into 3 groups according to the average ambient temperature:

Group 1: hot months (January/February/November/December)
Group 2: warm months (March/April/September/October)
Group 3: cold months (May/June/July/August)

Data were obtained from the review of the electronic medical records (EMR). Retrieved demographic data and clinical attributes were entered into an electronic database. Data entry was performed by five independent operators with their subsequent reconciliation by the principal investigator in order to detect missing information or inaccurate values. The protocol was approved by the Ethics Committee of Research Protocols of the *Hospital Italiano*.

Statistical analysis

Continuous variables were described as mean and standard deviation or median and interquartile range, based on the observed distribution. Categorical variables were described as absolute frequency and percentage. For the comparison of the categorical variables, the Fisher test was used and for the comparison of the continuous variables between multiple groups, the Kruskal–Wallis test was used, adjusted by Bonferroni. A value of $p < 0.05$ was considered significant. The STATA 13.1 statistical package was used for the analysis.

Results

We analyzed 972 samples of eyelashes from patients with chronic blepharitis and clinical suspicion of blepharitis due to *Demodex* spp. The age ranged from 5 to 96 years old. They were treated at the Ophthalmology, Allergy and Dermatology Departments of the *Hospital Italiano de Buenos Aires* and referred to the Dermatology Department to run the parasitological test (Rapitest). Patients assessed from 1/1/2016 to 12/31/2020 were included. In 60% (n = 585) of the assessed patients the presence of *Demodex* spp. was observed. The species found in 100% of the positive samples was *D. folliculorum*.

Table 1 describes the main clinical and epidemiological attributes of the patients assessed in the study. There was a predominance of female patients, who accounted for 75% of the total number of patients (n = 728). If we only consider the positive Rapitest results for *Demodex* spp. (n = 585), there were no significant differences in the prevalence associated with sex ($p = 0.38$). The median age at the time of the Rapitest sample collection was 67 years old (IQR 53–75.5). Sixty five percent (n = 411) of the patients with a positive result for the presence of *Demodex* spp. were over 60 years old.

In patients with a positive result for the presence of *Demodex* spp., the most frequently reported symptom was itching (n = 342), while the most frequently observed sign was meibomitis, observed in 21% (n = 202) of cases. A significant difference was observed in the presence of cylindrical dandruff ($p < 0.001$) in patients with blepharitis associated with the presence of the mite (n = 120). A statistically significant association was detected ($p = 0.03$) in patients with rosacea and positive results for the presence of *Demodex* spp.; history of seborrheic dermatitis/oily skin did not show statistically significant results for the presence of the mite ($p = 0.88$). A statistically significant decrease in the number of patients with a positive result for *Demodex* spp. during the months with lower ambient temperature (Group 3), was observed ($p = 0.006$). No significant difference was observed in the positive results throughout the different years of assessment: 2016 (55%), 2017 (57%), 2018 (61%), 2019 (59%), 2020 (66%).

Discussion

The prevalence of *Demodex* spp. in our population, patients with suspected blepharitis associated with *Demodex* spp. referred to the Dermatology Department of the *Hospital Italiano de Buenos Aires* to run a Rapitest of eyelashes in

Table 1 Clinical attributes, signs, and symptoms of patients with chronic blepharitis and parasitological study of eyelashes.

Clinical attributes, signs and symptoms of the assessed patients n = 972 (100%)	Rapitest (−) n = 387 (40%)	Rapitest (+) n = 585 (60%)	*p*-Value
Female 728 (75%)	284 (39%)	444 (61%)	0.380
60+ years old 628 (65%)	217 (35%)	411 (65%)	<0.001
Red eye 151 (16%)	60 (40%)	91 (60%)	0.076
Blurred vision 20 (2%)	8 (40%)	12 (60%)	0.079
Meibomitis 202 (21%)	60 (30%)	142 (70%)	0.001
Epiphora 199 (20%)	77 (39%)	122 (61%)	0.099
Gritty sensation in the eyes 259 (27%)	99 (20%)	160 (80%)	0.074
Photophobia 10 (1%)	2 (20%)	8 (80%)	0.047
Itching 342 (35%)	138 (40%)	204 (60%)	0.074
Soreness 143 (15%)	60 (42%)	83 (58%)	0.054
Cylindrical dandruff 160 (16%)	40 (25%)	120 (75%)	<0.001
Oily skin/seborrheic dermatitis 88 (9%)	37 (42%)	51 (58%)	0.88
Rosacea 191 (20%)	60 (31%)	131 (69%)	0.030

the period between January 1, 2016 and December 31, 2020, was 60%. This value is above that reported in similar studies. Velasco et al., documented 100 patients in 2017, observing an infection prevalence of 44%. On the other hand, Galvis et al., in 2011, reported a prevalence of 42% in 128 patients[3,11] and Demirkazık et al detected *Demodex* spp.in 42.6% of the 335 patients in 2020 publication[1].

In our study, a positive test was directly related to age, being close to 65% in patients over 60 years old. This data is consistent with the information reported by López-Ponce et al. in 2017, who observed that 94.55% of infected patients were over 71 years of age[6]. This may be associated with structural changes in the eyelid margin and decreased barrier function due to skin aging[9]. As reported by Mongi et al., in 2018, significant association was detected between the presence of the mite and patient gender[7].

The most frequent sign was meibomitis, followed by cylindrical dandruff. The associated symptom reported in the largest number of patients was itching (35%), followed by grittiness (27%) and epiphora (20%). These results are comparable with the cases analyzed in other studies[3,11]. However, in a study conducted by Laspina et al., in 2015, it was shown that the most frequent signs and symptoms were burning and papillary hypertrophy, both in the group of patients with *Demodex* spp., and without it[5].

The slight increase in positive tests for the presence of *Demodex* spp. in 2020 could be associated with the fact that only patients with severe symptoms attended the consultation because of mandatory isolation due to the COVID-19 pandemic. Ancillary studies were not carried out, the presence or absence of associated symptoms or signs referred to in the clinical history was considered.

The detection of *Demodex* spp. through the extraction of eyelashes by traction – selecting those presenting cylindrical dandruff, disorders in their arrangement (trichiasis, madarosis) and those easily detaching from the palpebral edge – has the advantage of being a simple and efficient method with minimal discomfort for the patient. Additionally, it optimizes the quality of the sample for microscopic examination, allowing a definitive diagnosis and adequate treatment[2,10]. A higher prevalence of mite presence was observed in patients with chronic blepharitis during the hot/warm months. This factor should be taken into account upon suspicion of this disease.

Our results show a high prevalence of *Demodex* spp. in patients older than 60 years old with symptoms of chronic blepharitis not associated with other infectious diseases. The relevance of a definitive diagnosis in this population is highlighted by carrying out a parasitological examination of the eyelashes (Rapitest), a technique that allows an adequate treatment and the improvement of the patients' quality of life[13].

Ethical disclosure

Protection of individuals and animals

The authors declare that no experiments have been performed on humans or animals for this research.

Data confidentiality. The authors declare that they have followed their institutional policy on the publication of patient data.

Right to privacy and informed consent. The authors declare that they have followed their institutional policy on the publication of patient data.

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Conflict of interest

The authors declare no conflicts of interest.

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