

Prevalence of *Demodex Folliculorum* and *Demodex Brevis* in Patients with Blepharitis and Chalazion

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Abstract

Purpose: *Demodex folliculorum* and *Demodex brevis* are common ectoparasites on skin that also can lead to blepharitis and chalazion. The aim of our study is to determine the prevalence of *Demodex spp.* in eyelashes of patients diagnosed with chronic blepharitis and chalazion.

Methods: This study included 330 patients diagnosed with chronic blepharitis, 70 patients diagnosed with chalazion and 130 volunteers without any ocular problems. Patient eyelashes were examined under a light microscope at magnifications of $\times 40$, $\times 100$ and $\times 400$. *Demodex spp.* were determined.

Results: Parasite prevalence was significantly higher in blepharitis (75.5%) and chalazion groups (70%) compared to the control group (16.2%) ($p < 0.001$). The prevalence of *D. folliculorum* in the blepharitis group and *D. brevis* in the chalazion group was found to be significantly higher compared to other groups ($p < 0.05$). The average number of mites per eyelash was found to be significantly higher in patients with Demodex positive blepharitis ($p = 0.001$) and in chalazion patients ($p = 0.047$) than in the control group. It has been determined that mite positivity increases with age in blepharitis and control groups ($p < 0.05$). In the group with blepharitis, it was found that mite positivity was significant in the presence of symptoms ($p = 0.0001$) and Demodex positivity decreased as the education level of individuals increased ($p = 0.039$).

Conclusion: The results of the study show that Demodex acari infestations should be considered in chronic blepharitis and chalazion.

Introduction

Blepharitis is an inflammatory disease of the eyelashes and eyelids. It usually appears bilateral and symmetrical. Although the etiopathogenesis is not known exactly, it is thought that environmental factors, bacteria and toxins, viruses and some parasites play a role in the etiology of the disease. The most common symptoms are itching, stinging, burning, redness and dandruff at the bottom of the eyelashes [1,2]. In addition to these ocular symptoms, it also causes visual problems such as photophobia and blurred vision. Although staphylococci and streptococci are the most common bacteria [3,4], it has been argued that Demodex mites also play a role in etiology [5,1].

Chalazion is a cystic, chronic granulomatous inflammation of the meibomian gland. It occurs around the sebaceous gland in the middle of the eyelid. Initially, the inflammation can be painful and manifest as an internal hordeolum. However, it becomes a painless granulomatous nodule and is considered an aseptic, chronic inflammation [6]. It has been reported that chalazion develops in association with bacterial infection, viral conjunctivitis and Demodex mites [7-9].

Demodex mites are obligate ectoparasites that can infect many host organisms. Two species, *Demodex folliculorum* (DF) and *Demodex brevis* (DB), have been identified in humans. While *Demodex folliculorum* lives in hair follicles, DB lives in sebaceous and meibomian glands [10,11]. Although Demodex infestation

is common in humans, it is known that it has been ignored as the cause of ocular problems for many years [12,5]. Recent studies indicate that ocular problems such as blepharitis and chalazion may be associated with mites. It supports the argument that settlement and multiplication of the *Demodex* mites in the hair follicles and sebaceous glands may be involved in the etiology of ocular infectious diseases [13,1].

We aimed to determine the prevalence of *Demodex* mites in patients with blepharitis and chalazion and also to determine the relationship between mites and these diseases in this study.

Materials And Methods

Study groups

330 patients with chronic blepharitis, 70 patients with chalazion and 130 volunteers without any eye disease or ocular symptoms who were admitted to Private Osmaniye Sevgi Hospital Ophthalmology Outpatient Clinic between 01.11.2018 and 15.11.2019 with various ocular complaints were included in the study. Those who were pregnant, under the age of 18, who received antiparasitic treatment in the last 1 month, who had eye surgery in the last 6 months, and who could not sign the consent form for any reason were not included in the study. Study approval numbered 2018/154 was obtained from the Clinical Research Ethics Committee of Hatay Mustafa Kemal University Tayfur Ata Sökmen Faculty of Medicine. A short questionnaire about age, gender, general eye complaints (burning, stinging, dryness, itching, blurred vision, dandruff at the eyelash base), systemic diseases (diabetes, cancer, kidney disease), pet feeding at home or in their gardens was applied to all participants included in the study and the consent form was filled out.

Sampling

Samples were taken from the problematic eyes of the patients with unilateral eye disease, the right eyes of the patients with bilateral eye disease or the volunteers without any eye problems. With the eyelash epilation method, a total of six eyelashes were taken from each individual's lower and upper eyelids, three each. The presence of *Demodex spp.* was examined under a light microscope at x40 and x100 magnifications. The distinction of demodex types was made according to the relevant literature [14,15].

Statistical Analysis

Kolmogorov Smirnov test was used to compare whether the data were normally distributed or not. One-way Anova, t-test, Chi-Square (χ^2), Fisher's Exact and Spearman Correlation tests were used for analysis. In the evaluation of the results, $p < 0.05$ was considered as statistically significant. All statistical analyzes were performed using the SPSS 22.0 for Windows (Statistical Package for Social Sciences, Chicago, IL) (SPSS Inc., Chicago, USA) package program.

Results

Demodex mite was found in 249 (75.5%) of 330 patients with blepharitis, 49 (70%) of 70 patients with chalazion, and 21 (16.2%) of 130 volunteers in the control group. The comparison of *Demodex spp.* according to the diagnosis groups is presented in **Table 1**. The parasite prevalence was significantly higher in both blepharitis and chalazion groups compared to the control group ($p < 0.001$). However, no statistically significant difference was found between blepharitis and chalazion groups ($p = 0.37$). Egg, larva, nymph and adult forms of the parasite in samples taken with the eyelash epilation method are presented in **Figure 1**.

Demodex folliculorum 74% (244/330) and 14.2% (47/330) DB in the blepharitis group; 55.7% (39/70) DF and 44.3% (31/70) DB in the chalazion group; 15.4% (20/130) DF and 0.8% (1/130) DB in the control group were detected. When the presence of DF and DB was compared in patients with blepharitis, the incidence of DF was significantly higher ($p < 0.05$). There was no significant difference between the frequency of DF and CD in patients with chalazion ($p > 0.05$). When the relationship between groups and Demodex types was examined, a significant difference was found ($\chi^2 = 203.3$; $p < 0.05$). The incidence of DF was significantly higher in the blepharitis group compared to the chalazion and control groups ($p < 0.05$). In the chalazion group, the incidence of DB was significantly higher than in the blepharitis and control groups ($p < 0.05$). The distribution of the average number of mites by species in Demodex infestation groups is presented in **Table 2**. The mean number of mites in the blepharitis group was found to be significantly higher in patients with both DF and DB compared to those with only DF ($p = 0.0007$) or those with only DB ($p = 0.04$) positive ($p = 0.005$). The mean mite count was significantly higher in patients with both DB and DF than patients with DB alone in chalazion group ($p = 0.02$). In the control group, there was no significant difference between the average mite number and mite species in the mite positive volunteers ($p = 0.48$).

The mean age of the volunteers included in the study was 49.62 ± 15.86 years (age range 19-85) in the blepharitis group, 41.86 ± 15.98 years (age range 19-79) in the chalazion group and 40.48 ± 13.97 (age range 18-82) years in the control group. Although the mean age was higher in the blepharitis group compared to the control group ($p = 0.010$) and the chalazion group ($p = 0.049$), there was no significant difference between the chalazion and control groups ($p = 0.527$). Each group was divided into seven age groups. According to the correlation coefficient (r) results, a positive correlation was found between age and presence of *Demodex spp.* in all groups. It was found that the correlation in the blepharitis and control groups was statistically significant ($p < 0.05$), while the correlation in the chalazion group was not significant ($p = 0.185$). The relationship between age groups and the positivity of *Demodex spp.* is presented in **Table 3**.

One hundred and thirty (39.4%) of 330 patients in the blepharitis group were women and 200 (60.4%) were men. In the chalazion group, there were 28 (40%) female and 42 (60%) male patients. In the control group, there were 55 (42.3%) female and 75 (57.7%) male volunteers. The relationship between gender

and the presence of *Demodex spp.* in the groups is presented in **Table 4**. In all groups, there was no significant difference between gender and *Demodex spp.* positivity ($p > 0.05$).

The presence of symptoms and the relationship between symptoms and mites in patients diagnosed with blepharitis and chalazion were given in **Table 5**. In the presence of symptoms, *Demodex spp.* positivity was found to be significant in the group with blepharitis ($p = 0.0001$), while no significant difference was found in the group with chalazion ($p = 0.308$).

The most common complaints in patients with blepharitis were 43.3% itching, 36.4% stinging (foreign body sensation) and 30.3% burning, respectively. However, among these complaints, a significant difference was found only between stinging and the positivity of *Demodex spp.* ($\chi^2 = 9.75$; $p = 0.002$). DF was found in 116 (96.6%) of 120 mite positive patients with stinging complaints, while 29 (24.1%) were found to have DB. There was a significant difference between the presence of DF and the complaint of stinging ($p < 0.05$). The most common complaints in patients with chalazion were 44.2% itching, 38.5% dandruff at the base of the eyelash, 35.7% stinging, 25.7% burning and 1.4% watering, respectively. No significant difference was found between the presence of *Demodex spp.* and symptoms in patients with chalazion ($p = 0.308$), and no complaints of dryness, blurred vision, redness, burring and pain were observed in any of the patients.

Thirtysix patients in the blepharitis group had systemic diseases, 33 of them were Type 2 diabetes, 2 were kidney failure and 1 cancer. Mites were positive in 84.8% (28/33) of diabetic patients, 2 patients with renal failure and 1 patient with cancer. However, there was no significant difference between the presence of systemic disease and mite positivity ($p = 0.150$). Two patients in the chalazion group had Type 2 diabetes, and these two patients also had mites. However, there was no significant difference between the presence of systemic disease and mite positivity in the chalazion group ($p > 0.05$).

The relationship between education level and mite positivity in the groups is given in **Table 6**. According to the correlation coefficient (r) results, a negative correlation was observed between the education level and the positivity of *Demodex spp.* in the blepharitis and the control group. However, while this negative correlation was not significant in the control group ($p = 0.213$), it was significant in the blepharitis group. It was found that the positivity of *Demodex spp.* decreased as the education level increased in patients with blepharitis ($r = -0.114$; $p = 0.039$). The positive correlation found in the chalazion group was not found to be significant ($p = 0.562$).

There was no significant difference between animal feeding and the positivity of *Demodex spp.* in all groups ($p > 0.05$).

Discussion

It is stated in some studies that *Demodex* mites, which are frequently detected in humans around the world, may be pathogenic in chronic eyelid diseases [16,17,1]. While *Demodex folliculorum* causes anterior blepharitis, DB positivity shows a strong correlation with the prevalence of posterior blepharitis,

meibomian gland dysfunction, recurrent chalazion, and keratoconjunctivitis [12,18]. In the pathogenesis of blepharitis, it has been reported that mites are vectors in the transport of some bacteria such as Bacillus, Staphylococcus and Streptococcus, and mites play a role with the bacteria especially in cases that are resistant to treatment and recurrent after treatment [12,19]. At the final point, mites are considered as a causative factor for blepharitis [20].

The etiology of chalazion has not been explained and is still unknown. The accumulated secretion causes an inflammatory reaction in the surrounding tissues. Another factor could be bacterial and / or Demodex infestation [7,6,13].

Considering the epidemiological studies on blepharitis and chalazion, it is seen that the prevalence of *Demodex spp.* ranges from 28.8% to 84% in patients with blepharitis, 63.2% to 91.67% in patients with chalazion, and 4.6% to 54.9% in the healthy control group. The results of our study are compatible with the literature [21-24,6,25]. In several studies, no significant difference was found between patients with blepharitis and the control group in terms of mite positivity [21,22]. However, it is stated in most of the literature that the presence of parasites is significantly higher in patients with blepharitis and chalazion [16,24,6,25,13]. In our study, it was found that the presence of *Demodex spp.* was statistically significantly higher in patients with blepharitis and chalazion.

It is known that the most common type in almost all studies showing the relationship between blepharitis and Demodex is DF [16,25-27]. Our study is similar to studies reporting that DF is significantly more common than DB in eyelash follicles in patients diagnosed with blepharitis.

Studies examining the relationship between chalazion and Demodex show that DF dominance is at the forefront, as in blepharitis studies [24]. In our study, it was determined that DF was the most common mite in the chalazion group. In addition, although there was no statistically significant difference between DF and DB in patients diagnosed with chalazion, the incidence of DB was found to be significantly higher than blepharitis and control groups. Based on the results, we think that both species may have a role in the pathogenesis of chalazion.

Although the eyelash hair removal method was commonly used in ocular demodicosis studies, which eye (right eye, left eye, both eyes, lower or upper eyelids), which eyelashes (cylindrical dandruff eyelashes or random) and how many eyelashes to be taken could not be made a standard method [28]. Apart from the eyelash epilation method, resection samples were also evaluated in patients with chalazion [24]. The average number of mites in the studies is not based on a standard method. In some studies, the average Demodex number was obtained by dividing the total number of mites by the number of Demodex positive volunteers [29,28], while in others the total number of mites was divided by the number of all volunteers [23,27]. The issues such as the different number of eyelashes, giving the average number of mites according to all patients or positive patients, giving the average per patient or per eyelash vary. This makes it difficult for researchers to interpret between studies. As a result of our observations and research, we think that positive individuals should be evaluated while giving the average number of mites. Because:

- a) When non-infested patients are included in the studied patient group, the average "mite density in infested patients in X group", which is the main focus, is reduced.
- b) The main goal in medicine is to develop a solution-treatment for the current problem. "Mite positive in X group" individuals should be evaluated in order to detect the change in the number of mites before and after treatment studies.
- c) Determining how the number of mites changes when species are found individually or together in "Mite positive in group X" will help to illuminate the pathogenesis between the mite and the disease.

In addition, we think that the average Demodex numbers should be given per eyelash. Although the researchers examine different numbers of eyelashes, by giving the results to the eyelash head, we provide the researchers with ease in interpretation and achieve a standard in the literature. If we list our two suggestions on this subject:

- 1) Positive individuals should be taken into consideration while giving average mite numbers.
- 2) Average mite counts should be given per eyelash.

In our study, we found it appropriate to give results with the average number of mites per eyelash. In the blepharitis group, the average number of mites when DF + DB coexists were found to be significantly higher compared to those with only DF or only DB and total mites. It was found that the number of mites increased in patients with blepharitis if both species were seen together.

Similarly, there is no standardization in the data in studies examining the relationship between chalazion and Demodex. Schear et al. found 0.804 ± 1.03 DF per eyelash epilled, while the average DF in the control group was found to be 0.487 ± 0.82 [24]. In our study, the DF number per eyelash was found by Schear et al. shows similarities with the results of their study. In addition, the average number of mites in DF + DB coexistence was found to be significantly higher in patients compared to those with DB alone.

It is reported that the increase in the activity of the sebaceous glands that occur with age and the changes in the sebum composition will facilitate the increase of mites in the elderly [26]. In many studies, it has been reported that ocular Demodex infestation increases with age, it is seen in 84% of the population in their 60s and in 100% of the population aged 70 and over [12,16,21,23]. Kasetuwan et al. reported the prevalence of ocular demodicosis as 70% over the age of 80 [30]. In other studies, it was reported that infestation was significantly higher in patients with blepharitis over the age of 50, those over 60 years of age, and those with ocular disease over the age of 70 [31,10,26]. However, in some studies, no significant difference was found between increasing age and the presence of mites [22,32]. This study supports the general literature. A statistically significant positive correlation was found between age and the presence of *Demodex spp.* in patients with blepharitis. We think that this is caused by the weakening immune system, increased sebum amount and weakened-deteriorating skin structure in the elderly.

Although there is no statistically significant difference, there are studies reporting that infestation is higher in women [10], as well as studies reporting that it is common in men [25]. Zeytun and Karakurt stated that the prevalence of mites is significantly higher in males [28]. Most of the studies argue that there is no significant difference between gender and *Demodex spp.* [16,22,23,26,32]. This study supports the literature. No significant difference was found between gender and mite positivity in patients with blepharitis.

As a result of the resection samples of patients with chalazion, no significant relationship was found between gender and the presence of mites. Tarkowski et al. reported that the infestation was in a similar distribution in men and women with chalazion [24,6]. Our study is the same as the results of the other two studies. There was no significant relationship between sex and mite positivity in the chalazion group.

It was reported that the symptoms of *Demodex spp.* positive blepharitis patients did not differ from those of other blepharitis patients. In some studies, it was reported that most of the patients were asymptomatic and their complaints generally increased in hot weather [33]. The most common complaints in patients with infected blepharitis were itching, foreign body sensation (stinging) and redness. Eyelash sticking, dandruff at the bottom of the eyelashes, mild papillary conjunctivitis, meibomian gland dysfunction and telangiectasia were seen in patients with chronic blepharitis [28]. In addition, it was stated that patients had ocular pain, contact lens intolerance, photophobia and crusting [23]. Symptoms were usually worse in the morning, and several flare-ups and remissions might occur [1]. In this study, a significant difference was found between the presence of symptoms and mite positivity in the blepharitis group. The most common complaints in patients with blepharitis were itching, stinging (foreign body sensation) and burning, respectively. Although itching, in our study, was the most common symptom as in other studies, this symptom was similar in patients with mite negative and positive blepharitis like the studies conducted by Inceboz et al [33]. In our study, among these symptoms, only a significant relationship was found between stinging complaint and the presence of DF.

While patients with chalazion have eyelid nodules showing pain, inflammation and sensitivity in the acute phase, there is a permanent, painless mass in the chronic phase [7]. Cylindrical dandruff was not common, as most patients with recurrent chalazion had regular eyelid hygiene [13]. In this study, the complaints seen in patients with chalazion were itching, dandruff at the base of the eyelashes, stinging, burning and watering, respectively. However, there was no significant difference between mite positivity and the presence of symptoms.

Studies show that DF is increased in immunocompromised patients such as diabetes, end-stage chronic renal failure, Behçet's disease, urological cancers and eyelid basal cell carcinomas [11,34]. It has been reported that DF is an important factor in eye diseases such as blepharitis and that the mite is more common in immune system disorders. In a study, DF was detected in 27.4% of eyelashes of 42 patients with Type 2 diabetes and 19% in 42 control group volunteers. It has been reported that infestation is significantly higher in patients with diabetes [35]. In our study, it was determined that the prevalence of infestation was higher in patients with systemic disease in both groups. However, no significant

difference was found between the presence of systemic disease and mite positivity in the blepharitis and chalazion groups. We think that this is due to the low number of patients with systemic diseases in our target study groups.

There is no information about the relationship between education level and mite positivity in the available literature. In our study, a negative correlation was observed between education level and the positivity of *Demodex spp.* in the blepharitis and control group. However, while this negative correlation was not significant in the control group, it was significant in the blepharitis group. It has been found that as the education level increases in patients with blepharitis, the mite positivity decreases. The reason for this may be the increase in personal hygiene practices and compliance with protection methods due to the increase in education level. There was no significant difference in the chalazion group.

It was reported that there was no significant relationship between pet feeding and demodicosis in patients with blepharitis [33]. In our study, no significant difference was found between animal feeding and mite positivity in blepharitis, chalazion and control groups. We think that this is due to the fact that mites transmitted from infested animals are host specific and can only cause a temporary dermatitis in the human body.

As a result, clinicians should warn especially the elderly patients against *Demodex* mites that infect eyelashes. Patients with recurrent blepharitis and chalazion who do not respond to the current blepharitis treatment procedure should be investigated and treated for *Demodex spp.*

Declarations

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We declare that there is no conflict of interest in this study.

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Tables

Table 1. Comparison of the *Demodex spp.* incidence in groups

	<i>Demodex spp.</i>		Total	p
	Negative	Pozitive		
	n (%)	n (%)	n	
Blepharitis	81 (24,5)	249 (75,5)	330	<0,001*
Chalazion	21 (30)	49 (70)	70	<0,001*
Control	109 (83,8)	21 (16,2)	130	
Total	211 (39,8)	319 (60,2)	530	

* Blepharitis and chalazion groups were compared with the control group using Fisher's Exact test.

Table 2. Distribution of the average number of mites by species in Demodex infestation groups.

Group	Species (n= number of <i>Demodex spp.</i> detected individual)	Number of <i>Demodex spp.</i>			Total mite (n)
		Min- Max (n/6 eyelash)	Average (n/6 eyelash) ±SD	Average mite per eyelash (n±SD)	
Blepharitis	DF (n:202)	2-45	7,3±5,7	1,2±0,9	1484
	DB (n:5)	2-4	3,6±0,8	0,6±0,1	18
	DF+DB (n:42)	4-56	11,6±9,5	1,9±1,6	490
	Total+(n:249)	2-56	8±6,6	1,3±1,1	1992
Chalazion	DF (n:18)	3-21	7±4,8	1,1±0,8	126
	DB (n:10)	2-5	3±1,1	0,5±0,2	30
	DF+DB (n:21)	4-19	8±4,7	1,3±0,8	168
	Total+ (n:49)	2-21	6,6±4,6	1,1±0,7	324
Control	DF (n:20)	1-10	3,4±2,3	0,57±0,38	69
	DB (n:1)	2	2	0,3	2
	DF+DB (n:0)	-	-	-	-
	Total+ (n:21)	1-10	3,3±2,2	0,56±0,37	71

Table 3. Relationship between age groups and the positivity of *Demodex spp.*

Age groups		Total	<i>Demodex spp.</i> negative	<i>Demodex spp.</i> positive	r	p	
		n	n	n			
50- 60->	Blepharitis	<20	6	4	2		
		20-29	29	14	15		
		30-39	45	15	30		
		40-49	53	17	36	0,301	<0,05
		50-59	73	19	54		
		60-69	75	8	67		
		≥70	49	4	45		
		Total	330	81	249		
		Chalazion	<20	5	2	3	
	20-29		18	5	13		
	30-39		14	6	8		
	40-49		9	4	5	0,160	0,185
	50-59		14	4	10		
	60-69		8	0	8		
	≥70		2	0	2		
	Control	<20	6	6	0		
		20-29	28	26	2		
		30-39	29	27	2		
		40-49	39	34	5	0,348	<0,05
50-59		18	12	6			
60-69		6	4	2			
≥70		4	0	4			
Total		130	109	21			

Table 4. Distribution of the relationship between gender and the presence of *Demodex spp* by groups.

	Sex	n (%)	<i>Demodex spp.</i>		Total	p
			Negative	Pozitive		
Blepharitis	Female	n (%)	30 (23,1)	100 (76,9)	130 (100)	0,358
	Male	n (%)	51 (25,5)	149 (74,5)	200 (100)	
	Total	n (%)	81 (24,5)	249 (75,5)	330 (100)	
Chalazion	Female	n (%)	7 (25,0)	21 (75,0)	28 (100)	0,318
	Male	n (%)	14 (33,3)	28 (66,7)	42 (100)	
	Total	n (%)	21 (30,0)	49 (70,0)	70 (100)	
Control	Female	n (%)	48 (87,3)	7 (12,7)	55 (100)	0,254
	Male	n (%)	61 (81,3)	14 (18,7)	75 (100)	
	Total	n (%)	109 (83,8)	21 (16,2)	130 (100)	

Table 5. Distribution of the relationship between the presence of *Demodex spp* and the presence of symptoms by groups.

Group	<i>Demodex spp.</i>	n (%)	Symptoms		Total	p*
			No	Yes		
Blepharitis	Negative	n (%)	17 (21,0)	64 (79,0)	81 (100)	0,0001
	Pozitive	n (%)	13 (5,2)	236 (94,8)	249 (100)	
	Total	n (%)	30 (9,1)	300 (90,9)	330 (100)	
Chalazion	Negative	n (%)	0 (0)	21 (100)	21(100)	0,308
	Pozitive	n (%)	4 (8,2)	45(91,8)	49 (100)	
	Total	n (%)	4 (5,7)	66 (91,8)	70 (100)	

* Fisher's Exact test was used.

Table 6. Distribution of the relationship between education level and *Demodex spp*.

Level of Education			<i>Demodex spp.</i>		Total	r	p
			Negative	Positive			
Blepharitis	Illiterate	n (%)	6 (18,8)	26 (81,3)	32 (100)	-0,114	0,039
	Primary Education	n (%)	36 (22,2)	126 (77,8)	162 (100)		
	High school	n (%)	17 (21,3)	63 (78,8)	80 (100)		
	University	n (%)	21 (38,9)	33 (61,1)	54 (100)		
	Postgraduate	n (%)	1 (50,0)	1 (50,0)	2 (100)		
	Total	n (%)	81 (24,5)	249 (75,5)	330 (100)		
Chalazion	Primary Education	n (%)	6 (40,0)	9 (60,0)	15 (100)	0,070	0,562
	High school	n (%)	11 (26,2)	31 (73,8)	42 (100)		
	University	n (%)	4 (30,8)	9 (69,2)	13 (100)		
	Total	n (%)	21 (30,0)	49 (70,0)	70 (100)		
Control	Primary Education	n (%)	14 (63,6)	8 (36,4)	22 (100)	-0,110	0,213
	High school	n (%)	43 (89,6)	5 (10,4)	48 (100)		
	University	n (%)	48 (90,6)	5 (9,4)	53 (100)		
	Postgraduate	n (%)	4 (57,1)	3 (42,9)	7 (100)		
	Total	n (%)	109 (83,8)	21 (16,2)	130 (100)		

Figures



Figure 1

Egg, larva, nymph and adult forms of the parasite; a) *D. folliculorum* egg (x400), b) *Demodex* spp., larvae (x400), c) *Demodex* spp., nymph (x100), d,e) *D. folliculorum* egg and adults (x100), f) *D. folliculorum* adult (x400), g) *D. brevis* adult (x400) (Original).