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Article in *Acta Parasitologica* · July 2014

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Demodex mites as potential etiological factor in chalazion – a study in Poland

Witold Tarkowski^{1*}, Marta Owczyńska¹, Anna Błaszczuk-Tyszka¹ and Daniel Młocicki^{2,3*}

¹Medical Centre KOL-MED SP ZOZ, Plac Dworcowy 6, 33-100 Tarnów, Poland; ²Department of General Biology and Parasitology, Medical University of Warsaw, ul. Chałubińskiego 5, Warsaw, Poland; ³W. Stefański Institute of Parasitology, Polish Academy of Sciences, ul. Twarda 51/55, 00-818 Warsaw, Poland

Abstract

The aim of the study was to investigate the presence of *Demodex* in the hair follicles of eyelashes and their potential participation in the aetiology of chalazion in patients in Poland. The study of the correlation between the presence of *Demodex* spp. and chalazion has never been performed in patients in Europe. There is, therefore, a justified necessity to check whether *Demodex* mites can be a potential risk factor in the development of chalazion in the European population. The samples were examined by light microscope, using standard parasitological methods. A positive result was assumed in the presence of *Demodex* spp. *Demodex* was detected in 91.67% of patients with a chalazion. The presence of *Demodex* was found in subjects from all examined age groups. The results of statistical analysis unambiguously determined the existence of an interrelationship between the presence of *Demodex* and chalazion. Our results clearly indicate the existence of a correlation between the occurrence of *Demodex* spp. and chalazion. Confirmation of the positive correlation between *Demodex* and chalazion in a European population provides further evidence for the pathogenic role of *Demodex* in the development of eye diseases.

Keywords

Demodex, *Demodex folliculorum*, *Demodex brevis*, inflammation, cilium hair follicle, chalazion

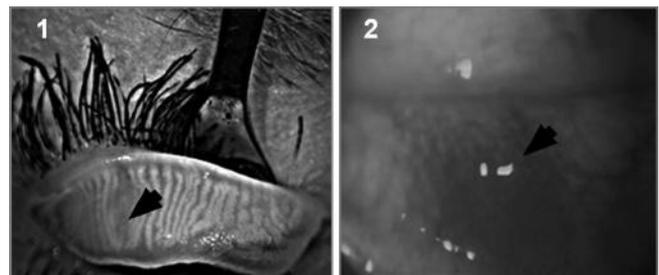
Introduction

Chalazion (Figs 1–2) is a chronic inflammation of a granulating nature caused by blockage of meibomian glands, with the result that there develops limited, mostly painless thickening of the tarsus causing a visible lump under the skin and hyperaemia of conjunctiva. Chalazion arises spontaneously or as a consequence of a sty (Bradford 2006; Kanski and Bowling 2011). Nodules develop slowly and are usually painless, and the skin in the area is reddened. It typically resolves over a period of weeks or months, when the contents of the chalazion drain to the outside through the skin, to the inside through the tarsus and palpebral conjunctiva, or when the phagocytized lipids are squeezed out under pressure into the surrounding tissues.

The etiology of chalazion is not explained and still remains unknown. The cause is believed to be changes in the secretion of the tarsal gland predisposing to the gradual closure of the opening of the tarsal gland (Kanski and Bowling 2011). Accumulated secretion causes the inflammatory reaction in the surrounding tissues. An additional factor may be a bacterial and/or *Demodex* infection (Liang *et al.* 2014; Yam *et al.* 2014).

The most common method of proceeding, in the absence of improvement and spontaneous decay of the chalazion, is surgical treatment (Figs 3A–3F). The eyelid is inverted with the blepharostat (Fig. 3C–3D), the chalazion is cut and its contents are curetted through the tarsus (Fig. 3D–3F).

As was mentioned above it is suspected that one of the potential factors influencing chalazion development and recurrence could be the infestation of hair follicles of eyelashes



Figs 1–2. Fig. 1 – Chalazion (arrowhead) of upper eyelid visible in the Meibography. Fig. 2. – Chalazion (arrowhead) of lower eyelid visible from the side of palpebral conjunctiva

*Corresponding authors: tarkowski@is.net.pl (W. Tarkowski); danmlo@twarda.pan.pl (D. Młocicki)

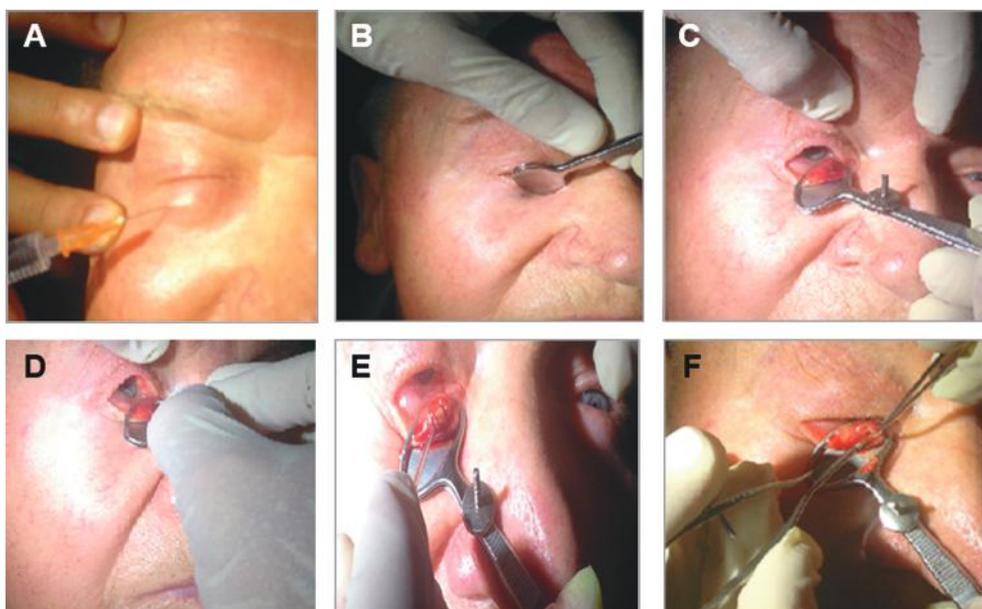


Fig. 3. Consecutive stages of surgical treatment in chalazion. **A** – Anaesthesia; **B** – Application of blepharostat; **C** – Inversion of eyelid; **D** – Incision of tarsus with scalpel along Meibomian gland; **E** – Curettage; **F** – Capsulectomy

by *Demodex* mites (Liang *et al.* 2014; Yam *et al.* 2014). On human skin, two species of *Demodex* have been found (Lacey *et al.* 2011) – *Demodex folliculorum* (Fig. 4A) and *Demodex brevis* (Fig. 4B). The existence of two distinct species of human *Demodex* was proved only in 1963, by Akbulatova (1963). Their accurate anatomical description was made by Desch and Nutting (1972, 1977); according to this morphological description both species have a worm-like shape and body covered with a thin cuticle. The bigger of them is *Demodex folliculorum* and it reaches a length of 0.3–0.4 mm; it is elongated and found in the opening of the hair follicles, where it creates clusters of a few mites. Alternatively, *Demodex brevis* is spindle-shaped and reaches a length of 0.2–0.3 mm and has shorter legs. It can be found mostly alone in the depths of the sebaceous glands in the skin of the face or in the Meibomian glands, located within the eyelids (Raszeja-Kotelba *et al.* 2004). It feeds itself on the gland cells and causes their destruction (Browning and Proia 1986).

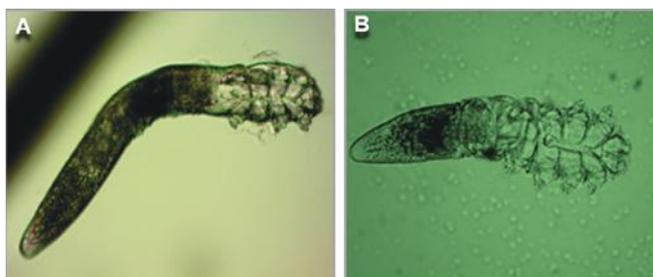


Fig. 4. Adult stages of two species of *Demodex* mites found in patients examined during the present studies. **A** – adult *D. folliculorum*; **B** – adult *D. brevis*

At all stages of *Demodex* development their main food is skin cells and sebum components, therefore, they inhabit the skin areas rich in sebaceous glands – the skin on the face, specifically the nose, cheeks, forehead and chin. They are also found in the external acoustic duct, and in the skin of the chest and genital areas (Raszeja-Kotelba *et al.* 2004).

It is believed that transfer of *Demodex* from person to person happens by direct contact, by using common toiletries and towels, and through dust. Colonization of the skin occurs in childhood, and they are not found only in the skin of newborns (Bonnar *et al.* 1993).

However, it should be pointed out that the mere presence of *Demodex* mites in the skin of the face does not lead to the development of disease symptoms in the majority of people (Akilov and Mumcuoglu 2003). It is probably this reason why it was believed that *Demodex* mites are commensals or symbiotes living in the human skin (Lacey *et al.* 2009). However, most recent research indicates a pathogenic role for *Demodex* in developing various skin and eye diseases (Lacey *et al.* 2007, 2009). This is why we decided to study if *Demodex* mites can be associated with the presence and development of chalazion in a European population in Poland.

Materials and Methods

Parasitological examination

In order to examine the presence of *Demodex* in the hair follicles of eyelashes and their potential participation in the etiology of chalazia, a study of 121 people was conducted (36 with the presence of chalazion and 85 without the pres-

ence of ill effects to the protective apparatus of the eye and its surface), including 80 women and 41 men. The average age of subjects was 34.81 (women – 34.02, men – 36.36). In all 36 patients with chalazion, the chalazion was found on the eyelid of one eye.

A minimum of ten random samples of the eyelashes were taken from each subject and the samples were examined by light microscope, using standard parasitological methods applied in suspicion of infestation by *Demodex* mites. Results were archived with a camera. A positive result was assumed in the presence of at least one individual adult, larvae, nymph or protonymph of *D. folliculorum* or *D. brevis*.

All patients signed a consent to the collection of hair samples from eyelashes for examination.

Archiving

Photographs were taken for patients with chalazion. Photographs were taken with the camera built into the biomicroscope fitted with a slit lamp and/or with the camera built into the Oculus Keratograph, as well as with the Oculus Keratograph’s Meibography, for storing the presence of chalazion in digital form. Photographs were not taken from subjects without the presence of chalazion.

Statistical analysis

In order to produce a statistical description of the examined population and to explore the relationships between the occurrence of *Demodex* and chalazion some statistical analysis was carried out. The independence test χ^2 was applied with usage of fourfold table. Test χ^2 does not give information about the strength of dependence, so Czaprow’s coefficient was calculated, which value is in the range of $<0; 1>$.

Results

All subjects were tested for the presence of *Demodex* and chalazion. It was found that in the whole examined population 4 groups could be identified:

- Group 1 – patients with *Demodex* present, chalazion absent,
- Group 2 – patients with *Demodex* and chalazion present,
- Group 3 – patients with *Demodex* absent, chalazion present,
- Group 4 – patients with *Demodex* and chalazion absent.

Among the patients with chalazion, 91.67% also had the simultaneous presence of *Demodex* spp. whereas only 12.94% of the patients without chalazion were infested with *Demodex* spp. Figures 5–6 show graphical presentations of the above dependencies.

The size of individual groups broken down by sex, fraction of the examined population, and average age of subjects in the groups, are shown in Table I. A graphical presentation of the data shown in Table I in the total examined population and broken down according to sex is included in Figures 7 and 8.

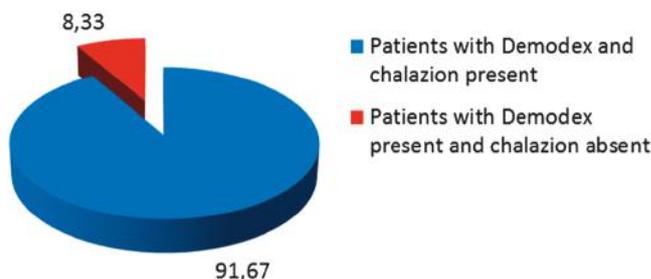


Fig. 5. Presence and absence of *Demodex* in patients with chalazion

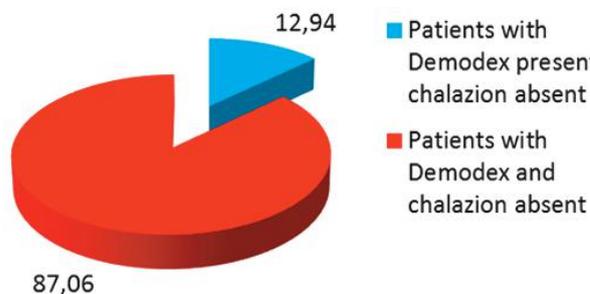


Fig. 6. Presence and absence of *Demodex* in patients without chalazion

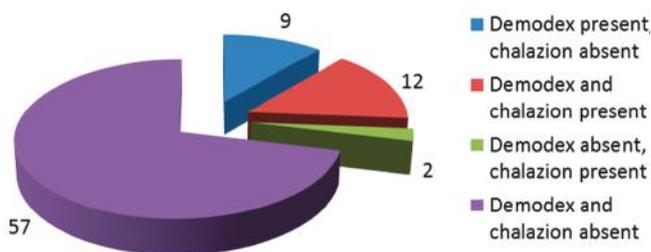


Fig. 7. Population of examined women with division into groups

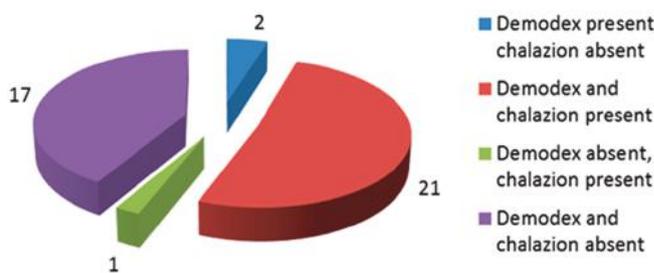


Fig. 8. Population of examined men with division into groups

Table I. The size of individual groups of subjects examined for the presence of *Demodex* broken down according to sex, percentage of the examined population and the average age of subjects included in the groups (F – female, M – male)

| | <i>Demodex</i> present, chalazion absent | <i>Demodex</i> and chalazion present | Chalazion present, <i>Demodex</i> absent | <i>Demodex</i> and chalazion absent |
|---------------------------------|---|---|---|--|
| Population | 11 (F:9; M:2) | 33 (F:12; M:21) | 3 (F:2; M:1) | 74 (F:57; M:17) |
| Percentage | 9.09% | 27.27% | 2.48% | 61.15% |
| Average age in the group | 37.63% | 47.54 | 19.00 | 28.56 |

Table II. The populations of individual groups of subjects, in whom the occurrence of *Demodex* and/or chalazion were found

| | Age ranges | | | | | | |
|-----------------|------------|-------|-------|-------|-------|-------|-------------|
| | 0–20 | 21–30 | 31–40 | 41–50 | 51–60 | 61–70 | 71 and more |
| IN TOTAL | | | | | | | |
| <i>Demodex</i> | 3 | 6 | 13 | 6 | 4 | 7 | 5 |
| Chalazion | 3 | 5 | 10 | 6 | 2 | 5 | 5 |
| WOMEN | | | | | | | |
| <i>Demodex</i> | 2 | 2 | 5 | 2 | 4 | 4 | 2 |
| Chalazion | 1 | 2 | 2 | 2 | 2 | 3 | 2 |
| MEN | | | | | | | |
| <i>Demodex</i> | 1 | 4 | 8 | 4 | 0 | 3 | 3 |
| Chalazion | 2 | 3 | 8 | 4 | 0 | 2 | 3 |

In order to carry out statistical analysis to confirm or exclude the existence of a cause-and-effect relationship between the presence of *Demodex* and occurrence of chalazion, the occurrence of *Demodex* and chalazion in individuals was divided into age ranges (see Table II): from 0 to 20 years old, from 21 to 30 years old, from 31 to 40 years old, from 41 to 50 years old, from 51 to 60 years old, from 61 to 70 years old, 71 years old and more.

Analysis of the data shows that the presence of *Demodex* was found in subjects of each examined age range (Table II). A correlation was noted between the prevalence of *Demodex* spp. and the age of the patients (Table II). Mites were most commonly found in subjects from the age ranges 31–40 and 41–50 years. Whereas, least occurrence was observed in patients of age groups 0–20 and 51–60 years and greater. A similar correlation in relation to age was observed in the case of chalazion occurrence (Table II).

Taking into account both the age and sex of the patients, *Demodex* was found most "commonly in women from age groups 31–40 and 51–60 years, while in men *Demodex* occurred more frequently in the age group 31–40 years (Table I). A similar distribution can be attributed to the presence of chalazia among men and women (Table I).

In order to examine the relationship between the occurrences of *Demodex* and chalazion a statistical inference was conducted using the Chi-square independence test and applying the fourfold table.

The following hypotheses were assumed: H_0 : "The occurrences of *Demodex* and chalazion are independent of each other"; and the alternative hypothesis H_1 : "There is a relationship between the occurrences of *Demodex* and chalazion". The significance level $\alpha = 0.05$ was adopted.

Among the 121 examined patients, chalazion occurred in 36 subjects, while the presence of *Demodex* was observed in 44 (Table III). The simultaneous presence of *Demodex* and chalazion was found in 33 subjects (27%), whereas only three subjects had the presence of chalazion in the absence of *Demodex*. These dependencies are shown in Table III.

Statistical analyses

The calculations gave the result: $\chi^2_{0.05,df} = 67.73$ – for the significance level $\alpha = 0.05$ and the number of independent variables $df = (k-1)*(w-1) = (2-1)*(2-1) = 1$. For these parameters,

Table III. Relationship between the occurrences of *Demodex* and chalazion in the examined group of patients

| | | <i>Demodex</i> | | Σ |
|------------------|----------------|----------------|-----------|------------|
| | | Present | Absent | |
| Chalazion | Present | 33 | 3 | 36 |
| | Absent | 11 | 74 | 85 |
| Σ | | 44 | 77 | 121 |

the critical value of distribution χ^2 , is $\chi^2_{0.05; df} = 3.841$; so the test result significantly exceeds the critical value, resulting in rejection of hypothesis H_0 in favour of adoption of the alternative hypothesis H_1 .

On the basis of the observations and the results obtained from statistical analysis we can unambiguously determine the existence of a relationship between the occurrence of *Demodex* and falling ill with chalazion – which confirms hypothesis H_1 .

Test χ^2 does not give information about the strength of dependencies, and in relation to this, Czaprow's coefficient was calculated, which value is in the range $<0; 1>$. For the analysed data its value is $T = 0.75$, which testifies for relatively large strength of dependency between the examined features and confirms the positive correlation between the presence of *Demodex* and chalazion.

Discussion

The role of bacterial factors (most frequently *S. aureus*) and *Demodex* in the etiology of chalazion is not exactly explained, therefore, each study providing new data to this problem contributes to a better understanding of the role of these organisms as possible causes of the development of chalazion.

First observations on the presence of *Demodex* in chalazion were made by von Hippel only in 1932. He found the presence of *Demodex* in the material collected from the chalazion of surveyed patients. Over ensuing years the contribution of *Demodex* in the development of chalazion has remained unknown, and numerous researchers considered *Demodex* to be commensal organisms that do not contribute in the etiology of skin and/or eyes diseases (Lacey *et al.* 2011). Nowadays, more and more researchers tend to acknowledge the role of *Demodex* as etiological factors of selected diseases of the skin and/or eyes (Humiczewska 1991; Kamoun *et al.* 1999; Pena and Andrade Filho 2000; Lacey *et al.* 2009; Kligman and Christensen 2011; Jarmuda *et al.* 2012), however, the discussion on this topic is still ongoing. Some of the other studies indicated that the cancer – and particularly breast cancer – is a risk factor for *Demodex* species infestation (Sönmez *et al.* 2013).

It is well known that infection with parasites can significantly vary in different human populations. Factors influencing prevalence of certain species are, *inter alia*, climatic, social, sanitary conditions, cultural determinants, the availability of treatment, the effectiveness of therapy, *etc.* Ascertainment of the pathogenic role of *Demodex* inclines researchers to analyse the correlation of the presence of diseases associated with it in a variety of patient populations, i.e. in populations from different age groups, backgrounds, continents and cultures. In this respect the topic taken by us seems justified, and finding a positive correlation between the presence of *Demodex* and chalazion, in the European population, proves their pathologic role.

In 2014, Yam *et al.* (2014) conducted a retrospective study of the presence of *Demodex* among 30 patients (48 eyes) with recurrent chalazion, simultaneously checking the effectiveness of treatment with application of tea tree oil (TTO). *Demodex* was detected in a microscopic study on randomly taken lashes. They observed the presence of mites in 35 eyes with chalazion (72.9%). On this basis, they found a positive correlation between the presence of *Demodex* and relapses of chalazion. The authors also confirmed a previously noted anti-*Demodex* effectiveness of the TTO. After applying TTO treatment to 31 patients with recurrent chalazion correlated with *Demodex* infection, they ascertained there was no recurrence of the disease with the exception of one case. The success rate of the TTO in prevention of relapse was 96.8%.

Similar studies were conducted by Liang *et al.* (2014) through analysing the presence of *Demodex* and chalazion in adult and paediatric patients; however, again in an Asiatic population. They observed a positive correlation between the occurrences of *Demodex* and chalazion, which was confirmed by statistical analyses. They claimed that *D. brevis* was much more present in the chalazion. On this basis, they came to the conclusion that the occurrence of *Demodex* increases the probability of the development of chalazion (Liang *et al.* 2014). They also observed that the prevalence of the occurrence of *Demodex* is significantly higher in patients with numerous chalazion. Similarly to the findings of Yam *et al.* (2014), in patients with *Demodex* the recurrences were more often recorded after surgical removal of chalazion.

Both studies presented above refer to groups of patients from China, Asia (Liang *et al.* 2014; Yam *et al.* 2014). As has been mentioned, the prevalence of infection of most species of parasites can vary greatly in different populations, even more on different continents. Therefore, it is important to determine the data on the prevalence of *Demodex* spp. in distinct populations, which increases the probability of confirmation of their importance in development of eye diseases including chalazion. From this point of view our results provide important additional evidence for the pathogenic role of this group of parasitic mites.

Our study of the group of 121 patients and statistical analysis clearly indicates the existence of a positive correlation between the occurrences of *Demodex* and chalazion in patients belonging to different age groups and living in European conditions. Our analyses show that the percentage of subjects with chalazion and simultaneous presence of *Demodex* was 91.67%. It appears that the percentage in Poland is higher than observed in patients in China, who were examined by Yam *et al.* (2014) and Liang *et al.* (2014), where it was 72.9% and 69.2%, respectively. The results of the present study indicate a statistically significant relationship between the occurrence of chalazion and the presence of *Demodex* in the hair follicles of eyelashes, in patients in Poland. In comparison to studies performed in Asia (Liang *et al.* 2014; Yam *et al.* 2014) we observed a more statistically significant interrelationship between the presence of *Demodex* and chalazion.

Concerning the pathogenesis of *Demodex* infestation, it is believed that *Demodex brevis*, which was observed in the centre of meibomian granulomas surrounded by epithelial cells, histiocytes, fibroblasts, lymphocytes and plasma cells, can be one of the causes of recurrent and treatment-resistant chalazion (Liang *et al.* 2014). In our studies we did not determine the species of *Demodex* as from our own experience we know that molecular PCR differentiation is necessary and this was not the ultimate goal of our studies.

Baima and Sticherling (2002) suggested that lesions which occur in the course of *Demodex* blepharitis are the consequence of:

1. obstruction caused by mites' blocking of hair follicles and the canaliculus orifices of sebaceous glands, excessive keratinization and hyperplasia of epithelium;
2. mechanical transmission of bacteria and fungi;
3. the host inflammatory response to the presence of parasite chitin, as a foreign body;
4. stimulation of humoral and cellular responses of the host under the influence of dust mites and their metabolites.

Probably, as suggested by Baima and Sticherling (2002), *Demodex* spp. block the orifices of the Meibomian glands, leading to stagnation of secretion in the holocrine glands and to the formation of chalazion. It also cannot be excluded that *Demodex* can be the vector for other pathogens that significantly affect the development of inflammatory states of the protective apparatus of the eye and its surface. In this case, mites may contribute to the development of inflammation directly, but also indirectly, as the vector for disease-causing bacteria and fungi. On the surface of *Demodex* mites there has been found the presence of strains of streptococci, staphylococci and fungi (Wolf *et al.* 1988; Lacey *et al.* 2007). It has been proven that one of the bacteria living in the digestive tract of *Demodex* – *Bacillus oleronius*, produces antigens which may stimulate the proliferation of peripheral blood mononuclear cells in patients with acne rosacea (Lacey *et al.* 2007). Similarly, *Demodex*'s antigens and their metabolism products might play a role in causing an inflammatory response in the host. Of course, we cannot rule out the possibility that the ongoing inflammation itself creates some favourable conditions for the existence of *Demodex* spp., which may stimulate the development of these mites in the hair follicles of eyelashes. Thus, the occurrence of *Demodex* would be the result of a secondary cause and not the primary one of the development of chalazion. However, more and more facts support the hypothesis that this group of mites are parasitic organisms. Also, our studies showing a positive correlation between *Demodex*-chalazion in various age groups indicates a pathogenic reaction to the presence of mites, rather than their existence as commensals. Nevertheless, to fully explore the role of *Demodex* in the pathogenesis of chalazion and other diseases of the protective apparatus of the eye, the eyes and skin, it is necessary to continue research on a variety of patient populations living in different countries, continents and climates. It is not out of the question that undertaking some multidisciplinary

research may be necessary, which will be performed by teams consisting of pathomorphologists, ophthalmologists, parasitologists, biochemists and molecular biologists. It would have fundamental importance for better understanding pathogenic mechanisms and reactions of humans to infestation by *Demodex* mites.

Conclusions

Parasitological examination of sampled eyelashes for the presence of *Demodex* in the hair follicles and statistical analysis of the obtained results clearly indicate the existence of a strong positive correlation between infestation by *Demodex* and the occurrence of chalazion in patients in Poland. Confirmation of the above-mentioned correlation in a European population provides further evidence for the possible pathogenic role of *Demodex* mites. The occurrence of chalazion should, therefore, be a signal to carry out parasitological diagnostics of possible demodicosis.

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Received: March 17, 2015

Revised: June 23, 2015

Accepted for publication: June 25, 2015