

Prevalence of *Demodex* spp. in students and employees of the Pomeranian Medical University in Szczecin*

Częstość występowania *Demodex* spp. u studentów i pracowników Pomorskiego Uniwersytetu Medycznego w Szczecinie*

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ABSTRACT

Introduction: *Demodex folliculorum* and *Demodex brevis* are mites living in the hair follicles and sebaceous glands of humans, with infestation usually being asymptomatic.

In the present study the prevalence and number of mites, together with influencing factors of *Demodex* infestation, were investigated in students and personnel of the Pomeranian Medical University in Szczecin in Poland (PUM). The prevalence of 2 *Demodex* species, *Demodex folliculorum* and *Demodex brevis*, was compared in epilated eyelashes and skin scrapings derived from healthy volunteers.

Materials and methods: The study was conducted on 217 healthy volunteers – females and males. The study group consisted of 114 students and 103 employees of PUM. From each study participant, 2–3 eyelashes from each lid were epilated. From 99 volunteers, skin scrapings and/or secretions from sebaceous glands were also obtained. Demodicosis was diagnosed when *Demodex* eggs, larvae or adult mites were identified under microscopic examination.

Results: The infestation rate of *Demodex* (in epilated eyelashes and skin) among the 217 volunteers was 21%. Prevalence of hair follicle mites among personnel and students were 28% and 14% respectively. The mean number of mites among the 217 patients was 2.52 ± 2.48 (3.16 ± 2.9 in PUM personnel and 1.46 ± 0.83 in students). The infestation rate increased with age ($p = 0.0005$). *Demodex folliculorum* infested 78% ($p = 0.005$) of the participants and 87.5% of epilated eyelashes ($p = 0.000001$). *Demodex brevis* was more frequent on the skin (67%; $p = 0.00001$). Hair follicle mites were detected more often in epilated eyelashes originating from the lower lid ($p = 0.000001$). Moreover, a weak correlation between the presence of selected symptoms (blepharodema, conjunctivitis, burning and itching of eyelids) and *Demodex* infestation was observed. There was no statistically significant association between *Demodex* infestation and an influencing factor: work using a microscope ($p = 0.92$).

Keywords: *Demodex* mite; demodicosis; *Demodex folliculorum*; *Demodex brevis*.

ABSTRAKT

Wstęp: Nużeńce *Demodex folliculorum* i *Demodex brevis* są roztozcami występującymi w mieszkach włosowych i gruczołach łojowych ludzi. Zarażenie nużeńcem może przebiegać bezobjawowo. Celem przeprowadzonych badań była ocena ekstensywności zarażenia *Demodex* spp. w rzęsach i skórze twarzy u studentów i pracowników Pomorskiego Uniwersytetu Medycznego w Szczecinie (PUM).

Materiały i metody: Badania przeprowadzono u 217 zdrowych ochotników – kobiet i mężczyzn – podzielonych na 2 grupy: 114 studentów i 103 pracowników. Od każdej osoby uczestniczącej w badaniu pobierano po 2–3 rzęsy z górnej i dolnej powieki obu oczu. Od 99 osób pobrano również zeszkrobiny skóry i/lub wydzielinę gruczołów łojowych twarzy. Wynik dodatni badania przyjmowano na podstawie stwierdzenia obecności postaci rozwojowych *Demodex folliculorum* i *Demodex brevis* – jaj, larw lub postaci dojrzałych.

Wyniki: Obecność *Demodex* spp. w rzęsach i/lub skórze twarzy stwierdzono u 21% badanych. Wśród pracowników odsetek osób

zarażonych *Demodex* spp. wynosił 28%, natomiast w grupie studentów 14%. Ekstensywność zarażenia *Demodex* spp. wzrasta wraz z wiekiem ($p = 0,0005$). Odsetek osób zarażonych *D. folliculorum* (rzęsy i/lub skóra twarzy) wyniósł 78% ($p = 0,005$), w przypadku infestacji samych rzęs – 87,5% ($p = 0,000001$). W skórze częściej stwierdzano obecność *D. brevis* (67%; $p = 0,00001$). W rzęsach średnia liczba osobników *Demodex* spp. wyniosła $2,52 \pm 2,48$, w tym u pracowników $3,16 \pm 2,9$, a u studentów $1,46 \pm 0,83$ ($p = 0,03$). U osób zarażonych stwierdzono częstsze występowanie *Demodex* spp. na rzęsach powiek dolnych niż górnych ($p = 0,000001$). Wykazano słabą korelację pomiędzy występowaniem niektórych objawów (obrzęk powiek, zapalenie spojówek, świąd i pieczenie powiek) a zarażeniem *Demodex* spp. Nie wykazano związku między pracą z mikroskopem a zarażeniem nużeńcem ($p = 0,92$).

Słowa kluczowe: nużeniec; demodekoza; *Demodex folliculorum*; *Demodex brevis*.

* Based on the Master's thesis accepted by the Council of the Faculty of Medicine and Biotechnology and Laboratory Medicine of the Pomeranian Medical University in Szczecin. Promoter: dr hab. n. med. Lidia Kołodziejczyk. The original includes 63 pages, 20 figures, 6 photographs, 14 tables and 103 references.

INTRODUCTION

Demodex spp. are external obligatory parasites found in hair follicles and sebaceous glands of mammalian skin. Two species – *Demodex folliculorum* and *Demodex brevis* – occur in humans [1]. In a study to determine the extensiveness of *Demodex* spp. it has been observed that most people are infested with this parasite [2]. Although the presence of *Demodex* spp. may be associated with numerous skin pathologies, they are also commonly detected in healthy individuals [3].

The symptoms accompanying demodicosis are slight redness and dryness of the skin, as well as conjunctivitis, dysfunction of Meibomian glands, and dry eye syndrome [4, 5, 6, 7]. *Demodex folliculorum* occur in clusters near the roots of eyelashes and hair follicles, whereas *D. brevis* usually occurs singly in sebaceous glands and Meibomian glands [8]. In men, the symptoms of infestation are more frequent than in women, probably due to the more abundant production of sebum. *Demodex folliculorum* is more likely to cause infestations, but *D. brevis* occurs over a larger area of the body [9]. *Demodex* spp. is rare in children and teenagers; it is believed that neonates may become infested with nebulae shortly after birth by direct contact, but their density remains low due to low sebum production in the first years of life [10, 11].

Demodex spp. probably plays an important role in the development and course of various dermatological diseases, such as follicular dandruff, perioral dermatitis, eyelid dermatitis, rosacea, rosacea-like rash, pustulosis and granulomatous dermatitis [12].

MATERIALS AND METHODS

The study was conducted on 217 healthy volunteers – women and men, divided into 2 groups: 114 students and 103 employees of the Pomeranian Medical University in Szczecin (PUM). In a research, 2–3 eyelashes were aseptically collected with tweezers from the upper and lower eyelids of both eyes from each person. They were placed between a slide and a cover slip and viewed under a light microscope (magnifications 100x, 400x). A positive result was obtained on the basis of the presence of developmental forms: eggs, larvae or mature forms of *D. folliculorum* and/or *D. brevis*.

Scrapings of the face skin (using a sharp spoon) and/or sebaceous gland secretion were also collected for diagnostic tests from 99 of the subjects. The tested material was placed on a slide, covered with 10% potassium hydroxide (KOH) and a cover slip. The preparation was viewed under a light microscope (magnifications 100x, 400x). Infestation was diagnosed when eggs, larvae or mature forms of *D. folliculorum* and/or *D. brevis* were observed.

RESULTS

Among the 217 participants, 21% (45) were infested with *Demodex* spp. Their presence was found on eyelashes and/or in the

skin of the face. In the group of students, the infestation rate was 14%, while among the employees it was twice as high (28%). A statistically significant correlation was found between the infestation rate and age ($p = 0.0005$) – Figure 1.

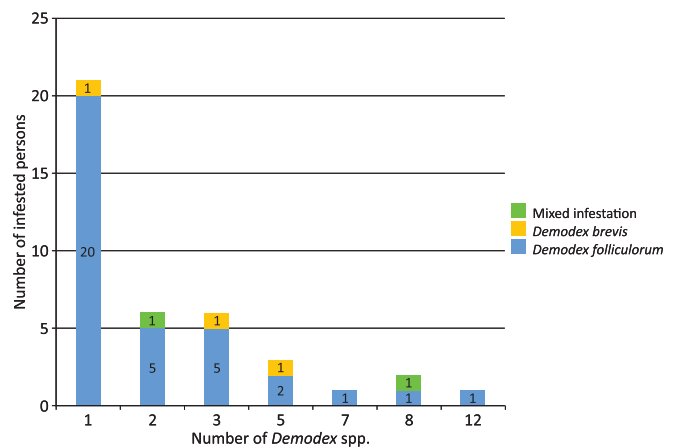


FIGURE 1. Number of *Demodex* spp. detected in infested persons, by species

The presence of *D. folliculorum* was more frequent in all infested subjects (78%) than *D. brevis* (11%; $p = 0.005$). *Demodex folliculorum* dominated in the eyelashes ($p = 0.000001$), whereas *D. brevis* dominated in skin scrapings and/or sebaceous secretions ($p = 0.00001$). During examination of the eyelashes, *Demodex* spp. were more frequent in the material collected from the lower eyelids than from the upper eyelids ($p = 0.000001$) – Figures 2 and 3.

Out of 217 participants, 164 declared symptoms that could potentially indicate the occurrence of ocular or dermal demodicosis. These symptoms included itching and burning of the eyelids, swelling of the eyelids, tearing of the eyes, loss of eyelashes, conjunctivitis, festering inflammation of the eye, skin

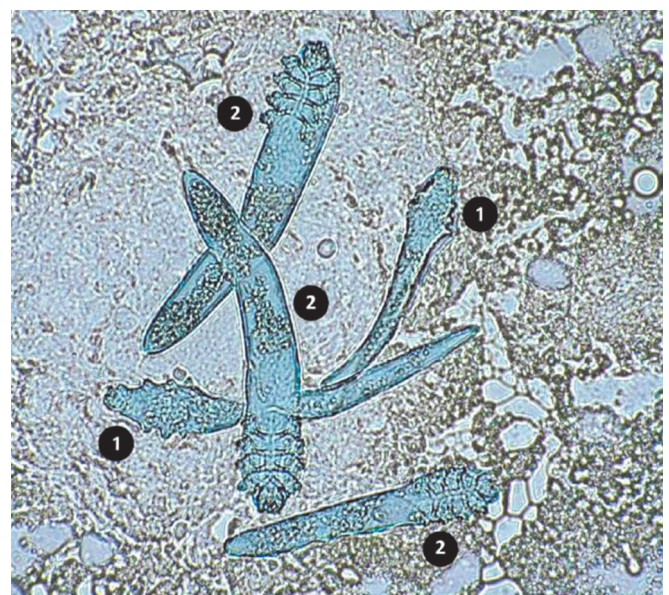


FIGURE 2. Larvae (1) and adult *Demodex folliculorum* (2) in facial sebaceous secretions (100x magnification)

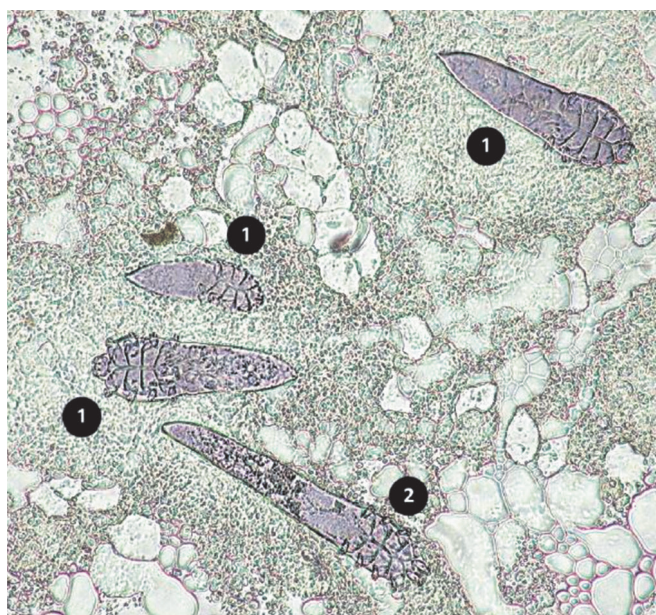


FIGURE 3. Mixed infestation in sebaceous secretions of the facial skin glands with adult *Demodex brevis* (1) and *Demodex folliculorum* (2) – 100× magnification

lesions (comedo) and itching of the skin around the forehead, mouth, nostrils and chin. A positive result of parasitological examination for *Demodex* spp. was found in 32 subjects (20%) declaring symptoms and 13 subjects (25%) not reporting any symptoms of demodicosis. No significant correlation was demonstrated between the demodicosis symptoms reported by the patients (at least one mentioned in the survey) and *Demodex* spp. infestation ($p = 0.43$).

Out of the 217 participants, 153 people worked with a microscope. In this group the percentage of people infested with *Demodex* spp. was 21% (32 persons), and in the group of people without any contact with a microscope the infestation rate was 20% (13 persons). There was no significant correlation between working with the microscope and *Demodex* spp. infestation ($p = 0.92$).

DISCUSSION AND CONCLUSIONS

Demodex folliculorum mites and *D. brevis* are common human parasites [2, 13, 14, 15, 16, 17]. Their prevalence increases with age, and in people over 70 may reach even 100% [18, 19, 20]. In this study, *Demodex* spp. was found in 21% of subjects, where among university employees it was higher (28%) than among students (14%).

In sociodemographic studies on healthy populations conducted in China [21, 22, 23, 24, 25, 26, 27, 28, 29, 30] and Turkey [31, 32, 33], the proportion of infested persons among primary, secondary and tertiary level students ranged 21.3–37.7%. Another study in China on students aged 18–22 years reported an exceptionally high prevalence (92.8%) of *Demodex* spp. infestation of the skin [29]. According to those researchers, that elevated rate was related to the multiple repetition of tests which increased

the reliability of diagnosis [34, 35]. Such a high percentage of infested young people may also be due to the type of *Demodex* spp. detection method commonly used in China – cellophane tape method (CTP). It consists of sticking several cellophane tapes to the face at night, then observing them under a light microscope the next morning. According to Zhao et al. [29], this method is the best for studying the extensiveness of *Demodex* spp. infestation in a healthy population and is more sensitive than other commonly used methods because of the movement of *Demodex* spp. at night to the mouth of the hair follicle for mating.

In studies conducted in patients with various facial skin diseases, skin biopsy and standard skin surface biopsy (SSSB) are recommended methods for detecting *Demodex* spp. Skin biopsy allows an observation of the damage caused by the pathological process in the histological fragment of the deeper skin layers. However, the invasiveness of this method requires the use of local anesthesia. In contrast, a standard skin surface biopsy is an easy and fast method that detects *Demodex* spp. in the follicle infundibulum and determines *Demodex* spp. density per 1 cm² of skin. In SSSB, cyanoacrylate is placed on the surface of the microscope slides, which is applied to the test person's skin for 1 min, and then detached and viewed under a stereomicroscope. The cellophane tape method, similar to surface skin biopsy, uses cellophane and cyanoacrylate. Standard skin surface biopsy is actually even a more sensitive method than CTP [29, 36]. Forton and De Maertelaer [37] attempted to increase the sensitivity of SSSB and performed 2 biopsies immediately after each other in the same place, after cleaning the slide and skin with ether. The obtained results were comparable to the detection of *Demodex* spp. by confocal microscope. The second superficial skin biopsy enabled the examination of deeper skin layers and the collection of about twice as many individuals [37].

In this study, *D. folliculorum* (78%) was the most frequently detected *Demodex* spp. species on eyelashes. Infestations caused by *D. brevis* or co-infestations were less frequent. These results confirm numerous reports [21, 22, 23, 27, 28, 29, 31, 38, 39] which indicate that *D. folliculorum* is the dominant *Demodex* spp. species on humans. More frequent detection of *D. folliculorum* may also be associated with the exact location of both species within the hair follicles. *Demodex folliculorum* occurs in the follicle infundibulum near its exit, whereas *D. brevis* occurs deeper, in the sebaceous glands [40].

In this research, *D. brevis* was a more frequently detected species (67%) on the skin. This was probably due to the type of method used to detect *Demodex* spp. on the skin. *Demodex* spp. were found mainly in sebaceous gland secretions (78%) and less frequently in skin scrapings (22%). This assumption is confirmed by research conducted by Akilov and Mumcuoglu [41], who, using the same method, obtained a higher prevalence of *D. brevis* infestation (41%). In a study in which a standard skin surface biopsy was performed, the main species detected was *D. folliculorum*, most likely due to the small amount of sebaceous secretions collected by that method [42].

This study did not show any correlation between *Demodex* spp. infestation and the presence of skin/eye demodicosis

symptoms. Similar results were obtained by Isa et al. [31] and Seyhan et al. [40], in contrast to earlier studies [43, 44], which showed a statistically significant correlation between these symptoms and infestation with *Demodex* spp. These discrepancies may be due to the fact that many factors (e.g. environmental, hormonal, individual) may cause similar symptoms to those caused by the presence of *Demodex* spp. The results may also be influenced by differences between the detection methods. In Poland, they are usually qualitative methods based on the examination of eyelashes, skin scrapings or sebaceous gland secretions. Cellophane tape method and SSSB are quantitative methods whose aim is to determine the number of individuals per 1 cm² of skin. In a superficial skin biopsy, demodicosis is confirmed by a concentration of >5 individuals per 1 cm² of skin and >10 individuals during the second SSSB, with symptoms suggesting *Demodex* spp. infestation [29, 37, 45, 46]. However, so far, there has been no clear indication of the minimum number of individuals responsible for the symptoms caused by the *Demodex* spp. infestation [8].

There was weak correlations between the occurrence of some symptoms of ocular demodicosis, such as eyelid swelling, conjunctivitis, itching and burning of eyelids, and the identification of *Demodex* spp. on the eyelashes. The research conducted among PUM employees also showed that the microscope as a working tool did not constitute a transmission factor for *Demodex* spp. On one hand, this may be due to the limited life expectancy of these mites outside the human body and the need for direct contact during transmission [8]. Garbacewicz et al. [47] demonstrated, however, in their study on a group working with the microscope on a daily basis, that the percentage of infested persons is similar to that found in the control group, whose mean age was 20 years higher. Due to the fact that the infestation rate among people working with a microscope was higher than their age might indicate, the microscope was indirectly considered as one of the transmission factors in that study.

Problems with maintaining eyelash hygiene result in a favorable environment for the spread and development of this parasite. However, the lack of specific symptoms of ocular demodicosis and the need for eyelash epilation to assess the infestation makes diagnostics problematic [48]. Detection of *Demodex* spp. is not very likely during random eyelash epilation. However, the detection of *Demodex* spp. on eyelashes accompanied by cylindrical dandruff increases the possibility of detection [7]. A study by Randon et al. [48] using *in vivo* confocal microscopes (IVCM) showed the reliability and effectiveness for the detection of ocular demodicosis. It also allows the evaluation of Meibomian glands and increases the possibility of detecting *D. brevis* larvae and adult individuals [48, 49].

SUMMARY

The effects of *Demodex* spp. infestations on human health are not fully understood. Studies carried out to determine the extensiveness of the occurrence of *Demodex* spp. show that

most people are infested with these parasites [2]. However, in most cases their presence does not cause any symptoms. The pathological role of *Demodex* spp. in humans is still a subject of numerous discussions and controversies. There is increasing evidence that they may become an opportunistic pathogen in conditions conducive to development and proliferation [50]. It is believed that one of the factors responsible for the transformation from clinically asymptomatic colonization of mites to dermatitis is primary or secondary immuno-suppression. Therefore, symptomatic infestation with *D. folliculorum* mites can be expected more often in immunocompromised patients, e.g. in AIDS, hematopoietic proliferative disease, and during immunosuppressive therapy (also topically) [12].

A recent study has implicated bacteria isolated from *Demodex* spp. mites in diseases such as rosacea or eyelid inflammation [51]. Further research to determine the role of *Demodex* spp. in skin and eyelid diseases and to improve the detection rate of these mites will help in designing new effective treatments.

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