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Original paper

Performance of the diagnostic test for *Demodex* spp. in the context of minimizing the risk of false negative results

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ABSTRACT. *Demodex* species are permanent parasites of pilosebaceous units infesting humans worldwide. Of the two species associated with the human host, *Demodex folliculorum* is much more commonly detected and occurs more abundantly in hair follicles and Zeiss glands. *Demodex brevis*, on the other hand, is mainly associated with the sebaceous and meibomian glands, all over the body. The infestation is usually chronic, and the troublesome ocular and skin symptoms often make it difficult for patients to function in both a social and professional context. Despite this, diagnostics for *Demodex* spp. are still not routinely performed during ophthalmological and dermatological examinations. We aimed to compare preparations of eyebrow hair, eyelashes, and skin scrapings, and to investigate the correlation with false negative results in the laboratory diagnosis of demodicosis. Direct microscopic examination was applied on 100 patients (81 women and 19 men). The most common ocular and skin symptoms reported by the study participants were eyelid itching and erythema, respectively. Significant differences were found between infested and uninfested patients with regard to the following symptoms: eyelid itching which was more common in infested patients (35.8%) (p = 0.0343) and chalazion occurring only in infested patients (13.2%) (p = 0.0285). The collection of diagnostic material from more than one site e.g. nasolabial folds, eyebrow hairs or eyelashes has a significant effect on the test result. Taking a single type of a sample from a single face zone can lead to false negative results.

Keywords: *Demodex*, demodicosis, diagnosis, eyelashes epilation, ocular symptoms, skin symptoms

Introduction

About 150 species of *Demodex* mites, collected from over 35 families of mammals, have been described [1]. So far two species have been recorded in humans: *Demodex folliculorum* (Simon, 1842) and *Demodex brevis* (Akbulatova, 1963).

In one host, co-infestation with both species of *Demodex* mites were noted. These species may differ in terms of the topography of lesions. The mean size of the adult forms is about 250 µm, although individuals reaching a length of up to 1000 µm have been described [2]. The body of *Demodex* mites is divided into three sections: gnathosoma, podosoma and opisthosoma.

Of the two, *Demodex folliculorum* (Fig. 1) is much more commonly detected and occurs more abundantly in human skin [3]. The mite inhabits hair follicles and Zeiss glands. Although it was first described in earwax, it is most often found in material collected from the eyelashes, nose, chin, cheeks, and forehead [4]. In contrast, *Demodex brevis* inhabits the sebaceous and meibomian glands [5–7]; it can be located practically all over the body, penetrating deep into sebaceous glands [8]. Unlike *D. folliculorum*, a single sebaceous gland usually contains a single *D. brevis* individual [9]. Finally, *D. brevis* (Fig. 2) has a very similar morphological structure to *D. folliculorum* but is shorter [9].

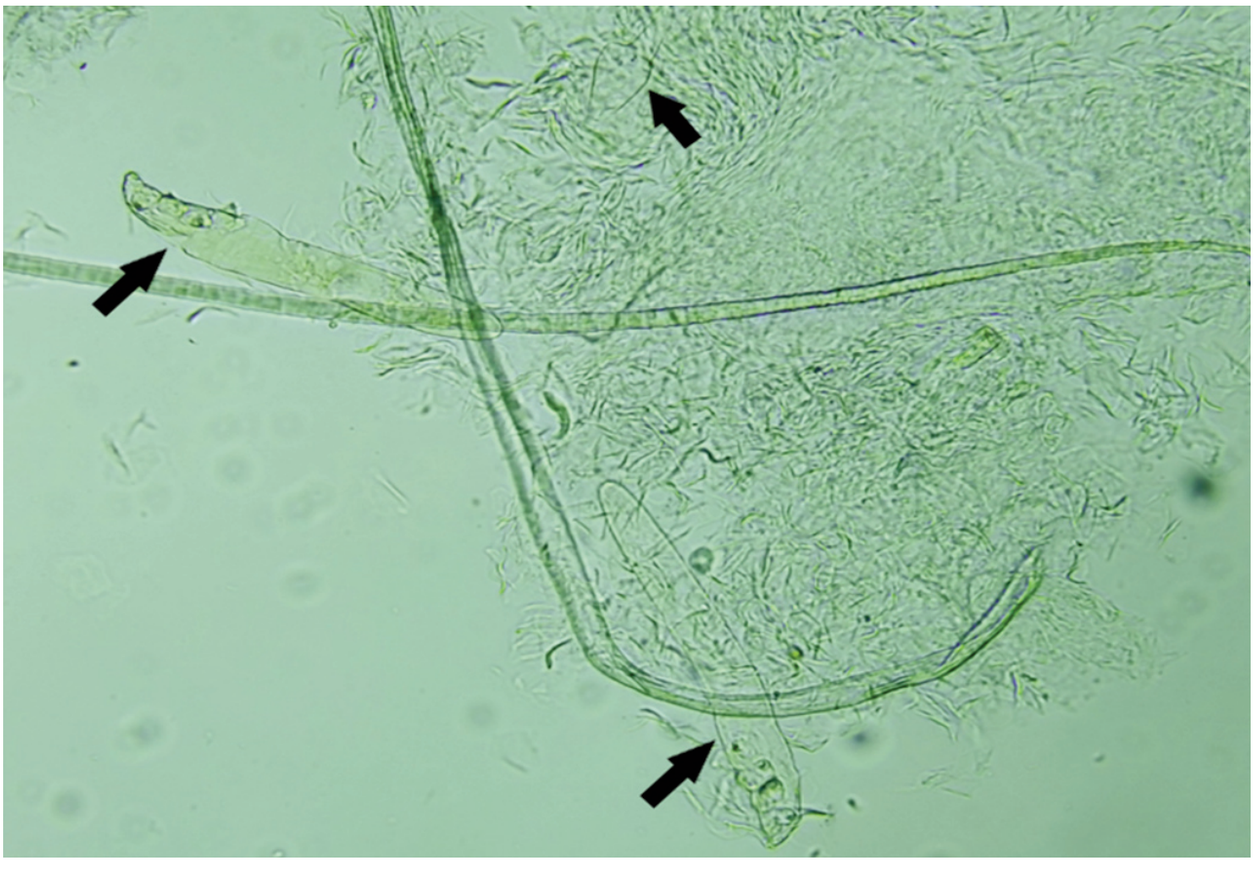

Figure 1. Two *Demodex folliculorum* adults and an egg marked with an arrow, in skin scraping preparation (original magnification 100×; photo by R. Przydatek-Tyrajska)

Demodex mites are usually detected using direct methods based on the analysis of eyelashes, scrapings of the facial epidermis or other affected areas under a light microscope. Validated methods used in the diagnosis of *Demodex* spp. also include standardized skin surface biopsy (SSSB), and less frequently, histopathological examination of a skin sample [10,11].

Eyelash examination involves taking several eyelashes from each eye using sterile tweezers. The eyelashes are placed on a glass slide and observed under a light microscope at 100–400× magnification

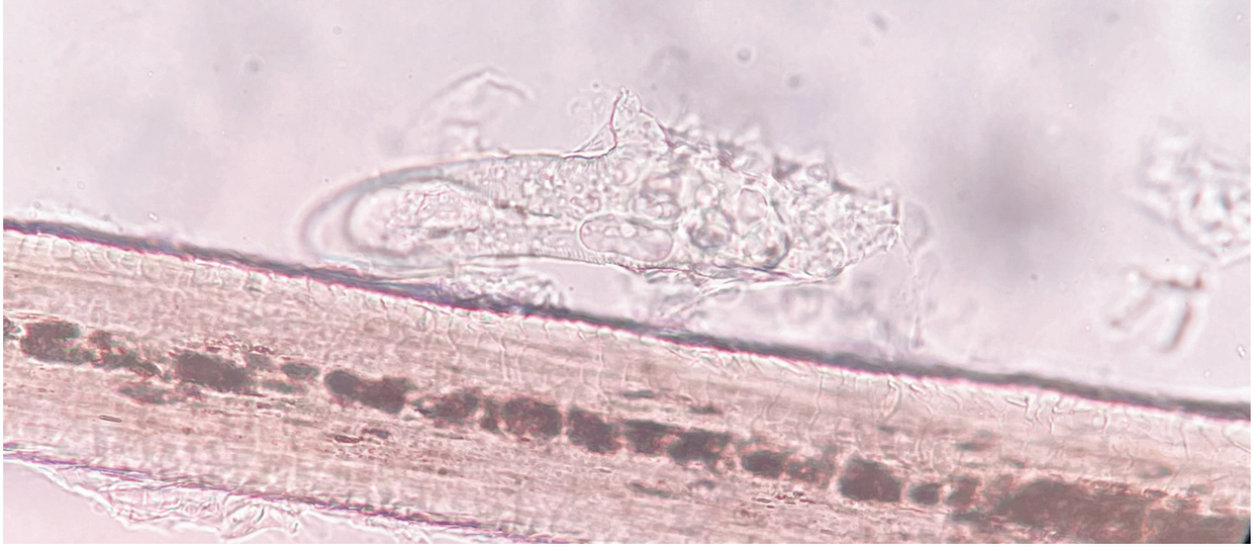

Figure 2. *Demodex brevis* adult in eyelashes preparation (original magnification 400×; photo by K. Bartosik)

Figure 3. Method of collecting diagnostic material for testing for the presence of *Demodex* mites: A – material collected from the eyebrows, B – collection of eyelashes, C – collection of skin scrapings from the nasolabial folds (photo by K. Kamińska)

[12]. The scrapings are examined by squeezing the contents of the sebaceous glands, then taking scrapings of the epidermis with sterile surgical blades [13]. In the SSSB method, a 1 cm^2 area is drawn on a microscope slide. A drop of cyanoacrylate glue is placed on the slide, the surface containing the glue is stuck to the skin, the glue is allowed to dry and the slide is then gently peeled off. After removal from the skin, 2–3 drops of immersion oil are dripped on the slide, which is then covered with a coverslip [14].

The aim of the study was to investigate the impact of diagnostic material collected (e.g., hair from eyebrows, eyelashes or epidermis scrapings) on the test result for the presence of *Demodex* spp.

Materials and Methods

Diagnostics were performed at RenoMed medical center in Warsaw. Direct microscopic examination (DME) was used to determine the presence and measure density of *Demodex* mites in obtained samples. The diagnostic material was collected from the eyebrows (Fig. 3A), eyelashes (Fig. 3B) and a scraping of the epidermis from the face (Fig. 3C). Eyelashes were collected using sterile tweezers, three from each eye, and scrapings from the face were collected using disposable sterile scalpel blades (size 10). Each sample was placed on a glass slide, then prepared with the addition of Hoyer's solution, and placed under a coverslip [15]. The preparations were studied under a light microscope at 100–400× magnification. A positive result was determined based on the presence of adult, juvenile forms or egg of *Demodex* spp. in the tested material.

The following patient inclusion criteria were applied: adults (i.e. aged at least 18 years), full legal capacity, complaining of chronic or recurrent skin and/or ophthalmological ailments (e.g. seborrheic dermatitis, rosacea, erythema, blepharitis, excessive loss of eyelashes and eyebrows).

Table 1. Ocular symptoms and diseases recorded in patients from the study group

Symptom/disease n = 100	n	%
Itchy eyelids	27	27.0
Dry eye sensation	24	24.0
Burning eyes	21	21.0
Watery eyes	9	9.0
Stye	13	13.0
Conjunctivitis	9	9.0
Chalazion	7	7.0
Eyelash loss	7	7.0
Blepharitis	7	7.0
Eyeball pain	1	1.0
Glaucoma	1	1.0
Photosensitivity	1	1.0
No symptomes/diseases	27	27.0
Total	154	154.9

n – number of responses; multiple responses – sum of % does not add up to 100

Table 2. Skin symptoms and skin diseases recorded in patients from the study group

Symptom/disease n = 100	n	%
Erythema	32	32.0
Papules	26	26.0
Pustules	18	18.0
Dry skin	17	17.0
Acne vulgaris	13	13.0
Rosacea	11	1.0
Excessive sebum production/seborrhoea	11	11.0
Pigmentation disorders	10	10.0
Itchy skin	7	7.0
Scars	4	4.0
Enlarged sebaceous glands	4	4.0
Dermographism	1	1.0
No symptoms/diseases	27	27.0
Total	186	186.0

n – number of responses; multiple responses – sum of % does not add up to 100

Table 3. The occurrence of skin symptoms in infested and uninfested individuals and the result of the Pearson chi-square test

	Demodex folliculorum infestation						
Skin symptoms	yes		no		χ^2	df	*p*
	n	%	n	%			
Occur	35	66.0	38	80.9			
Not occur	18	34.0	8	17.0	2.77	1	0.0958
Total	53	100.0	47	100.0			

n – number of people; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *p* – probability level

Table 4. The occurrence of skin allergies in infested and uninfested individuals and the result of the Pearson's chi-square test

	Demodex folliculorum infestation						
Skin allergies	yes		no		χ^2	df	*p*
	n	%	n	%			
Occur	5	9.4	11	23.4			
Not occur	48	90.6	36	76.6	3.62	1	0.0572
Total	53	100.0	47	100.0			

n – number of cases; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *P* – probability level

The study received the consent of the Bioethics Committee at the Medical University of Lublin (no. KE-0254/122/2018). All study participants gave their consent to participate by signing an „Informed Consent to Participate in a Scientific Research" form.

Table 5. The occurrence of inhalant allergies in infested and uninfested individuals and the result of the Pearson's chi-square test

	Demodex folliculorum infestation						
Inhalant allergies	yes		no		χ^2	df	*p*
	n	%	n	%			
Occur	13	24.5	13	27.7			
Not occur	40	75.5	34	72,3	0.13	1	0.7216
Total	53	100.0	47	100.0			

n – number of patients; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *p* – probability level

Table 6. The occurrence of inhalant and/or skin allergies in infested and uninfested individuals and the result of the Pearson's chi-square test

	Demodex folliculorum infestation						
Allergies	yes		no		χ^2	df	*p*
(inhalant/skin)	n	%	n	%			
Occur	16	30.2	19	40.4			
Not occur	37	69.8	28	59.6	3.15	1	0.2841
Total	53	100.0	47	100.0			

n – number of patients; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *p* – probability level

Table 7. The most common eye symptoms and eye diseases in infested and uninfested individuals and the result of the Pearson's chi-square test with Yates' correction

	Demodex folliculorum infestation						
	yes		no				
Ocular symptoms	n = 53		n = 47		χ^2	df	*p*
	n	%	n	%			
Itchy eyelids	19	35.8	8	17.0	4.48	1	0.0343
Dry eye sensation	12	22.6	12	25.5	0.11	1	0.7355
Burning eyes	14	26.4	7	14.9	1.99	1	0.1580
Watery eyes	6	11.3	3	6.4	0.26	1	0.6093
Stye	10	18.9	3	6.4	3.43	1	0.0639
Conjunctivitis	7	13.2	2	4.3	1.47	1	0.2258
Chalazion	7	13.2	0	0.0	4.80	1	0.0285
Eyelash loss	2	3.8	5	10.6	0.90	1	0.3420
Blepharitis	6	11.3	1	2.1	1.98	1	0.1598

n – number of patients; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *p* – probability level

Statistical analysis

Qualitative variables were described using counts (n) and frequencies (%). Age was described using basic parameters: arithmetic mean (M), standard deviation (SD), median (ME) and minimum and maximum values (Min. and Max.).

Pearson's chi-square test was used to examine the relationship between qualitative variables; where the expected counts were too small, the chi-square test with Yates' correction was used. The measurable variables were subjected to the Shapiro-Wilk test to confirm their normal distribution; where

Table 8. The most common skin symptoms in infested and uninfested individuals and the result of the Pearson's chi-square test with Yates' correction

Skin symptoms	*Demodex folliculorum* infestation						
	yes n = 53		no n = 47		χ^2	df	*p*
	n	%	n	%			
Erythema	14	26.4	18	38.3	1.62	1	0.2036
Papules	14	26.4	12	25.5	0.01	1	0.9200
Pustules	9	17.0	9	19.1	0.08	1	0.7782
Dry skin	11	20.8	6	12.8	1.13	1	0.2885
Pigmentation disorders	3	5.7	7	14.9	1.45	1	0.2293
Itchy skin	5	9.4	2	4.3	0.38	1	0.5350

n – number of patients; χ^2 – value of the chi-square statistic; df – number of degrees of freedom; *p* – probability level

they lacked a normal distribution, the nonparametric Mann-Whitney U-test was used to check the significance of the difference in two groups. A *p*-value of < 0.05 was considered statistically significant. Statistical calculations were performed using the STATISTICA 10 PL statistical package.

Results

In total, 100 patients were qualified for the study group (81 women and 19 men). The average age of the patients examined was 45 years both for women and men.

The ocular symptoms reported by the study participants are summarized in Table 1, with the eyelid itching as the most common symptom noted. The skin symptoms registered in the study participants are summarized in Table 2, with erythema as recorded most often. Various developmental forms of *D. folliculorum* were found in all infected patients. According to Pearson's chi-square test, no significant relationships were found between the occurrence of skin symptoms and *D. folliculorum* infestation (Table 3), the occurrence of skin allergies and the infestation (Table 4), the occurrence of inhalant allergies and the infestation (Table 5), or between the occurrence of inhalant and/or skin allergies and the infestation (Table 6) ($p > 0.05$).

Significant differences were found between infested and uninfested patients with regard to the following symptoms: eyelid itching which was more common in infested patients (35.8%) (< 0.05), and chalazion that affected only in infested patients (13.2%) (< 0.05). In the case of stye, the probability level was close to the significance limit ($p = 0.0639$) (Table 7). The skin symptoms associated with *Demodex folliculorum* infestation observed in the examined patients are presented in Table 8.

The relationship between the type of diagnostic material collected (the eyebrows, the eyelashes, and scraping of the epidermis from the face) and the number of *Demodex folliculorum* detected therein was also examined. In patients infested with

Table 9. Descriptive statistics of the number of *Demodex* mites for different preparations and the results of the Shapiro-Wilk test

Preparation	n	Number of *Demodex folliculorum*					W	*p*
		M	SD	Me	Min.	Maks.		
Eyebrows	52	0.8	1.9	0.0	0	11	0.62	< 0.0001
Eyelashes	52	3.6	3.3	3.0	0	13	0.88	< 0.0001
Skin	52	0.9	1.7	0.0	0	9	0.68	< 0.0001
Eyebrows+eyelashes+ skin	52	5.3	4.2	4.0	1	18	0.47	< 0.0001
Eyebrows+skin	52	1.7	2.8	0.0	0	12	0.89	0.0001

W – results of the Shapiro-Wilk normality test; *P* – probability level

Demodex folliculorum, the detection of mites was guaranteed by collecting skin scrapings from the nasolabial folds and eyebrow and eyelash hairs. Statistical analysis confirmed that collecting only one type of diagnostic material for testing, significantly reduced the probability of detecting of *Demodex folliculorum* in infested people (Table 9).

It was also investigated whether the number of *Demodex* mites detected in the eyelash preparation is related to the occurrence of ophthalmological symptoms (Table 9). The mean number of mites in the eyelash preparation was 4.1 (SD = 3.6) in patients without ophthalmological symptoms, and 5.6 (SD = 4.3) for those with ophthalmological symptoms. Despite the observed differences, no significant relationship was found between the intensity of *Demodex folliculorum* infestation and the occurrence of ophthalmological symptoms in patients ($Z = 1.03$; $p = 0.3007$; Mann-Whitney U-test).

Discussion

Typical symptoms of cutaneous demodicosis include lesions that present as papules, vesicles, or spots. In contrast, ocular demodicosis is characterized by redness in the conjunctiva and eyelids, exfoliation of the eyelid skin, watery eyes, burning sensations, itching, and loss of eyelashes [12,13]. Studies have indicated that among the ophthalmological symptoms, the most frequently reported by patients are itching of the eyelids and the presence of chalazion, both of which are significantly associated with *Demodex* spp. [13–17]. In some patients with demodicosis, the skin becomes dry or desiccated. Redness and characteristic peeling may occur as well as red spots and eczema [18,19]. Among the patients found to be positive for *Demodex folliculorum* in the present study, erythema occurred in 26.4%, dry skin in 20,8%, inflammatory papules in 26,4%, pustules in 17%; in other cases, pigmentation disorders, skin spots, acne scars and enlarged openings of the pilosebaceous follicles were less frequently noted. Furthermore, 3,8% of patients who had a positive result of the *Demodex folliculorum* eyelash test reported a problem with thinning eyelashes, i.e. an aesthetic discomfort, and were recommended preparations intended to stimulate eyelash growth. Other patients who reported accompanying eyebrow hair loss, were prescribed preparations to stimulate eyebrow growth. These cosmetics contain peptides and plant extracts, such as ginseng, green tea, saw palmetto, wheat, and marigold. Visible improvement, i.e. eyelash and eyebrow regrowth, occurred within three to four weeks of commencement. The effectiveness of preparations containing peptides and glycosaminoglucans in stimulating eyelash growth have been confirmed in previous studies [20].

The diagnosis of *Demodex* spp. infestation is straightforward and can usually be conducted in most ophthalmological or dermatological clinics. The location of sample collection (specifically the facial area) and the choice of diagnostic material are crucial for obtaining reliable results. The most effective method for detecting mites involves taking scrapings from the nasolabial folds and the hair of the eyebrows and eyelashes. Relying on only one type of sample significantly decreases the likelihood of parasite detection.

In conclusion, the performance of the diagnostic test for *Demodex* spp. has a significant effect on the result. Sample collection from the facial areas well as examination of different diagnostic materials minimizes the risk of obtaining a false negative result.

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