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RESEARCH ARTICLE

Prevalence and density of *Demodex* mites (Acari: Demodecidae) in patients with seborrheic dermatitis

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ABSTRACT: This study was conducted to determine the prevalence and density of *Demodex* mites in patients with seborrheic dermatitis. The study included 37 patients and 35 healthy controls who were diagnosed with seborrheic dermatitis by clinical examination. The sample materials were taken from the cheek, nasolabial and jaw areas of the participants using the standard superficial skin biopsy method and examined for the presence and number of *Demodex* mites under light microscopy. *Demodex* spp. mites were detected in 34 (91.9%) of the patients and in 20 (60%) of the controls. *Demodex folliculorum* was detected in 34 of 37 patients (mean 15.7/cm^2; total 535) and *D. brevis* (mean 0.6/cm^2; 20 total) in six patients. *Demodex folliculorum* was detected in 20 of 35 healthy controls (mean 2.7/cm^2; total 56) and *D. brevis* (mean 0.5/cm^2; total 1) in one of the 35 healthy controls. When patients and controls were compared in terms of *Demodex* prevalence and density, the differences were statistically significant. In conclusion, *Demodex* mites are more prevalent in patients with seborrheic dermatitis in Erzincan Province of Turkey. This condition may be related to the amount of sebum in patients with seborrheic dermatitis, however, this issue should be supported by further studies in which sebum levels are measured and ilarger number of patients are involved.

Keywords: *Demodex*, seborrheic dermatitis, sebum, epidemiology, Erzincan, Turkey.
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INTRODUCTION

Demodex mites (Acari) are microscopic organisms belonging to the family Demodecidae of the order Trombidiformes. Two species of *Demodex* mites are known from humans: *Demodex folliculorum* Simon and *Demodex brevis* Akbulutova (Desch and Nutting, 1972; Rufli and Mumcuoglu, 1981). *D. folliculorum*, which has a long opisthosoma, lives in hair follicles alone or in groups, while *D. brevis* which has a shorter and pointed opistosome, usually lives as a single in the sebaceous glands (Rufli and Mumcuoglu, 1981). *Demodex* mites have a cigar-shaped body, a chitinous exoskeleton, piercing mouthparts and four pairs of legs with a pair of claws at the terminal end. With their piercing mouthparts and their enzymes, they feed on the contents of the follicular epithelial cells and sebum (Desch and Nutting, 1977; Rufli and Mumcuoglu, 1981).

Seborrheic dermatitis is a chronic and inflammatory skin disease that affects parts of the body rich in sebaceous glands such as the scalp and face, manifested by erythematous, yellowish, oily and squamous plaques (Aksoy et al., 2012; Güleç, 2014). Most dermatologists have called seborrheic dermatitis ''dermatitis of areas with sebum" and have reported that an increase in the amount of sebum has an important role in the pathogenesis of seborrheic dermatitis (İnalöz and Kırtak, 2002; Güleç, 2014). Numerous epidemiological studies have been conducted in various dermatological patients (e.g., with acne vulgaris, rosacea, pityriasis folliculorum, perioral dermatitis, and eczema), in ophthalmological patients with symptoms such as blepharitis dandruf, and keratoconjunctivitis, in patients with diabetes, renal failure, and cancer), as well as in healthy individuals (Forton, 2012; Durmaz et al., 2015; Zeytun and Yazıcı, 2019; Sarı et al., 2019; Yılmaz and Akkaş, 2020; Zeytun and Karakurt, 2019; Erdal and Albayrak, 2022). However, studies on *Demodex* mites in patients with seborrheic dermatitis are quite limited (Basta-Juzbasic et al., 2002; Karıncaoğlu et al., 2009; Güleç, 2014; Tehrani et al., 2014; Aktaş Karabay and Aksu Çerman, 2020; Erdal and Albayrak, 2022). Since the main food sources of *Demodex* mites are follicular epithelial cells and sebum, there is a very high probability that there may be a relationship between seborrheic dermatitis and *Demodex* mites. The aim of this study was to determine the prevalence and density of *Demodex* mites in patients with seborrheic dermatitis in Erzincan province.

MATERIALS AND METHODS

The study included 37 patients diagnosed with seborrheic dermatitis and 35 healthy controls without any dermatological symptoms based on the clinical examination at the Dermatology Clinic of Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital. Patients who had dermatological or systemic diseases other than seborrheic dermatitis, who had undergone dermatological surgery, and who received systemic or topical treatment were not included in the study. Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University and all participants read and signed the informed consent form in accordance with the Helsinki Declaration.

Samples were taken from the cheek, nasolabial and chin areas of the participants using the Standard Superficial

Skin Biopsy (SSSB) method. The areas to be sampled were cleaned with alcohol and dried. An area of 1-cm^2 was drawn on one side of the glass slide, a drop of cyanoacrylate was dripped on the other side and lightly pressed the surface to be sampled, and after about a minute it was gently removed. A drop of Hoyer medium was placed onto the sample material, and sealed with a cover glass. The slides were examined by the same researcher at 4X, 10X, 40X magnifications under a light microscope (Leica DM750, Switzerland). The identification of *Demodex* mites was made by the same researcher using the relevant literature (Desch and Nutting 1972, 1977). *Demodex* mites were photographed using a DIC (Differential Interference Contrast) equipped research microscope (Olympus BX53, Japan). The mean density of *Demodex* mite was calculated by dividing the total number of *Demodex* mites by the number of participants where *Demodex* mites were found.

The statistical analysis of the data was performed using the SPSS 23.0 (Statistical Package for Social Sciences; Chicago, IL, USA) program. The Kolmogorov-Smirnov test was used to determine the suitability of the variables for normal distribution. The Mann-Whitney U and Kruskall-Wallis tests were used to comparisons between the groups. The Chi-square test was used to evaluate the categorical data. The prevalence and density of *Demodex* mites rates were calculated using maximum likelihood estimation method with 95% confidence intervals (CI). A P value of less than 0.05 was considered statistically significant.

RESULTS

A total of 72 participants, including 37 patients (23 female, 14 male, mean age 25.9), and 35 healthy controls (24 female, 11 male, mean age 25.8), were included in the study (Table 1).

Table1. Age and sex of patients and controls.

	Patients (n: 37)	**Controls (n: 35)**
Age (years)		
Mean ± SD	25.9 ± 11.3	25.8 ± 8.6
Median (min. – max.)	26 (12 - 56)	24 (13 - 45)
Sex		
Female	23/37 (62.2%)	24/ 35 (68.6%)
Male	14/37 (37.8%)	11/35 (31.4%)

SD: standard deviation; min: minimum; max: maximum.

Demodex spp. positivity was detected in 34 (91.9%) of the patients and in 20 (60%) of the controls. *D. folliculorum* was detected in 34 patients (total 535, mean 15.7/cm^2), *D. brevis* was detected in 6 patients (total 20, mean 0.6/cm^2). *D. folliculorum* was detected in 20 of the healthy controls (total 56, mean 2.7/cm^2) and *D. brevis* was detected in 1 (total 1, mean 0.5/cm^2) (Fig. 1). When patients and controls were compared in terms of *Demodex* prevalence and density the differences were statistically significant (Tables 2 and 3).

Figure 1. A. *Demodex* spp. (at 40x magnification), **B.** *D. folliculorum* (at 40x magnification.

Table 2. Prevalence of *Demodex* mites in patients and controls.

	Patients (n: 37)	**Controls (n: 35)**	***p* value[a]**
Prevalence of *Demodex*			
D. folliculorum	34/37 (91.9%) (95% CI: 83-100%)	20/35 (57.1%) (95% CI: 40-74%)	0.001
D. brevis	6/37 (16.2%) (95% CI: 4-29%)	1/35 (2.9%) (95% CI: 3-9%)	0.056
Demodex spp.	34/37 (91.9%) (95% CI: 83-100%)	21/35 (60.0%) (95% CI: 43-77%)	0.001

CI: confidence interval.
[a] Chi square test.

Table 3. Density of *Demodex* mites in patients and controls.

	Patients (n: 34)	**Controls (n: 21)**	***p* value[b]**
Mean density of *Demodex*[a] [mean (min-max)]			
D. folliculorum	15.7 (1-65) (CI: 9.02-22.45)	2.7 (0-8) (CI: 1.60-3.74)	0.002
D. brevis	0,6 (0-7) (CI: 0.04-1.13)	0.5 (0-1) (CI: 0.05-015)	0.147
Demodex spp.	16.3 (1-65) (CI: 9.39-23.26)	2.7 (1-8) (CI: 1.66-3.76)	0.002
Total density of *Demodex*[a]			
D. folliculorum	535	56	-
D. brevis	20	1	-
Demodex spp.	555	57	-

CI: confidence interval; min: minimum; max: maximum.
[a] For the calculation of the density of *Demodex*/cm^2 only the *Demodex* positive patients and controls have been taken into accounts.
[b] Mann-Whitney U test.

The relationship between the age and gender characteristics of the participants and the prevalence and density of *Demodex* are given in Tables 4 and 5. It was found that the prevalence of *Demodex* was almost the same in male and female patients, but the density of *Demodex* was greater in males than in females. In addition, the prevalence and density of *Demodex* were found to be higher in adolescent patients than in adult patients.

DISCUSSION

In the present study, *Demodex* spp. positivity was detected in 34 (91.9%) of the patients and in 20 (60%) of the controls. *Demodex folliculorum* was detected in 34 of 37 patients (mean 15.7/cm^2; total 535) and *D. brevis* (mean 0.6/cm^2; 20 total) in 6 patients. *D. folliculorum* was detected in 20 of 35 healthy controls (mean 2.7/cm^2; total 56) and *D. brevis* (mean 0.5/cm^2; total 1) in one of the 35 healthy controls. When patients and controls were compared in terms of *Demodex* prevalence and density, the differences were found to be statistically significant.

In a study conducted in Croatia and investigating the role of *D. folliculorum* in the development of rosettiform dermatitis, it was reported that *D. folliculorum* was detected in 59% of 132 patients. When oral tetracycline was administered to these patients for 1-4 months, a decrease in the number of *D. folliculorum* was achieved, and an improvement in papular and pustular lesions was reported (Basta-Juzbasic et al., 2002). In Iran, it was reported that 63.4% of patients with seborrheic dermatitis and 57.9% of controls were *Demodex* positive (Tehrani et al., 2014).

Table 4. The relationship between the prevalence of *Demodex* and age and sex of participants.

	Prevalence of *Demodex*		
	Patients (n: 37)	**Controls (n: 35)**	***p* value[a]**
Age (year)			
Adolescent (≤ 20)	15/15 (100%) (95% CI: 90-100%)	5/11 (45.5%) (95% CI: 10-81%)	0.005
Adult (≥ 21)	19/22 (86.4%) (95% CI: 71-100%)	16/24 (66.7%) (95% CI: 46-87%)	
Total	34/37 (91.9%) (95% CI: 83-100%)	21/35 (60.0%) (95% CI: 43-77%)	
Sex			
Female	21/23 (91.3%) (95% CI: 79-100%)	15/24 (62.5%) (95% CI: 42-83%)	0.015
Male	13/14 (92.9%) (95% CI: 77-100%)	6/11 (54.5%) (95% CI: 19-90%)	
Total	34/37 (91.9%) (95% CI: 83-100%)	21/35 (60.0%) (95% CI: 43-77%)	

CI: confidence interval.
[a] Chi square test.

Table 5. The relationship between the density of *Demodex* mites and age and sex of participants.

	Mean density of *Demodex*[a] [mean (min-max)]		
	Patients (n: 34)	**Controls (n: 21)**	***p* value[b]**
Age (year)			
Adolescent (≤ 20)	16.6 (1-64) (CI: 4.75-28.45)	2.2 (1-7) (CI: 1.13-5.53)	0.019
Adult (≥ 21)	16.1 (1-65) (CI: 6.86-25.35)	2.9 (1-8) (CI: 1.68-4.07)	
Total	16.3 (1-65) (CI: 9.39-23.26)	2.7 (1-8) (CI: 1.66-3.76)	
Sex			
Female	15.0 (1-48) (CI: 7.23-22.77)	1.8 (1-6) (CI: 0.86-2.74)	0.002
Male	18.5 (1-65) (CI: 3.76-33.17)	5.0 (3-8) (CI: 2,80-7.20)	
Total	16.3 (1-65) (CI: 9.39-23.26)	2.7 (1-8) (CI: 1.66-3.76)	

CI: confidence interval; min: minimum; max: maximum.
[a] For the calculation of the density of *Demodex*/cm^2 only the *Demodex* positive patients and controls have been taken into accounts.
[b] Kruskal-Walllis test.

In Turkey, Karıncaoğlu et al. (2009) reported that *D. folliculorum* was found in 50% of 38 patients with seborrheic dermatitis (mean 8.16/cm^2) and 13.1% of 38 controls (mean 1.03/cm^2). In the same study, it has been reported that the density of *D. folliculorum* is greater in patients than in controls, and *D. folliculorum* may play a direct or indirect role in the etiology of seborrheic dermatitis. In a study investigating the prevalence of *Demodex* in different

dermatological diseases, it was reported that *Demodex* positivity was detected in 6.7% of patients with seborrheic dermatitis (Erdal and Albayrak, 2022). In another study, it was reported that *D folliculorum* positivity was detected in 20 of 41 patients (48.8%) with seborrheic dermatitis and in two of 77 (2.6%) controls. In the same study, it was stated that *Demodex* mites tend to be found on facial areas with seborrheic dermatitis and *Demodex*-induced inflammation may contribute to the pathogenesis of seborrheic dermatitis (Aktaş Karabay and Aksu Çerman, 2020). In a study conducted to investigate the role of *D. folliculorum* in the etiology of seborrheic dermatitis, it was noted that *D. folliculorum* was detected in 25.5% of patients and 19.6% of controls. In the same study, the mean density of *D. folliculorum* was reported as 1.69/cm^2 in patients and 1.24/cm^2 in controls, and it was also noted that *D. folliculorum* was denser in non-lesion rather than in lesion areas (Güleç, 2014).

In our study as well as in studies conducted by other groups, it was found that *Demodex* mites are prevalent and more dense in patients with seborrheic dermatitis. These results indicate that there may be a relationship between seborrheic dermatitis and *Demodex* mites, and that seborrheic dermatitis may prepare the ground for *Demodex* infestation. This may be due to the fact that the main food source of *Demodex* mites is sebum. However, this theory needs to be supported by further studies, in which the amount of sebum is measured.

The fact that *Demodex* mites can also be found in healthy individuals and do not cause any clinical symptoms however, can lead to confusion. Many researchers have noted that *Demodex* mites destroy follicular and sebaceous epithelial cells with piercing mouthparts, disrupt the skin barrier and form lymphocyte infiltrates around the follicle, causing an immune response to the allergens of the mite when it penetrates the dermis. In addition, it has been reported that *Demodex* mites can increase in number and become opportunistic pathogens if the immune system is suppressed or insufficient (Forton 2012; Forton et al., 2015; Aytekin et al., 2017; Zeytun and Ölmez, 2017). Some researchers have reported that individuals with the HLA-A2 haplotype are three times more resistant to demodecosis, while individuals with the HLA-CW2 and HLA-CW4 haplotypes are five times more likely to develop demodecosis. It has also been reported that the density of *Demodex* increases due to an increase in lymphocyte and NK apoptosis in these individuals (Akilov and Mumcuoglu, 2003, 2004; Mumcuoglu and Akilov, 2005). Therefore, the fact that some patients and controls may be asymptomatic despite being *Demodex* positive may be related to the genetic characteristics of these individuals and the HLA haplotypes they have. However, additional studies are needed on this issue.

While *Demodex* mites are not found in newborns, they can be found widely in both gender in infantile, childhood, puberty and adult stages. In this study, it was found that the prevalence of *Demodex* was almost the same in male and female patients, but the density of *Demodex* was greater in males than in females. In other studies, it has been reported that the prevalence and density of *Demodex* is greater in males (Okyay et al., 2006; Durmaz et al., 2015; Tilki et al., 2017; Zeytun et al., 2017; Karakurt ve Zeytun, 2018), more in females (Özdemir et al., 2005; Zeytun 2017; Zeytun ve Ölmez, 2017) , or equal in males and females (Zhao et al., 2011). This situation which differs between the studies, may be related to the attention paid by the participants to personal care and hygiene.

In many studies, it has been reported that the prevalence and density of *Demodex* increases with age (Beekeeper et al., 2005; Inceboz et al., 2009; Kasemsuwan et al., 2017; Lopez-Ponce et al., 2017; Fox et al., 2017; Zeytun 2017; Zeytun and Ölmez 2017; Zeytun et al., 2017). However, in our study, it was found that the prevalence and density of *Demodex* were higher in adolescent than in adult patients. This may be associated with increased sebum secretion of adolescents.

As shortcomings of our study it can be mentioned that the sample size was small and the amount of sebum in patients was not measured. Therefore, it is necessary to support the issue with further studies in which sebum levels are measured and involving a larger number of patients.

Authors' contributions

Erhan Zeytun: Project manager, laboratory works (collection of samples, preparation of samples, microscopic examinations, identification of mites), writing - reviewing & editing, methodology, investigation, visualization, formal analysis (supporting), statistics. **Mustafa Yazıcı:** Clinical examination, selection of patients and controls, formal analysis (lead), writing - reviewing and editing, methodology, investigation.

Statement of ethics approval

Ethical approval was obtained from the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (Permission No: 2016-08/07).

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Conflict of interest

The authors declared that there is no conflict of interest.

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