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ORIGINAL ARTICLE

Demodex Species Frequency and Risk Factors in Patients With Rheumatoid Arthritis

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ABSTRACT

Objectives: This study aims to investigate the presence of Demodex species in rheumatoid arthritis (RA) patients, to identify the risk factors for developing Demodex infestation, and to determine the effect of immunosuppressant drugs on Demodex mite infestations.

Patients and methods: The study included 93 RA patients (16 males, 77 females; mean age 53.3±11.3 years; range, 27 to 83 years) and 76 healthy controls (19 males, 57 females; mean age 50.3±13.9 years; range, 19 to 86 years). Specimens were collected from face skin by using standardized surface skin biopsy. Demodex infestation was considered for ≥5 living parasites/cm^2 of skin while Demodex mite presence was defined as any Demodex larvae, adults, or eggs found in the specimen.

Results: The frequencies of Demodex mite presence were 44% for the RA patients and 15.7% for the healthy controls ($p<0.001$). The rates of Demodex infestation were similar between the two groups (18.3% versus 7.9%, $p=0.054$). There were no statistically significant differences between the groups regarding skin type, skin care, epilation, body washing, use of a moisturizer, personal towel use, the number of residents at home, or whether there were pets at home or in proximity. Itching in eyes was higher in RA patients, but the frequency of other skin symptoms was not different from healthy controls. Logistic regression analysis indicated that the diagnosis of RA was an independent risk factor for Demodex mite presence in this study population. Disease activity and duration, use of corticosteroids, conventional disease-modifying anti-rheumatic drugs (DMARDs) and biological DMARDs were not effective factors on Demodex mite presence in RA patients.

Conclusion: Although Demodex mite presence was 3.5-fold higher in RA patients, the rate of Demodex infestation was similar to that of healthy controls.

Keywords: Biological disease-modifying anti-rheumatic drugs, conventional disease-modifying anti-rheumatic drugs, Demodex folliculorum, infestation, mite, rheumatoid arthritis.

Demodex mites are permanent ectoparasites in mammalian pilosebaceous units. Although 140 species of Demodex have been identified in mammals, *Demodex brevis (D. brevis)* and *Demodex folliculorum (D. folliculorum)* have been found in humans.[1] These species occupy different glands in the skin: *D. folliculorum* is found in the follicles of simple hairs above the level of the sebaceous glands, whereas *D. brevis* is found in the sebaceous glands of the vellus hairs.[2]

Demodex infestation can be detected in healthy individuals, and its incidence increases with age.[1,3] The pathogenic potential of Demodex species in animals has been demonstrated; in humans, they can become harmful to the host as opportunistic pathogens in the immunosuppressive cases.[4]

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The relationship between Demodex species and the immune system remains unclear, and controversies and research on the pathogenesis of Demodex-related skin diseases continue.[5] Clinical studies have investigated the effect of immunosuppression on Demodex infestation,[6] which has been shown to be high in hematological malignancies treated with chemotherapy.[7] In a study by Kaya et al.,[8] demodex mites were found more often in patients with sickle cell anemia which is a compromised immune system.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent arthritis and systemic inflammation. RA affects more females than males, with an estimated prevalence of 0.5-1% in adults. The international recommendations for the treatment of RA include glucocorticoids; conventional disease-modifying anti-rheumatic drugs (cDMARDs), mainly methotrexate; and biological DMARDs (bDMARDs), such as antitumor necrosis factors, anti-interleukin-6, and anti-CD20. Patients with RA typically exhibit immune dysregulation and a predisposition to infections. Furthermore, drugs used to treat RA are immunosuppressive, and it was reported that the frequency of tuberculosis and opportunistic infections increased in RA patients treated with biological agents.[9]

Preliminary studies were conducted to estimate the prevalence of Demodex mites in RA patients.[10,11] In these studies, the number of patients was relatively low. Furthermore, the results of preliminary studies need verification. Therefore, in this study, we aimed to investigate the presence of Demodex species in RA patients, to identify the risk factors for developing Demodex infestation, and to determine the effect of immunosuppressant drugs on Demodex mite infestations.

PATIENTS AND METHODS

The study sample consisted of 93 patients with RA (16 males, 77 females; mean age 53.3±11.3 years; range, 27 to 83 years), who fulfilled the 2010 European League Against Rheumatism and the American College of Rheumatology criteria,[12] and 76 healthy controls (19 males, 57 females; mean age 50.3±13.9 years; range, 19 to 86 years). The RA patients included in this study were treated and followed-up at the rheumatology outpatient clinic of Antalya Training and Research Hospital between June 2017 and December 2018. The healthy control group consisted of volunteers who were checked from the department of internal medicine. Consecutive individuals without any systemic disease formed the healthy control group. Demographic characteristics of control individuals were similar to the RA patients. Exclusion criteria were patients with an autoimmune disease other than RA, chronic diseases associated with immune deficiency, diabetes mellitus, or any immunosuppressive drug use. The study protocol was approved by the Antalya Training and Research Hospital Ethics Committee (date: 05/09/2017, approval number: 12/08). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

A questionnaire was administered to ask the participants about the risk factors for Demodex mite presence. Samples were taken from the face area (i.e., cheeks, nose, chin, or forehead) by using a standard superficial skin biopsy (SSSB) method. SSSB was preferred particularly in areas with skin lesions such as redness or acne. The patients' faces were cleaned with alcohol (to remove any residual cream or lotion) and allowed to air dry. A drop of cyanoacrylic adhesive was placed on a microscope slide before applying the slide to

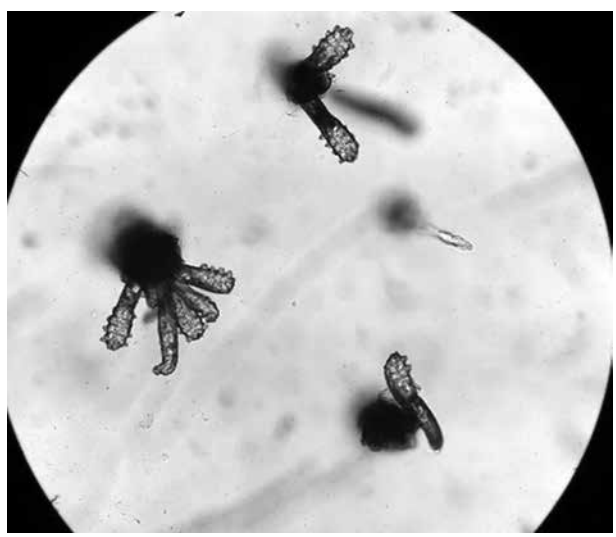

Figure 1. *Demodex species* on a sample from skin by using a standard superficial skin biopsy method.

Table 1. Clinical characteristics of patients with rheumatoid arthritis and healthy controls

	RA (n=93)			Healthy controls (n=76)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			53.3±11.3			50.3±13.9	0.119
Disease duration (month)			90.5±66.7			-	-
DAS28			2.9±1.0			-	-
Sex							0.213
Female	77	82.8		57	75.0		
Disease activity							
Remission (DAS28 ≤2.6)	46	49.5		-	-		-
Low (DAS28 2.6-3.2)	34	36.6		-	-		-
Moderate (DAS28 3.2-5.1)	8	8.6		-	-		-
High (DAS28 ≥5.1)	5	5.4		-	-		-
Drugs							
Corticosteroids	57	61.3		-	-		-
Conventional (cDMARDs)							
Methotrexate	77	82.2		-	-		-
Hydroxychloroquine	31	33.3		-	-		-
Sulfosalazine	9	9.7		-	-		-
Leflunomide	11	11.8		-	-		-
Biologic (bDMARDs)	14	15.1		-	-		-
Lifestyle							
Epilation*	4	4.6		4	5.6		0.799
Make-up*	6	6.8		7	9.6		0.524
Presence of pet*	12	13.6		13	17.8		0.534
Common towel use*	40	45.5		42	57.6		0.174
Use of a moisturizer*	16	18.2		14	19.2		0.678
Skin care*	1	1.13		4	5.6		0.118
Skin type*							
Oily	21	23.9		13	17.6		
Dry	37	42.0		34	46.6		0.617
Combination	30	34.1		26	35.6		
The number of residents at home							
1-2 person	34	36.6		21	27.6		
3-4 person	44	47.3		35	46.0		0.209
≥5 person	15	16.1		20	26.3		
Body washing							
1-2 times/week	18	19.4		23	30.3		
3-4 times/week	38	40.9		32	42.1		0.145
≥5 times/week	37	39.8		21	27.6		
Symptoms†							
Skin redness	35	39.7		20	27.0		0.120
Acne	17	19.3		19	25.7		0.352
Itching							
Eyes	40	45.5		22	29.7		**0.040**
Ears	29	37.8		28	37.8		0.517
Face	20	22.7		17	22.9		0.998
Demodex mite presence	41	44.1		12	15.7		**<0.001**
Demodex folliculorum	26	27.9		8	10.5		**0.006**
Demodex brevis	7	7.5		2	2.6		0.188
Demodex species	8	8.6		2	2.6		0.278
Demodex infestation (≥ 5 Demodex/cm^2)	17	18.3		6	7.9		0.054
Semiquantitative score							0.807
(<5 Demodex/cm^2) (+)	24	25.8		6	7.9		
(5-10 Demodex/cm^2) (++)	10	10.8		3	3.9		
(>10 Demodex/cm^2) (+++)	7	7.6		3	3.9		

RA: Rheumatoid arthritis; SD: Standard deviation; DAS28: Disease activity score 28; cDMARDs: Conventional disease-modifying anti-rheumatic drugs; bDMARDs: Biological disease-modifying anti-rheumatic drugs; * Eighty-eight rheumatoid arthritis patients and 73 healthy controls were included in the analysis; † Eighty-eight rheumatoid arthritis patients and 74 healthy controls were included in the analysis.

the skin surface. After approximately one minute, the slide was slowly removed. After a few drops of immersion oil material was applied to each slide, it was microscopically examined by a parasitology specialist at ×10 and ×40 objectives. Demodex mites are shown in Figure 1. *D. folliculorum* and *D. brevis* were distinguished by their apparent morphological features, as described by Akbulatova.[13] The number of parasites was evaluated using a semiquantitative scoring system: fewer than five (+), five-10 (++) and more than 10 (+++) mites/cm^2 of skin. Demodex infestation was considered for ≥5 living mites/cm^2; while Demodex mite presence was defined as any Demodex larvae, adults, or eggs found in the specimen.

Statistical analysis

Statistical analysis was performed using the SPSS for Windows version 13.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics was used to evaluate the characteristics of the study groups, and Chi-square and Fisher's exact tests were conducted to compare the categorical differences between the groups. The significance of differences was analyzed using Student's t test. Logistic regression was performed to ascertain the risk factors for Demodex mite presence, and $p<0.05$ was considered statistically significant.

RESULTS

The distribution of sexes was similar between the groups (p=0.213). The frequencies of clinical symptoms about Demodex mites, except itching in the eyes, were similar among groups. The questionnaire results yielded no statistically significant difference between the groups regarding

Table 2. Comparison of rheumatoid arthritis patients with and without Demodex mite presence and Demodex infestation

		Demodex mite presence (n=41)				Demodex infestation (n=17)			
	n	n	%	Mean±SD	p	n	%	Mean±SD	p
Age (year)					0.780				0.535
Positive	41			53.7±12.1		17		51.8±12.3	
Negative	52			53.0±10.7		76		53.7±11.1	
Disease duration (month)					0.274				0.719
Positive	41			99.1±77.5		17		85.2±40.3	
Negative	52			83.7±57.2		76		91.6±71.7	
Sex					0.256				0.171
Male	16	5	31.3			1		6.3	
Female	77	36	46.8			16		20.8	
Symptoms									
Skin redness	35	16	44.4		0.476	7		20.0	0.740
Acne	17	7	41.1		0.900	3		17.6	0.603
Itching									
Eyes	40	14	35.0		0.222	7		17.5	0.918
Ears	29	9	31.0		0.142	4		13.8	0.569
Face	20	9	45.0		0.799	4		20.0	0.710
Disease activity					0.985				0.578
Remission (DAS28 ≤2.6)	46	20	43.5			9		19.6	
Low (DAS28 2.6-3.2)	34	15	44.1			7		20.6	
Moderate (DAS28 3.2-5.1)	8	4	50.0			-		-	
High (≥5.1)	5	2	40.0			1		20.0	
Non-corticosteroids	36	18	50.0		0.361	7		19.4	0.817
Corticosteroids	57	23	40.4			10		17.5	
One cDMARDs	59	30	50.8			14		23.7	
Two cDMARDs combination	31	10	32.3		0.238	3		9.7	0.209
Three cDMARDs combination	2	1	50.0			-		-	
Non-bDMARDs	79	36	45.6		0.494	14		17.8	0.716
bDMARDs	14	5	35.7			3		21.4	

SD: Standard deviation; DAS28: Disease activity score 28; cDMARDs: Conventional disease-modifying anti-rheumatic drugs; bDMARDs: Biological disease-modifying anti-rheumatic drugs.

Table 3. Logistic regression analysis for Demodex mite presence in our cohort and patients with rheumatoid arthritis

	Odds ratio	95% CI	*p*
All of cohort (n=169)			
Rheumatoid arthritis	3.491	1.466-8.310	0.005
Living region	1.259	0.754-2.103	0.379
Bath number/week	1.137	0.846-1.530	0.395
Skin color	1.245	0.779-1.991	0.360
Skin type	0.883	0.426-1.485	0.640
Cream use	1.634	0.594-4.500	0.342
Make-up	0.318	0.051-1.914	0.211
Pet at home	1.469	0.496-4.351	0.487
Pet in proximity	0.309	0.079-1.209	0.091
Personal towel use	0.742	0.342-1.609	0.450
Number of residents (>50 years old)	0.578	0.260-1.019	0.187
Rheumatoid arthritis (n=93)			
Corticosteroids use	0.725	0.294-1.789	0.485
Methotrexate use	1.227	0.235-6.411	0.809
Leflunomide use	0.447	0.055-3.627	0.451
Hydroxychloroquine use	0.657	0.253-1.709	0.389
Sulfasalazine use	1.420	0.319-6.314	0.645
bDMARDs use	0.629	0.174-2.271	0.479
Disease duration	1.004	0.998-1.011	0.195
Disease activity	0.957	0.603-1.521	0.853

bDMARDs: Biological disease-modifying anti-rheumatic drugs; CI: Confidence interval.

skin type, skin care, epilation, body washing, use of a moisturizer, personal towel use, the number of residents at home, or whether there were pets at home or in proximity. All RA patients were using at least one cDMARD. The used drugs, rheumatoid factor and anti-cyclic citrullinated peptide positivity, disease activity and disease duration of RA patients are shown in Table 1.

Microscopic examination revealed that the frequencies of Demodex mite presence were 44% (n=41/93) for the RA patients and 15.7% for the healthy controls (n=12/76), with a statistically significant difference (p<0.001). *D. folliculorum* was significantly higher in RA patients (p=0.006), unlike the other Demodex species. The rates of Demodex infestation were similar between the groups (18.3% vs. 7.9%, p=0.054). *D. folliculorum* was detected in 34 RA patients and 10 healthy controls, while *D. brevis* was observed in seven RA patients and two healthy controls. The results of microscopic examination are also summarized in Table 1.

We compared some features of RA patients to detect any effect on Demodex mite presence and Demodex infestation. Age, sex, disease duration, disease activity and treatment agents such as corticosteroids, cDMARDs and bDMARDs were found to have no effect on Demodex mite presence or Demodex infestation. There was no correlation between the number of drugs used and Demodex species in RA patients Also, the frequencies of skin symptoms were not significantly different from RA patients without Demodex species (Table 2).

Logistic regression analysis was performed to determine the factors on Demodex positivity, which revealed that the diagnosis of RA was an independent risk factor for Demodex mite presence in the study population [odds ratio (OR)=3.491, 95% confidence interval (CI): 1,466-8,310, p=0.005]. However, there was no decisive factor determining the presence of Demodex mites in RA patients (Table 3).

DISCUSSION

Demodex mites are prevalent in many human skin disorders, and their pathogenic role continues to be debated.[1] In the skin flora, fewer than five mites/cm^2 is considered normal, whereas the presence of more than five mites indicates a positive diagnosis of demodicosis.[14-17] Demodex infestation can be detected in healthy individuals (total infestation rate=17%-72%) and the incidence increases with age, affecting 100% of people over the age of 96 years.[1,18] Also, some authors have claimed that Demodex mites are symbiotic inhabitants and can be a part of the microbiome within human skin.[19]

The results of this study have shown that Demodex mite presence (i.e., *D. folliculorum* and/or *D. brevis*) was higher in RA patients than healthy controls (44% vs. 15.7%). However, the rates of Demodex infestation (≥5 mites/cm^2 of skin) were 18.3% for the RA patients and 7.9% for the healthy controls, with no statistically significant difference between the groups. In previous studies, the prevalence of Demodex mites in RA patients has not been statistically significantly different from that in healthy controls.[10,11] This difference in study results may be attributed to several factors, such as age, sex, and the number of patients. In addition, the mite detection methods used in the cited studies may have affected the results. Çiftçi et al.[10] have reported that the prevalence of *D. folliculorum* infestation was 12% and 8% in their RA and control groups, respectively, and that there was no statistically significant difference between the groups in the density or incidence of Demodex. They considered ≥5 mites/cm^2 of skin as a positivity criterion. The results of our study support those of previous research regarding the prevalence of Demodex infestation. In a study conducted in Poland, the mean age was 61 years; 33% of patients in the RA group and 31% of the control group were found to be positive for Demodex infestation, with no statistically significant difference between the groups (p=0.98).[11] In the cited study, eyelashes were epilated to find mites and a positive result was recorded if any Demodex larvae, adults, or eggs were found. Although their study population and methods were different from those of our study, their results were similar to ours.

Our study has found Demodex infestation in 7.9% (n=6) of the healthy controls. Several studies have reported on the prevalence of Demodex species in the Turkish population.[20-29] Demodex species positivity was found in 6.7-74.7% of the healthy Turkish population living in various regions.[20-29] There may be several reasons for the differences among regions and many other factors may influence the prevalence of Demodex mite presence. It has been stated that the number of parasites increases, particularly in the summer months, with an increase in environmental temperature; this may occur through the seasonal activation of sebum production.[30] It was suggested that the high prevalence of Demodex mites in Brazil may be due to the humid subtropical climate.[31] In our study region, the summer season is quite long, with high temperature and humidity. In both the RA patients and healthy controls, the prevalence of Demodex mites was the same as those in patients from other cities in Turkey, even in the Antalya region.

Several methods are used for defining Demodex, such as the cellophane tape method, squeezing method, skin scraping, SSSB, eyelash epilation, comedone extraction, skin punch biopsy, extraction from acne through compression methods, dermoscopy, reflectance confocal microscopy, and confocal laser scanning microscopy. SSSB is the most commonly used method for comparing mite densities between patients and healthy controls. SSSB is a noninvasive and standardized method that enables determining the number of Demodex mites/cm^2 of skin. It is often painless and easily tolerated by the patient, even if the sample is removed from the contents of the upper pilosebaceous canals.[1,5,32,33] Likewise, in our study, we examined the skin by using SSSB to determine the number of mites/cm^2 (Table 1). Demodex mite presence was higher in RA patients than in healthy controls. The results of regression analysis showed that RA patients had a 3.5-fold increased risk of Demodex mite presence (OR: 3.491, 95% CI: 1,466-8,310, p=0.005). However, the rates of Demodex infestation and numbers of parasites on the skin were no different than those for the healthy controls. Based on our study findings, Demodex species can be easily located in the follicles of RA patients, while their spread and multiplication are not affected by RA. Further studies are necessary

to explain the spread factors that may be related to hosts and parasites regarding RA patients.

Most people exhibit the presence of Demodex mites without any clinical symptoms. Human demodicosis can be influenced by various factors, one of which is the immune system; immunosuppressed patients are particularly susceptible to such infections.[8,34] Furthermore, it has been claimed that the increased incidence of *D. folliculorum* in patients with immunosuppression is associated with systemic illnesses, such as hematologic malignancies, acquired immunodeficiency syndrome, and diabetes mellitus.[34-36] A previous study has reported that Demodex mites were observed more often in sickle cell anemia patients; in addition, authors have claimed that there was a relationship between the appearance of sickle cell anemia symptom attacks and the presence of Demodex mites.[8] Anti-rheumatic drugs are commonly used in the management of RA, and most of them have immunosuppressive effects. Increased rates of infections are reported in patients with RA treated with steroids, methotrexate, leflunomide, and biological drugs.[37] In our study, no relationship was found between the presence of Demodex mites and drugs, including corticosteroids, cDMARDs and bDMARDs.

Rheumatoid arthritis is mainly an autoimmune disease of the joints. However, it is not merely a joint disorder but also a systemic disease capable of involving a variety of major organ systems. Cutaneous manifestations related to RA are as follows: rheumatoid nodules; rheumatoid vasculitis; livedo racemosa; neutrophilic and/or granulomatous diseases, such as pyoderma gangrenosum; Sweet's syndrome; rheumatoid neutrophilic dermatitis; interstitial granulomatous dermatitis with arthritis; and palisaded neutrophilic and granulomatous dermatitis.[38] The etiopathogenesis of skin lesions remains unclear and may be related to immune changes in the pathogenesis of RA. The presence of Demodex mites was not associated with skin lesions in our RA patients.

The damage in the RA-affected joints cause disability and further inconvenience in patients' daily activities. It is suggested that age, physical function, and social support were correlated significantly with self-care behavior.[39] There is a negative relationship between disease severity and quality of life in RA patients.[40] Advanced disease and limitations of joint movements may cause insufficiency in personal care in RA patients. The relationship between the presence of Demodex species and personal hygiene ability in patients with RA can be speculated. We did not evaluate the physical functions of the patients; we evaluated the number of baths per week that can reflect the functional status and personal hygiene of the patients. There was no relationships between Demodex presence and the number of baths per week. Besides, our previous study has shown no correlation between Demodex infestations and body hygiene.[41]

Our study has some limitations. Firstly, SSSB was obtained only from face skin, and the other skin areas were not investigated and compared for Demodex mite presence. Secondly, the power of some statistical analysis, such as the effects of bDMARDs on Demodex mite presence, were not enought to comment. Thirdly, a disease control group receiving a similar profile of immunosuppressant drugs was not included in this study.

In conclusion, this study has revealed that while RA patients had a 3.5-fold increased risk of Demodex mite presence, treatment agents, disease duration and activity were not found to be independent risk factors for Demodex positivity in RA patients.

Declaration of conflicting interests

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