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Presence of Demodex in Seborrheic Dermatitis Lesions and its Relationship with Disease Severity: A Case Control Study

Seboreik Dermatit Lezyonlarında Demodeks Varlığı ve Hastalık Şiddeti ile İlişkisi: Bir Vaka Kontrol Çalışması

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ABSTRACT Objective: Seborrheic dermatitis (SD) is a common, chronic, recurring, inflammatory skin disorder. The cause of the disease is still completely unknown. Recent studies suggest that *Demodex* parasites may play a role in the etiopathogenesis of SD. The purpose of this study was, to investigate the presence of *Demodex* in SD and its association with disease severity. **Material and Methods:** A total of 40 patients over the age of 18 years, who were clinically diagnosed with SD, and 40 healthy control subjects were included in the study. The skin surface biopsy method was used to detect *Demodex* parasites on lesional and non-lesional skin of the patients and healthy skin of the control group. The Seborrheic Dermatitis Area Severity Index was used to calculate disease severity. The presence of 5 or more *Demodex* parasites per square centimeter was considered positive. **Results:** While *Demodex* parasites were found in 50% of the lesional skin and in 2.6% of the non-lesional skin of the patients, 12.5% of the control group (p<0.001) had *Demodex* parasites. No relationship was found between the presence of *Demodex* parasites in the lesional skin and the severity index of the SD area. **Conclusion:** In this study, we found that the presence of *Demodex* was more prevalent in SD lesions than in control skin and lesion-free skin. A member of the microbiota, *Demodex* may be a predisposing factor in the development of SD.

Keywords: *Demodex;* pathogenesis; seborrheic dermatitis; standardized skin surface biopsy

ÖZET Amaç: Seboreik dermatit (SD); yaygın, kronik, tekrarlayan, inflamatuar bir deri hastalığıdır. Nedeni hâlâ tam olarak anlaşılamamıştır. Son çalışmalar, *Demodeks* akarlarının SD etiyopatogenezinde rol oynayabileceğini düşündürmektedir. Bu çalışmada, SD'de *Demodeks* varlığını ve hastalık şiddeti ile ilişkisini araştırmayı amaçladık. **Gereç ve Yöntemler:** Çalışmaya, klinik olarak SD tanısı konan 18 yaş üstü 40 hasta ve 40 sağlıklı kontrol alındı. Hastaların lezyonlu ve lezyonsuz derileri ile kontrol grubunun sağlıklı derilerinden *Demodeks* akarlarını saptamak için deri yüzey biyopsisi yöntemi kullanıldı. Hastalık şiddetini hesaplamak için Seboreik Dermatit Alan Şiddet İndeksi kullanıldı. *Demodeks*'in cm2de 5 ve üzerinde olması pozitif kabul edildi. **Bulgular:** Hastaların lezyonlu ciltlerinde %50, lezyonsuz ciltlerinde %2,6 oranında *Demodeks* akarı saptanırken, kontrol grubunda bu oran %12,5 idi (p<0,001). Lezyonel deride *Demodeks* akarlarının varlığı ile SD bölgesinin şiddet indeksi arasında bir ilişki bulunmadı. **Sonuç:** Bu çalışmada, SD lezyonlarında *Demodeks* varlığı hem kontrol hem de lezyonsuz deriye göre daha fazla bulundu. Mikrobiyotanın bir üyesi olan *Demodeks*, SD gelişiminde predispozan bir faktör olabilir.

Anahtar Kelimeler: *Demodeks;* patogenez; seboreik dermatit; standardize deri yüzeyi biyopsisi

Seborrheic dermatitis (SD) is a common, chronic, recurring, inflammatory skin condition that affects areas where sebaceous glands are dense, such as the scalp, face, and chest.[1,2] It is characterized by scaly, erythematous patches with unclear borders. It peaks in the third and fourth decades of life.[3]

SD is a multifactorial disease. Many endogenous and exogenous factors are thought to play a role in its etiology.[1] Its cause is still completely unknown. Recent studies have emphasized microbiologic, immunologic and genetic causes.[4] Sebum production, presence of *Malassezia* species and individual sus-

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ceptibility are the most important factors in development of the condition.[5,6]

Demodex parasites are ectoparasites that are saprophytic to humans and live in hair follicles and sebaceous glands.[2,7] They are found in the parts of the body where sebaceous glands are dense, such as the face, scalp, and anterior chest.[7,8] Found at low densities in almost all healthy adults, 2 types of these parasites have been identified in humans: *Demodex folliculorum* and *Demodex brevis*. The more common species, *D. folliculorum*, lives in the follicular infundibulum. *Demodex brevis* lives in the sebaceous glands and ducts.[8,9] They feed on follicular cells and sebum.[2]

Generally asymptomatic, *Demodex* infestation is normally less than 5 per square centimeter and considered a member of the skin flora.[8,9]

When they increase in number and density or infest deeper layers of the dermis, they become pathological and cause inflammation.[2,8,9]

It has been suggested that *Demodex* parasites may play a role in the etiopathogenesis of inflammatory dermatoses. For this reason, numerous studies have investigated the relationship between *Demodex* and inflammatory skin diseases such as rosacea and acne. There are few studies on SD.[2,7,10-13]

The recurrent nature of SD and the fact that its regions of predilection are the same as those of *Demodex* parasites suggest that *Demodex* parasites may play a role in the etiopathogenesis of SD. In the present study, we investigated the presence of *Demodex* parasites in SD patients and the role of *Demodex* parasites in disease severity.

MATERIAL AND METHODS

This prospective case-control study included, 40 patients over 18 years of age, who were admitted to the dermatology outpatient clinic and clinically diagnosed with SD. Clinical and dermoscopic diagnoses were made by evaluating the presence of erythema and desquamation in the regions of SD predilection. For diagnostic accuracy, patients were evaluated separately by 2 different dermatologists.

There were no skin lesions such as papules and pustules suggestive of demodicosis. Pregnant and lactating women, patients with systemic diseases and skin diseases such as rosacea, patients using systemic medications, and patients who had received SD treatment in the preceding month were excluded from the study. Forty healthy subjects over the age of 18 years were included as a control group. Demographic characteristics and disease information of the patients were recorded. The Seborrheic Dermatitis Area Severity Index (SDASI) was used to calculate disease severity. The SDASI value is in the range of 0 to 12.6. Erythema, desquamation and itching in nine different anatomical regions were scored as follows 0=none, 1=mild, 2=moderate, and 3=severe. SDASI formula=forehead (0.1)+scalp (0.4)+nasolabial (0.1)+eyebrows (0.1)+postauricular (0.1)+ear (0.1)+chest (0.2)+dorsum (0.2)+chin and cheek (0.1).[14]

The skin surface biopsy method was used to detect *Demodex* parasites from the lesional and non-lesional skin of the patients and the facial cheek areas of the healthy control group. First, the skin surface to be sampled was cleaned with alcohol. A drop of cyanoacrylate adhesive was placed on a slide, and the adhesive surface was pressed against the patient's skin and held for a minute. A drop of immersion oil was applied to the sample, which was examined under a microscope. An area of 1 cm^2 was marked on the slide and the presence of *Demodex* in that area was observed. The presence of 5 or more *Demodex* per square centimeter was considered positive.

This study was conducted in accordance with the Declaration of Helsinki, and was approved by Yıldırım Beyazıt University Faculty of Medicine Clinical Research Ethics Committee (date: April 16, 2018, no: 26379996/105). The patients gave their informed consent.

All statistical analyses were performed on SPSS for Windows (IBM Corp., NY, USA, 21.0). All defined parameters were analyzed by comparing the case and control groups. Categorical variables were described as percentages and ratios, and differences between groups were determined by chi-squared or Fisher's exact test. Numerical variables were ex-

TABLE 1: Age and gender comparison in patient and control groups.

	Patients n=40	Controls n=40	p value
Age, mean (sd)	36.2 (11.9)	36.3 (11.9)	0.95
Gender, n (%)			0.65
Female	20 (50)	18 (45)	
Male	20 (50)	22 (55)	

TABLE 2: General characteristics of the disease.

Features	n (%)
Disease duration, median (interquartile range)	12 (6-36)
Pruritus	
Absent	12 (30)
Mild	22 (55)
Severe	6 (15)
Burning	
Absent	19 (47.5)
Mild	17 (42.5)
Severe	4 (10)
Family history	
Absent	38 (95)
Present	2 (5)
Drug history	
Absent	40 (100)
Present	0
Stress	
Absent	28 (70)
Present	12 (30)
Concomitant disease	
Absent	40 (100)
Present	0
SDSI, median (interquartile range)	1.8 (0.73-2.87)

SDSI: Seborrheic Dermatitis Area Severity Index.

pressed as mean (standard deviation, SD) and median (interquartile range, MAA). Student t-test or Mann-Whitney U test was used to compare numerical variables. Statistical significance was taken as $p<0.05$ in all analyses.

RESULTS

Forty patients and 40 healthy controls were included in the study. The patient and control groups were similar in terms of age and gender (Table 1). The SD characteristics of the patients are presented in Table 2.

DEMODEX DENSITY

In the non-lesional skin, 2.6% of the patient group had *Demodex*, while 97.4% did not have *Demodex*. On the other hand, 12.5% of the control group had *Demodex*, whereas 87.5% did not (p=0.096).

In the patient group, 50% of the patients with lesional skin had *Demodex*, while the incidence of *Demodex* was 2.6% in non-lesional skin. The presence of *Demodex* in lesional skin was higher than in non-lesional skin ($p<0.001$). While *Demodex* was present in the lesional skin of 50% of the patients, it was present in 12.5% of the controls. The difference was statistically significant ($p<0.001$) (Table 3).

RELATIONSHIP BETWEEN *DEMODEX* PRESENCE AND DEMOGRAPHIC CHARACTERISTICS

There was not any correlation between the presence of *Demodex* and gender (p=0.45) or age (p=0.31) in the patient or control group. A significant correlation was found between the presence of *Demodex* in lesional skin and age in patients with SD. While the mean age of patients with *Demodex* on their lesional skin was 40 years (sd=12.2), the mean age of patients without *Demodex* on their lesional skin was 32.3 years (sd=10.1) (p=0.036). There was not any relationship between the presence of *Demodex* on lesional skin and the gender of a subject (p=0.99).

There was no relationship between the presence of *Demodex* in the lesional skin and the duration of the disorder, itching, burning, family history, or stress. No association with SDASI was found either.

TABLE 3: Comparison of *Demodex* presence in patient and control groups.

	Patients		Controls n=40	p value
	Lesional skin % n=40	Nonlesional skin % n=39 (1 missing)	%	
Demodex positivity (5≥cm^{2})	50% (n=20)	2.6% (n=1)	12.5% (n=5)	p<0.001
Demodex negativity (5<cm$^{2)}$	50% (n=20)	97.4% (n=38)	87.5% (n=35)	

TABLE 4: Lesion localizations.

Localization	n (%)*
Scalp	12 (30)
Eyebrow	4 (10)
Nasolabial	28 (70)
Chin	10 (25)
Forehead	9 (22.5)
Cheek	9 (22.5)
Ear	4 (10)
Chest	7 (17.5)
Back	1 (2.5)

*In some patients, the total exceeds 100% because more than one localization is involved.

Locations of the SD lesions are shown in Table 4. *Demodex* parasites were found in 25% of the patients with scalp involvement, and in 60.7% of the patients with facial involvement and no scalp involvement. The *Demodex* presence rate was significantly higher in patients with facial involvement (p=0.038). Patients with and without eyebrow, nasolabial, chin, forehead, cheek, ear, chest and dorsal involvement had similar *Demodex* presence rates (p>0.05).

DISCUSSION

The present study investigated, the presence of *Demodex* in the skin of 40 patients with SD with and without lesions, and compared it to the presence of *Demodex* in the skin of 40 healthy control subjects. The *Demodex* positivity rate in the lesional skin of the patients with SD was significantly higher than in their non-lesional counterparts and in the normal skin of the healthy controls (50%, 2.6%, 12.5%, respectively).

Many exogenic and endogenic factors have been suggested to be involved in the etiopathogenesis of SD.[1] Host immunity and epidermal integrity, *Malassezia* and other microbiota, sebaceous gland activity, neurogenic and nutritional factors, emotional stress, medications, poor skin care, male sex and increased androgen activity, and seasonal changes are considered predisposing factors.[4,6,15]

Karakadze et al. reported that mutations in 11 gene encoding proteins involved in immune response and epidermal differentiation were shown to cause SD or SD-like lesions.[6] Genetics and the immune system play a key role in the development of SD.[1] Human leucocyte antigen (HLA) class I A 32, and HLA class II DQB1*05 and DRB1*01 alleles have been found very often in SD patients.[5]

Genetic predisposition; overgrowth of *Malassezia* yeasts as a result of abnormal host immunity; and, excessive or altered sebum levels may compromise the barrier function of the epidermis. Impaired barrier permeability causes *Malassezia* yeasts and their metabolites to stimulate the epidermis further, resulting in a disorder of epidermal differentiation. *Malassezia* yeasts break down triglycerides secreted by sebaceous glands with lipase enzymes, and cause inflammation and hyperproliferation with the formation of free fatty acids.[3,6,15,16] *Malassezia* yeasts are also thought to cause inflammation by releasing proinflammatory cytokines from keratinocytes.[3]

Demodex parasites are part of the normal human microbiota.[17] The exact role of these parasites in skin disease and healthy skin is still unknown.[18] It has been suggested that penetration into the dermis or disruption of skin integrity may stimulate the immune system.[17] *Demodex* clogs the hair follicles and sebaceous gland ducts, thereby disrupting and damaging the skin barrier. It has been suggested that the mite and their contents induce a natural immune response and type 4 hypersensitivity reaction, normally suppress the host toll-like receptor (TLR) pathway, and as they increase in number, cause inflammation in the skin by stimulating the host immune system via TLR 2.[19] Mite chitin and disruption of epithelial integrity can stimulate neutrophils and macrophages, leading to T helper 2 and T helper 17 responses. Suppression of the immune system for various reasons can lead to parasite proliferation and cause diseases.[18]

Chen and Plewig classified demodicosis into categories of primary demodicosis and secondary demodicosis. Primary demodicosis is characterized by an increase in parasites without inflammatory dermatosis, whereas secondary demodicosis is characterized by skin lesions associated with an abnormal increase in *Demodex* parasites in the presence of skin or systemic disease.[20] Rather and Hassan classified demodicosis as the etiological factor of dermatosis or as the cause of dermatosis-like lesions.[9]

Studies have shown that *Demodex* infestation is associated with the development of SD.[2,11,13,19]

Karincaoglu et al. reported *Demodex* positivity in 50% of the patients with SD but found pathological presence of *Demodex* exclusively in the lesional skin to be 34.2%.[2] In our study, *Demodex* positivity was higher (50%) in lesional skin but, very low (2.6%) in non-lesional skin. The present study was consistent with Karincaoglu et al. in terms of *Demodex* positivity detected in the skins of healthy controls (12.5% and 13.1%, respectively).[2] We found the highest rate of *Demodex* positivity (60.7%) in patients with facial involvement. SD lesions were also most frequently observed on the face. No study has investigated the relationship between *Demodex* density and SD severity so far. The SDASI score of our patients ranged from 0.73 to 2.87. We did not find any relationship between SDASI and *Demodex* positivity.

Yazısız et al., Aktaş Karabay et al. and Zhao et al. reported *Demodex* positivity in 57%, 48.8%, and 49.7% of patients with SD, respectively, which was consistent with our findings.[11,13,19] Zhao et al. claimed that oily and mixed skin types was associated with *Demodex* density, and that the movement of *Demodex* in the pilosebaceous unit increased sebum secretion by stimulating the sebaceous glands.[11] Dhingra also stated that *Demodex* infestation had the highest incidence between the ages of 20 and 40, when sebum secretion increased.[21] SD is also more common in oily skin and in young adults in the said age range. This may be because *Demodex* parasites stimulate sebum secretion and increase predisposition to SD. Demirdağ et al. showed that the levels of cholesterol esters in the serum of patients with demodicosis increased, and argued that this could create suitable conditions for the proliferation of *Demodex* species.[22] Both *Demodex* infestation and SD are more common in males.[2,10] However, the incidence of both is high in immunosuppressive patients.[1,10,18]

Genetic susceptibility is another important factor in *Demodex* pathogenicity. While people with HLA CW2 alleles are 5 times more susceptible to *Demodex*, people with CW4 alleles are one time more susceptible to *Demodex*. In addition, people with a HLA A2 alleles have been found to be 2.9 times more resistant to *Demodex*.[23] This suggests a relationship between *Demodex* and SD.

CONCLUSION

In the present study, we found that the presence of *Demodex* was more prevalent in SD lesions than in control and lesion-free skins. We believe that *Demodex*, a part of the microbiota, may be a predisposing factor in the development of SD.

New, multi-faceted studies including the investigation of the sebum content and acaricide treatments as well as genetic studies in larger series of patients with severe SD are needed.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Fadime Kılınç, Ayşe Akbaş, Yıldız Hayran, Akın Aktaş, Cansu Altınöz Günay, Zarif Tuçe Özkara Duman;* ***Design:*** *Fadime Kılınç, Ayşe Akbaş;* ***Control/Supervision:*** *Fadime Kılınç, Ayşe Akbaş, Yıldız Hayran;* ***Data Collection and/or Processing:*** *Fadime Kılınç, Ayşe Akbaş, Zarif Tuçe Özkara Duman, Cansu Altınöz Günay;* ***Analysis and/or Interpretation:*** *Fadime Kılınç, Ayşe Akbaş, Zarif Tuçe Özkara Duman, Cancu Altınöz Günay, Yıldız Hayran, Akın Aktaş;* ***Literature Review:*** *Fadime Kılınç;* ***Writing the Article:*** *Fadime Kılınç;* ***Critical Review:*** *Fadime Kılınç, Ayşe Akbaş, Zarif Tuçe Özkara Duman, Cancu Altınöz Günay, Yıldız Hayran, Akın Aktaş;* ***References and Fundings:*** *Fadime Kılınç.*

REFERENCES

1. Dessinioti C, Katsambas A. Seborrheic dermatitis: etiology, risk factors, and treatments: facts and controversies. Clin Dermatol. 2013;31(4):343-51. [Crossref] [PubMed]
2. Karincaoglu Y, Tepe B, Kalayci B, Atambay M, Seyhan M. Is Demodex folliculorum an aetiological factor in seborrhoeic dermatitis? Clin Exp Dermatol. 2009;34(8):e516-20. [Crossref] [PubMed]
3. Berk T, Scheinfeld N. Seborrheic dermatitis. P T. 2010;35(6):348-52. [PubMed] [PMC]
4. Adalsteinsson JA, Kaushik S, Muzumdar S, Guttman-Yassky E, Ungar J. An update on the microbiology, immunology and genetics of seborrheic dermatitis. Exp Dermatol. 2020;29(5):481-9. [Crossref] [PubMed]
5. Sampaio AL, Pôrto LC, Ramos-e-Silva M, Nunes AP, Cardoso-Oliveira J, Fabricio-Silva GM, et al. Human leucocyte antigen frequency in a miscegenated population presenting with seborrhoeic dermatitis. J Eur Acad Dermatol Venereol. 2014;28(11):1576-7. [Crossref] [PubMed]
6. Karakadze MA, Hirt PA, Wikramanayake TC. The genetic basis of seborrhoeic dermatitis: a review. J Eur Acad Dermatol Venereol. 2018;32(4):529-36. [Crossref] [PubMed]
7. Zhao YE, Wu LP, Peng Y, Cheng H. Retrospective analysis of the association between Demodex infestation and rosacea. Arch Dermatol. 2010;146(8):896-902. Erratum in: Arch Dermatol. 2010;146(12):1412. [Crossref] [PubMed]
8. Yazıcı AC, İkizoğlu G. Demodicosis. Turkiye Klinikleri J Med Sci. 2019;39(2):231-6. [Crossref]
9. Rather PA, Hassan I. Human demodex mite: the versatile mite of dermatological importance. Indian J Dermatol. 2014;59(1):60-6. [Crossref] [PubMed] [PMC]
10. Karincaoglu Y, Bayram N, Aycan O, Esrefoglu M. The clinical importance of demodex folliculorum presenting with nonspecific facial signs and symptoms. J Dermatol. 2004;31(8):618-26. [Crossref] [PubMed]
11. Zhao YE, Peng Y, Wang XL, Wu LP, Wang M, Yan HL, et al. Facial dermatosis associated with Demodex: a case-control study. J Zhejiang Univ Sci B. 2011;12(12):1008-15. [Crossref] [PubMed] [PMC]
12. Zhao YE, Hu L, Wu LP, Ma JX. A meta-analysis of association between acne vulgaris and Demodex infestation. J Zhejiang Univ Sci B. 2012;13(3):192-202. [Crossref] [PubMed] [PMC]
13. Aktaş Karabay E, Aksu Çerman A. Demodex folliculorum infestations in common facial dermatoses: acne vulgaris, rosacea, seborrheic dermatitis. An Bras Dermatol. 2020;95(2):187-93. [Crossref] [PubMed] [PMC]
14. Cömert A, Bekiroglu N, Gürbüz O, Ergun T. Efficacy of oral fluconazole in the treatment of seborrheic dermatitis: a placebo-controlled study. Am J Clin Dermatol. 2007;8(4):235-8. [Crossref] [PubMed]
15. Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: a comprehensive review. J Clin Investig Dermatol. 2015;3(2):10.13188/2373-1044.1000019. [Crossref] [PubMed] [PMC]
16. Schwartz JR, Messenger AG, Tosti A, Todd G, Hordinsky M, Hay RJ, et al. A comprehensive pathophysiology of dandruff and seborrheic dermatitis-towards a more precise definition of scalp health. Acta Derm Venereol. 2013;93(2):131-7. [Crossref] [PubMed]
17. Forton F, De Maertelaer V. Erythematotelangiectatic rosacea may be associated with a subclinical stage of demodicosis: a case-control study. Br J Dermatol. 2019;181(4):818-25. [Crossref] [PubMed]
18. Moran EM, Foley R, Powell FC. Demodex and rosacea revisited. Clin Dermatol. 2017;35(2):195-200. [Crossref] [PubMed]
19. Yazısız H, Çekin Y, Koçlar FG. The presence of demodex parasites in patients with dermatologic symptoms of the face. Turkiye Parazitol Derg. 2019;43(3):143-8. [Crossref] [PubMed]
20. Chen W, Plewig G. Human demodicosis: revisit and a proposed classification. Br J Dermatol. 2014;170(6):1219-25. [Crossref] [PubMed]
21. Dhingra KK, Saroha V, Gupta P, Khurana N. Demodex-associated dermatologic conditions--A coincidence or an etiological correlate. Review with a report of a rare case of sebaceous adenoma. Pathol Res Pract. 2009;205(6):423-6. [Crossref] [PubMed]
22. Demirdağ HG, Özcan H, Gürsoy Ş, Beker Akbulut G. The effects of sebum configuration on Demodex spp. density. Turk J Med Sci. 2016;46(5):1415-21. [Crossref] [PubMed]
23. Akilov OE, Mumcuoglu KY. Association between human demodicosis and HLA class I. Clin Exp Dermatol. 2003;28(1):70-3. [Crossref] [PubMed]