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Title: Association of non-melanocytic tumors of the eyelid with demodex folliculorum.
Short title: Demodex folliculorum effect on non-melanocytic eyelid tumors.

Abstract

Purpose: Demodex is a parasite that lives in the hair follicles or sebaceous glands of the eyelid. There are two species that live in humans. These are Demodex follicularum and Demodex brevis. Demodex mites have been found to be associated with many inflammatory diseases such as blepharitis, chalazion, and rosacea. The role of the inflammatory environment in the pathogenesis of cancer development is also known. Our aim in this study was to investigate the role of Demodex mites and inflammation in eyelid tumors

Materials and methods: Basal cell carcinoma, squamous cell carcinoma, and tumor-free control tissues were included in our study. In these cases, the presence and number of Demodex and the presence and severity of inflammation were evaluated. In addition, the demographic characteristics of the tumors and prognostic data such as tumor size and invasion depth were evaluated.

Results: No significant difference was found between the tumor and control groups in terms of the presence of Demodex. However, a significant relationship was found between the inflammation caused by Demodex mites in basal cell carcinomas and tumor size and invasion depth.

Conclusion: Demodex mites may create an inflammatory tumor environment and play a role in tumor growth and progression.

Keywords: Demodex mite, eyelid tumor, inflamation.

Makale başlığı: Demodex folliculorum varlığının, melanositik olmayan göz kapağı tümörleri ile ilişkisi.

Kısa başlık: Melanositik olmayan göz kapağı tümörlerinde demodex folliculorum etkisi.

Öz

Amaç: Demodex, göz kapağının kıl foliküllerinde veya yağ bezlerinde yaşayan bir parazittir. İnsanlarda yaşayan iki türü vardır. Bunlar Demodex follicularum ve Demodex brevis'tir. Demodex akarlarının blefarit, şalazyon ve rosacea gibi birçok inflamatuar hastalıkla ilişkili olduğu bulunmuştur. Kanser gelişiminin patogenezinde inflamatuar ortamın rolü de bilinmektedir. Bu çalışmadaki amacımız, Demodex akarlarının ve inflamasyonun göz kapağı tümörlerindeki rolünü araştırmaktır

Gereç ve yöntem: Çalışmamıza bazal hücreli karsinom, skuamöz hücreli karsinom ve tümörsüz kontrol dokuları dahil edildi. Bu vakalarda, Demodex'in varlığı ve sayısı ile inflamasyonun varlığı ve şiddeti değerlendirildi. Ek olarak, tümörlerin demografik özellikleri ve tümör boyutu ve invazyon derinliği gibi prognostik veriler değerlendirildi.

Bulgular: Demodex varlığı açısından tümör ve kontrol grupları arasında anlamlı bir fark bulunmadı. Ancak, bazal hücreli karsinomlarda Demodex akarlarının neden olduğu inflamasyon ile tümör boyutu ve invazyon derinliği arasında anlamlı bir ilişki bulundu.

Sonuç: Demodex akarları inflamatuvar bir tümör ortamı yaratabilir ve tümör büyümesinde ve ilerlemesinde rol oynayabilir.

Anahtar kelimeler: Demodex akarı, göz kapağı tümörleri, inflamasyon.

Introduction

Demodex is an ectoparasite that lives in or near the eyelash follicles and hair sebaceous glands of mammals. In humans, there are two subspecies: *Demodex folliculorum (D. Folliculorum)* and *Demodex brevis (D. Brevis)*. Both species are similar in shape, but differ in their length and anatomical location. *D. Folliculorum* is usually found in eyelash follicles on the eyelids, while *D. Brevis* is often found deep in the sebaceous glands and meibomian glands [1].

Demodex, which is commonly found in humans, usually does not cause any clinical problems. Studies have reported that approximately two-thirds of us have *D. Folliculorum* in our hair follicles, and one-quarter of us have *D. Brevis* in our sebaceous glands [2, 3]. In some cases, the number of demodex colonies may increase and cause disease, this is

called demodicosis [4]. Older age, male gender, crowded living conditions, malnutrition, immunosuppression, diabetes, corticosteroid use, and some skin diseases related to the sebaceous glands or some genetic HLA subtypes are at higher risk of developing demodicosis [5-7].

Demodex mites, which feed on sebum and skin cells on and around the eyelid, create mechanical blockages in the eyelash follicles and meibomian glands when their numbers increase [8, 9]. Demodex is the responsible agent in 60-70% of cases of blepharitis, which is inflammation of the eyelid margins [10]. It has been shown in the study that demodex, which causes meibomian gland dysfunction, causes ocular surface diseases such as recurrent chalazion, pterygium, corneal vascularization, corneal opacity and dry eye [11]. Demodex mites, which cause clinical symptoms such as itching and blurred vision due to chronic inflammation, play an important role in the formation of clinical conditions such as keratinization of the eyelid margins, hyperemia, scaling at the eyelash roots, shedding of the eyelashes and inversion [12, 13].

Demodex mites found on the eyelids increase the risk of infection as a bacterial vector, affecting the ocular microbiota and the stabilization of the immunological response. It also leads to the disruption of the epidermal barrier, increasing the risk of granulomatous inflammation and immune reactions [14, 15]. It is known that chronic inflammation is effective in the development of many diseases as well as in the pathogenesis of cancer development [16, 17]. Therefore, there are studies that demodex parasites may play a role in the development of both eyelid tumors and other tumors by causing chronic inflammation [18-20].

In this study, the presence of demodex in eyelid tumors was investigated and the relationship between the type of tumor and the presence of inflammation was investigated.

Materials and methods

In our study, pathological specimens of cases who underwent eyelid surgery for eyelid tumors and other reasons by the Department of Ophthalmology, Eskişehir Osmangazi University Faculty of Medicine, between 2011 and 2024 were retrospectively evaluated by the Department of Pathology. The eyelid tumor group consisted of basal cell carcinoma and squamous cell carcinoma samples, while benign lesions such as nevus and squamous papilloma and eyelid biopsies performed for blepharoplasty were evaluated as the control group. Demographic data such as gender and age of all cases, as well as lesion laterality and localization were recorded.

Our study was approved by Eskişehir Osmangazi University Non-Invasive Clinical Research Ethics Committee with decision number 25 dated 28.11.2023.

H&E stained preparations in the archives of the Department of Pathology were evaluated for the presence of Demodex. For this purpose, the eyelash follicles in the preparation were counted and the number of follicles in which D. folliculorum was detected was recorded. Inflammation was evaluated in two groups as mild (scattered and few inflammatory cells in the section) and moderate-severe (more grouped and dense cells). In addition, tumor size and invasion depth (in two groups as below and above 10 mm) and lymphovascular invasion, which have prognostic importance, were also recorded.

The relationships between the presence of Demodex folliculorum and gender, tumor localization, tumor size, tumor invasion depth, presence of inflammation and lymphovascular invasion were evaluated. The relationship between the number of positive follicles detected in tumors and inflammation and the relationship between tumor size and invasion depth were evaluated. Pearson Exact or Fisher Exact test Chi-Square analyses were used in the analysis of these relationships.

Statistical analysis

Continuous data are given as mean ± standard deviation. Categorical data are given as percentage (%). Shapiro Wilk test was used to investigate the conformity of the data to normal distribution. Mann-Whitney U test was used in cases where the number of groups was two in the comparison of groups that did not conform to normal distribution. Pearson Chi-Square test or Fisher Exact test were used in the analysis of the created cross tables. IBM SPSS Statistics 21.0 program was used in the application of the analyses. $p<0.05$ value was accepted as the criterion of statistical significance.

Results

A total of 144 cases were included in the study. Of these cases, 80 (55.6%) were basal cell carcinoma, 24 (16.7%) were squamous cell carcinoma, and 40 (27.8%) were the control group. Demographic data of all three groups are given in Table 1. Accordingly, no relationship was found between gender, laterality, localization on the eyelid, presence of lymphovascular invasion, and presence of Demodex (*p* values: $p=0.288$, $p=0.110$; $p=0.312$; $p=0.620$, respectively).

D. folliculorum was detected in a total of 53 cases, of which 31 (38.75%) were BCC, 7 (29.1%) were SCC and 15 (37.5%) were the control group (Figure 1 A-B).

The mean age of squamous cell carcinoma cases was 71.8±13 years; the mean age of basal cell carcinoma cases was 71±11.9 years and the mean age of the control

group was 60±14 years and D. folliculorum was statistically significantly higher in those over 50 years of age (p=0.030) (Table 2). In basal cell carcinoma cases, Demodex was detected in 5 out of 5 cases under 50 years of age. In those over 50 years of age, Demodex was detected in 26 out of 75 cases (p=0.007). In squamous cell carcinoma cases, Demodex was detected in 1 case under 50 years of age and in 6 out of 23 cases over 50 years of age (p=0.296). In the control group, Demodex was detected in 4 of 10 cases under 50 years of age and in 11 of 30 cases over 50 years of age (p=1.00).

Relationship between Demodex density, tumor prognostic parameters, and inflammation according to follicle number:

In squamous cell carcinoma cases, mean follicle count was 9.79 (1-40) and mean positive follicle count was 0.75 (0-8). Mean tumor size of squamous cell carcinoma cases was 11.6±10.4 mm; Depth of invasion was 6.6±7 mm and no correlation was found between both data and Demodex (p=0.169 and p=0.177, respectively). In addition, no significant correlation was found between increase in tumor size or depth of invasion as the number of positive follicles increased (p=0.293; p=0.798). No statistically significant result was found between Demodex and inflammation (p=1.000).

In basal cell carcinoma cases, the mean follicle count was 1.71 (1-5). The mean positive follicle count was 0.46 (0-2). In basal cell carcinoma cases, the mean tumor size was 8.7±5.1 mm; the depth of invasion was 3.6±2.6 mm, and no correlation was found between both data and the presence of Demodex follicles (p=0.435 and p=0.284, respectively). In cases with high positive follicle counts related to Demodex density, a statistically significant correlation was found between tumor size and depth of invasion (p=0.012; p=0.040) (Table 3). In addition, in BCC cases, inflammation was found to be statistically significantly higher in cases with Demodex (p=0.004). A significant correlation was also found between the number of positive follicles and the severity of inflammation (p=0.036). In conclusion, it was determined that the severity of inflammation increased in basal cell carcinoma cases in relation to Demodex density (Figure 2, 3).

Discussion

The most common tumors of the eyelid are basal cell carcinoma and squamous cell carcinoma. Basal cell carcinoma is more common in incidence. There are some common etiological factors in the development of these two types of cancer, such as chronic sun exposure and chronic damage [21].

The frequency of Demodex parasites increases with age. It is found in approximately 80% of people over the age of 60 [22]. In our study, it was found to be more common in older ages in all groups.

The presence of Demodex is not always associated with diseases. Patients are usually asymptomatic. Demodex is known to play a role in the etiology of diseases such as rosacea, acne formation and folliculitis [23]. However, individuals must have certain predisposing factors for these diseases to develop. For example, factors such as immune deficiency, sunlight, high body mass index, smoking and alcohol consumption, or stress also increase demodex colonization [24].

Erbağcı et al. [18] found a higher rate of Demodex parasites in 32 basal cell carcinoma cases compared to control groups and stated that this may play a role in the etiology of basal cell carcinoma cases. In addition, Sun et al. [25], in their study on the differences in Demodex incidence between basal cell carcinoma, squamous cell carcinoma, tricholemmoma and seborrheic keratosis in the facial region, found a higher rate of Demodex mites in basal cell carcinomas. In another study, they found no significant difference when comparing cancer types such as BCC, SCC and melanoma with the control group [26]. In this study, we also did not find a significant difference between the control group and BCC-SCC. However, when we compared the tumor size and invasion depth, which has not been previously examined in literature, with the number of positive follicles in BCC cases, we found a statistically significant result. We also saw that the severity of inflammation increased as the number of Demodex positive follicles increased. Inflammatory cells around the tumor affect tumor progression through various molecular pathways. Inflammation increases the tumor's invasive capacity by increasing epithelial-mesenchymal transition via the H-RAS gene [27]. In addition, some cytokines, such as Interleukin-17, are released from lymphocytes around the tumor and contribute to tumor progression and angiogenesis [28]. As a result, inflammatory cells that provide a pro-oncogenic environment for tumor growth contribute to tumor growth [29]. Therefore, it can be concluded that Demodex mites contribute to tumor progression by creating an inflammatory microenvironment.

There are also studies investigating the relationship between Demodex and other types of cancer. One study showed that the frequency of demodicosis increased in ovarian cancer cases among gynecological cancer types [30]. Another similar study found higher rates of Demodex mites in hematological cancer cases than in controls. This was generally associated with immunosuppression in the cases [31].

In this study, no significant relationship was found between Demodex and squamous cell carcinoma. This situation can be explained by the fact that factors such as chronic sun exposure, fair skin, and HPV infection are more dominant risk factors in the etiology of squamous cell carcinomas [32].

This study has some limitations. For example, the Demodex subtype could not be determined definitively, and further examination was not performed to determine the subtypes of inflammatory cells around the tumor.

In conclusion, it is known that the frequency of Demodex mites in the body increases in conditions such as old age and immunosuppression. The same etiological factors are valid for many types of cancer. In our study, no difference was found between the frequency of Demodex according to tumor types. It was thought that the relationship between Demodex mites, which are frequently found around the eyelid and face, and the tumor could be coincidental. However, a significant relationship was found between increased Demodex count and both the severity of inflammation and tumor size/invasion depth, especially in basal cell carcinoma cases, suggesting that it may be a factor in tumor growth. Basal cell carcinoma cases seen on the eyelid are a tumor group with high morbidity, and we believe that it is important to know the factors that affect the growth of these tumors.

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Author contributions: NSŞ and NY have constructed the main idea and hypothesis of the study. NSŞ and HT developed the theory and arranged/edited the material and method section. NSŞ and MB have done the evaluation of the data in the Results section. Discussion section of the article written by NSŞ, NY and HT, was also reviewed. In addition, all authors discussed the entire study and approved the final version

Conflict of interest: No conflict of interest was declared by the authors.

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Table 1. Tumor types and control group, and their relationships with demographic data

	BCC (n=80)	SCC (n=24)	CONTROL (n=40)	*p* values
Age	71.03±11.98 (45-92)	71.83±13.07 (47-92)	60.18±14.5 (20-85)	0.913 (z=0.109)
Gender				
Female	33 (41,25%)	8 (33.3%)	21 (52.5%)	0.288
Male	47 (58.75%)	16 (66.6%)	19 (47.5%)	(cs=2.487)
Localization				
Lower eyelid	50 (62.5%)	12 (50%)	13 (32.5%)	
Upper eyelid	20 (25%)	6 (25%)	19 (47.5%)	0.312
Inner canthus	8 (10%)	3 (12.5%)	5 (12.5%)	(cs=3.565)
External canthus	2 (2.5%)	3 (12.5%)	3 (7.5%)	
Laterality				
Right	43 (53.75%)	15 (62.49%)	15 (37.5%)	0.110
Left	37 (46.25%)	9 (37.49%)	25 (62.5%)	(cs=4.423)
Demodex foll.				
Yes	31 (38.75%)	7 (29.16%)	15 (37.5%)	0.69
No	49 (61.25%)	17 (70.83%)	25 (62.5%)	(cs=0.740)

z: Mann-Whitney U, Cs: Chi Square test
Descriptive statistics for the "Age": Mean ± Standard Deviation (Minimum- Maximum Age Range)

Table 2. Demodex counts by age groups

		Age Group		
D. Follıculorum		≤50	>50	*p*=0.030 * (cs=10.395)
	Yes	10 (62.5%)	43 (33.5%)	
	No	6 (37.5%)	85 (66.4%)	
Total		16 (100%)	128 0 0%)	

*Cs: Chi Square test *Pearson Chi Square test

Table 3. Relationship between positive follicle count and tumor size, invasion depth in basal cell carcinomas

		Tumor size			Depth of invasion		
		0-10 mm	**11 mm and above**		**0-10 mm**	**11 mm and above**	
Positive follicle count	0	37 (66.07%)	12 (50%)	*p*=0.012 * (cs=6.244)	49 (62.82%)	0 (0%)	*p*=0.040* (cs=6.427)
	1	18 (32.14%)	7 (29.16%)		24 (30.76%)	1(50%)	
	2	1 (1.78%)	5 (20.83%)		5 (6.41%)	1 (50%)	
Total		56 (100%)	24 (100%)		78 (100%)	2 (100%)	

Cs: Chi Square test *Pearson Chi Square test

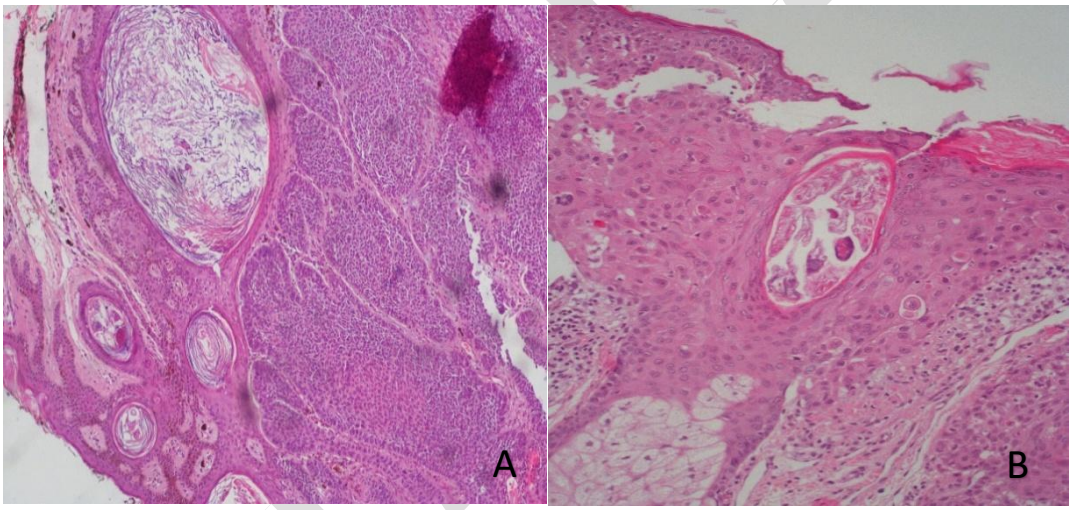

Figure 1- 1A. D. follıculorum with basal cell carcinoma (H&E, x100)

1B: D. follıculorum with squamous cell carcinoma and carcinoma in situ (H&E, x200)

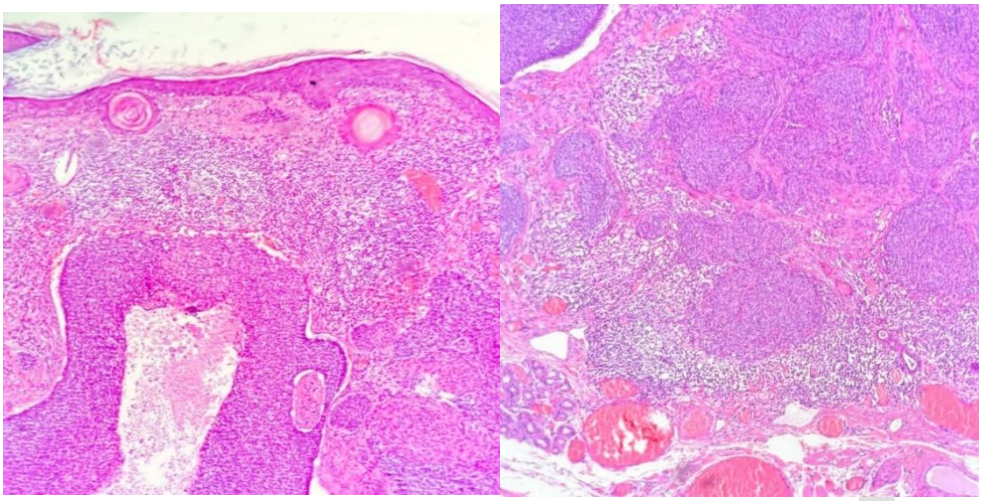

Figure 2 A-B. Severe chronic inflammation in the Demodex infected basal cell carcinoma cases (H&E, x100)

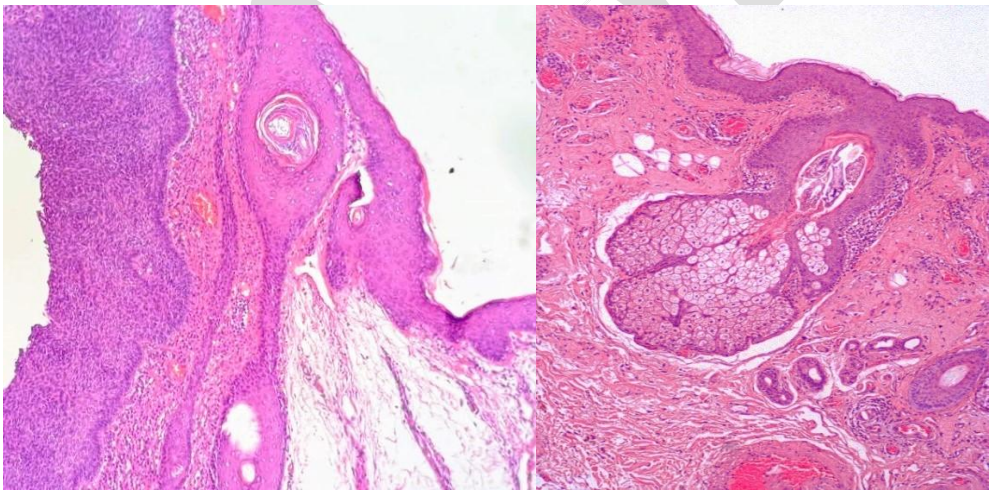

Figure 3- 3A. Basal cell carcinoma with mild inflammation (H&E, x100)

3B: D. follıcularum and perifollicular mild chronic inflammation (H&E, x100)

Seker NS, Erdogan H, Yildirim N, Bilgin M. Association of non-melanocytic tumors of the eyelid with demodex folliculorum. Pam Med J 2026;19:…-…

Şeker NS, Erdoğan H, Yıldırım N, Bilgin M. Demodex folliculorum varlığının, melanositik olmayan göz kapağı tümörleri ile ilişkisi. Pam Tıp Derg 2026;19:…-…

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