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Demodex folliculorum and Skin Disease: A Case-Control Study

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Demodex mites are commensals of the pilosebaceous unit in human. This study aimed to investigate possible association of *Demodex folliculorum* with Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC), melanoma, Discoid Lupus Erythematosus (DLE) and rosacea. In this case-control study, standardized skin surface biopsy samples were obtained from the cheeks of healthy individuals (n = 28); patients with BCC (n = 18), SCC (n = 13) and melanoma (n = 11) and patients with DLE (n = 28) and inflammatory rosacea (n = 34). Mite density (cm^{-2}) and the rate of infestation (density ≥5) were compared. The rate of mite infestation (%) was comparable between the controls (21.4) and the patients with BCC (22.2, p = 0.95), SCC (18.8, p = 0.83), melanoma (9.1, p = 0.37) and DLE (17.9, p = 0.74). Infestation was significantly more frequent in the rosacea group (47.1, p = 0.04) than in the controls. While, the mean mite density (cm^{-2}) was comparable between the control (4.07±2.06) and the groups with BCC (5.01±2.08, p = 0.68), SCC (3.17±1.29, p = 0.49) and DLE (3.26±1.04, p = 0.91); it was significantly higher in the group of rosacea (8.56±3.29, p = 0.03) and lower in the group of melanoma (1.45±0.58, p = 0.04) in comparison with the normal group. In conclusion, although *Demodex folliculorum* does not seem to play a role in the pathogenesis of BCC, SCC and DLE, it is apparently associated with rosacea and melanoma.

Key words: *Demodex*, melanoma, rosacea, basal cell carcinoma, squamous cell carcinoma

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INTRODUCTION

Hair follicle mites, *Demodex folliculorum* and *Demodex brevis* are obligatory commensals of the pilosebaceous unit and the most common ectoparasites in human (Lacey *et al*., 2009).

Although, the presence of these mites in the skin is asymptomatic when their density is not considerable (<5 cm^{-2}) (Dhingra *et al*., 2009), these two species of *Demodex* have been implicated in the pathogenesis of various skin diseases such as rosacea (Hsu *et al*., 2009), pityriasis (Baima and Sticherling, 2002), dermatitis (Karincaoglu *et al*., 2009), bacterial infections (Ozdemir *et al*., 2005), acne vulgaris (Karaman *et al*., 2008) and even more serious conditions such as Squamous Cell Carcinoma (SCC) and Basal Cell Carcinoma (BCC) (Erbagci and Erkilic, 2000; Sun *et al*., 2005) through provocation of inflammatory/immune reactions, mechanical obstruction of the follicles or acting as a vector for other pathogens (Powell, 2004).

Despite these evidences, there is still an ongoing debate on the connection between mites and skin diseases and a sheer coincidence is yet to be ruled out. These arguments are clinically critical, because the evidence linking *Demodex* to human disease has implications with regard to treatment (Elston, 2010).

The objective of this study is to investigate a possible association between the presence of *Demodex folliculorum* and its infestation with a number of malignant (BCC, SCC, melanoma) and nonmalignant (discoid lupus erythematosus, rosacea) skin diseases.

MATERIALS AND METHODS

In this study, 135 patients with histopathologic diagnosis of BCC (n = 18), SCC (n = 16), melanoma (n = 11), discoid lupus erythematosus (DLE, n = 28) and inflammatory (papulopustular) rosacea (n = 34) were recruited from three referral centers from October 2008 to March 2013.

In these patients, the main lesion of BCC, SCC and melanoma was on their cheeks and in the groups with DLE and rosacea, the cheeks were clinically affected.

Twenty eight age and sex-matched healthy individuals without any dermatological diseases or telangiectases served as the controls.

None of the participants had received any topical/systemic antibiotic, topical acaricides or corticosteroid/immunosuppressive therapy within one month prior to enrollment or radiotherapy/chemotherapy before skin sampling.

This study was approved by the ethics committee of a local university and written informed consents were obtained from all participants.

A non-invasive method for the Standardized Skin Surface Biopsy (SSSB) was used for detecting mites. For SSSB, a drop (about 0.05 mL) of cyanoacrylate glue was placed on a standard surface area of 1 cm^2 that was drawn by a waterproof pen on a slide glass. The adhesive bearing surface was pressed onto to the skin for 1 min and then peeled off gently. Two drops of immersion oil were used for clarification of the samples and after the application of cover-slides, the specimens were immediately examined for mites under light microscopy (X40 and X100 magnification) by a skilled dermatopathologist unaware of the cohorts.

For the patients with BCC, SCC and melanoma, the samplings were performed from the skin of the cheek just next to the lesions, as well as, from the corresponding site on the contralateral (intact) cheek.

For normal subjects and the patients with DLE and rosacea, the sampling was performed from both cheeks. For comparisons between group, the results were obtained arbitrarily from the right cheeks were used in these groups.

The skin and slides were cleaned with ether prior to sampling (Forton and Song, 1998). *Demodex* density was established as the number of mites per square centimetre of the skin (Erbagci and Ozgoztasi, 1998; Karincaoglu *et al*., 2009). Infestation (positivity) was reported when the mite density was ≥5 (Forton and Seys, 1993).

Statistical analysis: The mite density and positivity rate were compared between the patients and the controls (between group comparison), as well as between left and right cheeks in each group (intra-group comparison). The SPSS Software for Windows (ver.18.0, SPSS Inc., IL, USA) was used for analysis. One-way analysis of variance (ANOVA), Tukey *post hoc* test, the independent samples t-test and the Chi-square (χ^2) test were used for comparisons. A p-value ≤0.05 was considered statistically significant.

RESULTS

The patient groups were comparable with the normal group in terms of the participants' age and gender (Table 1).

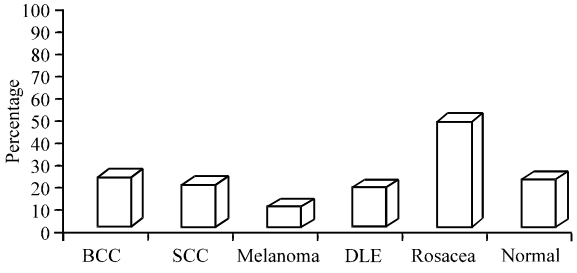

Fig. 1: Mite positivity on the cheeks of the studied individuals. BCC: Basal cell carcinoma, DLE: Discoid lupus erythematosus, SCC: Squamous cell carcinoma

Table 1: Age and gender of the individuals in the studied groups

		Gender			
Groups (n)	Age (year)	Male	Female	p*	p**
Basal cell carcinoma (18)	64.05±14.09	12 (66.7)	6 (33.3)	0.78	0.38
Squamous cell carcinoma (16)	64.58±11.56	13 (81.2)	3 (18.8)	0.66	0.07
Melanoma (11)	65.36±13.50	8 (72.7)	3 (27.3)	0.19	0.27
Discoid lupus erythematosus (28)	59.02±16.26	14 (50)	14 (50)	0.24	0.79
Rosacea (34)	59.50±19.23	16 (47.1)	18 (52.9)	0.49	0.61
Normal (28)	63.75±18.98	15 (53.6)	13 (46.4)	-	-

Significant at $p \leq 0.05$, *age, **gender. Data are presented as Mean±Standard deviation (age) and frequency (%) (gender)

Table 2: Mite density on the cheeks of the studied individuals

Groups (n)	Side	Density (cm^{-2})	p*	p**
Basal cell carcinoma (18)	Involved	5.01±2.08	0.68	0.97
	Intact	4.74±1.67		
Squamous cell carcinoma (16)	Involved	3.17±1.29	0.49	0.81
	Intact	4.80±2.45		
Melanoma (11)	Involved	1.45±0.58	0.04	0.77
	Intact	1.12±0.21		
Discoid lupus erythematosus (28)	Right	3.26±1.04	0.91	0.86
	Left	3.89±1.24		
Rosacea (34)	Right	8.56±3.29	0.03	0.97
	Left	8.14±2.94		
Normal (28)	Right	4.07±2.06	-	0.89
	Left	3.90±1.10		

Significant $p \leq 0.05$, *patient vs. normal, **two sides. Data are presented as Mean±Standard error of mean

Mite infestation was established in 4 patients (22.2%) with BCC, 3 patients (18.8%) with SCC, 1 patient (9.1%) with melanoma, 5 patients (17.9%) with DLE, 16 patients (47.1%) with rosacea and 6 subjected (21.4%) in the normal group (Fig. 1).

Based on the results of χ^2 test, the normal group was comparable with the groups of BCC ($p = 0.95$), SCC ($p = 0.83$), melanoma ($p = 0.37$) and DLE ($p = 0.74$), in terms of the mite infestation rate. The rate of mite infestation, however, was significantly higher in the group of patients with rosacea in comparison with that in the controls (χ^2 test, $p = 0.04$; odds ratio = 3.26 with 95% confidence interval of 1.06-10.05).

The mean mite densities on the cheeks of the studied individuals in different groups are summarized in Table 2. Comparing the mean mite density between two sides in each group using the independent samples t-test showed no significant difference.

According to the results of one-way analysis of variance (ANOVA), a significant difference was present between the six studied groups in terms of the mean mite density on the involved (in BCC, SCC and melanoma groups) or right (in DLE, rosacea and normal groups) cheeks ($p = 0.04$).

Based on the results of *post hoc* analysis, this significant difference was related to two comparisons; normal vs. melanoma group ($p = 0.04$) and normal vs. rosacea group ($p = 0.03$) (Table 2).

DISCUSSION

In the present study, both mite density and the rate of mite infestation were significantly higher on the cheeks of the patients with rosacea in comparison with those in their unaffected counterparts.

The role of *Demodex* in the pathogeny of rosacea is not a new concept (Abd-El-Al *et al*., 1997; El-Shazly *et al*., 2001; Zhao *et al*., 2010; Lazaridou *et al*., 2011; Parodi *et al*., 2011; Jarmuda *et al*., 2012; Sattler *et al*., 2012). Several mechanisms have been proposed in this regard such as the obstruction of the hair follicle or sebaceous duct by increased mite density, direct damage to the follicular epithelia, induction of foreign body/hypersensitivity reactions and playing the role of a vector for bacteria like *Staphylococcus albus* and *Bacillus oleronius* (Crawford *et al*., 2004; Yamasaki and Gallo, 2009; Rios-Yuil and Mercadillo-Perez, 2013).

It should be noted that the patients recruited in the present study were affected with papulopustular form of rosacea. However, the pathologic role of *Demodex folliculorum* in rosacea is apparently irrespective of rosacea subtype (Casas *et al*., 2012).

Comparing the two groups of patients with DLE and normal subjects, we did not find a significant difference in terms of mite density and the rate of infestation.

In a recent study by Perrigouard *et al*. (2013) while over 75% of the specimens obtained from patients with rosacea involved *Demodex*, no mite was seen in lupus erythematosus cases.

In another study by Moravvej *et al*. (2007) *Demodex* spp. was found in 38.6% of rosacea biopsies and only in 21.3% of DLE biopsies.

In line with these finding, Roihu and Kariniemi (1998) also reported no significant difference in mite counts of infested follicles between DLE patients and their healthy counterparts.

Unlike in rosacea and DLE, available data regarding possible association between *Demodex* mites and malignant skin diseases are more conflicting.

Erbagci and Erkilic (2000) reported a higher infestation rate and mean density of *Demodex* mites in patients with BCC comparing with a normal sex and age matched group.

Sun *et al*. (2005) examined specimens of the facial skin obtained from patients with BCC, SCC and nonmalignant conditions (seborrheic keratosis and trichilemmoma) regarding the rate of mite infestation. Comparing with nonmalignant conditions, the rate of mite infestation was significantly higher in the BCC group and lower in the SCC patients.

In contrast, neither BCC nor SCC cases in the current study differed significantly in mean mite density and the rate of mite infestation with the controls.

Various possible factors may underlie this heterogeneity, such as patients' age in different studies, lack of an appropriate control group, different time, site and method of sampling and dissimilar techniques in detecting mite and establishing its density.

It is thought that the likelihood of mite infestation and the mite density increase with advancing age (Erbagci *et al*., 2003; Aycan *et al*., 2007). In the present study, the groups of patients were matched with the normal subjects in terms of age. Although, there may not be a significant association between mite infestation and host gender, skin type, hygiene and use of cosmetics, (Andrews, 1982), the authors did their best to have homogenous groups in terms of these factors.

Since, incompetent immune system has been reported a risk factor for mite infestation (Akilov and Mumcuoglu, 2003, 2004), all samplings were performed before commencement of any treatment to ensure that the patients' immune system was untouched. Furthermore, all samplings were performed on the cheek, because the highest density of mites has been found on this area of the face (Bonnar *et al*., 1993).

There are different methods available for skin sampling to examine *Demodex* mites such as using adhesive tape, skin impression/scraping, comedo extraction, hair epilation, skin biopsy and skin surface biopsy (Bonnar *et al*., 1993; Forton and Seys, 1993; Erbagci and Ozgoztasi, 1998). We preferred SSSB because it is noninvasive and more sensitive comparing with similar approaches in this regard (Forton and Song, 1998; Kligman and Christensen, 2011).

By comparison of the melanoma and control groups, although, the infestation rate of *Demodex folliculorum* was less than half of that in the controls, the comparison did not reach a level of significance (9.1% vs. 21.4%; $p = 0.37$). On the other hand, however, the mean mite density was significantly lower in the melanoma group comparing with the controls (1.45±0.58 vs. 4.07±2.06 mites cm^{-2}; $p = 0.04$). To the best of our knowledge, the current study is apparently the first one that suggests an association between *Demodex* infestation and melanoma. This interesting finding may propose a shared point in immunological host defense against both melanoma and *Demodex* in human (Strohal *et al*., 1994; Thor Straten *et al*., 1999; Tsutsumi, 2004). Since the mite density was significantly higher in normal than in melanoma group, another possible hypothesis might be considering a protective role of infection with *Demodex folliculorum* against melanoma or vice versa. Further studies, particularly with larger sample size may better clarify this connection between mites and melanoma.

Performing an intra-group comparison of mite density and infestation rate between two sides of the face yielded no significant difference in all groups. This symmetry of mite distribution on the face has been also reported previously (Kligman and Christensen, 2011).

Although, this finding may indicate a systemic rather than a local phenomenon in relating skin diseases with *Demodex* mites, more studies merit to be carried out in this regard. The authors acknowledge small sample size in some groups particularly melanoma, as a limitation of the present study. This limitation is largely due to the criterion of including cases with involvement of a particular site on the face (the cheek) which was considered to the detriment of sample size and very time-consuming process of data collection. Nevertheless, the results of comparison between melanoma and normal groups are so interesting that make this limitation negligible.

Based on the findings on the present study, there is apparently no association between *Demodex folliculorum* and BCC, SCC and DLE. In conformity with previous reports, both *Demodex* mite density and infestation rate may be considered as risk factors for rosacea. Conversely, infestation rate and density of *Demodex folliculorum* are lower in patients with melanoma comparing with their healthy counterparts. Nevertheless, multicentric studies are required to establish the hypothesis.

The role of age in dermatological diseases (Babaeinejad *et al*., 2011; Amirnia *et al*., 2012; Khodaeiani *et al*., 2012, 2013; Babaeinejad and Fouladi, 2013) should also be considered in this regard.

CONCLUSION

According to the results of the present study, *Demodex folliculorum* doesn't play a significant role in the pathogenesis of basal cell carcinoma, squamous cell carcinoma and discoid lupus erythematosus. Infection with *Demodex folliculorum* may be associated with melanoma.

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