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The effects of sebum configuration on *Demodex* spp. density

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Background/aim: *Demodex* spp. are ectoparasites living in the pilosebaceous units, which feed on the host's sebum and cellular proteins. The protective barrier of the skin consists of sebum secretion, moisture, and the acid mantle. In this study, we aimed to determine the effects of skin sebum, moisture, pH levels, and sebum configuration on *Demodex* spp. density

Materials and methods: Forty-five patients who had demodicosis were enrolled in the study group, while the control group consisted of 40 subjects without demodicosis. Body fat percentage, serum triglyceride and cholesterol levels, skin sebum, moisture, and pH levels were measured. *Demodex* spp. density was determined with a standardized skin surface biopsy. Sebum samples were taken from the forehead and a high-performance thin-layer chromatography (HPTLC) method was performed on these samples. Subsequently, densitometric analyses were applied to the HPTLC plates.

Results: *Demodex* spp. were found on the cheeks and lived in an alkali environment. Skin sebum and moisture levels were low in all groups. The skin pH levels and cholesterol ester in the sebum configuration were determined to be significantly higher in the group with demodicosis.

Conclusion: We suggest that *Demodex* spp. may use cholesterol ester in the sebum as nutriment. In other words, cholesterol ester may be a suitable growth medium for the proliferation of *Demodex* spp.

Key words: *Demodex* spp., skin sebum, sebum configuration

1. Introduction

Demodex folliculorum and *Demodex brevis* are common ectoparasites of the pilosebaceous units in humans. *D. folliculorum* is usually found in the follicular infundibulum, while *D. brevis* is located in sebaceous ducts and meibomian glands (1–3). *D. folliculorum* is more common than *D. brevis* but *D. brevis* is distributed more extensively. They are frequently found on the face, especially in the nasolabial fold and on the nose, cheeks, forehead, and eyelids. *Demodex* spp. have a tendency to reside in vellus sebaceous follicles that have more sebum-producing cells (3). Demodicosis is a parasitic skin disease caused by these follicle mites (1,4). A standardized skin surface biopsy (SSSB) with a cyanoacrylic adhesive is the most recommended diagnostic tool for measuring the density of *Demodex* mites. Five or more mites counted on a surface area of 1 cm² on lesional skin is considered pathogenic (5–8).

Human sebum is a mixture of nonpolar lipids. It contains triglycerides, diglycerides, wax esters, squalene, fatty acids, cholesterol, and cholesterol esters (9). The skin surface lipid is derived from two sources: the epidermis and the sebaceous glands. Wax esters and squalene originate in the sebaceous glands, while cholesterol and cholesterol esters primarily originate in the epidermis. Triglycerides are derived from both the epidermis and sebaceous glands (10).

Demodex spp. feed on sebum and cellular proteins formed by epithelial and glandular cell destruction through their own enzymatic activity (11). Therefore, the type and the quantities of skin surface lipids may contribute to an increase in the number of *Demodex* mites. In addition, skin sebum, moisture, and pH levels may also affect mite proliferation. Thus, the aim of this study was to investigate the effects of skin sebum, moisture, pH levels, and sebum configuration on *Demodex* spp. density.

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2. Materials and methods

2.1. Subjects

A total of 45 patients with 5 or more *Demodex* spp. per cm² who had demodicosis were chosen as the study group, while the control group consisted of 40 subjects without demodicosis. The study was approved by the local ethics committee and informed consent was obtained from all patients. Subjects who were pregnant or breastfeeding; children; those taking drugs affecting skin sebum, moisture, or pH levels; those who had other dermatological diseases on the face (acne vulgaris, acne rosacea, seborrheic dermatitis), malignancy, or immunosuppression; and individuals who refused to participate in the study were excluded. Serum triacylglycerol (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) were analyzed. Body fat percentage (BFP) was measured with a Tanita Body Composition Analyzer (TBF 300).

2.2. Collection of the sebum samples

Sebum samples were taken from the forehead 3–4 h after cleaning it with wet material. All subjects were in an area with the same room temperature and air humidity. They were warned not to wash their faces or use cosmetics. The samples were collected with n-hexane-soaked cotton-tipped applicators and put into glass tubes containing 1 mL of n-hexane.

2.3. Measurement of skin sebum, moisture, and pH levels

A Sebumeter SM 810, Corneometer CM 825, Skin-pH-Meter pH 900 (Courage & Khazaka Electronic GmbH, Cologne, Germany) combined unit was used for the measurement of skin sebum, moisture, and pH levels. The sebum was measured as µg sebum/cm² in all groups. All of the measurements were performed using three different probes connected to this combined unit and taken from the forehead, right cheek, left cheek, nose, and chin at room temperature and at 40%–60% air humidity.

2.4. Investigation of *Demodex* spp. mites

The SSSB was performed to detect the presence of *Demodex* mites on the forehead, cheeks, nose, and chin. An area of 1 cm² was drawn to one side of the slide. A cyanoacrylate adhesive was put on the other side of the slide and pressed over the area for 1 min. The slide was then lifted gently and immersion oil or glycerol was dropped on the slide, and it was then closed with lamella. This method enabled the upper portion of the stratum corneum and its follicular contents to be collected. Following this, the samples were examined for the presence of parasites under a light microscope at 10× and 40× magnification. Five or more parasites in a 1 cm² area confirmed the presence of demodicosis. No further analysis was performed to differentiate mite species.

2.5. High-performance thin-layer chromatography (HPTLC)

Three different solvent mixtures and a standard lipid mixture were prepared for separation and identification of the lipids. A standard lipid mixture was composed of squalene, cholesteryl oleate, L-phosphatidylcholine, L-α-phosphatidylethanolamine, cholesterol-3-sulfate, galactocerebrosides (type I + type II), N-palmitoyl-D-sphingosine, cholesterol, palmitic acid, and glyceryl trioleate. HPTLC plates were activated in an autoclave at 100 °C for 30 min, and 10–30 µL of sebum samples and 10 µL of standards were applied for each component to 20 × 10 cm-sized HPTLC plates. Six different samples were applied to each plate. The development of the sebum samples occurred in a closed tank (Twin Through Chamber). After the development of each solvent was complete, the plate was removed from the tank and dried under a stream of warm air. The dried plates were sprayed with an aqueous solution of 10% copper(II) sulfate in 8% phosphoric acid and charred at 160 °C in the autoclave. In this way, the lipid bands became visible, as shown in Figure 1.

2.6. Densitometric analysis

All HPTLC plates were scanned with the Desaga Sarstedt Gruppe Densitometer CD 60 at 600 nm. The densitometric density of each lipid class was identified, and the peak heights were used to obtain the results shown in Figure 2. The percentage of each lipid class was calculated by measuring the peak height of each spot.

2.7. Statistical analysis

Statistical analysis was performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The data for quantitative variables were reported as arithmetic means (X) ± standard deviation (SD) and median (min–tandmax), while data for qualitative variables were shown as numbers and percentages. Quantitative variables were determined with a Shapiro–Wilks normality test. An unpaired t-test was used for the variables that showed normal distribution. A one-way analysis of variance and a least significant difference method were used in the independent groups. The variables that did not show normal distribution were determined with a Mann–Whitney U test and Kruskal–Wallis variance analysis. The assessment of qualitative data was performed using Pearson's chi-square test. P < 0.05 was accepted as statistically significant.

3. Results

3.1. Demographic data

The study included 45 patients with demodicosis, consisting of 26 females (57.8%) and 19 males (42.2%), and 40 normal individuals comprising 24 females (60%) and 16 males (40%). The mean age of the patients and controls was 46.5 ± 14.4 years (range: 19–90) and 45.9 ± 13.2 (range: 23–67) years, respectively. The age and sex distributions of



Figure 1. The sebum samples and standards are shown in one of the HPTLC plates.

the groups were not significantly different ($P > 0.05$). The smoking rate was 20% and 15% in the demodicosis group and control group, while the percentage of alcohol use was 6.7% and 7.5% in the demodicosis group and control group, respectively. There was no statistically significant difference in terms of these parameters.

3.2. Biochemical parameters and body fat percentage

LDL-C and TC were high in the patients with demodicosis but only LDL-C was found to be statistically significant, as shown in Table 1. BFP was not significantly different in the demodicosis patients compared to the control group.

3.3. The composition of skin surface lipids detected by HPTLC and densitometric results

The most prominent lipids in the chromatograms obtained by HPTLC were sphingosine + cholesterol (S + C), palmitic acid, glyceryl trioleate, cholesteryl oleate, and squalene. The sphingosine and cholesterol bands were not exactly separated from each other despite several attempts. Therefore, these lipid fractions were considered together (sphingosine + cholesterol). Other lipid standards such as L-phosphatidylcholine, L- α -phosphatidylethanolamine, cholesterol-3-sulfate, and galactocerebrosides (type I + type II), which are members of the phospholipid family, were detected in much smaller amounts and in only a few patients when compared to the other lipids. Therefore, these lipids were not taken into consideration in the sebum lipid configuration. The peak heights of the lipids were compared between the groups, as shown in Table 2. The cholesterol oleate peak was significantly higher in the demodicosis patients when compared to the control group ($P = 0.008$). Other lipid fractions did not show any statistically significant difference.

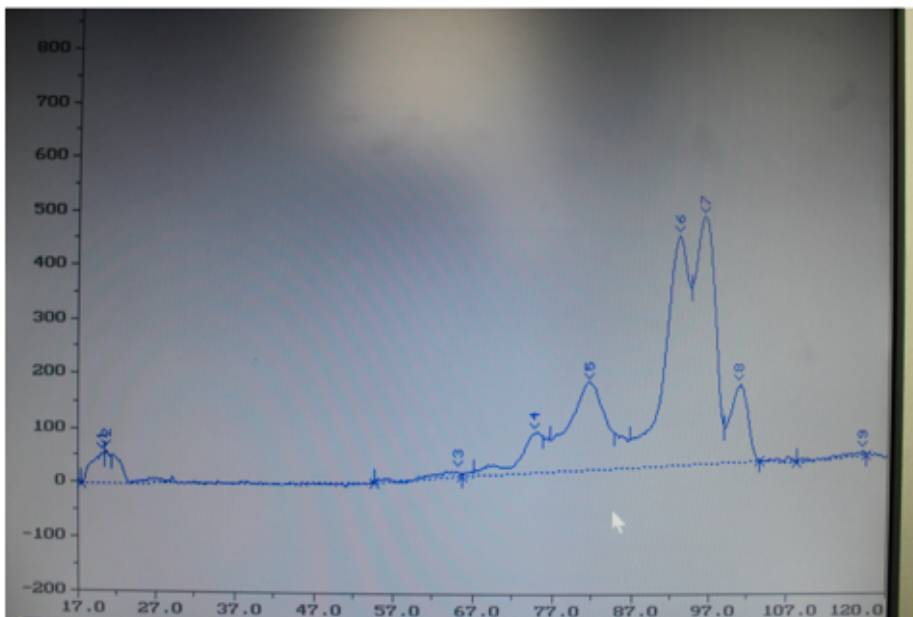


Figure 2. A sample of densitometric analysis in the HPTLC chromatogram of a patient.

Table 1. Biochemical parameters and body fat percentage in the groups.

	Demodicosis n: 45 X ± SD	Control n: 40 X ± SD	P-value
BFP %	28.8 ± 8.7	30.1 ± 9.5	>0.05
TG (mg/dL)	146 ± 95.5	160.0 ± 112.9	>0.05
TC (mg/dL)	190.6 ± 34.9	176.4 ± 33.3	>0.05
HDL-C (mg/dL)	45.0 ± 10.9	42.5 ± 12.2	>0.05
LDL-C (mg/dL)	117.9 ± 31.1	101.8 ± 26.3	0.021
VLDL-C (mg/dL)	29.2 ± 19.1	32.0 ± 22.5	>0.05

Table 2. Comparison of the sebum lipid peaks of the groups.

		Demodicosis n: 45	Control n: 40	P-value
Sphingosine + cholesterol	n X ± SD	31 80.1 ± 37.9	23 93.7 ± 75.9	0.636
	Median (min-max)	79 (20-169)	66 (12-297)	
Palmitic acid	n X ± SD	27 167.2 ± 127.4	32 203.8 ± 197.0	0.373
	Median (min - max)	146 (24-613)	133 (33-877)	
Glyceryl trioleate	n X ± SD	28 271.5 ± 176.3	31 249.4 ± 271.0	0.271
	Median (min-max)	253 (46-726)	137 (18-1133)	
Cholesteryl oleate	n X ± SD	34 338.5 ± 247.1	31 232.1 ± 156.4	0.008
	Median (min-max)	316 (31-1137)	179 (35-655)	
Squalene	n X ± SD	28 168.5 ± 121.2	27 220.2 ± 198.1	0.214
	Median (min-max)	137 (29-526)	196 (44-972)	

The lipid standards were classified according to the lipid classes to which they belonged. Palmitic acid, glyceryl trioleate, and cholesterol oleate represent the free fatty acids (FFAs), triacylglycerol (TG), and cholesterol ester (CE), respectively. Consequently, the percentage of lipid classes were 9% S + C, 20% FFA, 25% TG, 23% CE, and 22% squalene in the control group and 8% S + C, 16% FFA, 26% TG, 33% CE, and 16% squalene in the demodicosis group.

3.4. *Demodex* spp./cm² density of the groups

The density of *Demodex* spp./cm² is shown in Table 3. *Demodex* spp. were mostly found on the cheeks.

3.5. Sebum, moisture, and pH measurements of the groups

Skin types were determined according to sebum, moisture, and pH measurements. The right cheek, left cheek, forehead, and chin were dry, while the nose was normal with regard to skin surface sebum measured by sebumeter

Table 3. Density of *Demodex* spp. on facial areas.

<i>Demodex</i> spp./cm ²		Demodicosis n: 45	Control n: 40
Right cheek	X ± SD Median (min-max)	8.5 ± 7.5 7 (0-40)	0.2 ± 0.6 0 (0-3)
Left cheek	X ± SD Median (min-max)	9.0 ± 9.7 6 (0-60)	0.1 ± 0.4 0 (0-2)
Forehead	X ± SD Median (min-max)	6.8 ± 22.3 2 (0-150)	0 0 (0-0)
Nose	X ± SD Median (min-max)	1.4 ± 4.0 0 (0-25)	0.05 ± 0.31 0 (0-2)
Chin	X ± SD Median (min-max)	2.9 ± 4.7 0 (0-17)	0.07 ± 0.47 0 (0-3)

in the groups. Skin dryness was also detected according to the stratum corneum hydration measured by corneometer. The sebum and moisture quantities in the demodicosis group were lower than that of the control group, but the difference between the groups was not statistically significant ($P > 0.05$). The pH values of the right and left cheek in the demodicosis patients were found to be higher when compared to the control group. This difference was statistically significant ($P < 0.05$). The pH values of the nose, forehead, and chin were normal in both groups.

4. Discussion

Several studies have shown that *Demodex* mite numbers increased in patients with rosacea. The characteristics of the physical barrier on an individual's facial skin may facilitate the increase in *Demodex* mite populations (12). One study showed that patients with papulopustular rosacea had increased facial pH, transepidermal water loss, and reduced skin surface hydration levels. These patients frequently complain of dry and sensitive skin, and they also have abnormal sebaceous fatty acid composition with increased levels of myristic acid and reduced levels of long-chain saturated fatty acids. Palmitic acid and palmitoleic acid were at the highest concentration in the sebaceous fatty acids (13). Our study was performed during spring and summer. Although the air humidity was high because of the effect of hot air, skin dryness was detected in all of the groups. The stratum corneum serves as a barrier to evaporative water loss, and it also contains moisture within its substance. Stratum corneum lipids are very important for epidermal barrier function (14). A

loss of barrier function exists in skin dryness, and some skin disorders may easily occur because of this. This type of facial cutaneous microenvironment may facilitate mite proliferation (13).

The survival of *Demodex* mites has been shown to be influenced by temperature, humidity, pH, medium, and other factors. Zhao et al. concluded that *Demodex* mites survived longest at 16–22 °C and shortest at 36–37 °C, and serum was a suitable medium for the preservation of the mites (15). In our study, the room temperature and air humidity were stable. In terms of pH, it was concluded that the cheeks were more alkaline in the demodicosis group. This finding is consistent with the increased pH in papulopustular rosacea, the etiology of which *Demodex* spp. play a role in (12). The skin has long been known to have an acidic surface that is thought to play a key role in preventing infection. An increased stratum corneum pH could adversely affect permeability barrier homeostasis, stratum corneum integrity, and cohesion and could further exacerbate many skin conditions, resulting in more severe and/or more prolonged clinical manifestations (16). Thus, an increase in the skin pH value may have provided a suitable medium for reproduction of *Demodex* spp. mites although demodicosis may have also increased the skin pH level.

Serum lipid levels were also analyzed in our study. Güldür et al. reported that the relative proportion of wax esters and cholesteryl esters in skin surface lipids of type IV hyperlipoproteinemic (production of large VLDL with abnormally high triglyceride content) male subjects increased as compared to that in normolipoproteinemic

subjects. There was also a slight increase in the sebumetric measurements of hyperlipoproteinemic patients when compared to normolipidemic subjects. As a result, the researchers suggested that plasma triacylglycerol concentration may affect sebum wax ester and cholesteryl ester content (17). We found that TC and LDL-C levels were higher in the demodicosis patients. Therefore, the cholesterol levels possibly contributed to *Demodex* mite reproduction.

Demodex spp. take on different roles depending on host status, changing from commensals or even mutuals to parasites as the host's defenses are altered (12,18). Some reports have shown that diseases like diabetes mellitus, chronic renal failure, and acquired immune deficiency syndrome, all of which cause immunosuppression, may induce an increase in *Demodex* mite proliferation (11,19,20). Akilov et al. reported that the readiness of lymphocytes to undergo apoptosis increases parallel to the increasing density of *Demodex* mites. The number of natural killer cells with Fc receptors also increases with the increasing number of mites. This could be the result of local immunosuppression caused by the mites, which allows them to survive in the host's skin (21). Moreover, immune defense mechanisms may play a protective role by limiting mite numbers, although there are some conflicting results (11). Forton et al. found that patients with demodicosis appeared to be in good health (96%). Only 3 out of 115 (3%) patients had known immune deficiency. In the rare cases when patients have a serious immunodeficiency condition, *Demodex* proliferation is certainly easier, but this study shows that this condition is not essential at all (8). However, in our study, none of our patients had experienced immunodeficiency.

Human sebum is unique when compared to the sebum of other animals. The antioxidant and antibacterial effects, photoprotection, and hydration of the stratum corneum are some of the important functions of human sebum (9,22,23). Sebum is composed of 57% triglycerides, diglycerides, and free fatty acids; 26% wax esters; 12% squalene; and 4.5% cholesterol and its esters (22,23). In our study, the percentage results of the sebum lipid mixture of our groups were not compared to the human sebum lipid profile. One of the reasons for this was that sphingosine and cholesterol bands were not separated from each other, so the percentage of cholesterol appeared larger than normal. The other reason was that wax ester was not provided due to technical inadequacy, which accounts for approximately 26% of human sebum. Since wax ester was not present, the lipid percentage distribution of the groups could not be similar to that of the human sebum profile.

The composition of sebum is also influenced by commensal microorganisms inhabiting the

pilosebaceous apparatus such as *Malassezia* yeast and *Propionibacterium acnes*, which release free fatty acids from sebaceous triglycerides (13). Therefore, the presence of these commensal microorganisms may change sebum components.

Ayres proposed that failure to wash the face and overuse of oily or creamy preparations provide extra lipid nourishment to *Demodex* mites. In addition, this promotes reproduction of mites in large numbers, which plugs the pilosebaceous ducts, leading to a rosacea-like facial eruption (24). Forton et al. reported that the majority of patients with demodicosis (62%) did not use soap to wash their faces (8). Washing with soap and water probably helps fight mite proliferation by mechanical (face cloth) and chemical (soap) actions. This shows the importance of hygiene.

Demodex mites contain lipase enzymes. The presence of lipase may explain the predilection of this mite for the follicular canal and the sebaceous ducts. The lipolytic activity of *Demodex* lipase may cause or aggravate skin conditions through the hydrolysis of triglycerides in the sebum causing release of fatty acids with irritant properties (25). There is no study on the relationship between sebum configuration and *Demodex* spp. in the literature. The majority of the sebum on the forehead is under the influence of the sebaceous glands. Cholesterol is not unique to the sebaceous gland; it is found throughout the body as a component of cellular membranes. Cholesterol, with its esters, is the least abundant lipid in the serum (22,23).

The forehead area has been used as a standard sebum measurement region in studies on sebum configuration in the literature. In our study, sebum samples were obtained only from the forehead region. These sebum samples determined the sebum lipid profile of our groups. However, since *Demodex* spp. show a tendency to exist on the cheeks, we think that the cheek area may be used to obtain sebum samples.

In conclusion, our results show that an increase in CE can be seen in the sebum of demodicosis patients. We suggest that *Demodex* spp. may consume CE in the sebum as a form of nourishment, which means that CE may be a suitable growth medium for the proliferation of *Demodex* spp. We think that further studies involving larger groups are required to obtain more detailed information about this issue.

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