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RESEARCH ARTICLE

The role of skin biophysical properties in *Demodex* mites proliferation: A clinical study on acne, rosacea, seborrheic dermatitis, and blepharitis

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ABSTRACT: This study was conducted to determine how and to what extent differences in skin moisture and pH affect *Demodex* load in patients with acne, rosacea, seborrheic dermatitis, and blepharitis. A total of 318 patients clinically diagnosed with acne (81), rosacea (45), seborrheic dermatitis (22), and blepharitis (170), and found to be *Demodex*-positive (≥ 5), were included in the study. Sample materials were collected from the cheek region of participants using the standard superficial skin biopsy method and examined under a light microscope. Skin moisture and pH measurements were taken from the sampled facial areas and compared with *Demodex* load. It was found that *Demodex* load was significantly higher in patients with dry skin, and that *Demodex* load decreased substantially the higher the skin moisture was. Every 5% increase in skin moisture reduced the *Demodex* load by approximately 35% to 47%. Similarly, *Demodex* load was found to be lower in patients with lower skin pH levels (pH 4.5–5.0) and the *Demodex* load increased significantly when the skin pH was higher. Each 0.5-unit increase in skin pH resulted in an approximately 78% to 115% increase in *Demodex* load. As a result, it was determined that in *Demodex*-positive patients, particularly those diagnosed with rosacea and blepharitis, a lower skin moisture and a higher skin pH were significantly correlated with a higher *Demodex* load. Therefore, considering these biophysical properties of the skin in dermatology and ophthalmology clinics may significantly contribute to the diagnostic and treatment processes.

Keywords: *Demodex*, demodicosis, dermocosmetics, skin moisture, skin pH, skin barrier function

Zoobank: https://zoobank.org/8D580189-5BC7-4304-B3AA-935A67AEA70A

INTRODUCTION

Demodex mites (Acari) belong to the family Demodecidae within the order Trombidiformes (Mumcuoğlu et al., 2023). This family is currently represented by 8 genera and approximately 140 species that live as parasites exclusively on mammals (Tilki et al., 2017a, b; Sarı et al., 2019; Izdebska and Rolbiecki, 2020). There are two species that parasitize humans: *Demodex folliculorum* (Simon), which has a long opisthosoma, lives singly or in groups in hair follicles, while *Demodex brevis* Akbulutova, with a short opisthosoma, generally lives alone in sebaceous glands. *Demodex* mites utilize their specialized cheliceral and palpal structures to mechanically disrupt the epithelial lining of pilosebaceous units, thereby accessing and consuming intracellular components and lipid-rich sebum as nutrient sources (Desch and Nutting, 1972; Mumcuoğlu et al., 2023). *Demodex* mites are photophobic, therefore, they usually perform in the dark their behaviors such as reproduction, feeding and movement. Female and male mites mate at the follicle opening. The fertilized female then proceeds upside down, laying her eggs inside the follicle and into the sebaceous gland. From the egg, larva, protonymph, deutonymph and adult forms are formed respectively. Their average lifespan is about two weeks. Nymphs and adults have four pairs of legs, each with two claws, and can move at an average speed of 8–16 mm per hour (Desch and Nutting, 1977; Rufli and Mumcuoglu, 1981; Mumcuoğlu et al., 2023).

Demodex mites are found worldwide in people of all races and ages, often without causing any clinical symptoms. However, they can act as opportunistic pathogens when the immune system of the host is suppressed and may play a role in the pathogenesis of acne, rosacea, perioral dermatitis, seborrheic dermatitis, blepharitis, dandruff and hyperkeratosis (Rufli and Mumcuoglu, 1981; Sarı et al., 2019; Zeytun and Yazıcı, 2019, 2022, 2024). The damaged caused by *Demodex* mites to the follicular and sebaceous epithelial cells caused by their piercing mouthparts and claws, lead to the disruption of the skin barrier, and later to lymphocytic infiltration around the follicle, and when penetrating the dermis, lead to an immune response against the mite's chitinous exoskeleton (Forton, 2012; Forton et al., 2015; Zeytun and Ölmez, 2017; Zeytun and Karakurt, 2019).

Various dermatological (acne, rosacea, seborrheic dermatitis, pityriasis folliculorum, etc.), ophthalmological (blepharitis, hyperkeratosis and dandruff), other patient groups (diabetes, renal insufficiency, cancer, chronic obstructive pulmonary disease) and in healthy individuals, numerous epidemiological studies have been conducted to determine the prevalence and load of *Demodex mite* (Tilki et al., 2017a; Zeytun, 2017; Zeytun et al., 2017, 2023; Zeytun and Ölmez, 2017; Karakurt and Zeytun, 2018; Sarı et al., 2019; Zeytun and Karakurt, 2019; Zeytun and Yazıcı, 2019, 2022, 2024). However, there are a limited number of ex-vivo and in-vivo studies investigating the effects of biophysical skin factors such as moisture, pH, and

temperature on *Demodex* mite prevalence and load (Chen, 1985; Zhao et al., 2009; Demirdağ et al., 2016; Tilki et al., 2017a; Zeytun, 2017; Zeytun et al., 2017; Sarı et al., 2019). The present study was conducted to determine how and to what extent differences in skin moisture and pH affect the *Demodex* load in patients with acne, rosacea, seborrheic dermatitis, and blepharitis.

MATERIALS AND METHODS

Sample Group. The study was conducted with patients diagnosed clinically with acne, rosacea, seborrheic dermatitis, and blepharitis at the Dermatology and Ophthalmology Clinics of Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital. A total of 318 patients with *Demodex* positivity (≥ 5/cm^2) were included in the study: 81 with acne, 45 with rosacea, 22 with seborrheic dermatitis, and 170 with blepharitis. Patients with dermatological, ophthalmological or systemic diseases other than acne, rosacea, seborrheic dermatitis and blepharitis, patients who have undergone dermatological or ocular surgery or received systemic or topical treatment, and patients determined to be *Demodex* negative (<5/cm2) were excluded from the study. Ethical approval was obtained from the Erzincan University Clinical Research Ethics Committee, and informed consent forms were read and signed by all participants in accordance with the Helsinki Declaration.

Collection of Sample Materials. Samples were obtained from the cheek area of each participant using the Standard Superficial Skin Biopsy (SSSB) method. The facial areas to be sampled were cleaned with alcohol and dried. A clean glass slide was marked with a 1 cm square. On the opposite side of the slide, a drop of cyanoacrylate was placed at the center of the marked area, and the slide was gently pressed onto the designated skin surface. After approximately one minute, the slide was carefully removed. The participant's name and the sampling site were labelled on each sample.

Examination of Sample Materials. A drop of Hoyer's medium was applied onto the sample materials, and a cover slip was placed on the top of it. The preparations were examined under a light microscope (Leica DM500, Switzerland) at magnifications of 4X, 10X, and 40X. If 5 or more *Demodex folliculorum* and/or *Demodex brevis* larvae, nymphs, or adults were observed in the preparation, the sample was considered positive for *Demodex*. The average number of *Demodex* per cm^2 was calculated by dividing the total number of *Demodex* by the number of patients included in the study.

Skin Moisture and pH Measurements. Digital skin moisture meter (DMM, Türkiye) and digital skin pH meter (Hanna HI 14, Romania) devices were used for skin moisture and pH measurements. The measurements were obtained by placing the device probes in contact with the cheek area—specifically where the biopsy sample was taken—for about 30 seconds. To prevent contamination, the device probes were disinfected before each use. Measurements were conducted under normal room conditions, and care was taken to ensure patients had not applied moisturizer before measurements. Skin moisture values were classified as follows: ≤ 30.0 %RH as "dry skin", 30.1-35.0 %RH as "slightly moist skin", 35.1-40.0 %RH as "moderately moist skin" and > 40 %RH as "very moist skin". The skin moisture and pH measurement devices were calibrated periodically according to the criteria specified in their user manuals.

Data Analysis. Statistical analysis of the data was performed using SPSS 23.0 (Statistical Package for Social Sciences) software (Chicago, IL, USA). The normality of the variables was assessed using the Kolmogorov-Smirnov test. For comparisons between groups, the Kruskal-Wallis test, a non-parametric test, was used for data that did not follow a normal distribution. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 318 patients (230 females, 88 males; mean age 42.5) were included in the study: 81 with acne (67 females, 14 males; mean age 20.9), 45 with rosacea (38 females, 7 males; mean age 49.1), 22 with seborrheic dermatitis (16 females, 6 males; mean age 25.2), and 170 with blepharitis (109 females, 61 males; mean age 53.3) (Table 1).

Patients' mean skin moisture, pH, and *Demodex* load are detailed in Table 2. Skin moisture was highest in rosacea patients (mean 34.8 RH%) and lowest in seborrheic dermatitis patients (mean 30.9 RH%), while *Demodex* load was highest in rosacea patients (mean 103.6/cm^2) and lowest in acne patients (mean 21.7/cm^2). When the patient groups were compared in terms of *Demodex* load, the difference was found to be statistically significant ($p < 0.001$).

The patients' average skin moisture and the average *Demodex* load at those moisture levels are shown in Table 3. Among the 318 patients included in the study, 28.6% had "dry skin", 35.5% had "slightly moist skin", 20.5% had "moderately moist skin" and 15.4% had "very moist skin". It was determined that the *Demodex* load was significantly higher in patients with dry skin, and that it decreased significantly with increasing skin moisture. Each 5% increase in skin moisture reduced the *Demodex* load by an average of 35% to 47%. When patients were compared in terms of skin moisture and *Demodex* load at those moisture levels, the difference was statistically significant ($p < 0.001$).

The patients' average skin pH and the average *Demodex* load at those pH values are detailed in Table 4. A lower *Demodex* load was observed in patients with lower skin pH levels (pH 4.5-5.0), whereas a significant increase was noted with rising pH levels. Each 0.5 increase in skin pH was determined to increase the *Demodex* load by an average of 78% to 115%. When patients were compared in terms of skin pH and *Demodex* load at those pH values, the difference was statistically significant ($p < 0.001$).

DISCUSSION

Our study found that *Demodex* load was significantly higher in patients with dry skin than in those with higher skin moisture levels.

Table1. Age and sex of patients.

		Age (years)		Sex	
	n	**Mean ± SD**	**Median (min – max)**	**Female**	**Male**
Acne	81/318	20.9 ± 4.8	20 (12 – 39)	67/81	14/8
Rosacea	45/318	49.1 ± 15.4	50 (23 – 83)	38/45	7/45
Seborrheic dermatitis	22/318	25.2 ± 9.8	23 (12 – 48)	16/22	6/22
Blepharitis	170/318	53.3 ± 16.0	55 (11 – 86)	109/170	61/170
All patients	318/318	42.5 ± 19.7	42 (11 – 86)	230/230	88/88

SD: standard deviation; **min:** minimum; **max:** maximum

Table 2. Load of *Demodex* mites in patients.

	n	**Mean skin moisture (%RH) [median (min – max)]**	**Mean skin pH [median (min – max)]**	**Mean load of *Demodex* mites/cm² [median (min – max)]**	**P** *
Acne	81/318	33.6 (CI: 32.1 – 35.1) 32.7 (21.5 – 55.6)	5.5 (CI: 5.4 – 5.6) 5.5 (4.5 – 6.5)	21.7 (CI: 14.1 – 29.3) 12 (5 – 215)	0.000
Rosacea	45/318	34.8 (CI: 32.2 – 37.5) 31.3 (20.3 – 63.2)	5.6 (CI: 5.5 – 5.7) 5.75 (4.6 – 6.5)	103.6 (CI: 67.6 – 139.5) 83 (5 – 705)	
Seborrheic dermatitis	22/318	30.9 (CI: 28.8 – 33.0) 30.4 (25.4 – 41.5)	5.4 (CI: 5.3 – 5.6) 5.4 (4.9 – 6.3)	43.4 (CI: 17.9 – 68.9) 27 (5 – 238)	
Blepharitis	170/318	34.5 (CI: 33.5 – 35.4) 33.0 (22.4 – 66.2)	5.4 (CI: 5.3 – 5.5) 5.4 (4.5 – 6.5)	49.2 (CI: 37.0 – 61.5) 20 (5 – 625)	
All patients	318/318	34.0 (CI: 33.3 – 34.8) 32.7 (20.3 – 66.2)	5.5 (CI: 5.4 – 5.5) 5.5 (4.5 – 6.5)	49.5 (CI: 40.5 – 58.5) 18 (5 – 705)	

min: minimum; **max:** maximum**; CI:** 95% confidence interval

* Kruskal-Wallis test

Each 5% increase in skin moisture reduced the *Demodex* load by an average of 35% to 47%. These findings are consistent with our previous studies, which showed higher *Demodex* load in healthy individuals with low skin moisture (Zeytun et al., 2017; Tilki et al., 2017a; Zeytun, 2017; Sarı et al., 2019). Similarly, a study conducted by Turan et al. (2017) reported a higher *Demodex* load in patients with acne vulgaris and rosacea who had low skin moisture levels. In another study, it was reported that patients diagnosed with demodicosis had lower amounts of sebum and skin moisture (Demirdağ et al., 2016). Ni-Raghallaigh et al. (2012) found that patients with papulopustular rosacea generally complained of dry and sensitive skin, showed increased transepidermal water loss, and had decreased skin moisture levels. The same study also indicated that sebaceous fatty acids play a role in maintaining the integrity of the skin barrier that patients with papulopustular rosacea had an abnormal fatty acid composition, and that levels of long-chain saturated fatty acids were reduced. Sebum-modifying treatments were suggested for these patients. Since sebum is the primary nutrient for *Demodex* mites, changes occurring in the sebaceous microenvironment may have an influence on the *Demodex* load. Moreover, the stratum corneum (SC), the outermost layer of the epidermis, acts as a natural barrier that prevents transepidermal water loss and helps maintain the skin's moisture balance. When the integrity of this barrier is compromised, water loss increases and skin dryness occurs. This dryness reduces the protective function of the SC, facilitating the colonization of microorganisms, particularly *Demodex* mites, on the skin surface (Yosipovitch et al., 1998; Zeytun, 2017).

The SC also typically has an acidic pH level ranging between 4 and 6, which serves as another protective feature preventing the entry of microorganisms into the skin. Demirdağ et al. (2016) reported significantly higher skin pH levels in patients with demodicosis and noted that increased skin pH adversely affects the water permeability and barrier functions of the SC. Sarangua et al. (2013)

found that healthy individuals positive for *Demodex* had higher skin pH compared to those who were negative. An ex-vivo study reported that *Demodex* counts were higher in environments with near-neutral pH compared to acidic environments (Chen, 1985). Similarly, in our study, *Demodex* load was lower in patients with low skin pH (pH 4.5–5.0), and *Demodex* load significantly increased the higher the skin pH was. Each 0.5 unit increase in skin pH was found to increase *Demodex* load by an average of 78% to 115%. These findings are consistent with our previous studies conducted on healthy individuals (Zeytun et al., 2017; Tilki et al., 2017a; Zeytun, 2017; Sarı et al., 2019).

In our study, a lower skin moisture and an higher skin pH were identified as skin biophysical factors that increase *Demodex* load.

Table 3. The mean load of *Demodex* mites and the mean skin moisture values in patients.

		Skin moisture (RH%)						
	n	**≤ 30.0 (DS)**	**n**	**30.1 – 35.0 (SMS)**	**n**	**35.1 – 40.0 (MMS)**	**n**	**> 40.0 (VMS)**	**P ***
Acne	28/91		25/113		15/65		13/49		
Mean skin moisture (%RH)		27.1 (CI: 26.1 – 28.1)		32.5 (CI: 31.9 – 33.0)		37.4 (CI: 36.6 – 38.5)		45.2 (CI: 42.0 – 48.4)	0.000
Mean load of *Demodex* mites/cm²		34.1 (CI: 13.7 – 54.5)		21.8 (CI: 13.2 – 30.4)		11.5 (CI: 7.2 – 15.8)		6.8 (CI: 5.5 – 8.1)	0.000
Rosacea	15/91		11/113		10/65		9/49		
Mean skin moisture (%RH)		27.3 (CI: 25.8 – 28.7)		31.4 (CI: 30.7 – 32.2)		37.9 (CI: 36.7 – 39.1)		48.2 (CI: 41.7 – 54.7)	0.000
Mean load of *Demodex* mites/cm²		172.6 (CI: 81.9 – 263.3)		102.8 (CI: 38.7 – 167.0)		59.7 (CI: 23.0 – 96.5)		38.1 (CI: 12.2 – 64.0)	0.001
Seborrheic dermatitis	10/91		8/113		2/65		2/49		
Mean skin moisture (%RH)		26.9 (CI: 25.8 – 28.0)		32.0 (CI: 30.9 – 33.1)		36.4 (CI: 35.2 – 37.5)		41.3 (CI: 41.0 – 41.5)	0.001
Mean load of *Demodex* mites/cm²		67.7 (CI: 11.9 – 123.5)		30.3 (CI: 14.5 – 46.0)		12.0 (CI: 7.0 – 17.0)		6.0 (CI: 5.0 – 7.0)	0.048
Blepharitis	38/91		69/113		38/65		25/49		
Mean skin moisture (%RH)		27.9 (CI: 27.2 – 28.6)		32.3 (CI: 32.0 – 32.6)		37.3 (CI: 36.8 – 37.8)		46.2 (CI: 43.4 – 48.9)	0.000
Mean load of *Demodex* mites/cm²		95.7 (CI: 50.8 – 140.5)		47.3 (CI: 32.4 – 62.1)		27.0 (CI: 18.0 – 36.1)		17.7 (CI: 12.7 – 22.6)	0.001
All patients	91/91		113/113		65/65		49/49		
Mean skin moisture (%RH)		27.4 (CI: 27.0 – 27.9)		32.2 (CI: 32.0 – 32.5)		37.4 (CI: 37.0 – 37.8)		46.1 (CI: 44.2 – 47.9)	0.000
Mean load of *Demodex* mites/cm²		86.3 (CI: 60.5 – 112.2)		45.8 (CI: 34.5 – 57.2)		28.0 (CI: 19.9 – 36.0)		18.1 (CI: 12.5 – 23.7)	0.000

CI: 95% confidence interval; **RH:** Relative humidity; **DS:** Dry skin; **SMS:** Slightly moist skin; **MMS:** Moderately moist skin; **VMS:** Very moist skin

* Kruskal-Wallis test

Table 4. The mean load of *Demodex* mites and the mean skin pH values in patients.

	Skin pH								
	n	**4.5 – 5.0**	**n**	**5.1 – 5.5**	**n**	**5.6 – 6.0**	**n**	**6.1 – 6.5**	**P** *
Acne	11/56		30/121		29/108		11/33		
Mean pH		4.8 (CI: 4.6 – 4.9)		5.3 (CI: 5.3 – 5.4)		5.7 (CI: 5.7 – 5.8)		6.2 (CI: 6.1 – 6.3)	0.000
Mean load of *Demodex* mites/cm²		8.2 (CI: 5.4 – 10.1)		12.5 (CI: 9.4 – 15.7)		23.0 (CI: 11.9 – 34.2)		56.9 (CI: 9.3 – 104.6)	0.001
Rosacea	5/56		12/121		21/108		7/33		
Mean pH		4.8 (CI: 4.6 – 5.0)		5.3 (CI: 5.3 – 5.4)		5.7 (CI: 5.7 – 5.8)		6.3 (CI: 6.2 – 6.4)	0.000
Mean load of *Demodex* mites/cm²		17.8 (CI: 4.1 – 39.7)		55.3 (CI: 31.7 – 79.0)		111.8 (CI: 77.2 – 146.3)		222.9 (CI: 5.5 – 440.3)	0.001
Seborrheic dermatitis	3/56		12/121		5/10		2/33		
Mean pH		4.9 (CI: 4.8 – 5.1)		5.3 (CI: 5.2 – 5.4)		5.7 (CI: 5.6 – 5.8)		6.2 (CI: 6.1 – 6.3)	0.001
Mean load of *Demodex* mites/cm²		12.3 (CI: 7.0 – 13.0)		28.8 (CI: 17.6 – 39.9)		66.0 (CI: 18.6 – 150.4)		121.5 (CI: 5.0 – 238.0)	0.256
Blepharitis	37/56		67/112		53/108		13/33		
Mean pH		4.7 (CI: 4.6 – 4.8)		5.3 (CI: 5.2 – 5.3)		5.7 (CI: 5.7 – 5.8)		6.3 (CI: 6.2 – 6.3)	0.000
Mean load of *Demodex* mites/cm²		19.8 (CI: 13.6 – 26.0)		34.0 (CI: 25.5 – 42.5)		69.5 (CI: 44.4 – 94.5)		129.0 (CI: 14.3 – 243.5)	0.010
All patients	56/56		121/112		108/108		33/33		
Mean pH		4.8 (CI: 4.7 – 4.8)		5.3 (CI: 5.2 – 5.3)		5.7 (CI: 5.7 – 5.8)		6.2 (CI: 6.2 – 6.3)	0.000
Mean load of *Demodex* mites/cm²		17.0 (CI: 12.5 – 21.4)		30.3 (CI: 24.6 – 36.0)		65.1 (CI: 49.8 – 80.3)		124.4 (CI: 63.0 – 185.8)	0.000

CI: 95% confidence interval

* Kruskal-Wallis test

However, when the different patient groups included in the study were compared in terms of average *Demodex* load, it was found to be in the order from highest to lowest regarding rosacea (103.6/cm^2), blepharitis (49.2/cm^2), seborrheic dermatitis (43.4/cm^2), and acne (21.7/cm^2). When the patient groups were compared in terms of average skin moisture and pH, it was determined that the resulting order differed from the order formed according to *Demodex* load. The difference in the ranking of patient groups in terms of average *Demodex* load, average skin moisture and pH suggests that there may be other factors affecting *Demodex* load besides skin moisture and pH.

HLA (human leukocyte antigen) haplotypes, T and B lymphocytes, and NK (natural killer) cells play an important role in the development of the immune response. In studies investigating the relationship between demodicosis and HLA, it has been emphasized that the HLA-A2 haplotype is protective against demodicosis, and individuals with this phenotype are three times more resistant to demodicosis. Conversely, individuals with the HLA-CW2 and HLA-CW4 haplotypes have been reported to be five times more susceptible to

developing demodicosis, with increased *Demodex* load attributed to enhanced apoptosis (programmed cell death) of lymphocytes and NK cells in these individuals (Akilov and Mumcuoglu, 2003, 2004; Mumcuoglu and Akilov, 2005). Therefore, the discrepancy between the mean *Demodex* load ranking and the mean skin moisture and pH rankings among the patient groups in our study may be due to genetic differences among the participants and variations in their HLA haplotypes, and further studies are needed on this subject.

CONCLUSION

Our study showed that decreased skin moisture and increased skin pH lead to an increased *Demodex* load, particularly in *Demodex*-positive patients diagnosed with rosacea and blepharitis. Therefore, considering these biophysical properties of the skin in dermatology and ophthalmology may significantly contribute to diagnostic and therapeutic processes. Additionally, the use of dermocosmetic products that maintain the skin's acidic pH and moisture balance to support skin barrier function is believed to be beneficial. However, further studies are needed to confirm whether these topical treatments affect *Demodex* proliferation.

Authors' contributions

Erhan Zeytun: Project manager, laboratory works (collection of samples, preparation of specimens, microscopic examinations, identification of mites, etc.), writing - review & editing, methodology, investigation, visualization, formal analysis, statistics. **Mustafa Yazıcı** and **Yücel Karakurt:** Project administration, clinical examination, selection of patients and controls, formal analysis, writing - review & editing, methodology, investigation.

Statement of ethics approval

Ethical approval was obtained from the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (Decision no: 2016-08/07).

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Conflict of interest

The authors declared that there is no conflict of interest.

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