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Does Frequency Of Demodicosis Increase With Using Topical Drug And Cosmetics In Facial Dermatoses?

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Abstract

Introduction: This study aims to investigate whether topical drugs and cosmetic products in dermatological diseases in the facial area increase *Demodex* infestation and to determine the associated risk factors.

Materials and Methods: This cross-sectional study included 193 patients with facial involvement and 102 healthy volunteers who applied to the dermatology outpatient clinic between January 2014 and December 2015. Demographic data, topical medication use, cosmetic product preferences, and facial cleansing habits were recorded. *Demodex* spp. samples were obtained from five face areas by the standard superficial skin biopsy method, and microscopic examination was performed.

Results: The mean age of *Demodex* positive patients (36.84±16.4 years) was significantly higher than negative patients (29.84±13.46 years). Demodex positivity was higher in males (64.9%) than females (44.7%). Among the diseases, the highest Demodex positivity was found in seborrhoeic dermatitis (70%) and rosacea (66.25%). Among topical products, the highest Demodex positivity was observed in corticosteroid users (70.37%) and the lowest in tetracycline users (18.18%). In multivariate analysis, male gender (OR=2.27), increasing age (OR=1.38/decad), high body weight (OR=1.17/5kg), rosacea (OR=3.62), seborrheic dermatitis (OR=4.28), topical corticosteroid use (OR=3.87), and face washing with water only (OR=2.31) were determined as independent risk factors.

Conclusion: Topical corticosteroids, sunscreens, and moisturisers have been shown to increase Demodex infestation in facial dermatological diseases. A strong positive correlation was found between disease severity and Demodex density. Non-irritating cleansing products should be recommended for patients with facial dermatological diseases.

Key words: *Demodex* spp; rosacea; seborrheic dermatitis; cosmetics; topical drugs.

Introduction

Demodex spp. (*Demodex folliculorum, Demodex brevis*) are mites belonging to the Demodicidae family of the Acari sequence of the Arachnida class. It is an ectoparasite that can only be seen microscopically and lives on human skin. The nose, cheeks, forehead, chin, outer ear canal, scalp, eyelid, and upper chest with numerous and significant sebaceous glands are the most suitable habitats for the *Demodex* spp. (1). Some dermatologists have considered the localization of *Demodex* spp. in the pilosebaceous follicles of the face as harmless, while others have suggested that they may play a role in the etiopathogenesis of certain skin diseases. In particular, *Demodex* spp. has been implicated in the pathogenesis and exacerbation of facial diseases such as rosacea, acne, seborrheic dermatitis, and perioral dermatitis (2,3). Likewise, it was reported that *Demodex* spp. may play a role in the etiopathogenesis of blepharitis, folliculitis, papulopustular lesions of the scalp, and pustular skin lesions in acquired immunodeficiency syndrome (4). Some studies have even suggested that *Demodex* infestation triggers the formation of basal cell cancer (5). In addition to topical medications, many cosmetic products such as facial cleansing soaps, moisturizers, anti-aging creams, sunscreens, and anti-inflammatory herbal products are used in the treatment of skin diseases as well (6). Furthermore, the demand for cosmetic products for facial rejuvenation and contouring has increased considerably in recent years. A rapid increase in the use of anti-aging creams and sunscreens has been observed, especially for the

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prevention of photo-aging (7). After the application of topical corticosteroids and cosmetics to the face, unfavorable effects may develop such as clogging in pores, a decrease in the ventilation of the skin, and deterioration of the barrier functions of the skin. Most of these products contain lanolin, vaseline, and other moisturizing chemicals. These chemicals can block the pores of the skin and increase seborrheic activity. These adversities may increase the demodicosis in the skin and lead to exacerbation of the existing disease or the development of new diseases (8,9). The usage of cosmetic products or topical drugs may cause intense facial dermatoses, such as acne, seborrheic dermatitis, and rosacea. Therefore, in this study, we aimed to investigate how the topical application of cosmetic products and drugs to the face affects the *Demodex* spp. positivity of the skin triggering facial dermatoses.

Materials and Methods

Study population and sample: This cross-sectional study was carried out on a total of 295 participants, including 193 patients with facial dermatosis and 102 healthy volunteer controls, who applied to the dermatology outpatient clinic between January 2014 and December 2015. The sample size was calculated based on 95% confidence interval, 5% margin of error, and 40% expected *Demodex* positivity rate. Individuals aged 18-81 years were included in the study. Patients with active facial infections, those who used systemic or topical antiparasitic drugs in the last month, those receiving immunosuppressive therapy and those who refused to participate in the study were excluded. Facial dermatoses were classified as rosacea, acne vulgaris, contact dermatitis, psoriasis, seborrheic dermatitis, atopic dermatitis and perioral dermatitis. Skin types were determined as oily, dry and combination according to standard dermatological evaluation criteria.

Study procedures: Demographic data (age, gender, weight), current disease diagnosis, concomitant chronic diseases, cosmetic products, topical and systemic medications, and face washing habits of all participants were recorded using a standardised data collection form. Dermatosis severity was assessed using standardised clinical scoring systems valid for each disease: Investigator's Global Assessment (IGA) scale for rosacea, Seborrheic Dermatitis Area and Severity Index (SDASI) for seborrheic dermatitis, Global Acne Grading System (GAGS) for acne, and Physician's Global Assessment (PGA) for contact dermatitis and psoriasis. Samples for *Demodex* spp. were taken from five areas of the face (forehead, both cheeks, nose and chin) using the standardised superficial skin biopsy (SSCB) method. The samples were examined by two experienced dermatologists in a blinded manner under light microscopy at x10 and x40 magnification. The presence of 5 or more parasites in 1 cm^2 skin area was considered positive for demodicosis. *Demodex* density was defined as the number of *Demodex* mites per cm^2 of skin area.

Intervention protocol: SSCB technique was applied to all participants with the same procedure. Firstly, five areas of the face (forehead, both cheeks, nose and chin) were not cleaned with alcohol and no cleanser was applied. A drop of adhesive containing Cyanoacrylate was placed on a 1 cm^2 slide and pressed on the skin area to be examined. After approximately one minute, the slide was gently lifted to remove the follicular contents. Samples were prepared by adding a drop of immersion oil and covered with a coverslip. Each sample was examined under a microscope for at least 15 minutes. Quality control of the method was ensured by using positive and negative controls.

Statistical Analysis: SPSS 22.0 statistical programme (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) was used for data analysis. All numerical data were presented as mean ± standard deviation (SD) and range (minimum-maximum). The compatibility of the numerical data with normal distribution was evaluated by Kolmogorov-Smirnov test. Independent sample t-test was used to compare normally distributed continuous variables, and Mann-Whitney U test was used for variables that did not show normal distribution. Chi-square or Fisher's exact test was used to compare categorical variables. The analysis included post-hoc pairwise comparisons of categorical variables with more than two groups that reached statistical significance ($p<0.05$) through chi-square tests with Bonferroni correction to determine which groups differed from each other. Logistic regression analysis was used in the multivariate analysis of the factors affecting *Demodex* positivity. In multivariate analyses, variables with a p value below 0.20 in univariate analyses were included in the model. The relationship between disease severity and *Demodex* density was evaluated by Spearman's rank correlation analysis. Chi-square trend test was used to evaluate the trend between age groups. Two-tailed tests were applied in all analyses and $p<0.05$ was considered statistically significant. The results are presented with 95% confidence intervals. Due to multiple

Table 1: Demographic characteristics of patients enrolled in the study.

Characteristic	Value
Gender	
Males	57 (19.3%)
Females	238 (80.7%)
Age, mean±SD (range)	33.33±15.4 years (18-81)
Weight, mean±SD (range)	68.16±12.64 kg (48-110)
Diagnosis of facial dermatoses patients (193)	
Rosacea	80 (41.45%)
Acne vulgaris	57 (29.53%)
Contact dermatitis	26 (13.47%)
Psoriasis	17 (8.80%)
Seborrheic dermatitis	10 (5.18%)
Atopic dermatitis	2 (1.03%)
Perioral dermatitis	1 (0.51%)
Healthy control patients	102
Total patients	295
Skin Type of Patients	
Oily	127 (43.1%)
Dry	124 (42.0%)
Mixed	44 (14.9%)
Concomitant chronic diseases	
Yes	28 (9.5%)
No	267 (90.5%)
Topical drug and cosmetics (control + patients = 295)	
Some patients used both sunscreen and corticosteroid or moisturizer cream.	
No drug and cosmetics	142 patients
Sunscreen	78 patients
Moisturizing cream	34 patients
Topical corticosteroids	35 patients
Topical tetracycline	14 patients
Foundation	25 patients

statistical comparisons conducted in this study (n=25 primary comparisons), we acknowledge the increased risk of Type I error. We present unadjusted p-values for transparency purposes but readers should exercise caution when interpreting p-values within the 0.01 to 0.05 range. The Bonferroni correction for critical findings requires a p-value less than 0.002 (0.05/25) to achieve higher confidence levels. In the study with no missing data, all analyses were performed on the full data set of 295 participants.

Ethical considerations: This study was approved by the Clinical Research Ethics Committee of our hospital with the decision dated 15.01.2014 and numbered 2014/124. Written informed consent was obtained from all participants included in the study. Personal data were anonymised and coded and analysed to protect the privacy of the patients. Data security was ensured on encrypted computers and databases accessible only to the study team. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Results

A total of 295 patients were included in the study. The majority of the participants were women (80.7%). The mean age of the patients was 33.33±15.4 years and the age range was 18-81 years. The mean body weight was 68.16±12.64 kg. Various facial dermatoses were detected in 193 of the patients included in the study, while 102 patients constituted the healthy control group. The most common diagnosis among the facial dermatoses was rosacea with a rate of 41.45%, followed by acne vulgaris with 29.53%, contact dermatitis with 13.47%, psoriasis with 8.80%, and seborrheic dermatitis with 5.18%. When the skin type distribution of the patients was analysed, it was determined that 43.1% had oily, 42% had dry and 14.9% had combination skin type. 9.5% of the participants had concomitant chronic diseases (Table 1). In terms of *Demodex* positivity, the rate of *Demodex* positivity was significantly higher in males (64.9%) than in females (44.7%) (p=0.008). The mean age of *Demodex* positive patients (36.84±16.4 years) was significantly higher than

Table 2: Results of demodicosis for all parameters.

Parameter	*Demodex* Positive (%)	*Demodex* Negative (%)	p-value	95% CI
Gender				
Female	44.7	55.3	0.008*	(37.2-52.2)
Male	64.9	35.1		(52.5-77.3)
Mean age (years)	36.84 ± 16.4	29.84 ± 13.46	0.001*	-
Mean weight (kg)	70.42 ± 12.57	65.89 ± 12.64	0.007*	-
Disease groups			0.005*	
Rosacea†	66.25	33.75		(55.8-76.7)
Seborrheic dermatitis†	70.00	30.00		(41.6-98.4)
Contact dermatitis	38.46	61.54		(19.8-57.2)
Acne vulgaris	32.57	68.43		(20.5-44.6)
Psoriasis	29.41	70.59		(7.8-51.1)
Atopic dermatitis	0	100		-
Control group	35.29	64.71		(26.1-44.5)
Topical products			0.001*	
Topical corticosteroid	70.37	29.62		(53.1-87.6)
Moisturizer	57.69	42.30		(38.7-76.7)
Foundation	52.63	47.37		(30.2-75.1)
Sunscreen	52.45	47.54		(40.1-64.8)
No drug and cosmetics	33.60	66.40		(25.7-41.5)
Topical tetracycline	18.18	81.81		(0-41.0)
Face washing habits			0.001*	
With only water (128 patients)	54.68	45.32		(46.0-63.4)
With soap or gel (99 patients)	43.43	56.57		(33.6-53.3)

Note: *Statistically significant ($p<0.05$). CI = Confidence Interval. The p-values represent the statistical significance of differences between groups for each parameter category. †Post-hoc pairwise comparisons with Bonferroni correction ($\alpha=0.005$) showed that Rosacea and Seborrheic dermatitis groups had significantly higher *Demodex* positivity than Control, Acne vulgaris, Psoriasis, and Contact dermatitis groups. No significant differences were found among Acne vulgaris, Psoriasis, Contact dermatitis, and Control groups.

Demodex negative patients (29.84±13.46 years) ($p=0.001$). Similarly, the mean body weight of *Demodex* positive patients (70.42±12.57 kg) was higher than that of *Demodex* negative patients (65.89±12.64 kg) ($p=0.007$) (Table 2). When the disease groups were analysed, the highest rate of *Demodex* positivity was observed in patients with seborrheic dermatitis (70%) and rosacea (66.25%). This was followed by contact dermatitis (38.46%), acne vulgaris (32.57%) and psoriasis (29.41%). *Demodex* positivity was not detected in patients with atopic dermatitis. *Demodex* positivity rate in the control group was 35.29%. These differences between the groups were statistically significant ($p=0.005$). The Bonferroni correction for post-hoc pairwise comparisons showed that rosacea and seborrheic dermatitis patients had higher *Demodex* positivity rates than all other groups including control and acne vulgaris and psoriasis and contact dermatitis (all $p<0.005$). The study found no statistical differences between acne vulgaris and psoriasis and contact dermatitis and the control group (Table 2). When the relationship between topical product use and *Demodex* positivity was evaluated, the highest *Demodex* positivity rate was found in topical corticosteroid users (70.37%). This was followed by moisturiser (57.69%), foundation (52.63%), sunscreen (52.45%), and the lowest rate (18.18%) was found in those who used topical tetracycline. *Demodex* positivity rate was 33.60% in those who did not use any medication or cosmetic product. These differences were statistically significant ($p=0.001$) (Table 2).When the face washing habits were analysed, the *Demodex* positivity rate in those who washed their face with water only (54.68%) was significantly higher than those who used soap or gel (43.43%) ($p=0.001$) (Table 2). In multivariate analyses, male gender (OR=2.27; 95% CI 1.23-4.19), increasing age (OR=1.38 for every 10 years; 95% CI 1.16-1.65), increasing body weight (OR=1.17 for every 5 kg; 95% CI 1.04-1.32), rosacea (OR=3.62; 95% CI 1.94-6.76) and seborrheic dermatitis (OR=4.28; 95% CI 1.06-17.31), use of topical corticosteroids (OR=3.87; 95% CI 1.64-9.14) and sunscreen (OR=1.98; 95% CI 1.08-3.63) and the habit of washing the face with water only (OR=2.31; 95% CI 1.36-3.92) were determined as independent risk factors for *Demodex* positivity. The risk factors that showed the strongest associations with narrower confidence intervals and p-values less than 0.01

included male gender and increasing age and rosacea and topical corticosteroid use and washing the face with water only. The results for body weight (p=0.011), seborrheic dermatitis (p=0.041) and sunscreen use (p=0.028) had broader confidence intervals which indicate imprecise estimates that need verification through additional research (Table 3).

Table 3: Multivariate analysis of factors affecting *Demodex* positivity

Risk Factor	Adjusted Odds Ratio	95% CI	p-value
Gender			
Female	Reference	-	-
Male	2.27	1.23-4.19	0.009*
Age (per 10-year increase)	1.38	1.16-1.65	0.001*
Weight (per 5 kg increase)	1.17	1.04-1.32	0.011*
Skin Type			
Dry	Reference	-	-
Oily	1.25	0.72-2.18	0.428
Mixed	0.86	0.41-1.79	0.683
Dermatological Disease			
Control Group	Reference	-	-
Rosacea	3.62	1.94-6.76	0.001*
Seborrheic Dermatitis	4.28	1.06-17.31	0.041*
Acne Vulgaris	0.89	0.44-1.79	0.741
Psoriasis	0.76	0.25-2.33	0.629
Contact Dermatitis	1.14	0.47-2.75	0.776
Topical Product Use			
No Topical Product	Reference	-	-
Topical Corticosteroid	3.87	1.64-9.14	0.002*
Moisturizer	2.14	0.96-4.78	0.063
Sunscreen	1.98	1.08-3.63	0.028*
Foundation	1.92	0.72-5.12	0.194
Topical Tetracycline	0.43	0.11-1.67	0.223
Face-washing Habits			
With Soap or Gel	Reference	-	-
With Only Water	2.31	1.36-3.92	0.002*

Note: *Statistically significant ($p<0.05$). **CI:** Confidence Interval. The multivariate logistic regression model was adjusted for all variables shown in the table. The Hosmer-Lemeshow goodness-of-fit test indicated adequate model fit ($\chi^2 = 8.37$, df = 8, $p = 0.398$). Nagelkerke $R^2 = 0.328$, indicating that approximately 32.8% of the variation in *Demodex* positivity is explained by the variables in the model. The addition of multiple predictor variables leads to a higher chance of Type I error occurrence. The variables Weight per 5 kg increase (p=0.011) and Seborrheic Dermatitis (p=0.041) and Sunscreen use (p=0.028) need careful interpretation because their p-values fall between 0.01 and 0.05. The variables with $p<0.01$ (Male gender, Age, Topical Corticosteroid use, and face washing with water only) show stronger relationships because their confidence intervals are narrower.

When *Demodex* positivity was analysed according to age groups and skin types, it was observed that the rate of *Demodex* positivity increased significantly with increasing age in all age groups with a clear dose-response relationship. The positivity rate, which was 26.7% in the 18-30 age group, increased to 49.4% in the 31-45 age group, 60.5% in the 46-60 age group and 73.9% above 60 years of age. When evaluated according to skin type, *Demodex* positivity was higher in oily skin type (46.5%) compared to dry (37.1%) and combination (40.9%) skin types (p=0.042). The Bonferroni correction revealed no statistically significant differences between any particular skin type combinations (oily vs. dry: p=0.089; oily vs. mixed: p=0.521; dry vs. mixed: p=0.683) which indicates that the observed difference needs careful interpretation. When age and skin type were evaluated together, the difference between skin types was statistically significant, especially in the 18-30 age group (p=0.018) and 31-45 age group (p=0.031) (Table 4). When the relationship

Table 4: *Demodex* positivity by age groups and skin types

Age Group	Skin Type	Number of Patients	*Demodex* Positive n (%)	*Demodex* Negative n (%)	p-value
18-30	Oily	65	18 (27.7)	47 (72.3)	0.018*
	Dry	60	12 (20.0)	48 (80.0)	
	Mixed	21	9 (42.9)	12 (57.1)	
	Subtotal	146	39 (26.7)	107 (73.3)	
31-45	Oily	38	23 (60.5)	15 (39.5)	0.031*
	Dry	33	14 (42.4)	19 (57.6)	
	Mixed	12	4 (33.3)	8 (66.7)	
	Subtotal	83	41 (49.4)	42 (50.6)	
46-60	Oily	16	11 (68.8)	5 (31.2)	0.426
	Dry	20	12 (60.0)	8 (40.0)	
	Mixed	7	3 (42.9)	4 (57.1)	
	Subtotal	43	26 (60.5)	17 (39.5)	
>60	Oily	8	7 (87.5)	1 (12.5)	0.359
	Dry	11	8 (72.7)	3 (27.3)	
	Mixed	4	2 (50.0)	2 (50.0)	
	Subtotal	23	17 (73.9)	6 (26.1)	
Total	Oily	127	59 (46.5)	68 (53.5)	0.042*†
	Dry	124	46 (37.1)	78 (62.9)	
	Mixed	44	18 (40.9)	26 (59.1)	
	Overall	295	123 (41.7)	172 (58.3)	

*Statistically significant ($p<0.05$) †The overall difference in *Demodex* positivity by skin type ($p=0.042$) should be interpreted with caution due to multiple comparisons. Post-hoc pairwise comparisons with Bonferroni correction ($\alpha=0.017$) showed no statistically significant differences between any two skin types (Oily vs. Dry: $p=0.089$; Oily vs. Mixed: $p=0.521$; Dry vs. Mixed: $p=0.683$).

Table 5: Relationship between *Demodex* density and clinical severity

Disease	Severity	N	Mean *Demodex* Density (mites/cm²) ± SD	Range	Correlation Coefficient[1]	p-value
Rosacea	Mild	32	8.3 ± 3.2	5-15	r = 0.683	0.001*
	Moderate	38	14.6 ± 4.8	6-24		
	Severe	10	21.2 ± 6.5	12-36		
Seborrheic Dermatitis	Mild	4	7.5 ± 2.4	5-11	r = 0.712‡	0.021
	Moderate	5	12.2 ± 3.6	8-17		
	Severe	1	19.0‡	-		
Acne Vulgaris	Mild	25	5.2 ± 2.1	0-9	r = 0.418	0.036*
	Moderate	23	7.8 ± 3.4	0-14		
	Severe	9	11.4 ± 4.9	0-19		
Contact Dermatitis	Mild	11	4.9 ± 2.3	0-8	r = 0.386	0.052
	Moderate	12	7.2 ± 3.5	0-13		
	Severe	3	9.7 ± 4.1	6-14		
Psoriasis	Mild	8	4.3 ± 2.6	0-8	r = 0.324	0.204
	Moderate	7	6.1 ± 3.0	0-10		
	Severe	2	8.5 ± 3.5	6-11		
All Dermatoses Combined	Mild	80	6.3 ± 3.1	0-15	r = 0.621	0.001*
	Moderate	85	10.8 ± 5.2	0-24		
	Severe	25	15.6 ± 7.4	0-36		
Control Group	-	102	4.1 ± 2.3	0-9	-	-

Note: *Statistically significant ($p<0.05$). [1]Correlation coefficient (r) represents Spearman's rank correlation between disease severity scores and *Demodex* density. ‡Correlation analysis for Seborrheic Dermatitis should be interpreted with extreme caution due to small sample size (n=10) and the presence of only one patient in the severe category. This single data point may substantially influence the correlation coefficient. Further studies with larger sample sizes are needed to confirm this relationship.

Between *Demodex* density and clinical severity was analysed, it was observed that *Demodex* density increased with increasing disease severity.This relationship was more pronounced in cases of rosacea (r=0.683; p=0.001) and seborrhoeic dermatitis (r=0.712; p=0.021). The seborrheic dermatitis correlation needs careful evaluation because the study included only ten participants and one. A significant correlation was also found for acne vulgaris (r=0.418; p=0.036). However, this correlation was not statistically significant for contact dermatitis (r=0.386; p=0.052) and psoriasis (r=0.324; p=0.204). When all dermatoses were evaluated together, a strong positive correlation was observed between disease severity and *Demodex* density (r=0.621; p=0.001) (Table 5). When gender distribution and *Demodex* positivity according to gender were analysed, it was observed that the majority of the participants were women (80.7%), while the rate of *Demodex* positivity was significantly higher in men (64.9%) than in women (44.7%) (p=0.008) (Figure 1).

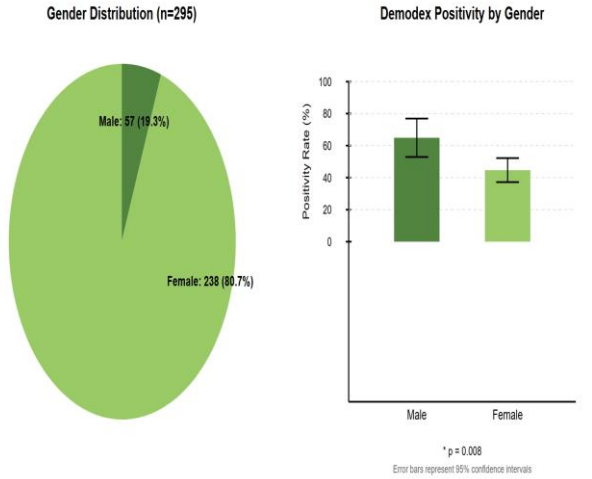

Figure 1: Gender distribution and *Demodex* positivity by gender: The pie chart on the left illustrates the gender distribution among participants (n = 295), with females comprising 80.7% and males 19.3%. The bar chart on the right compares *Demodex* positivity rates between genders, showing a significantly higher positivity in males (64.9%) than females (44.7%) (p = 0.008). Error bars represent 95% confidence intervals. Abbreviations: p: Probability value (indicates statistical significance) CI: Confidence Interval

When *Demodex* positivity was compared according to disease groups, topical medication use and face washing habits, the highest positivity rates were found in patients with rosacea and seborrhoeic dermatitis, those who used topical corticosteroids and those who washed their face with water only. These differences were statistically significant (Figure 2).

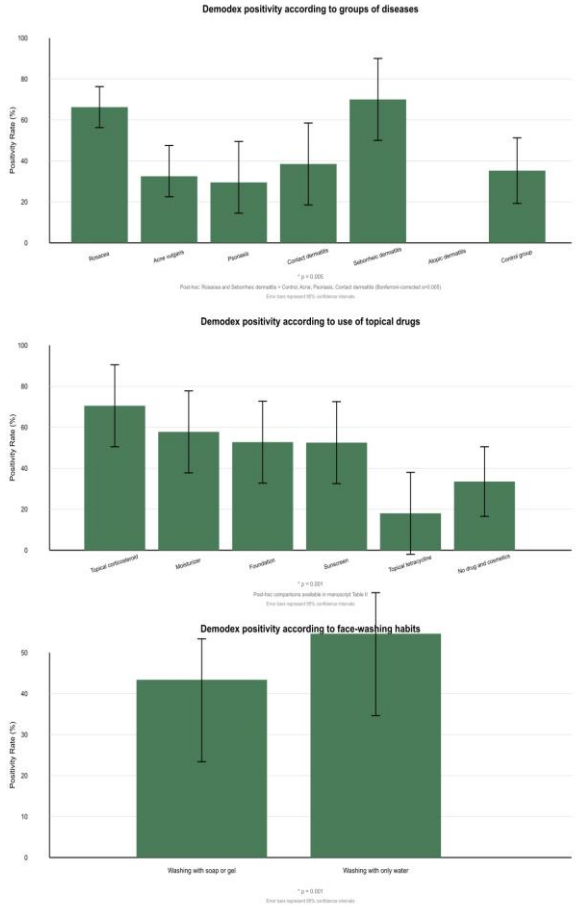

Figure 2: *Demodex* positivity according to disease groups, use of topical drugs, and face-washing habits: The figure consists of three bar charts displaying the prevalence of *Demodex* infestation in (i) different dermatological conditions, (ii) individuals using various topical products, and (iii) subjects with distinct face-washing routines. Error bars represent 95% confidence intervals. Statistical significance was observed across all comparisons (p values are indicated on each chart). Abbreviations: CI: Confidence Interval, p: Probability value (indicates statistical significance).

Discussion

In this study, we aimed to investigate the rates of *Demodex* infestation in patients with facial dermatological diseases and the factors affecting it. Our findings showed that *Demodex* positivity was higher in males than females. In addition, the mean age and body weight of *Demodex* positive patients were higher than negative patients. *Demodex* positivity was significantly more common in patients with rosacea and seborrhoeic dermatitis. In terms of topical cosmetic product use, the highest *Demodex* positivity was found in topical corticosteroid users. In addition, *Demodex* infestation rate was found to be higher in patients who had the habit of washing their face with water only. In our study, *Demodex* positivity was significantly higher in males than females (64.9%

vs. 44.7%), which is consistent with other studies in the literature. In a study conducted by Márquez et al. in a young population, *Demodex* infestation was found to be significantly higher in males than females (32.05% vs. 13.97%) (10). When the age factor was analysed, it was observed that the mean age of *Demodex* positive patients was higher in our study. This finding is in parallel with the result of Sun et al. who found that *Demodex* infestation was more common in elderly individuals than in young people (60.2% vs. 44.0%) (11). Similarly, Ferhatosmanoğlu et al. reported that *Demodex* positivity was significantly higher in individuals over 25 years of age (12). The association of high body weight with *Demodex* positivity found in our study was also confirmed in other studies in the literature. Eroğlu et al. showed that body mass index was significantly higher in *Demodex* positive patients (13). Nalbant et al. reported that obesity was an independent risk factor for *Demodex* positivity (OR=4.42) (14). Ferhatosmanoğlu et al. also emphasised that *Demodex* positivity was higher in obese acne patients (OR=4.06) (12). These findings suggest that demographic and anthropometric factors play an important role in *Demodex* infestation. The high *Demodex* positivity rates observed in patients with seborrhoeic dermatitis (70%) and rosacea (66.25%) in our study are consistent with the literature findings. Wei et al. reported that *Demodex* density was significantly higher in rosacea patients compared to healthy controls and the positivity rate could be up to 81% (15). Similarly, Chioveanu et al. reported that rosacea and seborrhoeic dermatitis are among the diseases with the highest *Demodex* density and reported positivity rates between 66.7% and 83.3% in rosacea subtypes (16). Aktas Karabay and Aksu Çerman also found *Demodex* positivity rates of 79.1% in rosacea patients, 48.8% in seborrheic dermatitis patients and 27.9% in acne vulgaris patients (17). The positivity rate of 32.57% found in acne vulgaris patients in our study seems to be partially compatible with the study of Paichitrojjana and Chalermchai, who found that *Demodex* positivity in acne vulgaris patients was similar to the control group (statistically insignificant difference) (18). *Demodex* positivity of 29.41% in psoriasis patients is remarkable. Aksoy et al. reported demodicosis with a rate of 61.8% in psoriasis patients receiving biological therapy and suggested that this may be due to suppression of immune elements such as IL-17 and TNF-alpha (19). In our study, rosacea (OR=3.62) and seborrhoeic dermatitis (OR=4.28) were found to be independent risk factors for *Demodex* positivity, suggesting that *Demodex* mites play an important role in the pathogenesis of these diseases. The high *Demodex* positivity (70.37%) found in topical corticosteroid users in our study is consistent with similar findings in the literature. Chudzicka-Strugała et al. reported that topical steroids may increase *Demodex* infestation due to immunosuppression (20). They emphasised that topical steroid use may delay the clearance of mites, especially in patients with rosacea (20). Similarly, Güder and Güder reported that the history of topical steroid use was significantly higher in rosacea patients (p=0.024) and *Demodex* positivity was more common in these patients (21). The relationship between moisturiser and sunscreen use and *Demodex* positivity that we found in our study overlaps with the findings of Pawełczyk. Pawełczyk stated that the use of cosmetic creams may increase *Demodex* infestation (22). Furthermore, Chudzicka-Strugała et al. reported that excessive use of daily cosmetics and facial care products may cause *Demodex* increase (20). The low *Demodex* positivity (18.18%) observed in topical tetracycline users in our study indicates the potential effect of antibiotics in reducing *Demodex* infestation. However, Pawełczyk stated that antibiotics such as metronidazole reduce symptoms because they are effective against bacteria, but do not kill mites directly (22). In our study, the high *Demodex* positivity rate (54.68%) found in those who washed their face only with water was in parallel with the findings in the literature. Song et al. reported that *Demodex* positivity rate (39%) was higher in those who washed their face with hot water (23). The effectiveness of soap or gel use in removing *Demodex* mites has also been emphasised in other studies. Tanabe et al. showed that the use of a special eye cleanser significantly reduced the number of *Demodex*, even completely eliminating the mites in 4 of 5 patients (24). This supports the efficacy of surfactants and antimicrobial components in cleansing products in *Demodex* eradication. Chudzicka-Strugała et al. emphasised that cleaning with agents such as soap, shampoo and tea tree oil is effective in the treatment of *Demodex* and physical removal of mites contributes to the treatment (20). Zhang et al. also stated that eye hygiene with traditional soap and similar products may be effective in controlling *Demodex*-related symptoms (25). Paichitrojjana and Paichitrojjana emphasised the importance of regular hygiene practices and suggested that cleaning practices are more common in individuals with high knowledge and awareness and this may be effective in the fight against *Demodex* (26). In our study, the strong positive correlation between

disease severity and *Demodex* density, especially in cases of rosacea (r=0.683; p<0.001) and seborrhoeic dermatitis (r=0.712; p=0.021), is consistent with other studies in the literature. Huang et al. reported that *Demodex* density increased as the severity of the disease increased, and this was especially remarkable in cases of rosacea and seborrhoeic dermatitis (27). Aktaş Karabay and Unay Demirel found that the rate of *Demodex* infestation was higher in the papulopustular rosacea group than in the erythematotelangiectatic rosacea group (p=0.018) and stated that this may be related to disease severity (28). Similarly, Forton emphasised that *Demodex* density increased in all clinical subtypes of rosacea, but reached the highest levels in the papulopustular form (29). This finding supports the strong correlation (r=0.683) found in our rosacea patients. Furthermore, Forton reported that the increase in *Demodex* was associated with the formation of a more pronounced inflammatory response over time (29). Márquez et al. also found that the total number of *Demodex* was significantly higher in individuals with eyelash and skin infections, potentially increasing disease severity (10). Ayres et al. stated in the *Demodex* Expert Panel on Treatment and Eyelid Health (DEPTH) report that mite density correlated with both clinical findings (collarette count) and symptom severity and that symptoms did not permanently resolve without mite eradication (30). Our study has some limitations. Firstly, it was not possible to determine the cause-effect relationship precisely due to its cross-sectional design. Detailed information about the duration and amount of cosmetic product use of the patients could not be collected. Furthermore, other factors that may affect *Demodex* colonisation, such as dietary habits and lifestyle factors, could not be evaluated. The research faces limitations because some subgroups have small participant numbers especially seborrheic dermatitis with only 10 patients which reduces statistical power and limits study generalizability. The correlation analysis for seborrheic dermatitis requires special attention because it only includes one patient in the severe category. The unadjusted multiple statistical tests (n=25) performed in this study increase the chance of Type I error. The results showing p-values near 0.05 (e.g., skin type differences with p=0.042) need verification through additional studies because they might lose significance after multiple testing correction. For future studies, the long-term effects of topical products can be examined using prospective designs and it is recommended to investigate molecular mechanisms that will reveal the relationship between *Demodex* density and disease severity more clearly.

Study Limitations: The cross-sectional design of this study may limit causal interpretation, and information regarding cosmetic product use and lifestyle factors that could influence Demodex colonisation could not be comprehensively assessed.

Conclusion

In conclusion, our study showed that the use of topical medication and cosmetic products may increase *Demodex* infestation on the face. Especially topical corticosteroids, moisturisers and sunscreens significantly increase *Demodex* positivity. Male gender, advanced age, high body weight, presence of rosacea and seborrhoeic dermatitis, and the habit of washing the face with water only were found to be independent risk factors for *Demodex* infestation. A positive correlation was found between *Demodex* density and disease severity. It is recommended that patients with dermatological diseases, especially rosacea and seborrheic dermatitis, should be made aware of the choice of cosmetic products and facial cleansing habits and non-irritant cleansing products should be preferred. These measures may reduce the aggravation of existing dermatological diseases by preventing excessive proliferation of *Demodex* mite.

Ethical considerations: This study was approved by the Clinical Research Ethics Committee of Van Yüzüncü Yıl University Faculty of Medicine with the decision dated 15.01.2014 and numbered 2014/124.

Conflict of interest: The authors declare that they have no conflict of interest to disclose.

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