

## COULD WEARING FACE MASKS DURING THE PANDEMIC HAVE CREATED AN ENVIRONMENT FOR DEMODEX MITES?

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**Abstract: Background:** During the COVID-19 pandemic, wearing face masks became mandatory in our country to prevent the spread of the virus, as in many other countries.

**Objective:** The study aimed to examine whether wearing face masks during the pandemic created a microenvironment for *Demodex* mites.

**Materials and Methods:** The study included three groups: (i) those who wore N95/FFP2 masks, (ii) those who wore a 3-ply surgical mask, and (iii) a control group (who rarely wore masks). The age, gender, occupation, smoking status, alcohol consumption, past medical history, and face-washing habits were questioned, and dermatological examination was performed. The presence of Demodex mites was detected by the standard superficial skin biopsy (SSSB) from three regions of the face.

**Results:** Sociodemographic characteristics and the findings of dermatological examination of the three groups were similar ( $p > 0.05$ ). There was no significant difference across the groups concerning the presence of Demodex mites in corresponding regions (the right cheek, nose, forehead) and corresponding numbers of Demodex mites ( $> 5 \text{ D/cm}^2$ ); the total number of Demodex mites in each region; presence of Demodex mites in any area on the face, number of Demodex mites in any region  $> 5 \text{ D/cm}^2$  on the face, the total number of Demodex mites found on the face, and the presence of Demodex mites in the areas under the mask (the nose and the right cheek together) ( $p > 0.05$ ).

**Conclusion:** We can conclude that wearing masks by healthy adults with no known skin diseases during the pandemic does not create a proper microenvironment for the lives of Demodex mites.

**Keywords:** COVID-19, *Demodex* mites, mask.

### INTRODUCTION

COVID-19 has profoundly impacted the world, leading to millions of deaths and causing sequelae

in various organs, particularly the lungs (1). To prevent the spread of the infection, which was declared a pandemic, wearing surgical masks was recommended and subsequently mandated in many countries (2). During the pandemic, N95/FFP2 and 3-ply surgical masks were among the most commonly worn types, with healthcare professionals, especially those treating COVID-19 patients, frequently using N95/FFP2 masks during work hours (3). These masks, especially N95/FFP2, provide a tighter fit to the face compared to three-ply surgical masks.

Various skin conditions like seborrheic dermatitis, acne vulgaris, acne rosacea, and contact dermatitis have been reported to worsen due to mask-wearing during the pandemic (3, 4). Demodex mites have also been implicated in exacerbating certain skin conditions post-mask-wearing (4, 5).

Demodex folliculorum and Demodex brevis are the main species residing in seborrheic areas such as the chin, cheeks, nose root, and forehead, and they can be found in healthy individuals, contributing to some skin diseases (5). These mites feed on sebum and epithelial contents, with Demodex folliculorum located in the follicular infundibulum and Demodex brevis residing in the sebaceous duct and meibomian glands (5, 6). Factors like humidity, temperature, and acidic pH are favorable environments for these mites, and their presence increases significantly with age (7-10).

The presence of a single mite is considered Demodex positive, and having more than five parasites in an area of  $1 \text{ cm}^2$  is considered sufficient to cause disease, although not all individuals with this parasite count develop symptoms (5, 11). Demodex mites are associated with the etiology of common skin diseases such as rosacea and seborrheic dermatitis (5).

Considering reports of an increase in demodex-related dermatoses due to mask-wearing, this study aims to investigate whether wearing masks by healthy in-

dividuals during the COVID-19 pandemic created an environment conducive to Demodex mites, compared with a control group.

## MATERIAL AND METHODS

The study was conducted on March 15, 2021, and data collection was completed within 6 months. Participants were randomized after stratification by age using a simple randomization table. Exclusion criteria included having dermatosis (such as acne, acne rosacea, seborrheic dermatitis) or other dermatological diseases associated with Demodex mites, receiving or having received treatment for Demodex-associated dermatosis (topical and/or systemic), using face wash gel or cologne that prevents the life of Demodex mites, using cosmetic cream with pore-clogging features, applying disinfectant, being obese (BMI > 30), regularly consuming alcohol, receiving oral therapy that suppresses the immune system, or using creams that may affect the immune system when applied topically on the face, as well as developing any skin complaints with wearing masks.

Participants were categorized into three groups: (i) those who wore N95/FFP2 masks during all working hours except for less than 1 hour; (ii) those who wore a 3-ply surgical mask during all working hours except for less than 1 hour; and (iii) people who wore a 3-ply surgical mask for less than 3 days a week, less than 2 hours a day, spending most of the time at home during the day, or those who worked alone all day in their rooms and wore a 3-ply surgical mask for less than 1 hour during the day as required. The third group represented the control group, consisting of individuals who presented to the hospital during the study period (as there were no individuals without masks during the day).

The sample size was calculated to be at least 48 people for each group, with a test power of approximately 80,151%. The study received approval from the Clinical Research Ethics Committee of Katip Çelebi University (approval number: 027, March 11, 2021) and was conducted following the principles of the Declaration of Helsinki. Participants provided informed consent before inclusion.

Sociodemographic characteristics including age, gender, and occupation, tobacco and alcohol use, past medical history, and face-washing habits were evaluated. Dermatological examination results, including skin type (oily or dry-normal) and Fitzpatrick skin typing, were also analyzed.

In order to detect the presence of Demodex mites, the standard superficial skin biopsy (SSSB) was performed on the face. Dry sterile gauze was used to wipe the face after dropping cyanoacrylate, marking

a 1 cm<sup>2</sup> area on the slide wiped with dry sterile gauze from three areas of the face: the right cheek, nose, and mid-forehead. After leaving the slide for 60 seconds and slowly removing it from the face, immersion oil was dripped onto the slide

The slide was covered with a coverslip, and Demodex mites were counted first under the light microscope at x10 and then x20 magnification (Demodex folliculorum and brevis were not differentiated). This process was repeated for each region. The presence of Demodex mites in each region and their number, if any, were recorded on the inquiry form.

SPSS 17.0 program was used for data analysis. Frequency and percentage values were determined for discontinuous variables, while mean and standard deviation values were calculated for continuous variables. Statistical analysis included the following tests: One-sample Kolmogorov-Smirnov test, Chi-Square test, Fisher's Exact Test and Kruskal-Wallis Test.

## RESULTS

The study included a total of 144 people who met the inclusion criteria, with 48 people in each group. Table 1 shows the distribution of the sociodemographic characteristics of the participants and the comparison of these characteristics, including smoking status, alcohol use, past medical history, facial washing habits (only water, water and soap, non-specific face wash), findings of dermatological examination, skin type (oily or dry-normal skin), and data on the distribution of Fitzpatrick skin type, along with comparisons of these data.

Table 2 presents data and comparisons categorized according to the presence and number of Demodex mites obtained from the right cheek, nose, and forehead regions of the participants using the SSSD technique. It also includes the results of SSSD regarding whether the number of Demodex mites was  $5 > D/cm^2$ .

## DISCUSSION

During the pandemic, wearing masks became mandatory in many countries, including our own, from August 9, 2020, to April 22, 2022, with recommendations for their use in closed working areas, public transportation, and entertainment venues (1, 2). Healthcare workers in COVID-19 departments, those in close contact with suspected cases, mostly wore FFP2/N95 masks for better protection, while others, including patients, primarily used single 3-ply surgical masks (4, 12). Studies indicate that mask use may lead to the occurrence or worsening of various facial conditions (12-16), including acne vulgaris, perioral der-

**Table 1.** Distribution of the sociodemographic characteristics of the participants

	<b>N95\FFP2 mask N (%)</b>	<b>A 3-ply surgical mask N (%)</b>	<b>Controls N (%)</b>	<b>P</b>
<b>Mean age (SD)*</b>	36.50 (8.91)	38.65 (7.81)	35.04 (9.59)	0.19
<b>Gender</b>				
Female	33 (68.8)	33 (68.8)	33 (68.8)	1.00
Male	15 (31.2)	15 (31.2)	15 (31.2)	
Total	48 (100)	48 (100)	48 (100)	
<b>Occupational group†</b>				
Health worker (doctor, nurse, staff)	48(100)	48(100)	15 (31.5)	0,00
Housewife	0 (0)	0 (0)	24 (50)	
Other	0 (0)	0 (0)	9 (18.75)	
Total	48(100)	48(100)	48(100)	
<b>Smoking</b>				
Yes	33 (61.8)	30 (62.5)	36 (75.0)	0.51
No	15 (31.3)	18 (37.5)	12 (25.0)	
Total	48 (100)	48 (100)	48 (100)	
<b>Alcohol consumption</b>				
Yes (social drinker)	29 (60.4)	37 (77.1)	36 (75.0)	0.14
No	19 (39.6)	11 (22.9)	12 (25.0)	
Total	48 (100)	48 (100)	48 (100)	
<b>Face washing habit</b>				
Water only	17 (35.4)	19 (36.6)	15 (31.3)	0.90
Water and soap	24 (50)	24 (50.0)	27 (56.3)	
Non-specific				
Face-wash product	7(14.6)	5 (10.4)	6 (12.5)	
Total	48 (100)	48 (100)	48 (100)	
<b>The number of face washes per day</b>				
Once	30 (62.8)	25 (52.1)	24 (50)	0.66
Twice	13 (27.1)	14 (29.2)	15 (31.3)	
Three times and more	5 (10.4)	9 (18.8)	9 (18.8)	
Total	48 (100)	48 (100)	48 (100)	
<b>Skin characteristic</b>				
Normal-Dry	17 35.4)	26 (54.2)	25 (52.1)	0.20
Oily	31 (64.6)	22 (45.8)	23 (47.9)	
Total	48 (100)	48 (100)	48 (100)	
<b>Fitzpatrick Skin Type</b>				
Type 2	16 (33.3)	24 50.0)	23 (47.9)	0.15
Type 3	32 (66.7)	24 (50.0)	25 (52.1)	
Total	48 (100)	48 (100)	48 (100)	

Participants had no past medical history.

\* Age was evaluated with the one-sample Kolmogorov-Smirnov test ( $p < 0.05$ ); since it was not consistent with the normal distribution, the Kruskal-Wallis test was used for analysis.

† Fisher’s Exact Test used.

The Chi-square test was used for other analyses in the table.

**Table 2.** *Datas and analysis related to demodex mites by groups*

	<b>N95\FFP2 mask N (%)</b>	<b>A 3-ply surgical mask N (%)</b>	<b>Controls N (%)</b>	<b>P</b>
1	2	3	4	5
<b>The presence of Demodex mites</b>				
The right cheek				
No	30 (62.5)	25 (52.1)	29 (60,4)	0.54
Yes	18 (37.5)	23 (47.9)	19 (39.6)	
The nose				
No	40 (83.3)	34 (70.8)	38 (79.2)	0.32
Yes	8 (16.7)	14 (29.2)	10 (20.8)	
The forehead				
No	38 (79.2)	35 (72.9)	33 (68.8)	0.50
Yes	10 (20.8)	13 (27.1)	15 (31.3)	
<b>The number of Demodex mites <math>5 &gt; D/cm^2</math></b>				
The right cheek				
No	39 (81.3)	36 (75.0)	41 (85.4)	0.09
Yes	9 (18.8)	12 (25.0)	7 (%4.6)	
The Nose*				
No	47 (97.9)	45 (93,8)	47 (97,9)	0.67
Yes	1 (2.1)	3 (6.3)	1 (2.2)	
The Forehead*				
No	46 (95.8)	45 (93.8)	45 (93.8)	0.93
Yes		3 (6.3)	3 (6.3)	
<b>The number of Demodex mites Mean (SD)†</b>				
The right cheek	2.33 (4.06)	3.53 (5.06)	1.92 (3.56)	0.29
The Nose	0,52 (1,36)	0,98 (2,46)	0,56 (1,64)	0.34
The Forehead	0.85 (2.34)	1.23 (3.52)	1.08 (3.52)	0.57
<b>The presence of Demodex mites on any part of the face (right cheek, nose, forehead)</b>				
No	29 (60.4)	24 (50.0)	25 (52.1)	0.55
Yes	19 (39.6)	24 (50.0)	23 (47.9)	
<b>The presence of Demodex mites on any part of the face (the right cheek, nose, forehead) <math>5 &gt; D/cm^2</math></b>				
No	37 (77.1)	31 (64.6)	40 (83.3)	0.90
Yes	11 (22.9)	17 (35.5)	8 (16.7)	
<b>The total number of Demodex mites on areas of the face (the right cheek, nose, forehead) Mean (SD)†</b>				
	3.71 (5.76)	5.71 (8.64)	3.54 (6.87)	0.48
<b>The presence of Demodex mites under the mask on any part of the face (the right cheek, nose)</b>				
No	29 (60.4)	25 (52.1)	28 (58.3)	0.69
Yes	19 (9.6)	23 (47.9)	20 (41.7)	

1	2	3	4	5
<b>The presence of <i>Demodex</i> mites under the mask on any part of the face</b> (the right cheek, nose) <b>5 &gt; D/cm<sup>2</sup></b>				
No	38 (79.2)	32 (66.7)	40 (83.3)	0.13
Yes	10 (20.8)	16 (33.3)	8 (16.7)	
<b>The number of <i>Demodex</i> mites on areas under the mask</b> (the right cheek, nose) <b>mean (SD)†</b>	2.85 (4.66)	4.47 (6.2)	2.45 (4.73)	0.46

\*Fisher’s Exact Test used

† The number of *Demodex* mites in each region of the face, and the total number of *Demodex* mites on the face, and the number of *Demodex* mites in the areas under the mask (right cheek and nose) were not consistent with the normal distribution when evaluated with the One-Sample Kolmogorov-Smirnov test ( $p < 0.05$ ). The Kruskal-Wallis test was used to analyze these data. The chi-square test was used for other analyses in the Table.

matitis, and acne rosacea, which are associated with *Demodex* mites (5, 15, 16). Some dermatoses exacerbated by mask use have been linked to *Demodex* mites (17, 18).

*Demodex* mites preferentially lay their eggs in the deep parts of hair follicles or sebaceous glands and are more commonly found in oily skin, feeding on sebum. They thrive in moist, warm, and acidic environments (5-8). Implicated in various skin conditions such as rosacea, non-specific facial dermatitis, androgenetic alopecia, *Demodex* mites are associated with factors like age, genetics, immunosuppression, increased sebum production, UV exposure, and hygiene habits (5, 7, 9). Although they can live on human skin without symptoms, an increase in their numbers may lead to inflammation, although density alone may not be the sole factor (5, 11).

The standard superficial skin biopsy (SSSB) is a common diagnostic method used to detect *Demodex* mites in clinical settings (5, 10, 11). In our study, SSSB was used to detect *Demodex* mites. No significant differences were found among the three groups regarding sociodemographic characteristics, skin type, gender, smoking, alcohol use, face washing habits, or skin condition (Table 1), indicating similarity between the groups.

During the pandemic, various skin conditions associated with mask use have been reported, including perioral dermatitis, acne vulgaris, rosacea, eczema, contact dermatitis, and seborrheic dermatitis (13-16). The term “maskne” has even been coined to describe these conditions related to mask-wearing during Covid-19. Factors such as changes in skin microbiota, duration of mask-wearing, textile properties, friction, and occlusion have been implicated (19). Textile dyes and formaldehyde found in masks may also contribute to dermatoses (4, 19, 20).

Some studies have suggested an increase in *Demodex* mites associated with mask use (9, 20, 21). For

instance, patients with acne have reported worsening of lesions with mask use, possibly due to an increase in *Demodex* mites along with other microorganisms (15, 16, 19). A prospective study investigating mask use and seasonal changes in individuals with rosacea and similar diseases found an association between these conditions, *Demodex* mites, and seasonal temperature (17).

Because *Demodex* mites thrive in a humid, warm, and acidic environment with increased sebum production, and wearing masks creates such an environment, our study aimed to investigate whether mask use contributes to the presence of *Demodex* mites (22). To our knowledge, no study has compared mask use and the presence of *Demodex* mites in healthy individuals without skin diseases with a control group.

We found no statistical difference across the three groups regarding the presence of *Demodex* mites on the right cheek, nose, and forehead; the number of *Demodex* mites exceeding 5 per square centimeter in these regions; the total number of *Demodex* mites on the face; or the presence of *Demodex* mites under the mask (Table 2). These findings suggest that mask use by healthy individuals without skin diseases does not create a favorable environment for *Demodex* mites.

In the context of our study, mask-wearing habits throughout the day, regardless of duration, might have influenced the presence of *Demodex* mites, potentially leading to no significant difference across the groups. Our control group consisted of individuals who predominantly stayed at home, wearing a surgical mask for less than 3 days a week, less than 2 hours a day or those who spent their entire day alone in their rooms. While we didn’t find any statistically significant difference across the groups, we observed that individuals wearing a single 3-ply surgical mask had proportionally more *Demodex* mites in almost all analyses compared to those wearing N95/FFP2 masks and the control group (Table 2).

This discrepancy might be attributed to unfavorable conditions created by N95/FFP2 masks for Demodex mites. The tight grip of N95/FFP2 masks creates a CO<sub>2</sub>-rich environment, potentially affecting the respiration of Demodex mites (21). Additionally, chemicals like formaldehyde and textile dyes left on N95/FFP2 masks were considered harmful to Demodex mites (4, 19). Makeup materials were also reported to block pores and prevent Demodex mites from feeding (9). The tight fit of N95/FFP2 masks might have led to pore-clogging, further affecting Demodex mites. Moreover, individuals wearing N95/FFP2 masks were often healthcare workers in high-risk environments, using more disinfectants, which could have affected the life of Demodex mites due to chemical exposure through evaporation or residual contact.

While a relationship has been established between the number of Demodex mites and the occurrence of dermatosis, recent studies have emphasized the association between HLA groups and the role of individual immunological mechanisms in triggering diseases caused by Demodex mites. Furthermore, it is well-documented that the parasite reproduces more easily under conditions of local or systemic immunosuppression. The exacerbation of Demodex-related dermatoses following mask usage may be attributed to mechanisms influenced by mask-wearing, particularly in individuals sensitive to Demodex mites. It's worth noting that our study was conducted with healthy volunteers devoid of any skin disease. However, the exclusion of individuals with skin complaints during the period of mask-wearing could have influenced our study results.

## Sažetak

# DA LI JE NOŠENJE ZAŠTITNIH MASKI TOKOM PANDEMIJE MOGLO STVORITI OKRUŽENJE POGODNO ZA DEMODEX GRINJA?

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**Uvod:** Tokom pandemije COVID-19 nošenje zaštitnih maski postalo je obavezno u našoj zemlji radi sprečavanja širenja virusa, kao i u mnogim drugim zemljama.

**Cilj:** Cilj studije bio je ispitati da li je nošenje zaštitnih maski tokom pandemije stvorilo mikrookruženje pogodno za Demodex grinje.

**Materijal i Metode:** Studija je uključivala tri grupe: (i) osobe koje su nosile N95/FFP2 maske, (ii) osobe koje su nosile troslojnu hiruršku masku i (iii) kontrolnu grupu (osobe koje retko nose masku). Ispitivani su se godine starosti, pol, zanimanje, pušački status, konzumiranje alkohola, medicinska istorija kao i navike

## Limitations

The control group consisted of individuals who presented to the hospital during the study period, as wearing a mask was mandatory in our country at that time.

## CONCLUSION

In conclusion, wearing masks by healthy adults without known skin diseases during the pandemic did not create an environment conducive to Demodex mites.

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**Data Sharing Statement:** Data were obtained with the consent of the participants.

**ClinicalTrial.gov Identifier:** NCT06185699.

**Note:** Artificial intelligence was not utilized as a tool in this study.

**Contribution Details:** Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor: Nurhan Doner Aktas.

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pranja lica, a obavljena je i dermatološka analiza. Prisustvo Demodex grinja otkriveno je standardnom površinskom biopsijom kože (SSSB) sa tri regije lica.

**Rezultati:** Sociodemografske karakteristike i nalazi dermatološkog pregleda tri grupe bili su slični ( $p > 0,05$ ). Nije bilo značajne razlike između grupa u vezi sa prisustvom Demodex grinja na odgovarajućim regijama (desni obraz, nos, čelo) i odgovarajućim brojevima Demodex grinja ( $> 5$  D/cm<sup>2</sup>); ukupan broj Demodex grinja u svakoj regiji; prisustvo Demodex grinja na bilo kojoj regiji lica, broj Demodex grinja u bilo kojoj regiji  $> 5$  D/cm<sup>2</sup> na licu, ukupan broj Demodex grinja prona-

đenih na licu i prisustvo Demodex grinja na područjima ispod maske (nos i desna obraz zajedno) ( $p > 0,05$ ).

**Zaključak:** Možemo zaključiti da nošenje maski od strane zdravih odraslih osoba bez poznatih kožnih

bolesti tokom pandemije ne stvara odgovarajuće mikrookruženje za Demodex grinje.

**Ključne reči:** COVID-19, Demodex grinje, maska.

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