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Original Article

Prevalence and Molecular Identification of *Demodex folliculorum* in Khuzestan Province, Iran: Associations with Demographic, Lifestyle and Clinical Factors

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Abstract

Background: *Demodex folliculorum* (Acariformes: Demodicidae) is a common human ectoparasite associated with various inflammatory skin and ocular disorders. This study investigated the prevalence, associated factors and molecular characteristics of *D. folliculorum* among individuals attending primary healthcare centers in Khuzestan Province, southwestern Iran.

Methods: In this cross-sectional study, 228 participants underwent cutaneous and ocular sampling for *Demodex* detection using a light microscope. Selected positive samples were identified molecularly by 18S rDNA sequencing. Demographic, behavioral and clinical data were collected using standardized questionnaires. Variables associated with infestation were evaluated by univariate analyses and multivariable binary logistic regression.

Results: Cutaneous *Demodex* infestation was detected in 20 participants (8.77%), while ocular infestation was identified in three participants. Molecular analysis confirmed all sequenced isolates as *D. folliculorum*, showing >99% similarity with reference sequences and only minor genetic polymorphisms. Multivariable analysis identified high psychological stress (adjusted odds ratio [AOR]=5.51, 95% confidence interval [CI]: 1.84–16.47; p=0.002) and active facial skin lesions (AOR=4.01, 95% CI: 1.22–13.20; p=0.022) as independent predictors of infestation. Ocular findings were reported descriptively because of the limited number of positive cases.

Conclusions: *Demodex folliculorum* infestation was relatively uncommon in this population but was independently associated with psychological stress and active facial skin lesions. Molecular findings demonstrated close genetic similarity between local and globally reported isolates. These results improve understanding of the epidemiology of demodicosis and support molecular identification in epidemiological investigations.

Keywords: Demodicosis; Follicle mites; Actinedida; Ectoparasites; Risk factors

Introduction

Demodex mites (Acariformes: Demodicidae) are obligate ectoparasites inhabiting human pilosebaceous units, particularly on the face and eyelashes. Two species are recognized in humans, *Demodex folliculorum* and *Demodex brevis*, which differ in morphology and microhabitat preferences but are frequently found as co-inhabitants of the same anatomical regions (1–3). Although often considered components of the normal skin microbiota, their pathogenic potential becomes apparent when local or systemic host factors allow proliferation, leading to inflammatory dermatoses, follicular obstruction and ocular surface disorders (4–7).

Global prevalence of *Demodex* infestation varies widely, ranging from 10% in younger adults to nearly 100% in elderly populations, reflecting the influence of age, immune func-

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tion, sebaceous activity and hygiene behaviors (8–11). Previous studies have associated high mite density with dermatological and ophthalmological disorders, including rosacea, blepharitis, perioral dermatitis, chalazion and chronic facial irritation (12–16). Blepharitis is one of the most common ocular disorders associated with *Demodex* infestation and is characterized by chronic inflammation of the eyelid margins, eyelash abnormalities, ocular irritation, itching, foreign-body sensation and tear-film instability (4). Numerous studies have demonstrated significantly higher *Demodex* densities in patients with chronic blepharitis than in healthy individuals, suggesting that the mites contribute to both the initiation and persistence of eyelid inflammation (17–18). Consequently, *Demodex* examination has become an important component of the diagnostic work-up for patients with chronic or treatment-resistant blepharitis.

Moreover, *Demodex*-related inflammation is thought to involve mechanical damage, disruption of the epidermal barrier, bacterial interactions and activation of innate immune pathways such as Toll-like receptor 2 signaling (19–22).

Despite increasing awareness of the clinical significance of *Demodex*, its epidemiology in many regions, including parts of the Middle East, remains insufficiently characterized. The tropical climate of southwestern Iran, along with variations in hygiene habits, cosmetic use and environmental exposures, may influence mite density and transmission. Previous regional studies have reported heterogeneous prevalence rates, yet data combining both dermatological and ocular evaluations with molecular identification remain limited (19, 23–25).

The present study investigates the prevalence of *D. folliculorum* among residents of Baghmalek and Ramhormoz, Khuzestan Province, Iran and examines associations with demographic characteristics, behavioral practices, clinical skin conditions and ocular symptoms. Additionally, the study incorporates molecular characterization of *Demodex* isolates using 18S rDNA sequencing to confirm species identity and assess genetic variability. Understanding these epidemiological and genetic aspects may help improve diagnostic approaches, identify high-risk groups and guide preventive strategies for demodicosis in both dermatological and ophthalmological settings.

Materials and Methods

This cross-sectional study included 228 participants attending comprehensive health centers in Baghmalek and Ramhormoz, Khuzestan Province, southwestern Iran. The required sample size was estimated before recruitment using the expected prevalence of *Demodex* infestation reported in previous studies (26–27): a 95% confidence level and a margin of error of 0.06%. Baghmalek and Ramhormoz health centers were selected because they are major referral primary healthcare facilities serving both urban and rural populations in eastern Khuzestan Province, allowing recruitment of participants with diverse demographic characteristics. Before sampling, all participants completed and signed an informed consent form and also completed a researcher-designed questionnaire that collected demographic information (age, sex, occupation, education level, marital status and place of residence) and lifestyle factors (use of shared hygiene items, cosmetic use, frequency of facial washing, history of skin lesions, skin type, active skin problems, stress level, use of immunosuppressive medications, sunlight exposure and dietary habits).

A second questionnaire focused specifically on ocular risk factors for *Demodex* infestation. It included items on sleep duration, daily use of electronic devices, income level, ocular surgery history, smoking and alcohol consumption and symptoms such as itching, pain, dryness, redness, blurred vision, foreign-body sensation, tearing and eyelid drooping. Only participants who completed both questionnaires underwent skin and ocular sampling.

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Skin sampling was performed using standard methods, including adhesive tape application, superficial skin scraping, and follicular expression. Ocular samples were obtained by gently removing three eyelashes from each eyelid using sterile forceps. For each participant, one microscopic preparation was prepared from each collected specimen and covered with a single coverslip before examination. All samples were treated with 10% potassium hydroxide (KOH) and examined under a light microscope at 40× and 100× magnifications. Mites were identified morphologically according to established taxonomic criteria. Because this study aimed to determine the prevalence and molecular identification of *Demodex* spp., rather than quantify mite density, the detection of at least one morphologically identifiable *Demodex* mite in any skin or eyelash specimen was considered a positive result. Quantitative diagnostic thresholds, such as ≥5 mites/cm², used with standardized skin surface biopsy, were not applied because the sampling techniques used in the present study (adhesive tape, skin scraping, follicular expression and eyelash epilation) are primarily qualitative methods.

DNA extraction was performed on a subset of microscopically positive samples using a commercial DNA extraction kit (Yekta Tajhiz Azma, Tehran, Iran) according to the manufacturer's protocol. A fragment of the 18S rDNA gene was amplified using primers described by Thoemmes et al. (21). PCR reactions (50 µL) were run under the following cycling conditions: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 min.

PCR products were visualized on 1.5% agarose gels stained with Safe Stain. Positive amplicons were purified and sequenced bidirectionally. Chromatograms were examined using Chromas software, manually corrected for ambiguous bases and aligned using Clustal Omega. Sequence identity was confirmed by BLAST analysis. Phylogenetic relationships were inferred using the Neighbor-Joining method in MEGA version 6.

Statistical analysis

Variables associated with *Demodex folliculorum* infestation at a significance level of $p < 0.10$ in the univariate analyses and those considered clinically relevant based on previous literature were evaluated for inclusion in a multivariable binary logistic regression model. Because the number of positive infestation cases was limited (20 events) and several categorical variables exhibited complete or quasi-complete separation, a parsimonious model was constructed to avoid model overfitting and unstable parameter estimates. Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were calculated. Statistical analyses were performed using SPSS version 16.0, and a two-sided p value< 0.05 was considered statistically significant.

Results

A total of 228 individuals were examined, among whom *Demodex* infestation was confirmed in 17 skin samples and 3 ocular samples. All detected mites were morphologically identified as *D. folliculorum*. *Demodex brevis* was not observed in any sample (Fig. 1).

Demographic characteristics

Among the participants, 118 (51.75%) were married and 160 (69.2%) were female. The prevalence of infestation was higher among single participants (10%) compared with married individuals (5.93%). Infestation also varied across age groups: rates of 6.55%, 9.72%, 14.28%, 00% and 15.38% were observed in individuals aged 11–17, 18–24, 25–31, 32–38 and 39–45 years, respectively, with no positive cases in the remaining age categories. The highest prevalence occurred in the 39–45 age group. Ocular *Demodex* infestation was detected in three participants**,** with one case each in

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the 32–38, 39–45 and >52-year age groups. Because only three ocular cases were identified, no percentage comparisons or statistical analyses were performed.

Females represented 70.2% of the study population and demonstrated an infestation rate of 8.75%, compared with 4.41% in males. Ocular *Demodex* infestation was detected in two female participants and one male participant. Education level also showed variation in prevalence: more than half of the participants held a diploma, among whom 7.81% were positive. The highest rate (8.82%) occurred in those with education below diploma level, while Ocular *Demodex* infestation was identified in two participants without formal education and one participant with below-diploma education. Occupational analysis indicated that students showed the highest relative rate of infestation (16.66 %), followed by office employees (12.5%), farmers (6.25%) and laborers (4.54%). Housewives represented the largest occupational group overall, among whom eight cases (7%) were detected. Ocular *Demodex* infestation was observed in two housewives and one freelance worker**.**

Lifestyle and clinical factors

Face-washing habits were associated with infestation. Individuals who washed their face once daily showed a prevalence of 3.57%, whereas those washing twice daily exhibited the highest rate (11.6%), followed by individuals washing three or more times daily (6.66 %). Participants who did not wash their faces (4.38% of the sample) showed no infestation. All three ocular infestation cases occurred among participants who reported washing their faces three or more times daily. Significant associations were found between infestation and both washing frequency (p=0.001) and the use of detergents for face washing (p=0.000).

A history of skin lesions or hyperpigmentation was strongly associated with higher infestation. Participants reporting such history had a prevalence of 21.62%, compared with 5.26 % among those without previous lesions, and all three ocular infestation cases occurred among participants with a history of facial skin lesions. Among individuals with clinically active skin conditions at the time of examination (12.28% of all participants), 28.57% were positive, while only 4.5% of those without active lesions were infested. Three participants with active facial skin lesions also had ocular infestation.

Skin type also affected prevalence. Individuals with normal skin exhibited an infestation rate of 11.66%, followed by those with oily skin (7%) and those with dry skin (4.41 %). Ocular *Demodex* infestation was detected in two participants with normal skin and one participant with oily skin. The use of oily cosmetics, lotions, or sunscreen was reported by 56.57% of participants and was significantly associated with infestation: 10.85% among users versus 3% among non-users (p= 0.004). All three ocular infestation cases occurred among participants who reported using oily cosmetics, lotions, or sunscreen.

Environmental and behavioral factors

Sunlight exposure appeared to influence prevalence; individuals with frequent exposure demonstrated a lower infestation rate (7.23%) than those with limited exposure (14%). All three ocular infestation cases occurred among participants reporting frequent sunlight exposure. Stress level showed a strong association with infestation: participants reporting high stress exhibited a prevalence of 16.19%, compared with 3.2% among those with low stress. All three ocular infestation cases were observed among participants reporting high psychological stress.

Use of immunosuppressive medications, including topical, oral, or injectable corticosteroids, was linked to a markedly higher prevalence. Skin infestation was more frequent among participants using immunosuppressive medications than among non-users. Of the three ocular infestation cases, all occurred in participants receiving immunosuppressive drugs. Because of the very small number of ocular cases,

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these findings should be interpreted descriptively. Sharing of personal items such as towels, soap, scarves, hats, or pillowcases was also associated with increased infestation. Participants who shared such items showed a prevalence of 14.28%, compared with 7.23% among those who used personal items exclusively. All three ocular infestation cases occurred among participants who reported sharing personal hygiene items.

Dietary factors could not be meaningfully assessed because 99.56% of participants reported balanced dietary habits and none met criteria for malnutrition.

Statistical analysis

A multivariable binary logistic regression model was performed to identify independent predictors of *Demodex folliculorum* infestation. After adjustment for the other variables in the model, psychological stress and the presence of active facial skin lesions remained independently associated with infestation. Participants reporting high psychological stress had approximately 5.5-fold greater odds of infestation than those reporting low stress (AOR= 5.51, 95% CI: 1.84–16.47, p=0.002). Likewise, participants with active facial skin lesions had approximately fourfold higher odds of infestation than those without active lesions (AOR= 4.01, 95% CI: 1.22–13.20, p=0.022). Female sex was not independently associated with infestation after adjustment (AOR= 1.25, 95% CI: 0.36–4.30, p=0.720).

Overall, the multivariable model was statistically significant (likelihood ratio test, p< 0.001), indicating that the included variables collectively improved prediction of *Demodex folliculorum* infestation compared with the null model.

Molecular analysis

PCR amplification targeting the 18S rDNA gene produced a 1630-bp fragment. Two skin-derived and two ocular-derived positive samples were sequenced; one low-quality sequence was excluded. A high-quality 1192-bp fragment was analyzed. BLAST comparison showed 99.08–100% identity with *D. folliculorum*. Multiple sequence alignment revealed nine polymorphic sites, four insertions or deletions, three transitions and two transversions. Similarity with other *Demodex* species ranged from 95.53% to 100%. The nucleotide sequences obtained in this study have been deposited in the NCBI GenBank database under accession numbers PZ675435 **(**participant01), PZ675436 (participant02), and PZ675437 (participant09).

Phylogenetic analysis using the Neighbor-Joining method demonstrated that all study isolates clustered closely with reference *D. folliculorum* sequences (EU861211.1, KY922187.1, KF745888.1) with minor divergence from KF 745876.1. One isolate formed a slightly distinct sub-branch while remaining within the *D. folliculorum* clade. Chinese reference sequences (JN885463.1 and GU377177.1) formed a separate sister cluster (Fig. 2). The GC content of the sequenced fragment was 44.2%, with base composition of T=27.2%, C=19.5%, A= 28.6% and G=24.7%.

Table 1. Multivariable binary logistic regression analysis of factors independently associated with *Demodex folliculorum* infestation

Variable	AOR	95% CI	P value
Female sex	1.25	0.36–4.30	0.720
High psychological stress	5.51	1.84–16.47	0.002
Active facial skin lesions	4.01	1.22–13.20	0.022

Abbreviations**:** AOR, adjusted odds ratio; CI, confidence interval. Variables with p< 0.10 in the univariate analysis and clinically relevant variables were considered for inclusion in the multivariable model. Statistical significance was defined as p< 0.05

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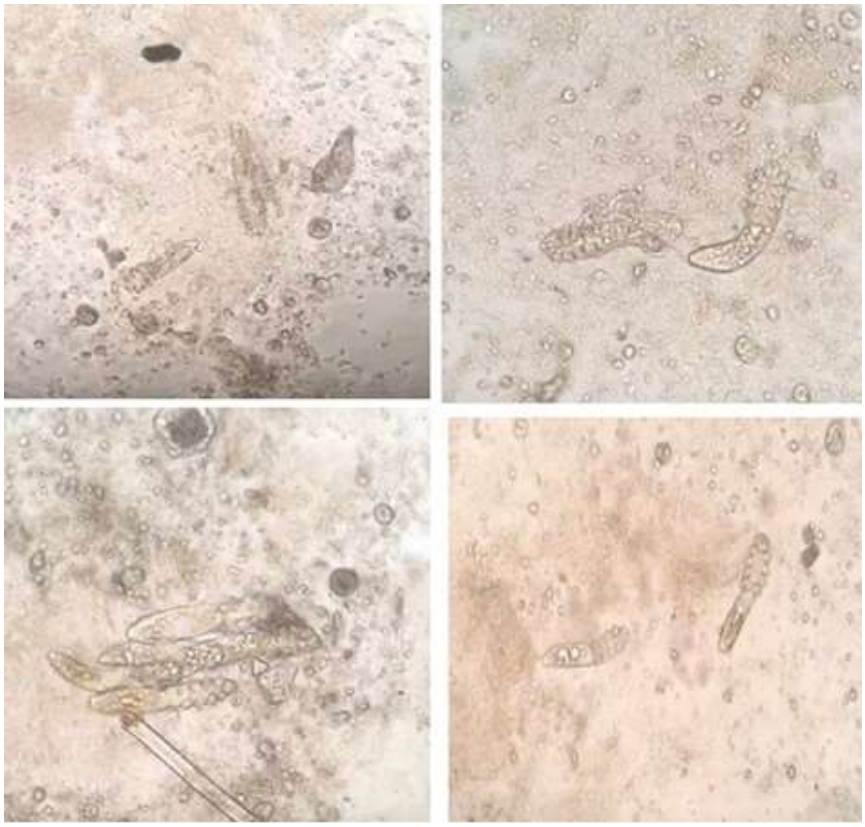

Fig. 1. *Demodex folliculorum* identified in samples taken from participants

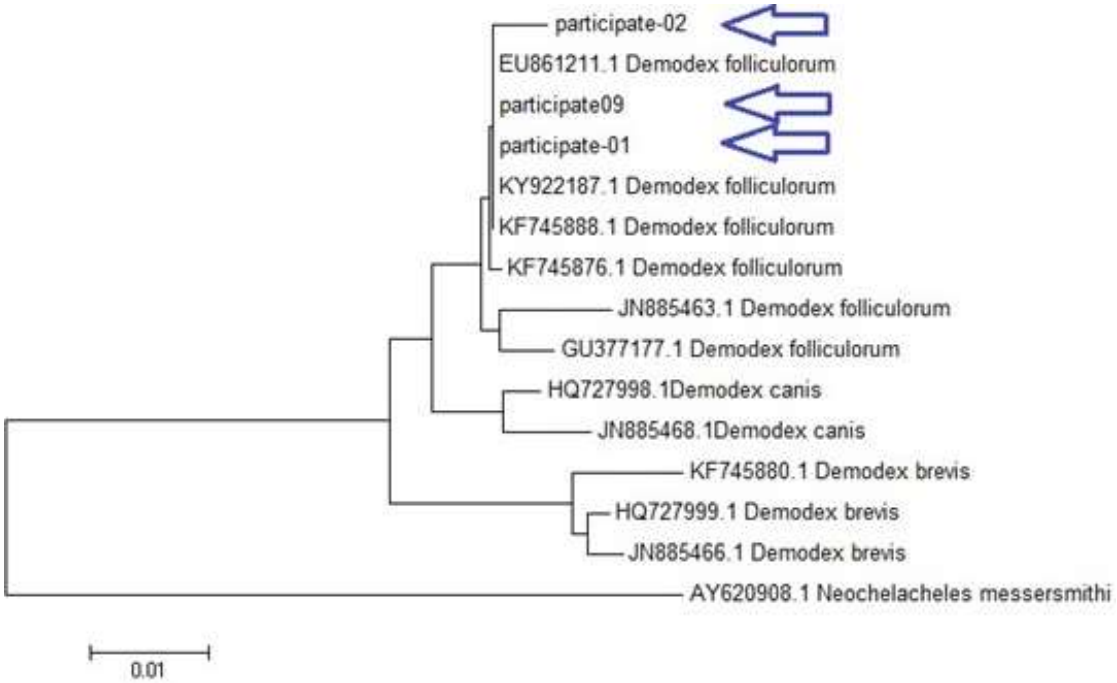

Fig. 2. Phylogenetic tree obtained by the Neighbor-Joining tree algorithm from comparing the sequences of the 18S-rDNA segment with a length of 1191 bp in 3 *Demodex folliculorum* samples from the study areas with the sequences of *Demodex* species registered in GenBank

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Discussion

The present study provides a comprehensive assessment of *Demodex folliculorum* infestation in a general population from Khuzestan Province, integrating clinical, behavioral, demographic and molecular data. The overall prevalence observed was lower than many global estimates, which commonly range from 20% to 80% depending on population characteristics and diagnostic techniques (9, 22). It was also slightly below several regional reports from Iran (15, 22). This comparatively reduced prevalence may reflect environmental characteristics of Khuzestan, particularly high ambient temperatures and intense sunlight exposure, together with widespread use of personal hygiene products and limited sharing of cosmetics or facial items. Previous studies have similarly reported that ultraviolet exposure, improved facial hygiene and avoidance of shared personal objects reduce mite survival and transmission (24–25).

The relatively low prevalence observed in the present study is broadly consistent with reports from neighboring Middle Eastern countries, although considerable variation exists according to study population, diagnostic method and clinical setting. Studies from Türkiye have consistently demonstrated higher *Demodex* prevalence among patients with rosacea, blepharitis and other inflammatory dermatoses than among healthy individuals (4, 17). Similar findings have been reported in Egypt, where *Demodex* infestation has been associated with chronic blepharitis and ocular surface disease (18). In Saudi Arabia, hospital-based investigations have likewise identified *Demodex* mites as important contributors to chronic eyelid inflammation, particularly among patients with persistent blepharitis or dry-eye symptoms (17). These regional observations support our findings that clinical skin lesions and ocular manifestations are closely associated with *Demodex* infestation despite differences in overall prevalence.

Despite the relatively low prevalence, several demographic and clinical associations were evident. The present study identified an apparent increase in *Demodex* infestation among middle-aged participants. However, this finding should be interpreted with caution because some age categories contained relatively few participants and the age intervals were not evenly distributed. Consequently, the prevalence estimates for individual age groups, particularly the 39–45-year category, may be unstable and should not be considered definitive. Nevertheless, the overall pattern of increasing infestation with advancing age is consistent with previous epidemiological studies (9, 12, 28).

Female participants exhibited higher infestation rates than males, a pattern reported in multiple epidemiological studies (17, 19). This difference may be influenced by more frequent cosmetic use, hormonal factors affecting sebum production, and repeated application of facial products that enhance follicular microenvironments favorable for mite colonization (10).

Lifestyle and behavioral characteristics also showed significant associations. Participants with active or previous facial lesions exhibited markedly higher infestation rates, supporting the well-documented relationship between *Demodex* overgrowth and inflammatory dermatoses such as rosacea, seborrheic dermatitis, acne vulgaris and blepharitis (9, 14). Increased mite density may exacerbate inflammation by disrupting the epidermal barrier, stimulating innate immune pathways, including Toll-like receptor-2 signaling, and interacting synergistically with bacteria such as *Bacillus oleronius* and *Staphylococcus epidermidis* (9, 29–30). The exclusive occurrence of ocular infestation among participants with prior dermatological conditions further highlights the importance of compromised cutaneous health in *Demodex*-associated ocular disease (17, 28).

Psychological stress was significantly associated with *Demodex* infestation. Similar findings have been attributed to stress-induced neuroendocrine changes, including cortisol-mediated immunosuppression and altered sebaceous gland

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activity (10, 14, 31).

Immunosuppressive therapy was associated with increased infestation, particularly ocular infestation. Similar observations have been reported among patients receiving corticosteroids and other immunomodulatory therapies (9, 12, 14). Similar associations have been reported in patients receiving corticosteroids, chemotherapy, or other immunomodulatory treatments (12, 28). These findings support routine consideration of demodicosis in immunocompromised individuals presenting with chronic facial or eyelid symptoms.

Multivariable analysis demonstrated that psychological stress and active facial skin lesions were the only independent predictors of *D. folliculorum* infestation after adjustment for potential confounders. The strong association with psychological stress is consistent with evidence that chronic stress alters cutaneous immune responses, impairs skin barrier function and influences sebaceous gland activity through neuroendocrine pathways, thereby creating conditions that may favor *Demodex* proliferation (10, 31–32). Similarly, active inflammatory skin lesions may compromise epidermal barrier integrity and alter the follicular microenvironment, facilitating colonization and proliferation of *Demodex* mites (14, 33–34). In contrast, although female participants exhibited a higher crude prevalence of infestation, sex was not independently associated with infestation after adjustment, suggesting that the observed difference may be explained by behavioral, hormonal, or other clinical factors rather than sex alone (22, 35).

Facial hygiene practices demonstrated a complex relationship with infestation. While moderate cleansing may reduce mite density, excessive washing, particularly with detergent-based products, can damage the lipid barrier and provoke compensatory sebum overproduction, thereby creating conditions favorable for mite survival (10, 36). The relationship between facial hygiene and *Demodex* infestation remains controversial. In the present study, infestation was observed most frequently among participants who washed their faces twice daily, whereas no infestation was detected among individuals who reported never washing their faces. Because the latter group represented only a very small proportion of the study population, this finding should be interpreted cautiously and should not be considered evidence that avoiding facial washing is protective. Previous studies have reported inconsistent findings regarding facial cleansing and *Demodex* density. Several investigations have shown that appropriate facial cleansing can reduce *D. folliculorum* density by removing excess sebum, keratin and debris from the skin surface (23–24). In contrast, other authors have suggested that excessive washing, particularly with alkaline soaps or harsh detergents, may disrupt the epidermal barrier, alter the cutaneous microbiome and stimulate compensatory sebaceous gland activity, thereby creating conditions that favor mite proliferation (36–37). Our findings therefore support the concept that the association between facial hygiene and *Demodex* infestation is likely non-linear. Washing frequency alone is unlikely to be an independent determinant of infestation risk; instead, the interaction between cleansing frequency, cleanser formulation, skin type, sebum production and host immune status probably determines the cutaneous environment in which *Demodex* mites survive. Consequently, moderate cleansing with non-irritating products may better preserve skin barrier homeostasis than either inadequate or excessive washing.

Environmental exposure was associated with differences in infestation prevalence. Participants with limited sunlight exposure showed higher infestation rates, supporting previous reports that ultraviolet exposure may reduce mite survival (9, 24). Sharing towels, pillowcases, and cosmetics has likewise been associated with increased transmission (24–25).

Molecular analysis confirmed that isolates from Khuzestan belonged to *D. folliculorum*, showing high genetic similarity to global ref-

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erence strains with only minor polymorphisms. These findings are consistent with previous molecular studies indicating modest geographic structuring within *D. folliculorum* populations rather than distinct taxonomic separation (21, 38). The observed genetic homogeneity supports the concept of long-term host association and global dispersion linked to human migration patterns.

Overall, the findings demonstrate that *Demodex* mites behave as opportunistic organisms whose proliferation is strongly influenced by host cutaneous integrity, immune status, behavioral habits and psychological factors. Even in populations with high ultraviolet exposure and generally good hygiene, specific vulnerable groups, including individuals with inflammatory dermatoses, cosmetic users, immunosuppressed patients and those experiencing chronic stress, remain at elevated risk.

This study has several strengths. It simultaneously investigated both cutaneous and ocular *Demodex* infestation in a community-based population rather than a dermatology clinic population, combined conventional microscopic diagnosis with molecular species confirmation, and evaluated a broad range of demographic, behavioral, environmental and clinical factors associated with infestation.

Several limitations should also be considered. First, the cross-sectional design precludes establishing temporal or causal relationships between the identified risk factors and *Demodex* infestation. Second, several variables, including hygiene practices, cosmetic use, stress level and previous dermatological history, were obtained through self-reported questionnaires and may therefore be subject to recall or reporting bias. Third, molecular characterization was performed on only a subset of positive samples because of financial and technical constraints, limiting the assessment of genetic diversity. Fourth, only three ocular-positive cases were identified; therefore, ocular findings should be considered exploratory and interpreted with caution. An additional limitation relates to the age-group distribution. The age categories were not equally spaced, and some groups contained relatively few participants, resulting in less precise age-specific prevalence estimates. Future studies should include larger samples with more evenly distributed age categories to improve the reliability of age-stratified analyses. Finally, participants were recruited by convenience sampling from two comprehensive health centers, which may limit the generalizability of the findings to the broader population. Future multicenter studies involving larger and more geographically diverse populations are warranted to validate these findings and further clarify the epidemiology and molecular diversity of *Demodex* infestation.

Conclusion

Demodex folliculorum was confirmed as the predominant species infesting the facial skin of individuals in Baghmalek and Ramhormoz, Khuzestan Province, Iran. Although the overall prevalence (8.77%) was relatively low, infestation was significantly associated with several demographic and lifestyle factors, including female sex, younger age, cosmetic use, psychological stress, immunosuppressive therapy, and facial skin lesions**.**

The findings support the hypothesis that *Demodex* mites may behave as opportunistic organisms under conditions associated with alterations in the cutaneous environment or host immune status. Routine screening for *Demodex* infestation may be beneficial in dermatological and ophthalmological practice, particularly among high-risk populations such as patients with rosacea-like dermatoses, immunosuppressed individuals and those reporting chronic facial irritation.

Further molecular studies involving larger, geographically diverse populations are recommended to elucidate the phylogenetic diversity and potential strain-level variation of *Demodex* mites in Iran. Understanding these genetic differences could enhance diagnostic precision and guide targeted management of demodicosis.

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Ethical considerations

The study was approved by the Ethics Committee of the Urmia University of Medical Sciences, approval number IR. UMSU.REC. 1403.179. All procedures involving human participants were carried out in accordance with relevant institutional guidelines and regulations, and in accordance with the Declaration of Helsinki.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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