Original Article

The Association between *Demodex* Infestation and Hair Loss Severity in a Referred Patient Population

Abozar Nasiri-Jahrodi[1], Hassan Bakhshi[2], Rezvan Rajabzadeh[3], Kamy Hosseinian-Khosroshahy[4], Hassan Reza Rokni[5], *Mehdi Barati[1], *Mitra Salehi[2]

[1]Department of Pathobiology and Medical Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran
[2]Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran
[3]Non-Communicable Diseases Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
[4]Department of Clinical Pathology, Dr. Khosroshahi's Pathobiology Laboratory, Tehran, Iran
[5]Student Research Committee, Gonabad University of Medical Sciences, Gonabad, Iran

***Corresponding authors:** Dr Mehdi Barati, E-mail: mehdi.barati10@yahoo.com, Dr Mitra Salehi, E-mail: misssalehi@yahoo.com

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Abstract

Background: Hair follicle mites of the genus *Demodex* (Prostigmata: Demodicidae) are the most common external obligate parasites in humans, which reside in the eyelids, sebaceous glands, and ear canal, and can cause clinical disease. The purpose of this study was to investigate the possible role of *Demodex* parasites in causing discomfort in patients referred to Tehran laboratories due to hair loss.

Methods: This cross-sectional study was conducted on 520 patients referred to a laboratory in Tehran, Iran, presenting with hair loss. Sampling was performed from the scalp and face, and samples were examined for *Demodex* mites using light microscopy.

Results: The results showed that among the 520 patients referred to the laboratory, 66.15% were infected with *Demodex*. Out of 344 positive cases, 83.43% were women and 16.57% were men. Among the affected patients aged between 7 and 70 years, most patients (59.01%) were in the age group of 25.4 to 39.2 years. A high prevalence of *Demodex* infestation (66.15%) was found among patients with hair loss.

Conclusion: Our findings establish a highly significant, direct correlation between the severity of hair loss and the density of *Demodex* mite infestation ($p < 0.001$). We propose that high *Demodex* mite density is a significant contributory factor in the etiology of severe hair loss.

Keywords: Mites; Demodicosis; Infestation; *Demodex*; Iran

Introduction

Ectoparasites are of significant medical and veterinary importance (1). Among ectoparasites, hair follicle mites of the genus *Demodex* (Actinedida: Demodecidae) are suggested to be the most common external obligate parasites in humans, inhabiting the eyelids, sebaceous glands, and auditory canal (2). Two species of *Demodex*, *D. folliculorum* (0.35 to 0.4 mm in length, living in the lash follicles) and *D. brevis* (0.15 to 0.2 mm in length, living deep in Meibomian glands and lash's sebaceous glands), are found as human parasites (2, 3). Although typically considered commensals, these species can cause dermatological diseases such as rosacea upon dermal penetration or excessive proliferation (4). In addition, several investigations have reported that these parasites play a critical role in causing blepharitis, pityriasis folliculorum, folliculitis (5) and hair loss (6). *Demodex* mites are generally considered commensal organisms residing in human pilosebaceous units. However, an increase in their density can lead to in-

flammatory conditions. The commonly accepted pathogenic threshold for demodicosis is a *Demodex* density greater than 5 mites per square centimeter (>5/cm^2), while a lower density (≤5/cm^2) is frequently observed in a healthy scalp.

This study aimed to investigate the prevalence of *Demodex* infestation and its potential association with hair loss severity in patients referred to a laboratory in Tehran, Iran.

Materials and Methods

This study was conducted from June 2019 to September 2021 in Tehran, the capital of Iran. Subjects were males and females aged between 7 and 70 years old. The study group consisted of 520 patients presenting with hair loss, who were referred to the laboratory (Doctor Khosroshahi laboratory, Moghadas-Ardabili St, Tehran, Iran) without other known causes of hair loss clinically confirmed by a dermatologist, including nutritional, hormonal (e.g., thyroid disease, polycystic ovary syndrome), fungal, or genetic etiologies (e.g., severe alopecia areata, androgenic alopecia not responding to standard therapy). Before sampling, patients were instructed to refrain from bathing and from using antifungal, antiparasitic, or cosmetic products for three days. After complying with the above, sampling from the head (and in case of inflammation and redness around the nose and cheeks, sampling was performed in these two points) was done using the disposable metal inoculation loop. In this regard, about one square centimeter of scalp and face was selected, and sampling was performed from three points on the head (left, middle, right). If inflammation was observed on the face, sampling was performed from around the nose and cheeks. Hair loss severity was categorized as 'low', 'moderate' and 'severe' based on the modified Basic and Specific (BAS) Classification scale, as assessed by a trained dermatologist (7). Specifically, the low category included patients with minimal or early visible thinning (equivalent to BAS scale B1-B2 or T1), moderate included patients with noticeable but manageable diffuse thinning or frontal/vertex recession (equivalent to B3 or T2), and severe included patients with extensive thinning, severe balding, or advanced pattern hair loss (equivalent to B4 or T3 and above). Samples were then placed on a glass slide and clarified using 10% potassium hydroxide (KOH). For enhanced visualization, samples were stained with methylene blue, which accentuates the mite's internal and external structures. For the observation of live mites, immersion oil was used instead of 10% KOH. The collected samples on slides were then examined for *Demodex* mites based on morphological characteristics using a light microscope at magnifications of 10 and 40. Finally, the number of parasites was counted per square centimeter. The final reported density was the average number of mites found across the three sampled scalp sites (Fig. 1).

Results

Of the 520 patients included in the study, 344 (66.15%) were positive for *Demodex* infestation. Among 344 positive cases, 287 (83.43 %) and 57 (16.57) patients were female and male, respectively. The infested patients ranged in age from 7 to 70 years, with the highest proportion (203; 59.01%) belonging to the 25.4-39.2 year age group. Furthermore, the least infestations were found between the age groups of 7–20.8 (22; 6.39 %) and 57.6–71.4 (7; 2.03 %) (Fig. 2).

There was no significant difference in *Demodex* infestation rates between males and females based on the Mann-Whitney test (p= 0/806).

The relationship between patient age and *Demodex* infestation density was assessed using Spearman's correlation coefficient. The analysis showed no statistically significant correlation between age and the mite density (r= 0.064; p-value=0.263). This indicates that infestation severity is independent of patient age within the range studied.

The density of *Demodex* infestation was compared across three categories of hair loss severity: low, moderate and severe. Descriptive statistics for the mite density in each group are presented in Table 1. A Kruskal-Wallis test revealed a statistically significant difference in *Demodex* mite density among the groups with varying severity of hair loss ($p<0.001$). Subsequent Bonferroni post-hoc pairwise comparisons indicated that the differences were significant across all pairs ($p<0.005$ for all comparisons): The mite density in patients with severe hair loss was significantly higher than those with moderate and low hair loss. The mite density in patients with moderate hair loss was significantly higher than that of those with low hair loss (Fig. 3). These findings indicate a positive correlation between mite density and the severity of hair loss (Table 2).

Table 1. Comparison of *Demodex* infestation density based on hair loss severity, 2019–2021, Tehran, Iran

Severity Category	Mean(SD#)	Median(IqR*)	Min-max	p-value
Low	1.61(1.06)	1.00(1.00)	1.00-6.00	<0.001
Moderate	5.93(4.73)	6.00(6.75)	1.00-25.00	
Severe	16.52(9.35)	12.00(10.00)	1.00-50.00	

SD: Standard Deviation; IqR: Interquartile Range

Table 2. Pairwise comparisons of hair loss severity groups, 2019–2021, Tehran, Iran

Sample 1-Sample 2	Test Statistic	p-value
low-moderate	-79.946	<0.001
low-Severe	-178.760	<0.001
moderate-Severe	-98.815	<0.001

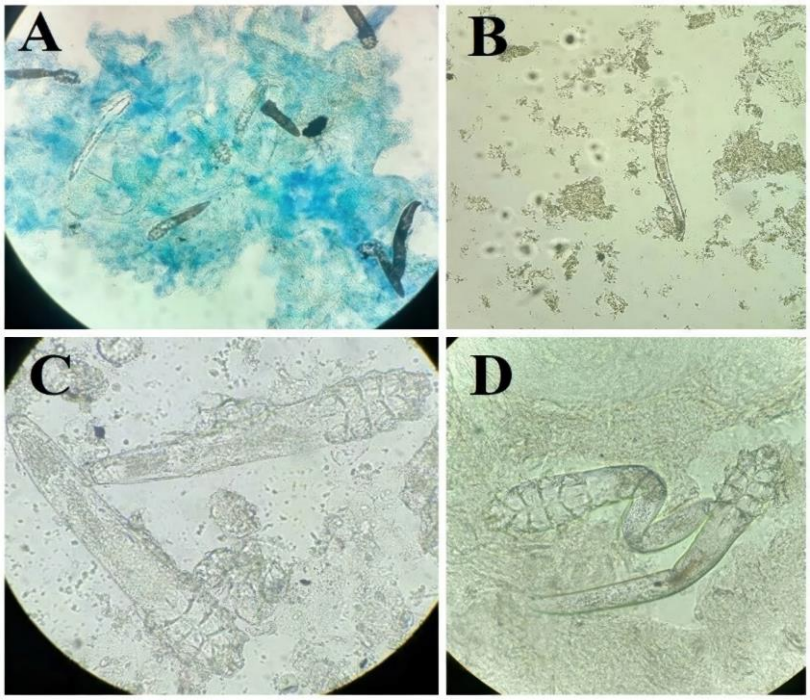

Fig. 1. *Demodex* on the light microscopy ×10 (A, B) and ×40 (C, D), 2019–2021, Tehran, Iran

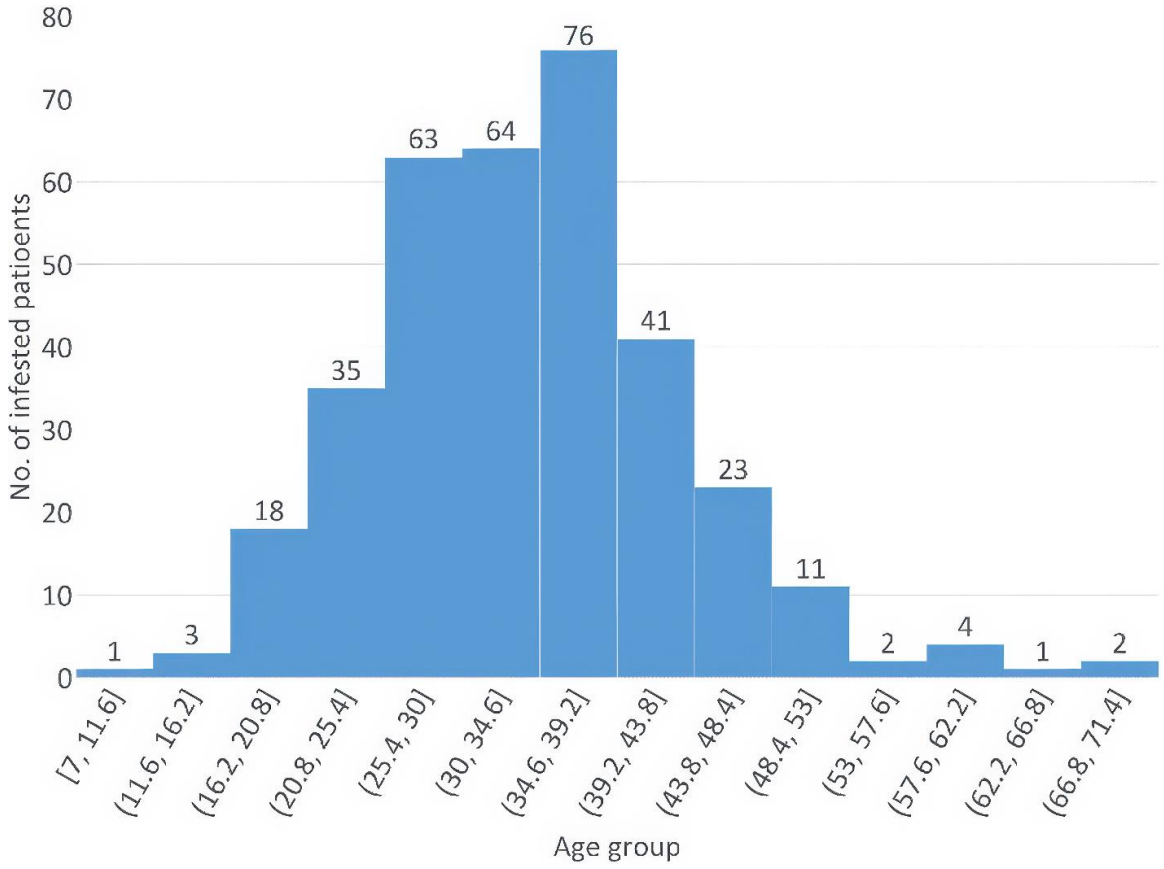

Fig. 2. The number of *Demodex*-infested patients among different age groups, 2019–2021, Tehran, Iran

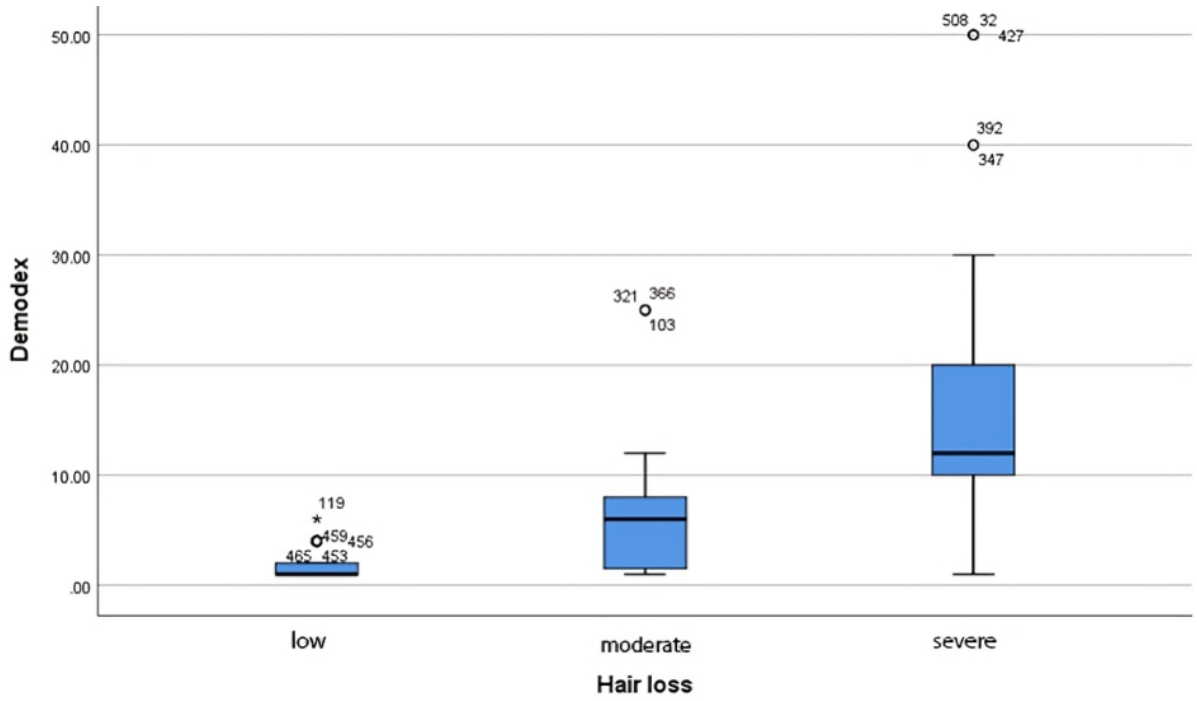

Fig. 3. Simple boxplot of *Demodex* by hair loss severity, 2019–2021, Tehran, Iran

Discussion

Demodex spp. infestation should be considered a serious health concern (8). To date, different investigations have been carried out on the prevalence and existence of *Demodex* in Iran. Our study found that 66.15% of patients referred to the laboratory were infested with *Demodex* mites. Our findings are in concordance with former investigations carried out in different regions of Iran: In a cross-sectional study conducted in Tehran, Iran, samples were collected from the skin around the nasal tip of patients, resulting in the report of demodicosis positivity in 68.3% of tested patients (9). The prevalence of ocular infestation of *Demodex* in male soldiers in a military base in Iran, using a cross-sectional study, has shown an infestation rate of 77.2 % (10), suggesting that the rate of infestation is higher in crowded places such as garrisons. Another cross-sectional study conducted on university students in the west of Iran (Lorestan Province) showed an overall prevalence of *Demodex* mites in different regions of the face in 55% of students, using the cellophane tape method (CTP) and skin pressurization (11).

In our study, the majority of infested patients were women (83.43%). While a female predominance has been reported elsewhere, the markedly high percentage in our cohort likely reflects factors beyond a higher biological prevalence. These may include greater healthcare-seeking behavior among women for cosmetic concerns such as hair loss. These factors may include higher healthcare-seeking behavior among women regarding cosmetic concerns like hair loss, as well as potential biological influences such as hormonal fluctuations or differences in skin lipid composition that may favor mite proliferation. This finding is consistent with a study by Maraghi et al. (2013), which reported an overall demodicosis prevalence of 20% in sebum expressed from the nasolabial folds of a youth group in northern Iran, with a higher prevalence in females (13.8%) than males (6.1%) (8). Contrary to studies that often report an increasing prevalence of *Demodex* infestation with age, our analysis found no significant correlation between patient age and the density of mite infestation ($p=0.263$). This suggests that, while exposure and initial colonization may be age-related, the transition to a high-density, potentially pathogenic state associated with hair loss may be driven more by localized scalp health, immune status, or other host factors rather than age alone.

The most significant finding of this study is the strong correlation between increasing *Demodex* mite density and the severity of hair loss ($p<0.001$). The Bonferroni post-hoc analysis confirmed a clear gradient, where patients with severe hair loss harbored a mean mite density substantially higher (16.52 per cm^2) than those with low hair loss (1.61 per cm^2).

This quantitative link provides stronger evidence supporting the hypothesis that *Demodex* is not merely a passive commensal organism in these patients. High density of mites can lead to follicular damage through several mechanisms, including: mechanical blockage of hair follicles, direct damage from feeding and inflammatory responses triggered by the release of bacterial products upon mite death. Our data support the consideration of a pathogenic threshold where mite densities exceeding a certain level significantly exacerbate or directly contribute to hair loss pathology. This finding underscores the necessity of quantifying mite density, not just presence, when screening patients presenting with severe hair loss. Other studies have also investigated human *Demodex* infestation. The prevalence of *Demodex* and its potential association with skin lesions has also been studied in two groups, showing the infestation of 44 % in the case group and 20 % in the control group (1). Furthermore, 5 cases of rosacea due to *D. folliculorum* have been reported in the south of Iran (Khuzestan Province) as well (12).

Most of the investigations have reported the presence of the *Demodex* mite in the head and face; however, other studies have shown the infestation of patients with the mite in other parts of the body. For example, in an investigation, the presence of *Demodex* was examined in a urine sample containing hematuria in a 44-year-old woman in northwest Iran (Kurdistan Province), resulting in the observation of the live parasite of *Demodex*. The presence of this parasite in the atypical areas close to hairy tissues may be one of the causes of allergic reactions and clinical symptoms in humans (13). A young woman referred to a dermatologist following severe itching and hyperkeratosis (abundant dandruff) of the cheeks in the northwest of Iran (East Azerbaijan Province) found to be infested with a large number the mites in the patient's cheeks (15). There have been different investigations carried out on the treatment of infested patients. In a survey purposing to evaluate the safety and efficacy of blepharoexfoliation in the treatment of blepharitis caused by *Demodex*, the positive patients were treated with in-office blepharoexfoliation (BlephEx LLC; Franklin, TN) using tea tree oil 2% shampoo, followed by eyelid scrubs with tea tree oil 2 % shampoo twice a day. At the 8-week visit, the *Demodex* count was 2.6±1.08 and 3.03± 1.27 in treated and control groups, respectively (p=0.025) (14). The relation between *Demodex* infestation and the prevalence of acne rosacea has also been investigated; the results have shown that the presence of this parasite increases the chance of expressing acne rosacea by 3.3 times (16). We propose that the existing molecular and morphological data on *Demodex* spp. in Iran are limited, warranting further investigation.

A limitation of the present study is that we did not perform molecular analysis on the specimens. However, the use of molecular methods would be very helpful in better identification and differentiation of the two different *Demodex* species. Molecular studies on 16S ribosomal DNA in *D. focullorum* isolates in Tehran Province showed an intraspecific similarity of 99.983% for the isolates studied (17). Mitochondrial 16S rDNA has been suggested to be one of the leading molecular barcodes for the identification of this species and intra-species characterization of *Demodex* mites (17). We suggest further molecular investigation in different provinces of Iran.

The most significant scientific limitation of this study is the absence of a control group of age- and sex-matched individuals without hair loss. While our findings establish a strong correlation between mite density and hair loss severity within the symptomatic population, they cannot definitively establish that *Demodex* density is higher in patients with hair loss compared to the general healthy population. Therefore, our conclusions are correlational and a well-designed case-control study is warranted to confirm the etiological role of *Demodex* in hair loss.

Conclusion

Our findings demonstrate a strong, direct correlation between the severity of hair loss and the density of *Demodex* mite infestation ($p< 0.001$). The mean mite density increased dramatically in parallel with hair loss severity categories (Low→Moderate→Severe). We therefore propose that high *Demodex* mite density is a significant contributory factor in the etiology of severe hair loss and recommend routine density assessment. We would emphasize that a well-designed case-control study in the future is necessary to confirm the etiological role of *Demodex*.

Acknowledgements

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Ethical consideration

Ethical approval for the study was obtained from the Ethics Committee of North Khorasan University of Medical Sciences (Ethical Code: IR.NKUMS.REC.1402.130). Written informed consent was obtained from all patients before sample collection.

Conflict of Interest Statement

The authors declare there is no conflict of interest.

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