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Research Article

Demodex Mites in Relation to the Degree of *Acne vulgaris* among Egyptian Patients

¹Ahmed Badawi Yousif, ^{2,3}Amany Ahmed Abdel-Aal, ¹Abd El-Hamid Abd El-Tawab Sabry, ¹Aya Ahmed Hashem El-Naggar, ⁴Mohamed Masoud, ⁵Shima Mohamed, ⁶Raafat M. Shaapan and ¹Faten A.M.M. Mohamed

¹Department of Medical Parasitology, Faculty of Medicine, Fayoum University, Egypt

²Department of Medical Parasitology, Faculty of Medicine, Cairo University, Egypt

³Department of Medical Parasitology, Armed Forces College of Medicine, Egypt

⁴Department of Public Health, Faculty of Medicine, Fayoum University, Egypt

⁵Department of Dermatology, Dermatology and Leprosy Hospital, Fayoum, Egypt

⁶Department of Zoonotic Diseases, National Research Centre, El-Tahrir Street, Dokki, Giza, Egypt

Abstract

Background and Objective: *Demodex* mites are considered to play a pathogenic role in facial dermatoses as *Acne vulgaris*. In this study the association between *Acne vulgaris* and *Demodex* spp. intensity, kind and activity were investigated. **Materials and Methods:** Two degrees of acne was included, mild acne (30 cases) and moderate to severe acne (30 cases), besides 30 healthy subjects as a control. Deep skin scraping followed by direct microscopic detection was applied to diagnose demodicosis. **Results:** All the detected mites in moderate to severe acne were *D. folliculorum* which was observed in a highly dynamic status with their entire life cycle stages with intensity >5 mites per cm². While very few inactive *D. brevis* mites were detected in the 2 subjects related to the control group. Despite that females comprised the majority of acne cases, males encompassed a statistically higher number than females among positive demodicosis cases. Itching and hair loss were the significant clinical signs within positive cases. Regarding risk factors, oily skins, repeated exposure to the sun, stressful lifestyle plus defective use of facial cleansers were found to be statistically significant. **Conclusion:** These results attained an association between moderate to severe acne and energetic *D. folliculorum* entire population. Thus, this study recommended lifestyle's modification for those cases suffering from *Acne vulgaris* with deep facial cleaning to avoid such infestation that worsens acne condition. Accordingly, once acne treatments are ineffective, consideration of *Demodex* mites with the needed acaricidal therapy is necessary.

Key words: Mites, *Demodex folliculorum*, *Demodex brevis*, *Acne vulgaris*, skin scrapping, facial cleansers, demodicosis

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Corresponding Author: Raafat Mohamed Shaapan Sayed, Department of Zoonotic Diseases, National Research Center, P.O. Box 12622, El-Tahrir Street, Dokki, Giza, Egypt Tel: 002-01005280571

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Demodex mites involved more than one hundred species¹. Yet, human skin is frequently colonized by only two of them, *Demodex folliculorum* and *D. brevis*. They are microscopic, obligate, elongated permanent ectoparasites². Usually, *D. folliculorum* inhabits the hair follicles while *D. brevis* lives in the sebaceous glands³. The exact ways of transmission are not fully identified but it may happen by direct contact or through dispersed dust. Indirectly, mites may be spread via contaminated cloths, towels, combs, coverlets in addition to bath sponges⁴. The responsibility of human *Demodex* species in certain skin disorders is still debated. Variable dermatological conditions, such as rosacea, perioral dermatitis, seborrhoeic dermatitis, blepharitis and chalazion, have been reported to have a significantly greater prevalence of mites⁵. *Demodex folliculorum* well is repeatedly detected in patients with insistent acne and recorded to provoke more severe clinical forms within such category⁶.

Acne vulgaris is a long-term skin disease that possibly occurs when hair follicles are blocked by dead epithelial cells and sebum. It is characterized by certain dermatological signs as white or blackheads and pimples⁷. It affects areas of the skin with a high number of sebaceous glands, including the face, upper part of the chest and the back⁸. Still, *Demodex* mites may inhabit the skin of any individual without causing any symptoms. This is why some scientists denied their role as factual dermal pathogens. However, this situation probably depends on variable factors including the status of mites' population⁹.

The main goal of the present study was on one hand to assess the link between the existence of the *Demodex* mites population and the degree of *Acne vulgaris*. On the other hand, to study the risk factors influencing the clinical manifestations in different cases.

MATERIALS AND METHODS

Study area and duration: The work was conducted over the period from July to October, 2021 and the study settings were outpatient clinics at the Department of Dermatology of Fayoum General Hospital in addition to Dermatology and Leprosy Hospital in Fayoum.

Study subjects: The current study comprised a total of 90 subjects that were divided into 3 groups, 30 patients with mild *Acne vulgaris*, 30 cases suffering from moderate to severe acne plus 30 healthy subjects as a control group. Calculation of sample size was done applying (G power version 3). A minimal sample size of patients was 30 in each

group required to get power level 0.80, alpha level 0.05 and 30.0% as an expected difference in the prevalence of *Demodex* between the two groups of acne patients vs. control.

Individual characteristics: Male or female patients diagnosed to have *Acne vulgaris* by a dermatologist and signed informed consent was eligible to join the study. Patients without come done as the special sign of *Acne vulgaris*, patients with skin disorders other than *Acne vulgaris* and patients lately under therapy against parasitic infections were excluded. All subjects were subjected to a medical questionnaire involving age, gender, duration of acne condition, previous treatment for acne, recurrence of acne after treatment, family history, chronic diseases, eating fast food, skin type and routine of skincare including moisturizers and facial cleansers plus exposure to sun or stress.

Dermatological examination: Affected patients skin examination was done by the dermatologist, applying the grading plan of Doshi *et al.*¹⁰ as the Global Acne Grading System (GAGS). This approach separates the face, chest and back into six sections and provides a size-based factor to each (forehead 2, each cheek 2, nose 1, chin, chest 3 and back 3). Each type of lesion is given a value depending on severity: No lesions = 0, comedones = 1, papules = 2, pustules = 3 and nodules = 4. The score for each area (local score) is calculated using the formula: Local score = Factor × Grade (0-4) and the global score then is the sum of local scores. A global score of 1-18 is considered mild, 19- 30, moderate, 31-38, severe and >39 is considered to be very severe.

The deep skin scraping method was performed according to the protocol of Omer *et al.*¹¹. Before scraping, one drop of liquid paraffin was spread over about 1.5×1.5 cm at the specific skin area. The skin then was pinched between thumb and index finger gently rolled and then the area was scraped using a blade held at a 45° angle to the skin surface. Scraping was continued till there was slight capillary ooze and at least 3 samples were smeared for each case. The scraped materials were then gently mixed with a drop of liquid paraffin on a slide and covered by a coverslip and microscopically examined.

Statistical analysis: The collected data were organized, tabulated and statistically analyzed using SPSS¹², software statistical computer package version 22 (SPSS Inc, USA). Data were presented as frequencies and percentages, chi-square (χ^2) or Fischer exact test, when appropriate, was used as a test of significance. For interpretation of results of tests of significance, significance was adopted at $p \leq 0.05$.

RESULTS

In the current study, the age of all 90 enrolled subjects ranged between 20 and 28 years with a mean of (23.2±2.9 SD). Females comprised 68.3% of the total acne cases (41 out of 60). Likewise, females constituted the bulk of the control group (70%). Students were the vast majority of the dermatologically affected cases, in which 19 (63.3%) and 25 (83.3%) out of the 30 cases/group had mild and moderate to severe acne respectively ($p < 0.0001$). Treatment was previously received by 21 and 25 out of mild and moderate to severe acne groups (30 cases, each). Recurrence was reported in all 25 cases of moderate to severe acne, indicating failure of therapy in such group of cases. In mild acne, recurrence was identified in a significantly lower number of cases, 5 out of 21 cases ($p < 0.0001$). Regarding clinical symptoms among acne

cases, itching and hair loss were found in a considerably higher number of cases with moderate to severe acne (12 and 7, respectively), compared to mild acne (4 and 1, respectively) and control subjects (1 and 0, respectively). The difference was statistically significant ($p = 0.001$ and 0.003) for itching and hair loss, respectively. Out of the 30 cases/mild and moderate to severe acne, 4 and 16 cases did not use facial cleansers, respectively to 9 out of the control group ($p < 0.0001$). Moisturizers as well were not applied in a significantly higher number of cases with moderate to severe acne (27 cases) than those of mild acne (15 cases) and control subjects (12 cases) ($p = 0.025$). Type of the skin, exposure to the sun, stress, chronic diseases and eating fast food didn't report significant differences concerning acne cases and control group ($p > 0.05$) (Table 1).

Table 1: Clinical manifestations and risk factors in different study groups

Variables	Mild acne (N = 30)		Moderate acne (N = 30)		Control (N = 30)		p-value
	Number	Percentage	Number	Percentage	Number	Percentage	
Itching							
Yes	4	13.3	12	40.00	1	3.3	0.001*
No	26	86.7	18	60.0	29	96.7	
Hair loss							
Yes	1	3.3	7	23.3	0	0.0	0.003*
No	29	96.7	23	76.7	30	100.0	
Use of sunscreen							
Yes	4	13.3	3	10.0	4	13.3	0.902
No	26	86.7	27	90.0	26	86.7	
Use of makeup							
Yes	8	26.7	4	13.3	9	30.0	0.271
No	22	73.3	26	86.7	21	70.0	
Use of moisturizers							
Yes	15	50	3	10.0	18	60.0	0.025*
No	15	50	27	90.0	12	40.0	
Use of facial cleanser							
Yes	26	86.7	6	20.0	21	70.0	<0.0001*
No	4	13.3	24	80.0	9	30.0	
Skin type							
Dry	1	3.3	0	0.0	1	3.3	0.712
Sensitive	2	6.7	2	6.7	2	6.7	
Oily	13	43.3	18	60.0	10	33.3	
Combination	10	33.3	8	26.7	13	43.3	
Normal	4	13.3	2	6.7	4	13.3	
Exposure to sun							
Yes	20	66.7	20	66.7	12	40.0	0.054
No	10	33.3	10	33.3	18	60.0	
Stress							
Yes	15	50.0	23	76.7	20	66.7	0.093
No	15	50.0	7	23.3	10	33.3	
Chronic diseases							
Yes	0	0.0	0	0.0	2	6.7	0.129
No	30	100.0	30	100.0	28	93.3	
Eating fast food							
Yes	17	56.7	20	66.7	19	63.3	0.718
No	13	43.3	10	33.3	11	36.7	

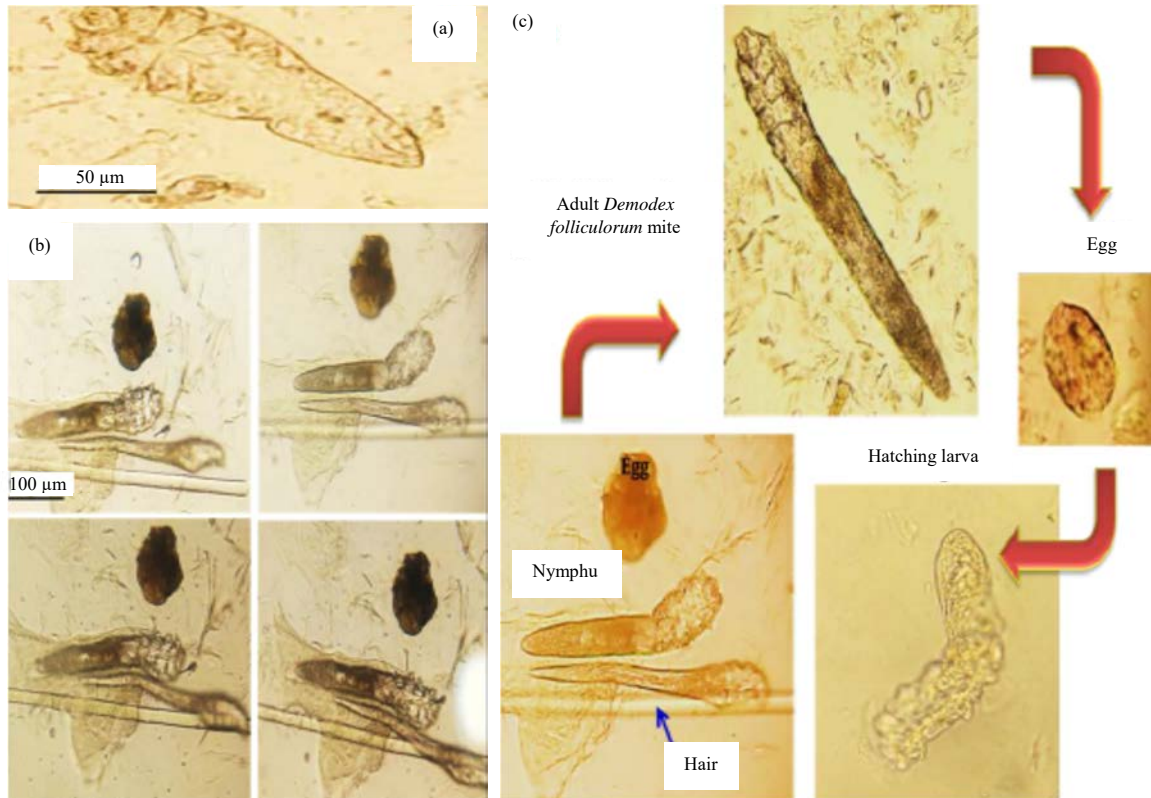


Fig. 1(a-c): Investigated *Demodex* species mites, (a) *Demodex brevis* mite which found solely and inactive, (b) Active nymph stages related to *D. folliculorum* mites, moving around a hair and (c) Entire life cycle of *Demodex folliculorum* mite

Concerning parasitological results, positive findings as regard *Demodex* species was obtained in (14) samples out of the total 90 collected samples. Twelve of them were related to moderate to severe acne group with density >5 mites per cm^2 and all of them were *Demodex folliculorum*. The remaining 2 positive cases were recorded among the control subjects and were *D. brevis* mites that were very few, only one and 2 mites were identified in these 2 positive control subjects correspondingly. On the contrary, adult *D. brevis* mites were detected solely (Fig. 1a) without any other stages, possibly indicating lethargic lifestyle and defective productivity of this kind of human mites. The active nymph stages related to *D. folliculorum* mites, appear moving around a hair (Fig. 1b) No positive cases were reported in patients with a mild degree of acne. The energetic lifestyle within the active mites' community in the 12 positive acne cases was also evident by the presence of all parasite stages (Fig. 1c). The motility of all investigated *D. folliculorum* mites was evidenced in Fig. 2. The energetic group of *D. folliculorum* mites, look as if they are helping each other to fix themselves into a hair (Fig. 2a-f). The rotatory movement of the mites detected (Fig. 2g-j) and the rest of the mites' activity are represented in Fig. 2k-m. On the

other hand, *D. brevis* mites did not demonstrate any activity. Regarding positive demodicosis cases, males comprised a statistically higher number than females when compared to acne cases which were free of *Demodex* (8 out of the 12 positive cases for demodicosis were males) ($p = 0.006$). Therapy for acne was received by all 12 positive demodicosis cases, compared to only 13 out of the 18 negative demodicosis cases within moderate to severe acne ($p = 0.046$) and subsequently, recurrence was reported in these 12 cases. 34 out of 48 acne cases with negative parasitological findings received previous treatment as well ($p = 0.033$). Sixteen out of the 34 negatives for demodicosis returned to the clinic manifested by mild acne without newly affected areas (no recurrence) (Table 2). This may indicate the unpleasant impact of demodicosis on the cure of acne cases following medicinal therapy. On the other hand, itching and hair loss reported a significantly higher number with positive demodicosis cases than those with negative findings ($p < 0.0001$). All the 12 positive demodicosis cases suffered from itching, while 7 out of these 12 positive cases suffered from facial hair loss ($p < 0.0001$) (Table 2).

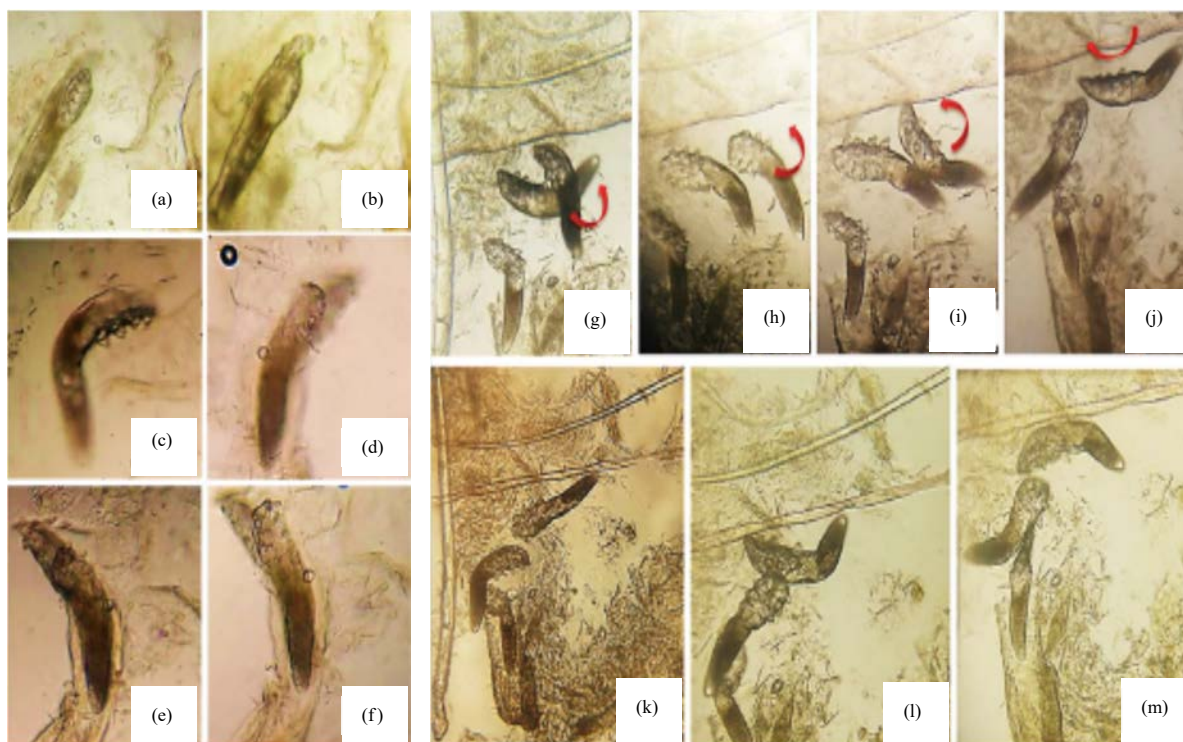


Fig. 2(a-m): Motility of all investigated *D. folliculorum* mites, (a-f) pairs of *D. folliculorum* appear alive within their sheaths. An energetic group of *D. folliculorum* mites, look as if they are helping each other to fix themselves into a hair, (g-j) Rotatory movement of one of the mites (red arrows) and (k-m) Rest of the mites's activity

Table 2: Clinical data on the prevalence of mites in acne groups

Variables	Moderate to severe acne patients				p-value	All acne patients without <i>Demodex</i> (N = 48)		
	Had <i>Demodex</i> (N = 12)		Without <i>Demodex</i> (N = 18)			Number	Percentage	p-value
	Number	Percentage	Number	Percentage				
Previous treatment								
Yes	12	100.0	13	72.2	0.046*	34	70.8	0.033*
No	0	0.0	5	27.8		14	29.2	
Recurrence								
Yes	12	100.0	13	100.0	-	1816	52.9-47.1	<0.0001*
No	0	0.0	0	0.0		1618	47.1-52.9	
Itching								
Yes	12	100.0	0	0.0	<0.0001*	4	8.3	<0.0001*
No	0	0.0	18	100.0		44	91.7	
Facial hair loss								
Yes	7	58.3	0	0.0	<0.0001*	1	2.1	<0.0001*
No	5	41.7	18	100.0		47	97.9	

*Significant

Concerning risk factors, oily skin type, exposure to the sun, stressful life and defects in using facial cleansers were reported in a statistically higher percentage of positive demodicosis cases than those with negative findings ($p < 0.05$) (Table 3).

DISCUSSION

In the current study, the deep scraping method was used to diagnose demodicosis among our cases instead of the standard skin surface biopsy method (SSSB) which is

Table 3: Risk factors to the prevalence of mites in acne groups

Variables	Moderate to severe acne patients				p-value	All acne patients without <i>Demodex</i> (N = 48)		
	With <i>Demodex</i> (N = 12)		Without <i>Demodex</i> (N = 18)			Number	Percentage	p-value
	Number	Percentage	Number	Percentage				
Skin type								
Dry	0	0.00	0	0.0	0.004*	1	2.1	0.007*
Sensitive	0	0.00	2	11.1		4	8.3	
Oily	12	100.00	6	33.3		19	39.6	
Combination	0	0.00	8	44.4		18	37.5	
Normal	0	0.00	2	11.1		6	12.5	
Exposure to sun								
Yes	11	91.70	9	50.0	0.024*	19	39.6	0.047*
No	1	8.30	9	50.0		29	60.4	
Stress								
Yes	12	100.00	11	61.1	0.014*	26	54.2	0.002*
No	0	0.00	7	38.9		22	45.8	
Use of facial cleansers								
Yes	2	16.7	12	66.7	0.007*	38	79.2	<0.0001*
No	10	83.3	6	33.3		10	20.8	

*Significant

considered as a gold standard method to diagnose dermatological lesions¹³. This is because SSSB is usually conducted on the superficial portions of the horny layer and follicles, but does not include the entire follicles. Besides its suboptimal results that may be obtained in patients with hyperkeratotic follicles or seborrheic skin due to poor adherence of mites to the applied microscopic slides¹⁴. The reason behind our choice is probably explained by See¹⁵, who remarked the better sensitivity of deep skin scraping procedure which is considerably improved by squeezing the skin area of interest between the thumb and a finger as performed in our study. This probably facilitates the emerging of mites from deeper skin areas to more superficial ones¹⁵. The procedure in addition to its feasibility and practicality enabled us, not only to observe the entire mites' populations and stages but also to investigate mites' viability. Thus for the same reason, stains and KOH were not used in this study to avoid killing or immobilization of the mites. The smears in this work were prepared using a drop of paraffin oil for processing skin scraping samples to improve the chance of discovering active mites as recommended by Kandi¹⁶.

In the current study, 14 out of the 90 enrolled subjects were found to be positive for *Demodex* infestation. Twelve of them were related to *D. folliculorum* with prominent intensity. They were noticed in a dynamic status with all life cycle stages in patients with moderate to severe degrees of acne in comparison to the remaining 2 positive cases belonging to the control group which were recorded to be inactive and very few *D. brevis*. While no positive cases were found in the mild group of acne. In general, *D. folliculorum* lives in the hair follicles that render its detection easier than *D. brevis*, which

lives in the sebaceous glands². In particular, chelicerae are less developed in *D. brevis* than *D. folliculorum* and this reflects on their lifestyle, type of nutrients and hence population size. The former type of mites feed only on the secretory element of the skin within the sebaceous glands, but can't feed on cellular elements as the latter one³.

Most of the acne cases enrolled in this study were young adults (students). This category was more or less in agreement with the chosen workgroups of Zhao *et al.*^{17,18}, in which most of the subjects involved in their work were university and middle school students who were susceptible to *Acne vulgaris* in the age of 15-25 years. Generally, students aged over 18 years old recorded at least 22 times higher risk of *Demodex* infestation compared to those less than 16 years as mentioned by Manolache¹⁹. In this study, *Acne vulgaris* was recorded in a statistically higher number of females, but a higher number of males was recorded to suffer from demodicosis than females. This was in agreement with Youssefi *et al.*²⁰, who revealed a higher infestation rate with *Demodex* in males compared to females, reflecting this to the presence of more proliferating sebaceous glands in males due to androgen, thus providing more food for *Demodex* mites, favouring their population's expansion. On the other hand, the use of anti-acne treatment in our 12 positive cases for demodicosis could not prevent recurrence and this may reflect the bad impact of demodicosis on the cure of acne condition. It is recorded that regular treatments for *Acne vulgaris* are ineffective if linked with demodicosis that should be carefully examined and treated²¹. In addition, mites can migrate from one follicle to another at a speed of 8-16 m hrs⁻¹ and hence, can repeat the dermatological disorder²².

More than 17 decades have passed since the discovery of *Demodex* mites, however, the probable roles in the pathogenesis of some human skin diseases are quite arguable. In general, most of the clinicians reject such possible association¹. To confirm or otherwise the link between *Demodex* infestation and *Acne vulgaris* in 2012²¹ published a meta-analysis revising a huge number of relevant scientific articles and conclusively verified the association between *Acne vulgaris* and *Demodex* infestation. This evidence-based information positively supports the findings obtained in the current study concerning the role of *Demodex* mites in the pathogenesis of *Acne vulgaris*. *Demodex* mites are thought to cause acne lesions through a variety of processes, including obstructing follicles mechanically¹⁻²¹, immunologically as a result of a foreign body or hypersensitivity reaction against mites relates or remnants²³. Additionally, bacterial infection was also suggested to cause the dermatologically inflamed lesions²⁴.

Regarding the risk factors, this study revealed that the detection rate of demodicosis in subjects with oily skin was much higher than that of subjects with other types of skin. Sebaceous glands in oily skin are more developed, thus may provide plenty of nutrition, suitable enough for mites' reproduction. In addition, chelae and claws of mites stimulate sebaceous follicles and enhance the secretions, thus worsening the condition²¹. Improper hygienic behaviours as well reported a significant correlation among our suffering cases from moderate to severe acne. Zeytun and Karakurt²⁵ reported that the majority of their demodicosis' cases did not use even soap to wash their faces. In general, facial cleansers probably help to fight mites' proliferation by mechanical and chemical actions, removing sebum and sloughs of epithelial cells essential for mites' survival. All cases with mild acne were recorded to use facial cleansers in the current study. So, this may explain the absence of positive cases with *Demodex* infestation in the patients suffering from a mild degree of *Acne vulgaris*.

Sun exposure recorded a significant correlation with moderate to severe acne as well. Although the connection between *D. folliculorum* mites and sunlight is still not completely understood, increased blood flow in dilated papillary dermal vessels due to solar heat may support multiplication or invasion of mites into the dermis, causing more complication²⁶. In our study as regards stress, all positive demodicosis cases were reported to be exposed repetitively to stresses. In a recent study, it is mentioned that the higher the stress level, the more severe the acne condition²⁷. However, the previous study did not consider mites as a possible factor that can affect the severity of acne condition.

Regarding clinical manifestations, according to these results, itching and hair loss reported significant association with acne complaints. In general, the most common symptoms associated with facial *Demodex* spp. are burning sensation, tightness and itching. When dynamic mites multiply, reaching a threshold number, they may lead to irritating skin lesions²⁸. As regard hair loss, this clinical sign reported an extremely significant higher number with positive demodicosis cases than those with negative findings. The cause of hair loss may be explained as a result of local inflammation within the sebaceous glands, which stimulate more collagen by the dermal fibroblasts. Later on, fibrosis occurred that replace the hair follicles, causing hair loss²⁹. In a more recent report, Rather and Hassan³⁰ stated that the mites feed on valuable nutrition needed for hair follicles, resulting in weaknesses up to the loss of hairs. Liang *et al.*³¹ added that the tiny skin abrasions caused by mites can cause epithelial hyperplasia and reactive hyper-keratinization which together with mites' populations and regurgitated undigested food particles cause the pathology that damage the hair follicle, causing hair loss.

CONCLUSION

From the result of this study, it is concluded that people with *Acne vulgaris* associated with itching and local hair loss are more likely to be positive for *Demodex* infestation. A cautious deep skin scraping method for at least 3 smears is a feasible method to detect all the stages of *Demodex* mites in the superficial skin as well as in the deeper skin portions. These results attained an association between moderate to severe acne and energetic *D. folliculorum* entire population. Oily skins, repeated exposure to the sun, stressful lifestyle plus defective use of facial cleansers were positively found to affect demodicosis. Accordingly, once acne treatments are ineffective, consideration of *Demodex* mites with the needed acaricidal therapy is necessary.

SIGNIFICANCE STATEMENT

Demodex mites play a pathogenic role in facial dermatoses as in *Acne vulgaris*. results attained an association between moderate to severe acne and energetic *D. folliculorum* entire population. Thus, this study recommended lifestyle's modification for those cases suffering from *Acne vulgaris* with deep facial cleaning to avoid such infestation that worsens acne condition. Accordingly, once acne treatments are ineffective, consideration of *Demodex* mites with the needed acaricidal therapy is necessary.

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