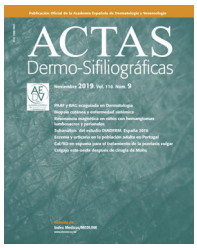
ACTAS Dermo-Sifiliográficas 113 (2022) T758–T764

ACTAS
Dermo-Sifiliográficas

Full English text available at
www.actasdermo.org



ORIGINAL ARTICLE

[Translated article] Association Between *Demodex* Infestation and Severe Acne Vulgaris: A Cross-Sectional Study of 168 Patients

W. Maldonado-Gómez[a,*], **E. Guevara-Sánchez**[a], **G. Guevara-Vásquez**[b], **K. Mera-Villasis**[b], **C.V. Munayco**[c]

[a] *Departamento de Áreas Clínicas, Hospital Regional Lambayeque Chiclayo, Peru*
[b] *Dirección de Investigación, Hospital Regional Lambayeque Chiclayo, Chiclayo, Peru*
[c] *Centro Nacional de Epidemiología, Prevención y Control de Enfermedades, Ministerio de Salud, Lima, Peru*

Received 3 June 2021; accepted 23 March 2022
Available online 16 July 2022

KEYWORDS
Acne vulgaris;
Demodex;
Infections;
Disease severity index

Abstract
Background and objectives: Infestation with *Demodex* mites has been associated with acne vulgaris. The aim of this study was to explore the association between *Demodex* infestation and severe acne vulgaris in outpatients seen at Hospital Regional Lambayeque in Chiclayo, Peru.
Material and methods: We conducted a cross-sectional study of 46 patients with severe acne and 92 patients with nonsevere acne. Severe acne vulgaris was diagnosed if the score was 3 or more on the Spanish Acne Severity Scale (EGAE, in its Spanish acronym). *Demodex* infestation was diagnosed when a skin surface biopsy showed more than 5 mites/cm^2.
Results: The patients had a median age of 18 years (interquartile range, 15–20 years), 60.9% were male, 81.9% lived in an urban area, and 29.7% were infested with *Demodex* mites. In the bivariate analysis, severe acne vulgaris was significantly associated with *Demodex* infestation ($P = .001$), sex ($P = .003$), residence ($P = .015$), a paternal history of acne ($P = .045$), a maternal history of acne ($P = .045$), and type of skin ($P < .001$). In the multivariate analysis, after adjustment for male sex, urban residence, previous treatment, maternal and paternal history of acne vulgaris, and an oily skin type, patients with *Demodex* infestation were 4.2 times more likely to have severe acne vulgaris (95% CI: 1.6–10.9, $P = .003$).
Conclusion: *Demodex* infestation was associated with severe acne vulgaris in outpatients at our hospital.

DOI of original article: https://doi.org/10.1016/j.ad.2022.03.011
* Corresponding author.
E-mail address: winstonmaldonado16@gmail.com (W. Maldonado-Gómez).

https://doi.org/10.1016/j.ad.2022.03.018

PALABRAS CLAVE
Acné vulgar;
Demodex;
Infecciones;
Índice de severidad de la enfermedad

Asociación entre la infestación por el *Demodex* sp. y el acné vulgar grave: estudio transversal de 168 pacientes

Resumen

Antecedentes y objetivos: Existen antecedentes de asociación de la infestación por *Demodex* sp. y el acné vulgar. El objetivo fue evaluar si la infestación por *Demodex* sp. se asocia a acné vulgar grave en consultas externas del Hospital Regional Lambayeque.

Material y métodos: Estudio transversal en 46 pacientes con acné grave y 92 pacientes con acné no grave. Se definió como acné vulgar grave al de los participantes que tuvieron un grado $\geq$ 3 con la escala de gravedad del acné en español (EGAE). La infestación por *Demodex* sp. se determinó mediante biopsia cutánea superficial, considerándose infestación si existieron más de cinco ácaros por cm^2.

Resultados: El 60,9% de los participantes fueron del sexo masculino, con una mediana de edad de 18 años y un rango intercuartílico de 15 a 20 años, provenían del sector urbano (81,9%) y se encontró infestación por *Demodex* sp. en el 29,7%. En el análisis bivariado, se encontró asociación estadísticamente significativa entre acné vulgar grave e infestación por *Demodex* sp. (p = 0,001), sexo (p = 0,003), procedencia (p = 0,015), antecedente paterno de acné (p = 0,045), antecedente materno de acné (p = 0,045) y tipo de piel (p < 0,001). En el análisis multivariado, la infestación por *Demodex* sp. estuvo 4,2 veces más asociada a acné vulgar grave (IC 95%: 1,6-10,9; p = 0,003) ajustado por sexo, procedencia urbana, tratamiento previo, antecedentes paterno y materno de acné vulgar y presencia de piel grasa.

Conclusiones: La infestación por *Demodex* sp. se asocia al acné vulgar grave.

Introduction

Acne vulgaris is one of the most common skin diseases and it has a major impact on quality of life. According to one study conducted in several European countries, it affects 58.7% of people aged 15 to 24 years.[1] Acne is also the second greatest contributor to the global burden of disease measured in disability-adjusted life years.[2] Other studies have linked it to anxiety, depression, and even suicidal ideation.[3] Acne vulgaris is thus a significant skin disease in terms of both prevalence and impact on quality of life.

Acne vulgaris is a multifactorial disease that has been associated with a paternal and maternal history of acne, consumption of chocolate and dairy products, and a high glycemic index diet.[1,4,5] *Cutibacterium acnes* also has a central role in the pathophysiology of acne vulgaris through activation of the innate immune system.[6] Acne is thus caused by genetic and lifestyle factors.

Demodex mites infest the sebaceous glands on the face. There are 2 species: *Demodex folliculorum* and *Demodex brevis*. Both can be found on the face, although *D. folliculorum* is the predominant species. *Demodex* mites are transmitted through close contact. Several publications have shown an association between *Demodex* infestation and a number of skin diseases that affect the face, including rosacea, blepharitis, and acne vulgaris.[7–15] *Demodex* mites have several mechanisms of action in the hair follicle, including obstruction, perforation leading to a foreign body–type inflammatory reaction, the release of cytokines and free radicals that induce inflammation, and alteration of the microbiome.[16–21] The same mechanisms are involved in the development of acne vulgaris and severe acne vulgaris.

Studies linking acne vulgaris to *Demodex* mites have been conducted in Turkey and China.[11,17,22] In our review of the literature, we found no such studies in Latin America.[23] The aim of this study was to evaluate the association between *Demodex* mites and severe acne vulgaris in patients seen at the outpatient clinic at Lambayeque Regional Hospital in northern Peru.

Material and Methods

We designed a prospective, cross-sectional, observational study of patients aged 12 to 30 years who received medical care at the Dermatology outpatient department of Hospital Regional Lambayeque, a high-complexity hospital in northern Peru, between May 2019 and April 2020.

Due to the little information available on the association between *Demodex* infestation and severe acne vulgaris, we conducted a pilot study with 30 patients to calculate the minimum sample size required; 60% of the patients with severe acne vulgaris and 33.3% of those with nonsevere acne vulgaris had a *Demodex* infestation. Using the Epidat 3.1 software program, and applying a confidence level of 95%, a power of 80%, and a ratio of 1:2 for people with and without the event, we calculated a minimum sample size of 138 patients: 46 with the event and 92 without.

Participants were recruited in the outpatient clinic at the end of routine practice. We included patients with severe and nonsevere acne vulgaris graded using the Spanish Acne Severity Scale (EGAE, in its Spanish acronym). We excluded patients who had been receiving acne treatment for more than 1 month, patients with a history of polycystic ovary syndrome, and patients under treatment with systemic

glucocorticoids, lithium, phenytoin, isoniazid, complex B, or halogenated compounds.

The details of the study were explained to all potential participants, who were assured that participation was voluntary and would not interfere with their care before, during, or after the study. Informed signed consent was obtained from all participants older than 18 years and from the parents or guardians of those aged 12 to 17 years. Each patient was then given a form containing 3 separate sections. The first contained a set of multiple-choice questions to be completed by the patient. The second was completed by a dermatologist at the outpatient clinic, while the third was completed by a microbiologist from the hospital laboratory blinded to the other information.

The first section was designed to collect information on sex, age (current age according to national identification card), highest level of education, place of residence (urban, rural, suburban), family history of acne vulgaris (father, mother, siblings), previous treatment of acne vulgaris (yes, no, type), personal history of rosacea, use of facial cleansers (yes, no), and number of times they washed their face a day (0, 1, 2, 3, >3).

In the second section, the dermatologist made a note of acne severity and skin type. Severity was assessed using the EGAE, which has an interobserver reliability of 0.773 (Kendall concordance coefficient) and a concurrent validity of 0.889 (Spearman correlation coefficient); it is also sensitive to change ($P < .001$). As the EGAE is a visual scale, acne lesions located in 3 areas of the body (face, chest, and back) were compared with the EGAE photographs ordered by degree of severity.[24] In this scale, severity is graded from 1 to 4 for lesions on the face and 1 to 3 for those on the chest and back. Severe acne vulgaris thus was diagnosed for EGAE scores of 3 or 4 accordingly. Skin type was classified as dry, mixed, or oily.

The third section of the form was used to record data on *Demodex* infestation. The tests were carried out independently and in a blinded fashion by a microbiologist at the hospital's parasitology laboratory. They therefore did not involve any risk for the patient. A standard superficial skin biopsy was performed[25] to collect a sample from the superficial stratum corneum and hair follicle. Cyanoacrylate adhesive placed on a microscope slide was applied to 1-cm^2 areas on the cheek, forehead, chin, and nose. Immersion oil was then placed on the slide and the *Demodex* mite count was calculated using an Olympus CX41RF binocular microscope. Infestation was considered to exist if at least 5 mites were observed in a low-magnification field (10×) or more than 5 mites were observed in a 1-cm^2 area.[26]

The data were entered into an Excel database and analyzed in Stata version 14. Normality of distribution for quantitative variables was checked using the Shapiro–Wilk test. Quantitative data are presented as median and interquartile range and qualitative variables as absolute and relative frequencies.

The χ^2 and Fisher tests were used in the bivariate analysis and logistic regression in the multivariate analysis. In both cases, significance was set at 5%.

The project was approved by the Research Ethics Committee at Hospital Regional de Lambayeque (code 0321-035-19 CIEI) and authorized for execution at said hospital. The authors supplied the study materials, while the hospital provided access to laboratory facilities.

Results

Overall, 138 patients who met the selection criteria were included; they were predominantly male (84, 60.9%), had a median age of 18 years (interquartile range, 15–20 years), largely lived an urban area (113, 81.9%), and were more likely to have a secondary level of education (74, 53.6%). Forty-one patients (29.7%) had a *Demodex* infestation, in all cases involving *D. folliculorum* (Table 1).

Sixty-five patients had received previous treatment for acne vulgaris (47.1%), the most common being doxycycline (32/65, 49.2%). Forty-five (69.2%) had not responded to treatment. Three patients (2.2%) reported a history of rosacea, but no clinical signs were observed at the time of this study (Table 1).

In the bivariate analysis, acne severity was significantly associated with *Demodex* infestation ($P = .001$), male sex ($P = .003$), urban residence ($P = .015$), a paternal or maternal history of acne vulgaris ($P = .045$) and an oily skin type ($P < .001$) (Table 2). In the multivariate analysis, patients with *Demodex* infestation were 4.2 times more likely to have severe acne vulgaris compared with those without an infestation (95% CI, 1.6–10.9; $P = .003$) following adjustment for age, sex, place of residence, previous treatment, a family history of acne (paternal or maternal), and skin type (Table 2).

Discussion

We detected a significant association between *Demodex* infestation and severe acne vulgaris in our study population. Significant differences between patients with and without severe acne vulgaris were observed for *Demodex* infestation, male sex, urban residence, a maternal and paternal history of acne vulgaris, and an oily skin type. Our findings are consistent with those of several previous studies.[11,12,16,25]

The participants largely lived in an urban area, probably because the study was performed in a tertiary care hospital, to which urban residents are more likely to be referred. Three participants reported a personal history of rosacea, but there were no clinical signs of the disease at the time of the study.

Our observation of an association between *Demodex* mites and oily skin supports findings by Porta[25] and Lacey et al.,[18] who found that oily skin favors the proliferation of these mites, greater inflammation, and, as a result, more severe acne vulgaris.

No significant associations were observed between acne severity and use of facial cleansers or frequency of face washing. Yuan et al.,[16] by contrast, found that facial cleansers used over a 7-day period reduced *Demodex* infestation in patients with mild or moderate acne. The differences between their findings and ours could be due to the fact that our patients had severe acne and that we did not analyze duration of facial cleanser use.

The association observed between *Demodex* mites and severe acne vulgaris is partly consistent with findings by Akçınar et al.[11] in Turkey. Using the Global Acne Grading

Table 1 Participant Characteristics According to Acne Severity.

Variable	No.	%	Not severe	Severe	*P*
Demodex infestation					
No	97	70.3	73 (79.4)	24 (52.2)	.001[a]
Yes	41	29.7	19 (20.7)	22 (47.8)	
Age (median). y	18 (15–20)	17 (15–20)	18 (16–21)	.265	
Sex					
Female	54	39.1	44 (47.8)	10 (21.7)	.003[a]
Male	84	60.9	48 (52.2)	36 (78.3)	
Level of education					
No schooling or primary only	2	1.5	1 (1.1)	1 (2.2)	.435
Secondary	74	53.6	47 (51.1)	27 (58.7)	
Higher	62	44.9	44 (47.8)	18 (39.1)	
Place of residence					
Rural	10	7.3	3 (3.3)	7 (15.2)	.015[b]
Suburban	15	10.9	8 (8.7)	7 (15.2)	
Urban	113	81.9	81 (88.0)	32 (69.6)	
Previous treatment					
No	73	52.9	54 (58.7)	19 (41.3)	.054
Yes	65	47.1	38 (41.3)	27 (58.7)	
Type of treatment					
Topical	13	20.0	9 (23.7)	4 (14.8)	.768
Doxycycline	32	49.2	18 (47.4)	14 (51.9)	
Minocycline	6	9.2	4 (10.5)	2 (7.4)	
Isotretinoin	14	21.5	7 (18.4)	7 (25.9)	
Treatment failure					
No	20	30.8	15 (39.5)	5 (18.5)	.102
Yes	45	69.2	23 (60.5)	22 (81.5)	
Rosacea					
No	135	97.8	91 (98.9)	44 (95.7)	.258
Yes	3	2.2	1 (1.1)	2 (4.4)	
Paternal history of acne vulgaris					
No	88	63.8	64 (69.6)	24 (52.2)	.045[a]
Yes	50	36.2	28 (30.4)	22 (47.8)	
Maternal history of acne vulgaris					
No	112	81.2	79 (85.9)	33 (71.7)	.045[a]
Yes	26	18.8	13 (14.1)	13 (28.3)	
History of acne vulgaris in siblings					
No	89	64.5	56 (60.9)	33 (71.7)	.208
Yes	49	35.5	36 (39.1)	13 (28.3)	
Facial cleansers					
No	76	55.1	54 (58.7)	22 (47.8)	0.226
Yes	62	44.9	38 (41.3)	24 (52.2)	
Hand washing					
Once a day	25	18.1	17 (18.5)	8 (17.4)	.987
2–3 times a day	95	68.8	63 (68.5)	32 (69.6)	
≥3 times a day	18	13.0	12 (13.0)	6 (13.0)	
Skin type					
Mixed	62	44.9	52 (56.5)	10 (21.7)	<.001[a]
Oily	76	55.1	40 (43.5)	36 (78.3)	

[a] χ^2 test.
[b] Fisher exact test.

Table 2 Risk Factors for Severe Acne Vulgaris.

Variable	Crude			Adjusted		
	OR	CI	*P*	OR	CI	*P*
Demodex sp.						
No						
Yes	3.5	1.6–7.6	0.001	4.2	1.6–10.9	.003
Age	1.1	1–1.2	0.133	1.1	1.0–1.3	.036
Sex						
Female						
Male	3.3	1.5–7.4	0.004	5.1	1.8–14.7	.003
Level of education						
No schooling or primary						
Secondary	0.6	0.0–9.6	0.699			
Higher	0.4	0.0–6.9	0.535			
Place of residence						
Rural						
Suburban	0.4	0.1–2.0	0.256	0.2	0.0–1.9	.170
Urban	0.2	0.0–0.7	0.014	0.2	0.0–0.8	.028
Previous treatment						
No						
Yes	2.0	1.0–4.1	0.055	1.2	0.5–3.1	.644
Type of treatment						
Topical						
Doxycycline	1.8	0.4–6.9	0.423			
Minocycline	1.1	0.1–8.9	0.911			
Isotretinoin	2.3	0.5–10.9	0.313			
Treatment failure						
No						
Yes	2.9	0.9–9.2	0.077			
Rosacea						
No						
Yes	4.1	0.4–46.9	0.252			
Paternal history of acne vulgaris						
No						
Yes	2.1	1.0–4.4	0.047	2.2	0.9–5.6	.088
Maternal history of acne vulgaris						
No						
Yes	2.4	1.0–5.7	0.049	2.8	0.9–8.4	.073
History of acne vulgaris in siblings						
No						
Yes	0.6	0.3–1.3	0.210			
Facial cleansers						
No						
Yes	1.6	0.8–3.2	0.227			
Hand washing						
Once a day						
2–3 times a day	1.1	0.4–2.8	0.874			
≥3 times a day	1.1	0.3–3.9	0.927			
Skin type						
Mixed						
Oily	4.7	2.1–10.6	*P* < .0001	3.3	1.3–8.6	.013

System (GAGS), they found that patients with severe acne vulgaris had a higher density of *Demodex* mites. The association, however, was not significant, possibly because they used a different scale to assess acne severity and a different cutoff for *Demodex* infestation.

Several studies have suggested that *Demodex* mites could favor higher rates of *Cutibacterium acnes* and *Staphylococcus aureus*[17,19,20,27] by acting as a vector for these bacteria.[26] *Demodex* mites can also induce the expression of toll-like 2 receptors and inflammatory infiltrates (macrophages, mast cells, T helper cells types 1 and 17, and eosinophils) and the release of inflammatory cytokines, such as tumor necrosis factor α and interleukin 1 and 6.[17,19,27]

Confirmation that these likely mechanisms are involved in acne vulgaris as they are in rosacea would mean that *Cutibacterium acnes* is not the only agent involved in the onset of acne, and would also explain why patients do not respond adequately to treatment.

One of the strengths of this study is that the EGAE scale has high validity and interobserver reliability. A range of methods exist for evaluating acne severity, but they have not been validated or undergone assessment of intraobserver or interobserver agreement.[28]

Our study also has some limitations. First, we excluded patients with a history of polycystic ovarian syndrome, which, in patients with a certain history, has been linked to *Demodex* infestation. We were therefore unable to evaluate the possible effect of polycystic ovarian syndrome on the association between *Demodex* infestation and severe acne vulgaris in our population. Second, our findings for a family history of acne and response to previous acne treatments may be influenced by recall bias or patient expectations, or in the case of treatments, subjective evaluations. Third, because of our study design, we were unable to determine whether *Demodex* infestation occurred before or after the onset of severe acne vulgaris. This is important and should be taken into account when interpreting results, especially in a disease such as acne, which has varying levels of severity.

Despite the above limitations, our study represents a preliminary approach to the evaluation of the association between *Demodex* mites and acne vulgaris in Latin America, where studies are lacking. The only study we identified in this regard, performed at our hospital, examined facial skin diseases.

Cohort studies are needed to evaluate possible correlations between acne severity (measured using properly validated quantitative scales) and both *Demodex* infestation and other potentially relevant variables. Clinical trials could also be designed to investigate the effects of *Demodex* treatment on acne severity

In conclusion, *Demodex* infestation is associated with severe acne vulgaris, a paternal and maternal history of acne vulgaris, age, sex, level of education, and skin type.

Funding

Hospital Regional Lambayeque provided access to the laboratory facilities, and the authors supplied the materials.

Conflicts of Interest

None.

References

1. Wolkenstein P, Machovcová A, Szepietowski JC, Tennstedt D, Veraldi S, Delarue A. Acne prevalence and associations with lifestyle: a cross-sectional online survey of adolescents/young adults in 7 European countries. J Eur Acad Dermatol Venereol. 2018;32:298–306.
2. Karimkhani C, Dellavalle RP, Coffeng LE, Flohr C, Hay RJ, Langan SM, et al. Global skin disease morbidity and mortality: an update from the Global Burden of Disease Study 2013. JAMA Dermatol. 2017;153:406–12.
3. Halvorsen JA, Stern RS, Dalgard F, Thoresen M, Bjertness E, Lien L. Suicidal ideation, mental health problems, and social impairment are increased in adolescents with acne: a population-based study. J Invest Dermatol. 2011;131:363–70.
4. Akpinar Kara Y, Ozdemir D. Evaluation of food consumption in patients with acne vulgaris and its relationship with acne severity. J Cosmet Dermatol. 2019, jocd.13255.
5. Fiedler F, Stangl G, Fiedler E, Taube K. Acne and nutrition: a systematic review. Acta Derm Venereol. 2017;97:7–9.
6. Beylot C, Auffret N, Poli F, Claudel J-P, Leccia M-T, Del Giudice P, et al. Propionibacterium acnes: an update on its role in the pathogenesis of acne. J Eur Acad Dermatol Venereol. 2014;28:271–8.
7. Zhao Y, Peng Y, Wang X, Wu L, Wang M, Yan H, et al. Facial dermatosis associated with Demodex: a case–control study. J Zhejiang Univ Sci B. 2011;12:1008–15.
8. Galvis-Ramírez V, Tello-Hernández A, Álvarez-Osorio L, Rey-Serrano JJ. Prevalencia de infección por Demodex folliculorum en pacientes que acuden a consulta general de oftalmología. Rev Salud Pública. 2011;13:990–7.
9. Rivera N, Molina P, Torres A. Determinación de índice de infestación por *Demodex* spp. en pacientes con blefaritis crónica y en pacientes sin otra patología ocular. Rev Chil Infectol. 2013;30:494–501.
10. Mucha ET, Ramirez LP, Salomón M, Anzardo MV, Chacaltana MD, Puccio FB, et al. Demodicidosis en pacientes con rosácea. Rev Medica Hered. 2012;18:15.
11. Akçınar UG, Ünal E, Doğruman Al F. *Demodex* spp. as a possible aetiopathogenic factor of acne and relation with acne severity and type. Postepy Dermatol Alergol. 2018;35:174–81.
12. Mera-Villasis KM, Valderrama-Ayén MY, Aguilar-Gamboa FR. *Demodex* sp. y dermatosis faciales en pacientes atendidos por consulta externa en un hospital de Perú. Rev Exp En Med Hosp Reg Lambayeque. 2019;5. Available from: http://rem.hrlamb.gob.pe/index.php/REM/article/view/346 [cited 2022 Feb 16].
13. Zhong J, Tan Y, Li S, Peng L, Wang B, Deng Y, et al. The prevalence of *Demodex folliculorum* and *Demodex brevis* in cylindrical dandruff patients. J Ophthalmol. 2019;2019:8949683.
14. Aktaş Karabay E, Aksu Çerman A. *Demodex folliculorum* infestations in common facial dermatoses: acne vulgaris, rosacea, seborrheic dermatitis. An Bras Dermatol. 2020.
15. Zhao Y, Guo N, Xun M, Xu J, Wang M, Wang D. Sociodemographic characteristics and risk factor analysis of Demodex infestation (Acari: Demodicidae). J Zhejiang Univ Sci B. 2011;12:998–1007.
16. Yuan C, Zheng S-L, Ma YF, Juliandri PH. Cleanser use could decrease numbers of *Demodex folliculorum* in mild to moderate acne patients. Dermatoendocrinology. 2017;9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5821153/ [cited 2019 Sep 17].

17. Zhao Y, Yang F, Wang R, Niu D, Mu X, Yang R, et al. Association study of Demodex bacteria and facial dermatoses based on DGGE technique. Parasitol Res. 2017;116:945–51.
18. Lacey N, Ní Raghallaigh S, Powell FC. Demodex mites: commensals, parasites or mutualistic organisms? Dermatol Basel Switz. 2011;222:128–30.
19. Steinhoff M, Vocanson M, Voegel JJ, Hacini-Rachinel F, Schäfer G. Topical ivermectin 10 mg/g and oral doxycycline 40 mg modified-release: current evidence on the complementary use of anti-inflammatory rosacea treatments. Adv Ther. 2016;33:1481–501.
20. Zhu M, Cheng C, Yi H, Lin L, Wu K. Quantitative analysis of the bacteria in blepharitis with Demodex infestation. Front Microbiol. 2018;9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6079233/ [cited 2018 Sep 18].
21. Gazi U, Gureser AS, Oztekin A, Karasartova D, Kosar-Acar N, Derici MK, et al. Skin-homing T-cell responses associated with Demodex infestation and rosacea. Parasite Immunol. 2019;41:e12658.
22. Zhao Y-E, Hu L, Wu L-P, Ma J-X. A meta-analysis of association between acne vulgaris and Demodex infestation. J Zhejiang Univ Sci B. 2012;13:192–202.
23. Palopoli MF, Fergus DJ, Minot S, Pei DT, Simison WB, Fernandez-Silva I, et al. Global divergence of the human follicle mite *Demodex folliculorum*: persistent associations between host ancestry and mite lineages. Proc Natl Acad Sci USA. 2015;112:15958–63.
24. Puig L, Guerra-Tapia A, Conejo-Mir J, Toribio J, Berasategui C, Zsolt I. Validation of the Spanish Acne Severity Scale (Escala de Gravedad del Acné Española – EGAE). Eur J Dermatol. 2013;23:233–40.
25. Porta Guardia CA. *Demodex folliculorum*: its association with oily skin surface rather than rosacea lesions. Int J Dermatol. 2015;54:e14–7.
26. Serrano-Grau P. Dermatosis inducidas por *Demodex folliculorum*. Piel. 2011;26:291–5.
27. Lacey N, Russell-Hallinan A, Zouboulis CC, Powell FC. Demodex mites modulate sebocyte immune reaction: possible role in the pathogenesis of rosacea. Br J Dermatol. 2018;179:420–30.
28. Agnew T, Furber G, Leach M, Segal L. A comprehensive critique and review of published measures of acne severity. J Clin Aesthetic Dermatol. 2016;9:40–52.