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Original Article

The association between *Malassezia spp.* and pruritus in facial acne vulgaris

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Abstract

Background Acne vulgaris (AV) is a chronic inflammatory condition involving the pilosebaceous unit. Recent studies have shown that itching of mild-to-moderate severity is relatively common in patients with acne. *Malassezia spp.* is normal flora of skin surface, and can be found in acne lesions. Aim: To clarify the relationship between *Malassezia spp.* in facial acne lesions and their association with pruritus symptom.

Methods This is a cross-sectional study conducted at dermatology and venereology outpatient clinic in a tertiary hospital in Indonesia. We recruited subjects with at least four inflammatory and four noninflammatory facial acne lesions. Acne was graded using Lehmann's criteria, and pruritus was graded using visual analog scale (VAS). Follicular content of acne lesions was collected for microscopic examination and fungal culture.

Results Of the 120 subjects, 46 (38.3%) reported experiencing itching in acne lesions. Pruritus was more common in patients with mild AV. Among those with pruritus, fungal spore was found in 60.6% subjects. There was no statistically significant difference in terms of the presence and severity of pruritus and presence of *Malassezia spp.* determined by culture (p=0.332; 0.317).

Conclusion Pruritus may accompany facial acne lesions. In this study, the presence and severity of pruritus did not correlate with the presence of *Malassezia spp.* in facial acne lesions.

Key words
Acne vulgaris, pruritus, *Malassezia*, fungal spores, skin disease.

Introduction

Acne vulgaris (AV) is a common inflammatory condition involving the pilosebaceous unit. Increased sebum production, abnormal follicular keratinization of the pilosebaceous duct, bacterial colonization, and host inflammatory response contribute to acne development.[1,2] Interaction between skin microbiome and host immunity has long been implicated in acne pathogenesis. Skin microbiome is composed of diverse microorganisms including bacteria, viruses, fungi, and mites.[2] Dysbiosis can have a detrimental effect on the host and may manifest as inflammatory diseases.[3] It is long known that *Propionibacterium acnes* significantly increases in the pilosebaceous unit of patients with acne and induces changes in the sebaceous gland activity, comedone formation, and host, yielding

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an inflammatory response.[2,4]

Numata *et al*.[5] studied the follicular content of acne lesion and reported the presence of not only *Propionibacterium spp.* but also *Staphylococcus* and *Malassezia spp.* Akaza *et al*.[4] also reported the correlation between the number of *Malassezia spp.* on the skin surface and that in follicular content and inflammatory acne. Malassezia folliculitis (MF) caused by *Malassezia spp.* is sometimes confused with or may occur with AV.[6] Kang *et al*.[7] reported that the coincidence of MF in patients with acne was 25%. A history including pruritus is usually the hallmark of MF, but Reitch *et al*.[8] also reported that itching of mild-to-moderate severity is relatively common in patients with acne and sufficient enough to cause distress.

Although the presence of *Malassezia spp.* in acne lesions has been confirmed, its exact role in the pathogenesis of acne is not clearly defined.[9] There are limited studies regarding the existence and role of *Malassezia spp.* in AV. The aim of this study is to clarify the relationship between *Malassezia spp.* in acne lesions and their association with pruritus symptom.

Methods

This study was conducted in parallel with another study titled "Comparison of *Malassezia spp.* Proportions in Inflammatory and Noninflammatory Facial Acne Vulgaris Lesions"[10] with ClinicalTrial.gov registration number NCT04367584 and was approved by the Health Research Ethics Committee of Universitas Indonesia (number 10969/UN2/F1/ETIK/2018).

Subjects This study included subjects from the dermatology and venereology outpatient clinic in a tertiary hospital in Indonesia during the period between October and November 2018. The inclusion criteria were as follows: an age between 15 and 49 years and the presence of at least four inflammatory and four noninflammatory acne lesions. Subjects with prior topical retinoid acid or topical antifungal medication use within the past two weeks, systemic antibiotic or antifungal use within the past month, and corticosteroid use within the past six months were excluded. We determined the sample size using a nonpaired numeric analytical equation with 80% power, which gave a proposed sample size of 120 subjects. The sample was divided into two groups of 60: subjects with inflammatory lesions and those with noninflammatory lesions. An informed consent was obtained from all subjects.

Data and Sample Collection The physical examination consisted of AV severity degree scoring based on Lehmann's criteria. The degree of pruritus was graded using visual analog scale (VAS) and categorized into mild (1–3), moderate (4–7), and severe (8–10). Pictures of the subjects' faces were taken in five positions (anterior, right lateral, right lateral oblique, left lateral oblique, and left lateral). The follicular content of four noninflammatory lesions (comedones) or four inflammatory lesions (papules, pustules, or nodes) was collected from each subject. The chosen lesions were decided by randomization. Samples were taken by incision using a 26½ gauge needle and then extracted using a sterile comedone extractor. One lesion from each subject's sample was mounted on a glass microscope slide for microscopic examination, while the other three lesions were placed on sterile plates to be sent for culture on the same day within two hours. The flowchart of data and sample collection is shown in **Figure 1**.

Isolation and Identification of Malassezia spp. Samples on microscope slide were stained using a drop of 20% potassium hydroxide (KOH)

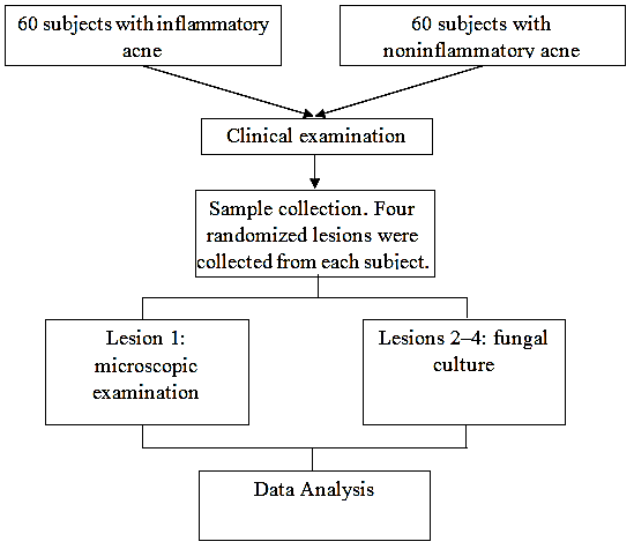

Figure 1 Flowchart of data and sample collection.

solution and Parker ink. Then, the slide was heated and examined under light microscope with 400x magnification. We used Jacinto-Jamora *et al*.[11] criteria to score the spore loads as follows: +1, one to two spores, no cluster; +2, small cluster of six or less spores or, if dispersed, 12 spores; +3, a large cluster of 7–12 spores or, if dispersed, 20 spores; +4, a cluster of more than 12 spores or, if dispersed, more than 20 spores.

Fungal cultures were performed in the Department of Clinical Microbiology of Universitas Indonesia. *Malassezia* samples were inoculated into CHROMagar *Malassezia*®, Dextrose Saboraud agar (DSA), and Tween 60-esculin agar.[11] Samples in CHROMagar *Malassezia*® were incubated at 32°C for 2 days. The grown colony was spread on the agar using streak technique and incubated again at 32°C for four days. The grown isolate was taken with a probe to be inoculated on DSA and Tween 60-esculin agar at 32°C for another 5-7 days. DSA was used to determine the fungal isolate's dependence on lipids for growth, while Tween 60-esculin agar was used to determine the fungal isolate's ability to hydrolyze esculin and utilize Tween 60. We also performed catalase test on the fungal isolates using 3% hydrogen peroxide.

The grown isolates on CHROMagar *Malassezia*® were observed on the 4th and 7th days. The size of colony was measured based on single isolate colony and categorized into small (<1 mm), medium (1-2 mm), or large (2-5 mm). The isolates on DSA were observed until the 14th day. Positive culture on DSA were defined as the presence of fungal colony growth with shiny creamy color, while positive culture on Tween 60-esculin agar was defined as the presence of fungal colony growth and blackening of the medium. Fungal colony morphological characteristic was described using revised criteria by Kaneko *et al*.[12]

Statistical Analysis Statistical analyses were performed using SPSS 20.0. Simple frequencies were tabulated, and association between factors was tested using chi-squared test. $P < 0.05$ was considered significant.

Results

Of the 120 subjects, 46 (38.3%) reported experiencing itching in facial acne lesions. **Table 1** showed the clinical characteristics of subjects between the two groups (inflammatory and noninflammatory). There was no significant difference between these two groups in terms of the clinical characteristics. The mean age of subjects was 22.7 (±6.2) years. There was also no significant difference between both groups in terms of the presence of pruritus ($p = 0.26$).

The presence of pruritus in correlation with lesion type and severity of AV was shown in **Table 2**. Pruritus was more common in mild facial AV compared to moderate-to-severe AV. The presence of pruritus in correlation with spore load and *Malassezia spp*. culture was shown in **Figure 2** and **Table 3**. Among the 120 subjects who underwent microscopic

Table 1 Clinical characteristics of subjects classified by acne vulgaris (AV) lesion type.

Parameter		*Total*		*Groups*				*p value*
				Non inflammatory lesions		*Inflammatory lesions*		
		n	*%*	*N*	*%*	*n*	*%*	
Age (mean ±SD)		22.7	(±6.2)	21	(15–40)	21	(15–36)	
Gender	Male	46	38.3	22	47.8	24	52.2	0.707
	Female	74	61.7	38	51.4	36	48.6	
AV severity	Mild	86	71.7	47	54.7	39	45.3	
	Moderate-to-severe	34	28.3	13	38.2	21	61.8	0.105
Pruritus	No	74	61.7	40	54.1	34	45.9	
	Yes	46	38.3	20	43.5	26	56.5	0.26
Pruritus severity	None	74	61.7	40	54.1	34	45.9	
	Mild	29	24.2	14	48.3	15	51.7	
	Moderate-to-severe	17	14.1	6	35.3	11	64.7	0.369
Prior medication	Yes	81	67.5	39	48.1	42	51.9	
	No	39	32.5	21	53.8	18	46.2	0.559

Table 2 Presence of pruritus in correlation with lesion type and severity of acne vulgaris (AV).

		Pruritus (n,%)		*p value*
		Yes	*No*	
Lesion type	Noninflammatory	20 (33.3)	40 (67.7)	0.260
	Inflammatory	26 (43.3)	34 (56.7)	
Severity of AV	Mild	30 (34.9)	56 (65.1)	0.216
	Moderate-to-severe	16 (47.1)	18 (52.9)	

Table 3 Presence and severity of pruritus in correlation with the presence of *Malassezia spp.* based on culture.

Pruritus		*Culture (n,%)*		*p value*
		Negative	*Positive*	
Pruritus	No	29 (39.2)	45 (60.8)	0.332
	Yes	14 (30.4)	32 (69.6)	
Pruritus severity	None	29 (39.2)	45 (60.8)	0.317
	Mild	7 (24.1)	22 (75.9)	
	Moderate-to-severe	7 (41.2)	10 (58.8)	

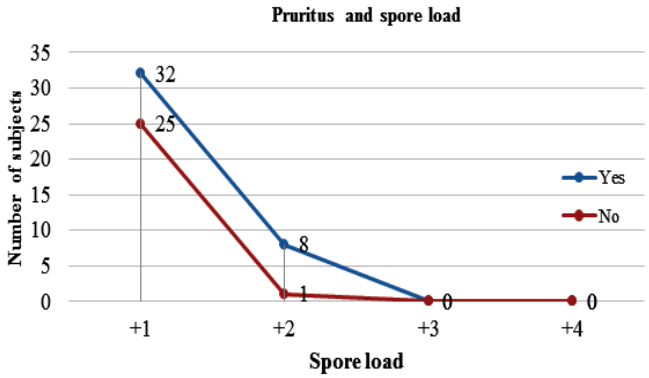

Figure 2 Presence of pruritus in correlation with spore load: +1, one to two spores, no cluster; +2, small cluster of six or less spores or, if dispersed, 12 spores; +3, large cluster of 7–12 spores or, if dispersed, 20 spores; +4, a cluster of more than 12 spores or, if dispersed, more than 20 spores.

examination, fungal spores were found in 66 subjects (55%), and pruritus was present in 40 subjects (60.6%). There was no significant difference in terms of spore load and the presence of pruritus in facial acne lesions (p=0.166). Pruritus was more common in culture positive group (69.6%), but there was no statistically significant difference in terms of the presence and severity of pruritus between subjects with positive and negative *Malassezia spp.* culture (p=0.332; 0.317). The most common *Malassezia spp.* identified in the pruritus group was *M. Dermatitis* (32.6%),

followed by *M Sympodialis* (10.9%) *and M. Slooffiae* (10.9%), as also reported in another study by Sutarjo *et al*.[10]

Discussion

One hundred and twenty subjects presenting with AV were included in this study. Most of the subjects had mild facial acne (71.7%), and pruritus occurred in 38.3% of subjects. A similar study conducted in Singapore, which has a climate similar to Indonesia, showed that pruritus is a common symptom in acne.[13] Lim *et al*.[13] reported a higher proportion of itch. Of the 120 patients, 80(70%) experienced itching in acne lesions, and 24(21%) had disturbed sleep due to the itching. Reich *et al*.[8] conducted a study among teenagers in Poland and showed that 13.8% of the subjects reported itching during the examination and 36.1% of the subjects reported experiencing itching in the past.

Fungal spore was found in 66 subjects (55%). Of those 66 subjects, 40 experienced pruritus in their acne lesions. The spore load was +1 (86.3%) in most subjects, and only 13.6% of the subjects had +2 spore load. When confirmed by fungal culture, *Malassezia spp.* were found in 69.6% of the subjects. *Malassezia spp.* are lipophillic yeasts, which are part of normal skin microbiota. *Malassezia* includes 17 species. Three of them are most frequently isolated from human skin: *M. globosa, M. restrica*, and *M. sympodialis.*[3] What determines the balance between commensalism and pathogenicity of *Malassezia spp.* are likely the changes in fungus, host, and environment, which promote the development of pathologies, such as interspecies variations, barrier defects, sebum production, and seasonal variation.[3]

MF is commonly misdiagnosed as acne vulgaris. *P. acnes* and *Malassezia spp.* have been known to induce keratinocyte to produce inflammatory cytokines via Toll-like receptor 2, which may explain the similarity between acne and MF lesions.[6] MF can also activate mast cells via the TLR-2 pathway to release inflammatory mediators and cytokines.[3,14] MF is characterized by lack of response to antibiotics, absence of comedones, and pruritic lesions which are findings that help in its differentiation from AV.[15] The diagnosis of MF can be supported by microscopic examination with KOH staining. Spore load of +4 is considered as diagnostic, whereas +3 and +2 are considered as highly suggestive of MF.[11] Low spore load can be misleading since *Malassezia spp.* are normal flora in the skin of healthy individual. Considering the low spore load in most of the subjects, we assume that pruritus in acne lesions in this study was not related to *Malassezia* spore load.

The mechanism of itching in acne is unclear. A previous study correlates between itching and acne severity, where inflammation plays a role.[16] Protease secreted by immune cells during inflammation activate protease-activated receptors (PARs) that induce inflammatory mediators and leucocyte recruitment.[17] Protease is also secreted by *P. Acnes*, which then leads to PAR-2 activation, a mediator in the nonhistaminergic itch pathway induction.[16-18] However, our study did not show a correlation between pruritus and acne severity as pruritus was more common in mild acne. Reich *et al*.[8] also reported that pruritus usually appears in mild and moderate acne. Heat and sweat were reported as exacerbating factors for itching as heat lowers the threshold of itching. Tropical humidity could also trigger *P. acnes* proliferation. The changes in microenvironment of follicle provide optimal environment for synthesis of histamine and histamine-like substances by *P. acnes*.[13,19]

Pruritus in acne may also aggravated by other pruritic skin diseases, such as atopic dermatitis, and may also be a complication resulting from acne therapy. The symptom usually appears between the second and sixth weeks of acne therapy.[8] However, this problem seems to be underestimated, since most of acne treatment guidelines do not discuss how to treat pruritus as a symptom related to AV, although it can lead to improper treatment compliance.[20] In this study, we did not assess the quality of life or disturbances caused by the itching, but previous studies mentioned that some patients were suffering from itching that was sufficient enough to cause distress, such as difficulty in falling asleep, feeling agitated, and depressed because of the itching.[8,13]

Conclusion

Even though acne is classified as a nonpruritic disease, our results showed that pruritus may accompany facial acne. The presence of pruritus in our study did not correlate with the severity of AV, presence of *Malassezia spp.*, and spore load in acne lesions. Nevertheless, since this is a cross-sectional study, we could not address direct causation. Further studies are needed to determine the causes of pruritus in facial acne lesions.

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