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## Prevalence of hair follicle mites, *Demodex folliculorum* and *Demodex brevis*, on the facial skin of Chiang Mai University Students, and the relationship with acne vulgaris

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### Abstract

The hair follicle mites, *Demodex folliculorum* and *D. brevis* are asymptomatic parasites of humans. To date, the genus *Demodex* has been implicated in the occurrence of many skin diseases. Thus, the main aim of this study was to determine the infestation rate of *Demodex* spp. in young adults and study the relationship between *Demodex* density and acne vulgaris. Furthermore, the mean densities of *Demodex* mites between clindamycin users and a control group were also observed in this study. Studied population consisted of 280 healthy volunteers from Chiang Mai University (age 20-22 years old). Skin samples were collected by a skin scraping technique and were examined for the parasite by direct microscopic examination. Both species were found in the resulting skin samples. The overall prevalence of *Demodex* mites on the facial skin of Chiang Mai University students was 40.36%. The mite prevalence in males (44.20%) was significantly higher than in female (36.62%) ( $P = 0.024$ ). *Demodex folliculorum* had a higher prevalence (31.1%) than *D. brevis* (26.1%). The mite density in males (0.89 *Demodex*/cm<sup>2</sup>) was also significantly higher than in female (0.49 *Demodex*/cm<sup>2</sup>) ( $P = 0.035$ ). We also found that males were approximately twice as likely to have acne vulgaris as females. Although *Demodex* has been involved in the etiology of many skin diseases, the parasite does not appear to be related to acne vulgaris in young adults ( $P = 0.313$ ). Thus, the eradication of *Demodex* mites is not necessary for the therapeutic treatment of acne vulgaris. The mites were found on all facial sites, the highest density being in the area of the nose followed by the forehead and cheeks, but there was no significant difference among these 3 areas. This study also determined the effect of clindamycin on *Demodex* mites. The results revealed that the use of clindamycin was not related to the density of *Demodex* mites. However, additional sample sizes for the clindamycin study are needed.

**Keywords:** *Demodex folliculorum*, *Demodex brevis*, acne vulgaris, skin scraping, young adults, clindamycin

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### Introduction

The hair follicle mites, *Demodex brevis* and *D. folliculorum* are asymptomatic parasites of

humans [1]. Both species were first recognized by Henle and Berger [2]. Subsequently, Akbutalova [3] proposed that *D. folliculorum* consisted of 2 subspecies, i.e., *D. folliculorum longus* and *D. folliculorum brevis* based on limited taxonomic criteria. However, Desch and Nutting [4-5] re-described those 2 subspecies by using statistical methods and morphological criteria, and the results revealed that they are distinct species. In addition, there are several studies on the biology and medical importance of these mites [1,6]

Although *Demodex* spp. are considered to be part of normal skin flora, the pathogenic role of *Demodex* is still a matter of debate. To date, the genus *Demodex* has been implicated in the occurrence of many skin diseases, such as rosacea, rosacea-like eruptions, some types of blepharitis and granulomatous dermal inflammation [7-10].

In Thailand, Sakuntabhai and Timpatanapong [11] found *D. folliculorum* from the skin lesions of a 39-year-old female patient who suffered from pruritic erythematous telangiectatic patches. Although the investigation of *Demodex* spp. in the laboratory has been reported in 2007 by Reangchainam [12], little is known about the prevalence of *Demodex* mites in this country as well as their relationship with acne vulgaris. Thus, the main aim of this study was to determine the infestation rate of *Demodex* spp. on the facial skin of Chiang Mai University students and study the relationship between *Demodex* density and acne vulgaris. In addition, the mean densities of *Demodex* mites between clindamycin users and control group were also observed in this study.

## Materials and methods

### Sample collection and microscopic examination

All facial skin samples were collected from a total of 280 healthy volunteers 20-22 years of age (140 each of male and female), who study at Chiang Mai University. This research has been approved by the Research Ethics Committee, study code no. PAR-12-1251-EX. All volunteers signed consent forms. They were asked to respond to the questionnaire concerning the gender, age,

cleaning face (soap, foam, soap and form or water) and drug treatment (e.g. clindamycin). Two groups of volunteers were classified based on the severity level of the level of acne, i.e., acne vulgaris patient (grade 4-8) and normal facial skin (grade 0-2) [13].

Skin samples were collected from 3 areas (forehead, cheeks and nose) of each volunteer using a skin scraping technique (Fig. 1) [12]. Briefly, the 1 cm<sup>2</sup> scaled-marking tape was applied on 3 areas of facial skin of each volunteer. The acne pressing-rod was used to scrape the skin in order to obtain skin debris (comedone). Then, each sample was smeared on a microscope slide with 1 drop of immersion oil and covered with a cover slip. The samples were examined for *Demodex* spp. within the area of 1 cm<sup>2</sup> under a light microscope (x40). Lastly, the prevalence and density of *Demodex* spp. were recorded.

### Data analysis

Chi-squared test was use to analyze the prevalence of *Demodex* mites between male and female while T-test was employed to compare (1) the mean densities of *Demodex* mites between each facial site; (2) the mean densities of *Demodex* mites between male and female; (3) the mean densities of *Demodex* mites between clindamycin users and the control group, and (4) the mean densities of *Demodex* mites between acne vulgaris patients and group with no acne vulgaris. The simple regression analyses were used to determine the relationship



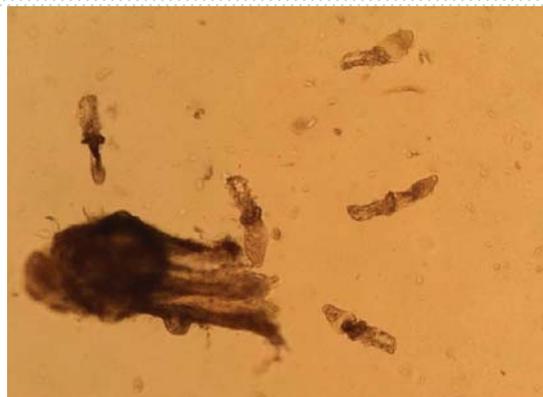
**Fig 1** Sample collection by skin scraping



**Fig 2** *Demodex brevis* (adult)



**Fig 4** *Demodex folliculorum* (adult)



**Fig 3** Group of *Demodex brevis* separated from hair follicle

between mean densities of *Demodex* mites with acne vulgaris. SPSS for windows version 16.0 was used for the analysis. The accepted level of significance was determined at 0.05% ( $P$ -value < 0.05).

## Results

Of 280 skin samples, *Demodex* mites were found in 113 (40.36%) samples. Only *Demodex brevis* (Figs. 2-3) was found in 26 (9.3%) samples, whereas *D. folliculorum* (Fig. 4) was found in 40 (14.3%) of both sexes. Both species were found in 47 (16.8%) samples. *Demodex* prevalence in males (44.20%) was significantly higher than in females (36.62%) ( $P = 0.024$ ). *Demodex folliculorum* had a higher prevalence (31.1%) than *D. brevis* (26.1%) (Table 1). The number of males with acne vulgaris (34/52) was significantly higher than in females (18/52) ( $P = 0.01$ ). However, the mite density was not significantly difference between the acne vulgaris and control group ( $P = 0.313$ ) (Table 2).

*Demodex* mites were found on all facial sites with the highest density being in the area of the

nose (3.87 *Demodex/cm*<sup>2</sup>) followed by the forehead (3.54 *Demodex/cm*<sup>2</sup>) and cheeks (3.19 *Demodex/cm*<sup>2</sup>), but there was no significant difference between these 3 areas ( $P > 0.05$ ) (Table 3).

The mites density in males (0.89 *Demodex/cm*<sup>2</sup>) was significantly higher than in females (0.49 *Demodex/cm*<sup>2</sup>) ( $P = 0.035$ ). Likewise, *D. folliculorum* density in males was also significantly higher than in females ( $P = 0.044$ ). Nonetheless, there was no significant difference in *D. brevis* density between them (Table 4).

Also, the mite density was not significantly difference between the clindamycin users (topical) and the control group ( $P = 0.838$ ) (Table 5).

## Discussion

Two species of follicle mites or *Demodex* (Greek: demos = fat; dex = woodworm) were found in humans, namely, *D. folliculorum* and *D. brevis* [14]. Morphologically, all stages of *D. folliculorum* are larger than the corresponding stages of *D. brevis*. The opisthoma end in *D. folliculorum* is rounded, while *D. brevis* is pointed. The length of opisthosoma in *D. folliculorum* is 7/10 of its body length, whereas that of *D. brevis* is 1/2 or 1/3. The eggs of *D. folliculorum* are arrow-shaped, whist the eggs of *D. brevis* are smaller and oval. Various studies used the "Standardized skin surface biopsy (SSSB) technique" to study the prevalence and the density of *Demodex* mites. This technique uses the application of cyanoacrylic adhesive to collect skin

**Table 1 Prevalence of *Demodex folliculorum* and *D. brevis* in male and female**

Species		Male	Female	Both sexes
<i>D. brevis</i>	n	15	11	26
	%	5.4	4.0	9.3
<i>D. folliculorum</i>	n	25	15	40
	%	9.0	5.4	14.3
Both species	n	21	26	47
	%	7.5	9.3	16.8
Host Total examined	n	138	142	280
<b>Total positive</b>	<b>n</b>	<b>61</b>	<b>52</b>	<b>113</b>
	<b>%</b>	<b>44.20</b>	<b>36.62</b>	<b>40.36</b>

**Table 2 Density of *Demodex folliculorum* and *D. brevis* in acne and control groups**

Studied group	n	<i>Demodex</i> density ( <i>Demodex/cm</i> <sup>2</sup> )		
		median	Q3-Q1	Mean
Control (acne grading < 4)	228	0	2	0.75
Acne vulgaris (acne grading > 4)	52	0	2	0.42
				0.313

**Table 3 Mean densities of *Demodex folliculorum* and *D. brevis* in each facial site (n = 113)**

Site of collection	<i>Demodex</i> density ( <i>Demodex/cm</i> <sup>2</sup> )
Forehead	3.54
Cheek	3.19
Nose	3.87
	<i>P</i> > 0.05

**Table 4 Mean densities of *Demodex folliculorum* and *D. brevis* in male and female (n =113)**

Sex	<i>D. folliculorum</i>	<i>D. brevis</i>	<i>Demodex</i> density ( <i>Demodex/cm</i> <sup>2</sup> )
Male	0.51	0.37	0.89
Female	0.23	0.26	0.49
<i>P</i> -value	0.044	0.784	0.035

**Table 5 Mean densities of *Demodex folliculorum* and *D. brevis* in clindamycin users and control group (n= 280)**

Sex	<i>D. folliculorum</i>	<i>D. brevis</i>	<i>Demodex</i> density ( <i>Demodex</i> /cm <sup>2</sup> )
control	0.38	0.31	0.70
clindamycin	0.14	0.22	0.36
<i>p</i> -value	0.795	0.640	0.838

samples for microscopic examination. However, the SSSB technique requires expensive imported chemicals and can lead to errors as the adhesion may be inadequate when attaching to the mites [15] and also has limitations in collecting *D. brevis* which inhabits a deep part of the pilosebaceous unit [16]. Thus, the "skin scraping technique" was employed in this study [12], and the positive results for *D. brevis* (26.1%) and *D. folliculorum* (31.1%) obtained from this study confirmed that this technique is effective for both species.

In the present study, the prevalence of *D. folliculorum* and *D. brevis* were 40.36%. In 66 (23.6%) samples, only 1 species was found, whereas in 47 samples (16.8%) both species were found, which was significantly higher than was found in people of the same age from various studies. For example, the study of Cao [17] in Chinese college student reported the prevalence of *Demodex* mites at 36.3% while 28.6% in the USA [18], 17.2 % in Malaysian medical students [19], and only 5% in the Polish young age group [20]. The results obtained in this study demonstrated that the rate of *Demodex* infestation is different in various sociodemographic sectors and also indicates that the young adults who study in Chiang Mai University with an age range of 20 to 22 years old has a high prevalence of *Demodex* mites when compared with other regions.

The prevalence and density of *Demodex* are related to gender, as the incidence is higher in male young adults. This result is in accordance with [21] which reported that the prevalence in males and females are 55.7% and 48.7% respectively. The reason why males have a higher incidence remains unknown, but it may be due to a higher sebum production in males and also

different facial hygiene practices.

Various studies have exhaustively investigated whether *Demodex* is involved in skin pathology. Ayres [7-8] found that *Demodex* was abundant in superficial vesicles and in the pustules of acne rosacea. Gmeiner's [22] study shows *Demodex* causes follicular dyskeratosis. *Demodex* was also shown to be a vector of some bacteria such as Leprosy sp. in Borrel's study [23]. Spickett [16] also found that where hair follicle were infected with *Demodex* (69%) there was a higher incidence of *Staphylococcus aureus* than in normal follicles (50%). However, this study shows no significant relationship between *Demodex* density and acne vulgaris. Some trials, such as Brown's [24] and Hervás's [25], found that ivermectin or ethyl ether are effective in *Demodex* eradication. The current study also consisted of questionnaires eliciting the efficacy of topical clindamycin. Compared to the control group, clindamycin does not reduce the incidence of *Demodex* mites.

In conclusion, the results obtained from this study showed that the mite prevalence and density in males were significantly higher than in females. The eradication of *Demodex* is not necessary for effective acne vulgaris therapy. However, the clindamycin group has a small sample size and the factors are not controlled as tightly as needed for a clinical trial, hence further study is needed for this result to be truly valid.

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