STANDARDIZED SKIN SURFACE BIOPSY - A BETTER DIAGNOSTIC OPTION FOR DEMODICOSIS IN MACULAR ROSACEA IN VIETNAMESE PATIENTS

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SUMMARY

Introduction: Demodicidosis is a chronic skin disease, caused by two species of Demodex *(D. folliculorum and D. brevis)*. *Demodex* infected individuals are mainly symptomless and may have pathogenic symptom only when mite density is high within the skin.

Objectives: To compare the value of the Standardized skin surface biopsy (SSSB) and direct microscopic examination with KOH (DME) for assessing *Demodex* density.

Methods: Fifty patients with demodicosis were determined *Demodex* by SSSB and DME. Comparision of the similarity of test results between the two methods was conducted by Cohen's Kappa statistic.

Results: The positive test rate of SSSB is 90.0%, of DME is 82.0%. The percentage of patients who tested positive for both methods was 76.0%, and the negative for both methods was 4.0%. *Demodex* density > 5/cm^2 of SSSB was 47.9% higher than DME of 29.2%. There was quite similarity of *Demodex* density between SSSB and DME with coefficient kappa = 0.62.

Conclusion: SSSB is more sensitive method for detecting Demodex than DME, particularly in patients with macular lesions, vasodilatation, U-shaped distribution, located on the nose and nasolabial sulcus.

Keywords: *Demodex, demodicosis, standardized skin surface biopsy, direct microscopic examination.*

1. INTRODUCTION

Demodex spp. is one of the most frequently mite lives in the hair follicle in human and malaria animals. Previous research has established that demodex mite is common in healthy human with 23% to 100% in the skin[1]. Although there are 65 species of demodex in natural world, but also it has two species found mostly in human included: D. folliculorum lives in the upper portion of the follicle unit, while D. brevis lives deeper[2]. Lacey et al suggested that Demodex can cause disease when increasing the normal mite population. There are four sides assosiated with changing from normal fauna to pathogenic activity: (1) High demodex density in the upper of the sebaceous hair follicles causes blockage; (2) granulomatous reaction with kitin; (3) The mite play role as a vector for bacteria and fungi for invasion; (4) immune responses to Demodex and their secretory products[3,4,5,6]. So, it is necessary to determine the density of demodex (Dd) to confirm the pathogenicity. There are some method for detection of Demodex mites such as standardized skin surface biopsy (SSSB),

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direct microscopic examination with potassium hydroxide, skin biopsy, and using confocal laser scanning microscopy[7]. SSSB method has been recognized as a gold standard for detecting Dd. However, the mite is difficult to visualize and stain. Kiuchi et al in the study blepharitis suggested that methylene blue was useful to assess the number of demodex[8]. Morever, direct examination microscopy (DME) with potassium hydroxide is a common method to test fungus on the skin. Bunyaratavej et al compared skin scraping versus SSSB to detect demodex mites found the sensivity and specificity of skin scraping with potassium hydroxide was 75% and 84.2%, but the author had not evaluate the number of Demodex of two method. This paper asseses the significance of SSSB with methylen blue and DME in evaluating Demodex density as well as factors related to it.

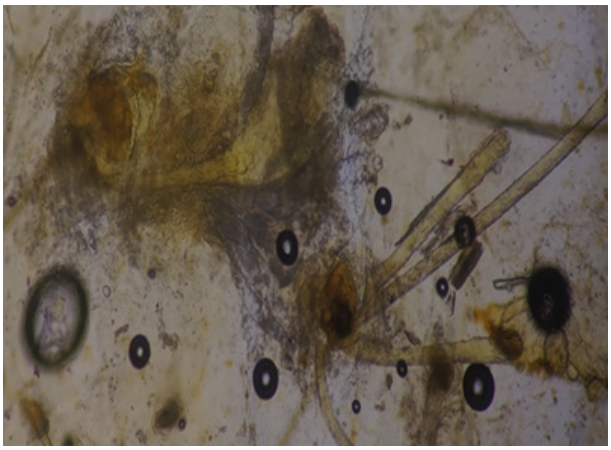
2. PATIENTS AND METHODS

2.1. Patients

Fifty patients (17 of rosacae, 12 of topical corticosteroid-included contact dermatitis and 21 of acne) with symptomes suspected Demodex infection were selected to participated in the study at the National Hospital of Dermatology and Venereology, Ha noi, Viet nam between June 2019 and June 2020. We also excluded patients who had used anti-parasitic drugs, sloughing off the scales for one month.

2.2. Standardized skin surface biopsy (SSSB) with metylene blue

A drop of cyanoacrylate glue was put on microscope slide, wait for 1 - 2 minutes until the glue reach a uniform density. The slide then was gently applied on the skin surface to be biopsied (facial skin areas: between the eyebrows, eyelids, nose, nasolabial folds, cheeks, chin and around the mouth and skin on back, chest, scalp...). Let the cyanoacrylate glue dried for 3 - 5 min, then gently removed the slide that carrying a biopsy skin layer. A drop of mixture (KOH 20%: Methylen blue = 1:2) was added on the slide. For patients who used cosmetics, or had a lot of oily skin, it is necessary to wipe off the outer layer of skin and then proceed to scrape to take samples.



Observation of *Demodex* was taken by microscopy (40X and 100X magnification).

Figure 1. Demodex mite in 40x microscope magnification power was detected by SSSB with methylene blue

2.3. DME with potassium hydroxit

One square centimeter sized skin area was scraped onto microscope slide. A drop of 10% KOH was added on the slide. Slide was covered and used for observation by microscopy (40x and 100x magnification).

Steps to conduct the study

Both methods were performed on the same lesion site on each patient. The SSSB was performed first on the first half of the lesion site and then the DME was performed on the other half.

Data processing

Data are entered and processed by SPSS 23.0 statistical software. Evaluating test results: comparing the similarity of test results between the two methods by Cohen's Kappa statistic: < 0.00: Poor; 0.00 - 0.20: Slight; 0.21 - 0.40: Fair; 0.41 - 0.60: Moderate; 0.61 - 0.80: Substantial; 0.81 - 1.00: Most perfect.

3. RESULTS

The result shows that with 50 patients in total, patients aged from 40 to 49-year-old are the majority (28%). The youngest patient was 16-year-old and the oldest patient was 76-year-old and patient average age is 37.7.

Table 1. General characteristics of the study subjects (n = 50)

Characteristics		n	%
Age		37.7 ± 15.0 (16 ÷ 74)	
Gender	Male	23	46.0
	Female	27	54.0
Physical symptom	Itching	22	44.0
	Burning	24	48.0
Skin lesions	Maculopapular	45	90.0
	Red papules	31	62.0
	Scab	9	18.0
	Pustules	7	14.0
	Vasodilation	17	34.0
Clinical diagnosis	Contact dermatitis	12	24.0
	Rosacea	17	34.0
	Acne	21	42.0

Table 2. The result of direct examination by SSSB and KOH (n = 50)

		SSSB				Total	
		Positive		Negative			
		n	%	n	%	n	%
KOH	Positive	38	76.0	3	6.0	41	82.0
	Negative	7	14.0	2	4.0	9	18.0
Total		45	90.0	5	10.0	50	100

The positive test rate of SSSB with metylene blue and DME was 90.0% and 82.0%, respectively. The percentage of patients who tested positive by both methods was 76.0%, and negative by both methods was 4.0%. There were 48 patients with direct examination to detect *Demodex* by either SSSB or KOH, the results were as follows:

Table 3. Comparison of demodex density with SSSB and DME (n = 48)

		SSSB				Kappa
		> 5 D/cm2		≤ 5 D/cm2		
		n	%	n	%	
DME	> 5 D/cm2	14	29.2	0	0	0.62
	≤ 5 D/cm2	9	18.7	25	52.1	

Table 4. Demodex density with clinical symptoms, lesion characteristics, location of lesion and clinical diagnosis (n = 48)

Characteristics		SSSB	DME	p
		(X ± SD)	(X ± SD)	
Clinical symptoms	Maculopapular	9.4 ± 9.5	7.3 ± 7.5	0.003
	Erythema papules	8.0 ± 8.2	6.7 ± 6.2	0.12
	Scab	10.0 ± 6.9	7.0 ± 7.2	0.12
	Pustules	8.1 ± 5.6	6.0 ± 6.0	0.09
	Vasodilation	12.6 ± 11.0	10.3 ± 9.0	0.04
Lesions distribution	U shape	7.4 ± 5.1	4.9 ± 4.4	0.001
	T shape	9.5 ± 13.0	6.8 ± 8.8	0.18
	Scattered	9.5 ± 9.4	7.8 ± 7.7	0.06
Location of lesion	Forehead	9.7 ± 9.6	7.8 ± 7.9	0.07
	Cheek	8.8 ± 8.8	7.3 ± 7.0	0.07
	Nose	8.4 ± 9.8	6.8 ± 7.9	0.01
	Nasolabial fold	9.8 ± 9.9	7.4 ± 8.0	0.001
	Around the mouth	8.6 ± 8.9	7.8 ± 8.0	0.21
	Chin	11.0 ± 10.6	9.0 ± 8.2	0.06
Clinical diagnosis	Contact dermatitis	11.1 ± 7.6	10.4 ± 7.9	0.29
	Rosacea	10.8 ± 11.7	6.5 ± 7.3	0.01
	Acne	6.7 ± 7.4	5.9 ± 6.9	0.12

The average number of *Demodex* detected by SSSB with methylene blue method was higher than that of DME method with maculopapular and vasodilation, the difference was statistically significant with $p < 0.05$. There was no difference between the two methods in red papules, scabs, and pustules. For lesion characteristics, number of *Demodex* detected by SSSB with methylene blue method is higher than that of DME method in focal lesions, known boundary and U-shaped distribution, the difference is statistically significant with $p < 0.05$. A similar phenomenon was observed with features: location of lesion and clinical diagnosis.

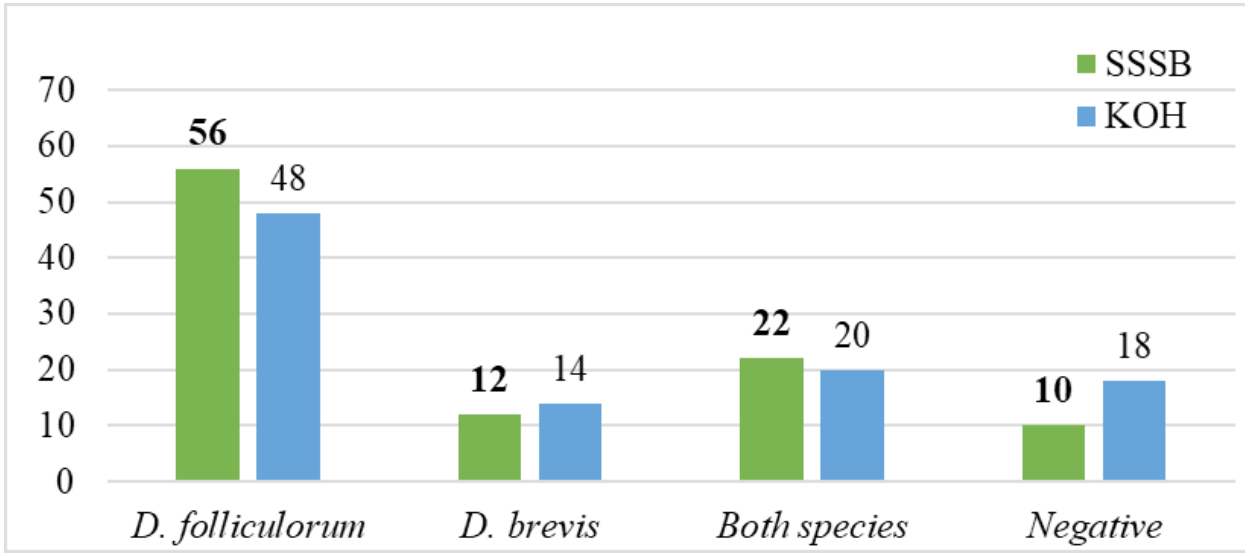


Figure 1. Distribution of Demodex species determined by two methods (n = 50)

4. DISCUSSION

Our result suggests that SSSB with metylene blue should be used to detect *Demodex* for rosacea. Askin et al found that the SSSB had a higher positive rate than skin scrapings for *D. folliculorum* species[9]. Positive detection rate of SSSB is higher than skin scrapings because it can collect hair follicles where *Demodex* mites live. Our data showed similar result to research of Bunyaratavej et al[10]. Direct microscopy examination with skin scrapings is a simple, high sensitivity and low false-negative rate method.

Many different views have been given to explain the pathogenic mechanism of the species, in which, the authors agree that *Demodex* density > 5/cm^2 is the standard to confirm disease caused by *Demodex*. Our results are consistent with researches of Bunyaratavej[10]. The skin scrapings is an efficient, saving of time and suitable technique for estimating *Demodex* density. Yun et al (2017) reported that skin scrapings is more effective than SSSB in patients with demodicosis[11]. Our data showed that both methods are equally effective in determining the presence of *Demodex* mites. However, the SSSB obtains lesions deeper in the hair follicle, so the number of *Demodex* per 1cm^2 of skin of this method is higher than that of skin scrapings. Other research using scraping technique also showed similar results with our study[9]. Our results suggested that SSSB has higher *Demodex* density results than skin scrapings. The difference between the two methods may be due to *Demodex*'s parasitic location in the hair follicle as well as the sample collection technique.

In this study, we selected three groups of common clinical form. The results indicate that the average number of *Demodex* detected by SSSB is higher than that of skin scrapings with rosacea diagnosis. According to Yun et al (2017), there is a difference in the *demodex* density by two methods with rosacea, but no difference with acne or pityriasis folliculitis[11]... A study by Georgala (2001) on 92 rosacea patients and 92 healthy individuals showed that D. folliculorum

was detected in 83 out of 92 rosacea patients (90.2%), while in the group control detected in 11 subjects (11.9%)[4]. *D. folliculorum* lives in superficial hair follicles, which often causes rosacea folliculitis, and *D. brevis* that lives in deep hair follicles, often causes rosacea. Most patients have severe papules and pustules, so it is more difficult for SSSB to detect *Demodex*[11].

5. CONCLUSION

Based on the results of the study, it is concluded that SSSB with methylene blue is more sensitive method for detecting Demodex than DME, particularly in patients with macular lesions, vasodilatation, U-shaped distribution, located on the nose and nasolabial sulcus.

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