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RESEARCH PAPER

Cleanser use could decrease numbers of Demodex Folliculorum in mild to moderate acne patients

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ABSTRACT

Objective: This study was to verify if the skin cleanser could help decrease the infection ratio of Demodex Folliculorum in acne patients. **Methods:** 132 participants with mild to moderate vulgaris acne participated in this monocentric, prospective, double-blind study. Dermatologists grading and Standardized Skin Surface Biopsy were performed in baseline and after using cleanser only 7 d later. **Results:** There was no significant difference between the 2 times for each type of acne, but the number of Demodex Folliculorum was significantly decreased compared with baseline. There was no relationship between the number of Demodex Folliculorum and the total number of acne lesions. **Limitations:** Short follow-up time in 7 d. **Conclusion:** Using the cleanser could decrease the average number of Demodex Folliculorum in only 7 d in mild to moderate acne patients. There is no relationship between Demodex and acne lesions number.

Introduction

Until now, the etiology of acne vulgaris has been uncertain.[1] Demodex mites are normal colonizers of the skin around the sebaceous glands, and these mites have been associated with several other skin conditions, including Papulopustular Rosacea[2] and Pityriasis Folliculorum.[3] Whether Demodex infestation plays a part in acne pathogenesis is therefore quite a relevant question. Although clinicians usually deny the association between Demodex infestation and acne vulgaris, Zhao et al confirmed the association between them through a meta-analysis from 63 publications.[4] However, to obtain more convincing data, it was necessary to conduct a clinical trial with a large sample size to confirm the relationship between Demodex infestation and acne vulgaris. In this study, Standardized Skin Surface Biopsy (SSSB) was used to measure Demodex Folliculorum density (Dd).[5]

Sociodemographic characteristics and risk factor for Demodex infestation were identified by a cross-sectional study in China.[6] Its results showed that the

Odd Ratio (OR) of using a facial cleanser was 1.0 time *versus* not using a cleanser. However another study demonstrated that a lower Demodex infestation rate was found to be associated with the use of skin care product.[7] Regrettably, these skin samples were all obtained using both skin scraping and the cellophane tape method, which are not optimal methods in searching for Demodex Folliculorum (DF).[5]

In this clinical trial, mild to moderate acne patients used a cleanser for 7 d. The SSSB technique was used to assess the Dd for each patient. The objective of the study was to verify if the skin cleanser could help decrease the infection ratio of Demodex in acne patients.

Results

Clinical scoring of different types of acne

The whole face of the acne patients was evaluated twice: before using the cleanser and after using it for 7 d. Two experienced Chinese dermatologists were

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Table 1. Number of lesions for each type of acne at both assessment times (Median (Q1, Q3)).

	Baseline	After 7 days
Open Comedones	2 (0, 6.75)	2 (0, 5.75)
Closed Comedones	4 (2, 8)	4 (2, 7)
Papules	14 (11, 20)	16.5 (11, 22)
Pustules	1 (0, 3)	1 (0, 2)
Number of Inflammatory Lesions[1]	16 (12, 22.75)	18 (11.25, 24)
Number of Non-Inflammatory Lesions[2]	7 (3, 13)	6 (3, 12.75)
Total Number of lesions[3]	24.5 (17, 34.5)	26 (16.25, 34)

Notes:
[1]Inflammatory lesions = papules and pustules;
[2]Non-inflammatory lesions = open comedones and closed comedones;
[3]Total number = adding numbers of lesions for all types of acne.

in charge of the evaluation and counted the different types of acne lesions. Closed comedones, open comedones, papules and pustules were counted respectively. There was no significant difference between the 2 time points for each type of acne ($P > 0.05$) (Table 1).

Dd in SSSB

Through SSSB, we assessed the Dd in each patient's cheek at both time points and we also calculated the Average number of DF, Positive Ratio (Dd $\geq$ 5DF/cm^2 was regarded as a positive case), Relevance Ratio (Dd $\geq$ 1DF/cm^2 was regarded as a relevance case) and Zero Ratio (Dd $=$ 0 DF/cm^2). After using the cleanser for 7 days, the number of DF was significantly decreased compared with baseline ($P < 0.05$). There was no statistical change for the Positive Ratio, but the Zero Ratio (ZR) was significantly increased after using the cleanser for 7 d only ($P < 0.10$) (Table 2 and Fig. 1).

Relationship between the number of acne lesions and Dd at baseline

Pearson's correlation coefficient showed that there was no relationship between Dd and the total number of

Table 2. Changes in Dd after using cleanser.

	Baseline	After 7 days	P value
Average number of DF (Mean $\pm$ SD)	1.77 $\pm$ 3.60*	1.50 $\pm$ 4.13*	**0.022**
Dd $\geq$ 5 DF/cm^2 (n)	14	12	
PR (%)	10.6	9.1	0.754
Dd $\geq$ 1 DF/cm^2	56	42	
ReR (%)	42.4	31.8	0.119
Dd $=$ 0 DF/cm^2(n)	76	90	
ZR (%)	57.6	68.2	**0.059**

Note: There was a statistical decrease in the Average number of DF after using the cleanser for 7 d only ($P < 0.05$). PR: Positive Ratio; ReR: Relevance Ratio; ZR: Zero Ratio.
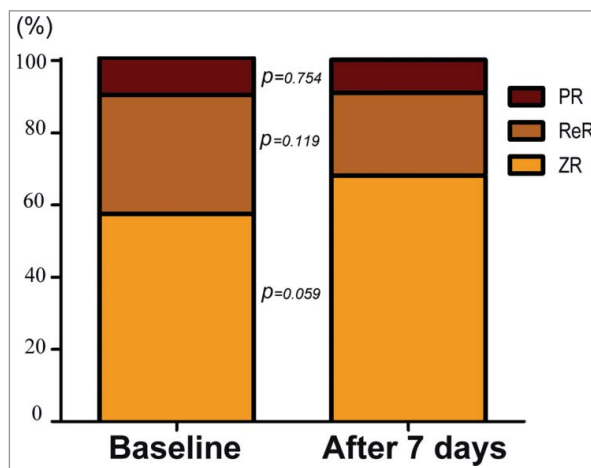

Figure 1. Changes in the different ratios in Baseline and after cleanser using 7 days. PR : Positive Ratio; ReR : Relevance Ratio; ZR : Zero Ratio.

acne lesions ($r = -0.23$, $P < 0.05$) or inflammatory lesions ($r = -0.17$, $P < 0.05$) or non-inflammatory lesions ($r = -0.21$, $P < 0.05$) (Fig. 2).

Discussion

The pathophysiology of acne is complex and multifactorial, and its etiology is considered as related to factors such as heredity, androgens, increase of sebum secretion, dyskeratosis of pilosebaceous duct, follicular orifice block up and proliferation of Propionibacterium. Recently further research has focused more on Demodex infestation and the development of acne. Zhao performed a meta-analysis of 63 studies, among them 43 found an association and 15 found no association[4] between Demodex infestation and the development of acne. The Odd Ratio (OR) of an association between Demodex infestation and the development of acne was significant at $P = 2.80$ (95% CI 2.34–3.36). Four different methods were described in the 63 studies: Cellophane Tape Method (CTP) over a short period of time, CTP overnight, extruded sebum smear microscopy and scraping method. Among the assessment methods for Demodex Folliculorum, the most efficient is Standardized Skin Surface Biopsy (SSSB), but no reference used SSSB in these 63 studies. SSSB is a non-invasive sampling method by which it is possible to collect the superficial part of the horny layer and the contents of the pilosebaceous follicle.

Demodex Folliculorum (DF) is a 0.3-mm long mite that parasitizes healthy skin with a density $\leq$ 5 Dd/cm^2 in adult population. In many skin diseases, such as Pityriasis folliculorum, Papulopustular rosacea
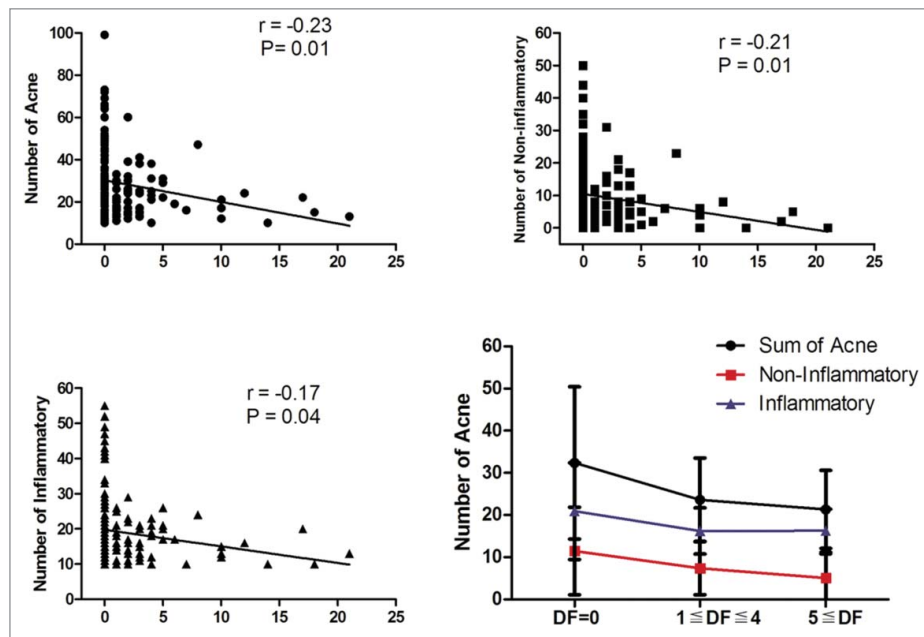
Figure 2. Relationship between the number of acne lesions and Dd at baseline.

(PPR), DF multiples and penetrates the dermis. There is a significant difference in the density of DF[8] between healthy skin and skin affected by disease. In this study, we tested DF's presence and mean density in acne vulgaris by SSSB, which helps to compare the different inflammation levels in various skin conditions (Table 3). Compared with healthy skin, assessments using SSSB show that DF's Presence and Mean Density in acne vulgaris are between healthy skin and PPR. It is well-known that the etiologies of rosacea and acne are completely different, now these findings show that the DF infestation situation is also different.

In the study of risk factors of Demodex infestation, questionnaires were given to healthy subjects and then a logistic regression analysis was performed. The results for the subjects using the facial cleanser had no statistical relationship regarding the Demodex infestation (P = 0.170).[6] Through our study, we found that using the cleanser could decrease the average number of DF in only 7 d in acne patients. These 2 conclusions are not contradictory because 2 different test subjects could not compare directly. Many clinical studies have

shown that the combined use of a facial skin cleanser and a moisturizer is safe and effective for the care of acne without skin irritation by intensive washing,[10] and that an acne cleanser could reduce both inflammatory and non-inflammatory acne lesions.[11] The use of a cleanser is recommended in the treatment of acne vulgaris. We also found that there was statistically no improvement in the Positive Ratio of DF within 7 days, only the Zero Ratio was increased (P = 0.059). These phenomena suggest that the infestations of DF in acne patients were at different levels, and that mild infestation (DF < 5 D/cm^2) is easy to clear in just 7 d. But if the infestation is more than 5 D/cm^2, more time may be necessary to wipe out Demodex. In a future study, we need longer follow-up time to see the details.

Regarding the finding of a missing relationship between Demodex and acne lesions number, we reached the same conclusion as many clinical dermatologists.[12,13] However, more clinical research is needed to determine whether the Demodex infestation situation could have an impact on the skin barrier function, and then have some impact on skin sensitivity in acne patients.

Materials and methods

Participants

This monocentric, prospective, double-blind study enrolled 132 participants with mild to moderate facial

Table 3. DF results by SSSB in different skin conditions.

	Presence (%)	Mean Density (DF/cm^2)
Healthy Skin	11.9	0.7[8]
Rosacea	/	10.8[8]
Papulopustular Rosacea	90.2	36[9]
Pityriasis Folliculorum	/	61[9]
Acne Vulgaris	42.4	1.77

acne vulgaris. Individuals with facial nodules and cysts or active psoriasis or a history of psoriasis, active allergic skin responses, or severe eczema were excluded. In addition, subjects that had been under treatment of any type of cancer within the last 6 months, or subjects that had used anti-inflammatory drugs, anti-acne drugs, immunosuppressive drugs, or antihistamine medications were also excluded. There were 92 females and 40 males, and the average age was 23.4 y with a standard deviation of 3.3 y. Each subject had signed a copy of the informed consent before joining the study. The study was conducted strictly in accordance with the instructions governed by the Ethic Committee of Shanghai Skin Disease Hospital (No. 2014–005).

Test materials

For the test, we used a cleanser without any pesticide or antimicrobial agent, which was widely available on the market (POND's intensive moisture cleanser). Each subject was exposed to the cleanser twice a day only for 7 days, once in the morning, once in the evening. The first application of the products was done at the testing facility under the supervision of the testing facility staff. Subjects subsequently self-administered the cleanser at home according to instructions and kept a diary for the product use.

Clinical scoring

Two well-trained dermatologists counted the number of lesions according to their types, including non-inflamed lesions (closed and open comedones) and inflamed lesions (papules and pustules), at baseline and 7 d later. All data were recorded on individual subject data forms.

Standardized skin surface biopsy (SSSB) for Demodex folliculorum density (Dd)

Firstly, the patient's cheek was cleaned with water before the test. Then cyanoacrylate glue (3S BIOKIT) was dropped on a marked 1 cm^2 area on a glass slide. The adhesive bearing surface of the glass slide was applied for 1 min on the cheek. The specimen was examined by light microscopy at $\times$ 40 and $\times$ 100 magnifications. After one week, the same SSSB procedure was repeated on the other cheek.

Statistical analysis

The SPSS13.0 software (Inc., Chicago, IL, USA) was used in the calculation of statistical data, which are expressed as Median (Q1, Q3), Mean $\pm$ SD and assessed for statistical significance. We used Wilcoxon Signed Ranks Test to compare the clinical scoring and Dd values at different times. McNemar Test was used to compare the Positive Ratio (PR), Relevance Ratio (ReR) and Zero Ratio (ZR) for the 2 different times. We used Pearson's correlation coefficient and conducted a linear regression analysis to determine correlations between Dd and acne lesions. A value of $P < 0.05$ was considered as being statistically significant.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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