High *Staphylococcus epidermidis* Colonization and Impaired Permeability Barrier in Facial Seborrheic Dermatitis

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Abstract

Background: Seborrheic dermatitis (SD) is a common inflammatory skin condition. The etiology is unclear, although overgrowth of *Malassezia* on the skin has been suggested to cause SD. This study investigated whether colonization with *Staphylococcus* plays a role in facial SD, which was not well addressed previously.

Methods: The study was conducted from September 1, 2011 to February 20, 2012 in the First Hospital of China Medical University. In the first phase, the study evaluated the level of transepidermal water loss (TEWL) and the number of colony-forming units (CFU) of *Staphylococcus* in defined skin areas of SD patients who were human immunodeficiency virus (HIV) seropositive (HIV [+] SD [+] group, $n = 13$), classical SD (HIV [−] SD [+] group, $n = 24$) patients, HIV seropositive-non-SD (HIV [+] SD [−] group, $n = 16$) patients, and healthy volunteers (HIV [−] SD [−] group, $n = 16$). In the second phase, we enrolled another cohort of HIV (−) SD (+) patients who applied topical fusidic acid ($n = 15$), tacrolimus ($n = 16$), or moisturizer ($n = 12$). Changes in the Seborrheic Dermatitis Area Severity Index (SDASI), TEWL, and *Staphylococcus* density were evaluated 2 weeks later. Comparisons of each index were performed using analysis of variance (ANOVA) and least significant difference method.

Results: The level of TEWL was greater through lesional sites in the HIV (+) SD (+) group than that in HIV (+) SD (−) and HIV (−) SD (−) groups (95% confidence interval [*CI*]: 18.873–47.071, $P < 0.001$ and 95% *CI*: 28.755–55.936, $P < 0.001$, respectively). The number of CFU of *Staphylococcus* was greater in the HIV (+) SD (+) group than that in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 37.487–142.744, $P = 0.001$ and 95% *CI*: 54.936–156.400, $P < 0.001$, respectively). TEWL was significantly more improved in patients treated with tacrolimus and fusidic acid than that in those treated with moisturizers (95% *CI*: 7.560–38.987, $P = 0.004$ and 95% *CI*: 4.659–37.619, $P = 0.011$, respectively). Topical tacrolimus and fusidic acid were significantly associated with decreased SDASI as compared with moisturizer (95% *CI*: 0.03–0.432, $P = 0.025$ and 95% *CI*: 0.033–0.44, $P = 0.024$, respectively).

Conclusions: High colonization with *Staphylococcus epidermidis*, along with impaired skin permeability barrier function, contributes to the occurrence of SD.

Key words: Human Immunodeficiency Virus; Seborrheic Dermatitis; *Staphylococcus*

Introduction

Seborrheic dermatitis (SD) is characterized by erythematous pruritic patches and plaques, with greasy scales, that occur in skin areas that contain a high density of sebaceous glands, such as the scalp, face, chest, and back. SD affects approximately 1–3% of the adult population and occurs in persons of all races.[1] There is a high prevalence of SD among persons with Parkinson's disease or human immunodeficiency virus (HIV) infection, which ranges from 30% to 83% in HIV-positive and AIDS patients.[2,3] The course of SD is characterized by outbreaks that may be triggered by emotional stress, depression, fatigue, and change of season.[4] The incidence and severity of SD is greatest during winter months.

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The etiopathological mechanism of SD remains elusive. The ages of its greatest incidence peaks (infancy, adolescent, and adults over 50 years of age) suggest that hormonal changes contribute to disease progression. In SD, sebum production is excessive,[5,6] and topically applied human sebum induces an irritant-like dermatitis in mice.[7] *Malassezia* yeasts tend to appear on the skin at the age of puberty. Among the known normal species of commensal *Malassezia* yeast of adult human skin, colonization with *Malassezia globosa* and *Malassezia restricta* is claimed to be associated with SD.[8,9]

A recent study by Tanaka *et al*.[10] analyzed bacterial microbiota on nonlesional and lesional sites of 24 patients with SD using pyrosequencing and quantitative real-time polymerase chain reaction. The results show a predominance of *Acinetobacter*, *Staphylococcus*, and *Streptococcus* on lesional sites. The authors suggest that, in addition to *Malassezia*, these commensal bacteria might contribute to SD development. Likewise, our preliminary study indicated a greater predominance of these phyla on SD lesional skin as compared to normal controls (unpublished data). Studies on both French and Chinese populations suggested that dandruff scalps, often associated with SD, are associated with a high incidence of *M. restricta* and *Staphylococcus epidermidis*.[11,12]

This study investigated whether changes in permeability barrier function and colonization with *Staphylococcus* were related to facial SD, to improve the management strategy for this incompletely understood condition.

Methods

Ethical approval

As a noninvasive study, the study was exempt from the ethical approval. The oral informed consent of patients was acquired. The study was conducted in accordance with the *Declaration of Helsinki*.

Study population

To best exclude the influence of climatic changes, the study was conducted from September 1, 2011 to February 20, 2012 in the First Hospital of China Medical University, a cool and cold season of the region. In the first phase of the study, 37 consecutive SD patients were enrolled, including 13 SD patients who were HIV seropositive (HIV [+] SD [−] group) and 24 patients with classical SD (HIV [−] SD [+] group). The controls included 16 HIV seropositive-non-SD (HIV [+] SD [−]) patients and 19 healthy volunteers (HIV [−] SD [−]). The HIV (+) SD (−) and HIV (+) SD (−) patients were from the Red Ribbon Clinic of the First Hospital of China Medical University. The HIV (−) SD (+) patients were from Dermatological Outpatient Clinic of China Medical University. Diagnosis of patients with facial SD was based on typical clinical manifestations, including the presence of erythematous pruritic patches and greasy scale in sebaceous areas. Enrolled patients should meet the following criteria: (1) erythematous pruritic patches with greasy scale present in sebaceous areas; (2) no history of taking anti-histamines, applied corticosteroid, calcineurin inhibitors, and antifungal drugs in the most recent 2 weeks. Twenty-one (72.4%) of the enrolled HIV-infected patients had one or more other comorbidities, including seven with xerosis, seven with syphilis, four with generalized eczema, three with folliculitis, three with condyloma acuminata, two with chronic urticaria, and one with psoriasis.

In the second phase of the study, we enrolled a cohort of patients with clinically diagnosed classical SD, who had not taken any systemic or topical antibiotics for at least the preceding 4 weeks and who consented to participate in an open-label trial of topical medications to treat SD, topical tacrolimus (Astellas Pharma Inc., Japan), fusidic acid cream (LEO Laboratories Limited, Ireland), or moisturizers (Stiefel, GSK, Hong Kong, China). The 43 patients who agreed to participate were randomly allocated to one of the three medications. The patients were asked to apply the topical drugs twice a day for 2 weeks and then returned for re-evaluation.

Scoring the severity of seborrheic dermatitis

We adopted the Seborrheic Dermatitis Area Severity Index (SDASI) scoring system.[13] In brief, the erythema and desquamation of nine different anatomic sites were graded from 0 to 3 (0 = none, 1 = mild, 2 = moderate, and 3 = severe). The score of each site was multiplied by the constant for the area (forehead = 0.1, scalp = 0.4, nasolabial = 0.1, eyebrow = 0.1, postauricular = 0.1, auricular = 0.1, intermammary = 0.2, back = 0.2, and cheek or chin = 0.1). The sum was determined as the SDASI score (range: 0–12.6). The pruritus score was based on subjective assessment by the patient (0 = none, 1 = mild, 2 = moderate, and 3 = severe itching).

A modified scoring was used for the second phase of the study. Four locations over the face, including forehead, nasolabial fold, and cheek or chin were scored and summed to calculate the facial SDASI score.

Evaluation of biophysical skin barrier function

Transepidermal water loss (TEWL) was measured on different parts of the face in all groups, using noninvasive AquaFlux 200 (Biox, London, UK). TEWL was measured of the forehead (frontal part), cheek (prominence of zygomatic bone), chin (submaxilla), and forearm (as control). Stratum corneum hydration and sebum content were measured by MPA6, Corneometer CM825 (CK, Germany). These evaluations were performed in a room at the standard temperature and humidity required for the measurement. After washing their face with tap water, all the patients were asked to stay calm for 30 min in the room to become accustomed to the environment. No skin moisturizers were applied 12 h prior to measurements.

Bacterial culture

In the first phase of the study, specimens were obtained from nasolabial folds of patients with or without SD lesions and forearm of all the patients, using the scrub

method. After incubation for 48 h at 37°C on blood agar plates (Baisibiology Technological Limited Company, Hangzhou, China), individual colony-forming units (CFUs) were counted from a defined area of 9 cm × 9 cm. Plates with "too many CFU to count" or those with confluent growth were defined as having >300 CFU (maximum). Microscopic examination of the colonies using Gram staining and the slide coagulase tests were performed in the Microbiology Laboratory. The species of *Staphylococcus* were identified based on their reaction profile in the 19 biochemical tests of the API STAPH system. For the second phase of the study, an easy-stamp method was employed to culture the bacteria, following the protocol in the product manual (KOMED, Seoul, Korea). This device has the advantage of easy collection of samples and instantaneous identification of *Staphylococcus* strains.

Statistical analysis

Data were entered into SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis. In the first phase of the study, differences of SDASI, pruritus score, TEWL, sebum, hydration, and the number of CFU of *Staphylococcus* in the HIV (+) SD (+), HIV (−) SD (+), HIV (+) SD (−), and HIV (−) SD (−) groups were analyzed by one-way analysis of variance (ANOVA), and comparisons of data between any two groups were completed with least significant difference (LSD) tests. In the second phase of the study, differences of SDASI, pruritus score, TEWL, sebum, hydration, and the number of CFU of *Staphylococcus* in tacrolimus, fusidic acid, and moisturizer groups were analyzed by one-way ANOVA, and comparisons of data between any two groups were completed with LSD tests. $P < 0.05$ indicated statistical significance. The results were reported as mean ± standard deviation when parameters were normally distributed and as median (interquartile range) when parameters were not normally distributed.

Results

Clinical parameters by study group in the first phase of the study

In general, the SDASI was higher in the HIV (+) SD (−) patients than the HIV (−) SD (+) patients (95% confidence interval [*CI*]: 0.035–0.494, $P = 0.024$) whereas the pruritus score did not differ significantly between HIV (+) SD (−) and HIV (−) SD (+) groups (95% *CI*: 0.021–0.623, $P = 0.066$).

The level of TEWL of SD patients was much greater than those of non-SD controls, both through lesional or nonlesional sites over the face. The TEWL through lesional sites in the HIV (+) SD (+) group was much greater than that through normal skin of HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 18.873–47.071, $P < 0.001$ and 95% *CI*: 28.755–55.936, $P < 0.001$, respectively). The level of TEWL through lesional sites in the HIV (−) SD (+) group was also greater than that in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 9.196–33.569, $P = 0.001$ and 95% *CI*: 19.161–42.351, $P < 0.001$, respectively). HIV (+) SD (+) group tended to have higher TEWL than HIV (−) SD (+) patients, though no statistical difference (95% *CI*: 1.414–24.592, $P = 0.080$) was reached. Greater TEWL was also observed though nonlesional sites and scalp areas of SD patients than in non-SD controls. SD patients also had higher levels of TEWL than the controls through nonseborrheic forearm skin, especially in the HIV (+) SD (+) group; levels of TEWL through the forearm skin were much greater in the HIV (+) SD (+) group than that in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 0.214–12.151, $P = 0.043$ and 95% *CI*: 2.348–13.855, $P = 0.006$, respectively) [Table 1].

The water-holding capacity of the stratum corneum affects its hydration level. The hydration level was similar among the four study groups, regardless of whether the facial area was inflamed or not (all $P > 0.05$). As a reference, we also evaluated the hydration level of the inner arms of all the enrolled patients; again, hydration levels did not differ among the four groups (data not shown). We also evaluated both the facial and scalp sebum levels. Sebum levels were much greater in both SD groups than in non-SD controls. The levels of sebum in the nasolabial fold were much greater in the HIV (+) SD (+) group than in the HIV (−) SD (−) group (95% *CI*: 1.128–63.868, $P = 0.043$). The levels of sebum in the nasolabial fold of the HIV (−) SD (+) group were also greater than in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 3.205–59.462, $P = 0.030$ and 95% *CI*: 10.116–63.643, $P = 0.008$, respectively). Higher levels of sebum were also observed in the scalp area of SD patients than in non-SD controls [Table 1].

Colonization with *Staphylococcus*

As shown in Table 2 and Figure 1, SD patients were colonized with more *Staphylococcus* CFUs than those without SD, and this difference was greater in HIV (+) SD (+) patients. The number of CFU of *Staphylococcus* on lesional sites in the HIV (+) SD (+) group was much greater than in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 37.487–142.744, $P = 0.001$ and 95% *CI*: 54.936–156.400, $P < 0.001$, respectively). The number of CFU of *Staphylococcus* on lesional sites in the HIV (−) SD (+) group was also greater than in HIV (+) SD (−) and HIV (−) SD (−) groups (95% *CI*: 16.135–107.115, $P = 0.009$ and 95% *CI*: 33.900–120.460, $P = 0.001$, respectively). On nonseborrheic forearm sites, HIV (+) patients tended to be colonized with more *Staphylococcus* than HIV (−) patients. The number of CFU of *Staphylococcus* in the HIV (+) SD (+) group was greater than in HIV (−) SD (+), HIV (+) SD (−), and HIV (−) SD (−) groups (95% *CI*: 2.687–7.833, $P < 0.001$; 95% *CI*: 1.657–7.237, $P = 0.002$; and 95% *CI*: 3.169–8.548, $P < 0.001$, respectively). The HIV (+) SD (+) group had significantly higher colonization numbers than the other groups, suggesting that their lower immunological status favored colonization with *Staphylococcus*. For colonies obtained from lesional skin in ten HIV (−) SD (+) patients, the bacterial species was identified. *Staphylococcus aureus* was isolated from

Table 1: Clinical parameters of different seborrheic dermatitis groups

Clinical parameters	HIV (+) SD (+) (n = 13)	HIV (−) SD (+) (n = 24)	HIV (+) SD (−) (n = 16)	HIV (−) SD (−) (n = 19)
SDASI	0.60 (1.00)	0.40 (0.40)*	0.00	0.00
Pruritus score	2.00 (1.00)	2.00 (0.75)	0.00	0.00
TEWL ($g \cdot m^{-2} \cdot h^{-1}$)				
Lesion	71.90 ± 16.63	60.32 ± 27.71	38.93 ± 11.53*,†	29.56 ± 8.75*,†
Cheek	43.60 (18.37)	30.40 (19.16)	28.86 (8.29)*	22.70 (10.88)*,†
Scalp	41.87 (18.22)	31.30 (19.12)	26.18 (11.83)*,†	29.37 (10.74)*
Forearm	12.24 (16.72)	12.54 (5.07)	10.98 (6.72)*	8.89 (4.72)*
Hydration in lesion (%)	34.65 ± 13.48	38.54 ± 12.81	38.94 ± 11.77	42.26 ± 16.91
Sebum ($\mu g/cm^2$)				
Nasolabial	72.08 ± 34.07	76.46 ± 50.61	45.13 ± 35.98†	39.58 ± 45.62*,†
Scalp	99.00 (69.50)	86.00 (56.50)	36.50 (74.75)†	31.00 (12.00)*,†

The data are presented as mean ± standard deviation when parameters are normally distributed and as median (IQR) when parameters are not normally distributed. *P<0.05 as compared with HIV (+) SD (+) group; †P<0.05 as compared with HIV (−) SD (+) group. SDASI: Seborrheic Dermatitis Area Severity Index; TEWL: Transepidermal water loss; IQR: Interquartile range; SD: Seborrheic dermatitis; HIV: Human immunodeficiency virus.

Table 2: Colonization density of *Staphylococcus* in different seborrheic dermatitis groups

Staphylococcus	HIV (+) SD (+) (n = 13)	HIV (−) SD (+) (n = 24)	HIV (+) SD (−) (n = 16)	HIV (−) SD (−) (n = 19)
Lesion of SD (CFU)	78.00 (169.00)	49.00 (91.50)	22.00 (11.50)*,†	9.00 (14.00)*,†
Forearm (CFU)	5.00 (8.50)	2.00 (3.00)†	2.00 (1.00)†	2.00 (2.00)†

The CFU numbers are presented as median (IQR). *P<0.05 as compared with HIV (−) SD (+) group; †P<0.05 as compared with HIV (+) SD (+) group. CFU: Colonization-forming unit; IQR: Interquartile range; HIV: Human immunodeficiency virus; SD: Seborrheic dermatitis.

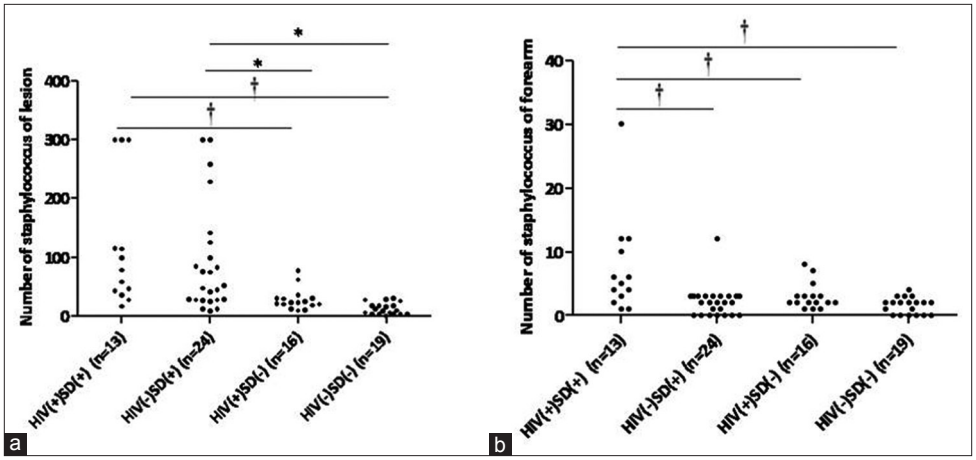

Figure 1: Colony-forming units of *Staphylococcus* of different seborrheic dermatitis patients. (a) the number of *Staphylococcus* of lesion. (b) the number of *Staphylococcus* of forearm. *P < 0.05 as compared with HIV (−) SD (+) group; †P < 0.05 as compared with HIV (+) SD (+) group. SD: Seborrheic dermatitis; HIV: Human immunodeficiency virus.

one SD patient with a high severity score (SDASI = 1.9), *S. epidermidis* was isolated from 8 (80%) SD patients, and *Staphylococcus haemolyticus* was isolated from one SD patient.

Effect of topical medications on Seborrheic Dermatitis Area Severity Index and permeability barrier

The above results indicate that most patients with SD were highly colonized with *Staphylococcus*, mostly with *S. epidermidis*. Therefore, we next evaluated whether topical antibiotics affected either clinical symptoms or permeability barrier in SD patients. Among the 43 SD patients who entered the trial, 16 patients applied tacrolimus ointment, 15 patients applied fusidic acid cream, and 12 patients applied moisturizer. All of them completed the 2-week study.

As shown in Table 3 and Figure 2, moisturizer alone decreased the average SDASI of patients moderately. Topical tacrolimus and fusidic acid decreased SDASI significantly more than applying moisturizer (95% *CI*: 0.030–0.432, P = 0.025 and 95% *CI*: 0.033–0.440, P = 0.024, respectively). TEWL through lesional sites was significantly more improved in patients treated with tacrolimus and fusidic acid than in those treated with moisturizers (95% *CI*: 7.560–38.987, P = 0.004 and 95% *CI*: 4.659–37.619, P = 0.011, respectively). Similar

Table 3: Clinical parameters of seborrheic dermatitis patients before and after the treatment with tacrolimus, fusidic acid, and moisturizers

Parameters	Tacrolimus (n = 16)			Fusidic acid (n = 15)		
	Before	After	Difference	Before	After	Difference
SDASI	0.65 (0.50)	0.20 (0.30)	0.50 (0.40)*	0.89 ± 0.53	0.30 (0.40)	0.48 ± 0.31*
Pruritus score	3.00 (1.00)	1.00 (1.00)	2.00 (1.00)	3.00 (1.00)	1.00 (1.00)	1.79 ± 1.05
TEWL ($g \cdot m^{-2} \cdot h^{-1}$)						
Lesion	69.04 ± 29.68	38.56 (16.44)	17.56 (38.17)*	70.54 ± 25.93	43.82 ± 10.28	26.72 ± 23.52*
Cheek	30.28 (23.21)	29.17 (14.31)	−0.41 (15.20)	42.78 (42.80)	37.67 ± 12.61	5.81 (21.20)
Forehead	43.12 (29.68)	29.43 (13.32)	10.01 (19.53)	59.68 ± 21.38	39.05 ± 10.12	20.63 ± 16.13
Chin	40.94 (12.93)	26.01 (8.95)	16.53 (19.49)*	51.19 ± 17.44	32.11 ± 9.01	19.07 ± 13.67*
Staphylococcus (CFU)						
Lesion (*S. epidermidis*)	117.50 (203.00)	48.00 (20.00)	42.00 (187.00)†	331.00 ± 137.00	18.00 (28.00)	251.00 (249.00)
Lesion (*S. aureus*)	1.50 (5.00)	2.50 (4.00)	0.69 ± 4.14	2.00 (3.00)	0.00 (10.00)	0.00 (2.00)
Forehead (*S. epidermidis*)	83.00 (248.00)	33.94 ± 19.52	35.00 (244.00)†	282.00 (232.00)	16.50 (15.00)	254.50 (263.00)
Forehead (*S. aureus*)	0.00 (2.00)	0.00 (0.00)	0.00 (2.00)	0.00 (2.00)	0.00 (0.00)	0.00 (1.00)

Parameters	Moisturizers (n = 12)		
	Before	After	Difference
SDASI	0.67 ± 0.34	0.42 ± 0.30	0.25 ± 0.18
Pruritus score	3.00 (1.00)	1.50 (1.00)	1.00 (1.00)
TEWL ($g \cdot m^{-2} \cdot h^{-1}$)			
Lesion	65.27 ± 25.05	61.32 ± 24.26	3.95 ± 3.69
Cheek	45.17 ± 17.75	42.52 ± 15.37	2.65 ± 7.48
Forehead	60.95 ± 22.56	55.62 ± 22.89	5.33 ± 5.67
Chin	60.24 ± 27.21	56.53 ± 22.96	3.71 ± 10.63
Staphylococcus (CFU)			
Lesion (*S. epidermidis*)	93.50 (99.00)	106.50 ± 88.42	0.00 (39.00)†
Lesion (*S. aureus*)	7.50 (56.00)	0.00 (2.00)	2.50 (15.00)
Forehead (*S. epidermidis*)	52.50 (154.00)	62.00 (149.00)	9.50 (15.00)†
Forehead (*S. aureus*)	0.00 (17.00)	0.00 (0.00)	0.00 (17.00)

The data are presented as mean ± standard deviation when parameters are normally distributed and as median (IQR) when parameters are not normally distributed. *P<0.05 as compared with moisturizer group; †P<0.05 as compared with fusidic acid cream group. SDASI: Seborrheic Dermatitis Area Severity Index; TEWL: Transepidermal water loss; CFU: Colonization forming units; *S. epidermidis: Staphylococcus epidermidis*; *S. aureus*: *Staphylococcus aureus*; IQR: Interquartile range; SD: Seborrheic dermatitis.

changes in TEWL through the chin area were observed (95% *CI*: 5.476–28.833, P = 0.005 and 95% *CI*: 2.713–26.401, P = 0.017, respectively). TEWL through the forehead skin did not change significantly (P > 0.05). The changes in TEWL after treatment did not differ between patients treated with tacrolimus and fusidic acid (P > 0.05).

S. epidermidis and a few *S. aureus* were identified in 43 patients before treatment. In all the three groups, the *S. epidermidis* count decreased after the 2-week treatment. In the fusidic acid group, the bacterial count decreased more than in those who applied tacrolimus ointment and moisturizer, both on the lesional and nonlesional sites. In one patient of the fusidic acid group, the colonization of *S. epidermidis* increased on the lesional site and changed insignificantly at the nonlesional site; however, he experienced moderate improvement in his SDASI and TEWL.

Further, colonization on the skin surface with a few CFU of *S. aureus* was detected in the three groups. Different treatment options produced no significant changes in the number of the colonies, as shown in Table 3.

Discussion

It is well acknowledged that the incidence of SD is higher in HIV-infected patients. In this study, the severity of SD did not reflect the severity and progression of HIV infection, although HIV (+) SD (+) patients tended to manifest more severe scores than HIV (−) SD (+) patients. TEWL through facial lesional and nonlesional sites in SD patients appeared greater than in those without SD. Another adjacent seborrheic site, the scalp, also showed a tendency toward greater TEWL in patients with SD than those without SD. We postulate that the permeability skin barrier function is lower at seborrheic sites, either with apparent or insidious clinical manifestation. HIV (+) SD (+) patients also had greater TEWL through a nonseborrheic site (forearm) than the controls. It seems that compromised immune function contributed to lowered skin barrier function.

Among several tentative etiological factors of SD, colonization of seborrheic sites with *M. globosa* and *M. restricta* is claimed to be the major culprit. These commensals have the ability to produce lipases and require an exogenous source of lipids to grow.[14] The lipases are

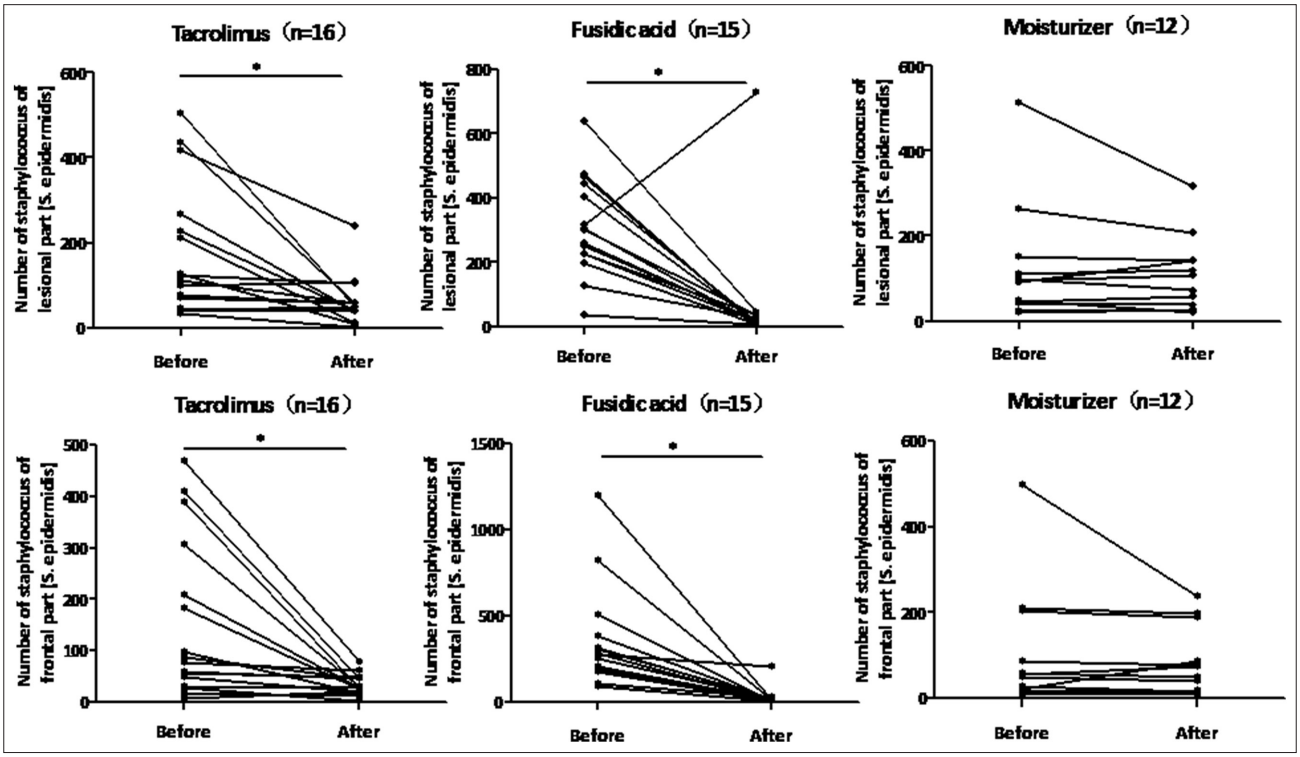

Figure 2: The number of *Staphylococcus epidermidis* before and after topical treatment with tacrolimus, fusidic acid, or moisturizers. *$P < 0.05$, according to before and after comparison within each group. *S. epidermidis*: *Staphylococcus epidermidis*.

involved in the release of arachidonic acid, which can cause inflammation of the skin.[15] Antifungal agents, such as ketoconazole, may benefit SD patients, though the efficacy is moderate.[16] However, several studies challenged the role of the yeast in SD. A study showed that pityrosporum cultures of HIV (+) SD (+) patients were either negative or with scant yeast growth, whereas significantly greater numbers of pityrosporum were cultured from HIV (−) SD (+) patients. These results suggest that HIV (+) SD (+) patients may have a pathogenetic mechanism distinct from the "classical" SD of immunocompetent individuals.[17] Detection of the *Malassezia* is affected by differences in medical conditions, and in healthy skin, by differences in age, body site, geographic location, season, and humidity.[18] A recent study of Chinese patients with SD showed no significant difference in the distribution of *Malassezia* species between Chinese SD patients and healthy individuals.[19] In the present study, we examined the presence of *Malassezia* by conventional KOH examination and fungal culture, and unexpectedly, found only two positive findings in 37 SD patients (data not shown). We speculate that factors such as the relatively cold temperature and low humidity at the time of the study, or the ethnicity of the cohort, might have affected the detection of *Malassezia*, and we suggest a less essential role of *Malassezia* in the pathogenesis of SD. Parallel to the situation in atopic dermatitis and unexpectedly, we observed high colonization with *Staphylococcus*, predominantly *S. epidermidis*, in both HIV (+) SD (+) and HIV (−) SD (+) patients, as compared to the controls. *S. epidermidis* was formally regarded as unharmful residents of the skin. Recently, they were reported to play a possible pathogenic role in the development of pustules in rosacea.[20] Recent research suggests that dysbiosis and *S. aureus* colonization drive inflammation in atopic dermatitis, demonstrating that $Adam17^{fl/fl}Sox9^{-Cre}$ mice, generated to model ADAM17 deficiency in human, developed eczematous dermatitis, similar to that observed in atopic dermatitis.[21] In the present study, we found that patients with SD were highly colonized with *Staphylococcus*, mostly *S. epidermidis*. We speculate that *S. epidermidis* may be pathogenic in SD as *S. aureus* is in atopic dermatitis. Of course, the role of other microorganisms cannot be definitely ruled out; as shown by Tanaka *et al*.,[10] *Acinetobacter*, *Staphylococcus*, and *Streptococcus* predominated on lesional sites of SD patients.

Fusidic acid is effective against most Gram-positive cocci, as well as some Gram-negative bacilli. In patients treated with topical fusidic acid, colonization of *S. epidermidis* significantly decreased after 2 weeks in all except one patient. In parallel, the SDASI and TEWL also improved, and the effect was superior to those who applied moisturizer. The patient in whom the *S. epidermidis* numbers increased after 2 weeks of treatment [as shown in Figure 2] might have been colonized with a fusidic acid-resistant strain or did not follow the application instructions properly. Moisturizer alone moderately affects inflammatory skin conditions, such as atopic dermatitis, by improving the permeability barrier and antimicrobial function of the skin.[22] Topical tacrolimus ointment is recommended for treating SD with more safety and efficiency, due to its immune suppressive and anti-inflammatory capabilities.[23] In the present study,

most of the patients who applied topical tacrolimus had satisfactory clinical improvement, as well as reduced TEWL and bacterial colonization numbers. The clinical efficacy of topical fusidic acid was similar to that of topical tacrolimus. The vehicles of the topical agents in the three groups were not similar. Fusidic acid cream contains glycerol, liquid paraffin, and other minor components. The moisturizer we used contains triglycerides, ceramides, phytosterol, phospholipid, and squalane. Tacrolimus ointment contains primarily paraffin wax, mineral oil, and petroleum. Thus, conclusions about the role of the active ingredients should be made cautiously.[24] Nevertheless, topical agents containing immunosuppressive agents and bactericidal agents appear to have an efficacy superior to moisturizer alone.

Tacrolimus has not been shown to have bactericidal capabilities. Local inflammation interrupts skin barrier function.[25-28] Breached skin barrier predisposes the skin to inflammatory responses by *S. epidermidis in vitro*, albeit the bacteria took no effect on the intact skin.[29] A combination of factors, such as high bacterial colonization, inflammation, and skin barrier disruption, as well as a genetic disposition, may explain the occurrence of this common skin condition.

One limitation of the present study was the inability to rule out the pathogenic roles of other resident microbials other than *S. epidermidis*. Second, we only investigated the changes in the physical barrier of the skin, while the antimicrobial barrier, as exemplified by antimicrobial peptides, may be more relevant to infectious skin diseases. Finally, the long-term effect of topical antibiotics was not investigated.

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Conflicts of interest

There are no conflicts of interest.

References

1. Crespo Erchiga V, Ojeda Martos A, Vera Casaño A, Crespo Erchiga A, Sanchez Fajardo F. *Malassezia globosa* as the causative agent of pityriasis versicolor. Br J Dermatol 2000;143:799-803. doi: 10.1046/j.1365-2133.2000.03779.x.
2. de Moraes AP, de Arruda EA, Vitoriano MA, de Moraes Filho MO, Bezerra FA, de Magalhães Holanda E, *et al.* An open-label efficacy pilot study with pimecrolimus cream 1% in adults with facial seborrhoeic dermatitis infected with HIV. J Eur Acad Dermatol Venereol 2007;21:596-601. doi: 10.1111/j.1468-3083.2006.01923.x.
3. Gary G. Optimizing treatment approaches in seborrheic dermatitis. J Clin Aesthet Dermatol 2013;6:44-9.
4. Berg M. Epidemiological studies of the influence of sunlight on the skin. Photodermatol 1989;6:80-4.
5. Ostlere LS, Taylor CR, Harris DW, Rustin MH, Wright S, Johnson M. Skin surface lipids in HIV-positive patients with and without seborrheic dermatitis. Int J Dermatol 1996;35:276-9. doi: 10.1111/j.1365-4362.1996.tb03001.x.
6. Harding CR, Moore AE, Rogers JS, Meldrum H, Scott AE, McGlone FP. Dandruff: A condition characterized by decreased levels of intercellular lipids in scalp stratum corneum and impaired barrier function. Arch Dermatol Res 2002;294:221-30. doi: 10.1007/s00403-002-0323-1.
7. Guo JW, Lin TK, Wu CH, Wei KC, Lan CC, Peng AC, *et al.* Human sebum extract induces barrier disruption and cytokine expression in murine epidermis. J Dermatol Sci 2015;78:34-43. doi: 10.1016/j.jdermsci.2015.01.010.
8. Greaves MW, Camp RD. Prostaglandins, leukotrienes, phospholipase, platelet activating factor, and cytokines: An integrated approach to inflammation of human skin. Arch Dermatol Res 1988;280:S33-41.
9. Schwartz RA. Superficial fungal infections. Lancet 2004;364:1173-82. doi: 10.1016/S0140-6736(04)17107-9.
10. Tanaka A, Cho O, Saito C, Saito M, Tsuboi R, Sugita T. Comprehensive pyrosequencing analysis of the bacterial microbiota of the skin of patients with seborrheic dermatitis. Microbiol Immunol 2016;60:521-6. doi: 10.1111/1348-0421.12398.
11. Clavaud C, Jourdain R, Bar-Hen A, Tichit M, Bouchier C, Pouradier F, *et al.* Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. PLoS One 2013;8:e58203. doi: 10.1371/journal.pone.0058203.
12. Wang L, Clavaud C, Bar-Hen A, Cui M, Gao J, Liu Y, *et al.* Characterization of the major bacterial-fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium. Exp Dermatol 2015;24:398-400. doi: 10.1111/exd.12684.
13. Cömert A, Bekiroglu N, Gürbüz O, Ergun T. Efficacy of oral fluconazole in the treatment of seborrheic dermatitis: A placebo-controlled study. Am J Clin Dermatol 2007;8:235-8.
14. Riciputo RM, Oliveri S, Micali G, Sapuppo A. Phospholipase activity in *Malassezia furfur* pathogenic strains. Mycoses 1996;39:233-5.
15. Ortonne JP, Lacour JP, Vitetta A, Le Fichoux Y. Comparative study of ketoconazole 2% foaming gel and betamethasone dipropionate 0.05% lotion in the treatment of seborrhoeic dermatitis in adults. Dermatology 1992;184:275-80.
16. Piérard GE, Ausma J, Henry F, Vroome V, Wouters L, Borgers M, *et al.* A pilot study on seborrheic dermatitis using pramiconazole as a potent oral anti-*Malassezia* agent. Dermatology 2007;214:162-9.
17. Peyrí J, Lleonart M; Grupo español del Estudio SEBDERM. Clinical and therapeutic profile and quality of life of patients with seborrheic dermatitis. Actas Dermosifiliogr 2007;98:476-82.
18. Pedrosa AF, Lisboa C, Gonçalves Rodrigues A. *Malassezia* infections: A medical conundrum. J Am Acad Dermatol 2014;71:170-6. doi: 10.1016/j.jaad.2013.12.022.
19. Lian CH, Shen LL, Gao QY, Jiang M, Zhao ZJ, Zhao JJ. Identification of *Malassezia* species in the facial lesions of Chinese seborrhoeic dermatitis patients based on DNA sequencing. Mycoses 2014;57:759-64. doi: 10.1111/myc.12229.
20. Whitfeld M, Gunasingam N, Leow LJ, Shirato K, Preda V. *Staphylococcus epidermidis*: A possible role in the pustules of rosacea. J Am Acad Dermatol 2011;64:49-52. doi: 10.1016/j.jaad.2009.12.036.
21. Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, *et al.* Dysbiosis and *Staphylococcus aureus* colonization drives inflammation in atopic dermatitis. Immunity 2015;42:756-66. doi: 10.1016/j.immuni.2015.03.014.
22. Czarnowicki T, Malajian D, Khattri S, Correa da Rosa J, Dutt R, Finney R, *et al.* Petrolatum: Barrier repair and antimicrobial responses underlying this "inert" moisturizer. J Allergy Clin Immunol 2016;137:1091-102.e1-7. doi: 10.1016/j.jaci.2015.08.013.
23. Kim GK, Rosso JD. Topical pimecrolimus 1% cream in the treatment of seborrheic dermatitis. J Clin Aesthet Dermatol 2013;6:29-35.
24. Lindh JD, Bradley M. Clinical effectiveness of moisturizers in atopic dermatitis and related disorders: A systematic review. Am J Clin Dermatol 2015;16:341-59. doi: 10.1007/s40257-015-0146-4.
25. Vestergaard C, Hvid M, Johansen C, Kemp K, Deleuran B,

Deleuran M. Inflammation-induced alterations in the skin barrier function: Implications in atopic dermatitis. Chem Immunol Allergy 2012;96:77-80. doi: 10.1159/000331889.
26. Mócsai G, Gáspár K, Nagy G, Irinyi B, Kapitány A, Bíró T, *et al.* Severe skin inflammation and filaggrin mutation similarly alter the skin barrier in patients with atopic dermatitis. Br J Dermatol 2014;170:617-24. doi: 10.1111/bjd.12743.
27. Takai Y, Hiramoto K, Nishimura Y, Ooi K .Relationship between biochemical factors and skin symptoms in chronic venous disease. Arch Dermatol Res 2017;309:253-8. doi: 10.1007/s00403-017-1721-8.
28. Borda LJ, Wikramanayake TC. Seborrheic Dermatitis and Dandruff: A Comprehensive Review. J Clin Investig Dermatol 2015;3. doi: 10.13188/2373-1044.1000019.
29. Duckney P, Wong HK, Serrano J, Yaradou D, Oddos T, Stamatas GN. The role of the skin barrier in modulating the effects of common skin microbial species on the inflammation, differentiation and proliferation status of epidermal keratinocytes. BMC Res Notes 2013;6:474. doi: 10.1186/1756-0500-6-474.