Changes in Lesional and Non-lesional Skin Microbiome During Treatment of Atopic Dermatitis

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The aim of this study was to evaluate changes in the skin surface microbiome in patients with atopic dermatitis during treatment. The effect of narrowband ultraviolet B phototherapy was also studied to determine the influence of exposure to ultraviolet. A total of 18 patients with atopic dermatitis were included in the study. Patients were divided into 2 groups based on treatment: 1 group treated with narrowband ultraviolet B phototherapy and topical corticosteroid, and the other group treated with topical corticosteroid only. Skin swabs and high-throughput sequencing of 16S ribosomal RNA bacterial genes were performed at 3 time-points. The microbial diversity of lesional skin increased greatly after treatment. The proportion of *Staphylococcus aureus* showed a significant positive correlation with eczema severity. In conclusion, a drastic increase in microbial diversity and decrease in *S. aureus* proportion were observed with eczema treatment. Narrowband ultraviolet B treatment did not exert additive effects on eczema improvement; however, it appeared to reduce the recurrence of eczema.

Key words: *Staphylococcus aureus*; atopic dermatitis; narrowband ultraviolet B; eczema; microbiome.

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The human skin microbiome consists of approximately 100 trillion microbial cells, which outnumber human cells by 10 to 1. The human skin microbiome refers to the entire communities of microbes, including bacteria, fungi, viruses, and mites, that reside in and on the human skin. Microbiomes influence the host immunity and sometimes protect the host from colonization by pathogenic organisms.

Several researchers have tried to identify the human microbiome and have developed various techniques for microbial characterization. Traditional culture-dependent microbial characterization focuses on the species that grow readily under standard culture conditions; thus, this method is limited to identifying less than 1% of bacterial species. However, the development of high-throughput sequencing techniques and bioinformatics has facilitated culture-independent and comprehensive identification of the microbiome. Bacterial microbiome analysis takes advantage of the universal presence of small-subunit (16S) ribosomal RNA gene in prokaryotes. The 16S rRNA gene plays an essential role in microbial characterization because it not only contains highly conserved regions, which facilitates PCR, but also has hypervariable regions, which can be used for phylogenetic categorization (1).

High-throughput sequencing has led to numerous findings concerning the human microbiome. Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease affecting 15–30% of children in industrialized countries (2). Patients with AD experience repetitive skin infection, and traditional culture-based studies have revealed that *Staphylococcus aureus* plays an important role in the pathogenesis of AD. Approximately 80–100% of patients with AD have *S. aureus* colonization, and the colonization density of *S. aureus* is correlated with disease severity (3, 4). Using high-throughput sequencing technologies, drastic changes in skin microbiome in AD flare have been identified (5).

However, how the skin microbiome changes with treatment and discontinuation of treatment in the lesional and non-lesional skin of the same individuals needs further study. Moreover, several patients with AD experience seasonal aggravation of eczema, but the effect of ultraviolet (UV) light on the skin microbiome remains poorly identified. In this study, bacterial 16S rRNA DNA sequencing was performed on lesional and non-lesional skin in patients with AD, before treatment, after treatment, and
after discontinuation of treatment. Furthermore, the skin microbiome of patients treated with topical corticosteroid (TCS) only and those treated with narrowband ultraviolet B (NBUBVB) plus TCS was compared in order to investigate the effect of exposure to UV.

**METHODS**

**Study design**

The study was designed to evaluate the skin surface microbiome of lesional and non-lesional skin of patients with AD at 3 time-points (before treatment, after 6 weeks of treatment, and after 3 weeks of discontinuation of treatment). The patients were divided into 2 treatment groups: TCS and NBUBVB+TCS. Changes in microbiome were compared between lesional and non-lesional skin in different treatment groups at 3 different time-points.

This study was performed between November 2014 and August 2015, from winter to summer, in Korea. The mean ambient temperature was between –0.5°C (December 2014) and 25.2°C (August 2015). The numbers of patients enrolled in the TCS and NBUBVB+TCS groups were kept similar by season, to minimize the seasonal differences between the 2 groups. The study was approved by Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-1310/222-004), and all subjects gave written informed consent.

**Subjects**

A total of 18 subjects was recruited, of whom 11 completed the study. Inclusion criteria for patients with AD were age 5–40 years, moderate disease, presence of at least one eczema on the antecubital or popliteal fossa, a score of > 3 in the Three-Item Severity Score (TISS) (6) at enrollment, and ability to tolerate > 3 weeks without topical corticosteroids. The diagnosis of AD was based on the AD criteria of Hanifin & Rajka (7). Subjects who used bleach baths or systemic or topical antibiotics or were exposed to strong UV light within the past 4 weeks, who needed systemic or topical antibiotics treatment, and who were treated with UV phototherapy within the past 8 weeks were excluded. During the study, the subjects who needed to switch treatment plan to systemic corticosteroid, immunosuppressant, and systemic or topical antibiotics due to aggravation were dropped out.

**Treatment and evaluation**

Of the 18 subjects, 13 and 5 subjects were randomly allocated to the NBUBVB+TCS and TCS groups, respectively. Randomization was performed using random tables.

All subjects were instructed to continue with basic skin care, including daily bathing and twice daily moisturizer use, throughout the study period. They were prescribed moderate potency TCS (methylprednisolone cream) and oral antihistamine. Patients were instructed to use TCS on the lesional skin only. Any substance (methylprednisolone cream) and oral antihistamine. Patients were instructed to use TCS on the lesional skin only. Any substance

**RESULTS**

All 5 subjects in the TCS group completed the study. However, only 6 out of 13 subjects in the NBUBVB+TCS
group finished the study. The 5 subjects in the NBUVB+TCS group were lost to follow-up. The other 2 subjects dropped out due to aggravation of symptoms and consequent treatment change. No significant side-effects occurred in either group. The subjects who dropped out were excluded from data analysis.

Differences in microbiome of lesional and non-lesional skin and change in microbiome in lesional skin with treatment

Fig. 1a shows the changes in disease severity assessed by TISS and EASI score. The mean TISS, which indicates the severity of eczema at the lesional sampling sites, decreased significantly during treatment periods. After 3 weeks of discontinuation of treatment, the mean TISS increased slightly; however, it was still significantly lower than its baseline level. The mean EASI score, which indicates severity of eczema on the whole body, was $12.4 \pm 5.0$ at week 0 and decreased significantly from week 3 and continuously throughout the study (Fig. 1a).

Fig. 1b shows the mean composition of bacterial communities obtained from lesional and non-lesional skin of patients with AD during the course of treatment. The proportion of the genus *Staphylococcus*, which was $> 80\%$ in baseline lesional skin, decreased drastically after treatment (week 6) and increased slightly after discontinuation of treatment (week 9). The proportion was much higher in lesional than in non-lesional skin, even after treatment. At the species level, the composition of

Fig. 1. Changes in the severity score and skin microbiome in inpatients with atopic dermatitis (AD). (a) Three-Item Severity Score (TISS) for lesional skin where skin swabs were performed shows significant improvement. Although TISS slightly increased at week 9, which is after 3 weeks of discontinuation of treatment, the difference in TISS at week 6 and week 9 was not significant. Eczema Area and Severity Index (EASI) showed significant and continuous improvement of eczema. (b) The mean composition of bacterial communities obtained from lesional skin and non-lesional skin of AD. Inner circle represents the mean relative abundance of 20 major phyla order in lesional and non-lesional skin. Phylum (inner circle) *Firmicutes*, where *Staphylococcus aureus* belongs, comprised the largest proportion, especially in lesional skin at week 0. Outer circle represents the mean relative abundance of genus order. The genus *Staphylococcus* was the most abundant species, and the proportion was the highest in week 0 in lesional skin. After 6 weeks of treatment, the proportion of genus *Staphylococcus* decreased on lesional skin; at week 9, it slightly increased again, but remained lower than its baseline level. (c) The mean relative abundance of 15 major species and Shannon diversity. The proportion of *S. aureus* was higher in lesional skin at all time points ($p=0.0014$, Wilcoxon rank-sum test). Shannon diversity of lesional skin increased at week 6 and remained increased level at week 9, and Shannon diversity of non-lesional skin was higher than that of lesional skin in all time-points. (d) The proportion of bacterial community drastically changed in both lesional and non-lesional skin. **$p < 0.005$ in McNemar test. LE0: lesion at week 0; LE6: lesion at week 6; LE9: lesion at week 9; NL0: non-lesion at week 0; NL6: non-lesion at week 6; NL9: non-lesion at week 9.
Skin microbiome changes with AD treatment

*S. aureus* by far exceeded other species in lesional skin, consisting of a much higher proportion than that in non-lesional skin (Fig. 1b). In baseline lesional skin, *S. aureus* comprised 72.5% of the whole species and *S. epidermidis* and *S. caprae* were the 2nd and 3rd most common species, respectively. In non-lesional skin, *Propionibacterium acnes* was the most common species, followed by *S. aureus* and *S. epidermidis*. The proportion of *S. aureus* on lesional and non-lesional skin was significantly different (*p* = 0.0014), and the proportion was higher in lesional skin at all time points, including week 6. The proportion of *S. epidermidis* in baseline lesional skin and non-lesional skin was 6.3% and 7.1%, respectively.

The Shannon diversity of lesional skin increased greatly after treatment (week 6), but was still lower than that of non-lesional skin at all time points, including week 6. The proportion of *S. epidermidis* in baseline lesional skin and non-lesional skin was 6.3% and 7.1%, respectively.

The Shannon diversity of lesional skin increased greatly after treatment (week 6), but was still lower than that of non-lesional skin. After discontinuation of treatment (week 9), Shannon diversity was decreased slightly, but was still much higher than its baseline level. Fig. 1d is an example of microbiome change during treatment. After treatment (week 6), a drastic decrease in the genus *Staphylococcus* and an increase in microbial diversity were observed in lesional skin.

**Correlation of eczema severity, microbial diversity, and proportion of *S. aureus***

TISS of lesional skin showed good positive correlation with EASI score (*r* = 0.76, *p* = 4.8e-07, Fig. 2a), which means that TISS can be used not only for evaluating severity of specific lesions, but also as a good indicator of eczema severity of the whole body, as reported in other studies (6, 15). Shannon diversity showed only a weak negative correlation with TISS (Fig. 2b). In contrast, the proportion of *S. aureus* showed a good positive correlation with TISS (*r* = 0.5, *p* = 0.003, Fig. 2c). *S. aureus* also correlated negatively with Shannon diversity in both lesional and non-lesional skin (Fig. 2d, 2e).

**Comparison between topical corticosteroid and narrowband ultraviolet B + topical corticosteroid treatment**

The mean ages of the TCS and NBUVB+TCS groups were 14.8 ± 4.9 and 14.8 ± 2.4 years, respectively. The mean...
baseline TISS were 5.4 ± 0.5 and 5.33 ± 1.0 in the TCS and NBUVB+TCS groups, respectively. The mean baseline EASI scores of the TCS and NBUVB+TCS groups were 11.6 ± 4.1 and 13.0 ± 6.0, respectively. No significant difference was observed in age, TISS, and EASI score between the 2 groups. As shown in Fig. 3, both groups showed significant improvement in the TISS and EASI score during the treatment period (weeks 3 and 6). After discontinuation of treatment, although the difference between the 2 groups was not significant, the NBUVB+TCS group showed continuous improvement of EASI, while the TCS group exhibited slight aggravation of eczema (Fig. 4).

Fig. 4. Comparison of topical corticosteroid treatment (TCS) vs narrowband UVB (NBUVB) phototherapy plus topical corticosteroid treatment (NBUVB+TCS). Both groups showed significant improvement in (a) Three-Item Severity Score (TISS) and (b) Eczema Area and Severity Index (EASI) scores during the treatment period. At week 9, which is after 3 weeks of discontinuation of treatment, the NBUVB+TCS group showed less aggravation both in TISS and EASI score, but the difference between NBUVB+TCS and TCS groups was not significant. *p < 0.05 in McNemar test, compared with week 0. LE0: lesion at week 0; LE6: lesion at week 6; LE9: lesion at week 9; NL0: non-lesion at week 0; NL6: non-lesion at week 6; NL9: non-lesion at week 9.

Fig. 5. (a) Shannon diversity of lesional and non-lesional skin of the topical corticosteroid treatment (TCS) and narrowband UVB (NBUVB)+TCS groups. No significant difference was observed in Shannon diversity of the 2 groups (p = 0.388, Wilcoxon rank-sum test). The TCS group showed an increase in lesional Shannon diversity. In the NBUVB+TCS group, lesional Shannon diversity did not increase at week 6, but increased slightly at week 9. Inter-individual difference was larger in the NBUVB+TCS group. While Shannon diversity of non-lesional skin remained unchanged in the TCS group, that of the NBUVB+TCS group increased at week 6. (b) The proportion of S. aureus in lesional and non-lesional skin of the TCS and NBUVB+TCS groups. The proportion of S. aureus showed significant differences between the 2 groups (p = 1.16e-4, Wilcoxon rank-sum test). The proportion of S. aureus decreased more drastically in the TCS group. The NBUVB+TCS group showed a large inter-individual difference. Interestingly, the proportion of S. aureus decreased in non-lesional skin of the NBUVB+TCS group, and kept decreasing until week 9. (c) Skin microbiome of lesional skin and non-lesional skin. The inner circle represents the mean relative abundance of 20 major phyla order in lesional and non-lesional skin. The outer circle represents the mean relative abundance of genus order. Genus Staphylococcus was the most abundant species, and the proportion was the highest in week 0 in lesional skin in both groups. The proportion of Staphylococcus decreased at week 6 in both groups as well as in lesional and non-lesional skin. The decrease in the proportion of Staphylococcus was more distinct in the TCS group than in the NBUVB+TCS group. TISS: Three-Item Severity Score; EASI: Eczema Area and Severity Index; LE0: lesion at week 0; LE6: lesion at week 6; LE9: lesion at week 9; NL0: non-lesion at week 0; NL6: non-lesion at week 6; NL9: non-lesion at week 9.
**Fig. 5** shows the comparison of microbiota in the 2 groups. No significant difference was observed in Shannon diversity of the 2 groups ($p=0.388$, Wilcoxon rank-sum test). The proportion of *S. aureus* was significantly different between the 2 groups ($p<0.001$, Wilcoxon rank-sum test). In contrast to our expectations, the proportion of *S. aureus* decreased more drastically in the lesional skin of the TCS group. The NBUVB+TCS group showed a large inter-individual difference. Interestingly, the non-lesional skin of the NBUVB+TCS group showed a continuous decrease in *S. aureus* composition, which was not observed in the TCS group.

**DISCUSSION**

The skin microbiome of healthy human skin provides a wide variety of environments, due to the heterogeneity of various human skin surfaces. There are 3 major categories of habitat: sebaceous areas, such as the forehead, retroauricular crease, and back; moist areas, such as the axillae; and dry area, such as the forearm and legs. *Propionibacterium* species dominate sebaceous areas, while *Staphylococcus* and *Corynebacterium* species dominate moist areas. Gram-negative organisms, which were previously considered as a rare population in the skin, were found in dry areas (16).

*Staphylococcus* and *Corynebacterium* are the main species of healthy moist skin. In our study, genus *Staphylococcus* comprised >80% of the baseline lesional microbiome. The proportion of *Corynebacterium* was below 1%, showing a dominance of *Staphylococcus* in lesional AD skin. Non-lesional skin samples were obtained from the volar forearm, which is a dry area, where in the healthy skin, the proportion of *Staphylococcus* is usually below 10% (17). However, the proportion of *Staphylococcus* on the non-lesional volar forearm observed in our study was 28.5%, which was considerably higher than in healthy skin, indicating that microbial characteristics of non-lesional AD skin were completely different from those of healthy skin.

At the genus level, *S. aureus* comprised >80% of the whole microbiome in baseline lesional skin, and its proportion decreased drastically with treatment. Our findings of *S. aureus* dominance of lesional skin microbiota are consistent with previous culture-based studies. *S. aureus* colonization is observed in 80–100% of lesional skin, and colonization density correlates with eczema severity. *S. aureus* colonization rate and density in non-lesional skin are significantly lower than those in lesional skin, but still higher than those in healthy skin (3, 18, 19). Kong et al. reported that the proportion of *S. epidermidis* was higher in patients with AD and higher in those with active disease, but our results showed that the proportion of *S. epidermidis* was not significantly different between lesional and non-lesional skin and between before and after treatment (5). A recent 16S rRNA DNA sequencing study reported that a decrease in microbial diversity correlated significantly with aggravation of eczema and increase in *S. aureus*. In our study, the decrease in microbial diversity showed only a weak correlation with eczema severity, while the proportion of *S. aureus* had a robust correlation with eczema severity. This suggests that the increase in *S. aureus* is not a collateral phenomenon accompanied by a decrease in microbial diversity, but it plays an important role in the pathogenesis of eczema.

*Corynebacterium* thrives in moist environments, as does *Staphylococcus*. Several studies have suggested a potential role of *Corynebacterium* in development of eczema (4, 20, 21).

However, in our study, *Corynebacterium* was less frequent in lesional than in non-lesional skin, and the proportion increased with improvement in eczema. Our results showing that *C. pseudogenitalium* was the most common *Corynebacterium* species and that the proportion of *C. pseudogenitalium* had a significant negative correlation with *S. aureus* and a strong negative correlation with eczema severity suggest a protective role of *Corynebacterium* species in AD. Further studies are necessary to investigate the role of *Corynebacterium* in AD.

In our study, NBUVB treatment did not show an additive effect in lesional eczema improvement and in the changes in lesional skin microbiome. Both TCS and NBUVB+TCS groups did not show any difference in EASI score change at week 6; the NBUVB+TCS group did not show a better result at reducing *S. aureus* proportion in lesional skin than the TCS group.

The high dropout rate of the NBUVB+TCS group may influence the results. For NBUVB treatment, the subjects had to visit the clinic twice weekly. Subjects who experienced sufficient improvement in eczema may have stopped NBUVB treatment, causing selection bias. However, a statistically insignificant difference in the disease severity between the 2 groups even after patient drop-out in the NBUVB+TCS group supports the validity of randomization in our study.

Although we could not find an additive effect of NBUVB therapy in eczema improvement, the NBUVB+TCS group showed a continuous decrease in EASI score at week 9, which is 3 weeks after the last treatment, while EASI score in the TCS group showed an increasing tendency (Fig. 2a). Shannon diversity of non-lesional skin in the NBUVB+TCS group increased at week 6, while that of the TCS group remained similar (Fig. 2b). This result may indicate the possible benefit of NBUVB in reducing eczema recurrence and increasing non-lesional microbial diversity.

The effect of NBUVB phototherapy in AD is well documented in various studies (22, 23), and some research findings suggest its protective role in restoring dysbiosis of AD skin. Previous studies have found that UV phototherapy reduces *S. aureus* colonization in lesional AD skin (24) and reduces toxin production of *S. aureus* (25).
UV exposure also induces cathelicidin peptide LL-37 production in AD skin (26), an antimicrobial peptide guarding skin from S. aureus. In our study, NBUVB-treated subjects appeared to have less recurrence. Other researchers also reported similar findings. In a British study, paediatric patients with AD treated with NBUVB phototherapy remained in a significantly improved state compared with controls even at 6 months after phototherapy (23). This long-lasting effect of the treatment is rarely observed in other treatment modalities. Among various mechanisms, restoration of dysbiosis, which was observed in non-lesional skin of patients in our study, may have played a protective role.

This study found a large difference in microbial communities between lesional and non-lesional skin of AD. Lesional skin harboured a much higher proportion of S. aureus and had less microbial diversity. With the improvement in eczema, a drastic reduction in S. aureus in lesional skin was observed. The proportion of S. aureus showed better correlation with eczema severity than microbial diversity. NBUVB treatment failed to exhibit additive effect in eczema improvement and lesional microbial diversity; however, it appeared to reduce recurrence of eczema and increase non-lesional microbial diversity.

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The authors have no conflicts of interest to declare.

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