Acta Dermatovenerol Croat 2019;27(4):245-249 CLINICAL ARTICLE

Human Skin Microbiota in Various Phases of Atopic Dermatitis

Elvina Murzina, Lydia Kaliuzhna, Katerina Bardova, Yana Yurchyk, Maria Barynova

Department of Dermatovenerology, Shupyk National Medical Academy of Postgraduate Education, Kiev, Ukraine

Corresponding author:
Assist. Prof. Elvina Murzina, MD, PhD
Department of Dermatovenereology
Shupyk National Medical Academy
of Postgraduate Education
Kiev
Ukraine
elvina2003@ukr.net

Received: July 1, 2019
Accepted: November 5, 2019

ABSTRACT Skin microbiota can be used to assess the macroorganism's overall health. The quantitative and qualitative microbiota composition depends on the macroorganism's state, while microbiota bacteria can cause and maintain cutaneous inflammation, in turn worsening the macroorganism's state. This leads to placing additional focus on determination of skin microbiota when studying the pathogenesis of allergic dermatoses. We present the results of our study on the microbiota of apparently healthy skin in children with atopic eczema (AE) exacerbation and skin microbiota in remission. The study revealed that the skin microbiota in children with AE significantly differs from that of healthy controls. The differences include not the quantitative but also qualitative skin microbiota composition both on AE lesions and apparently healthy skin, where the bacterial number exceeds that on the skin of the control group children by 2-4 times. We also observed qualitative bacterial imbalance and appearance and prevalence of microorganisms not typical for healthy skin, where saprophytic *Staphylococcus* is the basis of microbiota, while *Staphylococcus aureus* was the basis in patients with AE. The skin microbiota in children with AE in remission also differed significantly from the skin microbial flora in healthy children. The skin in remission was highly contaminated with microorganisms, in particular pathogens, which indicates sustained alterations of skin microbiota as an unfavorable prognostic factor that can provoke disease relapse.

KEY WORDS: atopic dermatitis, atopic eczema, skin microbial flora, opportunistic pathogens, skin microbiota

INTRODUCTION

Current epidemiological studies have found that 10-20% of children worldwide suffer from atopic dermatitis. In children, atopic dermatitis typically appears as the primary clinical manifestation in the "allergic march": most patients have a high risk of bronchial asthma and allergic rhinoconjunctivitis. Thus, 80% of children with atopic dermatitis suffer from allergic rhinitis or bronchial asthma, and the disease progression is of higher severity (1).

The 2018 European guidelines on atopic eczema (atopic dermatitis) treatment in adults and children defined the other characteristic features apart from the strong genetic effects (80% concordance in monozygotic twins, 20% in heterozygotic twins). They comprise immune abnormalities of the pathway involving T-helper cells (TH2) in the initiation phase with the subsequent increase of IgE production, the increased mediator production in various inflammatory cells,

Acta Dermatovenerol Croat
2019;27(4):245-249

skin barrier hypofunction ("dry" skin) due to abnormal lipid metabolism and the synthesis of the epidermal structural protein filaggrin and protease inhibitors, the abnormal microbial colonization by the pathogens such as *Staphylococcus S.* or *Malassezia* spp. (in comparison with *Staphylococcus epidermidis* in healthy subjects) and subsequently increased susceptibility to skin infections, and obvious profound psychosomatic effects (2).

Furthermore, studies have demonstrated that the skin microbiota impacts the progression of a variety of non-infectious skin diseases, such as atopic eczema, rosacea, psoriasis, acne, etc. Diseases can result from even minor alterations of skin microbial environment, while significant changes in immunomodulation and skin can result from major alterations (3).

In our previous studies we have shown that in children with atopic eczema (AE) a significant bacterial number can be plated from the lesions, with a mean (± Standard Deviation) of 617.14±100.48 colony-forming units (CFU). Although *Staphylococcus aureus* is prevalent (472.62±86.99 CFU/cm^2 of the skin) in microbiota of the skin lesions in children with AE, *Staphylococcus epidermidis* was also plated in large numbers: 132.19±20.29 CFU/cm^2 of the skin. *Micrococci* and *Bacilli* can find an enabling environment for their growth in AE. Their number reaches 28.64±3.14 and 80.16±19.78 CFU/cm^2 in the skin lesions. *Micrococci* occur in 21.43% of cases, *Bacilli* in 13.10%. *Sarcina*, *Streptococcus*, and *Acinetobacter* occur rarely (4).

Analysis of the qualitative microbiota composition of the AE skin lesions also demonstrated the prevalence of *Staphylococcus aureus*, 60.01±6.11%, in the total composition, and other microorganisms only served as a complement: *Staphylococcus epidermidis* at 14.87%±3.81% and *Micrococcus* at 23.27%±8.95%. The microflora analysis of the skin lesions in AE based on age demonstrated that the greatest contamination was present in the 12-16 age group, much higher than the values of the 3-7 age group and comparable with the skin contamination values in the 8-11 age group. It is worth noting that the skin lesion microbiota in children aged 3-7 was represented mainly by *Staphylococcus epidermidis*, whereas in the other age groups *Staphylococcus aureus* was the most prevalent in microbial flora.

The qualitative and quantitative skin microbiota alterations in patients with AE correlate with increased severity of the pathological process, determined according to the standard "SCORing Atopic Dermatitis" (SCORAD) tool designed by the European Task Force on Atopic Dermatitis (ETFAD). Studies have shown that the largest microorganism number per 1 cm^2 of the skin lesion is plated in severe AE, and the largest number of *Staphylococcus aureus* per 1 cm^2 of the skin lesion was also observed in severe AE. *Staphylococcus aureus* numbers in moderate AE are presumably higher than in mild AE. An imbalance in the composition of skin microbiota was reported as the severity increased. The number of *Staphylococcus epidermidis* and *Micrococci* on the AE lesion in mild and moderate severity can be comparable, but is considerably lower than in severe AE. The count of *Staphylococcus saprophyticus* in severe progression is significantly reduced due to *Staphylococcus epidermidis*, which can lead to pyogenic complications.

When comparing the ratio of each microorganism in the skin microbiota composition, depending on bacteriogenic cutaneous complications, it has been noted the mean number of *Staphylococcus aureus* per 1 cm^2 of the AE lesion in the absence of complications apparently outnumbers *Staphylococcus aureus* in the presence of pyoderma. Regarding *Staphylococcus epidermidis*, *Micrococci*, and *Bacilli*, their numbers in the AE lesions accompanied by bacteriogenic skin complications considerably exceed the corresponding value without bacteriogenic complications. It appears possible that the cause and the supporting force of bacteriogenic complications in AE is the association of opportunistic pathogens.

Study objective: Data on abnormal microbial colonization of the skin lesions in patients with atopic eczema (AE) in the exacerbation period led us to conduct a quantitative study of apparently healthy skin (outside of the lesions) in patients with AE, and analyze the skin microbiota in the course of AE regression.

MATERIALS AND METHODS

We conducted a microflora analysis of apparently healthy skin outside of the lesions in 34 children suffering from AE and aged 3-16. None of them had been exposed to immunomodulation or antimicrobial therapy within the last 6 months.

The most typical localizations of AE were the extensor surface of the forearm, the posterior surface of the neck and face, and cubital and popliteal fossae; therefore, the abdominal skin microbiota in sick children was taken as the condition of apparently healthy skin outside of AE lesions.

The forearm skin microbiota of 18 healthy children aged 3-16 were used as controls.

We prepared bacterial imprints with a blood agar or Sabouraud agar growth medium to identify and count the microorganisms located on the skin surface (5).

Acta Dermatovenerol Croat
2019;27(4):245-249

Tissue culture plates containing the growth medium were applied to the child's skin for 1-2 minutes to obtain the culture-imprints. They were subsequently placed into a thermostat and the counting of imprinted colonies was conducted a day later. This was followed by bacteriological study with species identification: genus *Staphylococcus* – S. *aureus*, *S. epidermidis*, *S. saprophitycus*; genera *Micrococcus*, *Sarcina*, *Bacillus*, *Streptococcus*. The number of colonies that grew on 1 cm^2 was re-counted and estimated as colony-forming units (CFU) per 1 square centimeter.

The statistical processing of the results is based on standard Quattro Pro software for Windows 2003 version 5.00, Data Analysis package Microsoft Excel for Windows 2003 version 2.0.5. Parametric and non-parametric variables were analysed using Wilcoxon-Mann-Whitney criteria, Kolmogorov-Smirnov test, Student test, and Spearman's correlation coefficient for detecting the dependence between variables. The arithmetic mean (M), average quadratic deviation (d), error of calculating average quadratic mean (m), level of probability difference (p), and correlation coefficient (r) were calculated.

RESULTS AND DISCUSSION

Analysis of skin microbiota outside of AE lesions in 34 children found that 27.26±5.58 CFU was plated from 1 cm^2 of apparently healthy skin in AE, which was higher than the values of the control group: 9.53±1.65 CFU/cm^2 (Table 1). Only in 10 of the 34 (32.25%) sick children the microorganisms plated from 1 cm^2 of apparently healthy skin did not outnumber the control. We identified a direct moderate correlation between the skin contamination in a lesion and apparently healthy skin, and the correlation coefficient was +0.6. This means that increased microorganism numbers in a lesion also indicated increased contamination of apparently healthy skin.

Staphylococcus aureus was not plated in the children of the control group, whereas *Staphylococcus aureus* was plated in 11 of the 34 (32.35%) examined children suffering from AE from apparently healthy skin, with a mean of 2.41±0.26 CFU/cm^2. It is worth mentioning that the intensified skin contamination of the lesions with *Staphylococcus aureus* occurs in parallel to the intensified contamination of apparently healthy skin, and the correlation coefficient was +0.5.

The mean number of *Staphylococcus epidermidis*, *Micrococci*, and *Bacilli* which were plated from 1 cm^2 of apparently healthy skin in patients with AE presumably exceeded the values of identical microorganisms in the control group, whereas the number of *Staphylococcus saprophyticus* was probably lower than the corresponding value in the control group.

The types of bacteria plated from apparently healthy skin were identical to the microorganisms plated from the lesions (genus *Staphylococcus*, *Micrococcus*, *Bacillus*). However, the basis of microbial flora of apparently healthy skin was comprised by *Micrococci* and *Staphylococcus epidermidis*, and the percentage

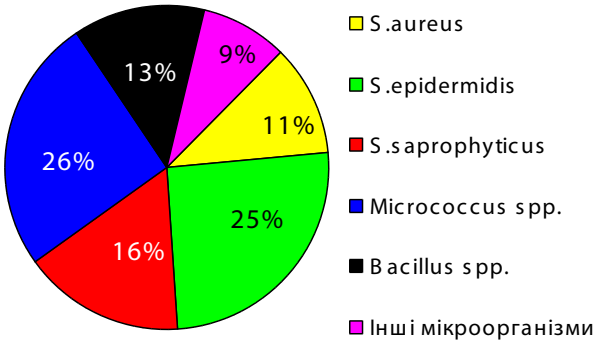

Figure 1. Percentage of bacteria composing the microbial flora of apparently healthy skin in children suffering from atopic eczema (AE).

Table 1. Quantitative microorganism content on skin and outside atopic dermatitis (AD) lesions

Cultured bacteria	Bacterial number per 1 cm^2 of skin M±m CFU/cm^2	
	Patients with AE (n=34)	Control group (n=18)
Per 1 cm^2	Outside of AD lesions	Abdominal skin
Total bacterial content	27.26 ±5.58*	9.53 ±1.65
S. aureus	2.41 ±0.26*	0
S. epidermidis	12.21 ±1.77*	2.35 ±0.46
S. saprophyticus	3.46 ±0.38*	8.42 ±1.53
Micrococcus spp.	23.69 ±3.24*	3.93 ±0.82
Bacillus spp.	3.34 ±0.69*	1.26 ±0.36
Other microorganisms	3.14 ±0.56*	0

AE: atopic eczema; *(*P* 0.05) divergence probability compared with the values of the abdominal skin

Acta Dermatovenerol Croat
2019;27(4):245-249

Table 2. Quantitative bacterial variety on the skin of patients with atopic dermatitis in remission

Cultured bacteria	Bacterial number per 1 cm^2 of skin (M±m CFU/cm^2)			
	Remission		Control group	
	Forearm skin	Abdominal skin	Forearm skin	Abdominal skin
Total bacterial content	78.78 ±14.78*	10.32 ±1.51***	4.58 ±0.89	9.53 ±1.65
S. aureus	19.25 ±1.59*	0.80 ±0.05**/***	0	0
S. epidermidis	52.65 ±2.98*	9.98 ±2.14**/***	4.05 ±0.90	2.35 ±0.46
S. saprophyticus	7.14 ±0.77*	1.75 ±0.41**/***	3.30 ±0.83	8.42 ±1.53
Micrococcus spp.	21.33 ±2.27*	5.25 ±0.74***	2.28 ±0.71	3.93 ±0.82
Bacillus spp.	3.60 ±0.95*	0.80 ±0.19***	0.28 ±0.04	1.26 ±0.36
Other microorganisms	2.63 ±0.31*	0.53 ±0.06**/***	0	0

* (*P* 0.05) divergence probability compared with the control values of the forearm skin; ** (*P* 0.05) divergence probability compared with the control values of the abdominal skin; *** (*P* 0.05) divergence probability compared with the control values of the forearm skin in remission.

of these microorganisms in the entire microbiota was 23.50%±6.89% and 23.38%±6.24%, respectively (Figure 1), whereas in the lesions *Staphylococcus aureus* was the most prevalent at 60.01%±6.11%.

When studying the skin microbiota of 12 children with AD in remission, we found that even in remission our patients' skin was highly colonized by microorganisms both in the typical sites and outside of them. From 1 cm^2 of the extensor surface of the forearm we plated 78.78±14.78 CFU/cm^2, which was higher the values of the control group, while 10.32±1.51 CFU/cm^2 was plated from the abdominal skin, which is comparable with the findings in the control group (Table 2).

It should be noted that the contamination of the forearm skin in patients with AD in remission was presumably higher than that of the abdominal skin, although the contamination of the abdominal skin was higher in the control group.

Microflora of the forearm skin in children with AD in remission was represented mainly by *Staphylococcus epidermidis* and *Micrococci* but was nevertheless of pathogenic nature because *Staphylococcus aureus* occurred in the microflora. The abdominal skin microbiota in patients with AE in remission was different from the skin microbiota composition in children from the control group. In children from the patient group, microflora was represented by *Micrococci* and *Staphylococcus epidermidis* as well as *Staphylococcus aureus*. The data, shown in Table 2, indicate that the overall bacterial count of the forearm skin microbiota in AE remission was higher than the corresponding values of the abdominal skin microbiota in remission and the bacterial number of the forearm skin in children from the control group. The levels of *Staphylococcus aureus* and *Staphylococcus epidermidis* in the abdominal skin microflora in AD remission was probably higher, and the number of *Staphylococcus saprophyticus* was presumably lower than the corresponding values in the abdominal skin of the control group. The number of *Micrococci* and *Bacilli* on apparently healthy skin in remission was comparable with the corresponding values in the control group.

CONCLUSION

The present study has shown that the skin microbiota in children with AE considerably differs from the microbiota in healthy children. The skin within and outside of lesions in children suffering from AE in remission is highly colonized not only with *Staphylococcus aureus* but also with saprophytic pathogens: *Staphylococcus epidermidis*, *Micrococci*, and *Bacilli*. High skin contamination was also observed on apparently healthy skin, outside of AE lesions the bacterial number was 2-4 times higher than on the skin of the control group. There is a qualitative bacterial imbalance in the incidence and prevalence of microorganisms not typical for healthy skin, whereas the microbiota basis in healthy skin comprises *Staphylococcus saprophyticus*, in patients with AE the basis is *Staphylococcus aureus*.

The microbial flora in children suffering from AE in remission also significantly differs from the skin microflora of healthy children's skin. The skin of patients with AE in remission is highly contaminated with bacteria, including pathogenic bacteria, which is indicative of persistent skin microbiota alterations, and in the case of their continuous persistence on the skin it tends to be an unfavorable prognostic factor and can provoke disease relapse.

References:

1. Atopic dermatitis in children (clinical lecture) Popovich Yu. A., Fedotov V. P. Available at: http://dspace.zsmu.edu.ua/bitstream/123456789/5248/1/2016%20Fedotov%20101-120.pdf
2. Wollenberg A, Barbarot S, Bieber T, Christen-Zaech S, Deleuran M, Fink-Wagner A, et al. Consensus based European guidelines for treatment of atopic eczema (atopic dermatitis) in adults and children: part I. J Eur Acad Dermatol Venereol. 2018;32:850-78.
3. W. C. Noble. Microbiology of Human Skin. – LLOYD-LUKE (Medical Books) LTD. 49 Newman street- London; 1981; pp. 496.
4. Murzina E. Features of skin microbiotes of children with atopic dermatitis. – Zbirnik the center of scientific publications "Veles" for the materials of the International Science and Practical Conference: Spring Science News , m. Kyiv, zbirnik articles (riven standard, academic riven). - K .: Center for Science Publications; 2019; pp. 51-61.
5. Methodical recommendations on the assessment of the immunological reactivity of people on the basis of autoflory of the skin and oral cavity. - Moscow; 1978:20.