

The Role of Staphylococcal Genotypes in the Clinical Course of Atopic Dermatitis

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Abstract: Recently, in the pathogenesis of atopic dermatitis, special attention has been paid to opportunistic skin microorganisms. Research has proven that the cause of the disease is a violation of the differentiation of granular cells of the epidermis due to a mutation in the gene responsible for the synthesis of filaggrin (FLG), which is the main component of the natural moisturizing factor of the skin. But even at the same time, the state of skin microbiotas plays an important role in the manifestation of the pathological process, as well as in the chronic course of dermatosis. The hot and dry climate of Central Asia leaves its mark on the clinical course and state of the skin microbiota in patients with atopic dermatitis.

Keywords: atopic dermatitis, genotypes of staphylococcal flora, filaggrin gene, colonization

1. Introduction

The article presents the results of molecular genetic studies of opportunistic microorganisms staphylococcus spp. taking into account the association of polymorphism of genotypes of the filaggrin gene 2282del4 FLG in children with atopic dermatitis in the conditions of Uzbekistan. The results of a PCR study of skin scales in children with AD revealed genotypes of Staphylococcus spp. in 54 of 57 patients, which accounted for 94.7% of cases. Whereas, taking into account the association of polymorphism of genotypes of the 2282del4 FLG gene with favorable genotypes, the MSSA genotype was detected in 53.6% of cases, and MRSA in 43.9% of cases, while in the group of children with heterozygous genotype variants, MRSA was detected in 70% of cases, and in 20% of cases - MSSA.

Recently, in the pathogenesis of atopic dermatitis, special attention has been paid to opportunistic skin microorganisms. [1,2,7,9] Research has proven that the cause of the disease is a violation of the differentiation of granular cells of the epidermis due to a mutation in the gene responsible for the synthesis of filaggrin (FLG), which is the main component of the natural moisturizing factor of the skin. But even at the same time, the state of skin microbiotas plays an important role in the manifestation of the pathological process, as well as in the chronic course of dermatosis. The hot and dry climate of Central Asia leaves its mark on the clinical course and state of the skin microbiota in patients with atopic dermatitis. [6]

The study of the genetic and microbiological aspects of atopic dermatitis will largely determine the nature of the clinical course and the tactics of pathogenetic therapy. [1,2,5]

The purpose of the study was to assess the detectability of genotyping of staphylococcal flora in patients with atopic dermatitis, taking into account the severity of the disease.

Material and research methods: 57 children with atopic dermatitis aged 1 to 18 years were examined. Among them, girls were 28 (49.1%) and boys were 29 (50.8%). All children underwent clinical, molecular genetic, microbiological and statistical studies. The diagnosis of atopic dermatitis was established according to the criteria of J. Hanifin and G. Rajka (1980).

Molecular genetic examination of biomaterials (DNA) was carried out on the basis of Geno-Technology LLC. The object and subject of the study were DNA samples from pregnant women, the filaggrin gene 2282del4 FLG. DNA samples were isolated from peripheral blood lymphocytes according to a modified procedure. The concentration and purity of isolated DNA were assessed by measuring the optical density of DNA-containing solutions at wavelengths of 260 and 280 nm against TE on a NanoDrop 2000 spectrophotometer (USA).

Microbiological studies were characterized by bacterioscopic and cultural studies of skin flakes from lesions in patients with allergic dermatoses. For cultural studies, 5% blood agar, Endo and Kligler medium, as well as salt agar with the addition of mannitol were used and incubation was carried out in a thermostat at 36.8 °C.

Molecular genetic study of *St. genotypes. aureus* isolated from biosubstrates in patients with FL were carried out on DNA material isolated from scrapings of the skin. For collection, disposable plastic tubes with saline were used. solution (500 µl) with a volume of 1.5 ml. Samples for further processing were stored at -20 °C.

Isolation of DNA from samples was carried out with a set of reagents “Proba GS” (manufactured by LLC “DNK-Technology”, Moscow, Russia). The method is based on the use of a strong chaotropic agent, guanidine thiocyanate (GuSCN), for cell lysis and subsequent sorption of DNA on a carrier (glass beads, diatomaceous earth, glass “milk”, etc.). The data obtained during the study were subjected to statistical processing on a personal computer using the Microsoft Office Excel-2010 software package, including the use of built-in statistical processing functions.

2. Research Results.

According to the clinical form, among 57 patients, 14 children were diagnosed with an erythematous-squamous form, an erythematous-squamous form with lichenification - 17, an exudative form - in 8, a lichenoid form - in 11 and a pruriginous form - in 7, respectively.

Microbiological studies of skin lesions in children with AD revealed the growth of opportunistic microorganisms of staphylococcal flora. (Table 1)

Table 1. Characteristics of the inoculation of opportunistic microorganisms on the skin of lesions in children with AD. (abs)

	st.aureus	st.epidermidis	st.haemolyticus	st.saprophyticusm	enterobacter
Infancy (from 0 weeks to 1 year) n= 3	1	2	0	0	0
Children's period 2-11 years n= 36	10	20	1	3	2
Teenage years 12-18 лет N=18	16	2	0	0	0
Total N= 57	27	24	1	3	2

As can be seen from the table, according to species identification, the main number of sown crops was *st.aureus* - 47.4% (27), *st. Epidermididis* - 42.1% (24), *St. Saprophyticus* - 5.3% (3), *St.Haemolyticus* - 1.7% (1) and *Enterobacter* - in 2, which accounted for 3.5% of cases.

The PCR study was used to determine the genotypes of staphylococcal flora isolated from children with AD. The results of a PCR study of skin scales in children with AD revealed genotypes of *Staphylococcus* spp. in 54 of 57 patients, which accounted for 94.7% of cases.

Taking into account the age of patients under 1 year, the MRSA genotype was detected in 2 children, at the age of 2-11 years - the MRSA genotype was detected in 22 children, which amounted to 61.1%, MSSA - in 9 (25%) and MRCoNS - in 2 (5.5%) respectively. Whereas at the age of 12-18 years, MSSA was most often detected - in 16 (88.8%) and MRSA in 2, which accounted for 11.1% of cases.

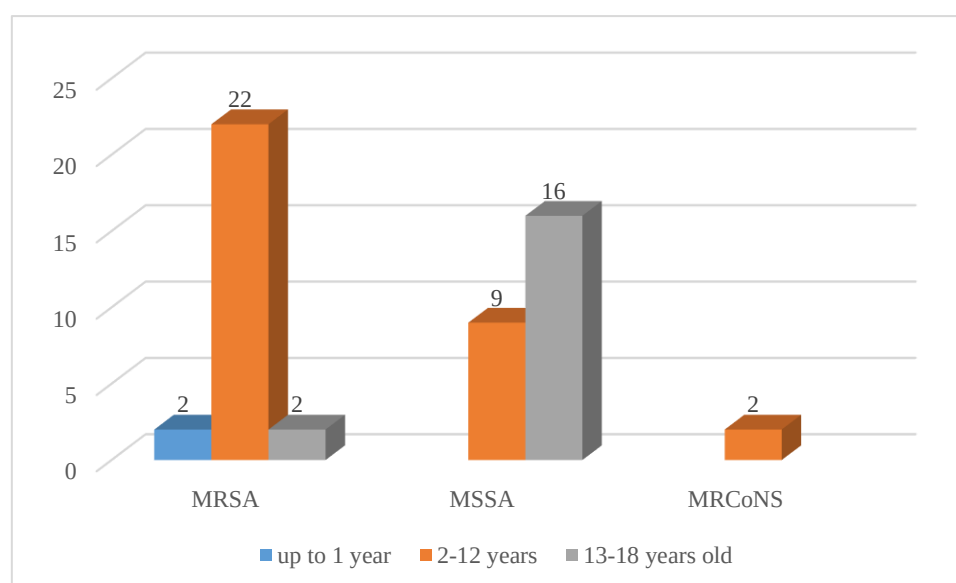


Fig. 2. Indicators of detection of genotypes of staphylococcal flora in patients with atopic dermatitis, taking into account age

In our opinion, the data obtained are important as factors contributing to the persistence of opportunistic microorganisms on the skin of children with atopic dermatitis.

The data obtained were compared with genetic analyzes of the fillagrin gene in children with AD. Analysis of the distribution of genotypic variants of the 2282del4 polymorphism of the FLG gene revealed the predominance of the homozygous genotype for the “wild” allele in the group of healthy children of population control - 100% (30/30), and in the main group of children with atopic dermatitis - 75.4% (43/57) cases, which was 1.3 times lower compared to the control group. ($\chi^2=7.73$, $P<0.005$; $OR=0.05$; $95\%CI$ 0.0-0.86). The heterozygous variant of the 2282del4 polymorphism of the FLG gene was detected in the main group of children with AD in 19.3% (11/57) of cases, while it was not detected in the control group. ($\chi^2=7.73$, $P<0.005$; $OR=15.09$; $95\%CI$ 0.86 – 265.5). The mutant homozygous variant of the 2282del4 polymorphism of the FLG gene was not detected in the control group, while in the group of children with AD it was 5.3% of cases (3/57), respectively. ($\chi^2=7.73$, $P<0.005$; $OR=3.92$; $95\%CI$ 0.20 – 78.4).

Thus, in children of 22 out of 41 patients with favorable genotypes of the 2282del4 FLG gene, 22 out of 41 patients had the MSSA genotype, which amounted to 53.6%, in 18 (43.9%) - MRSA and in 1 - MRCoNS, 2.4% cases accordingly. In the group of children with AD with heterozygous variants of the genotypes of the 2282del4 FLG gene, methicillin-resistant staphylococcus (MRSA) was most often detected in 7, which was 70% of cases, and methicillin-sensitive staphylococcus (MSSA) was detected in 2 (20%) cases.

We analyzed the results of the degree of colonization of staphylococci taking into account the association of polymorphism of genotypes of the fillagrin gene 2282del4 FLG (Table 2).

Table 2. The nature of the degree of colonization by staphylococcus spp., taking into account allelic variants of the fillagrin gene 2282del4 FLG(CFU)

	Healthy N=32	Patients with a favorable allele	Patients with an unfavorable allele (Mutation)
st.aureus	7,05± 1,2	49,05± 2,4 *	64,3± 3,2* **
st.epidermidis	9,1± 1,1	29,9± 1,5*	34,2± 1,6* **

Note: * - reliability indicator in relation to healthy individuals; ** - reliability indicator in relation to the indicators of the favorable allelic variant of the 2282del4 FLG gene ($P < 0.05$)

Severity analysis of staphylococcus spp. shows that with a favorable allelic variant of the fillagrin gene, the contamination of skin with st.aureus in the lesions was on average 49.05+ 2.4 CFU, and with an unfavorable

allelic variant - 64.3 ± 3.2 CFU, which is 1.3 times exceeded the indicators of patients with favorable alleles of the filaggrin gene. The same pattern was observed in the degree of colonization by st.epidermidis. ($P < 0.05$).

3. Conclusion.

Thus, in children with AD with unfavorable allelic variants of the filaggrin gene, the detection rate of methicillin-resistant staphylococcus (MRSA) is 70%, with an increased degree of colonization, which causes the development of persistent forms of bacterial infection on the skin in children with atopic dermatitis.

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