CHARACTERISTICS OF DETECTABILITY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS IN CHILDREN WITH ATOPIC DERMATITIS, TAKING INTO ACCOUNT THE FILAGGRIN (FLG) GENE.

Mavlyanova Shakhnoza Zakirovna–DSc, head of the scientific laboratory for the study of problems of dermatoses of the Republican Specialized Scientific and Practical Medical Center for Dermatovenereology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan;
e-mail: shahnoza_m@mail.ru
Alimukhamedova Yulduz Askarovna- PhD, researcher of the Republican Specialized Scientific and Practical Medical Center for Dermatovenereology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan;
e-mail: julia_alim@list.ru
Azimova Fotima Vahidovna –DSc, researcher of the Republican Specialized Scientific and Practical Medical Center for Dermatovenereology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan;
e-mail:Venera_15129@mail.ru
Jafarov Xondamir MurodÓgli - Researcher of the Republican Specialized Scientific and Practical Center for Dermatovenereology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan;
e-mail: xondamirdj@gmail.com

Key words: atopic dermatitis, filaggrin 2282del4 FLG gene, st.aureus, st.epidermididis, staphylococci, MRSA, MSSA, MRCoNS

SUMMARY

The article presents the results of molecular genetic studies of opportunistic microorganisms staphylococcus spp. taking into account the association of polymorphism of the 2282del4 FLG filaggrin gene genotypes in children with atopic dermatitis in Uzbekistan. The results of the study showed that in children with favorable genotypes of the 2282del4 FLG gene, 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 variants of genotypes, MRSA was detected in 70% of cases, and in 20% of cases - MSSA.

The study of the pathogenetic mechanisms of the clinical course of atopic dermatitis still remains an urgent problem in practical dermatology. Since recently there has been a high increase in the incidence among the child population. [1,3,4,7,] The hot and dry climate of the countries of Central Asia leaves its mark on the clinical course and the 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 aim of the study was to assess the clinical course of atopic dermatitis in children, taking into account the association of polymorphism of the genotypes of the filaggrin gene and the state of the skin microbiota.

Material and research methods. 57 children with atopic dermatitis aged from 1 to 18 years were examined. Among them, girls were 28 (49.1%) and boys - 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 performed on the basis of Geno-Technology LLC. The object and subject of the study were DNA samples of pregnant women, the gene filaggrin 2282del4 FLG. DNA samples were isolated from peripheral blood lymphocytes according to a modified method. The concentration and purity of the isolated DNA were evaluated by measuring the optical density of DNA-containing solutions at a wavelength 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 dermatosis. For cultural studies, we used 5% blood agar, Endo's medium, Kligler's medium, as well as salt agar with the addition of mannitol, and incubation was carried out in a thermostat at 36.8 °C.

Molecular genetic study of the genotypes of St. aureus, isolated from biosubstrates in FL patients, were carried out on DNA material isolated from kazhi scrapings.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.

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

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

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

	st.aureus	st.epidermidis	st.haemolyticus	st.saprophyticusм	enterobacter
infant period (from 0 weeks to 1 year) n= 3	1	2	0	0	0
Children's period 2-11 y.n= 36	10	20	1	3	2
Teenage years 12-18 y 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 method was used to determine the genotypes of staphylococcal flora isolated from children with AD. The results of PCR examination of skin scales in children with AD revealed the genotypes of staphylococcus spp. in 54 of 57 patients, which accounted for 94.7% of cases.

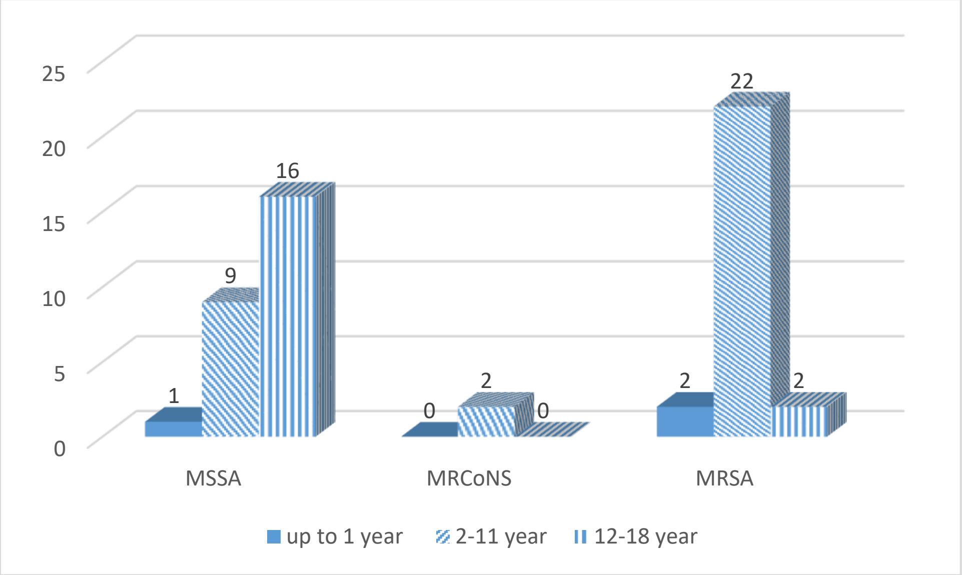

Fig.1. Indicators of st.aureus genotypes isolated from skin scales in children with atopic dermatitis.

As can be seen from the figure, the genotypes of methicillin-resistant staphylococcus - (MRSA) and methicillin-sensitive staphylococcus (MSSA) were found in 26 out of 57 DNA samples, which amounted to 45.6%, respectively, and methicillin-resistant coagulone-negative Staphylococcus spp (MRCoNS) - in 2 , which accounted for 3.5% of cases, respectively. 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 - in 22 children - the MRSA genotype was detected, 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 in 2 - MRSA, which accounted for 11.1% of cases.

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

The obtained data were compared with genetic analyzes of the filaggrin gene in children with AD. (table 3)

Table 3. Detection rate of genotypes of staphylococcus spp. taking into account the association of genotypes of the filaggrin 2282del4 FLG gene.

	Norm	heterozygous	homozygote
MSSAn=26	22	2	2
MRCoNS n=2	1	1	0
MRSA n=26	18	7	1
TotalN=54	41	10	3

As can be seen from the table, 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% of cases, respectively. 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 accounted for 70% of cases, and methicillin-sensitive staphylococcus (MSSA) - in 2 (20%) cases.

We analyzed the results of the degree of colonization of staphylococci, taking into account the association of polymorphism of the 2282del4 FLG filaggrin gene genotypes (Table 4).

Table 4. The nature of the degree of colonization of staphylococcus spp. taking into account allelic variants of the filaggrin 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; ** - significance indicator in relation to indicators of a favorable allelic variant of the 2282del4 FLG gene (P <0.05)

Analysis of the severity of staphylococcus spp. shows that with a favorable allelic variant of the filaggrin gene, the contamination of st.aureus of the skin in the lesions averaged 49.05+ 2.4 CFU, and with an unfavorable allelic variant - 64.3+ 3.2 CFU, which is 1.3 times exceeded those of patients with favorable alleles of the filaggrin gene. The same picture was observed in the degree of colonization of st.epidermidis. (P<0.05).

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.

Literature:

1. Zueva M.I. Mutations R501X and 2282del4 of the FLG gene in patients with allergic dermatoses. Kharkiv Medical Journal - Issue 13, No. 947, 2011 p.93-97.
2. Maksimov V.N., Kulikov I.V., Semaev S.E., MaksimovaYu.V., Prostyakova E.M., Malyutina S.K., Romashchenko A.G., Voevoda M.I. Deletion 2282del4 in the filaggrin gene in the Novosibirsk population and in patients with ichthyosis vulgaris. Medical genetics. 2007;8:21-23.
3. MaksimovaYu.V., Svechnikova E.V., Maksimov V.N., Lykova S.G. Mutations in the filaggrin gene and atopic dermatitis. Clinical dermatology and venereology. 2014;3:58-62.
4. Barker J.N.W.N., Palmer C.N.A., Zhao Y. et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007; 127: 3: p.564—567
5. Ginger RS, Blachford S, Rowland J, Rowson M, Harding CR. Filaggrin repeat number polymorphism is associated with a dry skin phenotype. Arch Dermatol Res. 2005 Dec;297(6):235-241.
6. Gulyamova, G., Mavlyanova, S.Z., &Boboev, K. T. (2015). The role of a polymorphic variant of the tumor-α necrosis factor gene in the development of atopic dermatitis in the population of uzbekistan. KlinicheskayaDermatologiyaiVenerologiya, 14(4), 79-83. doi:10.17116/klinderma201514479-83
7. Palmer CNA, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-offunction variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006 Apr;38(4):441- 446.