NEW GENE OF PSORIATIC ERYTHRODERMA AND PROBABLE PATHOGENESIS OF THE DISEASE

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Erythroderma represents one of the most complex problems of dermatology. First of all, it is explained by variety of the reasons, ambiguity of pathogenesis, rather monotonous clinical picture, tendency of many forms of erythroderma to a long and heavy current and resistance to therapy. Calcium – the key regulator of differentiation of keratinocytes, also participates in maintenance of a cellular homeostasis, especially in patients with erythroderma where damage of skin covers more than 80-90% of integument. Calcium ions in cells regulate the most different intracellular processes – muscular contraction, exocytosis (participates in delivery to cellular membrane of lipids, functional membrane proteins, such as receptors or proteins and also release of hormones, neurotransmitters), including secretion of hormones and neurotransmitters. In this pathology skin has the increased level of renewal of her layers. In this regard change of level of an expression of the genes coding the key proteins-connexins playing an important role in intercellular interactions and maintenance of a metabolic homeostasis and also emergence in them somatic mutations is possible. Different skin chronic diseases with the imposed phenotypes are caused by mutations in five various connexin genes. Some of these diseases are caused by mutations in GJB2 gene that implicated most of researchers to focus on the connexin 26.

Aim: search of mutations in the coding area of the gene of GJB2 among patients with various forms of erythroderma and also assessment of level of ions of Ca²⁺ in plasma of peripheral blood of patients of the same groups.

Materials and Methods. The prospective research was conducted in 56 patients with erythroderma undergoing examination and treatment on bases of dermatological offices: GBUZ Lenoblicentre of specialized types of medical care, GORKVD of St. Petersburg during the period from 2013 to 2018. Indicators of Ca²⁺ in patients with various forms of erythroderma are studied. In all examined groups the intake of peripheral blood in vacuetein with EDTA in the morning, on an empty stomach for the first days of arrival of the patient in a hospital has been carried out. Plasma of peripheral blood has been received from whole blood by centrifugation within 20 min. at 3000 rpm. Centrifugation was carried out no later than in 20 min. after blood sampling. The received samples of plasma were stored at – 70°C before carrying out a research. Determination of level of Ca²⁺ electrolyte in plasma of peripheral blood was defined by ionselective methods on the analyzer, Roche (Switzerland). The research was conducted triple. Genomic DNA was emitted from leukocytes of peripheral blood with method of phenolic and chloroformic extraction (Maniatis et al., 1984). Amplification of the coding area of the gene of GJB2 was carried out with the use of the primers described earlier. Statistical data processing was carried out with use of the SPSS 21.0 program. The reactionary mixes received as a result of amplification have been purified with use of the Cleanup Mini set (Evrogen, Moscow, Russia) according to the enclosed instruction. Assessment of concentration of genomic DNA and amplificated fragments of DNA was carried out with use of the NanoDrop 2000C spectrophotometer. Sequencing of the received amplicons was carried out with the use of the ABI3130xI device (Applied Biosystems, the USA) to the Sintol companies, Moscow, Russia. Processing of received chronograms was carried out with use of the BioEdit Sequence Alignment Editor 7.1.1.1 program, and comparison of the received sequence with reference – with the use of the Basic Local Alignment Search Tool (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). An inspection of the received variation ranks on compliance to normal distribution was carried out by Shapiro-Wilk method.

Results. In the real research all the patients were divided on the basis of the anamnesis, clinical picture, histological and immunohistochemical analyses into 4 groups. The first group of patients was made by patients with a psoriatic erythroderma (n = 19, middle age 51.25±14.92, 13 men and the 6 women). The second one was group of patients with atopic erythroderma (n = 17, middle age 46.6±11.1, 10 men and 7 women). The third studied group was made by 7 patients with drug induced erythroderma (n=7, middle age 72.6±8.73, 4 men and 3 women). The last, fourth group of 7 observations has included patients with an erythrodermic form of mycosis fungoides (n=7, middle age 71.57±8.75, 3 men and 4 women). Other 6 patients are excluded from a research in view of no specificity of histologic pictures and immunohistochemical reaction. The control group consisted of 20 people (middle age 54.3±15.51; 10 men, 10 women) not being close relatives, with absence of skin diseases. The age at the time of inspection didn’t differ when comparing groups of patients with psoriatic and atopic forms of erythroderma with control (p > 0.05). In plasma of peripheral blood of patients of the specified groups and control Ca²⁺ electrolyte level has been estimated. Decrease in the Ca²⁺ level in all the studied groups in comparison with individuals of control group is revealed. Also in the real research direct sequencing of the coding area of the gene of GJB2 among patients with various forms of an erythroderma has been carried out. Mutations of M34T, V37I, R127H in heterozygotic state in three separate cases in patients with psoriatic erythroderma have been revealed. Frequency of these mutations was 16.7% among patients with the psoriatic erythroderma and 8.8% among all the researched patients with different forms of an erythroderma. Frequency of each mutation separately was 5.6% among patients with psoriatic erythroderma and 2.8% among all the researched patients with different forms of erythroderma. In patients with other forms of erythroderma genetic changes in the coding area of the gene of GJB2 are not revealed. Statistically significant differences in the Ca²⁺ level
between group of patients with mutations in GJB2 gene with a psoriatic form of erythroderma and other patients of the same group were not revealed not \( (p = 0.43) \).

Discussion. In the real research we have carried out calcium level assessment in plasma of blood of patients with various forms of an erythroderma. We have shown for the first time the decrease in level of Ca\(^{2+}\) in patients with various forms of erythroderma \( (p<0.0001) \). More expressed decrease in level of calcium in group of patients with psoriatic erythroderma \( (more than 50\%) \) should be also noted. At the same time statistically significant distinctions when comparing this group of patients with patients with a dosage form of a disease weren’t revealed. This phenomenon is probably connected with feature of clinical course of the disease, namely — sharply expressed hyperkeratotic scaling more than 90\% of integument in patients with psoriatic erythroderma and peeling of epidermis in the form of large layers in erythroderma induced by intake of drugs. Epidermis supports the extracellular level of calcium which is settling down, it is most expressed in a granular layer and less expressed in a corneal layer, and, according to Elsholz et al., calcium is the key regulator of differentiation of keratinocytes \( (11) \). Electrophysiological researches, by means of scanning of cysteine have shown that regulation of calcium via hemi-channels in KID syndrome differs. Some mutations of Cx26 in KID syndrome become tolerant to suppression of activity of hemi-channels to extracellular Ca\(^{2+}\) whereas others have shown only insignificant malfunction of Ca\(^{2+}\) \( (2,3,33) \). However mutant hemi-channels with the expressed damage of function have shown gradually increasing permeability to Ca\(^{2+}\) that promotes change of the Ca\(^{2+}\) level and, therefore, to changes of a differentiation of keratinocytes \( (25, 26) \). On mouse models with KID syndrome it has been shown that mutations of hemi-channels break a calcium homeostasis under natural conditions. Also in mice increase inside - and the extracellular content of calcium in a corneal layer of epidermis was revealed \( (14) \). Many authors have noted that in KID syndrome of a mutation in the gene of GJB2 promote formation of active hemichannels with the increased passability of calcium or lead to change of regulation of calcium and pH of skin \( (2, 3, 4, 7) \). These changes in Ca\(^{2+}\) homeostasis have been connected with defects in an epidermal water barrier and the changed secretion of a lipid in the injured skin \( (8) \). The most reasonable pathophysiological process is the hyperactivity of hemichannels, leakage of Ca\(^{2+}\) or ATP and changing of a cellular homeostasis of skin, especially extracellular. The similar pathogenesis is observed also in our research where in the first three groups of patients with erythroderma significant decrease in the Ca\(^{2+}\) level in plasma of peripheral blood in comparison with the fourth and control groups is noted. Therefore, in the presence of mutations of connexin genes in the skin, probably release of ATP and inflow of Ca\(^{2+}\) into cells increases, thereby breaking these natural signals. Owing to penetration of Ca\(^{2+}\) into cells hemichannels are blocked, and the ionic homeostasis of keratinocytes can change and lead to accumulation of ions of calcium in a large number in cytoplasm of keratinocytes. Change in a homeostasis of cells of the Ca\(^{2+}\) level leads to changing of lipidic exchange and by that to changing of a skin barrier. This defect can lead to malfunction of keratinocytes and hyper activation of the last (to hyper proliferation, desquamation of epithelium). The similar hypothesis has been presented in KID syndrome (keratitis, ichthyosis, deafness ess) \( (9) \). Also in the real research when carrying out direct sequencing of the coding area of the gene of GJB2 in group of patients with the pointed forms of erythroderma three pro-gang, each of which bears a mutation of a gene of GJB2 M34T, V37I or R127H described earlier in sensorineural relative deafness, have been found for the first time \( (10) \). It should be noted that in one patient the most often found mutation 35delG this gene wasn’t revealed, found generally at the pathologies stated above \( (10-12) \). Now more than 200 various mutations in GJB2 gene, the majority of which are pathogenic are described and lead to development of sensorineural relative deafness \( (13) \). However, the pathogenicity of mutations of M34T, V37I, R127H is contradictory since their role in development of a hearing disorder is unclear \( (14-21) \). At the same time in a number of researches with the use of cellular cultures with an expression of mutant forms of connexin 26 it has been shown that mutations of M34T, V37I and R127H lead to decrease in functionality of intercellular hemichannels \( (22-24) \).

Conclusions: Our results mean that the mutation of the gene of connexin 26 – M34T, V37I, R127H (GJB2) probably is fundamental in development of psoriatic erythroderma. The found mutations, most likely, promote introduction of intercellular calcium into a cell and release of ATP. The last promotes changes of a cellular homeostasis and possibly brings to emergence of signs of erythroderma.

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Keywords: connexin 26, GJB2 gene mutation, M34T, V37I, R127H, calcium, erythroderma, psoriatic erythroderma, disturbance of skin barrier.

Accepted for printing on 20 Sept 2018

DOI: 10.29256/v.02.02.2018.escbm25

MEMBRANE-BOUNDING HEMOGLOBIN IN ERYTHROCYTES OF PREGNANT WOMEN WITH PREECLAMPSIA AND PREECLAMPSIA WITH HYPERTENSION

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The metabolic status of erythrocytes in women with preeclampsia has not been studied. Changes in the metabolism of red cells leads not only to a decrease in their basic function, but also to a violation of the physical and chemical properties of the membranes, which affects the charge of erythrocytes, the permeability of their...