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CLINICAL AND MOLECULAR CHARACTERISTICS OF 3 MOLDAVIAN CHILDREN WITH WISKOTT-ALDRICH SYNDROME

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Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency characterized by microthrombocytopenia, eczema, recurrent infections, and an increased incidence of autoimmunity and malignancies. The incidence of this rare X-linked primary immunodeficiency disorder is approximately one to four cases per 1,000,000 live male births, with an average age at diagnosis of 24 months in families without a previously affected family member [1]. Affected patients have mutations in the gene encoding Wiskott-Aldrich syndrome protein (WASp), a key regulator of signaling and reorganization of the cytoskeleton in hematopoietic cells [2]. Mutations that result in decreased, but not absent, protein expression cause the milder disease X-linked thrombocytopenia (XLT) that is characterized mainly by thrombocytopenia and sometimes is associated with milder eczema and immunodeficiency [3]. The absence of functional WASp leads to a severe clinical phenotype that can result in death if not diagnosed and treated early in life [4].

Materials and Methods. A detailed analysis of the clinical profiles, investigations and outcome of the 3 children diagnosed with WAS during the period 2016-2019 was performed. The number of T-cell receptor excision circle (TREC)/kappa-deleting recombination excision circle (KREC) copies were quantified by qPCR and were related to the albumin control gene. For the purpose of performing the genetic test, genomic DNA was isolated from peripheral blood leukocytes by using the Salting-out method. In order to examine mutations on the WAS gene, polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) was performed for all 12 coding regions of the WAS gene by using the primer sets from *Ariga T. et al.* [5]. To determine the sequences of the DNA samples showing mutant bands, direct sequencing was performed on the ABI 3500 DX Genetic Analyzer (Applied Biosystems). Data were analyzed using the bioinformatic software "Sequencing Analysis Software v6.0".

Results. During this period of 4 years, WAS was clinically suspected in 5 patients, all of Caucasian ethnicity. The diagnosis was genetically confirmed in 3 patients. No mutations could be demonstrated in the WASP gene in 2 children. The first case was a six months old boy with septicemia, thrombocytopenia, eczema and petechial rash. Immunological investigations have demonstrated essential humoral and cellular changes: a low serum level of IgM (0.3 g/L) and IgA (0.2 g/L), elevated IgE (767.6kU/L), low level of CD4(+) T cells (9%), elevated CD8(+) T cells (59%). Genetic analysis revealed A-to-G transition at complementary nucleotide 274 (c.274-2 A>G), located in intron 2. A review of family history did not reveal the presence of relatives clinically diagnosed as having WAS and since DNA analysis of the patient's mother revealed no mutation, we can assume that the mutation found in the patient must have occurred spontaneously. The second case was a 2 years old boy presenting with complaints of recurrent infections and thrombocytopenia with a low platelet volume. Immunological investigations showed a low serum level of IgM (0.2 g/L), normal IgA (1.39 g/L) and normal IgG (7.18 g/L), low level of CD3(+) (63%), low level of CD4(+) T cells (32%), elevated CD8(+) T cells (28%), elevated CD16(+) (32%). Quantification of TREC/KREC copies showed low TREC and KREC levels. Genetic testing detected a pathogenic mutation – c.391 G>A (p.

E131K) in exon 3 of *WAS* gene. The third case was a 16 years old boy who presented with thrombocytopenia and recurrent sinopulmonary infections. Immunological investigations indicated a normal immunoglobulin profile, low level of CD4(+) T cells (29,8%) and elevated CD8(+) T cells (36,6%). TREC and KREC copy counts revealed low TREC levels while KREC level was within the normal range. Molecular analysis of the *WAS* gene revealed two mutations – c.57 G>T (p. Q19H) in the first exon, and c.136 C>A (p. L46M) in the second exon. The presumed impact on the pacient phenotype was investigated on The Ensembl Variant Effect Predictor and as a result, c.57 G>T (p. Q19H) mutation has a severe phenotypic effect, while the impact of c.136 C>A (p. L46M) mutation is moderate.

Prospects for further research. In order to improve the diagnosis of primary immunodeficiency disorders and to achieve an efficient differential diagnosis, we intend for the future to broaden the spectrum of molecular-genetic diagnosis of immunodeficiency diseases in the Republic of Moldova.

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Key words: *primary immunodeficiency, Wiskott–Aldrich syndrome, thrombocytopenia.*

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ABOUT THE BIOMARKERS OF THE TRANSITION OF HUMAN INTERVERTEBRAL DISK FROM THE NORM TO PATHOLOGY

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It is known that tissue of intervertebral disc (ID) is characterized by intensive glycolysis, the marker of which may be components of the lactate dehydrogenase (LDH) system. As known, ID receives nutrients and O_2, and also carries out the outflow of metabolites by diffusion with use of vertebral bodies. Dystrophic-degenerative changes in ID, which can occur during stress, are the basis of osteochondrosis (OCh). The results of the experiment on white rats or different ages showed that during the response to stress, the components of the LDH system (total activity and isoenzyme spectrum of LDH, lactate, pyruvate content and their ratio) in the spinal tissues are characterized by phase changes. They consist in the switching of glycolytic processes in ID to the activation of aerobic metabolism, which may correspond to the depletion stage (according to G. Selye's theory of stress). If it is necessary to carry out an operation for a patient with osteochondrosis of the spine, we obviously deal with the depletion stage. It can not always be characterized by instrumental methods of examination. Therefore, it is relevant to search for biochemical markers of the transition of human ID in a pathological condition. The purpose of this work was to study the components of the LDH system in human ID tissues obtained during spinal cord surgery in OCh, as well as ID cadaveric material, to characterize the intensity of glycolysis in this tissue during its transition from norm to pathology.