Antinuclear antibodies in children with Wilson's disease

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Антинуклеарные антитела у детей с болезнью Вильсона

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ВВЕДЕНИЕ

Wilson-Konovalov's disease (WD) is a hereditary condition, transmitted as an autosomal recessive trait and associated with impaired excretion of cooper from the body. Excessive accumulation of cooper causes combined lesion of parenchymal organs (primarily liver) and brain [1, 2]. There is a description of over 700 *ATP7B* protein mutations (Cu^{++} transporting beta polypeptide; beta-polypeptide cooper-transporting ATPase), which may cause cooper metabolism disorder [1, 2, 3]. Missense variant is the most prevalent pathogenic variant, leading to replacement

of histidine by glutamine in 1069 position of the encoded protein *c.3207C>A (p.H1069Q)* [4]. It is also found that this H1069Q variant is associated with late neurological manifestations [5].

The clinical picture of Wilson's disease is distinguished by the polymorphism: in 40–45 % of cases the disease debuts with liver damage, developing at the age 5–18; rarer (in 30 %) — with the development of neurological and psychiatric disorders [6]. The symptoms of the disease include: jaundice, edema of shin, increased abdominal volume, esophageal varicose veins, propensity to form bruising and extended hemorrhage. Some patients with WD may have only minor abnormalities in biochemical parameters of liver function with no other symptoms over the years. Other patients with WD experience a fast progression of liver inflammation in the form of chronic hepatitis with high activity, pronounced jaundice, necrosis of hepatocytes and rapid transformation into cirrhosis [6]. Considering diversity of clinical symptoms, there are difficulties in differential diagnosis of Wilson's disease with other liver diseases [7]. In the early stages of hepatocellular damage, the involvement of endoplasmic reticulum, mitochondria, peroxisome and nucleoli in the process, combined with decreased activity of mitochondrial enzymes causes lipid peroxidation, the accumulation of triglycerides, and further — necrosis of hepatocytes. Malondialdehyde, produced by lipid peroxidation, stimulates collagen synthesis, contributing to fibrogenesis [8].

There is the description of disturbances in the immune system in patients with WD, progressing with increasing liver fibrosis stage and age of patients [9]. Subpopulation composition of lymphocytes in children with WD changes and is characterized by involvement of T-cellular immunity in the process of liver fibrosis, and changes are similar to those in other liver diseases [10]. For instance, different stages of fibrosis in non-alcoholic fat liver are accompanied by the accumulation of T-cell and NK-cell subpopulations with different functions and phenotypes in the liver tissues that usually causes pro-inflammatory effects [11].

The process of liver fibrosis is accompanied by changes in the profile of circulating cytokines in chronic liver disease, by the concentration of which it is possible to carry out differential diagnostics between fibrosis stages [12, 13].

The clinical picture of chronic inflammation of the liver in WD is less distinguishable from liver damage of another genesis and causes the development of liver cirrhosis without pathogenetic therapy. This points to the need for detecting WD in all patients with chronic damage of hepatic parenchyma [14].

Characteristic of chronic hepatitis in WD is a moderate increase in biochemical markers of cytolysis, cholestasis and bilirubin exchange with a high level of structural changes in hepatic parenchyma on the results of morphological examination of liver biopsy slides [15].

Clinically WD may occur like autoimmune hepatitis with a detected high level of serum immunoglobulins and nonspecific autoantibodies. Hence, it is also necessary to exclude Wilson's disease in patients with autoimmune hepatitis with inefficiency of corticosteroid therapy [15]. However, the detection rate of autoantibodies in WD has not been studies, only isolated cases of their detection are described that often causes a false positive diagnosis of "autoimmune hepatitis" [16].

STUDY OBJECTIVE: to evaluate the presence of antinuclear antibodies, parameters of cellular immunity and the composition of circulating cytokines in children with WD.

MATERIALS AND METHODS. As part of diagnostic measures for screening children with liver diseases, treated in FSAI "NMRC for Children's Health" of the Ministry of Health of the Russian Federation, there was examination of 46 children with clinical signs of autoimmune hepatitis. All the children got evaluation of the liver fibrosis stage by the method of transient liver elastography on FibroScan F502 (EchoSence, France). METAVIR scale was used to diagnose LF stage [17]. Complete blood test (automated hematologic Sysmex XN 550, Japan), biochemical blood test (AU680, USA), immunophenotyping of peripheral blood lymphocytes (CYTOMICS FC500, Beckman Coulter, USA), the determination of antinuclear antibody (ANA) on the cell line HEp-2 using the reaction of indirect immunofluorescence (RNIF, Immco Diagnostics, Inc, USA). Determining the level of circulating cytokines was performed using enzyme-linked immunosorbent assay MILLIPLEX Human Cytokine, based on Luminex technology (Merck Millipore, Germany). To confirm Wilson's disease, molecular genetic study was carried out by the Sanger method of sequencing on 3500xL Genetic Analyzer (Applied Biosystems, USA). All changes in the reference AT-

P7B gene sequence were described according to the HGVS nomenclature in accordance with the accepted recommendations [18]. Statistical calculations were carried out using Statistica 10.0 program (USA).

RESULTS AND DISCUSSION

Out of 46 examined children, 11 (24 %) were diagnosed with "Wilson's disease", based on molecular genetic testing. The following pathogenic variants of ATP7B gene were identified, causing the development of WD: *c.2304dup (p.M769HfsTer26), c.2998G>A (p.G1000R), c.3002T>G (p.V1001G), c.3036dup (p.K1013QfsTer15), c.3472_3482del (p.G1158FfsTer2)* и *c.3207C>A (p.H1069Q). The variant c.3207>A (H1069Q)* was more common as a part of compound-heterozygous mutation in the presented sample of patients, and as a part of homozygous mutation only in two children. 35 children (76%) got verified diagnosis of "autoimmune hepatitis".

ANA on the cell line HEp-2 were found in 4 out of 11 children with WD diagnosis, using RNIF, moreover, 3 children had a low titer of ANA (1/160), one patient had a high one (1/2560). Only the facts of low ANA titers in patients with WD were previously reported [15]. The type of fluorescence in all children with WD was similar— nuclear granular (AC-2,4). It is interesting to note that only in one child with revealed ANA, the pathogenic variant *c.3207>A (H1069Q)* was in homozygous state. *H1069Q* mutation in the homozygous state causes the development of severe hepatic insufficiency, depression, dysarthria and tremor at an earlier age that a mutation in the compound-heterozygous state [5, 19].

According to transient liver elastography, the child with a high ANA titer (1/2560) did not have liver fibrosis of F0 stage. Perhaps such a high ANA titer of the child is associated with sensitization to cow's milk protein (class 3), egg (class 2), wheat (class 3), total gE = 3043 IU/mL. In children with low ANA titer liver fibrosis ranged from F0 to F2. Hence, the presence of ANA in children with WD did not depend on liver fibrosis stage in this sample of patients.

The comparison of laboratory values of children with WD and the presence of ANA with no antibodies revealed that the former had a significantly lower concentration of albumin in serum than the latter: Me = 65,3 [63,55; 66,85] g/l versus Me = 68 [67; 69,3] g/l (p = 0,042).

Children with WD are characterized by increased concentration of T-cells through the population of T-helpers with a decrease in cytotoxic T-lymphocytes, B-lymphocytes and NK-cells against the backdrop of a rise in Thact, Th17-lymphocytes and Treg [20]. The level of Treg, Th17-lymphocytes, activated T-helpers in the group of children with and without ANA did not have statistical difference and was characterized by a large data range, especially in the group of children without ANA. Nevertheless, we can note tend to decrease the relative amount of Treg and to increase the relative amount of Th17-lymphocytes in the group of children with ANA. Perhaps a large spread of parameters of cellular immunity is due to the fact that the group of children without ANA had children with different fibrosis stages, including F3-4 stage, in which the level of Th17-lymphocytes is significantly higher than in the early stages of liver fibrosis in children with WB [9].

The evaluation of the level of circulating cytokines revealed that the level of IL27 was much higher in the group of children with ANA than in the one without ANA: Me = 12097 [11028; 13299] pg/ml versus

Me = 8338 [8,8; 10559] pg/ml (p = 0,024). Yet, IL22 and TNFα were significantly lower than in the group without ANA and were: TNFα — Me = 60 [53,55; 64,86] pg/ml versus Me = 130 [66,6; 164,5] pg/ml (p = 0,024); IL22 — Me = 20,3 [16,4; 22,4] pg/ml versus Me=41,9 [34,8; 592,9] pg/ml (p=0,012). In addition, there was tend to a higher concentration of IL4 and IL9 in children with ANA. It is worth noting a large spread in values of circulating cytokine levels that might also be associated with the fact that

the children with WD in this sample experienced different liver fibrosis stages, and the change in the cytokine profile of patients is related to liver fibrosis stages [12, 13].

The detection of antinuclear antibodies in patients with accumulation diseases is justified in terms of pathophysiology: damaged tissues of the body, including those associated with reactions to accumulating toxic components, are capable of inducing immune response to tissue damage. Liver damage as well as in Wilson's disease, can be a prime example of such reactions due to the fact that there is a considerable number of lymphocytes and macrophages in the liver tissue, which are the main producers of cytokines. Hence, assessment and monitoring of immune responses in WD is a promising marker of the severity of the condition and therapy efficacy. A promising area of research is broadening methods of drug therapy, with the inclusion of immunomodulators, metabolites and antioxidants, optimizing immune responses and the cytokine profile of patients [21].

CONCLUSION

The presence of ANA in children with Wilson's disease may indicate the attachment of an autoimmune component to the congenital genetic disease. A larger study is required regarding the frequency of ANA disclosure in children with WD, the association of ANA with liver fibrosis and the presence of specific pathogenic variants in *ATP7B* gene.

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