Following the X All-Russian Congress of the Association of Pediatric Allergologists and Immunologists of Russia (APAIR)

По следам X Всероссийского Конгресса Ассоциации детских аллергологов и иммунологов России (АДАИР)

Diagnostic significance of the determination of antinuclear antibodies in children with autoimmune hepatitis

SCO — краткое сообщение

https://doi.org/10.53529/2500-1175-2024-1-36-40

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Keywords: children, autoimmune hepatitis, liver fibrosis, antinuclear antibodies, HEp-2, autoimmunity, immunofluorescence.

For citation: Zhuzhula AA, Kurbatova OV, Petrichuk SV, Parakhina DV, Snovskaya MA, Movsisyan GB, Semikina EL, Potapov AS, Fisenko AP. Diagnostic significance of the determination of antinuclear antibodies in children with autoimmune hepatitis *Allergology and immunology in pediatrics*. 2024; 1: 36-40. https://doi.org/10.53529/2500-1175-2024-1-36-40

Диагностическая значимость определения антинуклеарных антител у детей с аутоиммунным гепатитом

https://doi.org/10.53529/2500-1175-2024-1-36-40

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Ключевые слова: дети, аутоиммунный гепатит, фиброз печени, антинуклеарные антитела, HEp-2, аутоиммунитет, иммунофлюоресценция.

Для цитирования: Жужула АА, Курбатова ОВ, Петричук СВ, Парахина ДВ, Сновская МА, Мовсисян ГБ, Семикина ЕЛ, Потапов АС, Фисенко АП. Диагностическая значимость определенияантинуклеарных антител у детей саутоиммунным гепатитом. *Аллергология и иммунология в педиатрии*. 2021; 1: 36-40. https://doi.org/10.53529/2500-1175-2024-1-36-40

INTRODUCTION

Autoimmune hepatitis (AIH) is an autoimmune inflammatory liver disease, causing the formation of autoaggressive antibodies to own hepatocytes [1]. AIH is considered a rare disease, its prevalence is 3–17 cases per 100000 population in Europe and the USA [2, 3, 4]. In Russia the percentage of AIH is 1,5–7 % [2, 4, 5] in the structure of chronic hepatitis in children. The disease often occurs in children aged 6–10, and it predominantly affects females [4, 5].

AIH etiology is not fully understood [4]. The violation of immunoreactivity in genetically predisposed individuals is considered a major cause of AIH development [2]. AIH triggers might be Epstein-Barr, hepatitis (A, B, C, D, G) and herpes simplex viruses, bacteria, drugs (interferon drugs, non-steroid anti-inflammatory drugs, nitrofurans, etc.), environmental factors [2, 4]. In the presence of a genetic predisposition there is impaired immune regulation, manifested by the defect in the function of T-cells [4]. The syn-

thesis of B-cells of the IgG class antibodies intensifies with AIH, which might cause the membrane destruction of normal hepatocytes [4].

One of the criteria for AIH diagnosis is the presence of antinuclear antibodies (ANA) in the blood serum, that are detected in 65– 78 %of patients with AIH [5, 6]. ANA is not detected in about 10 % of patients with AIH, but their absence does not exclude the diagnosis [6]. The "gold standard" and main screening method of laboratory diagnosis for detecting ANA in the blood serum is the reaction of indirect immunofluorescence (RNIF) on the cell line HEp-2 [3, 7]. The test result is information on the final titer of ANA and the type of nucleus fluorescence and cytoplasm of HEp-2 cells [7].

Detecting ANA in adults with AIH is accompanied by homogeneous (34–58 %), granule (21–34 %) and cytoplasmic (27 %) types of fluorescence or a combination of several types [6]. It is shown that the highest ANA titers in adults with AIH are noted in concomitant syndrome with primary biliary cirrhosis [6]. ANA studies in AIH against the backdrop of pathogenetic treatment in childhood have not been conducted.

Thus, the objective of our study was to define the diagnostic significance of a titer and types of ANA fluorescence in children with AIH during treatment.

MATERIALS AND METHODS

The study involved 77 children with AIH (42 girls and 35 boys), who were screened and treated in FSAI "NMRC for Children's Health" of the Ministry of Health of the Russian Federation. 65 of them were diagnosed with AIH type 1, 8 children — AIH type 2, 4 — seronegative AIH. Patients with AIH type 1 had comorbid diseases: AIH + primary sclerosing cholangitis (PSC) in 20 % of cases, AIH + autoimmune cholangitis (AIC) — in 12 %, AIH + multiple autoimmune pathologies — in 15 %. In the treatment dynamics 16 children were screened in 6 months — 1 year of standard therapy. The patient age changed from 1,91 to 17,97 years. All the children under treatment were examined on the standard protocol, including complete blood count (hematological automatic analyzer Sysmex XN 550, Japan), biochemical blood test (AU680, USA), ANA on the cell line HEp-2 by RNIF (Immco Diagnostics, Inc., USA).

RNIF method is based on incubation of patients' blood serum in serial dilutions with epithelial cells of adenocarcinoma in the person's larynx (HEp-2) in wells of a glass slide. After removing unbound components with the wash buffer, FITC conjugate was added to wells of a glass slide, which coloured bound antibodies. The result of the reaction was evaluated using fluorescence microscope Nikon Eclipse Ni (Japan) at a magnification of ×40. A maximum titer of detecting ANA and type of cell fluorescence were analyzed. ANA titers < 1/160 were considered as normal values, with the titer 1/160 the response was low-positive, 1/320–1/640 — moderate-positive, 1/1280 and higher — high-positive. We considered any detected type of ANA fluorescence as a positive result. To diagnose the liver fibrosis stage, the method of liver transient elastography was used on FibroScan F502 device (EchoSence, France). METAVIR scale was used to evaluate the severity of the degree of liver fibrosis: F0 stage— density in the range 1,5–5,8 kPa; F1 — 5,9–7,2 kPa; F2 — 7,3–9,5 kPa; F3 — 9,6–12,5 kPa; F4 stage (liver cirrhosis) — density 12,6 kPa and more [8, 9]. The distribution by the stages of liver fibrosis was: F0 — 11 patients (14 %), F1 — 14 (18 %), F2 — 13 (17 %), F3 — 9 (12 %), F4 — 30 детей (39 %).

Statistical processing of the results was carried out using Statistica 10.0 program (StatSoft, USA), Excel (Microsoft, USA), IBM SPSS Statistics 25 (USA). Descriptive statistics of quantitative traits is presented in the format: median [lower and upper quartiles] — Me [$Q_{0,25}$–$Q_{0,75}$].

RESULTS

Conducted analysis of ANA study results in children with AIH revealed that 8 children (10 %) had the titer 1/160, 14 (18 %) — 1/320, 9 (12 %) — 1/640, 19 (25 %) — 1/1280, 15 (20 %) — > 1/2560, and 12 patients out of 77 (15 %) did not have antibodies.

Analysis revealed that children with AIH often had the following types of ANA fluorescence: cytoplasmic (74 %), granular (63 %) and homogeneous (57 %). Combined, dots in the nucleus and nucleolar type of fluorescence were 32 %, 9 % and 8 % of incidence, respectively. There were more often cytoplasmic (63 %), homogeneous (57 %) and granular (54 %) types of fluorescence in the high-positive ANA titer (> 1/1280) than in the moderate-positive one (1/320–1/640). Nucleolar type of fluorescence (5 children — 8 %) was detected only in the moderate-positive titer. Dots in the nucleus were less common, but in any type of fluorescence (6 children — 9 %). High titers of ANA fluorescence in children were identified in 69 % with an isolated variant of

AIH type 1, while with a combination of AIH and PSC, AIC, multiple autoimmune pathologies high titers were detected in 34 % of children.

Analysis of titers and types of fluorescence with the severity by the degree of liver fibrosis has revealed phase dependence: from fibrosis stage F0 to F2 there is a decrease in the proportion of children with the high-positive ANA titer ($p < 0,001$), and from fibrosis stage F2 to F4 the proportion of patients with the high-positive ANA titer ($p < 0,05$) increases. We have not revealed the dependence of the fluorescence type on the liver fibrosis stage in this sample of patients.

Analysis of ANA content in 16 children during treatment showed that the titer of ANA fluorescence decreased in 10 patients: in 5 of them — became negative (< 1/160), in 4 — the fluorescence titer decreased to the minimum, in 1 — was negative at the onset of treatment and remained the same; in 6 children ANA titer did not change or became higher.

A decrease in the antibody titer in children (10 people — 63 %) was reliably ($p < 0,05$) associated with the reduction in measures of disease activity: ALT — from 43 [20; 156,7] to 19 [12; 50,3]; AST — from 35,7 [27,1; 75,8] to 25,7 [20; 33] and in the relative (from 0,45 [0,2; 0,6] to 0,1 [0,09; 0,11]) and absolute number of immature granulocytes (from 0,03 [0,01; 0,03] to 0,005 [0; 0,01]). However, there was tendency to decreasing CRP, GGT, ALP, WBC, neutrophils, ESR as well as a trend towards increasing albumin, MCHC, RDW-CV.

6 (37 %) children did not experience considerable dynamics in the titer of ANA fluorescence on the background of therapy; there was also a significant reduction during treatment: ALT — from 129,5 [92,3; 333,4] to 30,5 [16,5; 48]; AST —from 148 [74,4; 192,9] to 41,5 [24,4; 55,0], reduced absolute number of immature granulocytes, increased albumin — from 38,1 [36,8; 40,3] to 41 [40,8; 46]. There was tendency to decreased GGT, ALP, WBC, neutrophils, % of immature granulocytes, ESR, increased MCHC, RDW-CV. However, biochemical parameters in this group were significantly higher than in the children with decreased ANA titer.

All the children, who did not experience a decrease in the titer during treatment, had determined liver cirrhosis (F4) on METAVIR scale, the course of the disease was accompanied by the presence of other gastrointestinal diseases: erosive bulbit, gastroduodenitis, erosive gastritis, reflux esophagitis, ulcerative colitis. With remaining ANA titer, the type of fluorescence remained too, new types did not appear.

DISCUSSION

Thus, analysis of the study results shows that ANA have been identified in 85 % children with AIH that exceeds ANA detection in adults (78 %) with AIH [10]. Unlike adult with identified higher ANA titers in case of combined AIH and PBC, no such correlation was found in children with crossed syndrome [6].

It was found that children with AIH often had the following types of ANA fluorescence: cytoplasmic, granular and homogeneous. The comparison of the data obtained on prevailing types of fluorescence in children with AIH is consistent with the data on detected pathognomonic types of ANA fluorescence with AIH in adults obtained by K. L. Raykhelson [6]. The most frequently identified types of fluorescence with AIH are associated, according to the website https://www.anapatterns.org/, with the appearance of antibodies to the cytoplasm, to nucleoprotein and chromatin [11].

It is interesting to note that the proportion of children with the high-positive ANA titer ($p < 0,001$) decreases from fibrosis stage F0 to F2 and increases from fibrosis stage F2 to F4 ($p < 0,05$). The onset of the disease is apparently associated with a more inten-

sive formation of ANA that is confirmed by our data on detecting higher ANA titers in children with AIH in the absence of liver fibrosis (F0). In liver cirrhosis (F4) the proportion of children with high-positive ANA titers is also higher than in F1 — F3. This may be caused by the presence of a genetic predisposition to the disease and requires further study [12].

With a dynamic follow-up of patients during therapy, most children experienced a decrease in ANA titer one year after starting treatment that was associated with clinical efficacy of therapy. This fact is consistent with data, obtained by L.P. Ananyeva, on a decrease in ANA titers against the background of pathogenetic treatment in adults with autoimmune diseases [13].

CONCLUSION

Determination of ANA in children with AIH is of great clinical and diagnostic importance. ANA titer correlates with the severity of the disease. Determination of ANA titer can be used as additional criteria to evaluate efficacy of AIH therapy in children.

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