original article

Wien Klin Wochenschr
https://doi.org/10.1007/s00508-021-01907-x

Wiener klinische Wochenschrift
The Central European Journal of Medicine

Seronegative autoimmune hepatitis in children

A real diagnostic challenge

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Received: 21 September 2020 / Accepted: 11 June 2021

Summary
Background and aim Classical autoimmune hepatitis (AIH) is characterized by the presence of conventional autoantibodies (anti-smooth muscle, antinuclear and anti-liver-kidney-microsomal antibodies). The absence of such autoantibodies in some patients does not preclude AIH diagnosis or the need for its treatment. This group of patients was termed seronegative AIH. Whether non-conventional autoantibodies can identify this group of patients is still elusive. We aimed to study the prevalence of seronegativity of conventional autoantibodies and the occurrence of non-conventional autoantibodies in children with AIH.
Methods In this study, 55 children with AIH were investigated for non-conventional autoantibodies (anti-neutrophil cytoplasmic antibodies, antibodies to soluble liver antigen, anti-tissue transglutaminase and antiplatelet antibodies). All the patients received immunosuppressive therapy and were assessed for treatment response.
Results Of the patients 44 had classical AIH (type 1, 70.09%, type 2, 9.09%) and 20% were seronegative. The four studied non-conventional autoantibodies occurred in four patients, one for each. All non-conventional autoantibodies were exclusively associated with type 1 AIH. The clinical profile, ultrasonographic findings, liver biochemistry and histopathological findings were comparable in the classical and seronegative AIH. The majority of patients with classical (72.7%) and seronegative (54.5%) AIH were treatment responders.
Conclusion Seronegative AIH represents a substantial percentage of pediatric patients diagnosed with AIH. They were even negative for non-conventional autoantibodies. Furthermore, apart from autoantibodies, seronegative AIH is almost indistinguishable from the classical AIH and the majority of patients were treatment responders. This favorable response to immunosuppression deserves sustainable efforts for considering such a diagnosis and start therapy to halt disease progression is worthwhile.

Keywords Autoantibodies · Conventional · Non-conventional · Corticosteroids · Pediatrics

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Introduction

Autoimmune hepatitis (AIH) is a progressive inflammatory liver disorder that has no disease-specific causative agent [1]. The exact pathogenesis of AIH is still unclear and its diagnosis depends primarily on the detection of a characteristic clinical profile and excludion of other diseases with a similar clinical picture [2].

The suggested pathogenesis of AIH is complex and reflects unique interactions between tolerant liver, environmental stimuli (e.g., microbial products, drugs metabolites, and associated haptens), and dysregu-

Published online: 20 July 2021

lated immunological mechanisms that break this tolerance in genetically susceptible individuals leading to autoimmunity. The AIH manifestations have been described after hepatotropic viral infection such as Epstein-Barr virus [3], herpes simplex virus, and human herpes virus 6 [4]. In addition, acute hepatitis A [5] and hepatitis E viral infections have also been incriminated [6]. The loss of tolerance leads to cytotoxic T cell–mediated hepatocellular injury with important participation of autoreactive B cells that produce autoantibodies [7]. The AIH patients always show lack of immunoregulatory function, which is considered to be the cardinal cause of AIH [8].

The finding of non-organ-specific autoantibodies, high levels of gamma globulins and plasma cell infiltration of liver lobules in liver biopsy are hallmark features of this disorder and the favorable response to immunosuppressive therapy supports the diagnosis [1]. According to the autoantibody profile AIH is classified into two types. Patients with type I are positive for anti-nuclear antibody (ANA) and/or anti-smooth muscle antibody (ASMA) while patients who are positive for anti-liver-kidney-microsomal antibody type 1 (anti-LKM-1) are classified as type 2. These autoantibodies represent the standard/conventional serological markers of the disease [1]. New autoantibodies continue to be characterized in the hope of identifying relevant target autoantigens and markers that can reflect the treatment outcome [9].

Timely diagnosis with early immunosuppressive therapy is life saving. Being an aggressive disease, if left untreated it eventually progresses to cirrhosis and liver decompensation and occasionally even with treatment particularly if initiation is delayed [10]. None of AIH characteristics are imperative for the disease, and isolated deficiencies in the classical features of AIH, namely autoantibodies, do not restrain its existence. For that, an autoantibody negative AIH is possible [11].

Antibodies to soluble liver antigen (anti-SLA), actin (anti-actin), chromatin (anti-chromatin) and liver cytosol type 1(LC-1) have been linked to severe forms of the disease and/or poor treatment response [12]. Other autoantibodies that can support the diagnosis are investigational, such as asialoglycoprotein receptor, and uridine diphospho-glucuronosyltransferases [13]. They constitute non-conventional markers of AIH, and they may help in defining subsets of patients with a different disease course [12].

Perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), anti-lactoferrin and anti-myeloperoxidase (MPO) antibodies have been considered as additional markers of AIH [14, 15]. In addition, anti-tissue transglutaminase antibodies (anti-tTG), which are diagnostic for celiac disease, may be found in some patients with AIH [16].

The autoantibodies in AIH are widely used for its diagnosis and classification; however, their prognostic value needs to be elucidated. We aimed to study the prevalence of seronegativity of conventional autoantibodies and the occurrence of non-conventional autoantibodies in children with AIH.

Patients, material and methods

Study population and ethical statements

This prospective study included 55 patients with AIH presenting to the Pediatric Hepatology, Gastroenterology and Nutrition Department, National Liver Institute, Menoufia University, Egypt. An AIH was diagnosed according to the revised International Autoimmune Hepatitis Group (IAIHG) diagnostic score [17]. Children of both sexes with a diagnosis of AIH according to the revised AIH score, with age up to 18 years old and had a liver biopsy were included in the study. Patients with other associated liver diseases, such as metabolic or viral or other immunologic disorders were excluded from the study.

The study was approved by the research ethics committee of the National Liver Institute, Menoufia University. A signed informed consent was obtained from the parents or the legal guardians of the patients before starting the study.

Serum autoantibodies and protein electrophoresis

All the patients were investigated for the occurrence of serum autoantibodies and gamma globulin levels. The ANA, ASMA, anti-LKM-1, and anti-mitochondrial antibody (AMA) were evaluated by indirect immunofluorescence technique using a Fluoro-Kit™ Combo Pak (all from DiaSorin Inc, Stillwater, MN, USA). Myeloperoxidase ANCA (MPO-ANCA) immunoglobulin (Ig) G and anti-SLAIgG were tested by enzyme linked immunosorbent assay (ELISA) using QUANTALite™ Kit, (INOVA Diagnostics, San Diego, CA, USA). Anti-tTG IgA was performed by ELISA, ORG 540A (ORGENTEC Diagnostika GmbH, Mainz, Germany). Samples were interpreted as positive according to the following cut-off values; >10 units for anti-tTG, >20 units for MPO-ANCA and ≥25 units for anti-SLA. Anti-platelet antibodies (anti-PLT) were detected by ELISA kit (MyBioSource Inc., San Diego, CA, USA). Gamma globulins were measured by protein electrophoresis. Protein electrophoresis was performed using Titan III Cellulose Acetate Plate (Cat. No. 3013, 3023, 3024, 3033) and scanned using Helena QuickScan 2000 (Helena Laboratories, Beaumont, TX, USA). Hypergamma globulinemia was defined according to the reference ranges for age as described by Jolliff et al. [18].

Serum viral markers, ultrasonography and liver biopsy

Hepatitis B surface antigen (HBsAg), anti-hepatitis B core IgM and IgG types, were done by ELISA kit (all from SorinBiomedica Co, Spain.). Hepatitis C

virus antibody (anti-HCV) was done by 4th generation ELISA (Innogenetics, Ghent, Belgium). Real-time polymerase chain reaction for HCV-RNA was performed using COBAS®Ampliprep/COBAS®TaqMan®, Roche Molecular Systems, Branchburg, NJ, USA (detection limit is 15 IU/mL). Liver biopsy was performed for all the patients using an ultrasound (US) guided Tru-Cut needle. Hepatic fibrosis and inflammatory activity were evaluated according to Ishak staging and grading score [19].

Treatment regimens and treatment response

The majority of patients received combined low-dose steroids with azathioprine, while few patients received an alternative regimen due to failure or contraindication of the primary regimen. Treatment response was defined as responders, incomplete response or non-responders according to Manns et al. [1].

Statistical analysis

Quantitative variables were expressed as mean ± standard deviation (SD) or number (%). Statistical significance for quantitative data was tested by either independent samples *t*-test or by non-parametric Mann-Whitney U test accordingly. Additionally, significance for qualitative data was tested by χ^2-test or Fischer's exact test accordingly. A *P*-value of ≤ 0.05 was considered significant. Statistical analysis was performed using SPSS, version 13 (SPSS Inc, Chicago, IL, USA).

Table 1 Basic demographic, clinical and laboratory characteristics of the studied groups

Parameter	Classical AIH		Seronegative AIH (*n* = 11)	*P*1	Classical AIH (*n* = 44)	*P*2
	Type 1 (*n* = 39)	Type 2 (*n* = 5)				
Age, in years	8.97 ± 4.12	6.2 ± 4.32	6.72 ± 3.32	0.15	8.66 ± 4.19	0.187
Females, *n* (%)	23 (59)	4 (80)	8 (72.7)	0.512	27 (61.4)	0.483
History						
Acute hepatitis	19 (48.7)	3 (60)	4 (36.4)	0.643	22 (50)	0.418
Recurrent jaundice	10 (25.6)	2 (20)	4 (36.4)	0.672	12 (27.3)	0.553
Abdominal enlargement	9 (23.1)	0	3 (27.3)	0.444	9 (20.5)	0.624
Encephalopathy	1 (2.6)	0	0	0.811	1 (2.3)	1.0
Epistaxis	1 (2.6)	0	1 (9.1)	0.535	1 (2.3)	0.363
Hematemesis	1 (2.6)	0	0	0.811	1 (2.3)	1.0
Jaundice at presentation	17 (43.6)	3 (60)	2 (18)	0.199	20 (45.5)	0.99
Disease presentation						
Acute	10 (25.6)	1 (20)	3 (27.3)	0.358	11 (25)	0.769
Chronic	28 (71.8)	3 (60)	8 (72.7)	–	31 (70.5)	–
Fulminant	1 (2.6)	1 (20)	0	–	2 (4.5)	–
Liver (US)						
Normal	1 (2.6)	0	1 (9.1)	0.723	1 (2.3)	0.382
Enlarged	13 (33.3)	2 (40)	5 (45.5)	–	15 (34.1)	–
Cirrhotic	25 (64.1)	3 (60)	5 (45.5)	–	28 (63.6)	–
Splenomegaly (US)	37 (94.9)	5 (100)	9 (81.8)	0.273	42 (95.5)	0.175
Ascites (US)	7 (17.9)	0	2 (18.2)	0.584	7 (15.9)	0.855
Total bilirubin (mg/dl)	4.18 ± 5.22	5.64 ± 7.54	4.35 ± 3.65	0.733	4.35 ± 5.44	0.442
Direct bilirubin (mg/dl)	2.48 ± 3.44	3.59 ± 5.02	2.32 ± 2.54	0.992	2.6 ± 3.6	0.95
Alanine aminotransferase (U/L)	221.52 ± 296.89	473.6 ± 542.98	315.18 ± 318.16	0.446	250.17 ± 334.47	0.265
Aspartate aminotransferase (U/L)	305.31 ± 336.01	770.0 ± 888.56	273.45 ± 255.2	0.882	358.11 ± 442.13	0.825
Total proteins (g/dl)	7.46 ± 1.14	7.12 ± 1.71	7.02 ± 0.8	0.438	7.42 ± 1.19	0.283
Albumin (g/dl)	2.91 ± 0.65	3.12 ± 1.15	2.78 ± 0.62	0.82	2.93 ± 0.71	0.548
Gamma globulins (g/dl)	3.04 ± 1.36	2.76 ± 1.05	3.19 ± 1.67	0.986	3.01 ± 1.33	0.9
Hypergammaglobulinemia (>ULN)	34 (87.2)	5 (100)	8 (72.7)	0.305	39 (88.6)	0.181
Alkaline phosphatase (U/L)	307.92 ± 198.44	564.0 ± 476.95	333.64 ± 185.47	0.215	337.02 ± 250.43	0.793
Gamma-glutamyl transpeptidase (U/L)	81.67 ± 57.03	87.4 ± 72.25	89.1 ± 108.52	0.694	82.5 ± 57.99	0.394
Prothrombin time (seconds)	17.54 ± 6.28	18.5 ± 4.05	16.29 ± 5.54	0.385	17.65 ± 6.04	0.251

P1 significance between AIH type 1, type 2 and seronegative AIH, *P2* significance between classical and seronegative AIH groups. *ULN* upper limit of normal, *US* ultrasound

Results

Study population characteristics

The study included 55 children with AIH (35 females and 20 males and ages ranged from 2 years to 15 years). They were divided according to the occurrence of autoantibodies into classical AIH (type 1 AIH, $n=39$, type 2 AIH, $n=5$) and seronegative AIH groups ($n=11$). All groups were age and sex matched ($P=0.15$ and 0.512, respectively). The clinical presentation, ultrasonographic findings, liver function tests were comparable in all groups with no significant statistical differences (P values were >0.05 for all). Mean levels of gamma globulins were comparable in all the studied groups. Yet, hypergammaglobulinemia was found in the majority of the studied patients; in 87.2% (34/39) of type 1, in 100% (5/5) of type 2 and in 72.7% (8/11) of seronegative AIH patients (Table 1).

Occurrence of conventional and non-conventional autoantibodies in the studied groups

Regarding the conventional autoantibodies, ASMA was the commonest type of autoantibodies found in the sera of children with AIH type 1 (92.3%) and in one patient with AIH type 2. ANA was found exclusively in type 1 AIH. LKM-1 autoantibodies (type 2 AIH) were found in 5 patients representing 9.09% of the study population. AMA was found in two patients with AIH type 1.

Diagnosis of seronegative AIH was based on absence of the conventional autoantibodies (ASMA, ANA and anti-LKM-1). Based on these criteria, the diagnosis of seronegative AIH was established in 11 (20%) of the study population. Regarding the non-conventional autoantibodies, there was one patient positive for anti-SLA, one patient with MPO-ANCA, one patient with anti-PLT and one patient with anti-tTG. All non-conventional autoantibodies were exclusively associated with type 1 AIH patients. None of type 2 AIH or the seronegative patients was positive for non-conventional autoantibodies (Table 2).

Liver biopsy findings of the studied patients

The majority of patients showed moderate to severe liver fibrosis. Similarly, the majority had moderate to severe necroinflammatory activity with the exception of AIH type 2 which had minimal to mild necroinflammatory activity in 60% of patients. Nonetheless, there was no significant difference between the classical and seronegative AIH (Table 3).

Treatment regimens and treatment response in different groups

The majority of patients of the classical and seronegative AIH received combined regimen of low-dose

Table 2 Autoantibodies distribution in the studied groups

Autoantibody	Classical AIH		Seronegative AIH ($n=11$)
	Type 1 ($n=39$)	Type 2 ($n=5$)	
Anti-smooth muscle antibody	36 (92.3)	1 (20)	0
Antinuclear antibody	12 (30.8)	0	0
Anti-liver-kidney-microsomal antibody type 1	0	5 (100)	0
Antimitochondrial antibody	2 (5.1)	0	0
Antibodies to soluble liver antigen	1 (2.6)	0	0
Myeloperoxidase ANCA	1 (2.6)	0	0
Antiplatelet antibodies	1 (2.6)	0	0
Anti-tissue transglutaminase antibodies	1 (2.6)	0	0

Table 3 Histopathological findings in liver biopsy of the studied groups

Parameter	Classical AIH		Seronegative AIH ($n=11$)	*P1*	Classical AIH ($n=44$)	*P2*
	Type 1 ($n=39$)	Type 2 ($n=5$)				
Fibrosis						
Mild	2 (5.1)	1 (20)	1 (9.1)	0.571	3 (6.8)	0.907
Moderate	16 (41.0)	3 (60)	4 (36.4)	–	19 (43.2)	–
Severe	21 (53.8)	1 (20)	6 (54.5)	–	22 (50)	–
Activity						
Minimal	3 (7.7)	1 (20)	1 (9.1)	0.338	4 (9.1)	0.181
Mild	15 (38.5)	2 (40)	1 (9.1)	–	17 (38.6)	–
Moderate	18 (46.2)	1 (20)	6 (54.5)	–	19 (43.2)	–
Severe	3 (7.7)	1 (20)	3 (27.3)	–	4 (9.1)	–

P1 significance between AIH type 1, type 2 and seronegative, *P2* significance between classical and seronegative AIH groups

Table 4 Treatment regimen and response types in the studied groups

Parameter	Classical AIH		Seronegative AIH ($n=11$)	*P1*	Classical AIH ($n=44$)	*P2*
	Type 1 ($n=39$)	Type 2 ($n=5$)				
Type of treatment						
Low-dose steroid + azathioprine	35 (89.7)	4 (80)	7 (63.6)	0.303	39 (88.6)	0.073
Mycophenolate mofetil	1 (2.6)	0	1 (9.1)	–	1 (2.3)	–
Cyclosporin A	0	0	1 (9.1)	–	0	–
Steroid then azathioprine	0	0	1 (9.1)	–	0	–
Low-dose steroid	2 (5.1)	1 (20)	1 (9.1)	–	3	–
High-dose steroid	1 (2.6)	0	0	–	1 (2.3)	–
Response to treatment						
Responder[a]	27 (69.2)	5 (100)	6 (54.5)	0.192	32 (72.7)	0.131
Incomplete response[b]	7 (17.9)	0	1 (9.1)	–	7 (15.9)	–
Non-responder[c]	5 (12.8)	0	4 (36.4)	–	5 (11.4)	–

P1 significance between AIH type 1, type 2 and seronegative, *P2* significance between classical and seronegative AIH groups
[a]Responders included cases positive for SLA, MPO-ANCA and Anti-PLT
[b]Incomplete response included a case with AMA
[c]Non-responders included the case with anti-tTG and a case with AMA

steroids and azathioprine (88.6% and 63.6%, respectively) while the remaining patients received other alternative treatment regimens. There was no significant difference between the studied groups regarding the treatment regimen (Table 4). Treatment response was defined as responder, incomplete response and non-responders. The majority of patients of the classical and seronegative AIH were responders (72.7% and 54.5%, respectively) with no significant difference. The three patients with positive ant-SLA, MPO-ANCA and anti-PLT, were treatment responders, while the patient with positive anti-tTG was non-responder to therapy. In addition, one of the two patients with AMA was non-responder and the other had incomplete response (Table 4).

Discussion

The aim of the current study was to investigate the prevalence of seronegativity of conventional autoantibodies and the occurrence of non-conventional autoantibodies in children with AIH.

According to the occurrence of conventional autoantibodies, we divided the patients into classical AIH group (type 1 AIH, $n=39$; 70.9%, type 2 AIH, $n=5$; 9.09%) and seronegative AIH group ($n=11$; 20%). Type 1 AIH was found to be more prevalent than type 2 AIH [20]. ASMA was more common (92.3%) than ANA (30.8%) in AIH type 1. Oettinger et al. [21] reported a similar rate of 92% regarding the occurrence of ASMA in AIH type 1, and slightly lower rates have been reported by others [20]. The frequency of ANA positivity in type 1 AIH in the current study was 30.8%. Itis comparable to that reported by Delghani et al. that showed a frequency of22.6% [22]. The minority (9.09%) of our patients were of AIH type 2. Comparable frequencies ranging from 4% to 20% were reported by others [13].

Although detection of these autoantibodies offers a valuable tool in the diagnosis of AIH, they also can be detected in other liver disorders, such as drug-induced liver injury, non-alcoholic fatty liver disease, primary biliary cirrhosis, primary sclerosing cholangitis and chronic viral hepatitis B or C. In addition, their absence is not sufficient to exclude AIH. Another form of the disease with absent conventional autoantibodies has been described as seronegative AIH. The issue has not been sufficiently investigated in pediatric populations and publications are limited [23].

In this study 11 (20%) patients were seronegative for conventional autoantibodies (ANA, ASMA and anti-LKM-1) and all these seronegative patients were also negative for the studied non-conventional autoantibodies. Different studies have reported variable frequencies of seronegative AIH. In accordance with our results, Mehendiratta et al. [24] and Seo et al. [25] reported similar frequencies (19% and 22%, respectively). In addition, Delghani et al. [22] reported a higher frequency of 30%, while other studies reported lower frequencies of about 5–6% [26].

At disease onset, autoantibodies may be present at a low titer or even negative in both adults and children. Jain et al. [27] found that 20% (11/55) were seronegative at presentation, but only 5.5% (3/55) remained persistently seronegative, while 8/11 (72%) of seronegative patients became autoantibody positive during follow-up. The appearance of conventional autoantibodies may be delayed. So serial testing during the disease course may reveal the late appearance of the conventional autoantibodies [13].

The reported frequencies of autoantibody negative AIH suggest that there is lack of awareness of the disease that may be responsible for underestimations of its occurrence and missed opportunities for its treatment [28]. Even in recent studies the true prevalence of seronegative AIH in children needs rigorous prospective studies [29].

The pathogenic backgrounds of autoantibody negative AIH are proposed to be similar to those of classical AIH. This implies that the absence of autoantibodies in patients with otherwise classical phenotype of AIH is caused by the individual differences in the nature or expression of the autoantibodies, rather than the pathogenicity of the disease [13]. Host-specific factors may also influence autoantibody production. Concurrent disease state may affect the immune system to produce antibodies. Furthermore, genetic factors can also influence immune reactivity and autoantibody production. Human leucocyte antigen (HLA) DRB1*03 and HLA DRB1*07 have been associated with the production of anti-SLA and anti-LKM1, respectively [30].

Although anti-actin antibody has been suggested as subsidiary in diagnosing AIH type 1 and anti-LC1 has been suggested as subsidiary in diagnosing AIH type 2, they are neither considered a standard of care diagnostic tool nor incorporated into the revised AIH diagnostic score criteria until now [31]. In addition, both autoantibodies were sufficiently investigated in other studies [32–35]. For this we aimed to direct our financial resources to investigate other autoantibodies that were not sufficiently investigated before.

Regarding the non-conventional autoantibodies, in our study there was one patient positive for anti-SLA. Anti-SLA can help in the diagnosis of cases lacking the conventional autoantibodies. For this reason, they were included in the original and revised International Autoimmune Hepatitis Group (IAIHG) diagnostic scoring systems [17]. We found one patient with MPO-ANCA and another patient with anti-PLT. Maggiore et al. [23] reported that in children with AIH, anti-PLT antibodies were detected in 3 of the 4 studied children with thrombocytopenia and ANCA was found in an additional patient. If conventional autoantibodies are not detected, p-ANCA may be found. Furthermore, anti-SLA found in both type

1 and type 2 AIH may be helpful in diagnosing AIH lacking the classical autoantibodies [36].

In our study we reported one patient with positive anti-tTG. The association of celiac diseases with AIH has been described. El-Shabrawi et al. [37] reported a prevalence of 11.5% of celiac disease in children with AIH. In this work, all non-conventional autoantibodies were exclusively associated with type 1 AIH patients. P-ANCA that reacts with MPO was reported to be frequently present in AIH-1, primary sclerosing cholangitis, and inflammatory bowel disease [38]. In contrast to AIH-1, p-ANCA is virtually absent in AIH-2 [39].

The clinical presentation, ultrasonographic findings, and liver function tests were comparable in all our studied groups with no significant statistical differences ($P > 0.05$ for all). Mean levels of gamma globulins were comparable in all the studied groups. In addition, we found that the majority of patients had moderate to severe liver fibrosis. Similarly, the majority had moderate to severe necroinflammatory activity with no significant statistical difference between the classical and seronegative AIH. Similar results were reported by others [23, 26, 27] where there were no significant differences regarding the age, sex, clinical profile, liver function tests, liver biopsy parameters, response to therapy, and outcome between seronegative and seropositive AIH.

The majority of our patients with the classical and seronegative AIH were treatment responders (72.7% and 54.5%, respectively) with no significant statistical difference. Jain et al. [27] reported that the response to therapy and outcome of both seronegative and seropositive AIH groups studied were similar. Gassert et al. [26] and Manns et al. [36] reported also similar results.

In the current study, the three patients with positive anti-SLA, MPO-ANCA and anti-PLT were treatment responders, while the patient with anti-tTG was non-responder. Nastasio et al. [40] reported that the treatment-free sustained remission was significantly higher in patients with AIH and celiac disease when compared with patients with AIH without celiac disease, suggesting that gluten restriction in celiac disease patients has a possible long-term adjuvant effect. The patient with positive anti-tTG in our study was neither investigated for celiac disease nor restricted from gluten diet. Taken together, these results necessitate careful search for the possibility of celiac disease in patients with AIH that may get benefit from gluten restriction.

One of the two patients with positive AMA showed incomplete treatment response and the other was non-responder. Although the presence of AMA weighs against the diagnosis of AIH, rare cases of AMA positive AIH have been reported [41] and long-term follow-up of those patients into adult life is strongly recommended, as adults with AMA positive AIH have been shown to develop clinical, serological, and histological profile of primary biliary cholangitis up to 30 years after the first presentation [42].

Seronegative AIH is a challenging disease for which few data are available in the pediatric age group and early initiation of immunosuppressive therapy to suppress the progression of liver disease is worthwhile [43]. In children with clinical and biochemical signs of acute or chronic hepatitis of unknown cause, including patients who have undergone liver transplantation for autoimmune or non-autoimmune liver disease, liver biopsy is to be performed [13]. If liver histology is compatible with AIH, immunosuppressive treatment should be considered despite the absence of serological markers and even if the levels of serum gamma globulins are not elevated [23]. Czaja et al. [13] advised a 3-month trial with corticosteroids in all candidates for the diagnosis, regardless of the serological findings. Then, conventional serological markers can be assessed periodically to document an initially delayed or suppressed appearance. This may reveal the appearance of initially negative conventional autoantibody or the detection of a new autoantibody that helps definitive diagnosis of these cases [27].

The limitations in the current study are the small sample size, the lack of performing anti-actin and anti-LC1 autoantibodies and the lack of longitudinal follow-up of seronegative patients for the possible appearance of autoantibodies later in the course of the disease.

In conclusion, the current study demonstrates that seronegative AIH represents a substantial percentage of pediatric patients diagnosed as AIH. They were also negative for the studied non-conventional autoantibodies. Apart from autoantibodies, seronegative AIH is almost indistinguishable from the classical AIH and the majority of them were treatment responders. This favorable response to therapy deserves sustainable efforts and awareness for considering such diagnosis as early initiation of immunosuppressive therapy to halt the disease progression is life-saving.

Acknowledgements We would like to thank the working staff of the Pediatric Hepatology, Gastroenterology, and Nutrition Department and all physician staff of the Department of Immunology Research Laboratories, Microbiology and Immunology, Faculty of Medicine, Zagazig University, El-Sharkiya, Department of Microbiology and Immunology, Faculty of Medicine, Menoufia University, Department of Pediatrics, Ahmed Maher Teaching Hospital, Ministry of Health, Egypt for their contribution.

Funding The study was funded by National Liver Institute, Menoufia University, Egypt, without any particular role in the study design, recruitment of individuals, data analysis or the writing of the manuscript.

Conflict of interest M.A. Khedr, T.A. Salem, G.M. Boghdadi, A.S. Elharoun, A.A. El-Shahaway, H.R. Atallah and M.M. Sira declare that they have no competing interests.

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