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Autoantibodies: are they a clue for liver diseases?

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Abstract

Introduction: Autoantibody testing has contributed to both biological and clinical insights in managing patients with liver disease. These autoantibodies often have clinical value for the diagnosis, disease activity and/or prognosis.

Aim of the study: We aimed to investigate the potential application of auto-antibodies in different etiologies of non-autoimmune liver diseases.

Material and methods: This study was conducted on 53 infants and children with chronic liver diseases. The patients were subjected to clinical history and examination, laboratory investigations and abdominal ultrasound. Serum of all infants and children was tested for measurement of antiprothrombin antibody and anti-β2-glycoprotein I (aβ2GPI) and anticardiolipin (ACL) auto-antibodies using a fully-automated enzyme linked immunosorbent assay (ELISA) system.

Results: The mean age of the infants with cholestatic liver diseases was significantly lower than those with metabolic liver diseases, hepatitis C virus (HCV) and vascular liver diseases ($p < 0.05$). The gender distribution was proportionate in all groups ($p = 0.703$). Autoantibodies showed significant variations among different etiologies of chronic liver diseases. The incidence of aβ2GPI and ACL was significantly increased in both HCV (94.7% and 78.9%, respectively) and vascular liver diseases patients (90.9% and 72.7%, respectively) ($p < 0.05$). Antiprothrombin antibodies were found in 81.8% of vascular liver disease patients. Interestingly, all types of autoantibodies were deficient in cholestatic and metabolic liver diseases.

Conclusions: Testing for liver-related autoantibodies should be included in the workup of patients with chronic liver diseases. Further studies are needed to explain the cause-effect association of ACL, aβ2GPI and antiprothrombin with chronic HCV and vascular liver diseases.

Key words: antiprothrombin antibodies, anti-β2-glycoprotein antibodies, anticardiolipin antibodies, non-autoimmune liver diseases.

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Introduction

Testing for autoantibodies has contributed to great clinical and biological insights in managing chronic liver disease patients. Autoantibodies are produced by the humeral immune responses against self-cellular proteins and nucleic acids. It has been established as a serological hallmark of autoimmune liver diseases [1]. However, some of these autoantibodies are occasionally detected even in the sera of patients with non-autoimmune liver diseases [2]. These autoantibodies mostly have clinical value for the diagnosis, prognosis and/or disease activity. Such autoantigens are primarily engaged in essential cellular functions

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including DNA replication, DNA transcription, and RNA processing [3].

In liver diseases, it remains unclear whether auto-antibodies are primary or secondary consequences of the underlying processes. Some hepatotropic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) are capable of triggering autoimmune phenomena and manifest the features of autoimmune hepatitis (AIH) in the course of the disease. Meticulous attention is needed to differentiate between chronic viral hepatitis and AIH before the selection of treatment [4].

On the other hand, treatment for some liver diseases induces the production of several types of autoantibodies. In addition, elevations in antinuclear antibodies were found frequently inpatients under long-term d-penicillamine therapy [5].

We aimed to investigate the potential application of auto-antibodies in different etiologies of non-auto-immune liver diseases.

Material and methods

This study was conducted on 53 infants and children with chronic liver diseases consecutively admitted as inpatients of the Pediatric Hepatology, Gastroenterology, and Nutrition Department, National Liver Institute, Menoufia University between January 2020 and July 2020. The etiological diagnosis of the chronic liver diseases was confirmed by history, laboratory and pathological methods. Ethical committee approval of the National Liver Institute was obtained and informed written consent was taken from the patients' legal health authorities for participation in the study.

Patients with renal or liver failure or any disorder that caused reduced production and clearance of acute phase proteins, hypoxia, shock, patients receiving antibodies, anti-inflammatory drugs or corticosteroids, patients with sepsis and patients who are receiving total parenteral nutrition (chest infection, gastroenteritis, spontaneous bacterial peritonitis and urinary tract infection) were excluded from the study.

The patients were subjected to thorough clinical history and examination, complete blood picture with differential leucocytic count, liver function tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], total and direct bilirubin, serum albumin) and abdominal ultrasound.

Serum of all infants and children was tested for measurement of antiprothrombin antibody and anti-β2-glycoprotein I (aβ2GPI) and anticardiolipin (ACL) auto-antibodies using the Alergia fully-automated enzyme linked immunosorbant assay (ELISA) system (Orgentec Diagnostika GmbH, Germany).

Eight milliliters of venous blood was obtained under aseptic conditions from each subject, four milliliters of which were transferred into a plain vacutainer tube allowed to clot, and then centrifuged for 15 minutes at 3000 rpm to separate the serum for assessing liver function tests using a Cobas 6000 analyzer (c501 module) (Roche Diagnostics GmbH, D-68305 Mannheim, Germany).

Two milliliters of blood was delivered to an EDTA vacutainer tube for complete blood count (CBC) using an Automated Haematology Analyzer Sysmex XT 1800i (Sysmex Corporation, Kobe, Japan), and the reticulocyte count was assessed based on the property of ribosomal RNA to react with brilliant cresyl blue. The remaining sample was put in a 3.8% citrate solution in a dilution of 1 : 9 to determine prothrombin time (PT), INR and PTT by Sysmex CS-1600 automated hemostasis testing.

Statistical analysis

Data were collected and entered to the computer using the SPSS program for statistical analysis (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Data were entered as categorical or numerical data, as appropriate. Quantitative data were shown as mean, SD, median, minimum and maximum. Qualitative data were expressed in frequency and percentage. The chi-square (χ^2) test was used to evaluate the association between qualitative variables. Fisher's exact test was used for 2×2 qualitative variables when more than 20% of the cells had an expected count of less than 5. The oneway analysis of variance (ANOVA) test was used for comparison between three or more groups having quantitative normally distributed data, while the Kruskal-Wallis test was used when these data were not normally distributed. Pairwise multiple comparisons using the post hoc test tested the difference between each pair of means. The *p*-value was considered to be statistically significant when it was less than 0.05.

Results

Fifty-three infants and children with chronic liver diseases were included in the study; 34 patients were male and 19 patients were female; their mean age was 75.15 ±45 months. Thirteen patients were complaining of cholestatic liver diseases (11 biliary atresia and 2 progressive familial intrahepatic cholestasis), 10 patients had metabolic liver diseases (7 Wilson disease and 3 galactosemia), 19 with naive HCV and 11 had vascular liver diseases (7 portal vein thrombosis and

Table 1. Laboratory parameters among the different studied groups

Para-meter	Cholestatic liver diseases n = 13 mean ±SD median (min-max)	Metabolic liver diseases n = 10 mean ±SD median (min-max)	Chronic HCV n = 19 mean ±SD median (min-max)	Vascular liver disease n = 11 mean ±SD median (min-max)	P-value (Kruskal-Wallis test)	Post hoc P-values	
Total bilirubin (mg/dl)	9.95 ±6.71 8.1 (4-30)	12.03 ±2.41 11.9 (8.7-17.4)	1.00 ±0.08 1 (0.8-1.2)	1.07 ±0.101 1 (1-1.3)	0.0001	p1 = 0.899 p2 = 0.003 p3 = 0.003	p4 < 0.0001 p5 < 0.0001 p6 = 0.297
Direct bilirubin (mg/dl)	5.66 ±5.13 4.3 (2.3-22)	7.27 ±1.76 6.74 (4.8-10.1)	0.44 ±0.13 0.5 (0.2-0.6)	0.45 ±0.14 0.5 (0.2-0.6)	0.0001	p1 = 0.891 p2 = 0.019 p3 = 0.019	p4 < 0.0001 p5 < 0.0001 p6 = 1.000
AST (U/l)	208 ±212 130 (32-725)	249 ±85 227 (126-371)	42.31 ±25.75 37 (8-110)	36.63 ±19.87 35 (8-79)	0.0001	p1 = 0.990 p2 = 0.091 p3 = 0.077	p4 < 0.0001 p5 < 0.0001 p6 = 0.985
ALT (U/l)	204 ±270 112 (15-978)	137 ±61 130 (50-258)	44.6 ±27.96 34 (7-110)	41.4 ±28.38 32.0 (7-110)	0.001	p1 = 0.956 p2 = 0.289 p3 = 0.272	p4 = 0.005 p5 = 0.004 p6 = 1.000
ALKP (U/l)	567 ±300 588 (37-1065)	731 ±394 701 (291-1615)	147 ±19.19 147 (120-185)	155 ±25.45 157 (123-214)	0.0001	p1 = 0.872 p2 = 0.002 p3 = 0.002	p4 = 0.007 p5 = 0.007 p6 = 0.944
GGT (U/l)	237 ±357 102 (21-1329)	375.6 ±375 246 (125-1380)	95.47 ±19.05 98 (65-140)	100 ±24.45 98 (72-145)	0.001	p1 = 0.944 p2 = 0.694 p3 = 0.726	p4 = 0.230 p5 = 0.244 p6 = 0.994
Total protein (mg/dl)	5.53 ±1.22 5.4 (3.1-8.4)	5.34 ±0.76 5.25 (4.2-6.6)	6.22 ±0.51 6 (5.4-7)	5.87 ±0.30 5.9(5.40-6.50)	0.019 (A)	p1 = 0.998 p2 = 0.380 p3 = 0.930	p4 = 0.034 p5 = 0.320 p6 = 0.148
Albumin (mg/dl)	3.74 ±0.72 3.90 (2-4.5)	3.59 ±0.58 3.6 (2.3-4.5)	3.4 ±0.24 3.5 (3-3.8)	3.65 ±0.31 3.5 (3-4)	0.240 (A)	p1 = 0.995 p2 = 0.553 p3 = 0.999	p4 = 0.919 p5 = 1.000 p6 = 0.182
PT (s)	13.08 ±2.08 12.6 (10.4-18)	14.25 ±3.5 13.25 (10.5-22)	11.1 ±0.31 11 (11-12)	14.64 ±0.92 15 (13-16)	0.0001	p1 = 0.933 p2 = 0.030 p3 = 0.146	p4 = 0.111 p5 = 1.000 p6 = 0.000
PC (%)	81.15 ±16.2 85 (50-99)	78.5 ±22.68 76 (52-118)	99.16 ±2.52 100 (92-100)	65.18 ±6.45 63 (55-75)	0.0001	p1 = 1.000 p2 = 0.011 p3 = 0.029	p4 = 0.104 p5 = 0.477 p6 = 0.0001
INR	1.09 ±0.185 1.05 (0.87-1.50)	1.11 ±0.17 1.10 (0.88-1.40)	0.91 ±0.03 0.90 (0.90-1.00)	1.26 ±0.09 1.3 (1.10-1.40)	0.0001	p1 = 1.000 p2 = 0.022 p3 = 0.055	p4 = 0.030 p5 = 0.122 p6 = 0.0001
PTT (s)	44.67 ±8.86 43.35 (32.80-62.40)	43.83 ±5.34 44.9 (31.70-48.90)	32.0 ±2.49 31.0 (31.00-39.00)	39.09 ±2.7 39.0 (35.00-45.00)	0.0001	p1 = 1.000 p2 = 0.002 p3 = 0.301	p4 < 0.0001 p5 = 0.142 p6 = 0.000
Hb (g/dl)	9.85 ±1.13 10.10 (8.20-12.60)	10.56 ±1.06 10.75 (8.90-12.00)	12.35 ±1.36 12.4 (10.10-15.80)	12.61 ±1.44 12.30 (11.30-15.80)	0.0001 (A)	p1 = 0.584 p2 = 0.000 p3 = 0.000	p4 = 0.004 p5 = 0.009 p6 = 0.997
WBCs (×10⁹/l)	12.26 ±4.87 10.80 (6.50-25.00)	13.18 ±3.4 12.45 (8.50-20.00)	6104.74 ±2437.21 5900.0 (2590-11600)	6945.45 ±2700.87 6100 (2900-11600)	0.0001	p1 = 0.996 p2 = 0.000 p3 = 0.000	p4 < 0.0001 p5 < 0.0001 p6 = 0.956
Platelets (×10⁹/l)	311.08 ±128.08 318.0 (156.00-573.00)	382.6 ±174.24 322.5 (187.0-801.0)	298052.63 ±76464.57 310000.0 (164000-449000)	80909.09 ±16114.93 78000 (58000-112000)	0.0001 (A)	p1 = 0.873 p2 = 0.000 p3 = 0.000	p4 < 0.0001 p5 < 0.0001 p6 < 0.0001
Retics (%)	3.32 ±2.36 2.4 (0.7-7.5)	2.59 ±1.16 2.05 (1.5-4.9)	1.18 ±0.33 1 (1-2)	1.38 ±0.42 1.2 (1-2)	0.0001	p1 = 0.918 p2 = 0.039 p3 = 0.071	p4 = 0.022 p5 = 0.056 p6 = 0.717

A – ANOVA test, p1 – cholestatic liver diseases and metabolic liver diseases, p2 – cholestatic liver diseases and chronic HCV, p3 – cholestatic liver diseases and vascular liver disease, p4 – metabolic liver diseases and chronic HCV, p5 – metabolic liver diseases and vascular liver disease, p6 – chronic HCV and vascular liver disease

4 Budd-Chiari disease). The duration of disease presentations ranged from 7 days to 5 years.

The mean age of the infants with cholestatic liver diseases (2.69 ±2.19 months) was significantly lower than those with metabolic liver diseases (88.30 ±64.21 months), HCV (108.00 ±31.24 months) and vascular liver diseases (102.55 ±30.05 months) ($p < 0.05$). The mean ages were comparable among patients with metabolic liver diseases, HCV and vascular liver diseases ($p > 0.05$).

The gender distribution was proportionate in all groups ($p = 0.703$). Male patients accounted for 61.5%, 50.0%, 68.4% and 72.7% of the cholestatic liver diseases, metabolic liver diseases, HCV and vascular liver diseases; respectively.

Concerning the laboratory parameters, both cholestatic and metabolic liver diseases had significantly higher total bilirubin levels (9.95 ±6.71 and 12.03 ±2.41 mg/dl, respectively) than HCV and vascular liver disease (1.0 ±0.08, 1.07 ±0.101 mg/dl) ($p < 0.05$). Liver transaminases and alkaline phosphatase (ALKP) were significantly higher in metabolic liver diseases than HCV and vascular diseases. The glutamyl transpeptidase (GGT) and albumin levels were comparable among the different groups ($p > 0.05$) (Table 1).

The ultrasound findings differed significantly among the different studied groups ($p < 0.05$). Incidence of hepatomegaly was significantly higher in patients with metabolic liver diseases (100%) in comparison to its incidence in cholestatic liver diseases (7.7%), HCV (0%) and vascular liver diseases (36.4%) ($p < 0.05$). The incidence of splenomegaly was significantly higher in both patients with vascular liver diseases (100%) and metabolic liver diseases (100%) in comparison to its incidence in cholestatic liver diseases (30.8%) and HCV (0%) ($p < 0.05$).

Autoantibody expression showed significant variations among the different etiologies of chronic liver diseases. The incidence rates of anti-β2-glycoprotein I (aβ2GPI) and ACL were significantly increased in both HCV and vascular liver disease patients ($p < 0.05$). Antiprothrombin antibodies were significantly increased in vascular liver disease patients (81.8%). Interestingly, all types of autoantibodies were not found in cholestatic and metabolic liver diseases (Table 2). No significant correlation was found between the presence of the autoantibodies and the duration of the disease ($p > 0.05$).

Discussion

The liver is a very important immunological organ, having a major role in controlling systemic tolerance. The liver harbors unconventional and professional antigen-presenting cells which are crucial for induction of tolerance and its maintenance. Orchestrating the immune response depends on a healthy and well-toned immunological liver microenvironment [6], which is expected to be disrupted in patients with chronic liver diseases.

Disruption of tolerance towards microbial metabolites, self-antigens and lipids, in addition to alterations in bile acid composition, can result in changes in effector cell activation and polarization and may impair the protective anti-inflammatory regulatory T and B cell responses [7].

We investigated the predominance of antiprothrombin antibody, aβ2GPI and ACL in 53 patients with non-autoimmune liver diseases in the pediatric age group. Significant variations of autoantibody levels among cholestatic liver diseases, metabolic liver diseases, HCV and vascular liver diseases were found.

Table 2. Autoantibodies among the different studied groups

Parameter	Cholestatic liver diseases $n = 13$	Metabolic liver diseases $n = 10$	Chronic HCV $n = 19$	Vascular liver disease $n = 11$	P-value (χ^2 test)
Anticardiolipin antibody, n (%)					
Negative	13 (100.0)	10 (100)	4 (21.1)	3 (27.3)	
Positive	0 (0.0)	0 (0.0)	15 (78.9)	8 (72.7)	0.0001
Anti-β2-glycoprotein I antibody, n (%)					
Negative	13 (100.0)	10 (100)	1 (5.3)	1 (9.1)	
Positive	0 (0.0)	0 (0.0)	18 (94.7)	10 (90.9)	0.0001
Antiprothrombin autoantibody					
Negative	13 (100.0)	10 (100)	19 (100.0)	2 (18.2)	0.0001
Positive	0 (0.0)	0 (0.0)	0 (0.0)	9 (81.8)	(F)

F – Fisher's exact test

Patients with cholestatic and metabolic liver diseases were deficient in all the assessed autoantibodies. Children with HCV showed prevalence of aβ2GPI and ACL antibodies. Antiprothrombin antibodies were present only in patients with vascular liver disease. Interestingly, all the investigated autoantibodies were predominant in vascular liver disease patients.

In the current study, ACL antibodies, which are considered one of the serological hallmarks of antiphospholipid antibody syndrome, were detected in 78.9% of the sera of children with HCV. The frequency of ACL antibodies in our patients with chronic HCV was higher than that recorded by Melayah (51%) [8] and Leroy et al. (20.9%) [9].

The presence of ACL in viral or other infections may be induced by disturbances in the regulation of cellular and humoral immunity, as a consequence of infectious diseases. Moreover, stimulation of apoptosis by viruses can lead to plasma membrane phospholipids' redistribution and their overexpression on the apoptotic cell membrane surface, which result in formation of ACL antibodies. In patients with chronic viral hepatitis, especially HCV, induction of neoantigens may provoke antibody formation by rupturing the liver cell membrane [10].

Anti-β2-glycoprotein I antibody was highly prevalent in patients with HCV (94.7%). The significant expression of aβ2GPI and chronic HCV may be induced by different pathways, such as molecular mimicry between the β2-glycoprotein I molecule and different infectious pathogens. Blank et al. reported that the target epitopes of β2-glycoprotein I share homology with HCV. It is credible that the infectious particles which share the same determinant with β2-glycoprotein I are digested and presented by B cells, dendritic cells and macrophages to T cells, leading to an immune response and aβ2GPI synthesis [11]. In addition, HCV infection has been associated with dysbiosis of the gut microbiota [12-14]. Gut dysbiosis has been implicated in the induction and persistence of aβ2GPI through many mechanisms [15].

Anticardiolipin antibodies, aβ2GPI and antiprothrombin autoantibody were detected in the sera of 72.7%, 90.9% and 81.8% of our children with vascular liver diseases, respectively. Despite the strong association between antiphospholipid antibodies and thrombosis, the exact pathogenic mechanisms underlying thrombotic events have not been yet fully elucidated, and more than one mechanism may be involved, such as exposure to some environmental agents, e.g. infections, in susceptible individuals [16].

Beta-2-glycoprotein I has two essential roles in both the complement and coagulation systems. The proposed in vitro properties of β2-glycoprotein define it as a natural anticoagulant. Cumulative evidence suggests that aβ2GPI-IgA can predispose patients to venous or arterial thrombosis [17, 18].

The present study had some limitations. First, this was a cross-sectional study, so it could not explain the cause-effect association of ACL, aβ2GPI and antiprothrombin with liver diseases. Second, multiple measurements of the antibodies were lacking. Third, titers of the antibodies were not quantitatively measured. Fourth, IgM and IgG types were not clarified.

Conclusions

Testing for liver-related autoantibodies should be included in the workup of patients with chronic liver diseases. Further studies are needed to explain the cause-effect association of ACL, aβ2GPI and antiprothrombin with chronic HCV and vascular liver diseases.

Acknowledgements

Disclosure

The authors declare no conflict of interest.

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