Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 31 (1), 2024: 58–66.
www.Ejimmunology.org

Autoantibodies profile in autoimmune liver diseases and chronic viral hepatitis

Rehab A. Rabie[1], Alaa Hadhoud[1], Shymaa Abdelazim[1], Mohamed I. Radwan[2], Marwa H. Attia[1], and Mohammed A. Hasuna[2]

[1]Department of Medical Microbiology & Immunology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
[2]Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Corresponding author: Rehab A. Rabie, Department of Medical Microbiology & Immunology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
Email: dr.rehabrabie@yahoo.com.

Abstract

Despite their low prevalence, autoimmune liver diseases (AILD) cause liver cirrhosis, progress and leads to mortality from liver failure. Autoantibodies are confirmed to have significance in the early screening of AILD patients, especially in those who are asymptomatic before onset of clinical signs. This study aimed to assess levels of liver autoantibodies and their association with clinical manifestations of autoimmune liver diseases and chronic viral hepatitis (CVH) patients. This case-control study included 50 patients (case group of 25 patients with AILD and control group of 25 patients with CVH). They were investigated for presence of antibodies against LKM-1, AMA-M2, PML, M2-3E (BPO), gp210, Sp100, LC-1, Ro52 and SLA/LP using the line immune blot technique, and for the presence of antinuclear antibodies (ANA), as non-organ specific autoantibodies, using indirect immunofluorescence technique. Specific autoantibodies were detected in all AILD cases and some of their levels were significantly higher when compared with CVH group. Among AILD patients, 52% were positive for ANA, whereas 61.1% of chronic hepatitis C and 28.6% of chronic hepatitis B patients were positive for ANA with no significant difference (p=0.3). In conclusion, early diagnosis of autoimmune liver diseases has been linked to assessment of autoantibodies, allowing for prompt therapeutic intervention to stop the progression of liver cirrhosis and the accompanying complications.

Keywords: autoantibodies, autoimmune liver diseases, viral hepatitis, Immunoblot.

Date received: 23 May 2023; **accepted:** 10 November 2023

Introduction

Autoimmune liver diseases (AILD) are section of autoimmune diseases which occur as a result of dysfunction of the immune system and production of autoantibodies, which include autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). Usually, these diseases do not co-occur, but occasionally they may have similar symptoms in a condition called overlap syndrome, in which patients with AIH develop PBC either simultaneously or consecutively.[1]

Autoimmune hepatitis (AIH) involves in addition to autoantibodies, high serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and immunoglobulin G

(IgG).[2] Primary biliary cirrhosis is a chronic autoimmune cholestatic liver disease which may be complicated by cirrhosis and hepatocellular carcinoma and may require liver transplantation.[3] Primary sclerosing cholangitis is a rare liver disease affecting morbidity and mortality with poorly understood pathogenesis.[4]

Liver autoantibodies have two different categories, non-organ specific involving antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), smooth muscle antibodies and liver-kidney microsome type1 (LKM-1) antibodies, and disease specific autoantibodies which are important in diagnosis and classification of AILD such as anti-glycoprotein-210 (gp210) antibodies, anti-soluble liver antigen (SLA) antibodies, speckled protein-100 (Sp100) antibodies.[5]

Diagnosis of AILD, particularly in asymptomatic patients and before the onset of clinical symptoms, depends heavily on detection of liver autoantibodies, which with early therapeutic intervention may slow the progression.[6]

Additionally, loss of self-tolerance and promotion of antibody production were recently linked to hepatitis C virus (HCV) and hepatitis B virus (HBV) infections. This may add to liver damage and may even progress to a disease similar to AIH. Still, studies are needed to determine the prevalence and impact of autoantibodies on course of the disease.[7] In this study, we aimed to assess levels of liver autoantibodies and to determine their correlation with liver disease in AILD patients and chronic viral hepatitis (CVH) patients.

Subjects and Methods

Study design and setting

This case-control study was carried out at the Department of Medical Microbiology and Immunology, and Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt during the period from January 2021 to January 2022.

Patient enrollment

The study included 50 patients, divided into two groups, the case group included 25 patients with AILD. The control group included 25 patients with CVH (18 with chronic viral hepatitis C and 7 chronic viral hepatitis B). Their diagnosis was based on clinical and laboratory examinations.

Patients with AILD (case group) were selected according to the following inclusion criteria as reported in the hospital records; adult patients more than 18 years old, clinically diagnosed with autoimmune liver diseases (negative for viral hepatitis, elevated total IgG, marked elevation in liver enzymes) and patients with CVH (hepatitis B and C as the control group) with failure of antiviral treatment. On the other hand, pregnant females, patients with liver diseases other than hepatitis, and patients with hepatitis due to other causes such as drugs, toxins and alcohol were excluded.

Blood samples collection

A venous blood sample (5 ml) was collected from each study patient under complete aseptic condition. Blood samples were dispensed in commercially available plain tubes, allowed to clot, and centrifuged at 1000 x g for 15 minutes (Heraeus Sepatech Megafuge 1.0, Thermo Scientific, USA). Then sera were separated, aliquoted in microfuge tubes and stored at -20°C until used.

Specific liver autoantibodies were measured by immunoblotting technique using line immunoblotting test strips. The following antigens were included: AMA-M2 (pyruvate dehydrogenase complex E2 subunit), M2-3E (BPO-branched-chain oxoacid-, pyruvate- and oxoglutarate dehydrogenases), Sp100 (spot-pattern 100 kDa protein), PML (promyelocytic leukemia protein), gp210 (glycoprotein 210), LKM-1 (liver-kidney microsomes), LC-1 (liver cytosolic antigen type1), SLA/LP (soluble liver antigen/liver pancreas antigen) and Ro52. They were assessed using commercial kits (EUROLINE-Autoimmune Liver Diseases–(IgG)

(Euro immune, Germany), according to the manufacturer's instructions. At the end, test strips were scanned using a flatbed scanner (CANON EUROPA N.V, Vietnam) and evaluated with an automated scanner (DL 1300-5001-4G EURO Line scan order). The results were interpreted based on the relative signal intensity.[5]

Assessment of ANA, as a nonspecific autoantibody control, in serum samples was performed using indirect immunofluorescence assay kits (NOVA Lite HEp-2 ANA Kits/Substrate Slides, INOVA Diagnostics, USA), according to the manufacturer's instructions. Slides were examined by an immunofluorescent microscope (Leica DMLS Fluorescence Microscope, Leica Bio systems, Danaher, USA). The observation of apple green fluorescence was considered a positive reaction.[8]

Alpha fetoprotein (AFP) was quantitively detected in serum samples by an immunoassay system (ADVIA Centaur® CP Immunoassay System by Siemens, Canada), according to the manufacturer's instructions. The inflammatory marker C-reactive protein (CRP) was quantitively detected in serum samples by a clinical chemistry analyzer (Cobas c 501 module-Roche Diagnostics, Hungary), according to the manufacturer's instructions. Data for albumin, total bilirubin, prothrombin time (PT), international normalized ratio (INR), and complete blood count (CBC) were obtained from the hospital patients' records.

Patients were classified according to the following categories: AIH, a chronic liver inflammation associated with autoantibodies, was defined by positive ANA and autoantibodies against SLA/LP, LKM-1, LC-1, and Ro52. Type 2 AIH was defined specifically by (anti-LKM-1) and (anti-LC1) while type 1 was defined by ANA, SLA/LP and Ro52. PBC-associated autoantibodies were defined as autoantibodies against AMA-M2, Sp100, gp210, PML and M2-3E. Mixed autoantibodies were considered in overlap syndrome (AIH- PBC).[5,9,10]

Statistical Methods

Analysis of data was performed using the Statistical Package for the Social Sciences (SPSS version 20). Chi- square test or Fisher exact test (if any cell was equal to 5 or less) were used for comparing proportions. Independent t-test and Mann Whitney were used for quantitative variables. Pearson correlation between two quantitative variables was used to detect the dependency of one factor on another one. A p value ≤ 0.05 was considered significant.

Results

There was a statistically significant difference in age and sex between AILD patients and CVH ($p \leq 0.001$ for each). Young and middle- aged females were more common in AILD patients. There was a statistically significant increase in the albumin level ($p=0.01$), total bilirubin ($p \leq 0.001$), PT ($p=0.01$), CRP ($p=0.002$), and AFP ($p \leq 0.001$) in CVH patients more than AILD patients (Table 1).

Table 1. Characteristics of demographic and laboratory parameters in patients of the study groups.

Item	Autoimmune liver diseases (AILD) (N=25)	Chronic viral hepatitis (CVH) (N=25)	*p* value
Age (year) Mean ± SD	30.28± 10.57	55.0±10.99	≤0.001[1]
Sex N (%)			
Male	0 (0.0)	17 (68.0)	≤0.001[2]
Female	25 (100.0)	8 (32.0)	
Laboratory parameters			
-Albumin			
Median	2.76	2.5	0.01[3]
range	(1.57-5.8)	(1.5- 33)	
-Total bilirubin			
Median	3.35	4.35	≤0.001[3]
range	(1.11-20.5)	(0.89-163.0)	
-PT			
Median	40.2	54	0.01[3]
range	(18- 70.3)	(13.9- 100)	
-INR			
Median	1.79	1.28	NS[3]
range	(1.0-2.25)	(1.0-2.8)	
-CBC			
Pancytopenia	14 (56.0)	10(40.0)	NS[4]
Anemia	6 (24.0)	9(36)	
Anemia and leukocytosis	2 (8.0)	4(16.0)	
Anemia and thrombocytopenia	3 (12.0)	2(8.0)	
-CRP			
Median	15.5	50.48	0.002[3]
range	(6 -70.0)	(6.73-173.25)	
-AFP			
Median	6.30	560	≤0.001[3]
range	(4.20- 580.5)	(3.20-1000)	

[1]Independent t - test, [2]fisher exact test, [3]Mann Whitney, [4]chi square test. PT; prothrombin time, INR; international normalized ratio, CBC; complete blood count, CRP; c-reactive protein, AFP; alpha fetoprotein.
P > 0.05 is not significant (NS).

The following specific liver autoantibodies Ro52, liver cytosol antigen type 1 (LC-1), LKM-1, and anti-mitochondrial M2 (AMA-M2) antibodies were significantly increased the AILD group more than CVH (hepatitis B and C) group, (*p*≤0.001, *p*=0.01, *p*=0.05, and *p*=0.04, respectively). However, there was no significant difference in the level of ANA between these study groups (*p*=0.3) (Table 2). Certain specific liver autoantibodies were detected in different types of AILD as demonstrated in Table 3.

Table 2. Prevalence of specific liver autoantibodies as assessed by immunoblot and antinuclear antibodies (ANA) as non-specific antibodies as assessed by immunofluorescence (IF), in study groups.

Classification		AILD (N=25) N (%)	*p1	Chronic viral hepatitis (CVH)			*p3	*p4
				Hepatitis C (N=18) N (%)	*p2	Hepatitis B (N=7) N (%)		
Immunoblot	Ro52	14 (56.0)	≤0.001	2 (11.1)	NS	6 (85.7)	≤0.001	≤0.001
	SLA/LP	14 (56.0)	NS	7 (38.9)	NS	2 (28.6)	NS	NS
	LC-1	18 (72.0)	NS	12 (66.7)	0.005	1 (14.3)	0.01	0.01
	LKM-1	14 (56.0)	NS	5 (27.8)	0.05	1 (14.3)	NS	0.05
	gp210	8 (32.0)	NS	3 (16.7)	NS	2 (28.6)	NS	NS
	PML	8 (32.0)	NS	2 (11.1)	NS	2 (28.6)	NS	NS
	Sp100	7 (28.0)	NS	2 (11.1)	NS	1 (14.3)	NS	NS
	M2-3E	6 (24.0)	NS	3 (16.7)	NS	0 (0.0)	NS	NS
	AMA-M2	11 (44.0)	0.02	2 (11.1)	NS	1 (14.3)	NS	0.04
IF	ANA	13 (52.0)	NS	11 (61.1)	NS	2 (28.6)	NS	NS

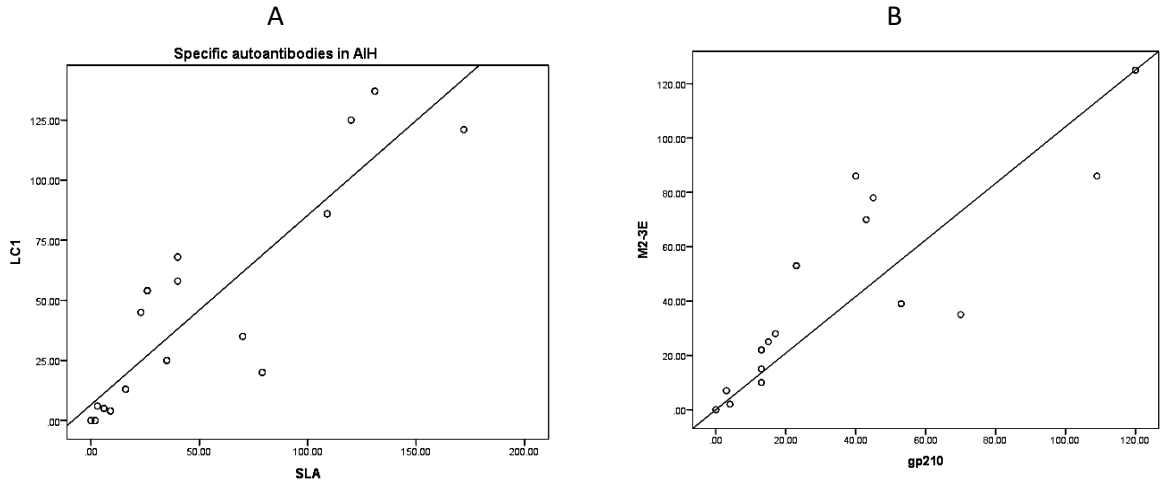
Chi square test or fisher exact test. *P*1; AILD versus Hepatitis C, *P2*; AILD versus hepatitis B, *P*3; hepatitis C versus hepatitis B, *P4*; AILD versus hepatitis C and B. **P* > 0.05 is not significant (NS).

Table 3. Prevalence of specific liver autoantibodies in different types of autoimmune liver diseases.

Autoantibodies	AIH (N=16)	Primary biliary cirrhosis (PBC, N=5)	Overlap Syndrome (N=4)
SLA/LP	11 (68.8)	1 (20.0)	2 (50.0)
LC-1	14 (87.5)	0 (0.0)	4 (100.0)
LKM-1	11 (68.8)	1 (20.0)	2 (50.0)
AMA-M2	6 (37.5)	3 (60)	2 (50.0)
Ro52	8 (50.0)	2 (40.0)	4 (100.0)
Sp100	2 (12.5)	3 (60.0)	2 (50.0)
gp210	2 (12.5)	2 (40.0)	4 (100.0)
PML	0 (0.0)	4 (80.0)	4 (100.0)
M2-E3	3 (18.8)	1 (20.0)	2 (50.0)

Of the 25 AILD patients, AIH was detected in 16 (64%), PBC in 5 (20%) and 4 (16%) overlap syndrome. AIH was divided into type 1 AIH (6 cases) and type 2 AIH (10 cases). LC-1 and LKM-1 autoantibodies were detected in all cases of type 2 AIH. While in type 1 AIH, LC-1 was detected in 53.5% and LKM-1 in 26.7% only.

A direct strong correlation was detected between cytosolic soluble liver antigen/liver pancreas antigen (SLA/LP) and LC-1 autoantibodies in the AIH group and between gp210 and mitochondrial type M2-3E (BPO), a recombinant fusion protein consisting of different subunits of the enzyme complex M2, autoantibodies in PBC group (p≤0.001 for each) (Figure 1).



r= 0.78 $p \leq 0.001$ r=0.85 $p \leq 0.001$

Figure 1. A) Direct strong correlation between SLA/LP and LC-1 autoantibodies in the autoimmune hepatitis (AIH) group. B) Direct strong correlation between gp210 and M2-3E autoantibodies in primary biliary cholangitis (PBC) group.

Discussion

Diagnosis of AILDs is a challenging issue and depends on histology of liver biopsies, serological markers, including specific autoantibodies and enzymes that indicate the liver damage nature. Investigating pattern and titer of autoantibodies is important for the diagnostic scoring system of AILD.[11] In CVH, autoantibodies are important markers for prediction of disease severity and indicate a disease similar to AIH in positive individuals.[12] Given the above, we were interested in investigating the level of liver autoantibodies and their association with clinical features in AILD compared to CVH patients as controls.

In this study, the range of age of studied patients was 18-80 years with a mean age of 30.28± 10.57 years in AILD patients and a mean age of 55.0±10.99 years in CVH patients. This seems to be logic because all ages could be affected by AIH including children, while CVH is usually restricted to adults and the diagnosis mostly from the fourth decade of life, with a peak at age 50–60 years.[13,14]

Among our studied AILD patients, there was a female predominance (100% were females). This agreed with data of two previous studies conducted by Velikova et al., 2019[5] and Invernizzi et al., 2022,[15] who reported that the effect of sex hormones on innate immunity; estrogen in high levels reduces the synthesis of interleukin (IL)-6, interleukin-1β, and tumor necrosis factor by macrophages and monocytes, also decreases the activity of dendritic cells, as well as that of natural killer cells. They further added that genetic factors linked to the X chromosomes affect the predominance of AILDS in females.

In our study, albumin level, bilirubin, PT, CRP, and AFP levels were statistically significantly increased in CVH patients than AILD patients. This observation agreed with that of Amin et al., 2017,[16] who reported that all hepatic markers and inflammatory markers were more in CVH patients than in AIH patients. Moreover, a study carried out by Gatselis et al., 2015,[17] reported that liver biochemistry is not characteristic in AILD patients and does not correlate with the disease severity. It may even normalize despite the continuing activity on liver histology, which may result in a delayed diagnosis. While in CVH patients, Zhu et al., 2017,[18] declared that inflammatory markers are important in disease prognosis as inflammation in the liver is mediated by cytokines which play an important role in the pathogenesis of CVH.

In our study, the most predominant autoantibodies in the AIH group as determined by the line immune blot assay, were anti LC-1

(87.5%), anti SLA/LP (68.8 %), anti LKM-1 (68.8%) and anti Ro52(50%). While in a study conducted by Toh, 2017,[19] anti SLA/LP (23.1%), anti LKM-1 (15.4%) and anti-Ro52 (15.4%) were the most common autoantibodies in AIH group with no positive cases for anti LC-1.

Previous studies by Chen et al., 2015[20] and Kirstein et al., 2015,[21] highlighted that the anti SLA/LP autoantibodies are diagnostic autoantibodies for type 1 AIH, and may indicate a more severe disease, correlate with a poor outcome and relapse after drug withdrawal. This observation may explain the difference between the percentage of SLA/LP in our study (68.8 %) and other studies.

In our study, among PBC patients, the most prevalent autoantibodies were antibodies against PML (80%) and SP100 (60%), and AMA-M2 (60%) while anti gp-210 and anti Ro52 (40%) (Table 3). Nearly similar results about antibodies against AMA-M2 were presented by Velikova et al., 2019,[5] who found that antibodies against AMA-M2 (83.4%) were the most prevalent autoantibodies in PBC patients.

In 2018, Lindor and his coworkers[22] reported data in line with ours that PBC patients have antibodies against some cellular components and that anti-mitochondrial antibodies (AMAs) are the most prevalent ones. Also, the study performed by Bauer et al., 2021,[23] demonstrated the significance of Sp100 and PML antibodies in the diagnosis of PBC specifically in patients negative for AMA, Sp100 and PML proteins act as transcriptional regulators.

In our study, we were not able to identify PSC using our panel of autoantibodies. The same was demonstrated in a study by Toh, 2017,[19] who reported no PSC disease-specific autoantibodies. However, when PSC is suspected, perinuclear anti-neutrophil cytoplasmic antibodies and cytoplasmic anti-neutrophil cytoplasmic antibodies could help in diagnosis.

In our hepatitis C patients, the most prevalent specific liver autoantibodies were antibodies against LC-1 (66.7%), SLA/LP (38.9%), and LKM-1 (27.8%). This finding indicated more liver damage and longer duration of disease in those patients. This was in accordance with results by Himoto and Nishoka, 2013,[10] who reported that (22%) of chronic hepatitis C patients were positive for anti- LKM-1 antibodies. Anti LKM-1 can recognize epitopes expressed on the surface of hepatocytes which share homology with proteins of HCV. Thereby induce T cells and poly-reactive B cells.[12]

In our study, the autoantibody profile in hepatitis B cases, as determined by the line immunoassay, included anti Ro52 antibodies (85.7%), and (28.6%) for each of anti GP-210, anti-PML, anti-SLA/LP antibodies (Table 2). This observation agreed with that described by Li et al., 2015,[24] who reported that the autoantibody panel of line immunoassay in hepatitis B patients included anti Ro52 antibodies (28%) as the most prevalent followed by anti GP-210 (12%) and anti- PML antibodies (11%). However, Velikova et al., 2019,[5] found that all chronic hepatitis B cases in their study were negative for specific liver autoantibodies by line immunoblot assay. Li et al., 2015,[24] attributed the presence of autoantibodies in chronic HBV patients to the activated humoral response with Th2 cells producing IL-4, IL-5, and IL-10, which in turn promote production of antibodies rather than viral clearance.

However, lacking disease specificity, antinuclear antibodies were positive in about 50–75% of AIH patients.[25] In our study, ANA was detected in 52% of autoimmune liver disease patients. This is lower than that reported by Velikova et al., 2019,[5] who detected ANA in 84.6% of autoimmune liver disease cases and Cha et al., 2021,[26] who attributed their high percentage of ANA to predominance of type 1 AIH cases in their studied patients. However, in our study, we had a predominance of Type 2 (62.5%).

In our study, ANA was found in 61.1% of chronic hepatitis C cases and in 28.6% of chronic hepatitis B cases. In a study conducted by Wei et al., 2020,[27] ANA was detected in 13.9% of chronic hepatitis C patients and in 19.1% of CHB patients. However, in another study by Velikova et al., 2019,[5] there were no positive cases of ANA in either hepatitis C or B patients.

This controversy could be explained by different factors. Daschakraborty et al., 2012,[28] attributed this to the difference between study

populations as regards antibody titer or association with old age and female sex. Another study by Muta et al., 2015,[29] attributed these variations to the use of different drug classes, and a third study by Hu et al., 2019,[30] ascribed this to association with clinical characteristics.

A study by Sebode et al., 2018,[9] declared that anti-SLA/LP had the highest specificity for autoimmune hepatitis among all autoantibodies and often associated with severe disease. Also, LC-1 antibodies were indicators for type 2, although being not specific for AIH and that the coincidence of SLA/LP and LC1 indicated severe type 2 AIH. In our study, such observation was confirmed with the strong positive correlation between anti-SLA/LP antibodies and anti-LC-1 antibodies. Also, we found a positive correlation between anti- gp210 antibodies and anti- M2-3E antibodies in PBC cases. The study by Sarcognato et al., 2021,[31] reported that anti-gp210 has high specificity for PBC and is associated with more aggressive disease and therefore, could help to detect the clinical course of the disease. In conclusion, assessment of autoantibodies helped to distinguish AILD from other liver diseases such as viral hepatitis. The use of line immunoblotting could be proposed as a diagnostic tool for patients with AIH and PBC.

Author Contributions

AH, MIR, SA, RAR; contributed to study design and conception. RAR, MHA; performed the laboratory work. RAR, MHA; made the analysis and interpretation of data. MIR, MAH; examined the patients. MHA, MAH; collected samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB), Faculty of Medicine; Zagazig University (approval number IRB#:6086-3-5-2020).

Informed consent

A written informed consent was taken from each patient (or his/her guardian) before being included in the study.

References

1. Kathuria P, Arora S, Karna R, Kumar N, Kumar S, Kar P. (2021). An Interesting Case of Autoimmune Liver Disease. Ann Natl Acad Med Sci (India); 57: 62-64.
2. Floreani A, Restrepo-Jiménez P, Secchi MF, *et al*. (2018). Etiopathogenesis of autoimmune hepatitis. J Autoimmun; 95:133–143.
3. Jeong SH. (2018). Current epidemiology and clinical characteristics of autoimmune liver diseases in South Korea. Clin Mol Hepatol; 24: 10-19.
4. Nakamoto N, Sasaki N, Aoki R, *et al.* (2019). Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. Nat Microbiol; 4:492-503.
5. Velikova T, Ivanova-Todorova E, Kancheva L, *et al.* (2019). Serological Differential Diagnosis of Autoimmune Liver Diseases by Line Blot Immunoassay for Parallel Detection of Nine Different Autoantibodies. Clin Exp Gastroenterol Hepatol; 1: 104.
6. Fernández MIC, Hernández DR, Cabrera Eugenio DE, Palanca W, Guridi ZD, González Fabián L. (2017). Diagnosis and treatment of autoimmune liver diseases in a tertiary referral center in Cuba. Curr Ther Res Clin Exp; 85: 8-14.
7. Zignego AL, Piluso A, Giannini C. (2008). HBV and HCV chronic infection: autoimmune manifestations and lymphoproliferation. Autoimmun Rev; 8: 107-111.
8. Park Y, Kim SY, Kwon GC, et al. *(2019).* Automated Versus Conventional Microscopic Interpretation of Antinuclear Antibody Indirect Immunofluorescence Test. *Ann Clin Lab Sci*; 49: 127-133.
9. Sebode M, Weiler-Normann C, Liwinski T, *et al.* (2018). Autoantibodies in Autoimmune Liver Disease-Clinical and Diagnostic Relevance. Front Immunol; 9:609.

10. Himoto T, Nishioka M. (2013). Autoantibodies in liver disease: important clues for the diagnosis, disease activity and prognosis. *Auto Immun Highlights*; 4: 39-53.
11. European Association for the Study of the Liver. (2017). EASL clinical practice guidelines: the diagnosis and management of patients with primary biliary cholangitis. J Hepatol; 67:145–172.
12. Yang DH, Ho LJ, Lai JH. (2014). Useful biomarkers for assessment of hepatitis C virus infection-associated autoimmune disorders. World J Gastroenterol; 20: 2962-2970.
13. Terziroli Beretta-Piccoli B, Mieli-Vergani G, Vergani D. (2022). Autoimmune hepatitis. *Cell Mol Immunol*; 19:158-176.
14. Oliveira AC, Bortotti AC, Nunes NN, El Bacha IA, Parise ER. (2014). Association between age at diagnosis and degree of liver injury in hepatitis C. Braz J Infect Dis; 18:507-511.
15. Invernizzi F, Cilla M, Trapani S, *et al*. (2022). Gender and Autoimmune Liver Diseases: Relevant Aspects in Clinical Practice. J Pers Med; 12:925.
16. Amin K, Rasool AH, Hattem A, Al-Karboly TA, Taher TE, Bystrom J. (2017). Autoantibody profiles in autoimmune hepatitis and chronic hepatitis C identifies similarities in patients with severe disease. World J Gastroenterol; 23: 1345-1352.
17. Gatselis NK, Zachou K, Koukoulis GK, Dalekos GN. (2015). Autoimmune hepatitis, one disease with many faces: etiopathogenetic, clinico-laboratory and histological characteristics. World J Gastroenterol; 21, 60-83.
18. Zhu S, Waili Y, Qi X, Chen Y, Lou Y, Chen B. (2017). Serum C - reactive protein predicts early mortality in hospitalized patients with HBV-related decompensated cirrhosis. Medicine; 96: e5988.
19. Toh BH. (2017). Diagnostic autoantibodies for autoimmune liver diseases. Clin Transl Immunology; 6: e139.
20. Chen ZX, Shao JG, Shen Y, Zhang J, Hua Y, Wang LJ. (2015). Prognostic implications of antibodies to soluble liver antigen in autoimmune hepatitis: a PRISMA-compliant meta-analysis. Medicine; 94: e953.
21. Kirstein MM, Metzler F, Geiger E, Heinrich E, Hallensleben M, Manns MP. (2015). Prediction of short- and long-term outcome in patients with autoimmune hepatitis. Hepatology; 62: 1524–1535.
22. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. (2018). Primary Biliary Cholangitis: Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology; 69:394–419.
23. Bauer A, Habior A, Wieszczy P, Gawel D. (2021). Analysis of Autoantibodies against Promyelocytic Leukemia Nuclear Body Components and Biochemical Parameters in Sera of Patients with Primary Biliary Cholangitis. *Diagnostics (Basel)*; 11:587.
24. Li BA, Liu J, Hou J, *et al.* (2015). Autoantibodies in Chinese patients with chronic hepatitis B: prevalence and clinical associations. World J Gastroenterol; 21: 283-91.
25. Liberal R, Mieli-Vergani G, Vergani D. (2013). Clinical significance of autoantibodies in autoimmune hepatitis. J Autoimmun; 46:17–24.
26. Cha HJ, Hwang J, Lee LE, Park Y, Song JJ. (2021). The significance of cytoplasmic antinuclear antibody patterns in autoimmune liver disease. PLoS One; 16: e0244950.
27. Wei Q, Jiang Y, Xie J, *et al.* (2020). Investigation and analysis of HEp 2 indirect immunofluorescence titers and patterns in various liver diseases. Clin Rheumatol; 39: 2425-2432.
28. Daschakraborty S, Aggarwal A, Aggarwal R. (2012). Non-organ-specific autoantibodies in Indian patients with chronic liver disease. Indian J Gastroenterol; 31:237-242.
29. Muta K, Fukami T, Nakajima MA. (2015). proposed mechanism for the adverse effects of acebutolol: CES2 and CYP2C19-mediated metabolism and antinuclear antibody production. Biochem Pharmacol; 98:659-670.
30. Hu J, Liu K, Luo J. (2019). HIV-HBV and HIV-HCV Coinfection and Liver Cancer Development. Cancer Treat Res; 177:231-250.
31. Sarcognato S, Sacchi D, Grillo F, *et al.* (2021). Autoimmune biliary diseases: primary biliary cholangitis and primary sclerosing cholangitis. Pathologica; 113: 170-184.